Indian Journal of Agricultural Sciences **82** (12): 1016–21, December 2012

Phylogenetic analysis of pigeonpea (*Cajanus cajan*) genotypes using *Panzee* retrotransposon based SSAP markers

PRAKASH PATIL[1], S DATTA[2], I P SINGH[3], ALOK DAS[4], K R SOREN[5], M KAASHYAP[6], A K CHOUDHARY[7], S K CHATURVEDI[8] and N NADARAJAN[9]

Indian Institute of Pulses Research, Kanpur, Uttar Pradesh 208 024

Received: 26 July 2011; Revised accepted: 5 July 2012

ABSTRACT

In the present study, twenty one pigeonpea [*Cajanus cajan* (L.) Millspaugh] genotypes representing three different gene pools were analysed for phylogenetic relationships using sequence specific amplification polymorphism markers. Four primers were screened involving selective AFLP primers in combination with primer specific to 5'LTR region of *Panzee* retrotransposon. A total of 183 bands were scored, out of which 166 (90.71%) bands were polymorphic with an average of 41.5 polymorphic bands per primer combination. Polymorphism information content (PIC) values ranged from 0.15 [*Panzee*-LTR/ *Eco*RI (+AAC)] to 0.26 [*Panzee*-LTR/ *Eco*RI (+AAG)] for four primer combinations. Resolving power (RP) values ranged from 10.14 [*Panzee*-LTR/ *Eco*RI (+AAC)] to 24.92 [*Panzee*-LTR/ *Mse*I (+CAC)], whereas marker index (MI) values ranged from 5.25 [*Panzee*-LTR/ *Eco*RI (+AAC)] to 14.25 [*Panzee*-LTR/ *Mse*I (+CAC)] exhibiting positive correlation (r^2=0.99 P<0.05). Primer *Panzee*-LTR/ *Mse*I (+CAC) with higher values of RP (24.92) and MI (14.25) was found most informative for analysing the phylogenetic relationships. NJ tree based on 166 polymorphic SSAP markers exhibited concordant relationships among pigeonpea genotypes supported by high bootstrap values. The genotypes belonging to tertiary gene pools and primary gene pool were placed at extremes, whereas one accession of *C. cajanifolius* (ICP 15632) was placed closer to primary gene pool, confirming *C. cajanifolius* is the closest progenitor of cultivated pigeonpea. SSAP markers produced high rate of polymorphism and revealed phylogenetic relationships among pigeonpea genotypes.

Key words: Phylogentic studies, Pigeonpea, SSAP markers

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is one of the important grain legume crops of tropical and subtropical regions of world. Being a second most important crop next to chickpea in India, it occupies an area of about 3.5 million ha with production of 3.1 million tonnes. Based on the vast natural genetic variability available in local germplasm and presence of numerous wild relatives, India is considered as the primary centre of origin (Van der Maesen 1980). Wild relatives of pigeonpea are considered to be rich reservoir of genes for resistance to biotic and abiotic stresses (Sharma *et al.* 2003). Introgression of these genes may be one of option in improving the yield, increasing production levels by imparting resistance to stresses of cultivated pigeonpea (Singh 2005). An understanding of phylogenetic relationships among pigeonpea genotypes is a prerequisite step for exploiting and molecular markers are known to play a very crucial role in thorough understanding of molecular diversity.

In many plant species various marker systems were used for diversity analysis, viz. RAPD in gladiolus (Pragya *et al.* 2010), STMS in chickpea (Bharadwaj *et al.* 2010), SSAP, AFLP and SSR in durum wheat (Mardi *et al.* 2011) and ISSR in fenugreek (Gurinder *et al.* 2012). Similarly, in pigeonpea various marker techniques have been used for diversity study among wild and cultivated species of pigeonpea. These studies revealed very low level of polymorphism among pigeonpea cultivars compared to wild species (Punguluri *et al.* 2006 and Yang *et al.* 2006). For phylogenetic studies, AFLP markers have proved most useful in many crops (Tatikonda *et al.* 2009 and Ganapathy *et al.* 2011). Recently Ty1-*copia* retrotransposon based marker system, e.g. sequence specific amplification polymorphism (SSAP) have been found extremely informative for phylogenetic studies in comparison to AFLP (Tam *et al.* 2005 and Bousios *et al.* 2007).

