Progressive Research 9 (Special) : 263-369 (2014) *Society for Sci. Dev. in Agric. and Tech.*

ASSESSMENT OF GENETIC DIVERSITY IN CAJANUS CAJAN L. CULTIVARS BY RAPD AND ISSR MARKERS

Shailesh Yadav[1] and Ravi Ranjan Kumar[2] and Varsha Shitole[2]

[1]*Institute of Biotechnology, Acharya N.G. Ranga Agricultural University, Hyderabad-500030, India*

[2]*Plant Biotechnology Laboratory, Vidya Prathisthan's College of Agricultural Biotechnology, Baramati-413133, India*

E-mail: ravi1709@gmail.com

ABSTRACT

Pigeonpea is an important grain legume crop ofrain-fed agriculture in the semi-arid tropics. The Indian subcontinent, eastern Africa and Central America are the world's three main pigeonpea producingregions. To evaluate the robustness of marker systems in detection of DNA polymorphism, it was aimed to studying genomic diversity using RAPD and ISSR markers in ten pigeonpea varieties which were differing in their seed color. For the study, 30 RAPD and 12 ISSR primers was screened in order to obtain the polymorphism among the cultivars. Among the 30 RAPD primers, 18 primers showed considerable polymorphism. These primers produced 86 loci with an average of band 3.59 per primer and percentage of polymorphism was 30%. The genetic diversity among the cultivars were in the range of 0.02 to 0.4 which showed a good polymorphism among pigeonpea. The ISSR primers showed better polymorphism than of RAPD primer. The 12 ISSR primers were able to generate polymorphic bands with an average of 5 bands per primer and 42.36% polymorphism. The range of diversity among the cultivars were in the range of 0.1210 to 0.4182.

Key words : *Genetic diversity, ISSR, RAPD, Polymorphism*

Pigeonpea is widely grown in the semi-arid tropics, particularly in the Indian subcontinent where it accounts for over 70% of the world's production and coverage (FAO, 2008). Despite past research efforts, productivity of this crop remained low at around 700–800 kg/ha (1) over the last five decades. This is mainly due to limited exploitation of available natural variability of genus Cajanus in breeding lines (2).

Determination of genetic diversity of any given crop species is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a breeding scheme. The early systematic studies of the genus Cajanus were based on phonological or morphological characters, which have been shown to have limited genetic resolution especially at species levels, as is required for Pigeonpea (3).

The use of DNA marker technology in plant breeding programmes can greatly facilitate the manipulation of gene movement along lines and analysis of polygenic character with a precision previously not possible (4). RAPD and ISSR technique have great potential in findings DNA marker for breeding programme. The integration of RAPD and ISSR technique into plant breeding programme promises to expedite the movement of desirable genes in crop plants through marker assisted selection.

The promotion of pigeonpea as a high value cash crop and the introduction and adoption of improved varieties is slowly reducing the genetic diversity of pigeonpea landraces (5). Limited pools of pigeonpea germplasm have been characterized previously by protein and isozyme electrophoresis (6), RFLP (Nadimpalli et al., 1992), RAPD (3), microsatellites (7) and simple tandem repeats (8). Although genomics research in Pigeonpea has gained momentum recently (9), the limited availability of genomics tools in the past has impeded progress in this important crop.

This study has the purpose of investigating possible advantages of the use of RAPD and ISSR, for identification and estimation of phylogenetic similarities among a set of ten commercial cultivars of pigeonpea.

MATERIALS AND METHODS

Tencultivated pigeonpea cultivars were selected for the analysis of genetic diversity using RAPD and ISSR markers. Among 10 pigeonpea cultivars; six red varieties (ICP-8863, ICPL-87119, TAT-9903, BSMR-736, G-RED and GC-11-39), three white

Table-1 : List of RAPD and ISSR primers and their sequences used for the amplification of genomic DNA of pigeonpea cultivars.

