Molecular genetic diversity in dual purpose and land races of pigeonpea (*Cajanus cajan* (L.) Millsp.)

S. E. Diwakar Reddy, S. Manju Devi and P. Jayamani*

Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641003

*E-Mail: jayamani1108@gmail.com

Abstract

Molecular genetic diversity was assessed among 32 dual purpose and land races of pigeonpea using 20 pigeonpea specific SSR markers. Among 20 markers, 15 markers revealed polymorphism, while five markers exhibited a monomorphic banding pattern. The polymorphic markers produced a total of 43 alleles with an average of 2.15 alleles per marker. The markers CcM 0008 and CcM 1026 produced the highest number of alleles (4). The Polymorphic Information Content (PIC) value of the markers ranged from 0.06 (PGM 16) to 0.64 (CcM 0008) with an average of 0.19. A dendrogram constructed using UPGMA distinguished 32 genotypes into 10 clusters. Cluster I was the largest with 15 genotypes followed by cluster V with five genotypes. The Neighbour-joining tree produced based on the weighted average for dissimilarity matrix grouped the 32 genotypes into eight groups. Among them group II was the largest comprising of 10 genotypes. Based on the molecular genetic diversity study using SSR markers, the genotypes Kunnathur local, CRG 13-01, BSR 1 and Pillayakothur local were found diverse among the genotypes and had good grain and vegetable pigeonpea traits could be used in the breeding programme. The results indicated that SSR markers provide a more definitive separation of clusters indicating a higher level of efficiency for determining the relationship among pigeonpea genotypes.

Key words: Pigeonpea, Dual purpose, Land races, Genetic diversity

INTRODUCTION

Pigeonpea (*Cajanus cajan*) known by the common names redgram, tur, arhar and gandul, is one of the foremost grain legumes of India. It is the second most important grain legume of India after chickpea. Pigeonpea is an often cross pollinated (20–70%) crop with diploid chromosome number 2n=2x=22, and genome size = 858 Mbp (Greilhuber and Obermayer, 1998). India is the largest pigeonpea growing country in the world, accounting for 5.39 m.ha with the production of 4.87 m.t and productivity of 903 kg/ha (FAOSTAT, 2019). In order to maintain, evaluate and utilize germplasm effectively in breeding, it is important to investigate the extent of genetic diversity available. Genetic diversity is an essential prerequisite in breeding programmes for identifying diverse genotypes for a further selection of parents. The availability of limited morphological markers and the environmental influence paved the way for utilization of molecular markers that were available in plenty. Among the molecular markers, Microsatellites or SSRs are stretches of tandemly arranged short sequence motifs which are abundant and highly polymorphic in several eukaryotic genomes. Assessment of genetic variability has been done using various molecular markers (Ratnaparkhe *et al*., 1995 and Yadav *et al*., 2010). SSR markers have been demonstrated to be a powerful tool in genotype identification and plant variety protection (Olufowote *et al*., 1997), seed purity evaluation, germplasm conservation (Powel *et al*., 1996), diversity studies (Xiao *et al*., 1996), pedigree analysis and marker assisted selection (Yang *et al*., 1994). SSR markers are highly polymorphic, reproducible, codominant and occur throughout the genome and have been used in assessment of genetic diversity in pigeonpea (Pushpavalli *et al*., 2016). The present investigation was to study the level of molecular genetic diversity among...
dual purpose and land races of pigeonpea using SSR markers.

MATERIALS AND METHODS
The molecular experiments for the current study were carried out for a total of 32 pigeonpea genotypes in the Marker Aided Selection Laboratory, Department of Pulses, Tamil Nadu Agricultural University, Coimbatore. Out of 32 genotypes, 25 were local genotypes collected from pigeonpea growing districts of Tamil Nadu. These genotypes are being used for vegetable and grain purposes by the local people. The list of genotypes used was represented in Table 1. A set of twenty pigeonpea specific SSR markers were used for the molecular analysis. Fresh leaves of 12 days old plants were collected and the CTAB method was followed for DNA extraction. The extracted DNA was treated with RNase to avoid RNA contamination present in the sample. The quality of DNA was checked by using 0.8 per cent agarose gel electrophoresis. The list of SSR primers used in the study is presented in Table 2.

