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Molecular characterization of wild and cultivated pigeonpea (*Cajanus* spp.) species by microsatellites

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Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a grain legume crop of the tropics and subtropics for its high protein (18–22%) seeds particularly in the Indian subcontinent where it accounts for 70% of the world's production and coverage. Analyzing genetic relationships in species is important for revealing diversity. In addition to displaying the existing variability among cultivars genetic diversity provides valuable information on target trait availability and diversity for successful breeding programmes. Molecular marker studies are increasingly important tools for genetic and genomic studies, breeding and biodiversity research. Currently several DNA-based molecular marker technologies are available for genetic diversity analysis. Among different types of molecular markers, markers based on simple sequence repeats (SSR) had been shown to be highly polymorphic even between closely related individuals within a species (Edwards *et al*. 1996) and tend to show more polymorphism than many alternative marker systems (Sarkar *et al.* 2020). Microsatellites have been increasingly used to assess genetic diversity and population structure among plants (Bohra *et al.* 2017 and Kinhoegbe *et al.* 2022). Keeping these factors in mind, current study was aimed to investigate the molecular diversity of wild and cultivated accessions that are frequently used for the creation of new breeding materials.

The study was carried out at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. A total of 55 germplasm accessions, including 5 wild species, 22 cultures and 28 cultivated species were choosen for the study which were obtained from the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu (Table 1).

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Genomic DNA isolation and PCR amplification: For genomic DNA isolation using a modified version CTAB method as described by Rogers and Bendich (1985). A total of 17 SSR markers were selected for PCR analysis based on their performance and reproducibility (Table 2).

Construction of dendrogram: NTSYSpc version 2.21 used with unweighted pair group method and arithmetic mean (UPGMA) (Excel software New York, USA).

Polymorphism information coefficient (PIC): PIC is a measure of the in formativeness of a genetic marker in any species Botstein *et al.* (1980). The PIC of a genetic marker is estimated by:

$$PIC = 1 - \sum(Pi)^2$$

Where Pi, Frequency of the 'i'th allele.

In the current study, the ability of the primers to resolve genotype variability varied greatly. The formed polymorphic bands were useful for assessing molecular diversity among the germplasm. A total of 17 SSR markers were screened across 55 pigeonpea germplasm. 10 polymorphic markers showed a total of 35 alleles, with an average of 3.5 alleles per locus. The average number of alleles per locus ranged from 2–5 alleles.

The highest number of alleles (five) were detected for the marker CCB 10, PGM 106, CCM 1263 followed by four alleles for CCM 0268 followed by three alleles for CCM 1538, CCM 1886 and CCM 2971 and the lowest number of alleles (two) was detected for the markers PGM 5, CCM 0583 and CCM 1026.

Polymorphism information content (PIC): The PIC (Polymorphism information content) value was used to calculate the discrimination strength of each locus. The SSR loci's PIC values varied from 0.51–0.80 (Table 3), with an average of 0.664. The highest PIC value was observed for primer CCB 10 (0.80) followed by CCM 1263 (0.73), CCM 0268 (0.70), PGM 106 (0.68), CCM 1538 (0.65), CCM 1886 (0.64), CCM 2971 (0.63), PGM 5 (0.62) and CCM 1026 (0.56). The lowest value of PIC was observed for the primer CCM 0583 (0.51).

PIC values of 0.5 or higher indicate that a molecular marker is highly informative for genetic studies and is incredibly helpful in determining the polymorphism rate of a marker at a particular locus. According to the PIC values of the markers used in the current study, CCB 10 would be the best choice for screening 55 pigeonpea genotypes. This was followed by CCM 1263, CCM 0268 and PGM 106. The PIC value thus signifies that all of these primers are highly informative and capable of genotype divergence.

Genetic relationship and cluster analysis: To understand the genetic relationships among the 55 pigeonpea genotypes, the UPGMA method (Unweighted paired group method using arithmetic averages) was used to construct a dendrogram using a total of 10 polymorphic SSR primers.

