



1 AFLP fingerprinting in pigeonpea (*Cajanus cajan* (L.) Millsp.) 2 and its wild relatives

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10 Abstract

11 Detection of DNA polymorphism in cultivated pigeonpea (*Cajanus cajan*) and two of its wild relatives
12 *Cajanus volubilis* and *Rhynchosia bracteata* is reported here for the first time using amplified fragment
13 length polymorphism (AFLP) fingerprinting. For this purpose, two *Eco*RI (three selective nucleotides) and
14 14 *Mse*I (three selective nucleotides) primers were used. The two wild species shared only 7.15% bands with
15 the pigeonpea cultivars, whereas 86.71% common bands were seen among cultivars. Similarly, 62.08%
16 bands were polymorphic between *C. volubilis* and pigeonpea cultivars in comparison to 63.33% poly-
17 morphic bands between *R. bracteata* and pigeonpea cultivars, and 13.28% polymorphic bands among
18 pigeonpea cultivars. The cluster analysis revealed low polymorphism among pigeonpea cultivars and very
19 high polymorphism between cultivated pigeonpea and its wild relatives. The AFLP analysis also indicated
20 that only one primer combination (*Eco*RI + ACT and *Mse*I + CTG), at the most any four primer pair
21 combinations, are sufficient for obtaining reliable estimation of genetic diversity in closely related cultivars
22 like pigeonpea material analyzed herein. AFLP analysis may prove to be a useful tool for molecular
23 characterization of pigeonpea cultivars and its wild relatives and for possible use in genome mapping.
24

25 Introduction

26 Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the
27 major grain legume (pulse) crops of the tropics and
28 subtropics. The Indian subcontinent, accounts for
29 about 90% of the global production. Of the dif-
30 ferent pulse crops, pigeonpea ranks sixth in area
31 and production but it is used in more diverse ways.
32 Its seed protein content (approximately 21%) is
33 also well comparable with that of other major
34 grain legumes (Nene and Sheila 1990).

35 Wild relatives of pigeonpea serve as a rich
36 source of disease resistance genes. For example,
37 *Cajanus volubilis* (Blanco) Blanco has the elite

character of resistance to sterility mosaic disease 40
(Remanandan 1980). Similarly, *Rhynchosia* 41
bracteata Benth. Ex Bek possesses resistance to 42
pod fly damage (Sharma et al. 2003). However, 43
development of improved types through hybrid- 44
ization and recombination of available variability 45
in pigeonpea and other species of *Cajanus* have 46
met with only limited success (Saxena and Sharma 47
1990). Attempts to improve production in both 48
traditional and intensive production systems and 49
to extend crop's adaptation beyond tropical and 50
subtropical regions have recently gained attention. 51
During the past three decades, breeders have 52
developed a large number of short duration, large 53

