Cross-genera amplification of informative microsatellite markers from common bean and lentil for the assessment of genetic diversity in pigeonpea

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ABSTRACT

A total of 24 pigeonpea (*Cajanus cajan* L. Millspaugh) cultivars representing different maturity groups were evaluated for genetic diversity analysis using 10 pigeonpea specific and 66 cross-genera microsatellite markers. Of the cross-genera microsatellite markers, only 12 showed amplification. A total of 45 alleles were amplified by the 22 markers. Nine markers showed 100 % polymorphism. Markers Lc 14, BMd 48 and CCB 9 amplified maximum number (5) of alleles each. One genotype specific unique band in Pusa 9 was generated by markers CCB 8. Maximum genetic diversity (74 %) was observed between cultivars MA 3 and CO 6, while the minimum diversity (12 %) was observed between NDA 1 and DA 11. The average diversity among the cultivars was estimated to be 45.6 %. SSR primers from pigeonpea were found to be more polymorphic (37 %) as compared to common bean and lentil markers. The arithmetic mean heterozygosity (H_{av}) and marker index (MI) were found to be 0.014 and 0.03, respectively, indicating the potential of common bean and lentil microsatellite markers for genetic mapping, diversity analysis and genotyping in *Cajanus*. [Physiol. Mol. Biol. Plants 2010; 16(2) : 123-134] E-mail : subhojit@email.com

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INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millspaugh) is one of the major grain legume crops of the tropics and subtropics. Globally, it is cultivated on 4.64 million ha area with annual production of 3.43 million tons (FAOSTAT, 2007, <http://faostat.fao.org>). Almost entire production of this nutritionally rich crop (22-25 % protein content) is confined to Asia and Africa where situation of undernourished population has been rising alarmingly. India, the primary centre of diversity, is the top producer of pigeonpea with 2.51 million tons production from 3.53 million ha area. In spite of tremendous yield potential, the average productivity of the crop remains low (740 kg/ha) as it is continuously challenged by various biotic and abiotic factors which are integral parts of its production base. Conventional breeding efforts could not make much progress in improving productivity

of pigeonpea mainly due to its narrow genetic base, highly sensitive nature to environmental factors and high genotype $\times$ environment (GE) interactions (Kumar and Ali, 2006). Moreover, limited research and pre-breeding efforts coupled with repeated use of a few parents in the current breeding programs have further narrowed down the genetic diversity of its cultivated gene pool (Singh *et al.*, 2006). Continuous cultivation of only few leading cultivars has led to erosion of a large number of land races, a major source for genes for stress tolerance in breeding programs. An assessment of genetic diversity among the released cultivars of pigeonpea will certainly help in deciding the prospective genotypes for conversion program and development of heterotic cross combinations besides of widening the genetic base of the cultivated germplasm.

Earlier studies on assessment of genetic diversity in pigeonpea using morphological characteristics and coefficient of parentage have indicated limited genetic diversity (Singh *et al.*, 2006; Upadhyaya *et al.*, 2007), and lack of correspondence between the results have

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further limited their utility. The phenotypic characterization has limited genetic resolution capacity and usually requires extensive multi-environment evaluation. Ladizinsky and Hamel (1980) detected very little polymorphism for seed protein and isozymes in pigeonpea. DNA fingerprinting can unambiguously establish the genetic relationships among crop cultivars with high precision as compared to morphological or biochemical attributes (Wilde *et al.*, 1992). Studies on molecular diversity in pigeonpea have been limited by the paucity of polymorphic PCR based markers. Though the molecular marker technologies such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) were occasionally employed in pigeonpea but they tend to present reproducibility problems and were inadequate in assessing within species diversity (Nadimpalli *et al.*, 1992; Ratnaparkhe *et al.*, 1995; Panguluri *et al.*, 2006). RAPD markers have also been used for characterization of cytoplasmic male sterility lines in pigeonpea for subsequent use in hybrid development (Souframanien *et al.*, 2003). Additionally, often cross-pollinated nature of this crop (up to 40 % out-crossing) poses a serious problem in maintenance of genetic purity of released cultivars (Gupta *et al.*, 1980; Ratnaparkhe *et al.*, 1995). Under such conditions, the use of molecular markers, particularly the neutral markers to unravel genetic relationships between cultivars becomes very important.

