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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *CAJANUS SCARABAEOIDES* AND *CAJANUS CAJANIFOLIUS* RELATED CMS LINES IN PIGEONPEA

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Abstract

With the aim to quantify diversity among 12 genotypes comprising six stable CMS lines and their maintainers derived from inter-specific crosses *viz*., *C. scarabaeoides* × *C. cajan* and *C. cajanifolius* × *C. cajan*. These lines were assessed with morphological features and 20 genic microsatellite markers. The selected SSR primers showed a high level of polymorphism, as their corresponding polymorphism information content value ranging from 0.58 to 0.72 with average PIC of 0.68. All the 12 genotypes representative of wild and cultivated species, clustered into five distinct major groups mostly based on the interspecific differences. This study showed ICPA 2043 and ICPB 2043 were most similar with 0.00 distances. While, GT 33A was showed high distance with all other genotypes. This study would accelerate the hybrid breeding program and improvement of both varieties and hybrids in pigeonpea. Particularly, this study would be helpful to restorer identification through marker assisted breeding as well as exploitation of cytoplasmic male sterile lines with three line breeding system in pigeonpea.

Key words : Pigeonpea, cytoplasmic male sterility, maintainer, SSR marker.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is the is one of the most suited crops under minimal resource farming system prevailing in semi-arid, and sub-tropical farming systems of the various countries (FAO, 2013). Pigeonpea is a diploid crop (2n = 2x = 22), with the genome size of 833.1 Mbp arranged in 11 pairs of chromosomes (Varshney *et al*., 2012). Globally, it covers 6.2 Mha area out of which 70% *i.e.* 4.6 Mha covered by India with a production of 3.49 Mt (FAO, 2013). It is a crucial source of dietary protein for vegetarians; its seeds contain about 21% protein and a good quantity of substantive amino acids. Since 1961, it has shown an exponential growth worldwide with the harvested area from 2.7– 6.2 Mha in 2013 (FAO, 2013). However, the productivity was stagnant around 762 kg/ha.

Despite the concerted efforts during the past few decades; pigeonpea yield has not increased considerably due to lack of enough genetic diversity for desirable traits in the germplasm used for its improvement. Genetic gain is also limited due to the narrow genetic base, which is a barrier to getting any new genetic variation for the breeding program. The available cultivars are not well adapted to varying growth conditions in different agro-ecological niches. To overcome the existing yield barriers, hybrid technology based on cytoplasmic male sterility (CMS) has been proved to be successful in pigeonpea (Saxena *et al*., 2010a). This cytoplasmic-genic male sterility (CGMS) consisting A line with S (rr), B line with F (rr) and R line with genotype S/F (RR), which was used in CGMS based hybrids development (Parmar and Tikka, 2001).

Availability of DNA markers has opened a new avenue for the improvement of pigeonpea cultivars and required for conducting the molecular breeding programme. Among different types of available molecular markers systems, simple sequence repeat (SSR) or microsatellite is favoured most for the molecular plant breeding and genetics studies because of co-dominant nature and in the case of legumes, such as pigeonpea, the SSR markers have already proven its valuable utility

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in molecular studies (Gupta and Varshney, 2000; Varshney *et al*., 2010). Therefore, the present study was carried out to assess the genetic diversity among the interspecific derivatives lines by using morphological and microsatellite markers.

Materials and Methods

Plant materials and data observation

Twelve pigeonpea genotypes consisted of six CMS lines along with its maintainer were belonged to two gene pool groups and traits wise explained (table 1). A first group had eight genotypes *viz*., MA CMS 25A, MA CMS 32A, GT 288A and GT 33A and their maintainers, which were derived from the A2 cytoplasm, *Cajanus scarabaeoides*. Two CMS lines namely MA CMS 25A, MA CMS 32A, were developed at Banaras Hindu University, Varanasi, while, rest two CMS GT 288A and GT 33A were originated by Gujarat Agricultural University. The second group consisted four genotypes likewise, ICPA 2043 and ICPA 2092 along with maintainers they are originated from the A_4 cytoplasm, *Cajanus cajanifolius* by ICRISAT, Hyderabad. The both groups of CMS lines were showed frost susceptible, when the temperature goes down about 10°C then pollen turned to sterile, flowers drop and initially pods also drop. When the temperature goes to about 26°C than plants comes in normal condition (Saroj *et al*., 2015).