Retrotransposons, widely distributed in the plant genomes are genetic elements comprising two broad classes: Class I

[1]Scientist (e mail: patilbt@gmail.com), [2]Senior Scientist (e mail: subhojit@email.com), [3]Principal Scientist (e mail: ipsingh1963@yahoo.com), [4]Scientist (e mail: adas@icar.org.in), [5]Scientist (e mail: sorenars@gmail.com), [6]Senior Research Fellow (e mail: mayankk2006@gmail.com), [7]Senior Scientist (e mail:arbindchoudhary@rediffmail.com), [8]Principal Scientist (e mail: sushilk.chaturvedi@gmail.com), [9]Director (e mail: n.nadarajan@gmail.com)

elements that transpose as a 'copy and paste' mechanism and, class II elements that transpose 'cut and paste' mechanism. Further, the first category includes two types of elements mainly LTR-retrotransposons and non-LTR-retrotransposons. The LTR-retrotransposon bear LTRs (long terminal repeats) at their terminal ends. Based on the order of internal domains (*gag* and *pol* ORFs encoding protease, endonulcease, reverse transcriptase and RNase H) LTR-retrotransposons are further divided into Ty1-*copia* and Ty3-*gypsy* groups. In *copia* group (e.g. *Panzee*) retrotransposons endonulcease domain positioned at 5' to the reverse transcriptase domain, whereas for *gypsy* group it was placed at 3' to the reverse transcriptase domain. LTR-retrotransposons are responsible for the vast differences in genomes size and genome arrangements in various plant species (Bennetzen 2000). The retrotransposon insertions are irreversible, high in copy numbers, well distributed throughout the genome and changes remain relatively fixed making them suitable candidates for analyzing phylogenetic relationships and recently as molecular tools in crop improvement programmes.

SSAP technique employs a primer which is specific to the long terminal repeat (LTR) region of particular retrotransposon in combination with selective AFLP primers during second round of selective amplification. It generates PCR bands between each insertion of a particular retrotransposon and a frequently occurring neighbouring restriction site (Bousios *et al.* 2007). This technique has been used successfully for phylogenetic and diversity studies and construction of linkage maps in many crops, viz. oat (Yu and Wise 2000), sweet potato (Berenyi *et al.* 2002), wheat (Queen *et al.* 2004), lettuce (Syed *et al.* 2006), cucurbits (Lou and Chen 2007), faba bean (Ali *et al.* 2012) and pea (Hamid *et al.*2012). In pigeonpea, understanding the phylogenetic relationship between species will be useful in exploiting the genetic resources for discovering novel traits for crop improvement. With this background, the present study was aimed at (i) development of SSAP markers in pigeonpea by utilizing sequence information of high copy number *Panzee* retrotransposon of pigeonpea, and (ii) understanding the phylogenetic relationship among *Cajanus* species by analyzing polymorphic, unique and shared banding patterns of SSAP markers. This study will provide an insight to understand phylogenetic relationships through the distribution of this particular retrotransposon at *Cajanus* genome.

MATERIALS AND METHODS

In the present study, 21 pigeonpea genotypes representing six species of genus *Cajanus,* viz. *C. cajan, C. scarabaeiodes, C. platycarpus, C. albicans, C. sericeus* and *C. cajanifolius* were raised during 2009–11. All the species are being maintained in the Crop Improvement Division, Indian Institute of Pulses Research, Kanpur (Table 1). Genomic DNA was extracted from the young leaves of 15 days old seedlings, using cetyl-trimethyl ammonium bromide (CTAB) method.