| | | |
|-----|--|------------|
| 54 | seeded, high yielding types, stable for sole crop- | 105 |
| 55 | ping under high levels of management, and disease | 106 |
| 56 | resistant types in different maturity groups (Saxe- | 107 |
| 57 | na and Sharma 1990). However, the initial limited | 108 |
| 58 | success of an intensive effort does not indicate the | 109 |
| 59 | ultimate efficiency of the breeding procedures, or | 110 |
| 60 | full utilization of the genetic potential of the crop. | 111 |
| 61 | Further research efforts need to concentrate on | 112 |
| 62 | developing good understanding of genetic systems | 113 |
| 63 | controlling qualitative and quantitative traits. | 114 |
| 64 | Knowledge about genetic diversity in available | |
| 65 | germplasm is very useful for plant breeders. It | |
| 66 | supports their decision on the selection of cross | |
| 67 | combinations from large sets of parent genotypes | |
| 68 | and is also helpful when they want to widen the | |
| 69 | genetic basis of a breeding program. Traditionally, | |
| 70 | morphological characters have been used for the | |
| 71 | identification of pigeonpea cultivars and its wild | |
| 72 | relatives, which necessitates growing the plants to | |
| 73 | full maturity prior to identification. Ladizinsky | |
| 74 | and Hamel (1980) used seed proteins electropho- | |
| 75 | resis to identify pigeonpea accessions, although | |
| 76 | very little polymorphism was detected. Later, | |
| 77 | restriction fragment length polymorphism (RFLP) | |
| 78 | technique was successfully used for detecting ge- | |
| 79 | netic diversity among the wild species of pigeonpea | |
| 80 | (Nadimpalli et al. 1992). Ratnaparkhe et al. (1995) | |
| 81 | have attempted randomly amplified polymorphic | |
| 82 | DNA (RAPD) to assess genetic diversity in pi- | |
| 83 | geonpea and its wild relatives. Another type of | |
| 84 | markers, amplified fragment length polymorphism | |
| 85 | (AFLP) markers, have proved as more reliable and | |
| 86 | reproducible as compared to RAPD markers and | |
| 87 | less cumbersome and time consuming than the | |
| 88 | RFLPs. Paran et al. (1998) reported that although | |
| 89 | percentage of polymorphic bands was lower for | |
| 90 | AFLP than RAPD analysis, AFLP primers were | |
| 91 | more efficient in detecting polymorphism as AF- | |
| 92 | LPs could detect polymorphism among closely | |
| 93 | related <i>Capsicum</i> cultivars that could not be de- | |
| 94 | tected by RAPDs. AFLP technique initially | |
| 95 | developed for fingerprinting plant genomes (Vos | |
| 96 | et al. 1995) has emerged as an important technique | |
| 97 | for genome mapping (Becker et al. 1995; | |
| 98 | Maheshwaran et al. 1997), gene tagging (Maksem | |
| 99 | et al. 1995), assessment of genetic diversity (Paul | |
| 100 | et al. 1997; Zhu et al.1998; Aggarwal et al. 2002; | |
| 101 | Bensnard et al. 2002), phylogenetic analysis of | |
| 102 | closely related plant species (Hill et al. 1996; | |
| 103 | Sharma et al. 1996; Aggarwal et al. 1999), and to | |
| 104 | assess somaclonal variation (Polanco and Ruiz | |
| | 2002). In barley, Schut et al. (1997) have tried to | 105 |
| | associate the relationship measures based on | 106 |
| | AFLP markers, pedigree data and morphological | 107 |
| | traits to decrease the effect of their individual | 108 |
| | independent errors. | 109 |
| | Here, we report for the first time, AFLP fin- | 110 |
| | gerprinting of some pigeonpea cultivars and two of | 111 |
| | its wild relatives to demonstrate the utility of this | 112 |
| | technique in assessing genetic diversity in this | 113 |
| | important pulse crop. | 114 |
| | Materials and methods | 115 |
| | <i>Plant material</i> | 116 |
| | The plant material comprised of 14 Pusa cultivars | 117 |
| | and 6 ICPL cultivars of pigeonpea, and two wild | 118 |
| | species, <i>Cajanus volubilis</i> (Blanco) Blanco and | 119 |
| | <i>Rhynchosia bracteata</i> Benth. ex Bek (For details | 120 |
| | see, (Table 1)). All the above material was ob- | 121 |
| | tained from The Pulse Research Laboratory, In- | 122 |
| | dian Agricultural Research Institute, New Delhi, | 123 |
| | India. | 124 |
| | <i>DNA extraction</i> | 125 |
| | Genomic DNA was extracted from young leaves | 126 |
| | by CTAB method (Rogers and Bendich 1988) with | 127 |
| | a few modifications. The extracted DNA was | 128 |
| | purified by RNase treatment for 1–2 h at 37 °C | 129 |
| | and then with phenol–chloroform extraction. The | 130 |
| | pellet was dried and dissolved in appropriate | 131 |
| | volume of TE buffer (10 mM Tris, 1mM EDTA, | 132 |
| | pH 8). | 133 |
| | DNA from different samples was quantified | 134 |
| | both by visual quantification (Agarose gel) and | 135 |
| | UV spectrophotometry. | 136 |
| | <i>AFLP reaction</i> | 137 |
| | AFLP assay was performed with AFLP analysis | 138 |
| | system-I (Invitrogen Life Technologies) as rec- | 139 |
| | ommended by the manufacturers. Genomic DNA | 140 |
| | (250 ng) was digested with <i>EcoRI</i> and <i>MseI</i> for | 141 |
| | 2 h at 37 °C and the enzymes were inactivated at | 142 |
| | 70 °C for 15 min. The DNA fragments were li- | 143 |
| | gated with 24µl of <i>EcoRI</i> and <i>MseI</i> adopter ligation | 144 |
| | mixture. For preselective amplification 5µl of | 145 |

Table 1. Pedigree and characteristics of pigeonpea cultivars and two of its wild relatives taken for AFLP analysis