Presently, a wide array of neutral genetic markers is available for assessment of genetic diversity in crop plants. Among different classes of molecular markers, SSRs (Simple Sequence Repeats) are more useful for assessment of genetic diversity in crops with low variability because of their reproducibility, multi-allelic nature, codominant inheritance, relative abundance and high genome coverage (Powell *et al.*, 1996; Varshney *et al.*, 2005). Despite its importance in nutritional security, pigeonpea has not benefited much from the advances made in the field of genomic tools and molecular markers (Datta *et al.*, 2009). As compared to large number of markers in common bean (L'taief *et al.*, 2008; Blair *et al.*, 2003), chickpea (Choudhary *et al.*, 2009; Sethy *et al.*, 2006), and lentil (Hamwieh *et al.*, 2009; Hamwieh *et al.*, 2005), only 109 microsatellite markers are reported so far in pigeonpea (Burns *et al.*, 2001; Odeny *et al.*, 2007; Odeny *et al.*, 2009). Therefore, there is an urgent need to increase the number of polymorphic microsatellite markers in pigeonpea for diversity analysis and mapping of important traits. Several reports have established transferability of SSR

markers from one genera/species to other (Peakall *et al.*, 1998; Pandian *et al.*, 2000; Choumane *et al.*, 2004; Gutierrez *et al.*, 2005; Datta *et al.*, 2009). Peakall *et al.* (1998) reported 65 % cross-species amplification within the genus *Glycine*. Pandian *et al.* (2000) observed high level of sequence conservation of microsatellite markers across vetch, lentil, chickpea and fieldpea. Datta *et al.* (2009) recorded 46 % transferability of chickpea specific SSRs in pigeonpea. Therefore, the present study was undertaken with an objective to assess genetic diversity among the 24 most widely grown pigeonpea cultivars in India using pigeonpea specific as well as transferable common bean and lentil specific SSR markers.

MATERIALS AND METHODS

Plant materials and DNA extraction

Twenty-four cultivars encompassing a broad range of genetic diversity of the cultivated germplasm (10 short, 5 medium and 9 long duration) were included in the present study. These are the commercial cultivars developed and released in India over the past 50 years using different breeding methods and are grown extensively in their area of adaptation. The details of the cultivars such as pedigree, crop duration, geographical adaptation and special features are given in Table 1. The study was carried out at Biotechnology Lab, Indian Institute of Pulses Research, Kanpur, India during 2007-08. Total genomic DNA was extracted from the leaves of three-week old plants grown in the Net house following the modified CTAB method (Abdelnoor *et al.*, 1995). DNA was purified with RNase treatment (10 µg/ml) for 1 hour at 37 °C followed by phenol: chloroform: isoamyl alcohol (25:24:1) treatment. Pellet was dissolved in T₁₀E₁ (Tris 10 mM, EDTA 1 mM) buffer. DNA was quantified both by visual quantification and UV spectrophotometer and finally diluted to a concentration of 25 ng/µl.

Microsatellite markers and PCR amplification

A total of 76 SSR primer pair sequences representing 10 from pigeonpea (*Cajanus cajan* L. Millsp.), 34 from common bean (*Phaseolus vulgaris*) and 32 from lentil (*Lens culinaris* ssp. *culinaris*) were used for PCR amplification to study transferability as well as their utility in genetic diversity analysis of pigeonpea cultivars. The pigeonpea microsatellite markers were based on the sequences published by Burns *et al.* (2001) whereas common bean and lentil SSR markers were