The PCR reactions of isolated genomic DNA were carried in a volume of 12 µl containing 7.0 µl of master mix, 3.0 µl of 5µM forward and reverse primer and 2.0 µl of 50 ng of genomic DNA and amplification was performed in Master cycler gradient PCR (Biorad). PCR conditions used for SSR amplification were follows. Initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing temperature at 53°C to

| S.No. | Genotype                  | Source                                      |
|-------|---------------------------|---------------------------------------------|
| 1     | Vazhavanthi local         | Yearcaud hills, Salem                      |
| 2     | Sengadu local             | Yearcaud hills, Salem                      |
| 3     | Kundakadu local           | Pachamalai hills, Trichy                   |
| 4     | Jambukuttaipatti Local    | Jambukuttaipatti, Krishnagiri              |
| 5     | Puliamatti local-1        | Puliamatti, Krishnagiri                    |
| 6     | Puliamatti local-2        | Puliamatti, Krishnagiri                    |
| 7     | Bendrahalli local-1       | Bendrahalli, Krishnagiri                   |
| 8     | Bendrahalli local-2       | Bendrahalli, Krishnagiri                   |
| 9     | Kunnathur local           | Kunnathur, Krishnagiri                     |
| 10    | Uthangarai local          | Uthangarai, Krishnagiri                    |
| 11    | Vandikarankottai local    | Vandikarankottai, Krishnagiri              |
| 12    | Singarapettai local       | Singarapettai, Krishnagiri                 |
| 13    | Pillayakothur local       | Pillayakothur, Krishnagiri                 |
| 14    | Irapputhuvanari           | Kalakuruchi, Vilupuram                     |
| 15    | Soolagiri local-1         | Soolagiri, Krishnagiri                     |
| 16    | Soolagiri local-2         | Soolagiri, Krishnagiri                     |
| 17    | Gengusettpatti local      | Gengusettpatti, Krishnagiri                |
| 18    | Periyavathal malai local  | Vathal malai hills, Dharmapuri            |
| 19    | Vathimalai local          | Vathal malai hills, Dharmapuri            |
| 20    | CRG 13-01                 | Department of Pulses.TNAU                  |
| 21    | Thondamuthur local        | Department of Pulses.TNAU                  |
| 22    | Coimbatore local          | Department of Pulses.TNAU                  |
| 23    | BSR 1                     | ARS, Bhavanisagar                          |
| 24    | BRG 1                     | GKVK, Bangalore                            |
| 25    | BRG 2                     | GKVK, Bangalore                            |
| 26    | BRG 3                     | GKVK, Bangalore                            |
| 27    | BRG 4                     | GKVK, Bangalore                            |
| 28    | BRG 5                     | GKVK, Bangalore                            |
| 29    | Yelagiri local            | Yelagiri, Vellore                          |
| 30    | Arur local                | Arur, Dharmapuri                           |
| 31    | Paiyur local              | Paiyur, Krishnagiri                        |
| 32    | Karyamangalam local       | Karyamangalam,                             |
61°C for 1 minute and extension at 72°C for 2 minutes and final primer extension at 72°C for 10 minutes. The amplified products as developed by the primers were separated by agarose (3.0 per cent) gel electrophoresis and documented in BIO RAD gel documentation unit.

