Received: 30 May 2022 | Revised: 14 Jun 2022 | Accepted: 28 Jun 2022

Electronic Journal of Plant Breeding

Research Article

Molecular genetic diversity analysis in short duration pigeonpea [*Cajanus cajan* (L.) Millsp.]

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Abstract
In the present study, 48 short duration pigeonpea genotypes were used to analyse molecular diversity employing 20 pigeonpea specific microsatellite markers. Among the 20 microsatellite markers, 11 markers produced polymorphism and nine markers showed monomorphic banding patterns. A total of 35 alleles were generated and the number of alleles produced by different markers ranged from one to three with an average of 1.75 alleles per marker. The polymorphic markers *viz*., PGM 82, CcM 1615, CcM 2857 and CcM 1277 produced a maximum of three alleles. The allele size ranged from 150 bp (CcLG08_RFQI4) to 290 bp (CcM 1277). The average PIC value obtained was 0.269. The data analysis for microsatellite markers showed high dissimilarity among 48 genotypes. A dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Average (UPGMA) analysis, 48 genotypes were grouped into seven clusters with the similarity coefficient ranged from 0.43 to 1.00. Among the seven clusters, subcluster 1 of cluster I was the largest with fifteen genotypes followed by nine genotypes in the subcluster 2 of cluster II. From this study, it is concluded that the diverse pigeonpea genotypes *viz*., ICPL 19009, ICPL 19019, ICPL 19030 (cluster I), ICPL 19002 (cluster VI) and CO 6R (cluster VII) could be utilized in the breeding program based on microsatellite marker and cluster analysis.

Keywords: Pigeonpea, microsatellite markers, molecular genetic diversity, Polymorphic Information Content, UPGMA clustering.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is also known as *tur*, redgram, Congo pea, no-eye pea, *kadios* and tropical green pea with the genome size of 858 Mbp (Greilhuber and Obermayer,1998). India ranked first in pigeonpea production of 38.80 lakh tonnes in an area of 48.24 lakh hectares with average productivity of 804 kg per hectare in 2020-2021 (agricoop.nic.in). Among the pulse crops, pigeonpea serves as a key source of dietary protein (18–25%) for the Indian population, in addition to rich in iron, iodine and sulphur and vitamins. The presence of genetic diversity in breeding material is a pre-requisite for successful crop improvement and germplasm conservation in any crop. In general, morphological and agronomic factors are being used to assess the crop's genetic diversity which is highly influenced by the environment. Assessment of genetic diversity with DNA markers will overcome this problem as the variability is studied at a molecular level. Among the DNA markers, microsatellite markers are used widely due to their high polymorphism, reproducibility, loci specificity and co-dominance in nature. The microsatellite markers used for this study are appropriate for assessing the molecular diversity in pigeonpea due to the cultivated pigeonpea is found to have low polymorphism (Costa and Santos, 2021). Hence, the aim of this present investigation was to explore the molecular genetic diversity among 48 pigeonpea inbreds by utilizing 20 microsatellite markers.

MATERIALS AND METHODS

The experimental materials comprising of 48 pigeonpea genotypes maintained at the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore was used for this study. The seedlings were raised using the roll towel method in a germination sheet. The DNA extraction was carried out in the Centre of Excellence in Molecular Breeding, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The DNA was isolated from a fresh leaf sample of 15-days old plant in each of the 48 inbreds by following cetyltrimethyl ammonium bromide (CTAB) method as described by Murray and Thompson (1980). The quality and concentration of DNA were checked through 0.8% agarose gel electrophoresis before PCR amplification. A set of 20 pigeonpea specific microsatellite markers were used for the molecular diversity analysis (Bohra *et al.,* 2012).

The microsatellite markers were selected based on the literature survey of several pigeonpea genomic investigations (Petchiammal *et al*., 2015a; Petchiammal *et al*., 2015b). The following were the thermal cycling conditions: Initial denaturation at 94 °C for 3 minutes, 35 cycles of denaturation (94 °C for 1 minute), 72 °C extensions, annealing (55–61°C) for 1 minute, primer extension (72 °C) for 2 minutes, final extension at 72 °C for 10 minutes, and an end hold at 4 °C. The amplified products were run on a gel electrophoresis unit using 3 per cent Ethidium Bromide stained agarose gel in 1X TBE buffer at 110 V for 2.30 hours before being photographed in a BIO-RAD gel documentation system using short wavelength transilluminating ultraviolet (UV) light. A 100-bp DNA ladder was used as a size fragment standard (Petchiammal *et al*., 2015a).