| Serial number | Accession | Pedigree | Plant type | Seed size (g/100 seeds) | Characteristics |
|---------------|---------------------|-------------------------------------|------------|-------------------------|--|
| 1 | Pusa 2001 | Progeny 27 cross no. 148 × upas 120 | IDT | 8.2 | Medium tall, semi spreading, early maturity |
| 2 | Pusa 2008 | Selection from ICPL 81 | IDT | 8.0 | Medium tall, semi spreading, extra early in maturity |
| 3 | Pusa 951 | EXN-5 × sel 90312 | IDT | 8.0 | Tall, semi spreading, extra early in Maturity |
| 4 | Pusa 2003 | Line 151 × Pusa 855 | IDT | 8.0 | Medium tall, semi spreading, early maturity |
| 5 | Pusa 2001-6 | Sel 90311 × Pusa 604 | IDT | 8.1 | Medium tall, semi spreading, early maturity |
| 6 | Pusa 2001-2 | ICPL154 × upas120 | IDT | 7.8 | Tall semi, spreading extra, early maturity |
| 7 | Pusa 2001-3 | EXN-5 × H88-25 | IDT | 8.2 | Medium tall, semi spreading, early maturity |
| 8 | Pusa 991 | Line 81 × sel 383 | | 8.1 | Tall, semi spreading, early maturity |
| 9 | Pusa 855 | Mutant of T-21 | IDT | 9.0 | Tall, semi spreading, early maturity |
| 10 | ICPL-182 | C11 × ICP-1-6-W3 WB1 | IDT | 9.2 | Short, semi spreading extra early maturity |
| 11 | Pusa 2002 | P945 × Pusa 78 | IDT | 8.0 | Tall, semi spreading, early maturity |
| 12 | Pusa 2001-1 | Sel9-5 × sel 90309 | IDT | 7.6 | Medium tall, bushy type, extra early maturity |
| 13 | Pusa 992 | Sel from 90306 progeny-11 | | 8.6 | Medium tall, semi spreading, early in maturity |
| 14 | Pusa 2001-7 | Sel 91031 × sel 90307 | IDT | 8.0 | Tall, semi spreading, early in maturity |
| 15 | Pusa 2006 | Pusa 604 × Pusa 78-1 | DT | 7.5 | Medium tall, semi spreading, early in maturity |
| 16 | <i>C. volubilis</i> | Wild from Philippines or Indonesia | Climber | | Climber on sal, teak or pine. |
| 17 | <i>R. bracteata</i> | Wild | Climber | | |
| 18 | ICPL-11953 | Germplasm line | | 8.5 | Tall, semi spreading, late maturity |
| 19 | ICPL-11961 | Germplasm line | | 6.6 | Medium tall, semi spreading, early maturity |
| 20 | ICPL-87119 | HY3C × PantA-2 | | 11.2 | Medium tall, semi spreading, early in maturity |
| 21 | ICPL-8858 | Germplasm line | | 10.8 | Medium tall, semi spreading, early in maturity |
| 22 | ICPL-11959 | Germplasm line | | 7.0 | Tall, semi spreading, late maturity |

DT indicates determinant, IDT indicates Indeterminate. Extra early maturity is 120–140 days, early is 160 days and late maturity is above 200 days. Tall means above 2 m height, medium tall is 150–190 cm and short is up to 130 cm height.

146 10-fold diluted ligation mixture was amplified by
 147 20 cycles of 94 °C for 30 s, 56 °C for 60 s, 72°C for
 148 60 s using *EcoRI* and *MseI* primers (each having
 149 one extra selective nucleotide) in a final volume of
 150 50 µl.

151 For selective amplification, *EcoRI* primers with
 152 three selective nucleotides were labeled using T₄
 153 polynucleotide kinase. ³³P labeled *EcoRI* (+3)
 154 primers (0.5 µl) was mixed with 5µl of 50-fold-di-
 155 luted preamplifier DNA, PCR buffer and the *MseI*
 156 (+3) primers in a final volume of 20µl. The reac-
 157 tion mixture was amplified for one cycle of 94 °C
 158 for 30 s, 65 °C for 30 s, 72 °C for 60 s and sub-
 159 sequently lowering the annealing temperature by
 160 0.7 °C for each cycle for 12 cycles followed by 23
 161 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C
 162 for 60 s. After completion of the cycle program, an
 163 equal volume of sequencing loading buffer [98%
 164 formamide (w/v), 10 mM EDTA, 0.25% xylene
 165 cyanol (w/v), 0.25% bromophenol blue (w/v)] was
 166 added to the reaction mixture. Prior to gel loading
 167 the mixture was heated for 3 min at 90 °C and
 168 then immediately placed on ice.

Electrophoresis

The amplified fragments were analyzed on 6%
 denaturing polyacrylamide gel containing (20:1)
 acrylamide:bisacrylamid, urea (7.5 M) and 1×TBE
 (100 mM Tris, 100 mM boric acid, 2 mM EDTA,
 pH 8.3). For each gel, 100 ml casting solution was
 prepared and mixed with 200 µl 10% (w/v)
 ammonium persulphate and 30 µl TEMED. The
 gel solution was poured into 44.5 × 34.5 cm casting
 cassettes (Tharmo EC). Spacers and combs were
 0.4 mm thick. TBE buffer was taken as electro-
 phoresis buffer. Usually a 3 µl sample of each
 reaction mixture was loaded on the gel. Gels were
 run using a Tharmo EC (EC4000P) power pack at
 a constant power of 60 W and maximum voltage of
 2000 V. Usually the gels were pre-electrophoresed
 at a constant power of 60 W for 30 min prior to
 sample loading. After electrophoresis, the gel was
 dismantled, taken on a Whatman paper sheet,
 wrapped with saran wrap and dried on a gel dryer.
 The dried gel was placed in a cassette and exposed
 to Kodak SB film at – 80°C overnight.