For morphological evaluation plants were grown at Agricultural Research Farm, Banaras Hindu University, Varanasi during crop season over three years (2009-10, 2010-11 and 2011- 2012). Each CMS line was grown in three rows and their maintainer was planted two rows, each row of maintainer was planted flanking the outer side of A-lines in the separate block. Each plot consisted 4-meter length and inter and intra-row spacing 75 × 25 cm. The morphological data of the 14 phenotypic traits *viz*. days to flowering, plant height, number of primary and secondary branches, days to maturity, pods per plant, seeds per pod, pod length, 100 seed weight, flower colour, plant type, pod colour, seed colour and seed yield per plant were recorded across the year and pooled for the analysis. The field trials were conducted under entomophilous proof nylon net cage (0.5 mm) to avoid out crossing.

Genotyping with SSRs

DNA was isolated from fresh young leaves collected after 2 weeks old plant. DNA extracted according to modified CTAB DNA extraction method by Doyle and Doyle (1987). Genomic DNA was quantified on 0.8% agarose gels. A set of 20 microsatellite primer pairs (table 1), as reported by Bohara *et al*. (2011) were chosen for the present study. Primers pair was chosen on the basis of amplification and polymorphism.

For Polymerase Chain Reaction (PCR), the final volume of 15µl of reaction mixture consisted of 1.5µl of 10x buffer, 0.20µl of 10mM dNTPs, 1.0µl each of forward and reverse primers (10pmol), 1.0 µl of template genomic DNA (30ng/µl), 0.2µL of Taq DNA polymerase (5U/µl) (Vivantis Technologies). The PCR amplification conditions were as follows, initial extended step of denaturation at 94°C for 4 minutes followed by 39 cycles of denaturation at 94°C for 30 seconds, primer annealing at respective annealing temperature for 30 seconds and primer extension at 72°C for 30 seconds and final elongation at 72°C for 5 minutes. The reaction product was mixed with 2.0µl of 6x loading dye (Bromophenol blue 0.25%; Xylene, cyanol0.25% and glycerol 30%) and spun briefly in a microfuge before loading. After loading of all samples, 5 µl of 50 bp (base pairs) DNA size ladder was loaded in first well as a reference for the molecular weight of amplified products. The amplicons were separated on 2.5% agarose gel at 95 V for 3 h for well separation of PCR fragments using an electric power (BIORAD, USA) in TAE buffer.

Scoring and data analysis

The amplicons were visualized under gel documentation system for analysis. The amplicons were scored as '1' for the presence and '0' for the absence of a band generating the 0 and 1 matrix. These binary data matrix was then utilized to generate genetic dissimilarity data among the 12 lines of pigeonpea through the DARwin software (Perrier and Jacquemoud-Collet, 2006) and PIC values were computed by Power Marker ver. 3.25 (Liu and Muse, 2005).

Results and Discussion

Morphological characterization

Visible contrasting traits are a robust tool for breeders, which can be directly used for selection or separation of desirable genotypes among the population. In the case of CMS based genotypes/lines have translucent anther or sterile pollen grains (unstained) as compared to normal genotypes. In the present study, several phenotypic characters were considered for selection of good general or specific combiner of stable CMS lines for further use in CMS based hybrid breeding programme. The hybrid technology based on stable CMS system has a potential of breaking the barrier of stagnant yield in pigeonpea.

Morphologically, CMS GT 288A line was very early in days to 50% flowering (80.50 days) followed by GT

33A (85.50 days), ICPA 2092 (102.50 days), ICPA 2043 (103.50 days), MA CMS 32A (110.00 days) and MA CMS 25A (120.50 days) along with maintainers (table 1). GT 288B line was very early in days to maturity (237.00 days) followed by MA CMS 25B (245.00 days), ICPB 2043 (247.50 days), ICPB 2092 (248.00 days), MA CMS 32B (251.00 days) and GT 33B (251.50 days). Low or medium plant height is desirable in pigeonpea. Plant height was ranged from 204.30 cm (MA CMS 25A) to 132.36 cm (MA CMS 32A). A number of primary and secondary branches were higher in CMS GT 33A *i.e.* 19.70 and 35.50, respectively. While low number of primary and secondary branches observed in GT 288A, 6.40 and 1.50, respectively. Pods per plant were ranged from 175.00 (ICPB 2092) to 24.97 (GT 288B). Seeds per pod were scored high in GT 33B (3.84) followed by MA CMS 25B (3.70), ICPB 2092(3.50), MA CMS 32B (3.42) and ICPB 2043(3.30). For pod length, ICPB 2092 was revealed (5.02) corresponds GT 33B (4.92), GT 288B (4.93), MA CMS 25B (4.93), ICPB 2043(4.66) and MA CMS 32B (4.07). ICPB 2043 was exhibited high 100 seed weight (11.77) followed by GT 288B (10.22), ICPB 2092 (10.19), MA CMS 32B (10.00) and GT 33B (7.92). For seed yield, ICPB 2092 was reported as high seed yield per plant (37.94) followed by MA CMS 25B (36.2), ICPB 2043 (34.10), MA CMS 32B (28.05), GT 288B (22.57) and GT 33B (22.27). According to this investigation, first three lines could better perform. These would be carried out as same in future as a good combiner for CMS based hybrid seed production in pigeonpea.

