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Efficiency of RAPD markers in estimating the genetic relationship and development of DNA fingerprint in popular pigeonpea [*Cajanus cajan* (L.) Millsp.] cultivars

P. Ray Choudhury[#], I. P. Singh*, B. George[1], A. K. Verma, Jyoti Kumari[2], Shiv Datt[3], G. Gupta and N. P. Singh

Indian Institute of Pulses Research, Kanpur 208 024; [1]Jawaharlal Nehru University; [2]National Bureau of Plant Genetic Resources; [3]IPTM, Krishi Anusandhan Bhawan I, New Delhi 110 012

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Abstract

In the present study, estimation of genetic diversity and identification of pigeonpea cultivars and breeding lines has been done using 121 RAPD primers, among which 20 were polymorphic. A total of 1193 bands were obtained and 900 (75.4%) showed polymorphism between cultivars. On an average 9.85 bands per primer were obtained. Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped all the cultivars into three major clusters. The clustering was strongly supported by high bootstrap values. The arithmetic mean heterozygosity (H_{av}) value, the average heterozygosity for polymorphic markers (H_{av}) p value and marker index (MI) was found to be 0.499, 0.661 and 4.917 respectively. The genotype specific bands can provide information to separate pigeonpea cultivars among the 24 studied, which can definitely be of great help in pigeonpea cultivar identification for cultivar-right-protection.

Key words: Pigeonpea, RAPD, molecular diversity, DNA fingerprinting

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp] belongs to the subtribe *Cajaninae* of the leguminous tribe *Phaseolae* of the family *Fabaceae*. The chromosome number of pigeonpea is $2n = 2x = 22$. It has the genome size 1C = 858 Mbp. Pigeonpea is a hardy, widely adapted and drought tolerant crop with a large temporal variation (90-300 days) for maturity. These traits allow its cultivation in a wide range of environments and cropping systems. It is grown in Asia, Eastern and Southern Africa, Latin America and Caribbean countries. Globally, pigeonpea is cultivated on 4.92 million hectares with an annual production of 3.65 million tons and productivity of 898 kg ha^{-1}. Pigeonpea occupies the second position in terms of area and production in India after chickpea. India occupies over 70% of world's area and production in pigeonpea. Pigeonpea accounts for around 15% of area (3.555 million hectare) under pulses and 17% of the total pulse production (2.619 million tones) in the country (http://faostat.fao.org/).

Among the variety of usage of pigeonpea, the dry seeds are cooked to make thick soup (*dhal*). It is one of the major sources of protein to the predominantly vegetarian Indian population. The protein content of pigeonpea seed is comparable with any pulse crop and ranges between 18.5 to 26.3%. The seed husks, pod walls and green leaves are used as cattle feed and leaves are used to feed silk worms. Being a legume it fixes atmospheric nitrogen. The leaf fallen at maturity not only adds to organic matter in the soil, but also provides additional nitrogen.

Estimation of genetic diversity and its exploitation in breeding programme is essential for crop improvement. Widely adopted PCR based marker technologies such as RAPD, ISSR and STMS amplify different regions of the genome as they are dispersed across the plant genome and, have their own advantages and disadvantages. RAPD representing the simplest and fastest detection technology, identifies multi locus markers [1] and used in numerous crops species for detecting genetic diversity including pulses

*Corresponding author's e-mail: ipsingh1963@yahoo.com
[#]Present address: ICAR, Krishi Bhawan, New Delhi
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and legumes. Limited pools of pigeonpea germplasm have been characterized previously through restriction fragment length polymorphism (RFLP) [2], randomly amplified polymorphic DNA (RAPD) [3], or microsatellites simple sequence repeats (SSRs) [4, 5], amplified fragment length polymorphism (AFLP) [6] and diversity arrays technology (DArT) [7]. In the current study we assessed the genetic diversity and relationships of twenty four popular cultivar/advance breeding lines developed in different national/ international research institutes/universities in India using RAPD marker. Attempts were also made to determine the efficiency of RAPD primers to identify the genotypes and to develop a DNA fingerprint of the selected cultivars.