RAPD PRIMER LIST			
Code	**Sequence (5'-3')**		
OPA 06	GGT CCC TGA C	OPE 01	CCC AAG GTG C
OPA 08	GTG ACG TAG G	OPE 02	GGT GCG GGA A
OPA 09	GGG TAA CGC C	OPE 04	GTG ACA TGC C
OPA 12	TCG GCG ATA G	OPE 11	GAG TCT CAG G
OPA 13	CAG CAC CCA C	OPE 14	TGC GGC TGA G
OPA 14	TCT GTG CTG G	OPE 16	GGT GAC TGT G
OPA 15	TTC CGA ACC C	OPE17	CTA CTG CCG T
OPA17	GAC CGC TTG T	OPE 18	GGA CTG CAG A
OPC 01	TTC GAG CCA G	OPE 19	ACG GCG TAT G
OPC 05	GAT GAC CGC C	OPE 20	AAC GGT GAC C
OPC 06	GAA CGG ACT C	OPH02	TCG GAC GTG A
OPC 07	GTC CCG ACG A	OPH 03	AGA CGT CCA C
OPC 09	CTC ACC GTC C	OPH 04	GGA AGT CGC C
OPC 17	TTC CCC CCA G	OPH 09	TGT AGC TGG G
OPC 18	TGA GTG GGT G	OPH 13	GAC GCC ACA C
ISSR PRIMER LIST			
Code no.	**ISSR primer sequence (5'-3')**		
UBC 807	AGA GAG AGA GAG AGA GT	UBC 840	GAG AGA GAG AGA GAG AYT
UBC 808	AGA GAG AGA GAG AGA GC	UBC 843	CTC TCT CTC TCT CTC TRA
UBC 816	CAC ACA CAC ACA CAC AT	UBC 844	CTC TCT CTC TCT CTC TRC
UBC 827	ACA CAC ACA CAC ACA CG	UBC 857	ACA CAC ACA CAC ACA CYG
UBC 832	ACA CAC ACA CAC ACA CYC	UBC 864	ATG ATG ATG ATA GAT GAT G
UBC 839	CAC ACA CAC ACA CAC ARG	UBC 873	GAC AGA CAG ACA GAC A

varieties (GRG-295, WRP-1 and TS-3) and one black variety (PG-12) were selected for present investigation.

Extraction and quantification of genomic DNA

Fresh and young leaf samples of equal quantity (~1 g) of all the cultivars were collected for isolation of genomic DNA. Genomic DNA was isolated and purified by using CTAB method (10) with few modifications. DNA concentration and purity was measured by using UV–Vis spectrophotometer with TE buffer (pH 8.0) as blank. For further confirmation the quantification of DNA was accomplished by analyzing the purified DNA on 0.8% agarose gel along with diluted uncut lambda DNA as ladder. DNA was diluted to concentration of 50ng/µl using TE buffer for the PCR analysis.

PCR amplification and data analysis

PCR was performed in a 20 ìl reaction volumes containing 50 ng of DNA, 45 pmol each forward and reverse primer, 25 ìM of each dNTPs (MBI Fermentas, La Jolla, USA) and 1U of Taqpolymerase (Bangalore Genei). For RAPD, the initial denaturation was done at 94 °C for 5 min followed by 30 cycles, each consisting of denaturation at 94 °C for 1 min, annealing at 37 °C for 45sec and elongation at 72°C for 1 min. A final extension step included 72 °C for 10 min followed by storage at 4°C. RAPD-PCR analysis of isolated DNA was performed by 30 random decamer primers, which were procured from Operon Technologies Inc., Alameda, USA (Table-1).

In case of ISSR, the PCR program consisted of initial denaturation at 94 °C for 5 min followed by 35 cycles, each consisting of denaturation at 94°C for 1 min, annealing at appropriate temperature for 1min standardized for each primer and elongation at 72 °C for 1 min. A final extension step included 72 °C for 10 min followed by storage at 4 °C.The ISSR screening of genomic DNA was performed by 12 microsatellites based UBC primers obtained from University of British Columbia, Vancouver, Canada (Table-1).

The amplified DNA fragments were resolved on ethidium bromide stained agarose gel (2%) in 1X TAE

Table-2 : Similarity matrix values among pigeonpea cultivars based on RAPD analysis

Genotypes	ICPL-87119	ICP-8863	G-RED	TAT-9903	BSMR-736	GC-11-39	PG-12	GRG-295	TS-3	WRP-1
ICPL-87119	0									
ICP-8863	0.0235	0								
G- RED	0.0849	0.0599	0							
TAT-9903	0.0976	0.0976	0.0599	0						
BSMR-736	0.1503	0.1236	0.1369	0.1236	0					
GC-11-39	0.1105	0.0849	0.1503	0.1369	0.1105	0				
PG-12	0.4113	0.3768	0.3939	0.3768	0.3768	0.2955	0			
GRG-295	0.2955	0.2955	0.3112	0.2647	0.2955	0.2202	0.28	0		
TS-3	0.2497	0.2497	0.2647	0.2497	0.3435	0.2348	0.2955	0.0849	0	
WRP-1	0.2955	0.2647	0.3112	0.2955	0.2955	0.2202	0.3435	0.1236	0.1369	0