The development of stable CMS systems is a boon to the breeders, and it has provided a platform to enhance the pace of research and development of hybrid (Saxena and Nadarajan 2010). For the stable hybrid breeding programme, it is thumb rule to have diverse CMS and fertility-restoring genotypes (Saxena et al. 2014a). Hitherto, eight CMS systems (A_1 - A_8) have been reported in pigeonpea (Saxena 2013). Based on the crossability with the cultivated species *Cajanus cajan* resides in the primary gene pool while the wild progenitors are laid in the secondary and the tertiary gene pool (Bohra *et al*., 2010). Saroj *et al*. (2015) also discussed adapted primary gene pool germplasm for the study of identification of good and stable new fertility restorer lines from for specific agro-climatic conditions of late-maturing pigeonpea hybrids carrying A_4 cytoplasm.

Characterization with SSR markers

A total of 20 SSR markers were screened (table 2). Four markers namely, CCB4, CCB5, CCB9 and CCtta011 were showed a total of 8 alleles, which were polymorphic with an average of 2.0 alleles per locus, the polymorphic profile of marker CCB5 and CCB9 are given in figs. 1 and 2. These markers were selected to characterize and assess the genomic level differences among 12 isogenic pigeonpea genotypes because of distinct amplification and highly reproducible bands (table 3). While, other primers *viz*. CCac003, CCac036, CCB1, CCB2, CCB7, CCB8, CCtc013 and CCtta015, CCttc006, CCttc007, CCttc008, CCttc033, ICPM103, ICPM127, ICPM128 and ICPM131 were not differentiated any allele. Probably this might be because the microsatellites were designed based on the genome of the cultivated *C. cajan* (Burans *et al*., 2001).

The selected SSR primers were highly polymorphic, as their corresponding polymorphism information content PIC value ranging from 0.58 to 0.72 with average PIC of 0.68. Maximum polymorphism information content was shown by two SSR primers CCB5 (0.72) and CCB9 (0.72) followed by primer CCB4 (0.69) and CCtta011 (0.58) respectively. In our investigation, 20 novel SSR primers were used to first time characterization of the mitochondrial genome of MACMS 25A and MACMS 32A along with their maintainers. Identification in the 20 SSR microsatellite markers could be helpful in detecting the genetically pure plant genotypes in a quick way. Markers could be deploy in marker assisted selection in early stage or seedling stages of growth; otherwise, it is very time-consuming and tedious job, with the land and labour cost especially in the long duration crop of pigeonpea (Metkar *et al*., 2010).

Unambiguous characterization of the parental lines in the CMS system alleviates concerns regarding the seed purity in hybrid seed production (Metkar *et al*., 2010). In this context, Choudhury *et al*. (2008) used RAPD marker for the identification of parental lines *i.e.* cytoplasmic genic male sterile, maintainer and restorer. Several earlier workers also studied of genetic diversity through microsatellite markers in A, B and R lines using SSR molecular marker (Saxena *et al*., 2010), in inter-specific crosses as well as cultivars of pigeonpea (Singh *et al*., 2008; Dutta *et al*., 2011). Genetic variability in Indian pigeonpea has been studied using restriction fragment length polymorphism (RFLP) (Nadimpalli et al. 1992) and random amplified polymorphic DNA (RAPD) (Ratnaparkhe *et al*., 1995). In a genetic variability study both within and between the diverse set of Cajanus advanced breeding lines, landraces and a large number of accessions from wild species, the number of polymorphic SNPs and comparative high level of cross-species transferability was reported by Saxena *et al*. (2014). Hitherto, by molecular characterization of CMS