Table 1. Pigeonpea cultivars used for molecular diversity assessment

S.No.	Cultivar	Pedigree	Area of adaptation	Crop duration	Special features
1	MA 3	Selection from MA 2	NEPZ	Long	Spreading, constricted pods
2.	MA 6	MA 2 × Bahar	NEPZ	Long	Spreading, resistant to SMD
3.	MAL 13	(MA 2 × MA166) × Bahar	NEPZ	Long	Spreading, resistant to SMD, large seeds
4.	NDA 1	Local selection from Faizabad	NEPZ	Long	Tolerance to fusarium wilt, SMD and PSB
5.	Pusa 9	UPAS 120 × 3673	NEPZ	Long	Indeterminate, tolerant to SMD and Alternaria
6	DA 11 (Sharad)	Bahar × NP (WR) 15	NEPZ	Long	Compact, tolerant to Alternaria and SMD, suitable for pre-rabi
7.	Azad	Bahar × KPBR 80-1	NEPZ	Long	Resistant to SMD
8.	Amar	Selection from Bahar	NEPZ	Long	Compact, resistant to SMD
9.	Bahar	Local selection from Motihari	NEPZ	Long	Compact, resistant to SMD
10.	BSMR 853	(ICPL7336 x BDN1) x BDN2	CZ	Medium	Resistant to SMD and wilt
11.	ICPL 87119 (Asha)	C11 × ICPL 6	CZ & SZ	Medium	Indeterminate, semi-spreading, resistant to fusarium wilt and SMD
12.	CO 6	Mutant of SA1	SZ	Medium	Indeterminate, tolerant to pod borer
13.	LRG 38	C11 × ICP7035	SZ	Medium	Tolerant to Helicoverpa
14.	LRG 30	Local selection from Lam	SZ	Medium	White large seeds
15.	TAT 10	TT2 × TT8	CZ	Short	Extra short duration
16.	GS 1	Local selection	SZ	Short	White seed
17.	UPAS 120	Selection from P4768	NWPZ & NEPZ	Short	Wide adaptability, tolerant to pod borer
18.	Pusa 992	Selection from 90306	NWPZ	Short	Tolerance to fusarium wilt
19	Manak	Type 21 × UPAS120	NWPZ	Short	Extra early
20.	Paras	EE76 × UPAS 120	NWPZ	Short	Resistant to SMD
21.	Pusa 33	C11x UPAS120	NWPZ	Short	Tolerant to fusarium wilt
22.	ICPL 87	Type 21 × JA 27	All zones	Short	Resistant to fusarium wilt, determinate
23.	GT 100	T15-15 × S5	CZ	Short	White seeds, determinate growth habit
24.	ICPL 84031	Prabhat × UPAS120	SZ	Short	Extra early, determinate

NEPZ= Northeast plain zone, NWPZ= Northwest plain zone; CZ= Central zone; SZ= South zone; SMD= Sterility mosaic disease; PSB= Phytophthora stem blight

Table 2. Properties of microsatellite markers used in the present study for assessment of genetic diversity in pigeonpea

Marker	Sequences of primers	Motifs	No. of alleles	Size (bp)	Polymorphism (%)	PIC	Average Heterozygosity (H_{av})	Marker index (MI)
CCB1	F AAGGGTTGTATCTCCGCGTG R GCAAAGCAGCAATCATTTTCG	(CA) ₁₀	1	200	0	0.00	0.00	0.00
CCB2	F CATAATCCAATCCAAATCC R AGAAGGCTTTTCATGTAACGC	(CA) ₂₁	1	180	0	0.00	0.00	0.00
CCB 3	F TCACAAAAACAAGTTGCCAC R ATGACCATGATTACGCCAAG	(CA) ₁₀	1	115	0	0.00	0.00	0.00
CCB 4	F GGAGCTATGTTGGAGGATGA R CCTTTTTCATGGGTTGTAT	(CA) ₃₁	2	250, 270	100	0.330	0.007	0.09
CCB 5	F GACAATTTTGCATGCATTGC R TTGCAAAAACACTTGGTTGG	(CT) ₂₂	3	200- 215	100	0.491	0.011	0.136
CCB 6	F ACAATGCTAGGGAACACCGC R TACCTTAACCCACAATGGCC	(CA) ₆	1	225	0	0.00	0.00	0.00
CCB 7	F CAACATTTGGACTAAAACTGV R AGGTATCCAATATCCAACCTTG	(CT) ₁₆	1	165	100	0.498	0.011	0.045
CCB 8	F TGC GTTTGTAAGCATTCTTCA R ACTTGAGGCTGAATGGATTG	(CT) ₃₀	3	80- 120	100	0.443	0.010	0.136
CCB 9	F CACTTGGTTGGCTCAAGAAC R GCCAATGAACTCACATCCTTC	(CT) ₂₂	5	150- 470	100	0.151	0.003	0.227
CCB 10	F CCTTCTTAAGGTGAAATGCAAGC R CATAACAATAAAAGACCTTGAAT	(CA) ₁₅	1	200	0	0.00	0.00	0.00
BMd29	F CTTACCGATCTGACAGCAG R TTTCTCCACTGGAACACTCG	(GAA) ₅	2	375, 480	100	0.594	0.013	0.09
BMd 47	F ACCTGGTCCCTCAAACCAAT R CAATGGAGCACCAAAGATCA	(AT) ₅	2	150, 325	50	0.750	0.017	0.045
BMd 48	F CCCACCAACTCTTTCTTCC R CAGAATTGACTTGGCGAGAA	(CT) ₅	5	140- 600	80	0.358	0.008	0.186
BMd 50	F TGGTGAGAGAAGGACAATAGCA R GCCGCTTGTGACGTTTATTT	(AAC) ₄	1	135	100	0.707	0.016	0.045
BMd54	F GGCTCCACCATCGACTACTG R GAATGAGGGCGCTAAGATCA	(CCT) ₆	2	70, 150	50	0.938	0.021	0.045
BMd 55	F GTCCGTTTCATGGGTTTGACT R TCGAGATCTACGGAGGAGTTC	(AT) ₅	1	200	100	0.234	0.005	0.045
BMd 11	F GCTCAACATTCCAGAGGCTAA R TCAAACCTACATAAATAAAACAAAACA	(GA) ₈	1	80	0	0.00	0.00	0.00
BMd 12	F CATCAACAAGGACAGCCTCA R GCAGCTGGCGGGTAAAACAG	(AGC) ₇	2	260, 410	50	0.082	0.002	0.045

