International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 9(5): 725-730, October 2016
DOI: 10.5958/2230-732X.2016.00094.2

BIOTECHNOLOGY

Molecular characterization of six pigeonpea varieties for drought tolerance by using RAPD markers

P.R. Damor[1*], A.V. Narwade[2], S.K. Jadav[2] and Y.A. Viradiya[2]

[1]Department of Plant Physiology, Anand Agricultural University, Anand, Guajarat-388001, India
[2]Department of Genetics and Plant Breeding, Navsari Agricultural University, Navsari, Gujarat- 396450, India

*Corresponding author: p.r.damor@gmail.com

Paper No. 497 Received: 18-2-2016 Accepted: 8-9-2016

Abstract

Genetic diversity among six pigeonpea genotypes with the four irrigation level in split plot design analysed by using 26 RAPD primer. The molecular characterization of six pigeonpea varieties showed genetically diverge condition forming four cluster groups i.e., A, B, C and D which showed that GNP-304 and Bharboot local formed the same cluster and they are susceptible varieties showing low seed yield. The varieties AGT-2 and C-11 formed the same cluster showing comparatively higher seed yield and they are drought tolerant variety. The variety GT-102 formed another cluster showing genetically divergence from other varieties. Similarly, the variety GT-1 formed another cluster showing genetically divergence from other varieties.

Highlights

- The molecular characterization of pigeonpea genotypes under water stress condition is studied
- Two pigeonpea genotypes GNP-304 and Bharboot local formed the same cluster which are drought susceptible
- Two pigeonpea genotypes AGT-2 and C-11 formed the same cluster which are drought tolerant
- Genetic diversity among six pigeonpea varieties is observed

Keywords: Water stress, pigeonpea, RAPD markers, genotypes, dendrogram

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is one of the major grain legume (pulse) crops of the tropics and subtropics. The Indian subcontinent, accounts for about 90% of the global production. Its seed protein content (approximately 21%) is also well comparable with that of other major grain legumes. It is considered as a drought tolerant crop and a post-rainy season crop is often subjected to water stress at one or several stages of crop growth and development, since it is a long duration crop with a large variation for maturity period. As a result, it is widely adapted to a range of environments and cropping systems. The variations for maturity have direct relevance on the survival and fitness of the crop in different agro-ecological niches (Choudhary, 2011). Determination of genetic diversity of any given cropspecies is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a Feature improvement. 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 atspecies levels, as is required for pigeon pea.

Randomly amplified polymorphic DNA (RAPD) markers have been used for numerous applications in plant molecular genetics research despite having

disadvantages of poor reproducibility and not generally being associated with gene regions [Welsh and McClelland (1990) and Williams *et al.* (1990)]. RAPD, being a multi locus marker [Karp *et al.* (1997)] with the simplest and fastest detection technology, have been successfully employed for determination of intraspecies genetic diversity in several grain legumes. These include *Vigna unguiculata* [Ba *et al.* (2004)], *Vigna radiata* [Souframanien (2004)], *Lens* sp. [Sharma *et al.* (1995) and Ahmad *et al.* (1996)], *Phaseolus* sp. [Beebe *et al.* (2000) and Chiorato *et al.*(2007)], *Glycine* sp. [Jeffrey *et al.* (1998) and Barroso *et al.* (2003)], *Cicer* sp. [Ahmad (1999)], *Pisum* sp. [Cheghamirza *et al.* (2002) and Taran *et al.* (2005)] and *Cajanus cajan* [Kotresh *et al.* (2006), Ratnaparkhe *et al.* (1995) and Choudhury *et al.* (2008)]. Genetic variability of pigeon pea has been studied using several other genetic markers such as RFLP [Nadimpalli *et al.* (1993) and Sivaramakrishnan *et al.* (2002)], AFLP [Wasike *et al.* (2005) and Panguluri *et al.* (2004)], microsatellite markers [Odeny *et al.* (2007)] and Diversity Array Technology [Yang *et al.* (2006)]. This paper reports assessment of genetic diversity among 6 pigeon pea genotypes with 26 RAPD primers for drought tolerance.