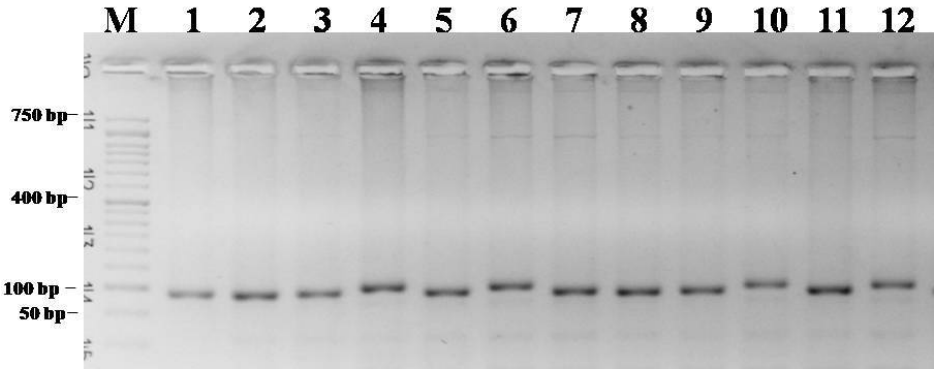

Fig. 1 : Agarose gel profile of CCB5 marker in pigeonpea genotypes M=Ladder (50 bp), 1=MA CMS 25A, 2=MA CMS 32A, 3=GT 288A, 4=GT 33A, 5=ICPA 2043, 6=ICPA 2092, 7=MA CMS 25B, 8=MA CMS 32B, 9=GT 288B, 10=GT 33B, 11=ICPB 2043 and 12=ICPB 2092.

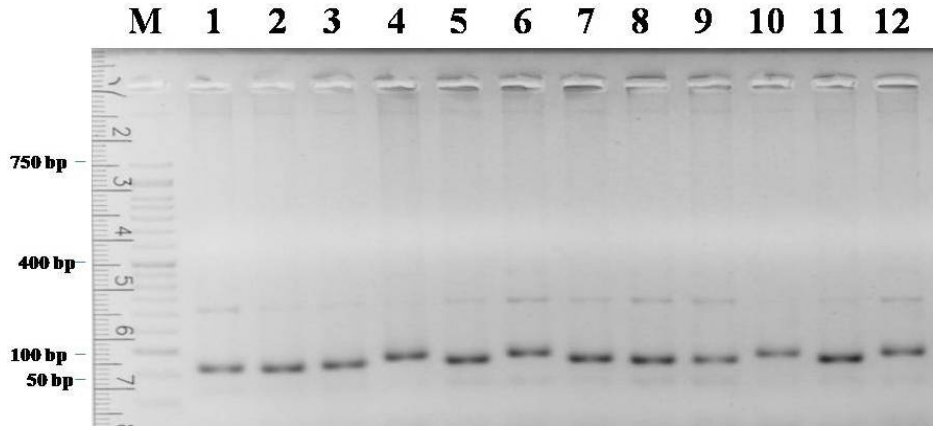

Fig. 2 : Agarose gel profile of CCB9 marker in pigeonpea genotypes M=Ladder (50 bp), 1=MA CMS 25A, 2=MA CMS 32A, 3=GT 288A, 4=GT 33A, 5=ICPA 2043, 6=ICPA 2092, 7=MA CMS 25B, 8=MA CMS 32B, 9=GT 288B, 10=GT 33B, 11=ICPB 2043 and 12=ICPB 2092.