Table 2. Continued

Marker	Sequences of primers	Motifs	No. of alleles	Size (bp)	Polymorphism (%)	PIC	Average Heterozygosity (H_{av})	Marker index (MI)
BMd 33	F TACGCTGTGATGCATGGTTT R CCTGAAAGTGCAGAGTGGTG	(ATT) ₉	1	110	0	0.00	0.00	0.00
Lc14	F GGTAGGTGAGATAGTTG R GGAGCAAGAAGAAGCAG	(TG) ₄	5	150-900	100	0.514	0.011	0.227
Lc24	F CTTGGAGCTGTTGGTC R GCCGCCTACATTATGG	(GT) ₉	3	180-490	66.6	0.387	0.009	0.09
Lc 31	F TTACGAAAAAGGCAAACATA R ATCTTCTTCTTCTTCTTCTCA	(TC) ₂₀	1	100	0	0.00	0.00	0.00
Avg. Range			2.1-4.5	70-600	0-100	0.292-0.938	0.006-0.021	0.01-0.042

based on the sequences reported by Blair *et al.* (2003) and Hamwieh *et al.* (2005), respectively. Primers were custom synthesized from Operon Technologies, USA.

PCR Amplification was conducted in 200 µl thin-wall PCR tubes using a PTC-200 gradient thermocycler (MJ Research, USA). The PCR was performed in a 25 µl reaction volume containing 1X PCR buffer (10 mM Tris HCl pH 9.0, 1.5 µM MgCl₂, 50 mM KCl, 0.01 % gelatin), 0.2 mM each of dNTP (Bangalore Genei), 0.6 U of *Taq* DNA polymerase (Bangalore Genei) and 40 pM each of forward and reverse primers. 25 ng of genomic DNA was added as template in PCR tubes. Amplifications were performed for 40 cycles programmed as initial denaturation of genomic DNA at 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 1 min each, primer annealing for 1 min, DNA amplification at 72 °C for 2 min and a final primer extension step at 72 °C for 7 min.

Amplicons were resolved by electrophoresis on 2 % agarose gel and visualised with ethidium bromide under UV illumination. GeneRuler 100 bp DNA ladder (MBI Fermentas) was used to estimate the allele size. The gels were run for 4 hours at 45 volts and SSR fingerprint profiles were recorded with BioRad Gel Doc XR version 2.0. Band scoring was performed by including only the strongest band as alleles. The SSR bands were scored as present (1) or absent (0) for each primer genotype combination and a binary matrix was generated for all banding patterns.