Materials and methods

A total of 24 elite cultivars of pigeonpea with different plant types, maturity group, pedigree and adapted to several agro-climatic zones were selected for genetic diversity analysis. All the cultivars and advanced lines used in the present study have been developed in National and International research institutes/ universities located in India. All of these germplasms (Table 1) were collected from core collection maintained at Indian Institute of Pulses Research, Kanpur, India.

Isolation of genomic DNA from young seedlings was carried out from each pigeonpea genotype based on a modified protocol without liquid nitrogen [8]. Purification of DNA was done by extracting with PCI (phenol: chloroform: iso-amyl alcohol, 25:24:1 respectively) and RNA was removed by RNAse treatment. Purified DNA was checked for its quality and quantity by 0.8% agarose gel electrophoresis using uncut lambda (λ) DNA as standard marker (300 ng/µl). Dilution of the DNA was done using TE buffer to a concentration of approximately 12.5 ng/ µl for use in PCR analysis.

A total of 121 RAPD primers (Operon Technologies, Alameda, CA, USA) out of 160 primers tested were employed in PCR analysis and 45 cycles were provided for amplification. PCR products were separated through electrophoresis on a 1.5% agarose gel. The amplified products were visualized and documented under UV light source using O'Gene Ruler ^{TM} 100 bp DNA Ladder Plus (Fermentas Life Sciences, USA) as molecular weight marker.

DNA bands were scored '1' for its presence and '0' for its absence for each primer genotype combination. Estimation of genetic similarity between the cultivars were done using Jaccard's similarity coefficient. Software NTSYS-pc [9] was used for clustering using UPGMA (unweighted pair group method using arithmetic averages). Support for clusters was evaluated by bootstrap analysis with Win Boot software [10]. Multidimensional 2D plot analysis was done to compare and support the clustering pattern obtained by UPGMA dendrogram. The expected heterozygosity for a genetic marker (H_n) was calculated by $H_n = 1-p_i^2$ (pi is the allele frequency of the ith allele; [11]. By using the values of H_n, H_{av} (the arithmetic mean heterozygosity) was calculated by $H_{av} = \Sigma H_n/n$ where, n = number of markers or loci analysed [12]. The average heterozygosity for polymorphic markers $(H_{av})p$ was derived as $(H_{av})p = \Sigma H_n/np$ (np = no. of polymorphic markers or loci). Marker index (MI) was also calculated as MI = E $(H_{av})p$; where E = effective multiplex ratio ($E = n_p \times (n_p/n)$ EMR (E) is the product of the fraction of polymorphic loci and the number of polymorphic loci.

Results and discussion

Detection of polymorphism

Out of a total of 160 primers tried, 121 primers showed unambiguous amplification and produced 1193 amplified products ranging from about 500 bp to 250 bp, among which 900 (75.44%) bands were found to be polymorphic. On an average 9.85 bands per primer were obtained and a representative of the amplified products obtained with the average products value (9.85 i.e. 10) or more has been described (Table 2). A total of 103 RAPD products produced by 59 RAPD primers were recorded as unique and genotype specific. DNA amplification pattern as detected by some of the RAPD primers has been provided in Fig. 1. Earlier, protein/ isozyme analysis was done to estimate variability in pigeonpea [13] but was not much informative due to limited polymorphism among the closely related cultivars. RAPD, being a dominant marker and its usefulness to detect multiple locus in a fast and cost effective manner, has been found to be an efficient tool to evaluate and reveal molecular diversity not only in pulses crops like chickpea [14], field pea [15] and mungbean [16], but also in cereals crops like rice [17], wheat [18] and maize [19]. Genetic variability obtained by RAPD was found very close to that obtained with marker systems like isozyme, SSR and ISSR and mean allelic frequency was found highest for RAPD [20]. Similarly RAPD has been found to be well correlated with other marker system in detecting diversity in pigeonpea. In our study, 75.44% fragments were found to be polymorphic with an average of 9.85 bands per

