# RAPD Analysis among Pigeon pea [*Cajanus cajan* (L.) Mill sp.] Cultivars for their Genetic Diversity

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**Abstract**

The genetic diversity of 17 cultivars of pigeon pea using 17 random amplified polymorphic DNA (RAPD) markers has been reported. A total of 198 bands were scored corresponding to an average of 11.6 bands per primer with 148 bands showing polymorphism (74.7%). Nine out of eighteen primers gave more than 80% polymorphism. Jaccard similarity coefficient ranged from 0.272 to 0.778. A dendrogram constructed based on the UPGMA clustering method revealed two major clusters. Cluster-I comprises of 12 cultivars which was further differentiated into two sub-clusters having six cultivars each. The cluster-II includes remaining five cultivars. The cultivar IPA-3088 was quite unique from the remaining cultivars as evident in the dendrogram.

**Keywords:** RAPD; Pigeon pea; Genetic diversity; Dendrogram; Cultivars.

## Introduction

Pigeon pea (*Cajanus cajan* (L.) mill sp.) is one of the major grain legume (pulse) crops of the tropics and subtropics. The Indian subcontinent, accounts for about 90% of the global production. Its seed protein content (approximately 21%) is also well comparable with that of other major grain legumes [1]. Determination of genetic diversity of any given crop species is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a breeding scheme. The early systematic studies of the genus *Cajanus* were based on phonological or morphological characters, which have been shown to have limited genetic resolution especially at species levels, as is required for pigeon pea [1, 2].

Randomly 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 [3, 4]. RAPD, being a multi locus marker [5] 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* [6], *Vigna radiata* [7, *Lens* sp. [8, 9], *Phaseolus* sp.[10, 11], *Glycine* sp. [12, 13], *Cicer* sp. [14], *Pisum* sp. [15, 16] and *Cajanus cajan* [17, 18, 19]. Genetic variability of pigeon pea has been studied using several other genetic markers such as RFLP [20, 21], AFLP [22, 23], microsatellite markers [24] and Diversity Array Technology [25]. This paper reports assessment of genetic diversity among 17 pigeon pea cultivars with 17 RAPD primers.

## Methods

### *Plant material*

Seventeen pigeon pea cultivars *viz*. Pusa 9, Bahar, AL-201, IPA-337, IPA-61, IPA-20, IPA-34, IPA 341, IPA-242, IPA-2013, IPA-285, T7, IPA-204, IPA-3088, IPA-234, IPA-98-3 and UPAS-120 collected from Indian Institute of Pulse Research, Kanpur; Punjab Agriculture University, Gurdaspur and G.B. Pant University of Agriculture and Technology, Pantnagar

were used in present study. Plants were grown in pots and leaf samples pooled from all plants of each cultivar were collected into labeled bags and stored in -20ºC freezer prior to genomic DNA isolation.

***Genomic DNA isolation***

Genomic DNA was isolated from germinating seedlings using CTAB based method [26], analyzed and quantified by standard methods [27].

***PCR amplification using RAPD primers***

Seventeen oligonucleotides primers selected from available literature (Table 1) were synthesized from Bangalore Genei, India Pvt. Ltd, India. PCR was carried out in 25µl reaction volumes containing 2.5 µl of 10 x *Taq* assay buffer (Tris with 15mM $MgCl_2$), 10 mM of each dATP, dCTP, dGTP and dTTP, 1U Taq polymerase, 16.5ng of primer and approx. 50ng of template DNA. Amplification were carried out in a thermo-cycler (Eppendorf, Germany) programmed for 45 cycles with an initial denaturation at 95$^0$C for 5 min followed by cycling conditions of denaturation at 92$^0$C for 1min, annealing at 3 min at 35$^0$C and extension at 72$^0$C for 2 min. After 45 cycles, there was a final extension step of 3 min at 72$^0$C. The amplicons were analyzed on 1.8% agarose gels and detected by staining with ethidium bromide. UV trans- illuminated gels were photographed with gel documentation system (Alpha Innotech, USA).