Table-3 : Similarity matrix values among pigeonpea cultivars based on ISSR analysis

Genotypes	ICPL-87119	ICP-8863	G-RED	TAT-9903	BSMR-736	GC-11-39	PG-12	GRG-295	TS-3	WRP-1
ICPL-87119	0									
ICP-8863	0.2751	0								
G- RED	0.121	0.2263	0							
TAT-9903	0.2106	0.2263	0.1648	0						
BSMR-736	0.2263	0.2751	0.2423	0.1798	0					
GC-11-39	0.309	0.2263	0.2919	0.2263	0.2423	0				
PG-12	0.4182	0.3992	0.4776	0.4776	0.4182	0.3992	0			
GRG-295	0.2919	0.3441	0.309	0.4182	0.3264	0.4182	0.1798	0		
TS-3	0.3621	0.309	0.4574	0.3805	0.2919	0.4574	0.309	0.1648	0	
WRP-1	0.2919	0.3441	0.3441	0.3441	0.3621	0.5408	0.2423	0.1648	0.1648	0

buffer at 50 V. The gels were visualized ontrans-UV illuminator and photographed.

Statistical Analysis

For data analysis of RAPD-PCR and ISSR profile, only distnict visible and reproducible bands were scored manually for presence (1), absence (2) and missing of bands (0) from the photographic profile and genetic distance was calculated by Tool for Population Genetic Analysis (TFPGA) package (11). Repeatability of the banding patterns was checked for each primer on several samples with independent DNA extractions and a repeatability test sample was included in each amplification reaction. Only those RAPD markers that reproduced consistently across successful PCR reactions and across DNA extractions were included in analysis. Differing band intensities were not taken into account to avoid errors introduced by competition among priming sites during the initial rounds of RAPD-PCR and ISSR analysis.

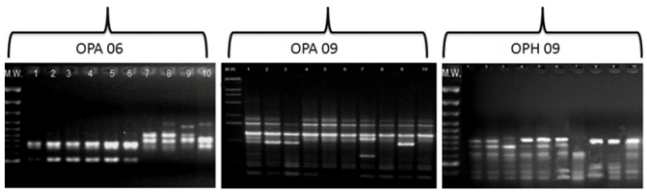

Fig1- Genomic DNA RAPD polymorphism analysis of 10 pigeonpea cultivars with various operon primers M.W.-200bp marker, **Lane 1-** ICPL 87119, **Lane 2-** ICP 8863, **Lane 3-** G-Red, **Lane 4-** TAT 9903, **Lane 5-** BSMR 736, **Lane 6-** GC-11-39, **Lane 7-** PG-12, **Lane 8-** GRG 295, **Lane 9-** TS-3, **Lane 10-** WRP 1

The similarity indexes were computed with the Genetic distance programme (TFPGA package) using the formula (12) to generate the pair wise genetic distance matrix

$$Sa.b = \frac{2Na, b}{Na + Nb}$$

Where, Sa.b is the similarity index between a and

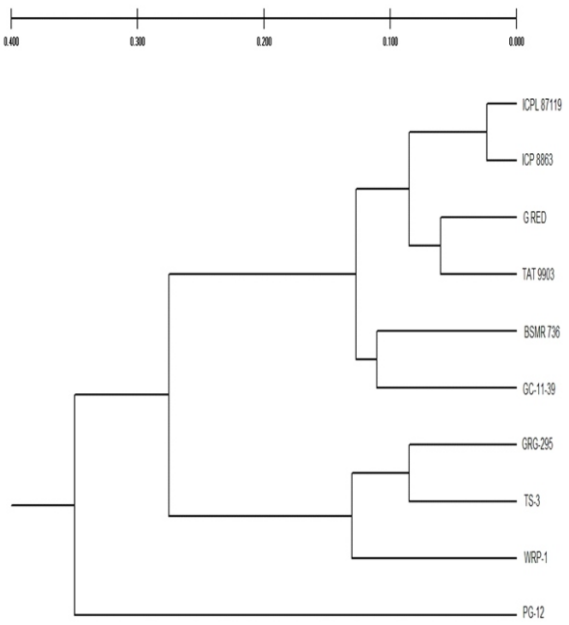

Figure 2 Phylogenetic analysis of RAPD data of genomic DNA in 10 pigeonpea cultivars

b genotypes, Na,b is the total of positive matching or common DNA bands between a and b genotypes and Na and Nb are the total of DNA bands present in each genotype a and b respectively.