Materials and Methods

Plant Material

The seed of six varieties of pigeon pea i.e. GT-102, Bharboot local, GNP –304, GT-1, AGT-2,C-11 were grown in Split Plot Design with three replication during *rabi*-2013-14 at College Farm, Navsari Agriculture University, Navsari. There was four treatments I_0- All irrigation given 25, 50 and 75 DAS, I_1-Two irrigation given 25 and 50 DAS, I_2- 25 DAS and I_3- Rainfed.

Genomic DNA Isolation

Young and tender leaves were plucked from different genotypes of the pigeon pea seedling in control and stressed condition both and DNA isolation was done using CTAB method as suggested by Saghai Maroof *et al.* (1984). Leaves were thoroughly washed with distilled water and from these 100 mg leaf tissues were ground into fine powder by crushing with liquid nitrogen in pestle and mortar. The ground sample was immediately transferred to 2.0 ml eppendorf tube. One ml of pre-warmed CTAB extraction buffer [50 mM Tris base (pH 8.0), 20mM EDTA (pH 8.0), 1.4 M NaCl, 0.1ml μ-mercaptoethanol, 2% CTAB and 1% PVP] was poured and the content was thoroughly mixed by inversion. Tubes were kept in water bath at 65°C for one hour and the contents were mixed after every 15 min. After cooling 800 μl of chloroform: isoamyl alcohol (24:1) was added and content was thoroughly mixed by inversion for one minute and centrifuged at 13000 rpm for 15 min. Clear supernatant was transferred in another clean eppendorf tube and equal amount of chloroform: isoamyl alcohol (24:1) was added, content was mixed by mild vortexing for 5-10 seconds and centrifuged at 13,000 rpm for 15 minutes.

After centrifugation upper phase was collected in fresh eppendorf tube and equal volume of chloroform: isoamyl alcohol (24:1) was added to these tubes, mixed well and centrifuged at 13,000 rpm for 15 min.The top aqueous phase appeared after centrifugation was transferred to a new 1.5 ml tube. To this solution, equal volume of ice cold isopropanol was added, mixed well by gentle inversion and kept at –20°C for 1 hour. After one hour of chilling treatment, tubes were centrifuged at 13,000 rpm for 20 min. Pellet of DNA was formed after spin. Supernatants were decanted and DNA pellet was washed with 500 μl of 70% ethanol and centrifuged again at 13,000 rpm for 5 minute. Pellet of DNA was recovered, air dried and dissolved in 100 μl of 1X TE and centrifuged for short run of 10 sec to collect DNA at bottom.

Electrophoretic quality check

To check the form of DNA (supercoiled, linear or sheared) and RNA contamination of isolated genomic DNA, DNA was run electrophoretically in 0.8% agarose gel and quality was judged by viewing the image of separated DNA fragments.

RAPD analysis

The genomic DNA was subjected to polymerase chain reaction. 26 oligonucleotide primers obtained from Bangalore GeNei, India (GeNeiTM) and Eurofins were used for RAPD analysis (Table 1). PCR tubes containing reaction mixture were tapped gently. The amplificationwas carried out in BIORAD thermalcycler set at following cycling regime.

Sl. No.	Component	Volume
1	10X reaction buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 15 mM $MgCl_2$ and gelatin)	2.5 µl
2	25 mM $MgCl_2$	1.0 µl
3	Primer (10 p moles/µl)	1.0 µl
4	dNTPs mix (2.5 mM each)	0.5 µl
5	*Taq* DNA polymerase (3 U/µl)	0.5 µl
6	Template DNA (20 ng/µl)	1.0 µl
7	Sterile distilled water	18.5 µl
	Total	25.0 µl

All the PCR products were run on 1.8% agarose gel containing 4µl of ethidium bromide (1 mg/ml). Running buffer containing Tris-buffer, boric acid and EDTA (pH 8.0) was used for electrophoresis. Twenty µl of PCR product was mixed with 4µl of 6x loading dye and loaded onto the well. The gel was run at a constant current of 80V to separate the amplified bands. The standard DNA marker (100bp to 3kb) was also run along with the samples. The separated bands were documented under UV transilluminator and photographed by Gel documentation system BIORAD and analyzed.