**Table 1:** List of RAPD primers used for RAPD profiling.

| S.No | Code of primers | Sequence (5'-3') | Percentage G+C content | Number of base pairs | Reference |
|---|---|---|---|---|---|
| 1 | 12ES10G23 | GTAGGCGTCG | 70 | 10 | [28] |
| 2 | 13ES10C24 | GGCTCGTACC | 70 | 10 | |
| 3 | 14ES10A25 | GACCCCGGCA | 80 | 10 | |
| 4 | 15ES10A26 | CAGGGGACGA | 70 | 10 | |
| 5 | 16ES10C27 | CGCCACGTTC | 70 | 10 | |
| 6 | 17ES10C28 | GCCTCCTACC | 70 | 10 | |
| 7 | 5ES23C16 | GCATCACAGCCTGTTATTGCCTC | 52.1 | 23 | |
| 8 | 19ES10T30 | CAGGGCCGCT | 80 | 10 | |
| 9 | 22ES10G33 | AGGCCCGATG | 70 | 10 | |
| 10 | 21ES10A32 | CTCGGCTGGA | 70 | 10 | |
| 11 | 20ES10A31 | CTCTCCGCCA | 70 | 10 | |
| 12 | 18ES10G29 | GGCGTCGGGG | 90 | 10 | |
| 13 | 9ES18G20 | ACTTACCTGCCTACGCGG | 61.1 | 18 | [29] |
| 14 | 11ES18T22 | GTAAGTCAGAGGGCCAGG | 61.1 | 18 | |
| 15 | 10ES18T21 | CCGGCAGGTCAGGTAAGT | 61.1 | 18 | |
| 16 | 6ES18C17 | ACTTACCTGAGGCGCGAC | 61.1 | 18 | |
| 17 | 8ES18G19 | ACTTACCTGCCTGCCGAG | 61.1 | 18 | |

***Data collection and analysis***

The amplification products were scored separately for each primer because of presence or absence of band corresponding to each cultivars i.e., use of binary code 1 and 0 for the presence or absence of band, respectively regardless of its intensity. Molecular size (bp) of amplified DNA fragment was determined by the 100 bp ladder & λ *Hin*d III/ *Eco*RI double digested marker that was loaded in separate well of agarose gel for each gel. DNA fragment analyses

were performed using the SPSS 12.0 computer software. Dendrogram was constructed from the dissimilarity matrix using UPGMA procedure [30] clustering method.

**Results and Discussion**

A total of 198 bands were scored for the 17 RAPD primers ranging from 7 to 15, corresponding to an average of 11.6 bands per primer, and 74.7% (148 bands) of these were polymorphic. Polymorphic bands for each primer ranged from 25% to 92.3%. Nine out of eighteen RAPD primers showed more than 80% of polymorphism. The size of the amplified fragment ranged from 89 bp to 7901 bp (Table 2).

**Table 2**: The number of loci detected in different cultivars of pigeon pea using random primers.

| Primer code | Total no. of loci | Monomor-phic loci | Polymor-phic loci | % polymorphism | Approxi-mate range of fragment size (bp) | Unique loci | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | No. | Germplasm | Size (bp) |
| 12ES10G23 | 13 | 2 | 11 | 84.6 | 6232 to 211 | - | - | - |
| 13ES10C24 | 13 | 1 | 12 | 92.3 | 5258 to 435 | - | - | - |
| 14ES10A25 | 8 | 1 | 7 | 87.5 | 2817 to 148 | - | - | - |
| 15ES10A26 | 15 | 2 | 13 | 86.6 | 7429 to 398 | - | - | - |
| 16ES10C27 | 14 | 8 | 6 | 42.8 | 7223 to 89 | - | - | - |
| 17ES10C28 | 14 | 3 | 11 | 78.6 | 7147 to 195 | 3 | IPA-3088 | 341, 277, 224 |
| 9ES18G20 | 7 | 2 | 5 | 71.4 | 4122 to 261 | - | - | - |
| 11ES18T22 | 14 | 2 | 12 | 85.7 | 4058 to 240 | - | - | - |
| 10ES18T21 | 12 | 1 | 11 | 91.6 | 3495 to 157 | 1 | IPA-3088 | 2234 |
| 6ES18C17 | 8 | 1 | 7 | 87.5 | 11549 to 256 | - | - | - |
| 7ES18A18 | 9 | 4 | 5 | 55.5 | 5110 to 381 | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 19ES10T30 | 12 | 9 | 3 | 25 | 5244 to 156 | - | - | - |
| 22ES10G33 | 10 | 4 | 6 | 60 | 4423 to 362 | - | - | - |
| 21ES10A32 | 15 | 2 | 13 | 86.6 | 7797 to 250 | 1 | IPA-98-3 | 257 |
| 20ES10A31 | 13 | 1 | 12 | 92.3 | 7901 to 149 | - | - | - |
| 18ES10G29 | 13 | 4 | 9 | 69.2 | 6651 to 150 | - | - | - |
| 8ES18G19 | 8 | 3 | 5 | 62.5 | 5283 to 217 | - | - | - |