Table 1. Brief information about pigeonpea germplasms used in the present study

S.No.	Name of genotype	Institution where developed	Distribution in India	Morphological characters	Pedigree
1.	UPAS 120	GBPUA&T, Uttaranchal	North-west and North east plain zone	EM, Ss, I, HY	Selection from P 4768
2	MAL 6	BHU (U.P.)	Northern and central zone	LM, S, I, resistant to SMD	MA-2 X Bahar
3.	MAL 13	BHU (U.P.)	North East Plain Zone	LM, S, I, resistant to SMD, LS	(MA-2 X MA 166) X Bahar
4.	PDA 10	IIPR (U.P.)	North East Plain Zone	LM, EC, I, purple stem, resistant to SMD, LS	Local Selection from Akbarpur, Kanpur Dehat (U. P.)
5.	PDA 92-1	IIPR (U.P.)	North East Plain Zone	LM, S, I, resistant to SMD & wilt	Bahar x ICP 8863
6.	IPA 402	IIPR (U.P.)	North East Plain Zone	LM, S, I, purple stem, resistant to SMD, LS	Local selection from Jaunpur (U.P.)
7.	IPA 602	IIPR (U.P.)	North East Plain Zone	LM, EC, I, resistant to SMD & wilt	Bahar x ICPL 84023
8.	IPA 3-1	IIPR (U.P.)	North East Plain Zone	LM, EC, I, green pods, resistant to SMD	Bahar x ICPL 96058 (ICRISAT line)
9.	IPA 3-2	IIPR (U.P.)	North East Plain Zone	LM, EC, I, green pods, resistant to SMD & wilt	Bahar X ICPL 96058 (ICRISAT line)
10.	ICPL 84023	ICRISAT (A.P.)	Southern zone	EM, Ss, D, resistant to SMD & wilt	ICP 6997 x ICP 7220
11.	ICPL 88039	ICRISAT (A.P.)	Southern zone	EM, Ss, I, resistant to SMD	ICPL 6 x Pant A-2
12.	ICP 8863	ICRISAT (A.P.)	Southern Zone	MM, S, I resistant to wilt, HY	Selection from land race of Maharashtra
13.	ICPL 87119	ICRISAT (A.P.)	Western and South-Central part of India	MM, Ss, I, resistant to wilt & SMD, HY	C-11 X ICPL 6
14.	Pusa 9	IARI (New Delhi)	North East Plain Zone	LM, EC, I, resistant to SMD, HY	UPAS 120 X 3673
15.	Pusa 992	IARI (New Delhi)	North-west Plain Zone	EM, Ss, I, HY, LS	Selection from ICPL 90306
16.	CO 5	TNAU (T.N.)	Southern Zone	EM, Ss, I, drought tolerant, bushy	Mutant of Co 1
17.	CO 6	TNAU (T.N.)	Southern Zone	MM, Ss, I, resistant to SMD, HY	Mutant of SA 1
18.	BSMR 853	ARS, Badnapur	Western & Central part of India	MM, S, I, red seeded, resistant to wilt and SMD, HY	(ICP 7336 X BDN-1) x BDN-2
19.	Amar	CSAUA&T, (U.P.)	North East Plain Zone	LM, EC, I, resistant to SMD, HY	Selection from Bahar
20.	Bahar	RAU, Pusa, Bihar,	North East Plain Zone	LM, EC, I, resistant to SMD, LS, HY	Selection from land race of Motihari, Bihar
21.	T-7	CSAUA&T, (U.P.)	Northern, Central and Eastern part of India	LM, EC, I, LS, HY	Selection from land race of Lucknow
22.	DA-11	RAU, Pusa, Bihar,	Eastern part of India	LM, EC, I, cream colour seeds, green pods, HY	Bahar x NP (WR) 15
23.	NDA-1	NDUA&T, Faizabad, U.P.	Northern part of India	LM, EC, I, resistant to SMD & tolerant to wilt, HY	Selection from land race of Faizabad, (U.P.)
24.	KPL 43	IIPR (U.P.)	North East Plain Zone	LM, Ss, I, resistant to SMD & wilt and tolerant to stem blight, LS	Selection from Bahar