191 *Data analysis*

192 DNA fragment profiles representing a consensus
 193 of two independent replicates were scored in a
 194 binary mode with '0' indicating the absence and '1'
 195 indicating presence of band. Using the binary
 196 data, a similarity matrix was constructed using the
 197 Jaccard coefficient which was further subjected to
 198 UPGMA clustering analysis and a dendrogram
 199 was generated. A cophenetic matrix was con-
 200 structed using the matrix that was used to generate
 201 the clusters. A correlation (mantel 't' test) between
 202 the cophenetic matrix and the similarity matrix
 203 was determined using MXCOMP module. All the
 204 above analysis was done using the software pack-
 205 age NTSYS-PC (version 2.02e).

206 **Results and discussion**

207 In the present study on pigeonpea, we have ana-
 208 lyzed 14 Pusa cultivars, 6 ICPL lines and two of its
 209 wild relatives using the AFLP fingerprinting
 210 approach.

211 *Identification of pigeonpea cultivars*

212 AFLP analysis revealed a large number of distinct
 213 scorable fragments per primer pair (Figure 1). A
 214 total of two *EcoRI* (with three selective nucleo-
 215 tides) and 14 *MseI* (with three selective nucleo-
 216 tides) primers were used to amplify DNA from
 217 different accessions of pigeonpea. Out of these
 218 combinations, *EcoRI* (+ACT) + *MseI* (+CAG)
 219 set shows monomorphic pattern in all these culti-
 220 vars, while other combinations like *EcoRI*
 221 (+AAG) with *MseI* (+CAG), (+CAC),
 222 (+CAT), (+CTA), (+CTG) and (+CTC),
 223 *EcoRI* (+ACT) with *MseI* (+CAA), (+CAC),
 224 (+CAT), (+CTA), (+CTG), (+CTC) and
 225 (+CTT) were found to be promising in detecting
 226 polymorphism.

227 The number of polymorphic bands among dif-
 228 ferent cultivars for each primer pair ranged from 1
 229 to 36 (Table 2). Since AFLP markers are domi-
 230 nant, a locus was considered to be polymorphic if
 231 the presence or absence of the band was observed
 232 in various cultivars and monomorphic if the band
 233 is present among all the cultivars. The primer set
 234 *EcoRI* (+AAG) + *MseI* (+CTA) yielded maxi-

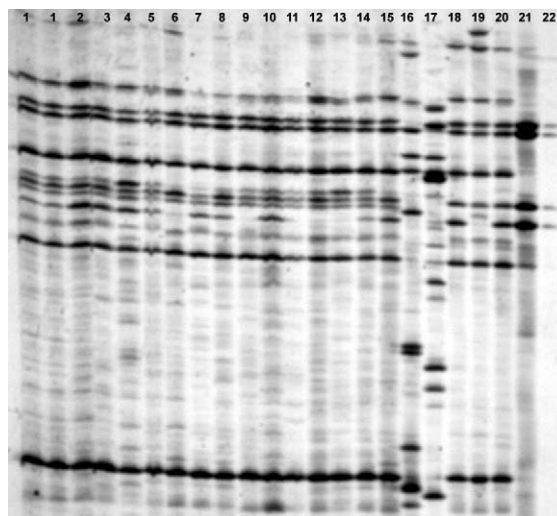


Figure 1. The autoradiogram of AFLP gel showing the pigeonpea cultivars and two wild relatives with the primer set *EcoRI* (+AAG) and *MseI* (+CTG). Lane 1–15, pigeonpea cultivars Pusa 2001, Pusa 2008, Pusa 951, Pusa 2003, Pusa 2001-6, Pusa 2001-2, Pusa 2001-3, Pusa 991, Pusa 855, ICPL-182, Pusa 2002, Pusa 2001-1, Pusa 992, Pusa 2001-7, Pusa 2006; Lane 16, *Cajanus volubilis*; Lane 17: *Rhynchosia bracteata*; Lane 18–22, pigeonpea cultivars ICPL-11953, ICPL-11961, ICPL-87119, ICPL-8858, ICPL-11959.