The pairwise matrix genetic distances was then employed to draw the precise relationship between the pigeonpea cultivars abd also for the cluster analysis for grouping the pigeonpea cultivars based on the dendrogram produced by Unweighted Pair Group Method with Arthimetic averages (UPGMA) of TFPGA package.

RESULTS AND DISCUSSION

To isolate high quality DNA suitable for RAPD-PCR and ISSR-PCR amplification from the Pigeonpealeaves CTAB protocol of (10) with necessary modificationswere used. The necessary modification was done using by changing the concentration of NaCl (5M) and including 1% activated charcoal in extraction procedure. Instead of precipitation using Isopropanol, as per (10) protocol, absolute ethanol was used. The isolated DNA was subjected to check their purity at A260/A280 which were found 1.91 that indicated the isolated DNA was contaminated with RNA which were further purified by RNase treatment. The DNA yielded from the pigeonpea plants was in the range of 80-100 ìg/g of leaf material.

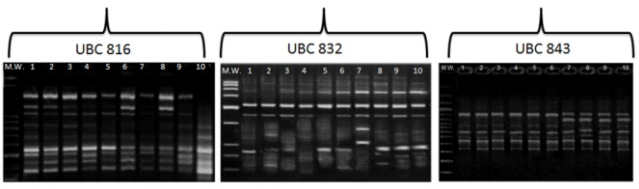

Fig3- Genomic DNA ISSR polymorphism analysis of 10 pigeonpea cultivars with various UBC primers M.W.-200bp marker, **Lane 1-** ICPL 87119, **Lane 2-** ICP 8863, **Lane 3-** G-Red, **Lane 4-** TAT 9903, **Lane 5-** BSMR 736, **Lane 6-** GC-11-39, **Lane 7-** PG-12, **Lane 8-** GRG 295, **Lane 9-** TS-3, **Lane 10-** WRP 1

In the present study pigeonpea genotypes were subjected for RAPD analysis with 30 random decamer primers, out of which 18 primers were produced considerable polymorphism while 12 primers were either not able to produce the bands or had weaker amplification. The high intensity and reproducible bands were only selected for polymorphism analysis among the cultivars.Differing band intensities were not taken into account to avoid errors introduced by competition among priming sites during the initial rounds of RAPD-PCR. The presence or absence of bands was scored as 1 and 2 respectively.

The primer OPA 06, OPA 08, OPA 09, OPA 12, OPA 13, OPA 15, OPA 17, OPC 01, OPC 05, OPC 06, OPC 07, OPC 09, OPE 01, OPE 02, OPE 04, OPH 02, OPH 03 and OPH 09 presented considerable polymorphism (Fig. 1), when compare to the other primers used for screening. Among the four series of primers OPA and OPC series developed more polymorphism than OPE and OPH series. These 18 primers produced 86 loci among 10 pigeonpea cultivars to which these 86 loci produced 646 bands with an average of 3.59 bands per primer. Among these 646 bands, it was observed that only 194 bands were polymorphic which shows about 30% polymorphism among the cultivars.

The scored data was used for calculating similarity indices by using the formula of (12) to generate pairwise matrix (Table 2). The pairwise matrix of genetic distances was then employed to draw the dendrogram produced by Unweighted Pair Group Method with Arthimetic Averages (UPGMA) of Tool for Population Genetic Analysis (TFPGA), in order to study the precise relationships between the pigeonpea

