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Cross-transferable microsatellite markers from *Vigna* Savi. are useful for assessing genetic diversity in wild and cultivated *Cajanus* Adans. species

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Abstract

We assessed 61 *Vigna* (azuki bean)-specific microsatellite markers for their cross transferability and efficiency in supporting diversity studies in *Cajanus*. Thirty *Cajanus* genotypes comprising 20 different cultivars of pigeonpea (*C. cajan*) and ten genotypes from five wild relatives of pigeonpea were examined in this study. Of the 61 SSR primer pairs screened, 18 amplified distinct and scorable amplicons 90–600 bp in size, revealing 32 alleles. The percentage transferability of these 18 markers ranged from 31.60% to 100%. Eight primer pairs (CEDG 56, CEDG 91, CEDG 139, CEDG 268, CEDG 275, CEDG 06, CEDG 88 and CEDG 257) amplified SSRs from the 30 genotypes examined, indicating 100% transferability. Except for CEDG 06, all markers with 100% transferability amplified di-nucleotide repeat motifs, with $(AG)_n$ as the only repeats. The Polymorphic Information Content (PIC) ranged from 0.24 in CEDG 176 to 0.69 in CEDG 127, with an average of 0.47. The UPGMA clustering method, which we used to assess the genetic distances and relatedness of the genotypes using the 18 successful markers, produced a dendrogram with two clusters, separating all 20 cultivars from the wild relatives at 67% similarity. *C. cajanifolius* (ICP 1629-1 and ICP 1629-2), which was previously reported to be a progenitor of *C. cajan*, clustered with the cultivars. The results of this study affirm the transferability of SSR markers within related legume genera. The identified *Vigna* SSRs will be useful for increasing the marker repository of pigeonpea and for diversity studies of pigeonpea cultivars.

Keywords: *Cajanus*, genetic diversity, microsatellites, pigeonpea, polymorphism, *Vigna*.
Abbreviations: CTAB_Cetyl trimethylammonium bromide; DNA_Deoxyribonucleic Acid; EDTA_Ethylenediaminetetraacetic acid; FW_*Fusarium* Wilt; ICRISAT_International Crop Research Institute for the Semi-arid Tropics; NCBI_National Center for Biotechnology Information; NTSYSpc_Numerical Taxonomy System; PCR_Polymerase Chain Reaction; PIC_Polymorphism Information Content; SAHN_Sequential Agglomerative Hierarchical Non-overlapping; SIMQUAL_Similarity for Qualitative data; SMD_Sterility mosaic disease; SNPs_Single Nucleotide Polymorphisms; SSRs_Simple Sequence Repeats; UPGMA_Un-weighted Paired Group Method of Arithmetic Averages; UV_Ultra violet.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] belongs to the subtribe Cajaninae of the tribe Phaseoleae in the family Leguminosae (Lackey, 1977, 1981; van der Maesen 2003). *C. cajan* is the only domesticated species under Cajaninae. Pigeonpea (2n = 22, genome size of 808 Mbp) is a hardy, widely adapted, drought tolerant pulse crop that is cultivated globally on 4.92 million hectares of land with an average annual production of 3.65 million tonnes. Pigeonpea represents a rich source of protein, carbohydrates and certain minerals (Gopalan et al., 1977). The high-protein genotypes contain a significantly higher level (approximately 25%) of sulphur-containing amino acids, namely methionine and cysteine, compared to the other genotypes (Singh et al., 1990). Pigeonpea represents a good source of dietary minerals such as calcium, phosphorus, magnesium, iron, sulphur and potassium as well as water-soluble vitamins, especially thiamin, riboflavin, niacin and choline (Ali and Kumar, 2005). As a legume crop, pigeonpea plays a major role in fixing atmospheric nitrogen through symbiotic nitrogen fixation with soil bacteria, which improves the nutrient status of the soil. Over the past few decades, the production of pigeonpea has not improved, despite concerted breeding efforts, due to poor exploitation of available natural diversity, poor crop husbandry and exposure to a number of biotic and abiotic stresses. In addition, narrow genetic diversity exists in the cultivated germplasm, which hampers the effective utilization of conventional breeding to address production constraints (Varshney et al., 2010). On the other hand, wild species have co-existed with pests and pathogens on an evolutionary time scale and have developed alleles conferring pest and pathogen resistance (Acosta-Gallegos et al. 1998). These natural defence mechanisms against diseases and pests have been lost during domestication and the intense selection for agriculturally desirable traits such as high yield, improved nutritional quality and other desirable agronomic traits (Kassa et al., 2012). Abiotic (e.g., drought, salinity) and biotic (e.g., diseases and pests) stresses constrain and adversely affect pigeonpea production and lead to huge economic losses. The major diseases affecting pigeonpea production include *Fusarium* wilt (FW), sterility mosaic disease (SMD) and *Phytophthora* blight disease, and the major pests that produce severe damage include pod borer