The dendrogram of the hierarchical cluster analysis (HCA) separated the 55 genotypes into nine main clusters (Supplementary Table 1 and Fig. 1) at 67% similarity within the range of 0.61–0.94 similarity coefficient based on UPGMA clustering. Among the nine clusters, the cluster size varied from 2 (cluster 2, 9) to 12 (cluster 6). The wild species were grouped separately into different clusters. One cluster comprised of CRG 20-001, CVP20-002, Agraharam local, APK1, CO9 and BDN 2013. The genotypes Guliyal red and BSMR846 formed another cluster. Another cluster had CRG20-012, CRG20-009, ICPL17116 and CVPP20-015. The genotypes *Rhynchosia bracteata*, CRG18-005, *Rhynchosia sublobata*, CRG20-011, CRG16-12, CRG18-010, *Cajanus scarabaeoides*, TJT 501 formed a distinct cluster. Another cluster contained BRG300, CRG18-007, CVPP20-022, CVPP20-014, CRG18-001, CVPP20-021, BRG1, CRG18-003, Nallur local and CVPP20-007 genotypes. The genotypes CRG20-004, CVPP20-005, CVPP20-012, CVPP20-029, ICPL7035, CRG20-006, CO8, *Cajanus cajanifolius*, CRG18-002, CRG189, *Cajanus sericeus* and CRG18-008 were grouped into separate large cluster. The genotypes CVPP20-001, CRG20-005, BSR1, CRG20-003, CRG20-002 and CRG20-010 were grouped into separate cluster. Another cluster contained CRG20-007, CVPP20-006, CRG18-007 and CRG20-004 genotypes. All the other genotypes (CRG20-008 and CRG18-006) were grouped separately from each other.

The primary objective of the current investigation was to use SSR markers to examine the degree of molecular genetic diversity among the pigeonpea germplasm using 17 SSR markers. Seven were monomorphic and 10 markers shown polymorphism. 10 polymorphic markers showed a total of 35 alleles, with an average of 3.5 alleles per locus in current study. The amplification of SSRs derived pigeonpea primers viz. CCB 10 was observed with an average of 2.15 alleles per primer, the number of alleles varied from 1–4. The PIC values calculated for these 35 polymorphic markers ranged from (0.22) CCM 2004 to (0.87) CCM 0494, with an average of 0.59. In the present study, the SSR loci's PIC values varied from 0.51–0.80, with an average of 0.66. The PIC value of 0.65 and the results was obtained by Songok *et al.* (2010). The higher PIC value implies the informativeness of the markers. The highest PIC value (0.80) was observed for primer CCB 10 followed by CCM 1263 (0.73), CCM 0268 (0.70), PGM 106 (0.68), CCM 1538 (0.65), CCM 1886 (0.64), CCM 2971 (0.63), PGM 5 (0.62) and CCM 1026 (0.56). The lowest value of PIC (0.51) was observed for the primer CCM 0583.

The markers CCB 10, PGM 10, PGM 106, CCM 1538, CCM 1886, CCM 2971, CCM 1263, CCM 0268, CCM 0583, PGM 5 and CCM 1026 showed polymorphism among the genotypes. Microsatellite profiling showed that maximum alleles with amplicon sizes of 150–260 bp. This indicated the effectiveness and higher resolution of such marker systems in detecting molecular diversity. Similar to this, a dendrogram was created using 55 pigeonpea genotypes and highly polymorphic 10 SSR markers which revealed 9 major clusters, ranging from clusters 1–9, with cluster 6 having the largest number of genotypes (12) and clusters 2, 9 having the smallest number of genotypes (2). This study showed the divergence among pigeonpea genotypes which can be further used in pigeonpea breeding programmes. All genotypes involved in this study exhibited a wide range of genetic variability due to different centres of origin, and