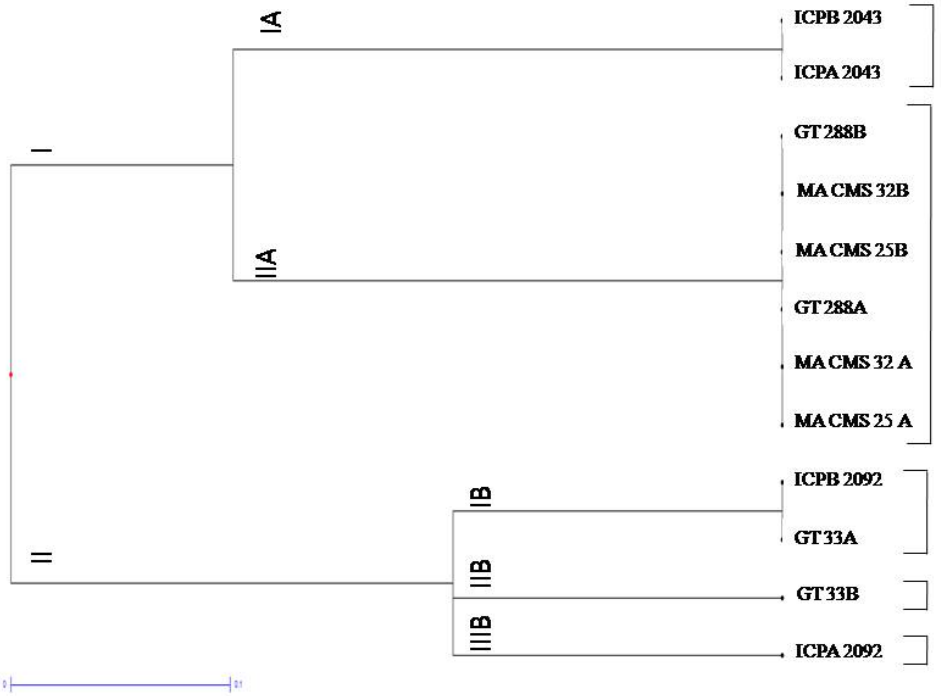

Fig. 3 : Dendrogram generated based on the Jaccard'scoefficient of genetic diversity among 12 pigeonpea genotypes.

Table 1 : Morphological characteristics of cytoplasmic male sterile lines and its maintainers.

S. no.	Lines	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches	Secondary branches	Pods per plant	Seeds per pod	Pod length (cm)	100 seed weight (g)	Plant type	Flower colour	Pod colour	Seed colour	Seed yield/ plant (g)
1.	MA CMS 25A	120.50	-	204.30	18.50	18.30	-	-	-	-	C	LRFS	PGS	W	-
2.	MA CMS 25B	124.00	245.50	188.36	15.80	7.13	141.00	3.70	4.93	10.00	”	”	”	”	36.02
3.	MA CMS 32A	110.00	-	142.77	14.80	16.54	-	-	-	-	C	RFRS	PGS	DR	-
4.	MA CMS 32B	113.00	251.00	132.36	12.80	4.00	156.80	3.42	4.07	7.63	”	”	”	”	28.05
5.	GT 288A	80.50	-	194.50	6.40	6.50	-	-	-	-	C	LWYF	PGS	W	-
6.	GT 288B	98.00	237.00	142.00	4.50	4.50	24.97	3.48	4.93	10.22	”	”	”	”	22.57
7.	GT 33A	85.50	-	165.81	19.70	35.50	-	-	-	-	C	Y	PGS	B	-
8.	GT 33B	99.00	251.50	134.47	14.30	3.75	43.20	3.84	4.97	7.92	”	”	”	”	22.27
9.	ICPA 2043	103.50	-	186.97	20.50	28.30	-	-	-	-	C	Y	G	B	-
10.	ICPB 2043	105.00	247.50	162.18	16.10	14.30	126.00	3.30	4.66	11.77	”	”	”	”	34.10
11.	ICPA 2092	102.50	-	180.00	20.20	12.90	-	-	-	-	S	Y	PGS	RB	-
12.	ICPB 2092	105.50	248.00	167.31	18.60	18.20	175.00	3.50	5.02	10.19	”	”	”	”	37.94
	Grand Mean	103.92	246.75	166.75	15.18	14.16	111.16	3.54	4.76	9.62	-	-	-	-	30.16
	SEm (±)	3.49	1.97	6.80	1.43	2.78	23.16	0.07	0.13	0.58	-	-	-	-	2.55
	CV%	11.62	1.95	14.13	32.69	67.89	51.04	5.06	6.94	14.88	-	-	-	-	20.74

C=Compact, LRFS= Light Reddish Flower with Strips, PGS= Pod Green with strips, W= Whitish, RFRS= Reddish Flower with Red Strips, DR=Dark Red, LWYF=Light Whitish Yellow Flower, S=Spreading Y= Yellow, G= Green, B= Brown, RB= Reddish brown.

Table 2 : List of primer name, sequence and their sources.