Table 1 Details of pigeonpea genotypes used for phylogenetic analysis

Pigeonpea	Species	Pedigree/origin	Maturity group	Plant type
		Wild accessions		
ICP 15632	*Cajanus cajanifolius*	Northern territory (Australia)	M	NDT
ICP 15637	*C. scarabaeoides*	Northern territory (Australia)	M	NDT
ICP 15748	*C. scarabaeoides*	Northern territory (Australia)	M	NDT
ICP 15760	*C. sericeus*	Western Ghats (India)	L	NDT
ICP 15761	*C. sericeus*	Western Ghats (India)	L	NDT
ICP 15921	*C. platycarpus*	Central (India)	E	NDT
ICP 15666	*C. platycarpus*	Central (India)	E	NDT
ICP 15622	*C. albicans*	Sri Lanka	L	NDT
ICP 15624	*C. albicans*	Sri Lanka	L	NDT
		Cultivars		
BSMR 853	*Cajanus cajan*	(ICPL7336 × BDN 1) × BDN 2	M	NDT
BDN 2	*C. cajan*	Local selection from Bori germplasm	M	NDT
ICPL 87119	*C. cajan*	C 11 × ICPL 6	M	NDT
Pusa 9	*C. cajan*	UPAS 120 × 3673	L	NDT
DA 11	*C. cajan*	Bahar × NP(WR) 15	L	NDT
NDA 1	*C. cajan*	Selection from landrace of Faizabad (UP), India	L	NDT
MA 6	*C. cajan*	MA2 × Bahar	L	NDT
Dholi dwarf	*C. cajan*	Selection from landrace of Darbhanga (Bihar), India	L	SDT
GT67B	*C. cajan*	India	E	SDT
Bahar	*C. cajan*	Selection from landrace of Motihari (Bihar), India	L	SDT
ICP 8863	*C. cajan*	Selection from landrace of Mharashtra, India	M	NDT
Type 7	*C. cajan*	Selection from landrace of Lucknow (UP), India	L	NDT

*E, Early duration; M, medium duration; L, late duration; SDT, semi determinate plant type; NDT, non-determinate plant type

DNA samples were quantified on 0.8% agarose gel electrophoresis by comparing them with known dilutions of lambda DNA and finally diluted to 0.1µg/µl for SSAP analysis. The sequence information of 5' end long terminal repeat (LTR) region of *Ty1/copia* like retrotransposon *Panzee* (Lall *et al.* 2002) was chosen to design retrotransposon specific primers. SSAP amplification was carried out, as described by Vos *et al.* (1995) with some minor modifications. Genomic DNA (0.5 µg) was double digested with *Eco*RI (10U) and *Mse*I (4U) enzymes at 37 °C for 3 hr. Restricted DNA was ligated with double stranded adapter [*Eco*RI (5 pmol) and *Mse*I (10 pmol)] in ligation buffer [(T_4 DNA ligase buffer (10X), T_4 DNA ligase enzyme (4U)] at 37 °C for 16 hr. These restricted and ligated DNA templates were further diluted (1:7) and used for pre-amplification (primers complimentary to the *Eco*RI and *Mse*I adapters, with one selective nucleotide adenine and cytosine respectively). PCR programmes followed for pre-amplifications were, 1 cycle at 94 °C for 3 min, 20 cycles at 94 °C for 30 sec, 56 °C for 1 min, and 72 °C for 1 min followed by 1 cycle of 72 °C for 10 min. Pre-amplification products were further diluted (1:8) and used for selective amplification (selective *Eco*RI or *Mse*I adapters specific primers, with three selective nucleotides were used in combination with one retrotransposon specific primer) (Table 2). PCR conditions followed for selective amplification were, 1 cycle at 94 °C for 3 min, 11 cycles at 94 °C for 30 sec, 65 °C for 30 sec reducing by 1 °C /cycle to 56 °C, 72 °C for 1 min and followed by 24 cycle at 94 °C for 30 sec, 56 °C for 30 sec, 72 °C for 1 min followed by 1 cycle of 72 °C for 10 min. Amplification products were separated on 6% denaturing polyacrylamide gels and bands were resolved by silver staining.