235 mum number of polymorphic bands (36). Only
 236 two lines, namely ICPL-8858 and ICPL-11959,
 237 were found to be monomorphic. The primer sets
 238 *EcoRI* (+AAG) + *MseI* (+CAC), *EcoRI*
 239 (+ACT) + *MseI* (+CAA) showed the fewest
 240 polymorphic bands (one), in which all the five
 241 ICPL lines except ICPL-182 showed only mono-
 242 morphic bands. Similarly, very low polymorphism
 243 was observed between the Pusa cultivars with all
 244 the primer sets except *EcoRI* (+AAG) with *MseI*
 245 (+CTG), *EcoRI* (+AAG) with *MseI* (+CAT)
 246 and *EcoRI* (+ACT) with *MseI* (+CAC). Till date
 247 there is a solitary report available on the identifi-
 248 cation of pigeonpea cultivars at the DNA level
 249 (Ratnaparkhe et al. 1995). As a result, pigeonpea
 250 breeding relies heavily on phenotypic selection
 251 methods. Moreover, pigeonpea is one of the
 252 exceptions among the grain legumes in that though
 253 it is a predominantly self-pollinating crop, out-
 254 crossing level also varies widely (Saxena and
 255 Sharma 1990). As a result of frequent out-crossing,
 256 existing standard cultivars have become heteroge-
 257 neous for several important agronomic characters
 258 such as disease resistance, maturity time, etc. The
 259 maintenance of germplasm in pigeonpea is very

Table 2. The number of polymorphic bands in *C. volubilis*, *C. bracteata* and with in pigeonpea cultivars with different combinations of *EcoRI* (three selective nucleotides) and *MseI* (three selective nucleotides) that are taken for AFLP analysis

| <i>EcoRI</i> primer selective nucleotides | <i>MseI</i> primer selective nucleotides | Polymorphic bands in <i>C. volubilis</i> | Polymorphic bands in <i>R. bracteata</i> | Polymorphic bands within pigeonpea cultivars |
|---|--|--|--|--|
| + AAG | + CAG | 60 | 70 | 7 |
| | + CAC | 75 | 77 | 1 |
| | + CAT | 69 | 70 | 2 |
| | + CTA | 68 | 80 | 36 |
| | + CTG | 66 | 59 | 36 |
| | + CTC | 47 | 51 | 7 |
| | + ACT | + CAA | 81 | 66 |
| | + CAG | 37 | 43 | 0 |
| | + CAC | 41 | 49 | 10 |
| | + CAT | 40 | 50 | 6 |
| | + CTA | 58 | 70 | 7 |
| | + CTG | 67 | 70 | 20 |
| | + CTC | 38 | 47 | 12 |
| | + CTT | 45 | 58 | 3 |

260 tedious, and problems of contamination have been
 261 enormous. The molecular identification of culti-
 262 vars will, therefore, be helpful in assessing the
 263 purity and stability of the genotypes entering into
 264 the breeding programs.

265 *Genetic relationships within Cajanus cajan*

266 In order to quantify the level of polymorphism
 267 detected by AFLP fingerprinting, Nei's estimate of
 268 similarity based on the probability that an ampli-
 269 fied fragment from one genotype will also be found
 270 in another was used to generate a similarity matrix
 271 (Nei and Li 1979). All the accessions fell in the
 272 range of 0.82–1.0 (86.71% of common bands).
 273 This indicates little polymorphism (13.28%) at the
 274 DNA level between various accessions and may be
 275 due to their predominantly self-pollinating nature
 276 of the cultivars that were taken here. Other self-
 277 pollinated species such as tomato and wheat also
 278 show little polymorphism among accessions (Joshi
 279 and Nguyen 1993; Williams and Clair 1993). At
 280 present, there is little information available about
 281 genetic diversity among pigeonpea cultivars. Pre-
 282 viously protein and isozyme electrophoresis were
 283 used to estimate variability in pigeonpea cultivars
 284 (Ladizinsky and Hamel 1980; Kollipara et al.
 285 1994). The major limitation of these techniques is
 286 an insufficient number of polymorphisms detected
 287 among closely related cultivars. Earlier, RAPD
 288 markers were used to detect the genetic variability
 289 among pigeonpea cultivars (Ratnaparkhe et al.

1995). But AFLP markers are considered to be
 more reliable and reproducible as compared to
 RADP markers. Our data demonstrate that the
 AFLP technique can be applied for estimating the
 genetic variability among closely related cultivars.

