www.gbtrp.com
International Journal of Biological Technology (2010) 1(2):31-37
ISSN: 0976 - 4313

Original Article

Genetic diversity of Velvet bean, *Mucuna pruriens* (L.) DC from Southern Western Ghats, India

C. Kalidass and V. R. Mohan*
Ethnopharmacology unit, Research Department of Botany,
V.O.Chidambaram College, Tuticorin-628008, Tamil Nadu.
Corresponding author e-mail: vrmohan_2005@yahoo.com
Received: 11.2.2010; Revision: 23.6.2010; Accepted: 16.7.2010; Published: 15.8.2010

Abstract

In the present study, genetic diversity assessment of wild population of *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex Burck has been done by employing five random amplified polymorphic DNA (RAPD) primers. Out of 43 amplified products, 28 showed polymorphism (65.12%) and an average of 5.6 bands were amplified per primer. The DNA samples extracted from velvet bean accessions were found to be relatively pure with the purity index (OD_{260}/OD_{280}) value of 1.807 to 1.850. Cluster analysis based on Nei and Li (1979) similarity coefficient using UPGMA grouped all the accessions into two clusters. The cluster 'A' consists of three accessions and cluster 'B' of two accessions.
Kewwords: *Mucuna pruriens* (L.) DC. var. *utilis*; DNA isolation; RAPD

Introduction

Documentation of genetic variability and its magnitude among natural population of *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex Burck is an important aspect to know the present status of this nutritive value little-known tribal pulse. Availability of reliable polymorphic markers, often limits the accurate estimation of genetic variation among individuals or different populations. The extent of genetic variation between geographical populations depends upon several factors, including gene flow between populations (Baker and Stebbin, 1965). The polymorphic chain reaction (PCR) based Randomly Amplified Polymorphic DNA (RAPD) has been a hardy and convenient alternative technique to examine the genetic variation (Bhat *et al*., 1999) and successfully employed in the construction of linkage maps. It has been extensively used for molecular fingerprinting and population diversity analysis (Albert *et al*., 1997, Saini *et al*., 2010).

Mucuna pruriens var. *utilis* commonly known as velvet bean or cow-itch is a self pollinated tropical legume known for its medicinal properties and reported to be indigenous to India and china. It tolerated a wide range of soil acidity and moisture. Other physical properties like high nitrogen fixing capability, aggressive growth habit and high productivity of vegetative matter make it an excellent soil- improving crop, pasture crop, green manure cover crop, source of food and weed controller (Padmesh *et al*., 2006). Recently, the Dravidian tribes in Western Ghats of Tirunelveli district, India have started large-scale cultivation of this wild legume for their food use. The seed materials of *Mucuna pruriens* var. *utilis* were reported to contain higher level of protein, desirable amino acids, fatty acids and mineral composition with good nutritional properties (Mohan & Janardhanan, 1995; Siddhuraju *et al*., 2000; Pugalenthi *et al*., 2005; Siddhuraju & Becker 2005; Vadivel & Pugalenthi 2008). This valuable wild species also needs attention for the characterization of its genetic diversity, protection and cultivation. The present work describes RAPD analysis to assess the genetic divergence among its five accessions collected from different agro-ecological regions of Southern Western Ghats, Tamil Nadu, India.

Materials and Methods

Plant Materials

Five different accessions of velvet bean (*Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex Burck) leaves were collected from different agro-ecological regions of Southern Western Ghats, Tamil Nadu. Leaves were stored at 4°C in zip-lock plastic bags till they were processed for DNA isolation. The locations from where the leaf samples are collected are

illustrated in Fig.1. List of populations, their altitude and other details are given in the Table1.