The SSR gels were scored and furnished as allelic data according to their allele size. A dendrogram was generated by cluster analysis using the UPGMA method by DARwin 5.0 software package. Neighbour joining tree was also created based on a weighted average for dissimilarity matrix using the DARwin 5.0 software package. Polymorphic Information Content (PIC) values were calculated to measure the ability of SSR markers to detect the polymorphism among the genotypes. The PIC value was calculated using the formula $\text{PIC} = 1 - \sum p_i^2$, where, $p_i$ is the frequency of the $i$th allele (Smith et al., 1997).

| S. No. | Marker | Forward and reverse sequence | Annealing temperature (°C) | Allele size (bp) | Number of alleles | PIC value | Remarks |
|-------|--------|-------------------------------|--------------------------|----------------|------------------|-----------|---------|
| 1     | CCB 1  | F: AAGGGTTGTATCTCCGCGTG      | 59.50                    | 190            | 1                | 0.00      | Monomorphic |
|       |        | R: GCAAAGCAGCAATCATTTTCG      |                          |                |                  |           |         |
| 2     | CCB 10 | F: CCTTTCTAAGGGTGAATGCAAGC   | 53.00                    | 200-210        | 2                | 0.28      | Polymorphic |
|       |        | R: CATACACATAAAAAGAGCCGGATGC  |                          |                |                  |           |         |
| 3     | CCB 7  | F: CAACATTTGGACTAAAAACTG      | 53.00                    | 180            | 1                | 0.00      | Monomorphic |
|       |        | R: AGGTATCCCAATATCACACTTG     |                          |                |                  |           |         |
| 4     | PGM 10 | F: TCAAGAGGACCACACCGAAG       | 61.00                    | 190-200        | 2                | 0.16      | Polymorphic |
|       |        | R: TGAGCATAGACATTGCGTGAAG     |                          |                |                  |           |         |
| 5     | PGM 102| F: ATCGGCTTTTGCCTTGTATGA      | 58.50                    | 180            | 1                | 0.00      | Monomorphic |
|       |        | R: AAGCTACAAGGGATACACATGC     |                          |                |                  |           |         |
| 6     | PGM 106| F: TGAATGAAACACCTCAATGG       | 58.50                    | 200-210        | 2                | 0.26      | Polymorphic |
|       |        | R: TGATTGCACATTTGCGTCTA       |                          |                |                  |           |         |
| 7     | PGM 109| F: ATTCCTCCCTCTATCTCAGACTTTT  | 60.50                    | 190-200        | 2                | 0.19      | Polymorphic |
|       |        | R: TCCTGTGGAATCTAGATACACT     |                          |                |                  |           |         |
| 8     | PGM 16 | F: CATTATTTCTCCTCTGGCATCACCT  | 60.00                    | 210-220        | 2                | 0.06      | Polymorphic |
|       |        | R: CGGCTGCAGATGCAAAC         |                          |                |                  |           |         |
| 9     | PGM 3  | F: ACACCACCATGCTAAAGAACAAG    | 60.50                    | 180-200        | 3                | 0.12      | Polymorphic |
|       |        | R: CCAAGCAAGACACAGGAATACATA  |                          |                |                  |           |         |
| 10    | PGM 45 | F: GGAAACTACACCTATATATACCAA   | 60.50                    | 200-220        | 2                | 0.19      | Polymorphic |
|       |        | R: CACTACGGCTCTACAGGCATCTC    |                          |                |                  |           |         |
| 11    | PGM 5  | F: ATGGGTGCTGTCTCTGCTCTAC     | 55.00                    | 200-210        | 2                | 0.36      | Polymorphic |
|       |        | R: TCCTACGGTACATTTGCTC        |                          |                |                  |           |         |
| 12    | PGM 82 | F: CAGATTCTCATGCGTGAG         | 61.00                    | 190-200        | 2                | 0.11      | Polymorphic |
|       |        | R: ACGGCTTCTCTGGAGG          |                          |                |                  |           |         |
| 13    | PKS 18 | F: ACCTGTCTGCTGTGTTG          | 60.00                    | 200            | 1                | 0.00      | Monomorphic |
|       |        | R: CATCAGCTACATGTTACC         |                          |                |                  |           |         |
| 14    | PKS 26 | F: ACCCATTATTTTGGTGTCTA       | 55.00                    | 190            | 1                | 0.00      | Monomorphic |
|       |        | R: CAAAATTTTACCAAAAGGAA       |                          |                |                  |           |         |
| 15    | PKS 30 | F: AAGGTGCAACCCCTCTACC       | 59.50                    | 190-200        | 2                | 0.19      | Polymorphic |
|       |        | R: TGACTCGGCCAGATAGATAGAA     |                          |                |                  |           |         |
| 16    | CcM 0257| F: GCCTTACGGGAATGTATG         | 60.00                    | 200-230        | 3                | 0.29      | Polymorphic |
|       |        | R: CTGTCTCAAAGGGACCTG         |                          |                |                  |           |         |
| 17    | CcM 0948| F: GCACAGGTCTGCTGTAACC       | 60.00                    | 160-180        | 3                | 0.21      | Polymorphic |
|       |        | R: CATTCTCCACCTTCTCTC         |                          |                |                  |           |         |
| 18    | CcM 0008| F: CGGTGAAAGGCTAAGT          | 58.00                    | 180-210        | 3                | 0.64      | Polymorphic |
|       |        | R: CAAAATTTAAGGCTTATTTTACGA  |                          |                |                  |           |         |
| 19    | CcM 0039| F: AGGAATATGTGCTGGG         | 59.00                    | 190            | 3                | 0.21      | Polymorphic |
|       |        | R: TTGATGTGGAAAGGCGG         |                          |                |                  |           |         |
| 20    | CcM 1026| F: TCATGGCAAAAGGACTCTAGC     | 59.00                    | 200-230        | 4                | 0.62      | Polymorphic |
|       |        | R: GGAAGTGATGATGAGTAACAGA    |                          |                |                  |           |         |