Table 1 Pigeonpea genotypes used in the present study

1	CRG20-001	29	CRG18-005
2	ICPL20137	30	CRG18-002
3	Guliyal red	31	*Rhynchosia sublobata*
4	BSMR846	32	*Cajanus scarabaeoides*
5	CRG20-012	33	*Cajanus cajanifolius*
6	CRG20-011	34	*Cajanus sericeus*
7	BDN2013	35	*Rhynchosia bracteate*
8	CRG20-007	36	CRG16-12
9	CRG20-009	37	BSR1
10	CRG20-010	38	CVPP20-021
11	BRG 00	39	TJT501
12	ICPL17116	40	CVPP20-029
13	CRG20-004	41	CVPP20-022
14	CRG20-003	42	ICPL7035
15	CRG20-005	43	CVPP20-015
16	CRG20-006	44	CVPP20-014
17	CRG20-002	45	CVPP20-012
18	CRG20-008	46	CVPP20-007
19	BRG1	47	CVPP20-006
20	CRG189	48	CVPP20-005
21	CRG18-007	49	CVPP20-002
22	CRG18-003	50	CVPP20-001
23	CRG18-008	51	Agraharam local
24	CRG18-004	52	Nallur local
25	CRG18-006	53	CO 9
26	CRG18-007	54	APK1
27	CRG18-001	55	CRG18-010
28	CO 8		

Table 2 List of SSR primers used and their sequence information

S.No.	Primer (SSR)	Sequence (5'–3')		Annealing temperature (°C)
1	PGM 106	F	TGAAATGAACAAACCTCAATGG	58.0
		R	TGTATTGCACATTGACTTGGCTA	
2	CCM 1538	F	AACAACAAACAAGCAAGGGC	59.0
		R	TCAAGTAAATGAATAGCTCATCGAA	
3	CCM 1886	F	CATGTATGTTCCCTGTATTTAATTTG	59.5
		R	AGGCTTTTGTACCACCGTGT	
4	CCM 2971	F	TTGATTTGAGTCTGCCCAATC	59.5
		R	AAAAGCTCCAACGTGTGTCC	
5	CCB 10	F	CCTTCTTAAGGTGAAATGCAAGC	53.0
		R	CATAACAATAAAAGACCTTGAATGC	
6	CCM 1263	F	CCCAAAATACACCCAATTCA	59.0
		R	GCATATCCTGCTAATGTCGATT	
7	CCM 0268	F	CCTTTTGGGTTAGGGTATCCA	60.0
		R	CCCCTAACGTAGCCTGTCAA	
8	CCM 0583	F	AGTTGGAAGCGATTGGATAAA	57.5
		R	ATCCCTAAAATAGGTCGATTAGATT	
9	PGM 5	F	ATCGCTTTGCATCCTTATC	55.0
		R	CTTCACGTACATTTTCGTTT	
10	CCM 0271	F	TGCTTCGCATTCCTCTTTTT	57.5
		R	AGGAAAATGCTGCTTTGCAC	
11	CCM 1026	F	TCAGTGCAAAGAAGCCTCAG	59.0
		R	GGAATGCATGATAGAGTAAACGA	
12	CCM 1982	F	TATCAAACCTGGCGATCACA	57.5
		R	ATTCCGCAAACACATCACAA	
13	CCM 0371	F	CACTCCGCACTCTCCTTCTC	61.5
		R	CCAAATCGAAGATTCCCTCA	
14	CCM 2280	F	TTTAAGAGTTGGATTGTTGGAATTT	59.5
		R	ATTGAGCGGGGTAGGTCTTC	
15	CCM 0257	F	GCCGTTACGAGGGTAATGAA	60.0
		R	CTGTCTCAAAGGGACCCTGA	
16	PKS 18	F	ACGCTTCTGATGCTGTGTTG	60.0
		R	CATCAGCATCATCGTTACCC	
17	CCM 1781	F	AAACATGCATATTGCAAATTTTATT	55.0
		R	TGTTCATTTATTTGATGTCTGTCAA	

different genetic constitutions. The genetic relatedness detected in this study may constitute the foundation for future systematic pigeonpea breeding programmes.

SUMMARY

The primary objective of the current investigation was to use SSR markers to examine the degree of molecular genetic diversity among the 50 cultivated genotypes and five wild species using 17 SSR markers. Seven were monomorphic and 10 markers showed polymorphism. The markers CCB 10, PGM 10, PGM 106, CCM 1538, CCM 1886, CCM 2971, CCM 1263, CCM 0268, CCM 0583, PGM 5 and CCM 1026 showed polymorphism among the genotypes. Microsatellite profiling showed that maximum alleles with amplicon sizes of 150–260 bp. This indicated the effectiveness and higher resolution of such marker systems in detecting molecular diversity. Similar to this, a dendrogram was created using 55 pigeonpea genotypes and highly polymorphic 10 SSR markers which revealed 9 major clusters, ranging from Clusters 1–9, with cluster 6 having the largest number of genotypes (12), and clusters 2, 9 having the smallest number of genotypes (2). This study showed the divergence among pigeonpea genotypes which can be further used in pigeonpea breeding programs. All genotypes involved in this study exhibited a wide range of genetic variability due to different centres of origin,