The UPGMA dendrogram (Figure 2) analysis
 of pigeonpea cultivars shows three main clusters,
 which are further divided into subgroups. First
 cluster consists of 'ICPL-8858' and 'ICPL-11959',
 while the second cluster consists of 'ICPL-87119',
 'ICPL-11961' and 'ICPL-11953'. Among them
 'ICPL-11961' and 'ICPL-11953' form a subgroup
 and are more closely related to each other than to
 'ICPL-87119'. The third cluster consists of six
 subgroups in which Pusa 2002 forms its independ-
 ent subgroup as in case of Pusa 2001-3. The third
 subgroup has ICPL-182 and Pusa 855, which are
 very similar to each other. The fourth subgroup
 contains 'Pusa 2008', 'Pusa 2001-1' and 'Pusa
 2001-2', out of which 'Pusa 2001-1' and 'Pusa
 2001-2' are very similar to each other. The fifth
 subgroup contains Pusa 992, Pusa 2001-6, Pusa
 2003, Pusa 991 and Pusa 951. Here Pusa 992 and
 Pusa 2001-6 are very similar to each other, and
 Pusa 991 and Pusa 951 are similar to each other
 leaving Pusa 2003 out. The sixth subgroup con-
 tains Pusa 2001-7, Pusa 2006 and Pusa 2001, in
 which Pusa 2006 and Pusa 2001 are more similar
 to each other than to Pusa 2001-7.

The clustering pattern generated through UP-
 GMA analysis was validated by generating a
 cophenetic similarity value matrix from a set of
 nested clusters and comparing the cophenetic

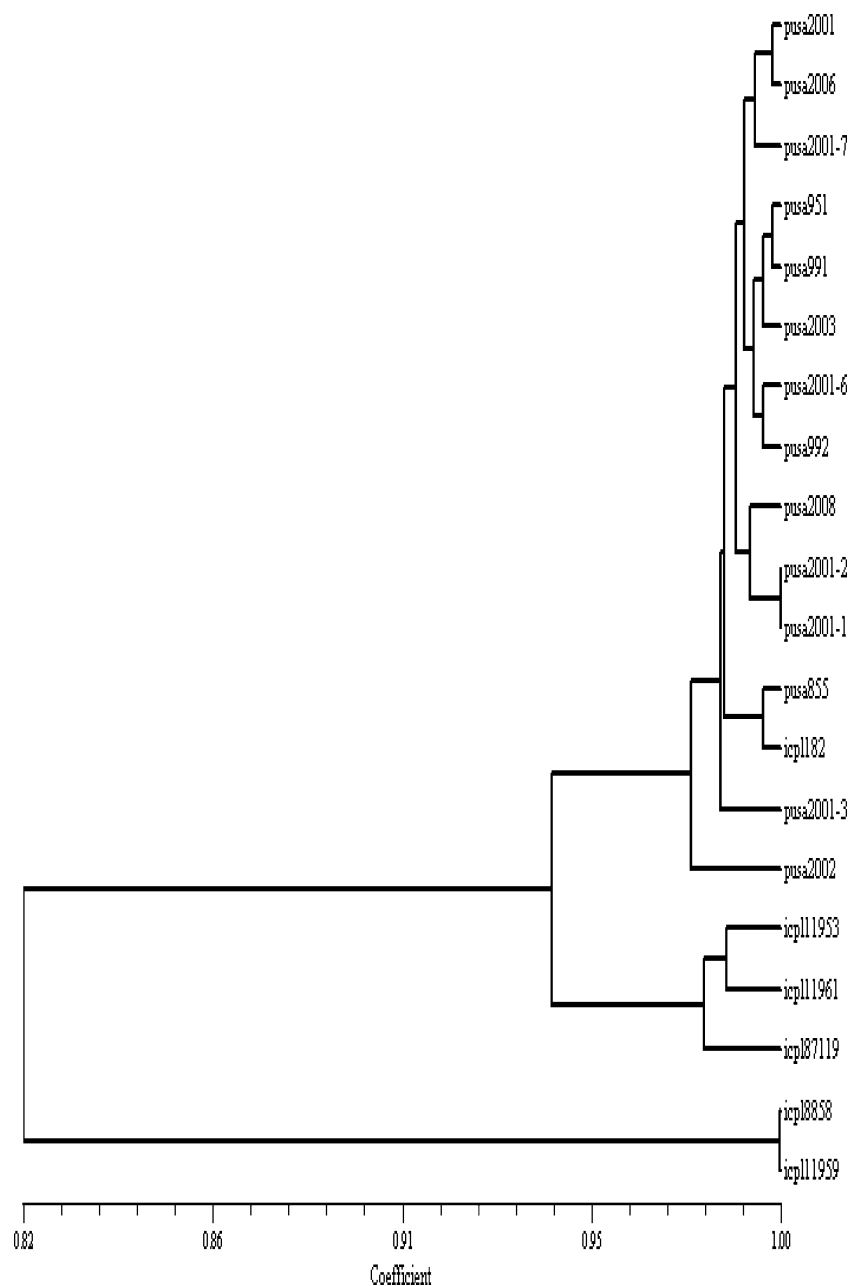


Figure 2. The UPGMA dendrogram of pigeonpea cultivars.