**Total** 43
RESULTS AND DISCUSSION

To quantify the genetic diversity among the genotypes, simple sequence repeats (SSR) are the marker of choice for genetic studies viz., genetic diversity assessment, genetic mapping and marker assisted selection by virtue of their extreme polymorphism, ubiquitous presence and codominant inheritance (Rafalski and Tingey, 1993; Gupta et al., 1996; Jarne and Lagoda, 1996) and robust, reproducible, hypervariable, informative and reasonably easy to use properties (Powell et al., 1996). In the present study, 20 SSR markers were used for diversity analysis in pigeonpea genotypes. Among the 20 SSR markers, 15 markers showed polymorphism and five markers showed a monomorphic pattern. The amplification of pigeonpea derived SSR primers viz. CCB 7, CCB 10 and CcM 0257 were observed by Sharma et al. (2018). The number of alleles ranged from one to four with an average of 2.15 alleles per marker. Muniswamy et al. (2019) reported 44 alleles with an average of 2.44 alleles per marker in 196 pigeonpea genotypes. Sarkar et al. (2017) reported that 52 alleles with an average of 1.6 alleles per locus in pigeonpea. Njung’e et al. (2016) reported 212 alleles with an average of 5.58 alleles per locus in pigeonpea genotypes. Pigeonpea marker viz. CcM 0008 (Plate 1) and CcM 1026 (Plate 2) recorded the highest number of alleles (4) followed by 3 alleles for PGM 3, CcM 0257, CcM 0948, CcM 0039 and the lowest number of alleles (2) were detected for the markers CCB 10, PGM 10, PGM 106, PGM 109, PGM 16, PGM 45, PGM 5, PGM 82, PGS 30. The allele size varied from 160 – 230 bp. Sarkar et al. (2017) recorded an allele size ranged from 100 – 200 bp among 138 pigeonpea genotypes. Polymorphic Information Content (PIC) value measures the discriminatory power of a marker based on the number and relative frequency of alleles expressed among the genotypes. In the present study, PIC value of the SSR markers ranged from 0.06 to 0.64 with an average of 0.19 (Table 2). Kimaro et al. (2020) reported the PIC value from 0.08 to 0.84 with an average of 0.46 in 48 pigeonpea genotypes. Sarkar et al. (2017) reported the PIC value from 0.01 to 0.38 with an average of 0.22 in pigeonpea. Sousa et al. (2011) reported the PIC values ranged from 0.11 to 0.80 with an average of 0.49 in pigeonpea. The markers CcM 0008 and CcM 1026 recorded high PIC values and are highly informative for genetic studies and are tremendously useful in distinguishing the polymorphism among the pigeonpea genotypes.