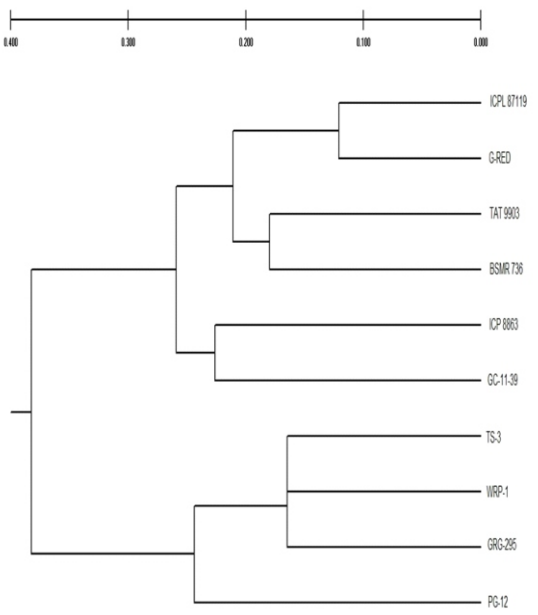

Figure 4: Phylogenetic analysis of ISSR data of genomic DNA in 10 pigeonpea cultivars

genotypes and also for cluster analysis for grouping the pigeonpea cultivars.

The banding pattern of all the primers opted for data analysis resolved significant level of polymorphism and consistent reproducible banding profiles. The phylogenetic tree generated by UPGMA revealed much more clear analysis and showed the genetic variation with some extent among the pigeonpea cultivars. The 10 cultivars were clustered into 2 populations and 3 subpopulations (Fig. 2). Further, the cultivars specific putative banding pattern was detected, which could be used for the identification of cultivars.

Among the wild relatives of pigeonpea cultivars, only Cajanusgenera is the most widely cultivated through out the world. The limited genetic diversity has been reported in pigeonpea due to its self pollinating nature. RAPD techniquewas used to identify parents from hybrids of thecross *C. platycarpusx, C. cajan* by (13)) who suggested that although RAPDs are not favored as compared to othermarkers, they can still be effectively used to distinguish parents and hybrids. (14) used RAPD marker for identification of pigeonpea cultivars and its related wild species. The level of polymorphism among the wild species was extremely high, while a low polymorphism was detected with in Cajanus cajanaccessions. (14) used RAPD marker for molecular characterization of 13 somaclonal varients of pigeonpea CV ICPL 87 in order to identify their variant agronomic nature such as seed color, high seed mass, reduced plant height high and low incidence of Helicoverpa damage etc. (16) assessed the genetic diversity and identification of pigeonpea cultivars by employing 76 RAPD primers and observed a significant higher polymorphism among cultivars. (17) investigated identifying and tagging of RAPD marker linked with plant type trait in F1 and F2 genotype obtained by crossing the open tall and compact dwarf pigeonpea.

In our present investigation, the RAPD primers were used to access the genetic diversity among 10 pigeonpea cultivars which were differing in their seed color and seed biomass. Among ten cultivars; six cultivars seed (ICPL 87119, ICP 8863, G-RED, TAT-9903, BSMR-736 and GC-11-39) were red in color, three cultivars seed (GRG-295, TS-3 and WRP-1) were white while one cultivar (PG-12) seed was black in color.18 out of 30 RAPD markers showed a considerable marker in the present study and the phylogenetic analysis results suggested that the red cultivars were clustered in one group, white cultivars in other and the black cultivar showed a highly genetic distance than red group but nearer to the white cultivars. An average of 3.59 bands per primer per cultivar was obtained and the range of amplified band obtained by the RAPD primer was 3 to 9. Our results are in strongly supported by the earlier work of (14, 15) and also suggest that the RAPD can be used to access genetic diversity with some extent among pigeonpea cultivars differing in their agronomic characters.The significant level of polymorphism was obtained by OPA set of primer followed by OPC series.

For the analysis of ISSR markers on the genomic DNA, the microsatellite based ISSR primers (UBC, Vancouver, Canada) were used for assessing the genetic variation among ten pigeonpea cultivars. Due to constraints in obtaining large number of primers, only 12 ISSR primers which produced polymorphic bands were used for the analysis on 10 pigeonpea cultivars. It was observed that all individual primers used for screening were able to generate the

considerable polymorphism with standardized condition.

The criteria for data scoring, analysis and calculation of genetic distance were followed as that of RAPD data analysis. The amplified ISSR fragments were ranged from 200 bp to 4kb and the number of bands produced by each ISSR primer was ranged from 5 to 12. The pairwise genetic similarity matrix was generated using the formula of (12) by the genetic distance programme TFPGA package. The genetic distance among the pigeonpea cultivars were found in the range of 0.1210 to 0.4776 (Table 3).