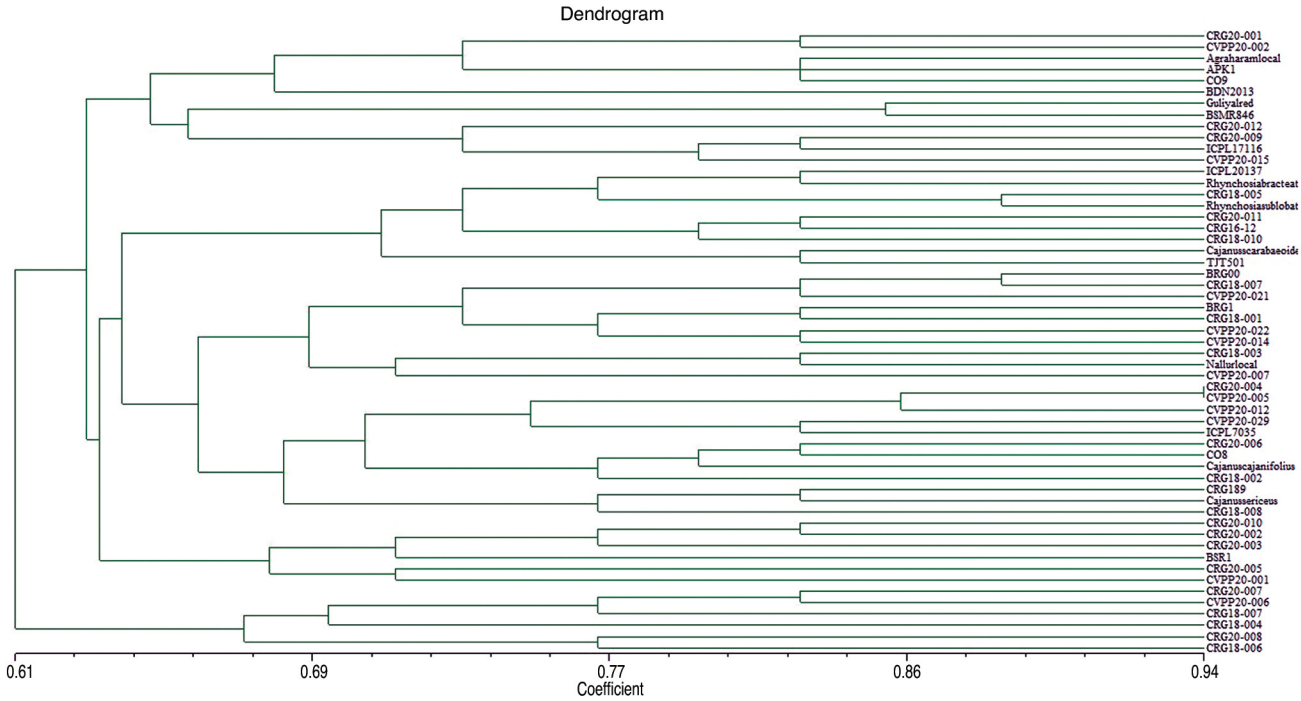

Fig. 1 Dendrogram of pigeonpea germplasm based on molecular data.

Table 3 PIC of SSR loci across various germplasm analyzed

S.No.	Primers	No. of alleles	PIC
1	PGM 106	5	0.68
2	CCM 1538	3	0.65
3	CCM 1886	3	0.65
4	CCM 2971	3	0.64
5	CCB 10	5	0.88
6	CCM 1263	5	0.73
7	CCM 0268	4	0.70
8	CCM 0583	2	0.51
9	PGM 5	2	0.64
10	CCM 1026	3	0.56
Total		35	-
Average		3.5	0.66

PIC, Polymorphism infamation content.

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