The microsatellite alleles scored for all the 48 genotypes were analysed in the NTYSYS software (Rao *et al*., 2021*)*. The polymorphic information content (PIC) value is a measure of molecular heterogeneity. Polymorphic information content values were calculated for SSR markers in order to characterize the capacity of each marker to detect polymorphic loci among the genotypes. PIC value was calculated using the formula PIC =1-©pi^2, where, pi is the frequency of the '*i*' th allele (Devi and Jayamani, 2020). Based on the DICE coefficient and the Unweighted Pair Group Method with Arithmetic Average, cluster analysis and dendrogram were constructed (UPGMA) using the SIMQUAL programme of NTYSYSpc 2.02i (Rao *et al.,* 2021).

RESULTS AND DISCUSSION

Forty-eight pigeonpea inbreds were analysed using 20 microsatellite markers to assess the genetic diversity. Out of 20 markers used, eleven showed polymorphism, while nine (PGM 45, PKS 25, CCB1, PKS 26, CcM 120, PGM 10, CcM 0207, PGM 102, CcM 0008) were found to be monomorphic (**Table 1**). Bohra *et al*. (2017) reported that out of 421 microsatellite markers, only 401 markers were amplified and 217 markers showed polymorphism in pigeonpea. Twenty microsatellite markers were not amplified. Among the polymorphic markers, four markers were found to have a maximum of three alleles each (PGM 82, CcM 1615, CcM 2857, CcM 1277), seven markers showed two alleles each (PGM 3, CcLG08_RFQI1, CcLG08_RFQI4**,** CcM 1891, CcM 1459, CcM 0252, CcM 0271). The number of alleles generated by these eleven polymorphic markers was 26 alleles whose allele size ranged from 150 (CcLG08_RFQI4) to 290bp (CcM 1277) with an average of two alleles per marker. Kimaro *et al*. (2020) and Manju *et al*., (2017) reported an average number of 4.78 and 3.4 alleles, respectively in pigeonpea.

Polymorphic information content of (PIC) value of microsatellite marker gives the information on the capacity to produce more number of alleles. A higher PIC value indicates the higher number of alleles detected. Generally, the PIC value ranges from 0 (monomorphic) to 1 (polymorphic). The PIC value of the present study ranged from the lowest value of 0.223 for the marker CcM 1891 to the highest value of 0.687 for the marker PGM 82 with an average PIC value of 0.269 (**Table 1**). The markers *viz.,* PGM 82, CcM 2857 and CcM 0271 recorded high PIC value, found to be more informative and aid to determine the genetic difference among the pigeonpea genotypes. Similar findings were reported in the earlier studies that the PIC value ranged from 0.60 to 0.87 with an average of 0.74 (Rao *et al.,* 2021), 0.11 to 0.71 with an average of 0.38 (Bohra *et al.,* 2017), 0.04 to 0.84 with an average of 0.44 (Kimaro *et al*., 2020) and 0.24 to 0.86 with an average value of 0.50 (Manju *et al*., 2017) in pigeonpea.

The dendrogram was constructed with 35 alleles generated by 20 microsatellite markers and 48 pigeonpea inbreds were grouped into seven clusters at 65 per cent similarity level within the range of 0.43 to 1.00 similarity coefficient based on UPGMA clustering (**Table 2 & Fig. 1)**. Oinam *et al.* (2015) reported similar results that 40 genotypes were categorized into seven clusters. The clusters and sub clusters formed by using 20 microsatellite markers provide information on the genetic diversity at molecular level and observed a high level of diversity among the genotypes studied. Among the seven clusters, the cluster size varied from 1(cluster V, VI, VII) to 15 (cluster I- subcluster 1). The subcluster I of cluster I was highly heterogenous where, more number of genotypes (15) were grouped which was followed by subcluster 2 of cluster II which had 9 genotypes at 80 % similarity coefficient. The genotypes ICPL 19012 and ICPL 19049 had a high similarity coefficient (97% similarity) were grouped together. The genotypes showed high dissimilarity were grouped distantly in a different cluster. The mono genotypic or solitary clusters were observed in the clusters V, VI ad VII, respectively. Hemavathy *et al*., (2017) also reported solitary cluster