Results and Discussion

In recent years molecular markers have attained great significance in evaluating plant material. Scientists have invented numerous marker systems depending upon their need and material to be handled to explore the hidden information stored in the DNA as variation in nucleotide sequences. The beauty of these markers is that they are devoid of environmental interaction.

Table 1: Details of RAPD primers

Sl. No.	Name of Primers	Primer Sequence
1	OPA1	5¹CAGGCCCTTC3¹
2	OPA2	5¹TGCCGAGCTG3¹
3	OPA3	5¹AGTCAGCCAC3¹
4	OPA4	5¹AATCGGGCTG3¹
5	OPA5	5¹AGGGGTCTTG3¹
6	OPA6	5¹GGTCCCTGAC3¹
7	OPA7	5¹GAAACGGGTG¹
8	OPA8	5¹GTGACGTAGG3¹
9	OPA9	5¹GGGTAACGCC3¹
10	OPA10	5¹GTGATCTAGG3¹
11	OPA12	5¹TCGGCGATAG3¹
12	OPA13	5¹CAGCACCCAC3¹
13	OPA14	5¹TCTGTGCTGG3¹
14	OPA15	5¹TTCCGAACCC3¹
15	OPO2	5¹ACGTAGCGTC3¹
16	OPO3	5¹CTGTTGCTAC3¹
17	OPO4	5¹AAGTCCGCTC3¹
18	OPO5	5¹CCCAGTCACT3¹
19	OPO6	5¹CCACGGGAAG3¹
20	OPO7	5¹CAGCACTGAC3¹
21	OPO8	5¹CCTCCATGAC3¹
22	OPO9	5¹TCCCACGCAA3¹
23	OPO10	5¹TCAGAGCGCC3¹
24	OPO11	5¹GACAGGAGGT3¹
25	OPO12	5¹CAGTGCTGTG3¹
26	OPO13	5¹GTCAGAGTCC3¹

Six genotypes of pigeonpea were examined for DNA polymorphism using 26 primers out of which seven primers showed amplification. Out of 26 primers used, amplification could be obtained with 7 primers, whereas 19 primers failed to show any amplification. The NTSYSpc programme was used to calculate Jaccard's similarity coefficient. Similarity coefficient of different pigeonpea genotypes is presented in (Table 2).

Table 2: Jaccard's similarity coefficient among different pigeonpea variety based on RAPD data analysis

Genotype	G_1	G_2	G_3	G_4	G_5	G_6
G_1	1.00					
G_2	0.81	1.00				
G_3	0.85	0.95	1.00			
G_4	0.78	0.87	0.83	1.00		
G_5	0.85	0.95	0.90	0.91	1.00	
G_6	0.85	0.95	0.90	0.91	1.00	1.00

Note: **G_1**= GT-102, **G_2**=Bharboot, **G_3**= GNP-304, **G_4**= GT-1, **G_5**= AGT-2, **G_6**= C-11

A, **B**, **C** and **D** are clusters

Genetic similarity matrix revealed, similarity values ranging from 0.83 to 1.00. Maximum genetic similarity (1.00) was observed between G_5 and G_6. And least was observed in G_1 (0.83) and G_4 (0.89). The clustering pattern of dendrogram constructed by Jaccard's coefficient of similarity indicated differences among the different six pigeonpea genotypes. Fig. 1. The dendrogram showed four major clusters such as A, B, C and D formed on the basis of their similarity coefficient. The cluster A

consists of only one genotype G_1. Cluster B consist of G_2 and G_3. Cluster C consist of G_5 and G_6 and Cluster D consist of G_4.