SSAP bands were scored manually for presence (1) or absence (0), to obtain a binary matrix. The statistical analysis was carried out using DARwin programme version 5.0 (Perrier *et al.* 2003). Pair wise comparison was done to obtain genetic dissimilarity matrix by reciprocating similarity values obtained from Jaccard's similarity coefficient. Dissimilarity matrices were finally subjected to UPGMA (unweighted pair-group method with arithmetic averages) based Neighbour-joining tree construction with 1 000 times bootstrap resampling. The basic marker feature for determining the in formativeness of given primer combinations, i e polymorphism information content (PIC) and marker index (MI) were calculated. PIC was calculated using POWERMARKER programme version 3.0 (Liu and Muse 2005) using formula, $PIC = 1 - [\sum_{i=1}^{n} p_i^2] - [\sum_{i=1}^{n=1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2]$ where p_i is the frequency of '1'th allele and p_j is the frequency of (i+1)th allele. Marker index was calculated as given by Tatikonda *et al.* (2009) using formula, MI = PIC × EMR, where EMR (effective multiplex ratio) - is the product of total number of bands per primer (*n*) and the fraction of polymorphic bands (β) (EMR = n β). Apart from these, discriminatory power of a given primer combination, i e resolving power (RP) was calculated using formula, $RP = \sum I_b$, where, I_b, is the band in formativeness $Ib = 1 - [2 \times |0.5 - p|]$, where p is the proportion of the 21 pigeonpea genotypes containing the bands.

RESULTS AND DISCUSSION

Panzee is Ty1-*copia* like retrotransposon present in very high copy numbers in pigeonpea genome (Lall *et al.* 2002) and might have played active role in evolution of pigeonpea genome. The present study was carried out in order to develop and utilize the SSAP markers related to the *Panzee* retrotransposon in pigeonpea. Four primer combinations were employed to generate SSAP markers (Fig 1). A total of 183 scorable bands were obtained, out of which 166 (90.71%) were polymorphic (Table 3). LTR specific primer in combination with *Mse*I selective primers produced higher number of bands, compared to that of *Eco*RI selective primers. The number of bands obtained by different primer combinations ranged from 25 [*Panzee*-LTR/ *Eco*RI (+AAG)] to 65 [*Panzee*-LTR/ *Mse*I (+CAC)] with an average of 45.75 per primer combination. *Panzee*-LTR/ *Mse*I (+CAC) showed highest polymorphism (95.38 %), followed by *Panzee*-LTR/ *Mse*I (+CAG) (92.00%) and *Panzee*-LTR/*EcoRI* (+AAG) (92.00%). In contrast to this, least polymorphism was

Table 2 SSAP adapter and primer sequences

Type	Primer/adapter name	Sequence
SSAP retrotransposon primer	*Pnzee*-LTR	5'- AAG TTT TTC CTT TCG GCC AA - 3'
SSAP adapters	Double stranded	5'- GAC GAT GAG TCC TGA G- 3'
	*Mse*I adapters	5'- TAC TCA GGA CTC AT - 3'
	Double stranded	5'- CTC GTA GAC TGC GTA C - 3'
	*Eco*RI adapters	5'- AAT TGT ACG CAG TC - 3'
SSAP adapters	M+C (*Mse*I adapters specific primers)	5'- GAT GAG TCC TGA GTA AC - 3'
Primers	E+A (*Eco*RI adapters specific primers)	5'- GAC TGC GTA CAA TTC A - 3'
	*Mse*I(+ CAG)	5'- GAT GAG TCC TGA GTA ACA G - 3'
	*Mse*I(+ CAC)	5'- GAT GAG TCC TGA GTA ACA C - 3'
	*Eco*RI (+AAG)	5'- GAC TGC GTA CAA TTC AAG - 3'
	EcoRI(+AAC)	5'- GAC TGC GTA CAA TTC AAC - 3'