(*Helicoverpa armigera* and *Maruca vitrata*) and pod fly (*Melanagromyza obtusa*) (Minja et al., 2000). Molecular breeding has recently gained importance as a method for supplementing pigeonpea breeding efforts aimed at addressing these challenges. However, the lack of genetic and genomic resources for this crop has hampered these efforts. The development of numerous genomic and genetic resources for genetic mapping and reverse genetic analysis (Varshney et al., 2009), and the recent independent sequencing/decoding of the whole genome of a single pigeonpea genotype (ICPL 87119) by two separate groups (Singh et al., 2011; Varshney et al., 2011), have opened up a new era in the molecular breeding of pigeonpea. This sequence information has tremendous potential to increase our understanding of the genetics of important traits that currently limit the yield potential of this crop. While we applaud the scientific prowess and immense contributions of these teams, it is important to point out that such genomic resources, which are housed in the NCBI database, are only useful to a few specialised scientists with the expertise required to query, understand, interpret and apply information resulting from this resource. According to Jackson et al. (2011), "Several barriers prevent rapid and effective deployment of these tools to a wide variety of crops. Because of the complexity of crop genomes, *de novo* sequencing with next-generation sequencing technologies is a process fraught with difficulties that then create roadblocks to the utilization of these genome sequences for crop improvement". In other words, decoding the genome of a single pigeonpea genotype does not solve the biotic or abiotic problems affecting this crop; it simply creates an avalanche of useful information for finding solutions to these problems. One potential use of whole genome sequences is the large-scale mining of a preferred class of markers such as SSRs and SNPs in a cost effective and time saving manner. For example, for pigeonpea, sets of SSRs comprising 309,502 and 189,895 SSRs were identified through a microsatellite survey of two draft genome sequences, respectively. In the present study, we examined the ability of 61 *Vigna* SSR primer pairs to amplify PCR products from 30 *Cajanus* genotypes belonging to six pigeonpea species. This study is based on several earlier studies of legumes and other crops, which revealed the high level of sequence conservation of microsatellite markers across different genera and their transferability from one genus/species to another (Dayanand et al., 1997; Peakall et al., 1998; Pandian et al., 2000; Choumane et al., 2004; Eujayl et al., 2004; Datta et al., 2010a, b; 2011; 2013). The transferable markers from *Vigna* that were examined in this study can be combined with the existing SSR markers from *Cajanus*. These new markers will also be useful for introgressing desirable alleles, and they provide a basis for assessing the genetic diversity and relationship between *Vigna* and *Cajanus*, both members of the tribe Phaseoleae.

Results

Cross transferability and diversity studies using Vigna microsatellites

Of the primer pairs targeting 61 *Vigna*-specific SSRs (Table 1), 29 pairs (representing 47.54% of the total) yielded amplification products in the 30 *Cajanus* genotypes examined in this study. Eighteen (29.51%) of these 29 primer pairs amplified unique bands 90–600 bp in size (Table 2), while 11 (CEDG 014, 050, 115, 118, 149, 173, 204, 228, 282, 290 and 294) produced multiple banding and inconsistent patterns and were therefore excluded from further analysis. Details of the sequences of the 18 SSR pairs, their repeat motifs, annealing temperatures and number of alleles are provided in Table 2. Also included are the transferability percentage, polymorphism information content (PIC) and expected heterozygosity of each SSR pair. The percentage of transferability ranged from 31.60% to 100% (Table 2). The amplification patterns of four of the SSR markers are shown in Figure 1 (a–d). Eight primer pairs (CEDG 56, CEDG 91, CEDG 139, CEDG 268, CEDG 275, CEDG 06, CEDG 88 and CEDG 257) amplified SSRs in all 30 genotypes, indicating 100% transferability. Twelve of the 18 markers had transferability values between 70% and 100%, while the transferability of three markers ranged from 50% to 69%. The lowest transferability percentages were observed for CEDG 292 (31.6%) and CEDG 180 (38.3%; Table 2). Except for CEDG 06, the primer pairs for all 100% transferable markers amplified di-nucleotide repeat motifs with $(AG)_n$ as the only repeats. Some markers failed to be amplified in the wild genotypes. Among the ten wild genotypes examined, CEDG 180 was only amplified in *C. scarabaeoides* (ICP 15724), while among the cultivars, it was not amplified in MAL 13, MA 6, PARAS or MANAK. Except for two of the *C. cajanifolius* genotypes (ICP 1629-1 and ICP 1629-2), CEDG 27 was not amplified in any wild *Cajanus* species. Similarly, CEDG 43 was only amplified in genotypes 21 and 30 (*C. cajanifolius*: ICP 1629-1 and *C. albicans*: ICP 15622), and CEDG 292 was amplified in *C. cajanifolius* (ICP 1629-1 and ICP 1629-2) and *C. scarabaeoides* (ICP 15685 and ICP 15724). Among the cultivars, CEDG 01 failed to be amplified in six genotypes (ICPL 87119, Type 7, BSMR 853, PUSA 9, NDA1 and PUSA 33), CEDG 27 was not amplified in cultivar PUSA 9, GT1 or PUSA 33 and CEDG 292 was not amplified in UPAS 120, PARAS or MANAK. In total, all 18 *Vigna* SSRs were amplified in more than 50% of the genotypes examined.