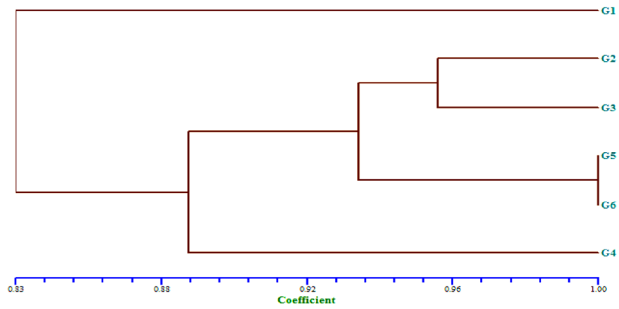

Fig. 1: Dendrogram depicting the genetic relationship among the different pigeonpea varieties based on RAPD data

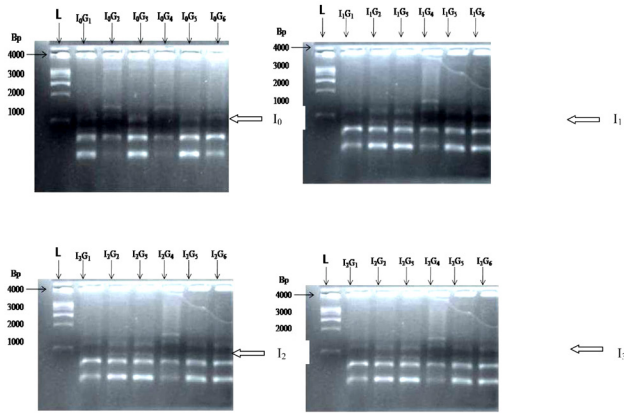

Fig. 2: Amplification patterns of different pigeon pea genotypes produced by primer OPA 1

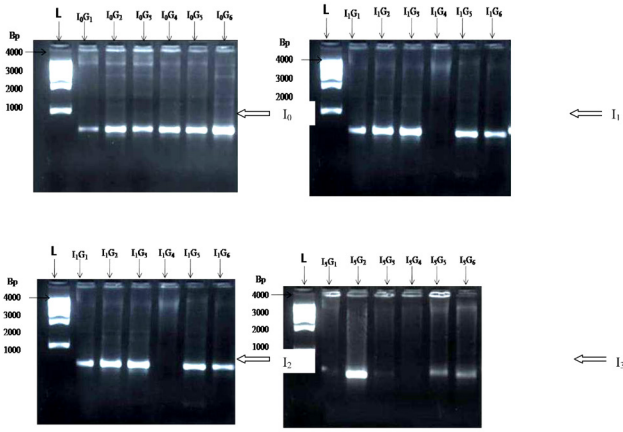

Fig. 3: Amplification patterns of different pigeon pea genotypes produced by primer OPA 8

The dendrogram denotes that cluster A shows clear genetical divergence from other cluster. Cluster B near to cluster A shows the genetic similarity between the genotypes G_2 and G_3 which are drought susceptible varieties. Whereas, cluster C encompasses the genetic similarity between the genotypes G_5 and G_6, which are drought resistant varieties.

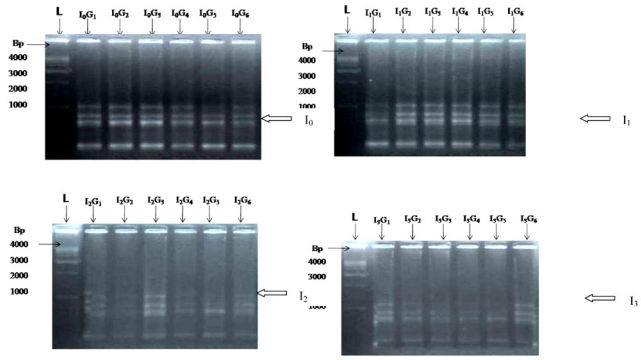

Fig. 4: Amplification patterns of different pigeon pea genotypes produced by primer OPA 10

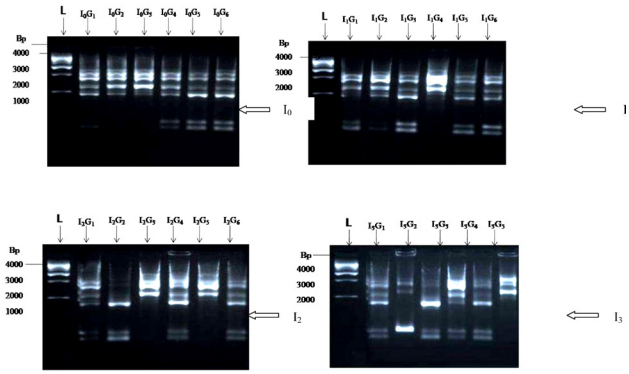

Fig. 5: Amplification patterns of different pigeon pea genotypes produced by primer OPA 12