DNA Extraction

The genomic DNA was extracted from the different accessions of velvet bean by the modified CTAB method (Doyle and Doyle 1987). Fresh young leaves were taken, weighed and grinded with liquid nitrogen in prechilled sterile pestle and mortar. The transfer this powder to a 50 ml Oakridge tube containing 15 ml of pre-warmed (65°C) extraction buffer (100 mM Tris chloride, 20.0 mM EDTA, 1.4 mM NaCl, 2% CTAB, 1% β-mercaptoethanol). Tubes were then incubated at 65°C for an hour and centrifuged for 15 min at 12,000 rpm (27°C). Supernatant was transferred to fresh tubes. Equal volume of chloroform: isoamyl alcohol in the ratio of 24:1. Mix the contents by gentle swirling and centrifuged the tubes for 15 min at 12,000 rpm (4°C). Solution gets separated into two layers. Remove the upper layer gently into separate Oakridge tube. Now add 6ml of prechilled isopropanol to each tube and mixed the contents by gentle swirling and kept the tubes for 2 hours or overnight at 4°C. Centrifuge these tubs for 10min, 10,000rpm at 4°C and pellet so obtained was washed with 70% ethanol. Then drain off ethanol very gently and dry the pellet, at least for half an hour at 37°C. Add 100 µl of TE (5:1 of 1M Tris buffer and 0.5 M EDTA) buffer to DNA pellet. DNA thus obtained is subjected to purification treatment and then quantified using UV spectrophotometer. The quality of DNA was analyzed spectrophotometrically by measuring the absorbency at 260 and 280 nm and the purity index (OD_{260}/OD_{280}) was calculated according to Sambrook *et al*. (1989) method.

Optimization of PCR

The protocol for PCR was optimized by varying the concentration of template, Taq DNA polymerase and $MgCl_2$. Various protocols were tested for obtaining the best amplification for this species. The following protocol was Optimized-Template DNA zong. Taq DNA polymerase (Genei) – 76 unit, Assay buffer 10x (Genei) containing 1.5 mM of $MgCl_2$, dNTP's mix (Genei) -2.0 µM each. The reaction volumes were 25µl. The polymerase chain reaction was performed using Eppendorf thermocycler with following temperature profile 92°C for 5 min, 37°C for 1 min and 72°C for 2 min, followed by 40 continuous cycles of 92°C for 1 min, 37°C for 1min and 72°C for 2 min with an elongation of 72°C for 10 min. Primer survey was carried out 5 primers (Table 2) using DNA from all the five lines.

Agarose Gel Electrophoresis

Submerged gel electrophoresis unit was used for fractionating RAPD markers on agarose gel. Agarose gel (1.5%) was prepared by dissolving appropriate amount of agarose in 1xTAE buffer (Sambrook *et al*., 1989). For each well, DNA dye and DNA sample were thoroughly mixed in a ratio of 5:1 and loaded with a micropipette. Electrophoresis was done at 75 V for 4 h in 1xTAE electrophoresis buffer. The gel was then stained in ethidium bromide solution. After destaining in distilled water, the gel image was viewed under UV light and stored in a gel documentation system. The size of each band was estimated using the DNA molecular weight marker (EcoRI/HindIII double digest and 100 bp DNA ladder).

Scoring of Bands and Data Analysis

All the gels were scored twice manually and independently. Presence of bands was representedby 1 and its absence by 0. All monomorphic bands were also scored and included in analysis. Presence or absence of unique and shared polymorphic as well as monomorphic products were used to generate similarity coefficients, and then similarity coefficients were used to construct a dendrogram by UPGMA (Unweighted Pair Group Method of Arithmetic Average) and cluster analysis using the NTSYS pc 2.02e (Numerical System, Applied Biostatistics, Inc., New York, USA) computer programme according to the method proposed by Nei and Li (1979).

Results and Discussion

The purity analysis of the genomic DNA extracts from five different accessions of velvet bean was given to Table 2. The purity of DNA was determined by calculating the ratio between OD_{260} and OD_{280} which ranges from 1.807 – 1.850, which is an indicator of very good quality of plant DNA and the ratio was almost consistent irrespective of the accessions. The good quality of DNA was also supported by appearance of single, compact, sharp band that was not sheared on 1.5% Agarose gel electrophoresis corresponded to the high molecular weight DNA compared with standard

www.gbtrp.com
International Journal of Biological Technology (2010) 1(2):31-37
ISSN: 0976 - 4313

DNA marker (1kb) (Henry 1997, Sambrook and Russell, 2001).

