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Research Article

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

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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 for 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 94^0 C for 3 minutes, followed by 35 cycles of denaturation at 94^0 C for 1 minute, annealing temperature at 53^0 C to

Table 1. List of pigeonpea genotypes used in the study

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	Puliampatti local-1	Puliampatti, Krishnagiri
6	Puliampatti local-2	Puliampatti, 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	Irapputhuvarai	Kalakuruchi, Villupuram
15	Soolagiri local-1	Soolagiri, Krishnagiri
16	Soolagiri local-2	Soolagiri, Krishnagiri
17	Gengusettipatti local	Gengusettipatti, Krishnagiri
18	Periyavathal malai local	Vathal malai hills, Dharmapuri
19	Vathalmalai 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,

61^0 C for 1 minute and extension at 72^0 C for 2 minutes and final primer extension at 72^0 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 PIC $=1-\Sigma pi^2$, where, pi is the frequency of the '*i*'th allele (Smith *et al.*, 1997).

Table 2. List of microsatellite markers used in the study

S. No.	Marker	Forward and reverse sequence	Annealing temperature (^{0}C)	Allele size (bp)	Number of alleles	PIC value	Remarks
1	CCB 1	F: AAGGGTTGTATCTCCGCGTG R: GCAAAGCAGCAATCATTTCG	59.50	190	1	0.00	Monomorphic
2	CCB 10	F: CCTTCTTAAGGTGAAATGCAAGC R: CATAACAATAAAAGACCTTGAATGC	53.00	200-210	2	0.28	Polymorphic
3	CCB 7	F: CAACATTTGGACTAAAAACTG R: AGGTATCCAATATCCAACTTG	53.00	180	1	0.00	Monomorphic
4	PGM 10	F: TCACAGAGGACCACACGAAG R:TGGACTAGACATTGCGTGAAG	61.00	190-200	2	0.16	Polymorphic
5	PGM 102	F: ATCGGCTTTTGTCTTGATGA R: AAGCTACAAGGGATACACATGC	58.50	180	1	0.00	Monomorphic
6	PGM 106	F: TGAAATGAACAAACCTCAATGG R: TGTATTGCACATTGACTTGGCTA	58.50	200-210	2	0.26	Polymorphic
7	PGM 109	F: ATTCCCTCTCTATCTCAGACTTTT R: TCGTGATGGAACTCAAGATACACT	60.50	190-200	2	0.19	Polymorphic
8	PGM 16	F: CATTTATTTCTCTCTGGCATTCAC R: CGAGCTGCAAGCATAAACG	60.00	210-220	2	0.06	Polymorphic
9	PGM 3	F: ACACCACCATGCTAAAGAACAAG R: CCAAGCAAGACACGAGTAATCATA	60.50	180-200	3	0.12	Polymorphic
10	PGM 45	F: GGGAAACTCACCTATATTACCAA R: CACTACCGTCTACAGCCATCTC	60.50	200-220	2	0.19	Polymorphic
11	PGM 5	F: ATCGCTTTGCATCCTTATC R: CTTCACGTACATTTTCGTTT	55.00	200-210	2	0.36	Polymorphic
12	PGM 82	F: CACGATTCCATTGGTGGAG R: ACGGTTTCTGGGAGGGTCTA	61.00	190-200	2	0.11	Polymorphic
13	PKS 18	F: ACGCTTCTGATGCTGTGTTG R: CATCAGCATCATCGTTACCC	60.00	200	1	0.00	Monomorphic
14	PKS 26	F: ACCCATTATTGATTTGGGTA R: CCAAATTTCACCCAAGAAA	55.00	190	1	0.00	Monomorphic
15	PKS 30	F: AAGTGTGACACCCTCTACCC R: TGACATCGGGACATAGATAGAA	59.50	190-200	2	0.19	Polymorphic
16	CcM 0257	F: GCCGTTACGAGGGTAATGAA R: CTGTCTCAAAGGGACCCTGA	60.00	200-230	3	0.29	Polymorphic
17	CcM 0948	F: GCACAGGTCACGTCTGTACC R: CATTTTCCCACCTTTCCTGA	60.00	160-180	3	0.21	Polymorphic
18	CcM 0008	F: CGGTGAAAAGGGTCAATGAG R:CAAAATTAAAGCCTACTTATTTTACGA	58.00	180-210	4	0.64	Polymorphic
19	CcM 0039	F: AGGAATAATGTTTGCTGCGG R: TTGGTATGTGGAACGATTGC	59.00	190-200	3	0.21	Polymorphic
20	CcM 1026	F: TCAGTGCAAAGAAGCCTCAG R: GGAATGCATGATAGAGTAAACGA	59.00	200-230	4	0.62	Polymorphic
			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, PKS 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 (Irapputhuvarai local), IX (Puliampatti 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 similarity 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