Abbreviation of the institutions/universities and morphological features:
BHU: Benaras Hindu University, IIPR: Indian Institute of Pulses Research, ICRISAT: International Crops Research Institute for the Semi-Arid Tropics, IARI: Indian Agricultural Research Institute, TNAU: Tamil Nadu Agricultural University, ARS: Agricultural Research Station, CSAUA&T: Chandra Shekhar Azad University of Agriculture and Technology, RAU: Rajendra Agricultural University, NDUA&T: Narendra Dev University of Agriculture and Technology, GBPUA&T: Govind Ballabh Pant University of Agriculture and Technology. Early maturing (EM), Spreading (S), Semi-spreading (Ss), Indeterminate (I), Determinate (D), Late maturing (LM), Medium Maturing (MM), Sterility mosaic disease (SMD), Erect and compact (EC), High yield (HY), Large seeded (LS).

primer, whereas, Ratnaparkhe *et al.* [3] obtained 7.93 bands per primer using 16 polymorphic RAPD primers in 10 cultivars. In the present study, the range of genetic diversity using 121 RAPD primers was found to be higher as compared to AFLP primers, where the value was observed to be 0.82-1.00 with little polymorphism of 13.28% in 20 cultivars [6], however, narrow genetic background of pigeonpea used in the study could be a probable reason for the low levels of genetic polymorphism estimated by AFLP. Yadava *et al.* [21] studied genetic diversity of pigeonpea (*Cajanus cajan* (L.) Millsp.) cultivars and its wild relatives using RAPD markers that could delineate between cultivated and wild species. DArT analysis could not show clear differentiation among cultivars from different regions, It is worth noting that Yang *et al.* [7] in their high throughput diversity analysis (DArT) of 46 cultivated and 50 wild accessions of pigeonpea using 700 markers only 64 markers detected variation in 48 accessions and 50% of these had one of the two markers present at low frequency (below 5%).

Detection of heterozygosity and marker index

Marker efficiency and utility in terms of quantitative estimation and polymorphism can be expressed in mean heterozygosity and marker index [12]. Polymorphism within a population is detected by the number of alleles present at a locus and their frequency, whereas heterozygosity is the probability that two alleles taken at random from a population can be distinguished using a marker system [22]. Heterozygosity was calculated for the 1193 amplified products obtained by employing 121 RAPD primers across the varieties. The Hav and (Hav)p were found to be 0.499 and 0.661, respectively, whereas the marker index (MI) value was obtained to be 4.917. Based on biochemical marker system allozyme, the Hav was found to be 0.027 in cowpea [23] and 0.342 in wild lentil [24]. When RAPD was used as marker system, the Hav value was found to be 0.20 and 0.35 in two trigonella species respectively [22]. In our present study, the Hav and (Hav)p values were found to be higher (0.499 and 0.661 respectively), whereas the marker index (MI) was obtained to be 4.917 and thus proving the usefulness of RAPD as a marker system in detecting heterozygosity in pigeon pea.

Genetic relationship and genotyping

Similarity coefficient data among pairs of pigeonpea cultivars found to vary considerably (0.556 to 0.805, Table 3) with an average value of 0.74. The range of genetic similarity obtained in our study found to be wider as compared to 0.7 to 0.9 obtained by Ratnaparkhe *et al.* [3] because of diversified geographical distribution, varied maturity group and different pedigree of the selected genotypes. The pair of genotypes indicated maximum similarity was CO5 and CO6 with a similarity value of 0.805. Other genotypes showed high degree

Table 2. Primer name and amplified products obtained with the average value of 10 bands (approx.) or more per primer across the germplasms tested for molecular diversity analysis

Primer name	Total no.of band	No. of poly-morphic band	Primer	Total no.of band	No. of poly-morphic band
OPA1	12	12	OPBB13	10	10
OPAQ 04	15	12	OPBB14	13	13
OPAQ 05	19	9	OPBB16	19	19
OPAQ 06	11	0	OPBB17	11	8
OPAQ 07	12	11	OPX 03	10	8
OPAQ 12	15	4	OPX 04	10	8
OPAQ 13	14	4	OPX 11	12	8
OPAQ 14	12	10	OPH 01	13	13
OPAQ 15	17	13	OPH02	10	9
OPAQ 18	21	20	OPH 03	14	12
OPAQ 19	20	19	OPH 04	10	8
OPAQ 20	18	14	OPH 08	10	4
OPAZ 05	10	9	OPH 09	16	15
OPAZ 08	11	8	OPH 10	11	10
OPAZ 09	12	5	OPH11	17	16
OPAZ 11	10	6	OPH 12	11	5
OPAZ 12	11	5	OPH 13	11	7
OPAZ 18	14	14	OPH 17	12	9
OPBA6	13	6	OPP 04	15	15
OPBA8	11	9	OPP 05	15	14
OPBA 19	14	13	OPP 06	10	4
OPBA20	14	14	OPP 07	11	9
OPBB 4	12	8	OPP 08	17	15
OPBB5	15	14	OPP 09	14	12
OPBB6	11	10	OPP 10	19	17
OPBB7	15	15	OPP 11	10	9
OPBB8	13	12	OPP 12	16	10
OPBB9	14	5	OPP 13	10	5
OPBB10	15	15	OPP 14	15	10
OPBB12	16	7	OPP 15	12	10