For the present study, bulk leaf samples of velvet bean were collected from 20 sites representing 5 agro-ecological regions distributed in Southern Western Ghats Tamil Nadu. Further, to study the variation between the samples from the plants located within 2 km distance. Then, to know about the genetic relationship between the accessions located at the same site but at a distance of more than 2 km less 50 km. Hence, a total 5 samples were collected; 5 from different accessions and five each from the same location. The samples were analyzed for genetic diversity using five random primers. The picture of RAPD PCR gel amplified by primer OPB-01, OPB-12, OPB-18, OPM-06 and OPB-04 are shown in fig 3. Out of a total of 43 fragments generated by random decamer primers, 28 (65.12%) were polymorphic with an average of four polymorphic products per primer.

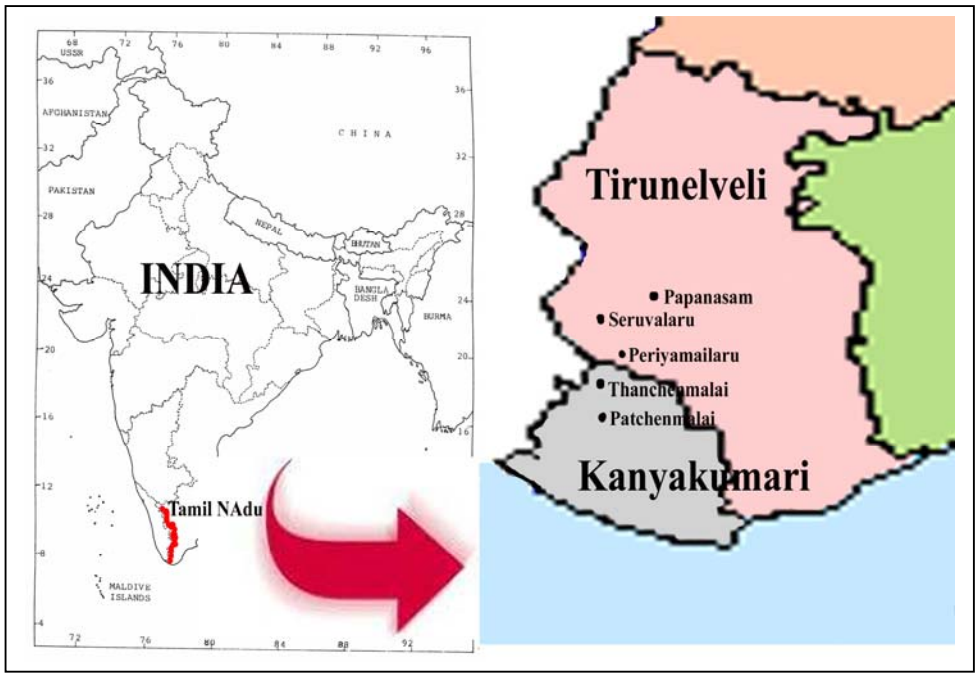

Fig. 1: Assession of *Mucuna pruriens* (L.) DC var *utilis* collected area map.

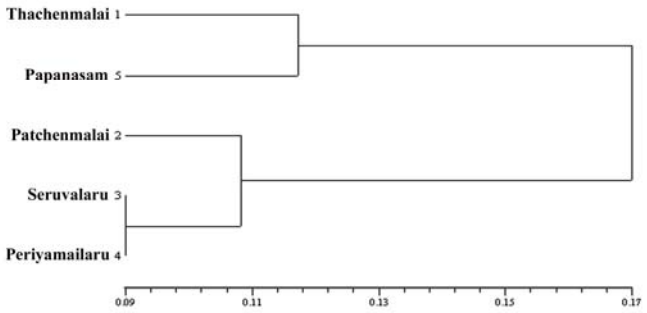

Fig. 2. UPGMA dendrogram based on Nei and Li (1979) genetic distance, summarizing the data on differentiation between five *Mucuna pruriens* (L.) DC var *utilis* germplasms according to RAPD analysis.