Data analysis for genetic diversity and PIC

The amplification data generated by transferable primers were analyzed using SIMQUAL route to generate Jaccard's similarity coefficient (Jaccard, 1908) using software NTSYS-PC version 2.1 (Rohlf, 1998). These similarity coefficients were used to construct a dendrogram depicting genetic relationships among the cultivars by employing the Unweighted Paired Group Method of Arithmetic Averages (UPGMA) algorithm and SAHN clustering. The robustness of the dendrogram was evaluated with a bootstrap analysis performed on the binary dataset using software WINBOOT version 2.0. Gene diversity (H_j), also termed as the polymorphism information content (PIC) (Anderson *et al.*, 1993) and average heterozygosity (H_{av}) (Powell *et al.*, 1996), was calculated for all the amplifying SSR markers using the following formula:

$$H_{av} = \frac{\sum (1 - \sum P_{ij}^2)}{n}$$

where $(1 - \sum P_{ij}^2)$ is the expected heterozygosity estimated for each individual locus, $j = 1 - n$ and P is the frequency of the i^{th} allele of each marker and n is the number of alleles (Nei and Li, 1979). Marker index (MI) was calculated according to Powell *et al.* (1996).

RESULTS

Transferability of SSR markers

Of the 34 common bean markers assayed, only nine markers (26.4 %) amplified fragments in all the 24 cultivars of pigeonpea. The transferability of lentil microsatellite markers was very low (9.3 %) as only three out of 32 markers showed amplification in pigeonpea. The remaining markers specific to common bean and lentil did not amplify even with changes in PCR conditions and, therefore, these SSR primers were not included for the assessment of genetic diversity.

DNA polymorphism

Total 22 SSR markers (pigeonpea-10, common bean-9, and lentil-3) amplified easily scorable bands of 70 to 900 bp size range in all the pigeonpea cultivars (Table 2). Of the 22 markers, 17 were di-nucleotide repeats and 5 tri-nucleotide repeats. Of the 22 markers, 11 (50 %) were polymorphic and remaining (50 %) were monomorphic. A total of 45 alleles were amplified by the 22 microsatellite markers (Table 3). We detected 1 to 5 alleles per microsatellite locus with an average of 2.1 alleles per marker. Pigeonpea primers amplified 19 alleles with 1.9 alleles/locus whereas common bean primers detected 17 alleles with 1.9 alleles per marker and lentil primers detected 9 alleles with 3 alleles per locus. Out of these 45 alleles, only 32 (71 %) were polymorphic. Three markers, namely CCB 9, BMd 48 and Lc 14 amplified five alleles each, while other three markers, namely CCB 5, CCB 8 and Lc 24 amplified three alleles each. Two alleles each were amplified by five markers, CCB 4, BMd 29, BMd 47, BMd 54 and BMd 12. Nine markers *viz.*, CCB 4, CCB 5, CCB 7, CCB 8, CCB 9, BMd 29, BMd 50, BMd 55 and Lc 14 exhibited 100 % polymorphism. Minimum level of

polymorphism by any marker was 50 % as shown by BMd 47, BMd 54 and BMd 12. Out of 10 SSR markers from pigeonpea, 5 (50 %) were polymorphic. However, only 7 out of 34 (20 %) common bean SSR markers and 2 out of 32 (6.25 %) lentil SSR markers exhibited polymorphism in pigeonpea.

Among the polymorphic markers, the maximum PIC value was obtained with primer BMd 54 (0.938) and minimum with BMd 12 (0.082), the average being 0.462 (Table 2). Similarly, the average heterozygosity (Hav) and marker index (MI) had a range of 0.002-0.021 and 0.045-0.227 with mean values of 0.006 and 0.03, respectively. Highest Hav was recorded with marker BMd 54 (0.021) and lowest with BMd 12 (0.002). Similarly, high marker index (0.227) was recorded with marker CCB 9 and Lc 14 whereas low value (0.045) was observed with many markers like CCB 7, BMd 47, BMd 50, BMd 54, BMd 55, BMd 12.

Diversity and cluster analysis

In order to quantify the level of polymorphism, Jaccard's estimate of similarity based on the probability that an amplified fragment from one plant will also be found in another was used to generate a similarity matrix. The estimated similarity coefficient values between varietal combinations ranged from 0.26 to 0.88 with a mean of 0.51 over the 276 varietal combinations in the present study (Table 4). With microsatellite markers derived from lentil and common bean, the similarity coefficient values between cultivars ranged from 0.43 to 1.00 with average genetic diversity of 32 %. Similarly, with pigeonpea derived SSR markers, the similarity coefficients between cultivars ranged from 0.31 to 1.00 with 37 % genetic diversity. Pigeonpea derived SSRs could not differentiate three cultivars MAL 13, DA 11