Polymorphism of Vigna SSR markers in Cajanus

A total of 32 alleles were detected in the wild and cultivated *Cajanus* lines using the 18 transferable *Vigna* SSR markers (Table 2). Ten of the primer pairs (CEDG 06, 43, 56, 88, 91, 139, 257, 268, 275 and 292) produced completely monomorphic amplicons with product sizes ranging from 90 bp (CEDG 257) to 380 bp (CEDG 292). Of the remaining eight markers (44.44%), two alleles each were detected with three markers (CEDG 27, CEDG 176 and CEDG 180), three alleles with four markers (CEDG 111, CEDG 01, CEDG 20 and CEDG 08) and four alleles with CEDG 127. The product sizes of the polymorphic SSRs ranged from 100 bp (CEDG 27 and CEDG 176) to 600 bp (CEDG 180). The Polymorphism Information Content (PIC) value ranged from 0.24 in CEDG 176 to 0.69 in CEDG 127, with an average of 0.47. The highest expected heterozygosity value (0.73) was recorded for CEDG 127.

Phylogenetic relationship among Cajanus species

We produced a dendrogram to assess the genetic distance and relatedness among the *Cajanus* species based on the 32 alleles detected by the 18 transferable *Vigna* SSRs, as shown in Figure 2. Two clear clusters, designated 1 and 2, separate the 20 *C. cajan* cultivars (Cluster 1) from eight established genotypes of four wild relatives (Cluster 2). *C. cajanifolius* (represented by ICP 1629-1 and ICP 1629-2) is grouped with

Table 1. List of *Cajanus* species (cultivated and wild genotypes) used in this study.

Sl. No	Pigeonpea Genotype	Species	Pedigree/Origin	Maturity Group	Plant Type	Disease Reaction *Fusarium* Wilt	Sterility Mosaic Disease
1	UPAS 120	*C. cajan*	Selection from P4768	E	NDT	S	S
2	ICPL 87119	*C. cajan*	C11 x ICPL 6	M	NDT	R	R
3	ICP 8863	*C. cajan*	Selection from landrace of Maharashtra, India	M	NDT	R	S
4	Type 7	*C. cajan*	Selection from landrace of Lucknow	L	NDT	S	S
5	BDN 2	*C. cajan*	Local selection from Bori germplasm	M	NDT	R	S
6	BSMR 853	*C. cajan*	(ICPL 7336 x BDN1) x BDN2	M	NDT	R	R
7	MAL 13	*C. cajan*	(MA2 x MA166) x Bahar	L	NDT	S	R
8	PUSA 9	*C. cajan*	UPAS120 x 3673	L	NDT	S	R
9	DA 11	*C. cajan*	Bahar x NP (WR)15 x PS16	L	NDT	S	R
10	NDA 1	*C. cajan*	Selection from landrace of Faizabad (Uttar Pradesh), India	L	NDT	S	R
11	MA 6	*C. cajan*	MA2 x Bahar	L	NDT	S	R
12	MA 3	*C. cajan*	Selection from MA 2	L	NDT	S	S
13	GT 1	*C. cajan*	Selection from Dabhali	L	NDT	NA	NA
14	PUSA 992	*C. cajan*	Selection from 90306	E	NDT	S	R
15	Paras	*C. cajan*	EE76 x UPAS 120	E	NDT	S	R
16	PUSA 33	*C. cajan*	C11 x UPAS 120	E	NDT	R	S
17	Manak	*C. cajan*	Type 21 X UPAS 120	E	NDT	S	S
18	GT 100	*C. cajan*	T15-15x S5	E	DT	R	R
19	Bahar	*C. cajan*	Selection from landrace of Motihari (Bihar), India	L	SDT	S	R
20	ICPL 20116	*C. cajan*	ICRISAT line	M	NDT	R	R
21	ICP 1629-1	*C. cajanifolius*	ICRISAT collection	M	NDT	S	S
22	ICP 1629-2	*C. cajanifolius*	ICRISAT collection	M	NDT	S	S
23	ICP 15685	*C. scarabaeoides*	ICRISAT collection	E	NDT	R	R
24	ICP 15724	*C. scarabaeoides*	ICRISAT collection	E	NDT	R	S
25	ICP 15661	*C. platycarpus*	ICRISAT collection	E	NDT	NA	R
26	ICP 15666	*C. platycarpus*	Central (India)	E	NDT	NA	R
27	ICP 15760	*C. sericeus*	Western Ghats (India)	L	NDT	NA	R
28	ICP 15761	*C. sericeus*	Western Ghats (India)	L	NDT	NA	R
29	ICP15624	*C. albicans*	Sri Lanka	L	NDT	R	R
30	ICP 15622	*C. albicans*	Sri Lanka	L	NDT	R	R