The primer UBC807, UBC808, UBC816, UBC827, UBC832, UBC839, UBC840, UBC843, UBC844, UBC857, UBC864 and UBC873 presented considerable polymorphism. These 12 primers produced 79 loci among 10 pigeonpea cultivars to which these 79 loci produced 608 bands with an average of 5.07 bands per primer for each cultivar. Among these 608 bands, it was observed that 263 bands were polymorphic which shows about 43.26% polymorphism among the cultivars (Fig. 3). The polymorphism obtained by the ISSR primers was considerably greater than that of RAPD primers.

The banding pattern of all the primers opted for data analysis resolved significant level of polymorphism and consistent reproducible banding profiles. The phylogenetic tree generated by UPGMA revealed much more clear analysis and showed better genetic variation as compare to RAPD primers among the pigeonpea cultivars (Fig. 4). Further, the cultivars specific putative banding pattern was detected, which could be used for the identification of cultivars.

The ISSR markers have been used to access genetic diversity and to establish phylogenetic relationship in many plant species like Blackgram (18), Chickpea (19) but the ISSR analysis in pigeonpea cultivar is till reported by (20). In their report, the ISSR analysis was done both in Cecer aretinum and Cajanus cajan genotypes selected for wilt susceptibility/ resistant and observed unexpected considerable high polymorphism of 95% with an average of 7.9 bands per primer. In their report, it was concluded that ISSR dendrogram was similar to SSR dendrogram and thus these marker system could be effectively used individually in determination of genetic relationships among chickpea and pigeonpea. As compare to SSR; RAPD and ISSR markers were more polymorphic but SSR still have advantage as co-dominant arker to detect homozygotes and heterozygote's more efficiently (20).

In our present investigation, it was observed that ISSR markers have considerable higher polymorphism than that of RAPD marker but the genetic distance among the cultivars were narrow. The ISSR polymorphism among the pigeonpea cultivars observed in the present study was 43.26%. The limited/ narrow genetic similarity was observed because all the pigeonpea cultivars selected for the present study are predominantly cultivated in Gulbarga region and thus these cultivars are closely related to each other. Even though a narrow genetic diversity was observed by ISSR marker, the pigeonpea cultivars were clustered according to their seed color. The red cultivar was well differentiated in phylogenetic tree and clustered in one group while white and black cultivars were clustered in another group. The clustering pattern of ISSR and RAPD was similar however the position of the cultivars within the cluster was not found at similar position.

The evaluation of ISSR and RAPD markers with important agronomic values was studied in cotton genotypes by (21) and reported ISSR as superior marker over RAPD because of the length of the primer, higher annealing temperature and greater reproducibility. The usefulness of ISSR marker and its correlation with agronomic characters are not only restricted to genomic DNA but also chloroplast and mitochondrial DNA diversity was evaluated in sugarcane by (22).

For identification purposes (fingerprinting), microsatellites (both SSR and ISSR markers) are moreappropriate since they provide a higher level of polymorphism. The results obtained in this study indicatethat microsatellites provide a powerful tool for cultivaridentification and diversity studies in pigeonpea, exhibitingadvantages over RAPD analyses.The markers generating simple patterns as RAPDs or ISSRs can be desirable for phylogenetic analysis. In this case, ISSR is advantageous over

RAPD analysis due to its higher percentageof polymorphism. Furthermore, an easy and reliabletransfer of information across laboratories is possible.