International Journal of Biological Technology (2010) 1(2):31-37
ISSN: 0976 - 4313

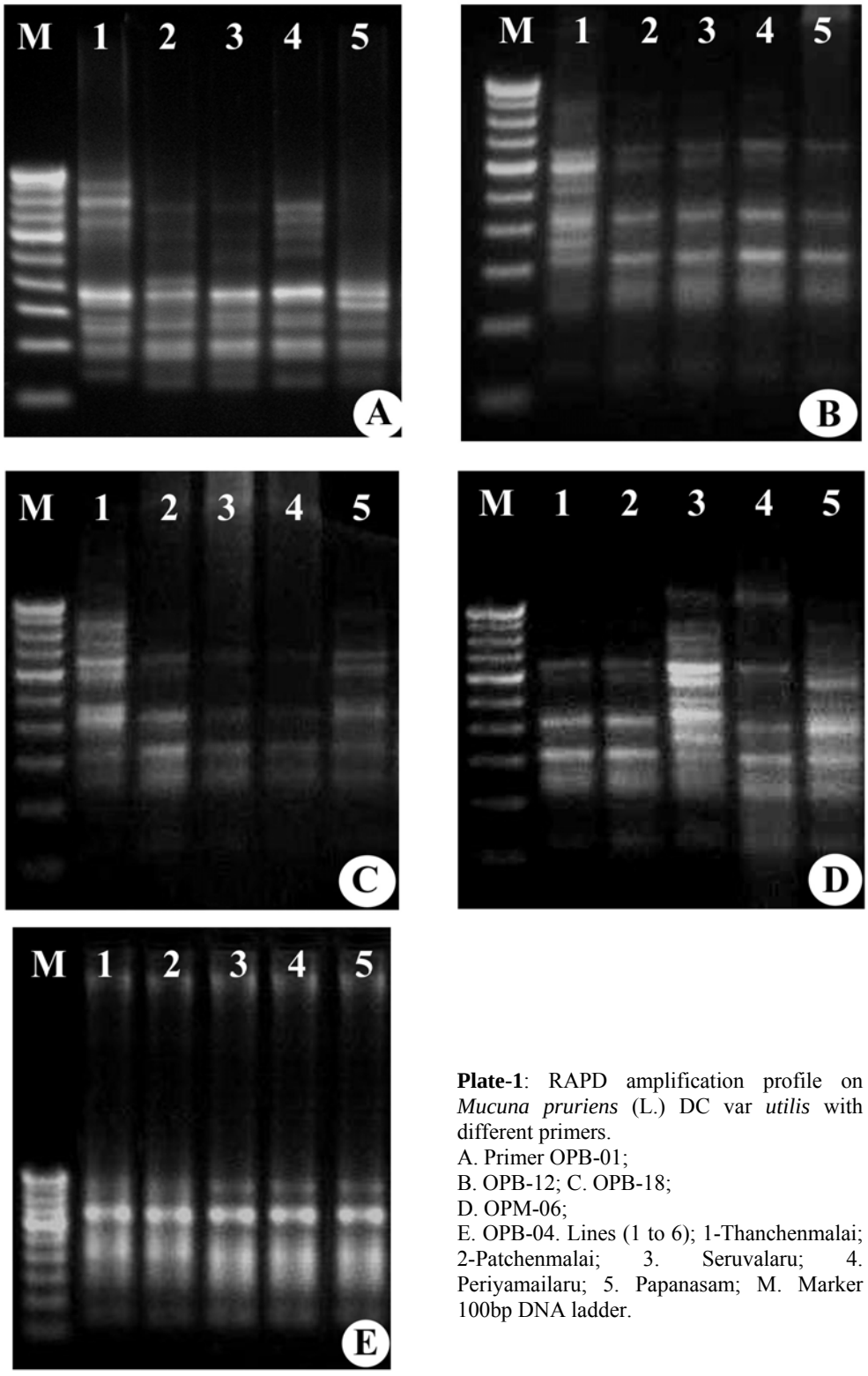

Plate-1: RAPD amplification profile on *Mucuna pruriens* (L.) DC var *utilis* with different primers.
A. Primer OPB-01;
B. OPB-12; C. OPB-18;
D. OPM-06;
E. OPB-04. Lines (1 to 6); 1-Thanchenmalai; 2-Patchenmalai; 3. Seruvalaru; 4. Periyamailaru; 5. Papanasam; M. Marker 100bp DNA ladder.

Table- 1: Five different accessions of *Mucuna pruriens* var. *utilis* been collected from different agro-ecological climates of Southern Western Ghats, Tamil Nadu.