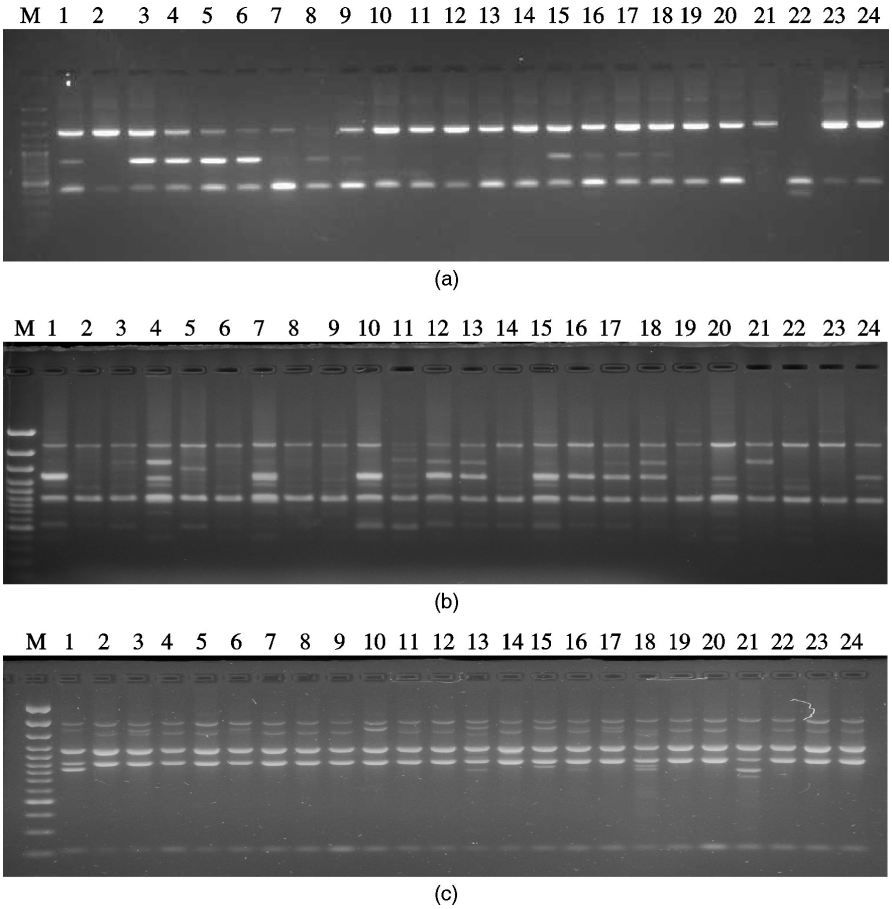

Fig. 1. RAPD profile of pigeonpea cultivars obtained with primers OPA12 (a), OPP 09 (b) and OPA 08 (c). Serial number of the varieties corresponds to table 1. M=Standard DNA marker, 100 bp DNA ladder plus

of similarity was IPA 602 and PDA 10; Pusa 992 and ICPL 84023; Pusa 9 and ICPL 87119 and IPA 3-1 and IPA 3-2. Least similarity (0.556) was found between UPAS 120 and T7. It was also observed that T7 showed considerable amount of diversity with ICPL 84023, ICPL 8863 and ICPL 88039. NDA-1 also found to show high diversity with most of the genotypes. Multivariate (cluster) analysis of the genetic similarity data grouped the cultivars into three major clusters (I, II and III, Fig. 2). Cluster II is further divided into two sub-clusters (i, ii) with two sub-groups in each sub-cluster (*viz*., II(i)a, II(i) b and II(ii) a, II (ii) b). However, two cultivars (NDA-1 and T7) showed considerable diversity and could not be included in any cluster. Bootstrap analysis was used to evaluate the degree of support for clusters within the dendrogram. It was observed that clusters and sub-clusters within the dendrogram were supported by high bootstrap values, thus further indicated that RAPD system, if standardized properly, could be used in a precise manner to classify the genotypes properly.