The RAPD cluster pattern is presented Figure 1. It showed two major clusters namely Cluster-I and Cluster-II comprising of 12 and 5 cultivars respectively. Cluster I includes 12 cultivars namely IPA-337, IPA-61, T7, IPA-204, IPA-98-3, IPA-234, IPA-341, IPA-242, IPA-20, IPA-34, IPA-2013 and IPA-285. This major cluster was further differentiated into two subclusters with subcluster I having cultivars IPA-337, IPA-61, T7, IPA-204, IPA-98-3, IPA-234 and subcluster II with cultivars IPA-341, IPA-242, IPA-20, IPA-34, IPA-2013, IPA-285. The major cluster-II includes only 5 cultivars namely Pusa 9, Bahar, AL-201, UPAS-120 and IPA-3088. The cultivar IPA-3088 occupies a distinct place as revealed in the dendrogram constructed (Figure-1) with four unique loci (Figure 2a, 2b). The amplification pattern revealed five unique loci with three RAPD primers namely 17ES10C28, 10ES18T21 and 21ES10A32 for cultivars IPA-3088 and IPA-98-3 (Table-2).

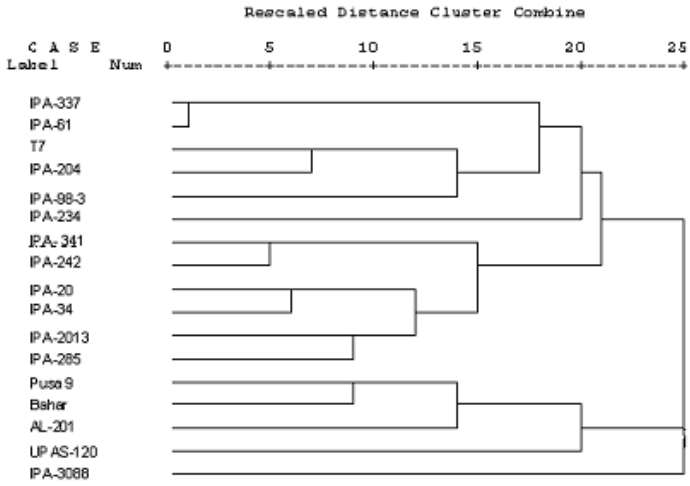


**Figure 1:** Dendrogram constructed using UPGMA cluster analysis.

**Table 3**: Similarity index of RAPD banding patterns among 17 pigeon pea cultivars.

| | Pusa 9 | Bahar | AL-201 | IPA-337 | IPA-61 | IPA-20 | IPA-34 | IPA-341 | IPA-242 | IPA-2013 | IPA-285 | T7 | IPA-204 | IPA-3088 | IPA-234 | IPA-98-3 | UPAS-120 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pusa 9** | | .653 | .509 | .534 | .456 | .374 | .432 | .362 | .365 | .347 | .459 | .471 | .493 | .335 | .331 | .286 | .460 |
| **Bahar** | | | .626 | .535 | .499 | .417 | .371 | .280 | .355 | .425 | .444 | .497 | .535 | .306 | .316 | .310 | .530 |
| **AL-201** | | | | .536 | .461 | .276 | .289 | .322 | .392 | .361 | .387 | .420 | .454 | .385 | .383 | .272 | .414 |
| **IPA-337** | | | | | .778 | .515 | .514 | .443 | .474 | .473 | .538 | .508 | .575 | .381 | .429 | .507 | .449 |
| **IPA-61** | | | | | | .717 | .576 | .424 | .379 | .477 | .536 | .526 | .515 | .408 | .446 | .505 | .418 |
| **IPA-20** | | | | | | | .697 | .505 | .502 | .639 | .576 | .567 | .434 | .428 | .446 | .505 | .481 |
| **IPA-34** | | | | | | | | .545 | .619 | .615 | .599 | .488 | .433 | .463 | .388 | .466 | .407 |
| **IPA-341** | | | | | | | | | .713 | .565 | .466 | .437 | .383 | .454 | .377 | .395 | .315 |
| **IPA-242** | | | | | | | | | | .672 | .572 | .420 | .454 | .427 | .362 | .375 | .393 |
| **IPA-2013** | | | | | | | | | | | .664 | .513 | .534 | .476 | .414 | .531 | .497 |
| **IPA-285** | | | | | | | | | | | | .444 | .518 | .435 | .363 | .464 | .494 |
| **T7** | | | | | | | | | | | | | .690 | .345 | .514 | .576 | 486 |
| **IPA-204** | | | | | | | | | | | | | | .422 | .510 | .568 | .618 |
| **IPA-3088** | | | | | | | | | | | | | | | .389 | .300 | .432 |
| **IPA-234** | | | | | | | | | | | | | | | | .515 | .493 |
| **IPA-98-3** | | | | | | | | | | | | | | | | | .438 |
| **UPAS-120** | | | | | | | | | | | | | | | | | |