Dendrogram based on Unweighted Pair Group Method with Arithmetic mean, the 32 genotypes were grouped into 10 clusters (Fig. 1). Among the 10 clusters, cluster I was the largest with 15 genotypes followed by cluster V with five genotypes and the clusters IV (Periyavathal malai local), VI (Kunnathur local), VIII (frapputhuvanari local), IX (Puliamppatti local-2) and X (Bendrahalli local-1) were solitary. In cluster I, the genotypes, Coimbatore local, BSR 1 and BRG 1 had a high similarity. Similarly, Yelagiri local and Arur local also had similarly at DNA level. In cluster III, the genotypes Thondamuthur local and BRG 4 were observed to be similar however, a clear morphological difference was observed in the above genotypes. Cluster II showed two genotypes, cluster III showed three genotypes and cluster VII showed two genotypes. Manju et al. (2017) by using SSR markers, 40 accessions of pigeonpea were grouped into two clusters. Hullur et al. (2018) by using SSR markers, 20 genotypes of pigeonpea were grouped into two clusters. The neighbour-joining tree developed based on weighted
average for dissimilarity matrix grouped the 32 genotypes into eight groups (Fig. 2). Group II was the largest group comprising ten genotypes with four sub groups and Group I comprised of eight genotypes, group III comprised of three genotypes, group V comprised of three genotypes, and group VIII comprised of five genotypes. The groups IV, VI and VII were monogenetic containing BRG 2, Kunnathur local and BRG 5, respectively. Based on UPGMA and neighbour-joining methods, the genotype Kunnathur local was observed solitary indicating its distinctiveness and diverse nature among the genotypes. The genotypes Kunnathur local (Cluster VI) had high value for the traits viz. number of pods per plant and single plant yield and the genotype Pillayakothur local (Cluster I) with bold seeds, high pod length, pod width, shelling percentage, protein, fibre and TSS can be used in the breeding programmes for developing genotypes with high yield and yield attributing traits.

Based on the molecular genetic diversity study using SSR markers, the genotypes Kunnathur local, CRG 13-01, BSR 1 and Pillayakothur local were found diverse among the genotypes and had good grain and vegetable pigeonpea traits could be used in the breeding
Fig. 2. Neighbour-joining tree of 32 pigeonpea genotypes based on SSR marker data
programme The results indicated that SSRs provide more definitive separation of clusters indicating a higher level of efficiency for determining the relationship among pigeonpea genotypes.

REFERENCES

Food and Agriculture Organization of the United Nations., FAOSTAT statistical database. [Rome]: FAO, 2019.

Greilhuber, J. and Obermayer, R. 1998. Genome size variation in Cajanus cajan (Fabaceae): a reconsideration. Plant Systematics and Evolution, 212(1):135-141. [Cross Ref]

Gupta, P.K., Balyan, H.S., Sharma, P.C. and Ramesh, B. 1996. Microsatellites in plants: a new class of molecular markers. Current science, 1996 Jan 10: 45-54.

Jarne, P. and Lagoda, P.J. 1996. Microsatellites, from molecules to populations and back. Trends in ecology & evolution, 11(10): 424-429. [Cross Ref]

Kimaro, D., Melis, R., Sibiya, J., Shimelis, H. and Shayanowako, A. 2020. Analysis of genetic diversity and population structure of pigeonpea [Cajanus cajan (L.) Millsp.] accessions using SSR markers. Plants, 9(12): Pp 1643. [Cross Ref]

Manju, Y., Kumar, Y.Y., Pushpendra, K., Kumar, S.R., Renu, Y., Pawan, K., Shaily, J., Mahesh, R., Neelam, Y., Upadhyaya, H.D. and Rajendra, K. 2017. Molecular diversity analysis as an improvement tool for pigeonpea [Cajanus cajan (L.)]. Research Journal of Biotechnology, 12(9):130-136.