Table 3. Comparison of efficiency of different SSR markers in detecting polymorphism in pigeonpea

Description	Pigeonpea SSR	Common bean and lentil SSR	Total SSR
Total markers used	10	66	76
Number of amplified markers	10	12	22
Total alleles amplified	19	26	45
Number of loci amplified	258	374	632
Similarity coefficient values	0.31-1.00	0.43-1.00	0.26-0.88
Genetic diversity (%)	37	32	44.6
Genotypes not differentiated	MAL 13, DA 11, Azad	MA3, Paras	-

Table 4. Similarity coefficient values among different pigeonpea cultivars based on UPGMA

Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
MMA 3	1.00																							
MMA 6	0.69	1.00																						
MAL 13	0.63	0.63	1.00																					
NDA 1	0.40	0.60	0.63	1.00																				
PUSA 9	0.32	0.48	0.50	0.80	1.00																			
DA 11	0.42	0.63	0.65	0.88	0.77	1.00																		
AZAD	0.63	0.82	0.76	0.63	0.50	0.65	1.00																	
AMAR	0.38	0.58	0.48	0.79	0.68	0.80	0.63	1.00																
CO 6	0.26	0.38	0.43	0.68	0.71	0.65	0.43	0.67	1.00															
BSMR 853	0.32	0.42	0.43	0.45	0.45	0.46	0.50	0.60	0.64	1.00														
ICPL 87119	0.28	0.48	0.38	0.50	0.55	0.52	0.50	0.59	0.69	0.70	1.00													
TAT 10	0.44	0.40	0.50	0.33	0.27	0.35	0.50	0.47	0.43	0.67	0.57	1.00												
GS 1	0.37	0.48	0.30	0.44	0.41	0.41	0.43	0.48	0.43	0.38	0.64	0.42	1.00											
LRG 38	0.40	0.50	0.39	0.52	0.47	0.48	0.52	0.44	0.50	0.46	0.73	0.53	0.68	1.00										
BAHAR	0.33	0.38	0.33	0.42	0.34	0.38	0.40	0.40	0.37	0.40	0.48	0.47	0.47	0.67	1.00									
LRG 30	0.35	0.50	0.46	0.57	0.56	0.59	0.59	0.56	0.66	0.58	0.78	0.52	0.52	0.76	0.50	1.00								
UPAS 120	0.28	0.48	0.38	0.50	0.56	0.57	0.50	0.65	0.63	0.70	0.75	0.50	0.50	0.52	0.42	0.58	1.00							
PUSA 992	0.47	0.42	0.45	0.46	0.42	0.42	0.45	0.50	0.40	0.33	0.52	0.44	0.70	0.55	0.42	0.42	0.40	1.00						
MANAK	0.47	0.50	0.45	0.46	0.52	0.48	0.52	0.56	0.45	0.40	0.58	0.45	0.60	0.55	0.36	0.48	0.52	0.72	1.00					
PARAS	0.57	0.42	0.53	0.35	0.28	0.36	0.53	0.43	0.34	0.53	0.45	0.77	0.53	0.47	0.41	0.41	0.45	0.56	0.56	1.00				
PUSA 33	0.38	0.48	0.50	0.45	0.45	0.52	0.57	0.60	0.57	0.80	0.63	0.58	0.43	0.40	0.29	0.52	0.77	0.39	0.46	0.61	1.00			
ICPL 87	0.47	0.57	0.60	0.46	0.47	0.53	0.68	0.63	0.50	0.67	0.58	0.53	0.39	0.36	0.25	0.48	0.58	0.48	0.56	0.56	0.75	1.00		
GT 100	0.59	0.68	0.55	0.43	0.39	0.50	0.63	0.58	0.47	0.55	0.48	0.47	0.41	0.32	0.21	0.44	0.54	0.49	0.50	0.50	0.70	0.73	1.00	
ICPL 84031	0.45	0.62	0.43	0.45	0.45	0.52	0.57	0.60	0.53	0.57	0.63	0.43	0.43	0.40	0.29	0.52	0.63	0.39	0.46	0.38	0.57	0.67	0.70	1.00

Fig. 1. SSR profile of 24 pigeonpea cultivars obtained with primer CCB05. Lanes M = Molecular weight marker, 100bp DNA ladder, 1-24 = 24 pigeonpea genotypes as per the serial number in Table 1.