Table 3 Degree of polymorphism and infromativeness as revealed by SSAP primer combinations

Primer combinations	TNB	NPB	%P	NUB	PIC	MI	RP
Panzee-LTR/*Mse*I (+CAG)	50	46	92	3	0.2	9.2	17.24
Panzee-LTR/*Mse*I (+CAC)	65	62	95.38	4	0.23	14.25	24.92
Panzee-LTR/*EcoRI* (+AAG)	25	23	92	3	0.26	5.98	13.18
Panzee-LTR/*EcoRI* (+AAC)	43	35	81.39	10	0.15	5.25	10.14
Total	183	166		20			
Minimum	25	23	81.39	3	0.15	5.25	10.14
Maximum	65	62	95.38	10	0.26	14.25	24.92
Average	45.75	41.5	90.19	5	0.21	8.67	16.37

*TNB, Total number of bands produced; NPB, no. of polymorphic bands: %P, per cent polymorphism; NUF, no. of unique bands; PIC, polymorphism information content; MI, marker index; RP, resolving power

observed for primer combination *Panzee*-LTR/*Eco*RI (+AAC) (81.39%). The rate of polymorphism for SSAP markers was higher as compared to AFLP markers as reported by Punguluri *et al.* (2006) using AFLP in pigeonpea. These results are similar to the earlier findings on the basis of comparative study of SSAP markers in relation to AFLP marker system (Lou and Chen 2007).

The informativeness and discriminatory power of four primer combinations were also estimated (Table 3). All the polymorphic bands generated by a primer combination were considered to get an average PIC values for the corresponding primer combinations and PIC values ranged from 0.15 [*Panzee*-LTR/ *Eco*RI (+AAC)] to 0.26 [*Panzee*-LTR/ *Eco*RI (+AAG)]. Similarly, MI values ranged from 5.25 [*Panzee*-LTR/ *Eco*RI (+AAC)] to 14.25 [*Panzee*-LTR/ *Mse*I (+CAC)] and RP values ranged from 10.14 [*Panzee*-LTR/ *Eco*RI (+AAC)] to 24.92 [*Panzee*-LTR/ *Mse*I (+CAC)]. Correlation between PIC, MI and RP values revealed stronger positive correlation between MI and RP features (r^2=0.99, P<0.05), as compared to PIC and Rp (r^2=0.41, P>0.05). A similar observation was reported for AFLP markers in jatropha (Tatikonda *et al.* 2009). In our study, *Panzee*-LTR/*Mse*I (+CAC) is having high values for PIC (0.23), MI (14.25) and RP (24.92). Based on correlation values, this primer may be considered as the best primer combination for analyzing the phylogenetic relationships among pigeonpea genotypes. RP or MI could be considered as real marker attributes in choosing the best SSAP primer combination.

Unique bands were surveyed for SSAP markers across 21 *Cajanus* genotypes. Out of 166 polymorphic bands, 20 bands were found to be unique insertions in the pigeonpea genome. *Panzee*-LTR/*EcoRI* (+AAC) primer has detected maximum number of unique bands (10), in comparison to other primers combinations used. It is interesting to note that all the 20 unique bands were found only in wild accession of pigeonpea. The maximum numbers of unique bands were specific to *C. sericeus* (ICP 15760) (7), followed by 3 unique bands each in accession of *C. cajanifolius* (ICP 15632), *C. scarabaeiodes* (ICP 15748) and *C. sericeus* (ICP 15761). Whereas, one unique band was specific to each of the three wild species, viz. *C. scarabaeiodes* (ICP 15637), *C. platycarpus* (ICP 15921 and ICP 15666) and *C. albicans* (ICP 15624). In contrast to this, all the culivars of pigeonpea were having many shared SSAP bands. These results clearly demonstrated that *Panzee* retrotransposon might have played a significant role during evolution of wild pigeonpea species to the present day cultivars.