Sl.No	Location	Latitude	Longitude	Altitude	District
1	Thachenmalai	08º 28" 39.62" N	077º 18" 45.41" E	390 ft	Kanyakumari
2	Patchenmalai	08º 29" 34.92" N	077º 17" 05.72" E	1732 ft	Kanyakumari
3	Seruvalaru	08º 41"20.53" N	077º 36" 77.42" E	1732 ft	Tirunelveli
4	Periyamailaru	08º 39" 16.19" N	077º 18" 52.30" E	854 ft	Tirunelveli
5	Papanasam	08º 38" 49.25" N	077º 17" 39.59" E	1387 ft	Tirunelveli

Table- 2: Purity analysis of the DNA samples extracted five different accessions of *Mucuna pruriens* var. *utilis* for RAPD analysis**.**

Sl.No	Accessions	Optical density value of the DNA		Purity index (OD_{260}/OD_{280})
		260 nm	280 nm	
1	Thachenmalai	1.102	0.604	1.8245
2	Patchenmalai	0.983	0.543	1.8103
3	Seruvalaru	1.038	0.561	1.8502
4	Periyamailaru	1.046	0.574	1.8222
5	Papanasam	0.978	0.541	1.8077

Table- 3: Polymorphism of RAPD primers analysis.

Primer Code	Operon	Sequence5'-3'	Scorable bands (a)	MW of bp	Total no. of polymorphic band (b)	Polymorphism (%) b/a X 100
P1	OPB-01	GTTTCGCTCC	10	100, 200, 300, 400, 500, 600, 800	7	70.00%
P2	OPB-12	CCTTGACGCA	9	100, 300, 500 600, 700	5	55.56%
P3	OPB-18	CCACAGCAGT	8	200, 300, 400, 500, 600, 700	6	75.00%
P4	OPM-06	CTGGGCAACT	10	100, 200, 300, 500, 600, 800	6	60.00%
P5	OPB-04	GGACTGGAGT	6	100, 400, 500, 600	4	66.67%
Total			**43**		**28**	**65.12%**

Table-4: Summary of Nei and Li (1979) genetic distance value between five *Mucuna pruriens* var. *utilis* accessions.

Locality	Thachenmalai	Patchenmalai	Seruvalaru	Periyamailaru	Papanasam
Thachenmalai	1				
Patchenmalai	0.8529	1			
Seruvalaru	0.8219	0.8955	1		
Periyamailaru	0.8406	0.8889	0.9118	1	
Papanasam	0.8824	0.8387	0.8060	0.7937	1

RAPD data were used to make pair wise comparison of the accessions based on shared and unique amplification products to generate a similarity matrix with NTSYS-Pc 2.02e. Similarity value for all the five accession ranged from 79.37% to 91.18% (Fig 3). Of the five samples analyzed, two accessions, 'Seruvalaru' and 'Periyamailaru' displayed the maximum genetic similarity, with a 91.18 similarity coefficient value.

The similarity matrix representing Nei and Li (1979) coefficient was used to cluster the data following the UPGMA algorithm. As per fig 3. five accessions into major cluster 'A' and 'B' at a demarcation having 82.19% similarity. Major cluster 'A' comprised of two accessions 'Thatchamalai' and 'Papanasam' and is having 88.24% similarity. The major cluster 'B' comprises of three accessions 'Patchamalai',

'Seruvalaru' and 'Periyamailaru'. 'Seruvalaru' and 'Periyamailaru' were closely related with 91.18% similarity and they together showed a similarity of 89.20% with 'Patchamalai'. The minimum similarity was exhibited by the accessions from 'Periyamailaru' and 'Papanasam' with a similarity value of 79.37% as were belonged to different agro-ecological regions and are geographical far apart from each other.

RAPD techniques have been found to be more useful and accurate for the determination of both inter-specific and intra-specific genetic variations in plants. In particular, RAPD markers have been successfully employed for the determination of intra-specific genetic diversity in several species (Wolfe and Liston, 1998). In contrast, only few reports were available on the determination of inter-specific diversity in plants using the RAPD technique, which yield information about the phylogenetic of the plant (Singh *et al*., 2006; Carlos *et al*., 2006; Ranade *et al*., 2006; Lim *et al*., 2007; Singh *et al*., 2007; Parab *et al*., 2008; Pawel *et al*., 2009; Dhanasekar *et al*., 2010).