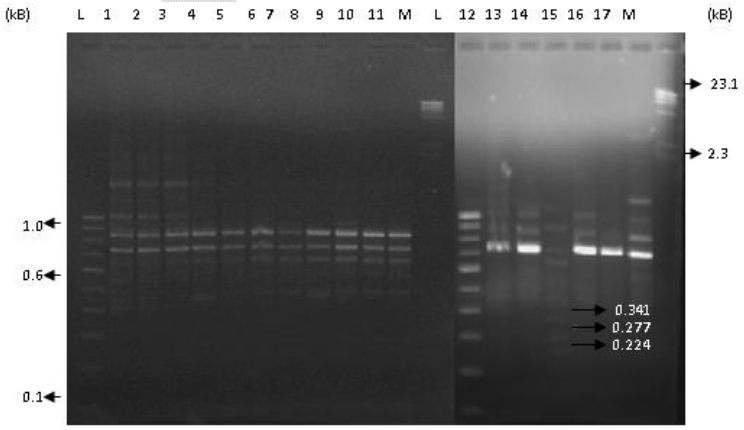


Figure-2a: RAPD profile of different cultivars of *Cajanus cajan* (L) Millsp. using primer 17ES10C28 . Lane L: 1 kb DNA ladder; Lane M: Lambda *Hind* III marker DNA; Lane1-17: Pusa 9, Bahar, AL-201, IPA-337, IPA-61, IPA-20, IPA-34, IPA 341, IPA-242, IPA-2013, IPA-285, T7, IPA-204, IPA-3088, IPA-234, IPA-98-3 and UPAS-120 respectively.

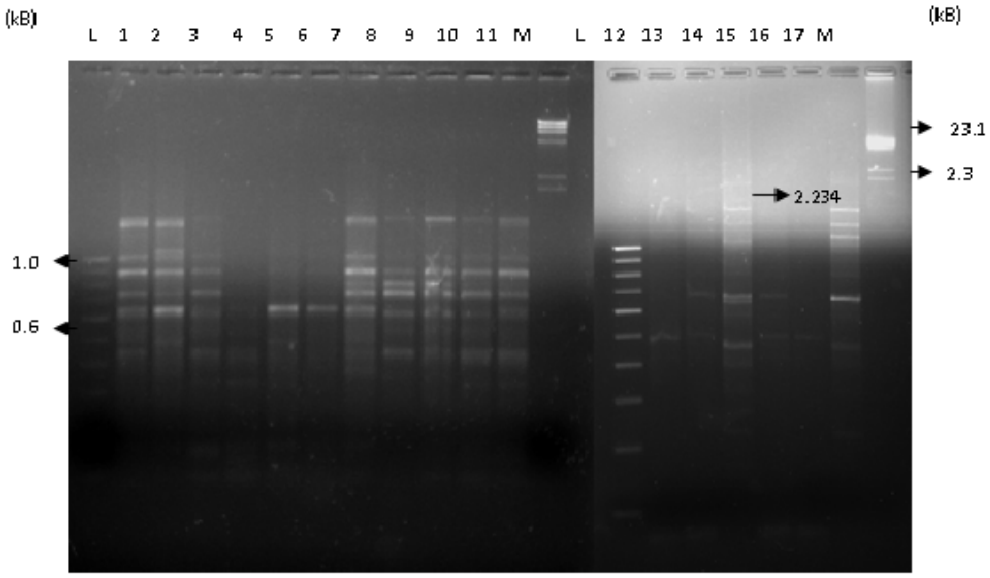


Figure-2b: RAPD profile of different cultivars of *Cajanus cajan* (L) Millsp. using primer 10ES18T21 . Lane L: 1 kb DNA ladder; Lane M: Lambda *Hind* III marker DNA; Lane1-17: Pusa 9, Bahar, AL-201, IPA-337, IPA-61, IPA-20, IPA-34, IPA 341, IPA-242, IPA-2013, IPA-285, T7, IPA-204, IPA-3088, IPA-234, IPA-98-3 and UPAS-120 respectively.