Cluster I comprises of seven genotypes *viz.,* UPAS 120, PDA 10, IPA 602, ICP 84023, Pusa 992, ICP 8863 and Bahar. These genotypes are being mostly grown in high rainfall areas of north-east plain zone and south zone. Plant type of these genotypes is semi-spreading/ compact along with indeterminate growth habit. Bahar and ICP 84023 are parents of IPA 602. PDA 10 and ICP 8863 are selections from the land races of their respective zones. Pusa 992 and UPAS 120 both are selections from the ICP germplasm lines of ICRISAT. In the sub-cluster II (i), all the five genotypes possess one common parent, Bahar. The sub-group II (i) a contains two genotypes (IPA 3-1 and IPA 3-2) and both have been developed from the same cross (Bahar x ICPL 96058). In the sub-group II(i)b, KPL 43 and Amar both are selections from Bahar, whereas, another cultivar DA-11 has Bahar as one of its parent (Bahar x NP(WR) 15). In the sub-group II (ii) a, there are three genotypes *viz.,* ICPL 88039, ICPL 87119 and Pusa 9. These genotypes have indeterminate growth habit, and spreading/ semi-

Table 3. Genetic similarity matrix (Jaccard's coefficient) of the pigeonpea cultivars analysed in the present study

	UPAS-120	MAL-6	MAL-13	PDA-10	PDA 92-1	IPA 402	IPA 602	IPA 3-1	IPA 3-2	ICP 84023	ICP 88039	ICP 8863	ICP 87119	PUSA 9	PUSA 992	CO5	CO6	BMSR 853	AMAR	BAHAR	T-7	DA11	NDA 1	KPL 43
UPAS-120	1.000																							
MAL-6	0.703	1.000																						
MAL-13	0.641	0.756	1.000																					
PDA-10	0.737	0.706	0.719	1.000																				
PDA92-1	0.639	0.742	0.755	0.716	1.000																			
IPA402	0.671	0.757	0.762	0.746	0.767	1.000																		
IPA602	0.721	0.687	0.685	0.796	0.717	0.746	1.000																	
IPA3-1	0.644	0.703	0.715	0.752	0.722	0.754	0.752	1.000																
IPA3-2	0.673	0.679	0.686	0.733	0.697	0.722	0.736	0.777	1.000															
ICP84023	0.727	0.652	0.647	0.759	0.661	0.679	0.796	0.705	0.707	1.000														
ICP88039	0.677	0.695	0.673	0.718	0.683	0.699	0.713	0.727	0.755	0.735	1.000													
ICP8863	0.694	0.663	0.646	0.745	0.667	0.681	0.739	0.668	0.690	0.738	0.718	1.000												
ICP87119	0.666	0.674	0.650	0.687	0.667	0.680	0.689	0.692	0.724	0.677	0.738	0.709	1.000											
PUSA9	0.656	0.699	0.669	0.714	0.678	0.688	0.694	0.722	0.724	0.699	0.754	0.695	0.778	1.000										
PUSA992	0.735	0.672	0.649	0.762	0.679	0.707	0.763	0.688	0.688	0.788	0.715	0.755	0.721	0.751	1.000									
CO5	0.663	0.688	0.688	0.699	0.696	0.706	0.709	0.738	0.738	0.683	0.729	0.686	0.768	0.747	0.739	1.000								
CO6	0.691	0.718	0.707	0.722	0.703	0.726	0.735	0.729	0.746	0.701	0.756	0.722	0.765	0.747	0.738	0.805	1.000							
BMSR853	0.642	0.680	0.674	0.672	0.671	0.695	0.677	0.697	0.671	0.663	0.693	0.677	0.719	0.699	0.704	0.773	0.746	1.000						
AMAR	0.664	0.713	0.687	0.709	0.697	0.713	0.722	0.751	0.739	0.695	0.751	0.698	0.745	0.759	0.726	0.743	0.769	0.732	1.000					
BAHAR	0.684	0.682	0.664	0.723	0.656	0.691	0.735	0.703	0.683	0.703	0.701	0.696	0.678	0.689	0.734	0.677	0.703	0.682	0.755	1.000				
T-7	0.556	0.607	0.614	0.591	0.605	0.628	0.589	0.602	0.572	0.557	0.570	0.565	0.577	0.591	0.589	0.582	0.594	0.604	0.608	0.599	1.000			
DA11	0.638	0.653	0.657	0.688	0.652	0.674	0.699	0.724	0.719	0.657	0.708	0.653	0.702	0.714	0.675	0.697	0.719	0.681	0.749	0.699	0.594	1.000		
NDA 1	0.597	0.693	0.655	0.636	0.674	0.695	0.629	0.665	0.644	0.617	0.629	0.621	0.610	0.638	0.635	0.640	0.671	0.639	0.683	0.636	0.619	0.637	1.000	
KPL 43	0.634	0.698	0.689	0.693	0.694	0.698	0.700	0.733	0.710	0.683	0.698	0.671	0.711	0.726	0.687	0.727	0.749	0.698	0.756	0.698	0.594	0.733	0.696	1.000