REFERENCES

1. FAO (208). Food and Agriculture Organization of USA (http://www.fao.org/).
2. Yang, S.Y.; Saxena, R.K.; Kulwal, P.L.; Ash, G.J.; Dubey, A.; Harper, J.D.I.; Upadhyaya, H.D.; Gothalwal, R.; Kilian, A. and Varshney, R.K. (2011). The first genetic map of pigeonpea based on diversity arrays technology (DArT) *Markers, J. Genet, 90 :* 103-109.
3. Malviya, N. and Yadav, D. (2010). RAPD Analysis among Pigeon pea [*Cajanus cajan* (L.) Mill sp.] Cultivars for their Genetic Diversity, *Genetic Engineer Biotechnol J. :* 1-9.
4. Malik, T.A.; Pric, A. and Wright, D. (2000). Bulked segregant analysis and RAPD markers for drought resistance in wheat, *Pak J Agri Res, 2 :* 82-88.
5. Bramel, J.; Kiran, S.; Reddy, J.; Ford Lloyd, B. and Chandra, S. (2004). Degree and distribution of pigeonpea landrace mophological diversity in traditional cropping systems in Andhra Pradesh.In: Assessing the risk of losses of biodiversity in traditional cropping systems: A case study of pigeonpea in Andhra Pradesh, Bramel PJ (ed). *International Crops Research Institute for the Semi-Arid Tropics,* Pantacheru, Andhra Pradesh, India, (2004)1-45.
6. Kollipara, K.P.; Singh, L. and Hymowitz, T. (1994). Genetic variation of trypsin and chymotrypsin inhibitors in pigeonpea (*Cajanus cajan* (L.) Millsp.) and its wild relatives, *Theor Appl Genet, 8 :* 986-993.
7. Litt, M. and Luty, J.A. (1989). A hypervariable microsatellite revealed by in vitro amplification of dinucleotide repeat within the cardiac muscle actin gene, *Am J Hum Genet, 44 :* 397-340.
8. Edwards, J.; Barker, A.; Daly, A.; Jones, C. and Karp, A. (1996). Microsatellite libraries enriched for several microsatellite sequences in plants, *Biotechniques, 20 :* 758-759.
9. Varshney, R.K.; Penmetsa, R.V.; Dutta, S.; Kulwal, P.L.; Saxena, R.K.; Datta, S. and Sharma, T.R. (2010). Pigeonpea genomics initiative (PGI): an international effort to improve crop productivity of pigeonpea (*Cajanus cajan* L.), *Mol Breeding, 26 :* 393–408.
10. Doyle, J.J. and Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue, *Focus, 12 :* 13-15.
11. Miller, M.P. (1997). Tools for Population Genetic Analysis, Version 1.3. Department of Biological Sciences, *Northern Arizona University,* Flagstaff.
12. Nei, M. and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases, *Proc Natl Acad Sci, 76 :* 5269-5273.
13. Mallikarjuna, N.; Saxena, K.B. and Jadhav, D.R.; Cajanus, C. Kole (ed.). Wild Crop Relatives : Genomic and Breeding Resources, *Legume Crops and Forages :* 21-33.
14. Ratnarpakhe, M.B.; Gupta, V.S.; Ven Murthy, M.R. and Ranjekar, P.K. (1995). Genetic fingerprinting of pigeonpea (*Cajnus cajan* (L.) Millsp.) and its wild relatives using RAPD markers, *Theor Appl Genet, 91 :* 893-898.
15. Prasannalatha, C.H.; Kaur, P. and Bhalla, J.K. (1999). Molecular characterization of somaclonal variants in pigeonpea, *Current Sci, 76 :* 693-695.
16. Choudhury, P.R.; Singh, I.P.; George, B.; Verma, A.K.; Singh, N.P. (2008). Assessment of genetic diversity of pigeon pea cultivars using RAPD analysis, *Biologia Plantarum, 52 :* 648-653.
17. Dhanasekar, P.; Dhumal, K.N. and Reddy, K.S. (2010). Identifications of RAPD markers linked to plant type gene in pigeonpea, *Indian J Biotech, 9 :* 58-63.
18. Souframanien, J. and Gopalakrishna, T.A. (2004). Comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers, *Theoretical Applied Genet, 109 (8) :* 1687-1693.
19. Chowdhury, M.A.; Vandenberg, B. and Warkentin, T. (2002). Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.), *Euphytica, 127 :* 317-325.
20. Datta, J. and Lal. N. (2011). Characterization of genetic diversity in *Cicer arietinum* L. and *Cajanus cajan* L. Millspaugh using random amplified polymorphic DNA and simple sequence repeat markers, *Genomics and Quantitative Genetics, 3 :* 30-41.
21. Dongre, A.; Parkhi, V. and Gahukar, S. (2004). Characterization of cotton (*Gossipium hirsatum*) genotypes by ISSR, RAPD and agronomic values, *Indian J Biotech, 3 :* 388-393.
22. Virupakshi, S. and Naik, G.R. (2008). ISSR Analysis of chloroplast and mitochondrial genome can indicate the diversity in sugarcane genotypes for red rot resistance, *Sugar Tech, 10 :* 65-70.