E = Early, M = Medium, L = Long, DT = Determinate plant type, NDT = Non-determinate plant type, SDT = Spreading and determinate plant type, R = resistant, S = susceptible; NA = Not applicable

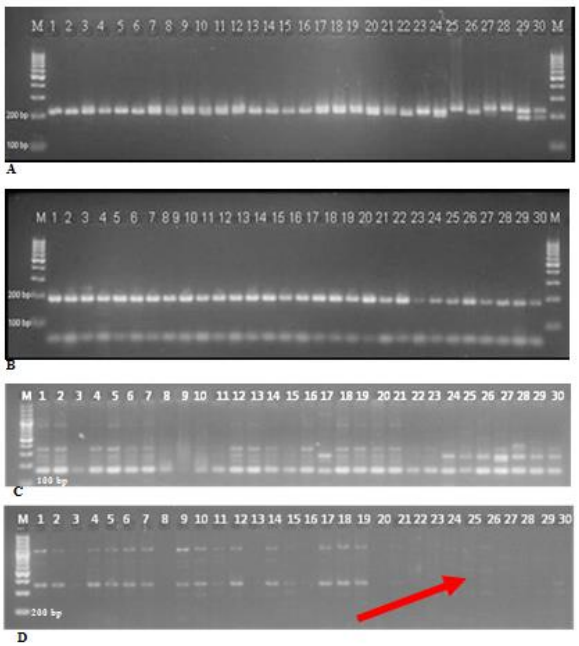

Fig 1. SSR profile of *Cajanus* genotypes obtained with *Vigna* SSR markers. Lanes M: 100 bp ladder, 1 -30 *Cajanus* genotypes listed in Table 1. M: 100 bp ladder. d – Arrow points to a section of the profile of CEDG 43 showing non-amplification of wild species of *Cajanus* due to inherent differences between *Vigna* and *Cajanus*. A – CEDG 139; B – CEDG 127; C – CEDG 111; D – CEDG 43.

Table 2. *Vigna* SSRs that amplified distinct and scorable amplicons in *Cajanus*.

S. No.	Primer Name	Sequence (5'–3')	Repeat motif	Tm (ºC)	Allele Size (bp)		No. of alleles	*** % Transferable	PIC	**** He
					*Exp.	**Obs.				
1	CEDG56	F TTCCATCTATAGGGGAAGGGAG R GCTATGATGGAAGAGGGCATGG	(AG)14	60.1	190	180	1	100	0	0
2	CEDG 91	F TGGTGGAACAAAGCAAAAGAGT R TGCGTCTTGGTGCAAAGAAGAA	(AG)7	53	-	190	1	100	0	0
3	CEDG 139	FAAACTTCCGATCGAAAGCGCTTG RGTTTCTCCTCAATCTCAAGCTCCG	(AG)19	55	190	190	1	100	0	0
4	CEDG 268	FCATCTCCCTGAAACTTGTG R GCTATCAATCGAGTGCAG	(AG)16	57.1	168	150	1	100	0	0
5	CEDG 275	F CACACTTCAAGGAACCTCAAG R TAGGCAACCTCCATTGAAC	(AG)14	53	250	250	1	100	0	0
6	CEDG 111	F GGAAGTTTCCAAGAGGGTTTTC R TCTCACCACCTTTTACCTTCTCA	(AT)7(AG)14	55	225	180-350	3	70	0.57	0.64
7	CEDG 43	F GGATTGTGGTTGGTGCATG R CTATTTCCAACCTGCTGGG	(AG)14	55	200	390	1	50	0	0
8	CEDC 27	F ACTTGGATGAGGGTTTAGTGCG R CTGTCTTGTCTTGTGGGTTCGTTC	(AC)8	57.1	128	100,350	2	66.6	0.29	0.34
9	CEDG 127	F GTTAGCATCTGAGCTTCTTCGTC R CTCCTCACTTGGTCTGAAACTC	(TG)3(AG)9	60.1	220	120-215	4	51.6	0.69	0.73
10	CEDG 6	F ATTGCTCTCGAACCAGCTC R GTGTACAAGTGTGTGCAAG	(AG)10 AA(AG) 18	53	90	180	1	100	0	0
11	CEDG 176	FGGTAACACGGGTTCAGATGCC R AAGGTGGAGGACAAGATCGG	(AG)12	53	150	100,500	2	98.3	0.24	0.28
12	CEDG 292	F GTGGTTTTGTTGACCTTGTC R GTAATGCTCCAATGGCTTC	(AG)6	55	450	380	1	31.6	0	0
13	CEDG 88	FTCTTGTCATTTAGCACTTAGCACG RTTGTTGTTTACTAAGAGCCCGTGT	(AG)7	55	100	120	1	100	0	0
14	CEDG 180	F GGTATGGAGCAAAACAATC R TGCGTGAAGTTGTCTTATC	(AG)11	55	200	400,600	2	38.3	0.49	0.57
15	CEDG 1	F ACTATGCAGAAAGACGCTCC R GGCTCTCTCTTTCTCCATTC	(AG)26	60.1	180	120-500	3	43.3	0.50	0.59
16	CEDG 257	F ACTACTCTCAAGACCAAAG RGATGGTTGTAGATAACACTCC	(AG)12	57.1	250	90	1	100	0	0
17	CEDG 20	F ATCCATACCCAGCTCAAGG R GCCATACCAAGAAAGAGG	(AT)18(AG)20	55	450	180-520	3	87.7	0.53	0.61