Table 1. List of microsatellite markers used for molecular diversity studies

S.No.	Marker name	Forward primer (5'-3') / Reverse primer (5'-3')	Annealing Temp(°C)	Allele size range (bp)	Number of alleles	PIC value
1	PGM 82	F: CACGATTCCATTGGTGGAG R: ACGGTTTCTGGGAGGGTCTA	61	190-210	3	0.687
2	PGM 3	F: ACACCACCATGCTAAAGAACAAG R: CCAAGCAAGACACGAGTAATCATA	60	180-190	2	0.530
3	PGM 45	F: GGGAAACTCACCTATATTACCAA R: CACTACCGTCTACAGCCATCTC	60	230	1	0.000
4	CcLG08_RFQI1	F: AGGGAGAATCCCTTGTTTGG R: AGACATCAACACCCGATTCA	56	160-170	2	0.538
5	CcLG08_RFQI4	F: GAATGCATTACTAGCACTCCTCAA R: GCTGAGGGTCTGAAGGTTTG	56	150-160	2	0.352
6	CcM 1615	F:TTCAAAGTTTGCATTATCGCT R: GTTCTCAGCCGAGAGCATTC	58.5	200-220	3	0.578
7	CcM 1891	F: AATGATTCAAGGTGCAAGGG R: CCATCCAATCCAATTAAAGGC	58.5	200-210	2	0.223
8	CcM 1459	F:TTGGGATTGACCTTCCAAAG R:CAAGATCAAGAAATAATAAGACACGA	58.5	180-190	2	0.311
9	CcM 2857	F:TCCACGAATTCTCTATGCCC R: CCCTTTTCTTGATTGGTTTCA	58.5	240-260	3	0.605
10	CcM 0252	F:CATAGAAGCCCACCTTCCAA R: CTGCATGCAAAACGAAGAAG	58	220-230	2	0.444
11	PKS 25	F: TACAGCAGCCACATCAAAGC R: TGAACCGTGAAAGTGGGATT	58	220	1	0.000
12	CCB1	F: AAGGGTTGTATCTCCGCGTG R: GCAAAGCAGCAATCATTTCG	59	200	1	0.000
13	PKS 26	F: ACCCATTATTGATTTGGGTA R: CCAAATTTCACCCAAGAAA	55	210	1	0.000
14	CcM 1207	F:TTCTCCCCAAATTTCCACAG R: TTTTTGGCATTCTTTTTGGA	55	210	1	0.000
15	CcM 0271	F:TGCTTCGCATTCCTCTTTTT R: AGGAAAATGCTGCTTTGCAC	57.5	250-260	2	0.617
16	CcM 1277	F:TACCTTTGGAGGCTTTGGTG R: TTGCGACAACCCTGTCAATA	59.5	270-290	3	0.514
17	PGM 10	F: TCACAGAGGACCACACGAAG R: TGGACTAGACATTGCGTGAAG	61	250	1	0.000
18	CcM 0207	F:TTTTGGCGGTCATTTTAACC R: TTAGTCGGGAGCAACACTGA	58.5	210	1	0.000
19	PGM 102	F: ATCGGCTTTTGTCTTGATGA R: AAGCTACAAGGGATACACATGC	58	210	1	0.000
20	CcM 0008	F:CGGTGAAAAGGGTCAATGAG R:CAAAATTAAAGCCTACTTATTTTACGA	58	200	1	0.000
		Total			35	5.399
		Average			1.75	0.269

among three main clusters while analysing molecular diversity in pigeonpea. Similar ranges of coefficient were reported by Rao *et al. (*2021) (0.28 to 0.91) and Manju *et al*. (2017) (0.45 to 0.93) in pigeonpea.

The results from this study showed the potentiality of microsatellite markers for estimating the genetic diversity among pigeonpea genotypes. Based on field performance and cluster analysis, the diverse genotype *viz.*, ICPL 19009, ICPL 19019, ICPL 19030 (cluster I), ICPL 19002 (cluster VI) and CO 6R (cluster VII) can be utilized further in the pigeonpea improvement program.