In this study, 21 pigeonpea genotypes represented three gene pools, viz. primary gene pool (*C. cajan*), secondary gene pool (*C. scarabaeiodes, C. albicans*, *C. sericeus* and *C. cajanifolius*) and tertiary gene pool (*C. platycarpus*). The NJ tree obtained for 166 polymorphic SSAP markers depicted, well resolved phylogenetic relationships among pigeonpea genotypes with high bootstrap values (Fig 2). Genotypes belonging to primary and tertiary gene pools were placed at the extreme ends of the tree. The genotypes representing primary gene pool (Pusa 9, DA 11, BDN 2, ICPL 87119, NDA 1, BSMR 853, Bahar, Dholi Dwarf, Type 7, ICP 8863, MA 6 and GT67B) were grouped together with higher bootstrap support value (99%). Similarly, accessions representing tertiary gene pool (ICP 15921 and ICP 15666) were grouped together with 100% bootstrap support value. In this cluster one accession belonging to secondary pool *C. sericeus* (ICP 15761) was found, which is also evident from the earlier studies using SSR markers in pigeonpea (Datta *et al*. 2010 and Saxena *et al.* 2010). The genotypes belonging to secondary gene pool were well distributed in the middle of tree, that is *C. albicans* (ICP 15622 and ICP 15624), *C. scarabaeoides* (ICP 15637 and ICP 15748) grouped separately with 100% bootstrap value, whereas one accession each of *C. sericeus* (ICP 15760) and *C. cajanifolius* (ICP 15632) was placed with 75% and 83% bootstrap values, respectively. It was interesting to note that one of the accession of *C. cajanifolius* (ICP 15632) belonging to secondary gene pool was placed closer to genotypes of primary gene pool. This result confirmed the earlier findings that *C. cajanifolius* is the closest wild relative and possible progenitor of cultivated pigeonpea (Van der Maesen 1990).

NJ-tree depicted pigeonpea genotypes with varying branch lengths. The more branch lengths were observed for

all the wild relatives, whereas all cultivars depicted less branch length. Branch length is directly proportional to the number of SSAP bands unique to a particular genotype. One of the wild accession of *C. sericeus* (ICP 15760) showed maximum number of unique bands (7) and was having more branch length followed by *C. cajanifolius* (ICP 15632), *C. scarabaeiodes* (ICP 15748) and *C. sericeus* (ICP 15761) as reflected from the NJ-tree. SSAP analysis is not only useful in differentiating genotypes with branch lengths, but also attaches bootstrap confidence values to the branching patterns (Bousios *et al.* 2007). In this study, more branch length with confidence bootstrap values (ranging from 42% to 100%) was observed. These results indicated the reliability of phylogenetic tree obtained with SSAP markers in pigeonpea. Similar kinds of results were reported by Kalender *et al.* (2000) in wild barley (*Hordeum spontaneum*) using SSAP markers.

In the present study, 12 pigeonpea cultivars with two different plant types were used. Three genotypes (Bahar, Dholi Dwarf and GT67B) possessed semi-determinate and 9 genotypes (Pusa 9, DA 11, BDN 2, ICPL 87119, NDA 1, BSMR 853, Type 7, ICP 8863 and MA 6) non-determinate plant type. In NJ tree, all 12 pigeonpea cultivars were clustered as a one major cluster having two main clusters (Cluster I and Cluster II). Cluster I comprised 7 genotypes, out of which 5 genotypes (BSMR 853, NDA 1, ICPL 87119, BDN 2 and DA 11) representing non-determinate plant type and 2 genotypes (Bahar and Dholi Dwarf) representing semi-determinate plant type were grouped separately. Similarly, cluster II with 4 genotypes, of which 3 genotypes (Type 7, ICP 8863 and MA 6) representing non-determinate plant type and one genotype (GT67B) representing semi-determinate plant type were grouped separately. One of the genotype (Pusa 9) representing non-determinate plant type is placed separately as a single node (with more branch length). Apart from this, four genotypes (BSMR 853, BDN 2, DA 11 and Bahar) were placed in cluster I with exception of MA 6 in relation to pedigree of origin. Results indicated that clustering of these had no geographical basis, while grouping of genotypes within the clusters was based on plant types used.