18	CEDG 8	F GCTTGCATCACCCATGATTC R AGTGATACGGTCTGGTTCC	(AT)12(AG)14	55	123	120-220	3	71.1	0.47	0.52
Total/ Average							32		0.47	

*Expected Allele Size; **Observed Allele Size; ***% Transferability; **** Expected Heterozygosity

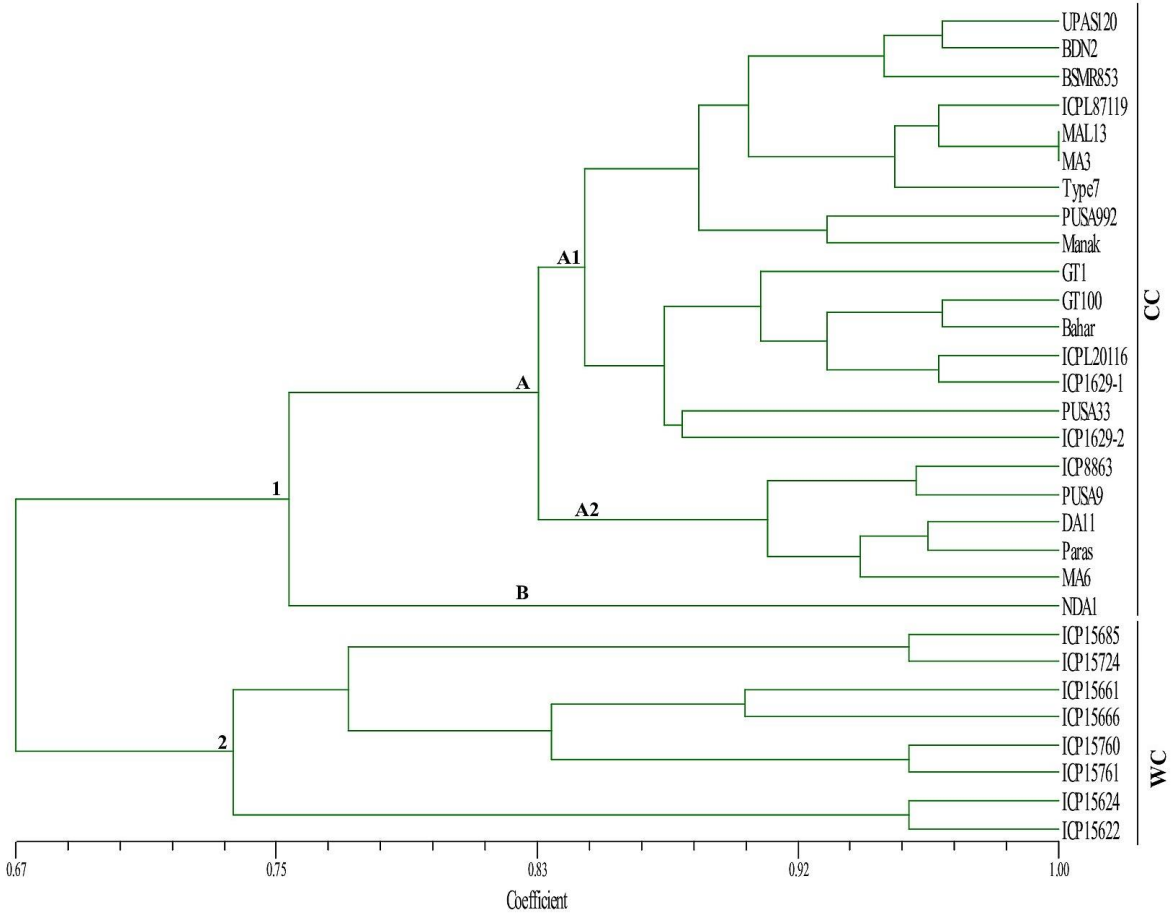

Fig 2. Dendrogram produced by Jaccard's coefficient and the unweighted pair group method with arithmetic average (UPGMA) clustering method based on *Vigna* SSR primers. CC – Cultivated *Cajanus* (i.e. pigeonpea); WC – Wild *Cajanus* species.