S. no.	Primer name	Primer Sequence	References
1	CCac003	F: TGCTTCAAGTTGCCTACCAG R: TCAAGGGAGGTGGACTACAAA	Odeny *et al.* (2009)
2	CCac036	F: ATCGGCTTTTGTCTTGATGA R: AAGCTACAAGGGATACACATGC	Odeny *et al.* (2009)
3	CCB1	F: AAGGGTTGTATCTCCGCGTG R: GCAAAGCAGCAATCATTTCG	Burns *et al.* (2001)
4	CCB2	F: CCATAATCCAATCCAAATCC R: AGAAGGCTTTCATGTAACGC	Odeny *et al.* (2007)
5	CCB4	F: GGAGCTATGTTGGAGGATGA R: CCTTTTTGCATGGGTTGTAT	Burns *et al.* (2001)
6	CCB5	F: GACAATTTTGCATGCATTGC R: TTGCAAAAACACTTGGTTGG	Odeny *et al.* (2007)
7	CCB7	F: CAACATTTGGACTAAAAACTG R: AGGTATCCAATATCCAACTTG	Odeny *et al.* (2007)
8	CCB8	F: TGCGTTTGTAAGCATTCTTCA R: ACTTGAGGCTGAATGGATTTG	Odeny *et al.* (2007)
9	CCB9	F: CACTTGGTTGGCTCAAGAAC R: GCCAATGAACTCACATCCTTC	Odeny *et al.* (2007)
10	CCtc013	F: CTTCTCCCTGCCTCTTTTCC R: CAAGTGGAGGGGAGTGAAGA	Odeny *et al.* (2009)
11	CCtta011	F: TCAGGGGTAAATGCGGTATC R: GAATTGCTTTTTGCTTCCTCA	Odeny *et al.* (2009)
12	CCtta015	F: AACACGCACCTCAATTCCA R: GAATGAGGAATGAAGGGACAAA	Odeny *et al.* (2009)
13	CCttc006	F: TAGAGGAGGTTCCAAATGACATA R: ATCTGTCTGGTGTTTTAGTGTGCT	Odeny *et al.* (2009)
14	CCttc007	F: CTCTTGCTTACGCGTGGACT R: CTTTTGCTTTTGCGTGCTT	Odeny *et al.* (2009)
15	CCttc008	F: TCACAGAGGACCACACGAAG R: TGGACTAGACATTGCGTGAAG	Odeny *et al.* (2009)
16	CCttc033	F: ATTCCCTCTCTATCTCAGACTTTT R: CGTGATGGAACTCAAGATACACT	Odeny *et al.* (2009)
17	ICPM103	F: ATCCCGTAATGCACCTTTTG R: TTGGTCTGAATTGTGGCCTAT	R. K. Saxena *et al.* (2010)
18	ICPM127	F: CGAGCTCGAATTGACCCTAT R: TTTGTTTTTGGGCTCATTCC	R. K. Saxena *et al.* (2010)
19	ICPM128	F: CCAATCCTGGGCAGTTTCT R: GCGGGCTTCATGACAACTT	R. K. Saxena *et al.* (2010)
20.	ICPM131	F: CTACCTTGGCCAACCATTCT R: GGCACAGTTCTTCCACCATT	R. K. Saxena *et al.* (2010)

and their maintainer were very less studied. In hybrid technology era, the utility of molecular markers increases when we work with CMS based heterosis for identification of good general and specific combiner parents for hybrid seed production.

Cluster analysis and genetic interrelationship

The SSR amplification pattern was used to assess the degree of genetic variation among species i.e., *Cajanus scarabaeoides*, *C. cajanifolius* and *Cajanus cajan* cultivars by cluster analysis and to detect the varietal diagnostic markers. The cross genera

Table 3 : Name, sequence number of allele and PIC value for each of the 4 primers

S. no.	Primer	Sequences	Total number of alleles	PIC
1.	CCB4	F: GGAGCTATGTTGGAGGATGA R: CCTTTTTGCATGGGTTGTAT	2	0.69
2.	CCB5	F: GACAATTTTGCATGCATTGC R: TTGCAAAAACACTTGGTTGG	2	0.72
3.	CCB9	F: CACTTGGTTGGCTCAAGAAC R: GCCAATGAACTCACATCCTTC	2	0.72
4.	CCtta011	F: TCAGGGGTAAATGCGGTATC R: GAATTGCTTTTTGCTTCCTCA	2	0.58