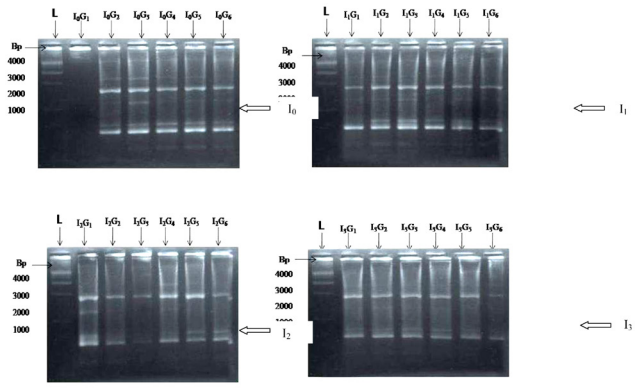

Fig. 6: Amplification patterns of different pigeon pea genotypes produced by primer OPA 15

Cluster D consisting of G_4 shows genetic divergence. The similar results were observed by Neha Malviya and Dinesh Yadav (2010), Yadav *et al.* (2012), Mishra *et al.* (2013) and Walunjkar *et al.* (2014). All of the cultivars and wild species under study could be easily distinguished with the help of different primers, thereby indicating the immense potential

of RAPD in the genetic fingerprinting of pigeonpea (Ratnaparkhe *et al.*, 1995).

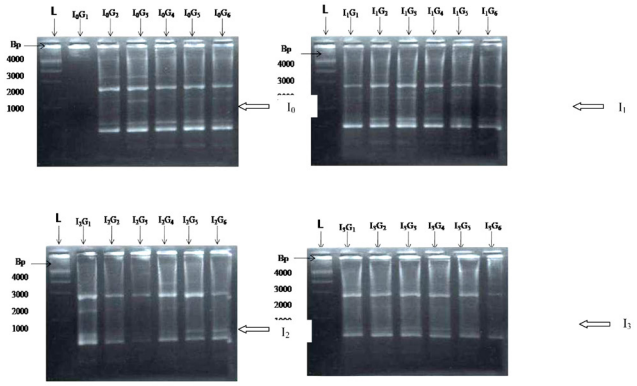

Fig. 7: Amplification patterns of different pigeon pea genotypes produced by primer OPO

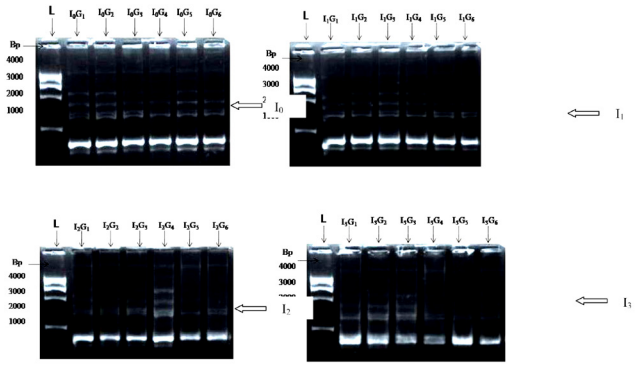

Fig. 8: Amplification patterns of different pigeon pea genotypes produced by primer OPO 11 34

Note: Lane-1 DNA Ladder, I1**G**$_1$= GT-102, **G**$_2$=Bharboot, **G**$_3$= GNP-304, **G**$_4$= GT-1, **G**$_5$= AGT-2, **G**$_6$= C-11, and I$_0$= all irrigation 25,50 and 75 DAS, I$_1$= Two Irrigation 25 and 50 DAS, I$_2$= one irrigation at 25 DAS and I$_3$= Rainfed Condition

The molecular characterization showed that there was genetic diversity among the six pigeonpea varieties. There was genetic similarity among the drought tolerant varieties forming the same cluster and genetic similarity among the drought susceptible varieties forming the same cluster.