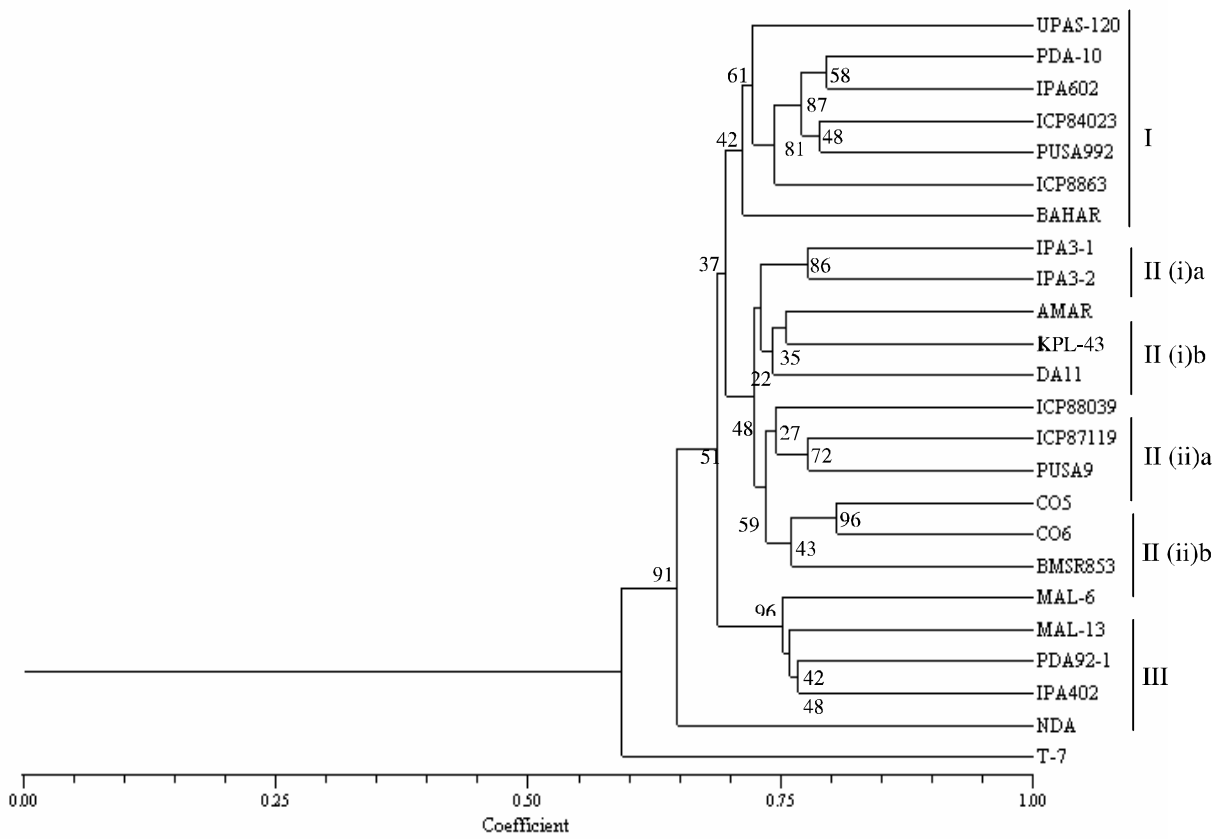

Fig. 2. Dendrogram of pigeonpea varieties constructed using UPGMA based on 121 RAPD primers. The major clusters and sub-clusters are indicated on right margin. Numbers at branch points indicate support for varieties clustered; values are percent of bootstrap sample that exhibited the cluster

spreading plant type. ICPL 87119 and Pusa 9 both have been developed through hybridization. All the three genotypes possess resistance to sterility mosaic. All the three genotypes *viz*., CO5, CO6 and BMSR 853 of the sub-group II (ii) b are being grown in the same agro-climatic and geographical region i.e. southern and south-western part of India. CO 5 and CO 6 both is mutant varieties and developed from local land race from south India. Moreover, BSMR 853 is also a progeny of local land race from south-west part of India. These varieties possess semi-spreading/spreading type of plant. The cluster number III consists of four genotypes *viz.,* MAL 6, MAL 13, PDA 92-1 and IPA 402. All these four genotypes belong to long duration group, possess indeterminate growth habit and are being grown in north-east plain zone of India. The other two genotypes (IPA 402 and Bahar) in the cluster are local collection from farmer's field of north-eastern part of India i.e. Jaunpur district of U.P. and Motihari district of Bihar, respectively. Two cultivars NDA 1 and T-7 was not included in any cluster and developed from diverse land races of northern part of India.

Three genotypes MAL 6, Mal 13 and PDA 92-1 have one common parent 'Bahar'. However, few other genotypes such as DA-11 IPA 3-2, IPA 3-1, IPA 602 and PDA 92-1which also include Bahar as one of the parent are not included in cluster III. Such low level of correlation between genetic similarity based on pedigree and DNA profiles has been reported earlier in different crop species [25]. There can be several possible explanations for such results. Selection and genetic drift, which play a significant role in variety development, are not taken into account in pedigree records. It is also possible that the molecular markers employed in these studies are insufficient to assay a significant proportion of the genome. Similar results were obtained when morphological traits