Based on the above results, it can be concluded that SSAP markers based on *Panzee* retrotransposon produce high quality polymorphic profile among pigeonpea genotypes used. These markers revealed highly reliable phylogenetic relationships among pigeonpea genotypes with more branch length and bootstrap supports and will have greater potential in increasing the number of markers for linkage analysis in pigeonpea. Further, there is need to identify such potential high copy number Ty1-*copia* like retrotransposons in order to develop robust SSAP marker system in pigeonpea.

REFERENCES

Ali O, Safia E B, Naeem H S, Raoudha A, Mustapha R, Andrew J F and Mohamed E G. 2012. Genetic diversity of faba bean (*Vicia faba* L.) populations revealed by sequence specific amplified polymorphism (SSAP) markers. *African Journal of Biotechnology* **11**: 2162–8.

Bharadwaj C, Chauhan S K, Rajguru G, Srivastava R, Satyavathi C T, Yadav S, Rizvi A H, Kumar J and Solanki R K. 2010. Diversity analysis of chickpea (*Cicer arietinum*) cultivars using STMS markers. *Indian Journal of Agricultural Sciences* **80**: 947–51.

Bennetzen J L. 2000. Transposable element contributions to plant gene and genome evolution. *Plant Molecular Biology* **42**: 251–69.

Berenyi M, Gichuki T, Schmidt J and Burg K. 2002. Ty1-copia retrotransposon-based S-SAP (sequence-specific amplified polymorphism) for genetic analysis of sweet potato. *Theoretical and Applied Genetics* **105**: 862–69.

Bousios A, Oyarzabal I S, Ana G. Zapata V, Wood C and Pearce S R. 2007. Isolation and characterization of Ty1-*copia* retrotransposon sequences in the blue agave (*Agave tequilana* Weber var. azul) and their development as SSAP markers for phylogenetic analysis. *Plant Science* **172**: 291–98.

Datta S, Kaashyap M and Kumar S. 2010. Amplification of chickpea-specific SSR primers in *Cajanus* species and their validity in diversity analysis. *Plant Breeding* **129**: 334–40.

Ganapathy K N, Gnanesh B N, Byre Gowda M, Venkatesha S C, Gomashe S S and Channamallikarjuna V. 2011. AFLP analysis in pigeonpea (*Cajanus cajan* (L.) Millsp.) revealed close relationship of cultivated genotypes with some of its wild relatives. *Genetic Resources and Crop Evolution* **58**: 837–47.

Gurinder J R, Monika S, Gangopadhyay K K, Gunjeet K and Sunil A. 2012.Genetic analysis of fenugreek (*Trigonella foenum-graecum*) accessions using morphometric and ISSR markers. *Indian Journal of Agricultural Sciences* **82**: 393–01.

Hamid M, Waseem S, Barkat A, Ashiq M, Ijaz A and Abdul S M.2012. Genetic assessment of the genus *Pisum* L. based on sequence specific amplification polymorphism data. *Journal of Medicinal Plants Research* **6**: 959–67.

Kalendar R, Tanskanen J, Immonen S, Nevo E and Schulman A H. 2000. Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. *Proceedings of the National Academy of Sciences*, USA **97**: 6 603–7.

Lall I, Maneesha and Upadhyaya K C. 2002. *Panzee*, a copia-like retrotransposon from the grain legume, pigeonpea (*Cajanus cajan* L.). *Molecular Genetics and Genomics* **267**: 271–80.

Liu K and Muse S V. 2005. PowerMarker: integrated analysis environment for genetic marker data. *Bioinformatics* **21**: 2 128–9.