323 value matrix with the similarity matrix of the ori-
 324 ginal data. The goodness of fit of the clusters tes-
 325 ted using an MXCOMP module through a mantel
 326 't' test, gave a very high correlation coefficient of
 327 0.997, thereby indicating the validity of the clusters
 328 generated from the present study. The cophenetic
 329 correlation coefficient measures the agreement
 330 between the similarity values implied by the

331 dendrogram and those of the original similarity
 332 matrix (Sneath and Sokal 1973).

333 Another important aspect of this investigation is
 334 to know the minimum possible number of AFLP
 335 markers needed for reliable grouping of the pi-
 336 geonpea cultivars having closely related genetic
 337 background. Information on this line will help in
 338 reducing the labor and cost of an AFLP project.

339 For this purpose, we developed dendrograms from
 340 each primer pairs independently as well as from
 341 four primer pairs randomly in addition to the final
 342 dendrogram that was developed utilizing data from
 343 all the primer combinations. In this way, we found
 344 that the dendrograms developed from the primer
 345 pairs *EcoRI* (+ AAG) with *MseI* (+ CTC) and
 346 *EcoRI* (+ ACT) with *MseI* (+ CAC), respectively,
 347 have the same pattern with some minor differences.
 348 In these two dendrograms all the Pusa cultivars are
 349 grouped in a cluster leaving all ICPL cultivars as
 350 separate cluster with no subgrouping within the
 351 clusters. These primers can serve as good markers
 352 to distinguish the Pusa cultivars and ICPL cultivars
 353 from a pool. The dendrogram developed from the
 354 primer pair *EcoRI* (+ AAG) with *MseI* (+ CTA)
 355 is almost similar to the final dendrogram that was
 356 developed from all the primer combinations. This
 357 clearly shows that single primer pair *EcoRI* (+
 358 AAG) with *MseI* (+ CTA) is sufficient for reliable
 359 grouping of closely related pigeonpea cultivars.
 360 The dendrograms that were developed from any
 361 four-primer pair combinations randomly also
 362 showed the same pattern with minor variations
 363 with the final dendrogram that was raised from all
 364 the primer pairs taken in this study. This clearly
 365 indicates that at the most any four of the tested
 366 primer pairs are sufficient for reliable estimation of
 367 genetic diversity in closely related cultivars like
 368 pigeonpea material analyzed herein. Recently,
 369 Aggarwal et al. (2002) have also reported a similar
 370 observation in rice.

371 We have also exploited the utility of RAPD
 372 markers (unpublished data) on these lines. The
 373 preliminary results established the utility of RAPD
 374 markers in assessing the genetic diversity in
 375 pigeonpea; however, the number of primers re-
 376 quired to reveal a meaningful estimate of diversity
 377 is relatively high (~40).

378 *Genetic relationship between wild species* 379 *and cultivars*

380 The wild relatives of pigeonpea viz; *Cajanus volu-*
 381 *bilis* and *Rhynchosia bracteata* were selected to find
 382 the genetic relationships with the cultivars. Unlike
 383 in pigeonpea cultivars, extensive polymorphism
 384 was found between the two wild species and the
 385 cultivars. Many bands were common between the
 386 two wild species, even though few unique bands

were also found. The percentage of polymorphism
 between *C. volubilis* and pigeonpea cultivars was
 found to be 62.08%, whereas between *R. bracteata*
 and pigeonpea cultivars it was 63.33%. The
 number of polymorphic bands between *C. volubilis*
 and pigeonpea cultivars varied from 37 to 81 for
 each primer set. Similarly, the number of poly-
 morphic bands between *R. bracteata* and pigeon-
 pea cultivars varied from 43 to 80 for each primer
 set (Table 2). From the UPGMA dendrogram
 based on wild species and pigeonpea cultivars
 (Figure 3), the similarity matrix between the wild
 species and cultivars ranged from 0.22 to 1.0,
 indicating a large amount of genetic variation be-
 tween them. We also made an attempt to correlate
 the relationship measures based on AFLP mark-
 ers, pedigree data and morphological traits in pi-
 geonpea accessions. Due to insufficient
 morphological data and inclusion of some of the
 mutant genotypes (Pusa 855), some germplasm
 lines (ICPL-11953, 11961, 8858 and 11959) and
 wild species, we were unable to correlate these
 measurements with our fingerprint data.