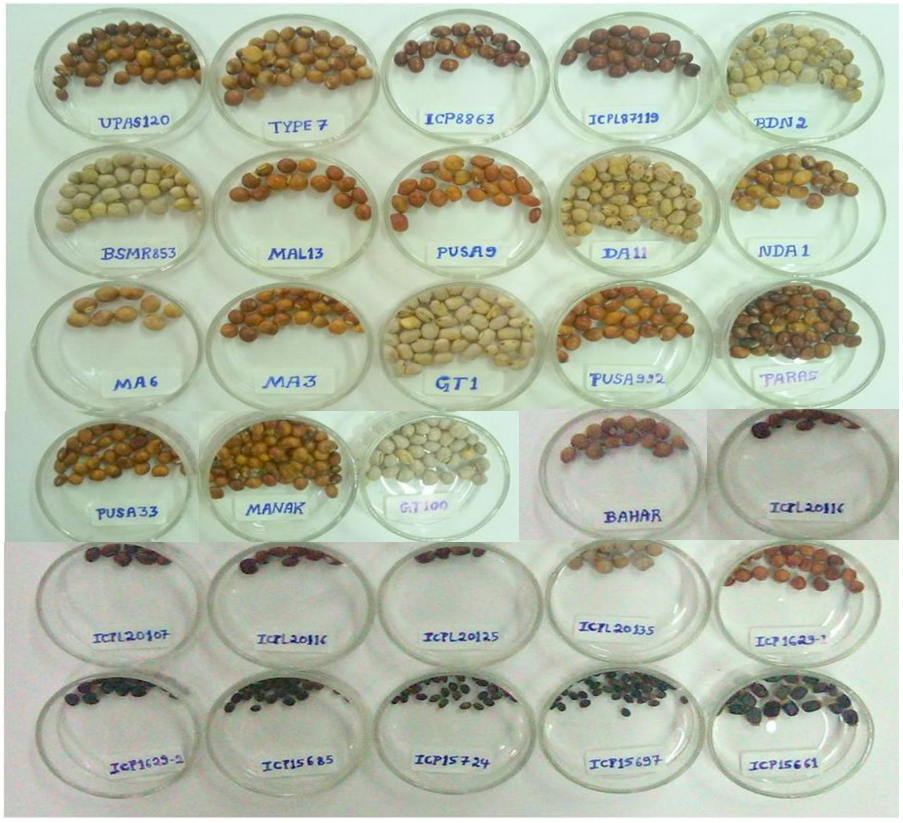

Fig 3. Seed samples of *Cajanus* genotypes used for the study showing seed morphological diversity.

the cultivars. Thus, Cluster 1 contains 22 cultivars. Notably, two subclusters (Subcluster A and B) emerge from the *C. cajan* cultivars (Cluster 1) with NDA 1 (Subcluster B), a long maturity cultivar selected from a core traditional landrace. Subcluster A is further separated into subgroups A1 (16 cultivars) and A2 (five cultivars). In subgroup A1, two medium duration varieties, BDN 2 and BSMR 853 (BDN 2 is one of the parents of BSMR) are clustered together, along with UPAS 120. Three long duration varieties, MAL 13, MA 3 (with Bahar as one of the parents in their pedigrees) and Type 7 are clustered together, along with medium duration variety ICPL 87119. MAL 13 and MA 3, however, tied. Similarly, two early duration varieties, PUSA 992 and Manak (adapted to the northwest plain zone) are clustered together. Furthermore, five varieties, including two long duration varieties, GT1 and Bahar, with the exception of the early duration variety GT100, are clustered together. This cluster also contains one cultivar (ICPL 20116) grouped together with a wild species (ICP 1629-1). ICPL 20116 and ICP 1629-1 (*C. cajanifolius*) are medium duration crops from the ICRISAT collection. PUSA is clustered with ICPL 1629-2 (wild species); both are susceptible to SMD. Six of the seven SMD-susceptible cultivars (Table 1) are clustered in A1. Subgroup A2 contains five varieties (ICP 8863, PUSA 9, DA 11, MA 6 and Paras); of these five varieties, DA 11 and MA 6 share Bahar as a pedigree parent and both are long duration crops, while Paras and PUSA 9 share UPAS 120 as a common parent. Except for ICP 8863, which is resistant to FW, the four other cultivars are resistant to SMD.

Cluster 2 contains the wild species, which are strictly grouped together in their order of pedigree relationship (Figure 2). However, the two *C. scarabaeoides* cultivars (ICP 15685 and ICP 15724), which are early maturity, FW-resistant cultivars from the ICRISAT collection, are separated into a subcluster.