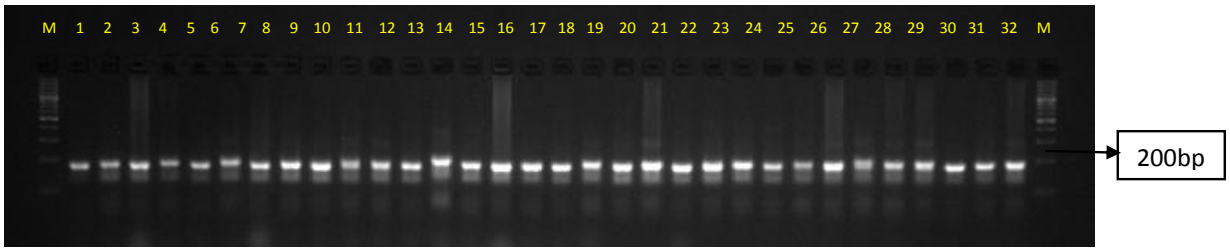

Plate 1. SSR marker profile of 32 pigeonpea genotypes by CcM0008

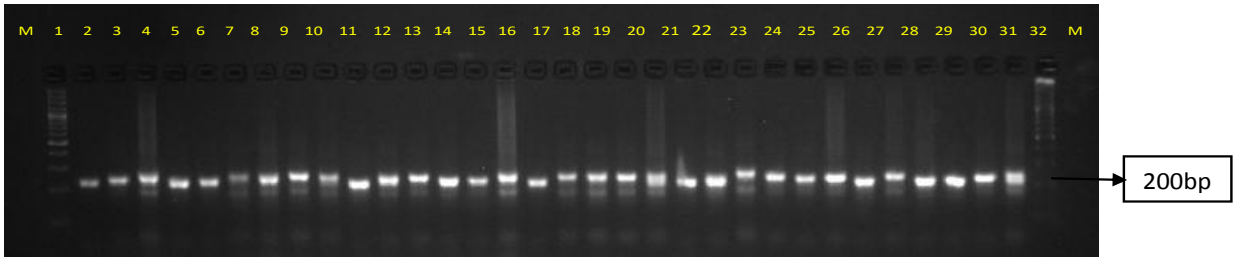

Plate 2. SSR marker profile of 32 pigeonpea genotypes by CcM1026

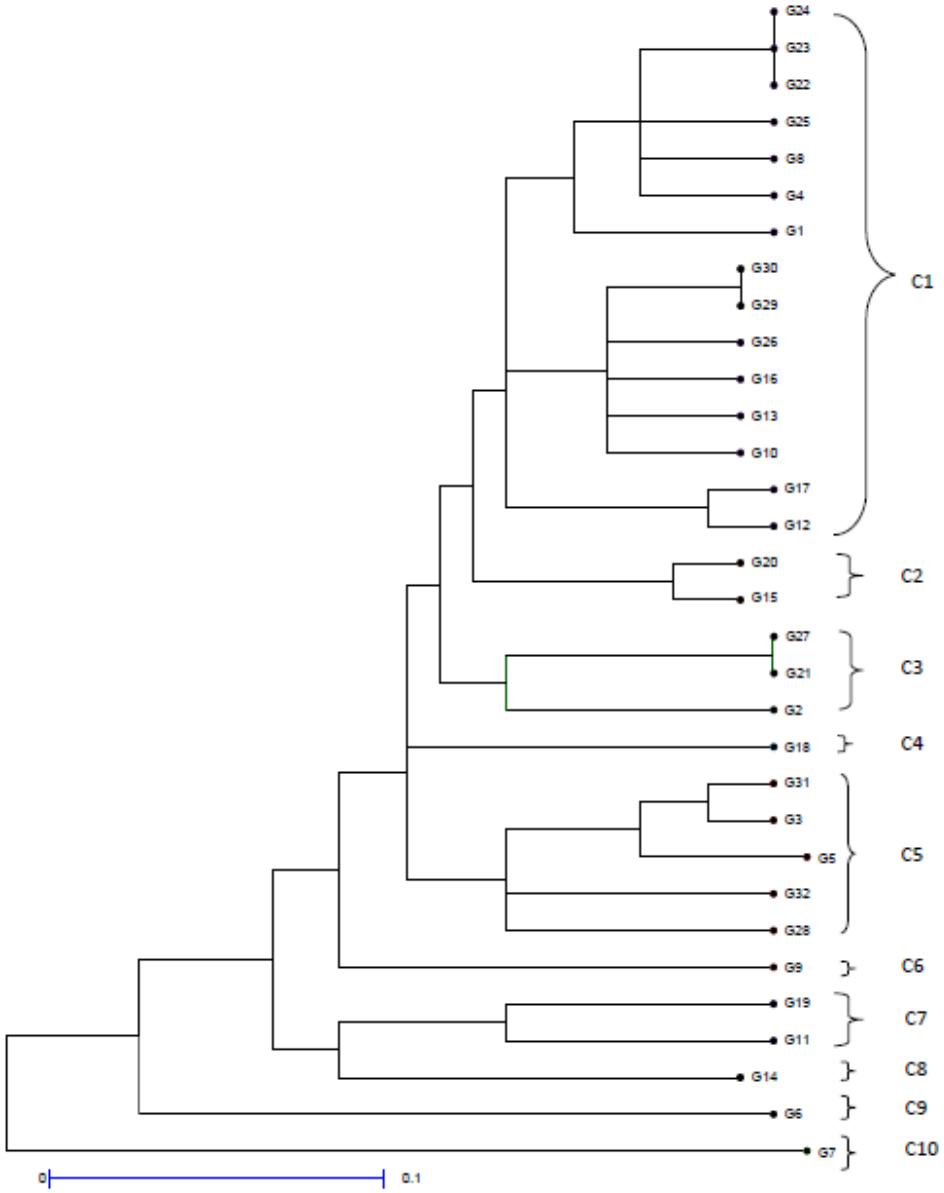

Fig. 1. Dendrogram of 32 pigeonpea genotypes based on SSR marker data