microsatellite markers produced amplifications and powerful enough to separate distinct interspecific groups. The dendrogram revealed two major clusters at dissimilarity coefficient (fig. 3). The maximum 8 genotypes were grouped in Cluster I and four genotypes grouped in Cluster II. The GT 33B lines were showed high 0.80% genetically dissimilarity with ICPA 2043 and their ICPB 2043. These lines are also dissimilar in cytoplasmic level and separated in Cluster I and II. Cluster I was again divided into two sub-clusters IA and IIA. In sub-cluster IA, there are two genotypes including CMS and their B lines whereas six genotypes were included in cluster IIA. The sub-cluster IA had CMS ICPA 2043 and its maintainer. The CMS ICPA 2043 was revealed superiors in primary and secondary branches, 100 seed weight and good in seed yield per plant after ICPB 2092 and MA CMS 25B. The sub-cluster IIA, three CMS (GT 288A, MA CMS 32A and MA CMS 25A) and their maintainer lines had similar gene pool relationship together. The cluster II was divided into three sub-clusters IB, IIB, and IIIB. The sub-cluster IB had two CMS lines *viz*., ICPB 2092 and GT 33A that was also phenotypically slightly similar to each other. The ICPA 2043 and ICPB 2043 were most similar with 0.00 distances. While, GT 33A was showed high distance with MA CMS 25B with 0.80 distances and showed high distance with all genotypes with 0.75 distance except GT 288A had distance 0.40.

Very few reports are available to characterize CMS and their maintainers due to long duration pigeonpea crop. Metkar *et al.* (2010) characterized some CMS and their maintainer with the help of SSR markers. The set of primers we used in this study could be able to distinguish the CMS systems derived from *C. cajanifolius* and *C. scarabaeoides* adds to the new information. Differences between CMS lines and its maintainers are mainly due to the presence of sterile and fertile cytoplasm. Therefore, A and B lines are assumed to be near isogenic except for the genomic portion related to male sterility. Further, the primers distinguishing the CMS lines from their maintainers might be linked to the cytoplasmic genome (mt-DNA) as earlier experienced by Choudhury *et al*. (2008) in CMS system of pigeonpea. This undetectable variability might have been magnified by molecular markers that were not expressed at the phenotypic level. Another possible reason for the detected variability at the molecular level might be due to the conserved sequence of the isogenic lines which may not express at the morphological level. The earlier worker reported similar results in soybean (Brown-Guedira *et al*., 2000).

The results would be very helpful for utilization of CMS lines to develop highly heterotic hybrids using diverse CMS lines and restorer combinations. Further, the primers identified in the present investigation would be useful in the identification of CMS lines, their maintainers, and restorers at the molecular level during early growth stages, which in turn, would be an alternative to grow out test of these lines in the commercial seed production programme.

References

Bohra, A., N. Mallikarjuna, K. B. Saxena, H. D. Upadhyaya, I. Vales and R. K. Varshaney (2010). Harnessing the potential of crop wild relatives through genomics tools for pigeonpea improvement. *J. Plant Biol.*, **37** : 83-98.

Brown Guedira, G. L., J. A. Thompson, R. L. Nelson and M. L. Warburton (2000). Evaluation of genetic diversity of soybean introductions and North American ancestors using RAPD and SSR markers. *Crop Sci*., **40** : 815-823.

Burns, M. J., K. J. Edwards, H. J. Newbury, B. V. Ford-Lloyd and C. D. Baggot (2001). Development of simple sequence repeats (SSR) markers for the assessment of gene flow and genetic diversity in Pigeonpea (*Cajanus cajan* L.). *Mol. Ecol. Notes*, **1(4)** : 283–285.

Choudhary, P. R., I. P. Singh, S. Verma, N. P. Singh and S. Kumar (2008). RAPD markers for identification of cytoplasmic genic male sterile, maintainer and restorer lines of pigeonpea. *J. Food Legumes*, **21** : 218-221.

Doyle, J. J. and J. L. Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull*, **19** : 11–15.

Dutta, S., G. Kumawat, B. P. Singh, D. K. Gupta, S. Singh, V. Dogra, K. Gaikwad, T. R. Sharma, R. S. Raje, T. K. Bandhopadhya, S. Datta, M. N. Singh, F. Bashasab, P. Kulwal, K. B. Wanjari, R. K. Varshney, D. R. Cook and N. K. Singh (2011). Development of genic- SSR markers by deep transcriptome sequencing in pigeonpea [*Cajanus cajan* (L.) Millspaugh]. *BMC Plant Biol.*, **11** : 17.

Gupta, P. K. and R. K. Varshney (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, **113** : 163–185.

Liu, K. and S. V. Muse (2005). Power Marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, **21(9)** : 2128-9.

Metkar, A. P., V. L. Gawande and S. J. Gahukar (2010). Molecular characterization of CMS lines in pigeonpea. *J. Food Legumes*, **23(3&4)** : 234-237.