Fig. 2. Dendrogram showing genetic relationship among the pigeonpea cultivars based on 22 cross-species microsatellites. Scale indicates Jaccard's coefficient of similarity.

and Azad while SSRs derived from lentil and common bean showed 100 % similarity between MA 3 and Paras.

The highest similarity value was observed in varietal combination of NDA 1- DA 11 (0.88) followed by MA 6-Azad (0.82) and NDA 1-Pusa 9 (0.80). Interestingly, all these cultivars belong to the long maturity group and are adapted to agro-climatic conditions of northeast plain zone of India. Further, the three of them, namely DA 11, MA 6 and Azad have 'Bahar' as one of the parents in their pedigrees. However, such relationships were not universal. For example, Amar is the selection from Bahar but similarity index value based on SSR primers was only 0.40. The low level of correlation between genetic similarity based on pedigree and DNA profiles has been reported earlier in different crop species (Barrett *et al.*, 1998). This could be due to the fact that the pedigree records do not take into account selection and genetic drift, which play a significant role in variety development. It is also possible that the molecular markers employed in these studies are insufficient to assay a significant proportion of the genome. The most diverse varietal combination was MA 3-Co 6 with 74 % diversity, which is expected as these two cultivars belong to different maturity groups and grown in different agro-ecological conditions. MA 3 is a long duration cultivar with adaptation to northeast plain zone whereas Co 6 is a medium duration cultivar with adaptability to extreme south zone of India.

The dendrogram constructed based on the similarity index resulted in two main clusters of 24 pigeonpea cultivars (Fig. 2). High bootstrap values were recorded for many nodes which indicated the robustness of this clustering. The first main cluster A had three sub-clusters: the first sub-cluster A1 contained four long duration cultivars (MA 3, MA 6, Azad and MAL 13) which are adapted to northeast plain zone of India. Interestingly, all four cultivars have 'Bahar' as one of the parents in their pedigrees. The second sub-cluster A2 grouped five cultivars (NDA 1, DA 11, Amar, Pusa 9 and Co 6) together. This was a diverse cluster with regard to parentage, maturity and area of adaptation for the member cultivars. The third sub-cluster A3 comprised six cultivars (BSMR 853, Pusa 33, UPAS 120, ICPL 87, GT 100 and ICPL 84031). All but BSMR 853 and GT 100 have 'UPAS 120' in their pedigrees and belong to short duration group. The second main cluster B had four sub-clusters: B1 containing three medium duration cultivars (ICPL 87119, LRG 30 and LRG 38) adapted to South India; B2 comprising three short duration cultivars (GS 1, Pusa 992 and Manak)

adapted to northwest plain zone; B3 with two extra short duration cultivars (TAT 10 and Paras); and B4 with one cultivar Bahar. In the present study, one unique band was obtained in Pusa 9 with marker CCB 8. The presence of unique band in a particular cultivar with specific marker can be used for varietal identification and will be of immense value to establish genetic purity of the seed.

DISCUSSION

Transferability of markers from common bean and lentil to pigeonpea demonstrated that the regions flanking these microsatellites are conserved enough to allow locus amplification. We found that the transferability rate of SSR markers from common bean and lentil to pigeonpea was lower than the earlier reports in other legumes (Peakall *et al.*, 1998; Choumane *et al.*, 2004; Gutierrez *et al.*, 2005; Datta *et al.*, 2009). Peakall *et al.* (1998) reported 65 % cross-species amplification within the genus *Glycine*. Gutierrez *et al.* (2005) reported 40 %, 36.4 % and 37.6 % transferability of *Medicago truncatula* microsatellites in fababean, chickpea and pea, respectively. In another study, Choumane *et al.* (2004) observed 54.4 % sequences conservation among chickpea, lentil and pea. Datta *et al.* (2009) observed 46 % transferability of chickpea microsatellites in pigeonpea. The lower rate of marker transferability observed by us, however, compared well with the results of Pandian *et al.* (2000) who reported 5 % and 18 % transferability of microsatellite markers from chickpea to lentil and pea. Variation in SSR transferability depends on factors including the number of markers tested, amplification conditions and plant species involved. The transferability of genomic microsatellite markers across genera and beyond is generally low (Peakall *et al.*, 1998; Roa *et al.*, 2000). Amplification of a particular locus in one genome with primers designed from another crop depends not only on the evolutionary distance between the two species but also on the rate of evolution of the genome sequence where the primer binding sites are located (Roa *et al.*, 2000). Hence, the low level of transferability of lentil SSRs might be attributed to the fact that pigeonpea and lentil belong to two distinct tribes, Phaseoleae and Viceae and the divergence time between these two tribes is estimated to be 37-38 million years ago (Choi *et al.*, 2004). Therefore, the transferable SSRs from common bean and lentil showing positive amplification in pigeonpea can be useful genomic resources for comparative genome analysis of legumes and will add