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

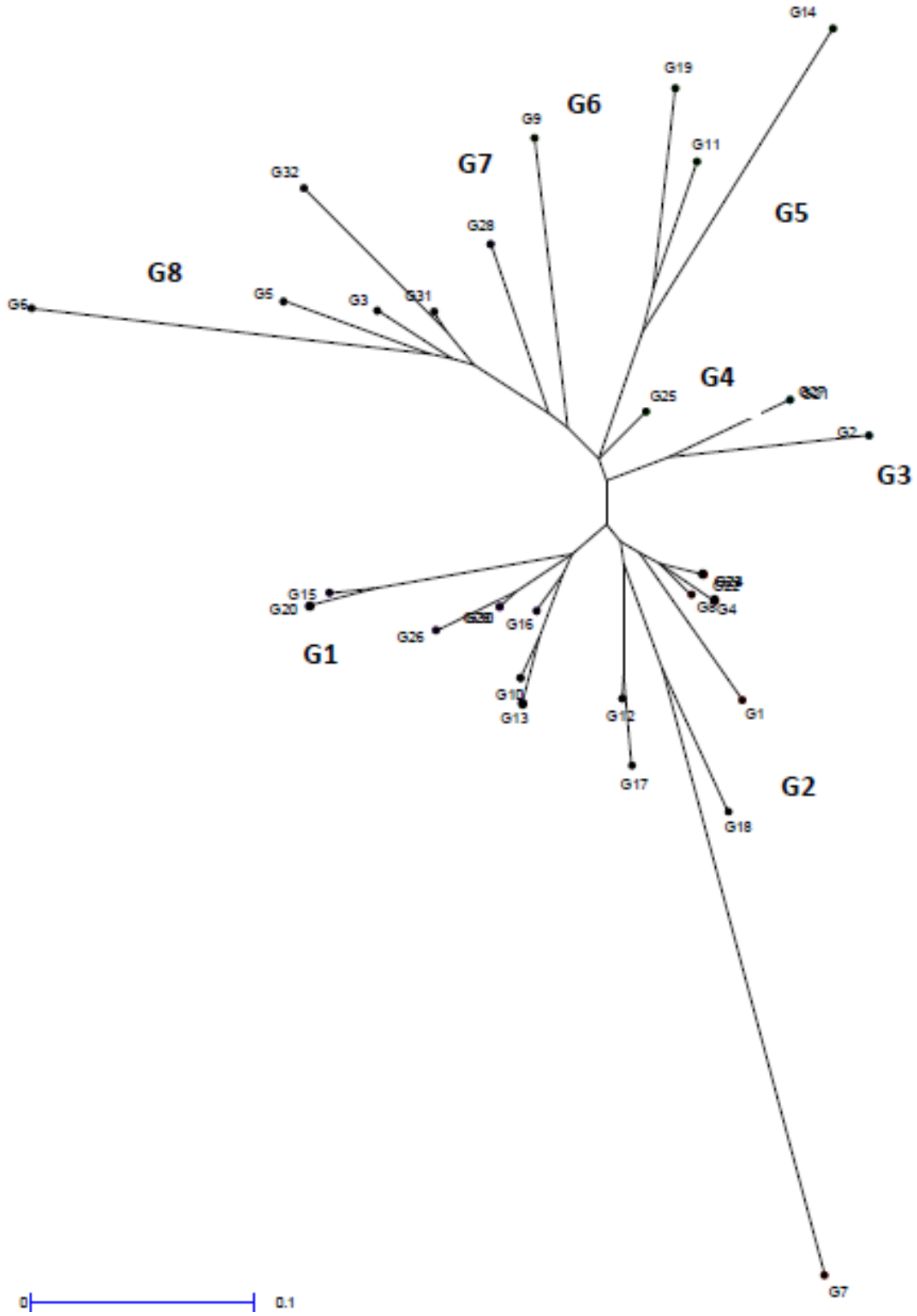

Fig. 2. Neighbour-joining tree of 32 pigeonpea genotypes based on SSR marker data

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.

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