Discussion

Cross transferability and diversity studies using Vigna microsatellites

The results of the current study show that 29 of the 61 *Vigna*-specific SSRs examined were amplified in 30 pigeonpea genotypes. Eighteen of these 29 primer pairs produced unique bands 90–600 bp in size. The transferability of markers from *Vigna* to *Cajanus* demonstrates that the regions flanking these microsatellites are conserved to the extent that they permit locus amplification between genera. This fact, which was previously noted by Datta et al. (2010a) in a study examining the cross transferability of common bean and lentil SSRs to pigeonpea, is most clearly demonstrated by the 18 primer pairs that amplified unique bands. Earlier studies by Datta et al. (2010a, b) examining the transferability of 100 *Cicer* (chickpea) and 34 *Phaseolus* (common bean) SSRs to *Cajanus* yielded transferability rates of 46% and 26.4%, respectively. Considering the differences in the number of microsatellite markers and genotypes used in the current versus previous studies, it is important to note that the transferability rate of 29.51% from 61 *Vigna* SSRs observed in the current study compares favourably with earlier findings. Datta (2010a) reported that variations in SSR transferability depend on such factors as the number of markers tested, amplification conditions and plant species involved. It is also noteworthy that the transferability of genomic microsatellite markers across genera and beyond is generally low (Peakall et al., 1988; Rao et al., 2000). Comparing the results of previous studies with those of the

present study, it is plausible that cross-genera transferability rates range from 0.4% to 1%. The 18 transferable *Vigna* SSRs showing positive amplification in *Cajanus* identified in the current study add to the growing pool of markers available for diversity analysis, mapping of useful genes and marker-assisted selection in pigeonpea. The results are also useful for the assessment of phylogenetic relationships between *Vigna* and *Cajanus*.

The 11 primer pairs that displayed multiple banding patterns were excluded from further analysis. The transferable microsatellites produced amplicons in *Cajanus* with a wide range of sizes, from 90 bp with CEDG 257 to 600 bp with CEDG 180, although the expected product size for CEDG 180 was only 200 bp. Aside from CEDG 139 and CEDG 275, which yielded products at the expected size range, the other primer pairs, like CEDG 180, yielded products either above or below the expected allele size. CEDG 43 failed to be amplified in all wild species of *Cajanus*. Considering that these primers were developed for *Vigna* species (azuki bean), perhaps the differences in amplicon size between the expected and observed values originated from the inherent differences between the two genera (i.e., *Vigna* and *Cajanus*), which are phenotypically observable. According to Choumane et al. (2004), the generation of amplification products from a defined locus requires the 3' terminal nucleotides of the target sequence to be perfectly complimentary to the primers. If amplification across genera boundaries is possible, the respective loci are expected to be conserved between the two genera. However, amplification of an SSR locus in one genus/species with primers from the other species does not necessarily confirm the conservation and identity of the loci. Datta et al. (2010a) reported that in cross transferability studies, failure of SSRs from one genus to produce amplification products in a related genus may stem from a mutation in the primer binding site or the complete absence of the locus in the genus under investigation; the results of the current study support this notion.

Polymorphism of Vigna SSR markers in Cajanus

The 18 transferable *Vigna* SSRs uncovered a total 32 alleles in the wild and cultivated *Cajanus* genotypes examined, with an average of 1.8 alleles per locus. The core polymorphic markers identified in this study include CEDG 01, CEDG 08, CEDG 20, CEDG 27, CEDG 111, CEDG 127, CEDG 176 and CEDG 180. The microsatellite markers with PIC values of ≥0.50, such as CEDG 1, CEDG 20, CEDG 111 and CEDG 127, exhibited high informativeness, making them useful for taxonomic and genetic studies and extremely useful for distinguishing the polymorphism rate of the marker at a specific locus (De Woody et al., 1995). The range of PIC values obtained are similar to those reported in previous cross transferability studies in legumes by Datta et al. (2010a,b).

Phylogenetic relationships among Cajanus species

We constructed a dendrogram based on the Unweighted Paired Group Method of Arithmetic Averages (UPGMA) clustering method, which separated the 30 genotypes into two clusters. Cluster 1 contains all *C. cajan* cultivars and the two *C. cajanifolius* genotypes (i.e., 20 genotypes), and Cluster 2 contains all wild species; the species in these clusters exhibited 67% similarity. *C. cajanifolius* was previously reported to be the probable progenitor of *C. cajan* (van der Maesen, 1980, 1990) and hence its clustering with the *C. cajan* cultivars is expected. The clear separation of NDA 1 from a single subcluster in Cluster 1 containing all of the cultivars is noteworthy. This finding may be explained by the origin of NDA1 (selection from a landrace of Faizabad, Uttar Pradesh, India), which may have limited gene flow between it and the other cultivars. Most subclusters were grouped based on pedigree relationship, disease reaction to FW and SMD or both factors. For instance, of the five cultivars in a single subcluster (DA 11, MA 6, Paras, PUSA 9 and ICP 8863), DA 11 and MA 6 share a common pedigree parent (Bahar) and both are long duration crops, while the common parent of Paras and PUSA 9 is UPAS 120. Except for ICP 8863, which is resistant to FW, the four other cultivars are resistant to SMD. These facts and others shown in Table 1 and Figure 2 demonstrate the utility of *Vigna* SSRs in elucidating the genetic relationships among different pigeonpea cultivars based on origin, maturity group and disease response. The cross-genera microsatellite markers produced amplification products and were effective enough to separate wild species of *Cajanus* from all cultivars of pigeonpea, i.e., the cultivated species.