Conclusions

The present investigation demonstrates the po-
 tential of AFLP fingerprinting in detecting poly-
 morphism among pigeonpea cultivars, which have
 narrow genetic background particularly in case of
 Pusa lines. The AFLP analysis clearly indicated
 that only one primer pair *EcoRI* (+ AAG) with
MseI (+ CTA) or at the most any four primer pair
 combinations are sufficient for reliable estimation
 of genetic diversity, while with RAPD markers
 nearly 40 primers are required for the same in
 closely related cultivars like pigeonpea material
 analyzed herein. In addition to this, the markers
 generated via AFLP assay can provide practical
 information for the management of genetic re-
 sources. For the selection of good parental mate-
 rial for breeding program the genetic data
 produced through AFLP can be used to correlate
 with the relationship measures based on pedigree
 data and morphological traits to minimize the
 individual inaccuracies in pigeonpea. Further, a
 large amount of genetic variation exists between
 pigeonpea cultivars and its wild relatives, which
 can be used efficiently for gene tagging, and
 genome mapping of wild and cultivar crosses to

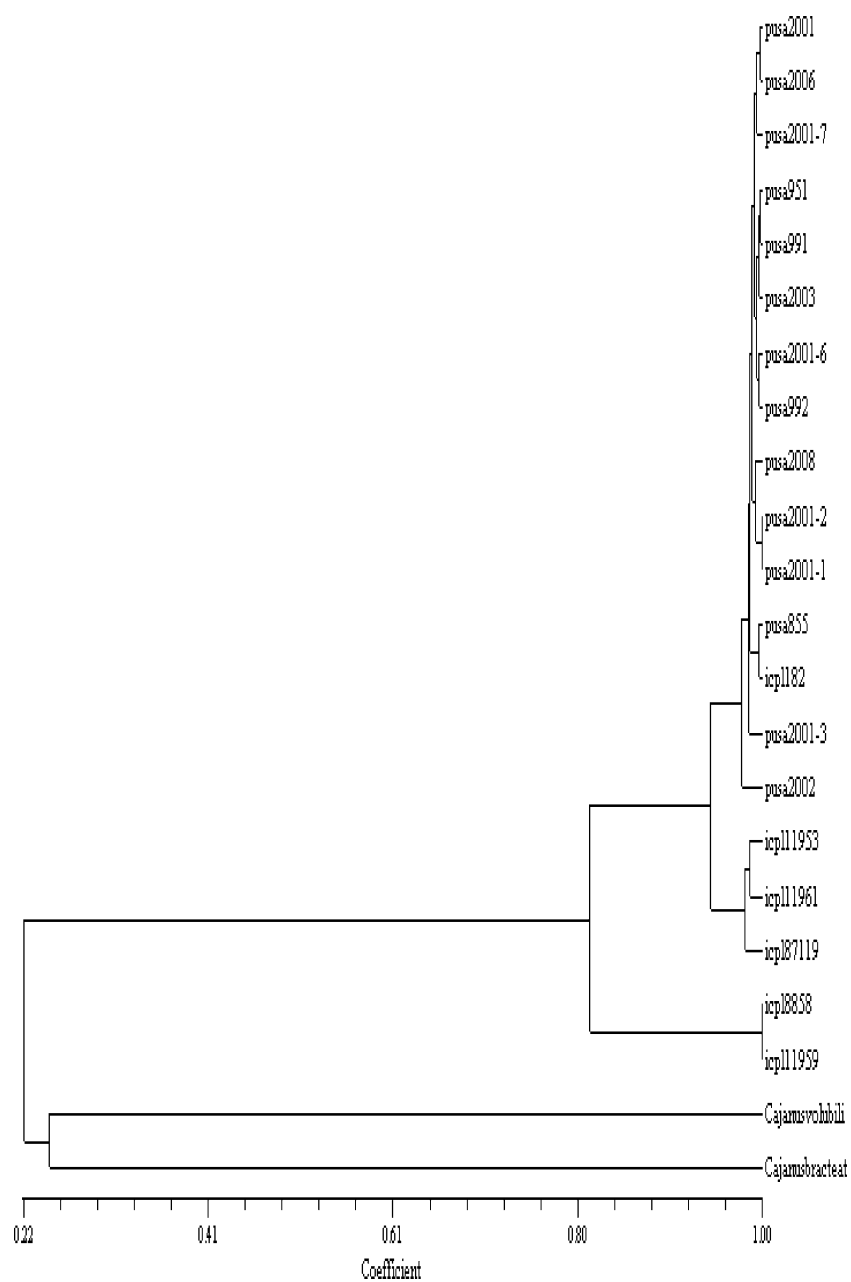


Figure 3. The UPGMA dendrogram of pigeonpea cultivars and two of its wild relatives.

435 introgress the disease and insect resistance into the
436 cultivated genotypes.

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