Conclusion

The molecular characterization investigation showed the genetic diversity among the six pigeonpea genotypes. The drought susceptible variety GNP-304 and Bharboot local showed the similarity forming the same cluster while the varieties AGT-2 and C-11 which are drought tolerant showed the genetic similarity forming the same cluster. It was observed that the similarity was also observed in the seed yield in these varieties. The primers (OPA 1, OPA 8, OPA 10, OPA 12, OPA 15, OPO 4, and OPO 11) which is depicted in the Fig. 2 to 8 showed the amplification. Thus, this primers can be used for the further screening of the drought tolerant varieties of pigeonpea.

References

Ahmad, F. 1999. Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. *Theoretical and Applied Genetics* **98**: 657-663. http://dx.doi.org/10.1007/s001220051117

Ahmad, M., McNeil, D.L., Fautrier, A.G., Armstrong, K.F. and Pearson, A.M. 1996. Genetic relationship in *Lens* species and parentage determination of their interspecific hybrids using RAPD markers. *Theoretical and Applied Genetics* **92**: 1081-1098. http://dx.doi.org/10.1007/s001220050235

Barroso, P.A.V., Geraldi, I.O., Vieira, M.L.C., Pulcinelli, C.E., Vencovsky, R. and Santos Dias, C.T. 2003. Predicting performance of soybean populations using genetic distances estimated with RAPD markers. *Genetics and Molecular Biology* **26** (3): 343-348. http://dx.doi.org/10.1590/s1415-47572003000300020

Beebe, S., Skroch, P.W., Tohme, J., Duque, M.C., Pedraza, F. and Nienhuis. 2000. Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. *Crop Science* **40**: 264-273. http://dx.doi.org/10.2135/cropsci2000.401264x

Cheghamirza, K., Koveza, O., Konovalov, F. and Gostimsky, S. 2002. Identification of RAPD markers and their use for molecular mapping in Pea (*Pisum sativum* L.). *Cellular and Molecular Biology Letters* **7**: 649-655.

Chiorato, A.F., Carbonell, S.A.M., Benchimol, L.L., Chiavegato, M.B., Santos Dias, L.A.D. and Colombo, C.A. 2007. Genetic diversity in common bean accessions evaluated by means of morpho-agronomical and RAPD data. *Scientia Agricola (Piracicaba, Brazil)*, **64**(3): 256-262. http://dx.doi.org/10.1590/s0103-90162007000300007

Choudhary, A.K. 2011. Effects of pollination control in pigeonpea and their implication. *Journal of Food Legumes* **24**: 50-53.

Choudhury, P.R., Singh, I.P., George, B., Verma, A.K. and Singh, N.P. 2008. Assessment of genetic diversity of pigeon pea cultivars using RAPD analysis. *Biologia Plantarum* **52**(4): 648-653. http://dx.doi.org/10.1007/s10535-008-0126-0

Jeffrey, A., Thompson, Randall, L. and Nelson. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Science* **38**(5): 1348-1355. http://dx.doi.org/10.2135/cropsci1998.0011183x003800050033x

Karp, A., Edwards, K., Bruford, M., Funk, S., Vosman, B., Morgante, M., Seberg, O., Kremer, A., Boursot, P., Arctander, P., Tautz, D. and Hewitt, G. 1997. Molecular technologies for biodiversity evaluation: opportunities and challenges. *Nature Biotechnology* **15**: 625-628. http://dx.doi.org/10.1038/nbt0797-625

Kotresh, H., Fakrudin, B., Punnuri, S.M., Rajkumar, B.K., Thudi, M., Paramesh, H., Lohithasw and Kuruvinashetti, M.S. 2006. Identification of two RAPD markers genetically linked to a recessive allele of *Fusarium* wilt resistance gene in pigeon pea (*Cajanus cajan* (L.) Millsp.). *Euphytica* **149**: 113-120. http://dx.doi.org/10.1007/s10681-005-9059-2

Mishra, R.R., Behera, B. and Panigrahi, J. 2013. Elucidation of genetic relationships in the genus *cajanus* using random amplified polymorphic dna marker analysis. *Journal of Phylogenetics and Evolutionary Biology* **1**(1): 1-7.