Muniswamy, S., Lokesha, R., Saxena, R.K., Fakrudin, B. and Patel, K. 2019. Molecular dissection of genetic diversity in pigeonpea [Cajanus cajan (L) Millsp.] minicore collection. Legume Research-An International Journal (TSI), 42(1): 32-38. [Cross Ref]

Njung’e, V., Deshpande, S., Siambi, M., Jones, R., Silim, S. and De Villiers, S. 2016. SSR genetic diversity assessment of popular pigeonpea varieties in Malawi reveals unique fingerprints. Electronic journal of Biotechnology, 21: Pp 65-71. [Cross Ref]

Olufowote, J.O., Xu, Y., Chen, X., Park, W.D., Beachell, H.M., Didlay, R.H., Goto, M. and McCouch, S.R. 1997. Comparative evaluation of within cultivar variation of rice (Oryza sativa L.) using microsatellite and RFLP markers. Genome, 40: 370-278. [Cross Ref]

Powell, W., Machray, G.C. and Provan, J. 1996. Polymorphism revealed by simple sequence repeats. Trends in plant science, 1(7): Pp 215-222. [Cross Ref]

Pushpavalli, S. N. C. V. L., Rajeswari, R. R. and Kumar, C. V. 2016. Assessment of Genetic Diversity among Pigeon pea Male Sterile lines and Popular Cultivars using SSR Markers. Electronic Journal of Plant Breeding, 7(3): 564-573. [Cross Ref]

Rafalski, J.A. and Tingey, S.V. 1993. Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends in genetics, 9(8): Pp 275-280. [Cross Ref]

Ratnaparkhe, M.B., Gupta, V.S., Murthy, M.R. and Ranjekar, P.K. 1995. Genetic Fingerprinting of Pigeonpea [Cajanus cajan (L.) Millsp.] and its Wild Relatives Using RAPD Markers. Theor. Appl. Genet., 91: 893-898. [Cross Ref]

Sarkar, B., Chakravarthy, V.S.K., Varalaxmi, Y., Yadav, S.K. and Vanaja, M. 2017. Genetic diversity among Pigeonpea (Cajanus cajan (L. Millsp.) genotypes usinggeneric SSRs with putative function for drought tolerance. International Journal of Current Microbiology. App.Sci., 6 (4): 1804-1814. [Cross Ref]

Sharma, P., Singh, I. and Singh, S. 2018. Studies on genetic diversity and inheritance of fertility restoration in pigeonpea [Cajanus cajan (L) Millsp.]. Journal of Food Legumes, 31(3): Pp 135-138.

Smith, J.S.C., Chin, E.C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L., Mitchell, S.E., Kresovich, S. and Ziegle, J. 1997. An evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RFLPs and pedigree. Theoretical and Applied Genetics, 95(1): Pp 163-173. [Cross Ref]

Sousa, A.C.B.D., Godoy, R., Sforça, D.A., Campos, T.D., Zucchi, M.I., Jank, L. and Souza, A.P.D. 2011. Genetic diversity analysis among pigeonpea genotypes adapted to South American regions based on microsatellite markers. Scientia Agricola, 68(4): 431-439. [Cross Ref]

Xiao, J., Li, J., Yuan, L., McCouch, S.R. and Tanksley, S.D. 1996. Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR based markers. Theor. Appl. Genet., 92: 637-643. [Cross Ref]

Yadav K., Singh, B.D., Srivastava, C.P., Chand, R. and Yadav, A. 2010. Analysis of Genetic Divergence in Pea (Pisum sativum L.) Using Quantitative Traits and RAPD Markers. Indian Journal of Genetics and Plant Breeding, 70: 363-369.

Yang, G.P. et al., 1994. Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. Mol. Gen. Genet., 245: 187-194. [Cross Ref]