Materials and Methods

Plant materials

Twenty genotypes of pigeonpea (*Cajanus cajan*) cultivars and two genotypes from each of five wild relatives (*C. cajanifolius*, *C. scarabaeoides*, *C. platycarpus*, *C. sericeus* and *C. albicans*), comprising a total of 30 genotypes (Table 1, Figure 3), were used in this study. The study was carried out at the Biotechnology Laboratory, Indian Institute of Pulses Research (IIPR), Kanpur, India.

Microsatellite markers

Sixty-one genomic *Vigna* SSR markers used for transferability and diversity studies in the cultivated and wild *Cajanus* genotypes were based on the primer sequences reported by Wang et al. (2004). These primers were synthesised by IDT Technologies, Coralville, IA, USA.

DNA extraction, PCR amplification and SSR scoring

Seeds of the 30 accessions were planted in the screen house at the Biotechnology Unit, IIPR, Kanpur, India. Total genomic DNA was isolated from young leaves of each accession after approximately 10 days using a modified CTAB method (Agbagwa et al., 2012). The extracted DNA was purified by RNase treatment (10 µg/ml) for 1 hour at 37°C, followed by treatment with phenol: chloroform: isoamyl alcohol (25:24:1). The pellet was dissolved in an appropriate amount of $T_{10}E_1$ (Tris 10 mM, EDTA 1 mM) buffer. DNA from different samples was quantified by both visual quantification and UV spectrophotometry and diluted to a concentration of 20 ng/µl. PCR was performed in a 20 µl volume containing 20 ng of template DNA, 1X PCR buffer (10 mM Tris HCl pH 9.0, 1.5 µM $MgCl_2$, 50 mM KCl, 0.01% gelatin), 20 pM each of forward and reverse primers, 0.2 mM each of dNTPs and 3 U *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd). The reactions were conducted in a tetrad thermocycler (G-Storm, Gene Technologies Ltd., UK) for each primer set using the following reaction conditions: initial denaturation for 3 min at 94°C followed by 35 cycles of 94°C for 2 min, 53°C to 60°C (depending on the primer set) for 1 min and 72 for 1 min, with a final elongation at 72°C for 7 min. All PCR amplicons were resolved by electrophoresis on 3% agarose gels and visualised by

ethidium bromide staining under UV illumination to identify the informative SSR loci across all 30 genotypes. A GeneRuler 100 bp DNA ladder (MBI Fermentas) was used to estimate the allele sizes. The gels were run for 4 hours at 45 volts, and SSR fingerprint profiles were recorded with BioRad Gel Doc XR version 2.0 (BioRad Laboratories, Hercules, CA, USA). Band scoring was performed by including only the strongest bands as alleles. The SSR bands were scored as present (1) or absent (0) for each primer/genotype combination, and a binary raw matrix was generated for all banding patterns.

Data analysis

The amplification data generated using the transferable markers were analysed using SIMQUAL route to generate Jaccard's similarity coefficient (Jaccard, 1908) using NTSYS-PC software version 2.1 (Rohlf 1998). These similarity coefficients were used to construct a dendrogram depicting the genetic relationships among the cultivars by employing the UPGMA algorithm and Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering. The Polymorphism Information Content (PIC) (Anderson et al., 1993) was calculated for each marker using the following equation:

$$\text{Polymorphism information content } (PIC_i) = 1 - \sum_{j=1}^{n} P_{ij}^2$$

, where P_{ij} is the frequency of the j^{th} allele for the i^{th} marker, summed over 'n' alleles.

Conclusions

This study demonstrates the utility of *Vigna* SSRs for elucidating the genetic relationships among different cultivars of pigeonpea based on origin, maturity group and disease response. The *Vigna* markers produced amplification products and were effective enough to separate wild species of *Cajanus* from all cultivars of pigeonpea (cultivated species). This study has once again demonstrated convincingly that microsatellites are transferable across related genera. Therefore, introgression of desirable alleles, diversity and phylogenetic studies and molecular breeding of lesser-known crops can continue to be performed using microsatellites developed for the more highly studied relatives of these crops, which is applicable not to only legumes but to all crops. This study supports the inclusion of *Vigna* and *Cajanus* into the tribe Phaseoleae on a phylogenetic basis. The use of *Vigna* markers, which are readily available, has increased the number of SSRs available for pigeonpea studies.

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