Nadimpalli, B. G., R. L. Jarret, S. C. Pathak and G. Kochert (1992). Phylogenetic relationships of the pigeonpea (*Cajanus cajan* L.) based on nuclear restriction fragment length polymorphism. *Genome*, **36** : 216-223.

Odeny, D. A., B. Jayashree, M. Ferguson, D. Hoisington, J. Crouch and C. Gebhardt (2007). Development, characterization and utilization of microsatellite markers in pigeonpea. *Plant Breed*., **126** : 130–137.

Odeny, D. A., B. Jayashree, C. Gebhardt and J. Crouch (2009). New microsatellite markers for pigeonpea (*Cajanus cajan* (L.) Millsp.). *BMC Res Notes*, **2** : 35.

Parmar, L. D. and S. B. S. Tikka (2001). Genetics of cytoplasmic-genic male sterility in pigeonpea [*Cajanus cajan* (L.) Millsp]. Proceedings National Symposium on Pulses and Oilseed for Sustainable Agriculture, held during 29–31 July 2001, 28. Tamil Nadu Agric. Univ., Coimbatore, India.

Perrier, X. and J. P. Jacquemoud-Collet (2006). DARwin software http://darwin.cirad.fr/

Ratnaparkhe, M. B., V. S. Gupta, M. R. Murthy and P. K. Ranjekar (1995). Genetic Fingerprinting of Pigeonpea [*Cajanus cajan* (L.) Millsp.] and Its Wild Relatives Using RAPD Markers. *Theor. Appl. Genet*., **91(6-7)** : 893-898.

Saroj, S. K., M. N. Singh, M. K. Vishwakarma, T. Singh and V. K. Mishra (2015). Identification of stable restorers and genetics of fertility restoration in late-maturing pigeonpea [*Cajanus cajan* (L.) Millspaugh]. *Plant Breed*., **134** : 696-702.

Saxena, K. B. (2013). A novel source of CMS in pigeonpea derived from *Cajanus reticulatus*. *Indian J. Genet*., **73** : 259-263.

Saxena, K. B. and N. Nadarajan (2010). Prospects of pigeonpea hybrids in Indian Agriculture. *Elect. J. Plant Breed*., **1** : 1107-1117.

Saxena, K. B., R. Sultana, N. Mallikarjuna, R. K. Saxena, R. V. Kumar, S. L. Awargaonkar and R. K. Varshney (2010a). Male-sterility systems in pigeonpea and their role in enhancing yield. *Plant Breed*., **129** : 125–134.

Saxena, K. B., R. V. Kumar and M. Bharathi (2014a). Studies on fertility restoration of A_4 cytoplasm in Pigeonpea. *Euphytica*, **198** :127-135.

Saxena, R. K., W. E. Von, H. D. Upadhyaya, V. Sanchez, S. Songok, K. B. Saxena, P. Kimurto and R. K. Varshney (2014). Genetic Diversity and Demographic History of *Cajanus* spp. Illustrated from Genome-Wide SNPs. *PLOS One*, **9(2)** : 1-9.

Saxena, R. K., K. B. Saxena and R. K. Varshney (2010). Application of SSR markers for molecular characterization of hybrid parents and purity assessment of ICPH 2438 hybrid of pigeonpea [*Cajanus cajan* (L.) Millspaugh]. *Mol. Breed*., **26** : 371–380.

Singh, S., K. N. Singh, R. Kant, S. Mehfooz and S. Dutta (2008). Assessment of genetic diversity among pigeonpea genotypes using SSR markers. *Indian J. Genet.*, **68(3)** : 255-260.

Varshney, R. K., J. C. Glaszmann, H. Leung and J. M. Ribaut (2010). More genomic resources for less-studied crops. *Trends in Biotech.*, **28** : 452–460.

Varshney, R. K., W. Chen, Y. Li, A. K. Bharti, R. K. Saxena, J. A. Schlueter, M. T. A. Donoghue, S. Azam, G. Fan, A. M. Whaley, A. D. Farmer, J. Sheridan, A. Iwata, R. Tuteja, R. V. Penmetsa, W. Wu, H. D. Upadhyaya, S. P. Yang, T. Shah, K. B. Saxena, T. Michael, W. R. Mc Combie, B. Yang, G. Zhang, H. Yang, J. Wang, C. Spillane, D. R. Cook, G. D. May, X. Xu, and S. A. Jackson (2012). Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource poor farmers. *Nat. Biotechnol.*, **30** : 83–89.