to the scarce pool of available markers in pigeonpea for diversity analysis, mapping of useful genes and marker aided selection.

The number of alleles per locus varied according to the origin of the marker. Markers with PIC values of ≥ 0.50 , such as, BMd 29 (0.594), BMd 47 (0.750), BMd 50 (0.707), BMd 54 (0.938), and Lc 14 (0.514) will be highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of the marker at specific locus (De Woody *et al.*, 1995). High levels of polymorphism associated with microsatellites are expected because of the unique mechanism responsible for generating microsatellite allelic diversity by replication slippage (Tautz and Renz, 1984; Tautz, 1989) rather than by simple mutations or insertions/deletions. Microsatellites have been used in many crop plants for detection of genetic diversity (Zeng *et al.*, 2006; Stepien *et al.*, 2004). Ford *et al.* (2002) reported the use of *Pisum sativum* specific microsatellite primers to assess genetic relationships among pea cultivars. Doldi *et al.* (1997) evaluated genetic diversity among 18 soybean genotypes with 12 microsatellite primers pairs which amplified 2-6 alleles per marker. Similarly, Sethy *et al.* (2006) observed 78 % diversity among chickpea accessions on the basis of microsatellite markers.

High selection intensity for a few traits during selection may have contributed to low diversity within the cultivated germplasm. However, these values still present a wide range of similarity index values among the pigeonpea cultivars as compared to previous reports of narrow range of 0.70 to 0.90 with RAPD and 0.82-1.00 with AFLP markers (Ratnaparkhe *et al.*, 1995; Panguluri *et al.*, 2006). The mean estimate of genetic diversity was higher with SSR primers (49 %) than the diversity reported with RAPD (<30 %) and AFLP (<14 %) in previous reports in pigeonpea. This suggests that SSR markers have better discriminatory powers in genetic diversity analysis.

It was surprising that Bahar formed a separate cluster in the second cluster inspite of being a common parent to many of the cultivars included in the study. This supports the earlier report questioning the genetic purity of Bahar due to out-crossing (Gupta *et al.*, 1980). This is quite possible that the Bahar used as parent in development of cultivars might be different from the one used in the present study. Interestingly, only the medium duration cultivars were often placed in different clusters, indicating that these cultivars have more plasticity and diversity, supporting the view of

Upadhyaya *et al.* (2007) that medium maturity group has greater diversity compared to late and early maturity groups. This may be due to the fact that medium duration cultivars have overlapping period with short as well as long duration cultivars particularly during the pollination. Since pigeonpea is an often cross pollinated crop, there is every chance of out-crossing of medium duration cultivars from both the groups, thus encompassing the wide diversity in the medium maturity group. Placement of medium duration cultivars in different clusters may also be attributed to high polymorphism detected in the analyzed loci and the occurrence of homoplastic alleles being identical by descent.

The present study clearly demonstrates the utility of common bean and lentil specific microsatellite markers in establishing genetic relationships among different cultivars in pigeonpea. Although the number of useful markers was low, all commercial cultivars could be differentiated from each other. The high discriminatory powers exhibited by these PCR compatible markers identified as polymorphic can easily be put on the linkage map of pigeonpea which is being jointly developed in many laboratory including ICRISAT, IIPR and UC Davis, USA and then be linked with genes/QTLs determining the trait of interest. One of the most informative primers (BMd 54) identified in this study can be utilized for unambiguous identification of the commercial cultivars of pigeonpea, which is one of the major challenges in often cross pollinated crops. Assessment of genetic diversity among the pigeonpea cultivars provided an opportunity to understand the present structure of genetic base and will be helpful to breeders to embark on the widening of the genetic base of cultivated germplasm by involving new germplasm in hybridization and conversion programs.

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