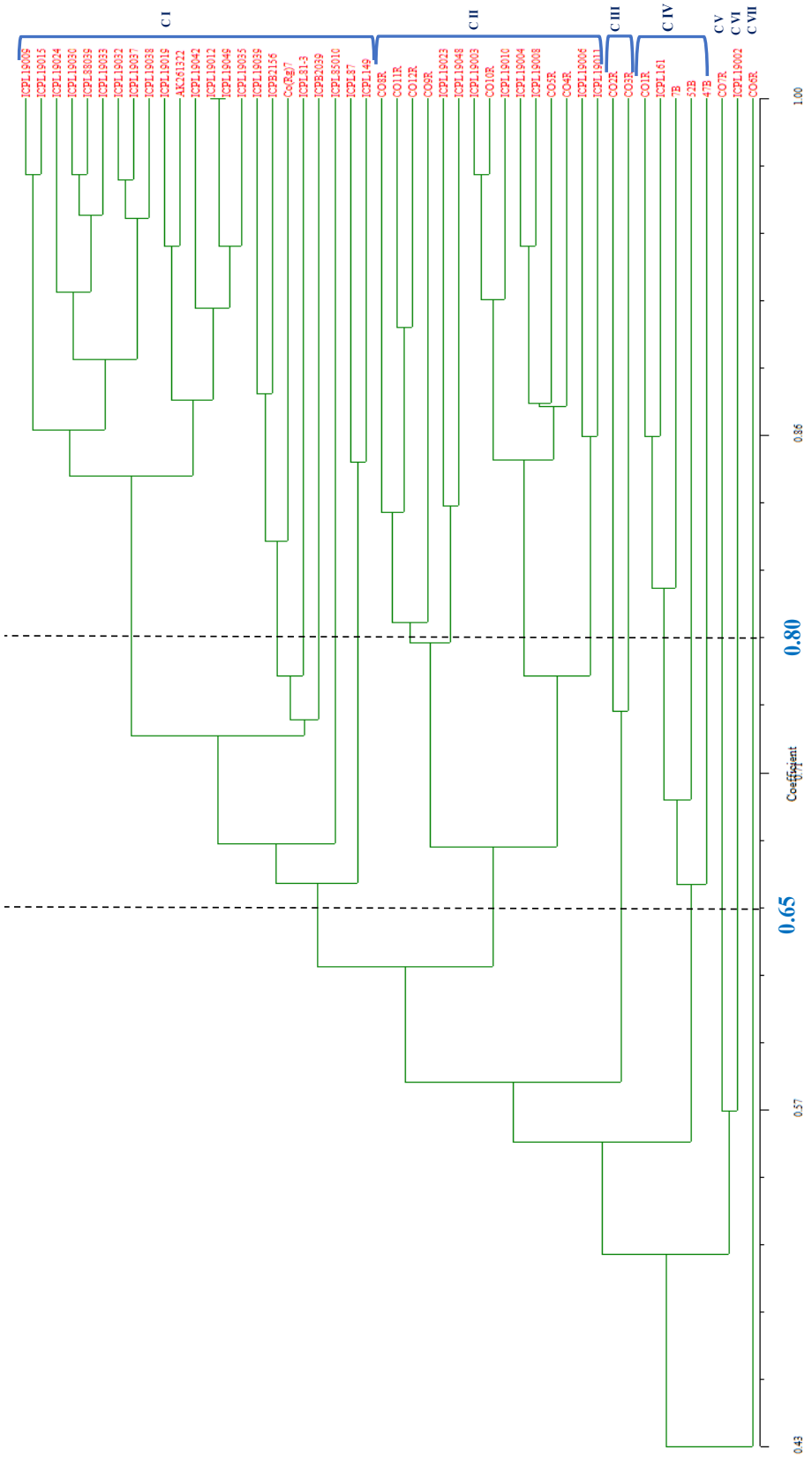

Fig. 1. Classification of genotypes based on dendrogram.

Table 2. Classification of genotypes into clusters based on dendrogram with 20 microsatellite markers

Cluster	Number of genotypes	Genotypes
I	15	ICPL 19009, ICPL 19015, ICPL 19024, ICPL 19030, ICPL 88039, ICPL 19033, ICPL 19032, ICPL 19037, ICPL 19038, ICPL 19019, AK 261322, ICPL 19042, ICPL 19012, ICPL 19049, ICPL 19035
	8	ICPL 19039, ICPB 2156, CO (RG) 7, ICPL 81_3, ICPB 2039, ICPL 85010, ICPL 87, ICPL 149.
II	6	CO 8R, CO 11R, CO 12R, CO 9R, ICPL 19023, ICPL 19048
	9	ICPL 19003, CO 10R, ICPL 19010, ICPL 19004, ICPL 19008, CO 5R, CO 4R, ICPL 19006, ICPL 19011
III	2	CO 2R, CO 3R
IV	5	CO 1R, ICPL 161, 7B, 52B, 47B
V	1	CO 7R
VI	1	ICPL 19002
VII	1	CO 6R

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