were compared among genotypes in the cluster. Since, quantitative morphological traits used for the genetic diversity analysis are influenced by environmental conditions and can show considerable variation among genotypes studied. We also made an attempt to correlate the relationship measures based on RAPD markers, Distribution pattern, pedigree data and morphological traits in pigeonpea accessions. However, we were unable to correlate these

measurements with our fingerprint data, probably due to insufficient morphological data. Similar attempts to correlate AFLP markers with pedigree and morphological features went unsuccessful because of the same reasons [6]. Under such situations where the cultivars could not be differentiated with specific morphological features the fingerprint pattern obtained in this analysis could be useful for preliminary identification of closely related cultivars.

Identification and development of DNA fingerprint of pigeonpea genotypes

Appropriate identification of cultivars is pre-requisite to duplicate detection, variety registration and protection of plant breeders' right. For defining DUS (distinctiveness, uniformity and stability), DNA data is well accepted along with the morphological data. The genotype specific bands can provide information to separate pigeonpea cultivars among the 24 studied, which can definitely be of great help in pigeonpea cultivar identification for cultivar-right-protection. In the present study, creation of a basic fingerprint pattern using RAPD as a marker system has been done. In this present study, a total of 1193 amplified product was generated out of which 102 products were recorded as genotype specific and could be used as a ready reference for fingerprint. Similar strategy was used in the identification of number of grapevine cultivars [26], pisum lines [27] and rice [28]. Unique banding pattern specific to all the cultivars except MAL 13 and IPA 402 were produced, out of which a maximum of 12 unique products were produced by the cultivar ICP 8863 followed by 10 products by UPAS 120 and 9 products by BMSR 853. These bands in due course could be converted into CAPS or SCAR marker for varietal confirmatory tests.

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