Nadimpalli, R.G., Jarret, R.L., Phatak, S.C. and Kochert, G. 1993. Phylogenetic relationships of the pigeon pea (*Cajanus cajan*) based on nuclear restriction fragment length polymorphism. *Genome* **36**: 216-223. http://dx.doi.org/10.1139/g93-030

Neha Malviya and Dinesh Yadav. 2010. RAPD Analysis among pigeonpea [*Cajanus cajan* (L.) Millsp.] Cultivars for their Genetic Diversity. *Journal of Genetic Engineering and Biotechnology* 1-9.

Odeny, D.A., Jayshree, B., Ferguson, M., Hoisington, D., Crouch, J. and Gebhardt, C. 2007. Development, characterization and utilization of microsatellite markers in pigeon pea. *Plant Breeding* **126**: 130-136. http://dx.doi.org/10.1111/j.1439-0523.2007.01324.x

Panguluri, S.K., Janaiah, K., Govil, J.N., Kumar, P.A. and Sharma, P.C. 2004. AFLP fingerprinting in pigeon pea (*Cajanus cajan* (L.) Millsp.) and its wild relatives. *Genetic Resources and Crop Evolution* **00**: 1-9.

Ratnaparkhe, M.B., Gupta, V.S., Murthy, M.R. and Ranjekar, P.K. 1995. Genetic fingerprinting of pigeon pea (*Cajanus cajan* (L.) Millsp.) and its wild relatives using RAPD markers. *Theoretical and Applied Genetics* **91**: 893-898. http://dx.doi.org/10.1007/bf00223897

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. 1984. Ribosomal DNAsepacer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences* **81**: 8014–8019. http://dx.doi.org/10.1073/pnas.81.24.8014

Sharma, S.K., Dawson, I.K. and Waugh, R. 1995. Relationship among cultivated and wild lentils revealed by RAPD analysis. *Theoretical and Applied Genetics* **91**: 647-654. http://dx.doi.org/10 .1007/bf00223292

Sivaramakrishnan, S., Kannan, S. and Reddy, L. 2002. Diversity in selected wild and cultivated species of pigeon pea using RFLP of mtDNA. *Euphytica* **125**: 24-28. doi: 10.1023/A:1015759318497

Souframanien, J. and Gopalakrishna, T. 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theoretical and Applied Genetics* **109**: 1687-1693. http://dx.doi.org/10.1007/s00122-004-1797-3

Taran, B., Zhang, C., Warkentin, T., Tullu, A. and Vanderberg, A. 2005. Genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on molecular markers, and morphological and physiological characters. *Genome* **48**: 257-272. http://dx.doi.org/10.1139/g04-114

Walunjkar, B.C., Palihar, A., Kumar, S.N. and Parmar, L.D. 2013. Genetic diversity of wild and cultivated genotypes of pigeonpea through RAPD and SSR markers. *Journal of Environmental Biology* **36**: 461-466.

Wasike, S., Okori, P. and Rubaihayo, P.R. 2005. Genetic variability and relatedness of the Asian and African pigeon pea as revealed by AFLP. *African Journal of Biotechnology* **4**(11): 1228-1233.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18**: 7213-7218. http://dx.doi.org/10.1093/nar/18.24.7213

Williams, J.G.K., Kublelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphism's amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531-6535. http://dx.doi.org/10.1093/nar/18.22.6531

Yadav, K., Yadav, S.K., Yadav, A., Pandey, V.P. and Dwivedi, U.N. 2012. Genetic Diversity of pigeonpea (*Cajanus cajan* (L.) Millsp.) Cultivars and its Wild Relatives Using Randomly Amplified Polymorphic DNA (RAPD) Markers. *American Journal of Plant Sciences* **3**: 322-330. http://dx.doi.org/10.4236/ajps.2012.33038

Yadav, V.K., Yadav, N. and Singh, R.D. 1996. Metabolic changes and their impact on yield in chickpea under water stress. *Plant Physiology and Biochemistry* **23**(1): 49-52.

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 pigeon pea compared to its wild relatives is revealed by diversity arrays technology. *Theoretical and Applied Genetics* **113**: 585-595. http://dx.doi.org/10.1007/s00122-006-0317-z