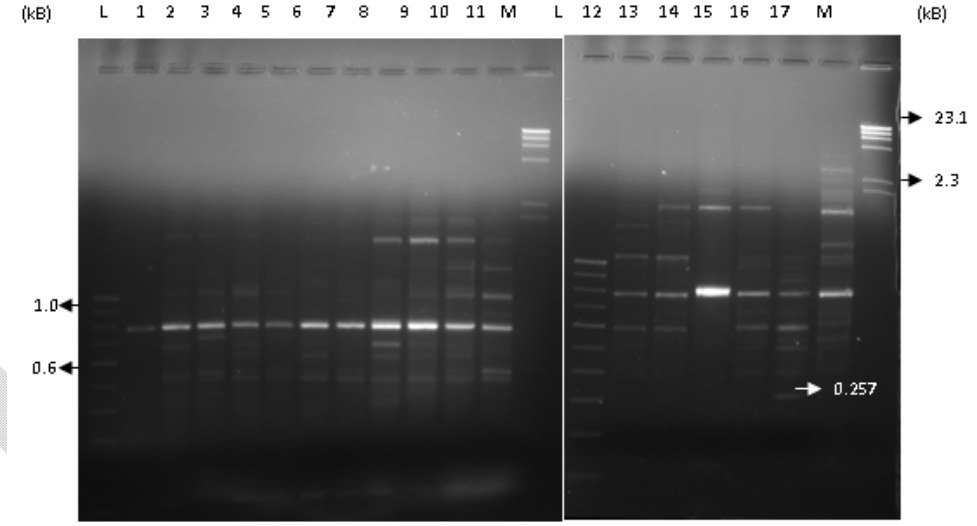


Figure-2c: RAPD profile of different cultivars of *Cajanus cajan* (L) Millsp. using primer 21ES10A32 . Lane L: 1 kb DNA ladder; Lane M: Lambda *Hind* III marker DNA; Lane1-17: Pusa 9, Bahar, AL-201, IPA-337, IPA-61, IPA-20, IPA-34, IPA 341, IPA-242, IPA-2013, IPA-285, T7, IPA-204, IPA-3088, IPA-234, IPA-98-3 and UPAS-120 respectively.

Further, the similarity index as shown in Table 3 revealed the maximum similarity of cultivar IPA337 with IPA61 (similarity indices 0.778) while distantly related cultivars were AL201 and IPA98-3 (similarity indices 0.272). In the present study also, the average similarity index of 0.463 between cultivars along with the average number of bands developed per primer (11.6) with the average percent polymorphism (74.7) as revealed by 17 RAPD primers is quite significant. Five amplified bands found to be specific to a given cultivar, i.e. they were present in only one cultivar but absent from remaining cultivars (Figure2 a, b, c) as shown in Table 3 could be used as ready reference for cultivar identification and could also be converted into CAPS or SCAR marker after its subsequent cloning and sequencing for cultivar identification.

**Conclusions**

Though there has been great advancement in the marker technology with the advent of different DNA markers like amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), single nucleotide polymorphism (SNPs and diversity arrays technology (DArT) still RAPD is quite convenient to apply provided the problem of reproducibility is minimized. The only option left over is to validate the RAPD based assessment of genetic diversity by using maximum number of random primers for the samples provided. The preliminary work carried out with 17 random primers selected from literature revealing the genetic diversity among 17 pigeon pea cultivars could be exploited further by increasing the number of random primers and by validating it with other available DNA markers.

**List of Abbreviations**

CTAB, Cetyltrimethylammonium bromide; RAPD, Randomly Amplified Polymorphic DNA; RFLP, Restriction Fragment Length Polymorphism; PVP, Poly vinyl pyrrolidone; UPGMA, Unweighted Paired Group Method using Arithmetic Averages.

**Competing Interests**

The authors declare that they have no competing interests.

**Authors' Contributions**

DY developed the project and supervised preparation of manuscript; all the lab work was done by NM.

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