Lou Q and Chen J. 2007. Ty1-*copia* retrotransposon based SSAP marker development and its potential in the genetic study of cucurbits. *Genome* **50**: 802–10.

Mardi M, Naghavi M R, Pirseyedi S M, Alamooti M K, Monfared S R, Ahkami A H, Omidbakhsh M A, Alavi N S, Shanjani P S and Katsiotis A. 2011. Comparative Assessment of SSAP, AFLP and SSR Markers for Evaluation of Genetic Diversity of Durum Wheat (*Triticum turgidum* L. *var. durum*). *Journal of Agricultural Science and Technology* **13**: 905–20.

Pragya, Bhat K V, Misra R L and Ranjan J K. 2010. Analysis of diversity and relationships among *Gladiolus* cultivars using

morphological and RAPD markers. *Indian Journal of Agricultural Sciences* **80**: 766–72.

Perrier X, Flori A and Bonnot F. 2003. Data analysis methods. (*in*) *Genetic Diversity of Cultivated Tropical Plants*, pp 43–76. Hamon P, Seguin M, Perrier X and Glaszmann J C. (Eds) Enfield, Science Publishers, Montpellier.

Punguluri S K, Janaiah K, Govil J N, Kumar P A and Sharma P C. 2006. AFLP fingerprinting in pigeonpea (*Cajanus cajan* (L.) Millsp) and its wild relatives. *Genetic Resources and Crop Evolution* **53**: 523–31.

Queen R A, Gribbon B M, James C, Jack P and Flavell A J. 2004. Retrotransposon-based molecular markers for linkage and genetic diversity analysis in wheat. *Molecular Genetics and Genomics* **271**: 91–7.

Saxena R K, Prathima C, Saxena K B, Hoisington D A, Singh N K and Varshney R K. 2010. Novel SSR markers for polymorphism detection in pigeonpea (*Cajanus* spp.). *Plant Breeding* **129**: 142–8.

Sharma H C, Pampathy G and Reddy L J. 2003. Wild relatives of pigeonpea as a source of resistance to the pod fly (*Melanogromyza obtuse* Malloch) and pod wasp (*Tanaostigmodes cajaninae* La Salle). *Genetic Resources and Crop Evolution* **50**: 817–24.

Singh N. 2005. Management of pigeonpea genetic resources. (*in*) *Advances in Pigeonpea Research*, pp 23–38. Ali M and Kumar S. (Eds). Indian Institute of Pulses Research, Kanpur.

Syed N H, Sorensen A P, Antonise R, Wiel C, Linden C G, Vant Westende W, Hooftman D A, Den Nijs H C and Flavell A J. 2006. A detailed linkage map of lettuce based on SSAP, AFLP and NBS markers. *Theoretical and Applied Genetics* **112**: 517–27.

Tam S M, Mhiri C, Vogelaar A, Kerkveld M, Pearce S R and Grandbastien M A. 2005. Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon based SSAP, AFLP and SSR. *Theoretical and Applied Genetics* **110**: 819–31.

Tatikonda L, Suhas P W, Kannan S, Beerelli N, Sreedevi T K, Hoisington D A, Devi P and Varshney R K. 2009. AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., a biofuel plant. *Plant Science* **176**: 505–13.

Van der Maesen L J G. 1980. India is the native home of the pigeon pea. (*in*) *Libergratulatorius in honerem HCD De Wit*, pp 257–62. Arends J C, Boelema G, De Groot C T and Leeuwenberg A J M (Eds). Wageningen Agricultural University, The Netherlands.

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407–14.

Yang S, Pang W, Ash G, Harper J, Carling J, Wenzl P, Huttner E, Zong X and Kilian A. 2006. Low level of genetic diversity in cultivated pigeonpea compared to its wild relatives is revealed by diversity arrays technology. *Theoretical and Applied Genetics* **113**: 585–95.

Yu G X and Wise R P. 2000. An anchored AFLP and retrotransposon based map of diploid *Avena*. *Genome* **43**: 736–49.