Ecological and geographical differentiational are important factors which is influencing strategies for breeding and conservation of the wild species. In this background, various accessions of velvet bean, were collected from various locations as described earlier. To access the genetic diversity of velvet bean samples from different agro-ecological regions in Tamil Nadu, five random primers were used of which gave amplification and of them. These primers generated 43 fragments of which 28 (65.12%) were polymorphic. Goswami and Ranade (1999) reported only 29% polymorphic primers with 357 bands in *Prosopis* species. It has been reported that the ability to resolve genetic variation may be more directly related to the number of polymorphisms detected by the marker system (Sivaprakash *et al*. 2004).

In the present investigation, the average number of fragments amplified by RAPD primers among the accessions were 86.00 with a range of 6 to 10. Ratnaparkhe *et al*. (1995) reported an average of 8 markers per primer in *Cajanus cajan*. However, Maciel *et al*. (2001) reported the generation of RAPD fragments ranging from 7 to 31 in common beans. Such a high variation in the number of fragments produced by these arbitrary primers may be attributed to the differences in the binding sites throughout genome of the accessions included. In RAPD analysis, the velvet bean genotypes were grouped into two sub clusters revealing sufficient amount of diversity within the cluster. Similar results have been found by Dikshit *et al*. (2007) for *Vigna umbellata* accessions and estimated 53 per cent of genetic similarity based on RAPD and SSR markers data also.Inclusion of these accessions for specific objectives like resistance and quality parameters over different agro-ecological regions resulting in narrowing of genetic base. The relationships between the agro-ecological regions are not necessarily reflecting the breeding traits. Molecular markers are scattered throughout the genome and their association with various breeding traits is influenced by the cultivator under selection pressure induced by domestication. Exploration and evaluation of diversity among these agro-ecological would be of great significance for *in situ* conservation and velvet bean breeding programmes.

Acknowledgement

The first author expresses heartfelt thanks to Secretary, V.O Chidambaram Educational Trust, Tuticorin, and also thank to Principal, VOC College, Tuticorin for their valuable helps.

References

Albert, M.J., Bhuiyan, N.A., Talukder, K.A., Faruque, A.S.G., Nahar, S., Faruque, S.M., Ansaruzzaman, M. and Rahman, M. 1997. Phenotypic and genotypic changes in *Vibrio cholerae* 0139 Bengal. *J. Clini. Microbio,* 35: 2588–2592.

Baker, H.G. and Stebbins, G.L. 1965. The Genetics of Colonizing Species. Academic Press, New York, New York, USA.

Bhat, K.V., Babrekar, P.P. and Lakhanpaul, S. 1999. Study of genetic diversity in Indian and exotic sesame (*Sesamum indicum* L.) germplasm using random amplified polymorphic DNA (RAPD) markers. *Euphytica*, 110: 21-34.

Carlos, F.V., Victor, P., Peter, F. and Jorge, L. 2006. Genetic diversity and structure in fragmented populations of the tropical Orchid *Myrmecophila christinae* var *christinae*. *Biotropica*, 38: 754 – 763.

Dhanasekar, P., Dhumal, K.N. and Reddy, K.S. 2010. Identification of RAPD markers linked to plant type gene in pigeonpea. *Indian J. Biotech,* 9: 58 – 63.

Dikshit, H.K., Jhang, T.. Singh, N.K., Koundal, K.R., Bansal, K.C., Chandra, N., Tickoo, J.L. and Sharma, T.R. 2007. Genetic differentiation of *Vigna* species by RAPD, URP and SSR markers. *Biologia Plantarum*, 51: 451-457.

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochem Bull*, 19: 11-15.

Goswami, M. and Ranade. 1999. Analysis of variations in RAPD profiles among accessions of *Prosopis*. *Journal of Genetics*, 78: 141 -147.

Henry, R.J. 1997. *Practical Applications of Plant Molecular Biology*. Published Chapman & Hall, pp. 280.

Lim, A., Yue, G.H., Lim-Ho, C.L. and Hong, Y. 2007. Development and characterization of microsatellites in *Vanda* varieties. *Mol Ecol Notes,* 7: 461 – 463.

Maciel, F.L., Garald, L.T.S. and Echevarrigaray, S. 2001. RAPD markers variability among cultivars and landraces of common beans (*Phaseolus vulgaris* L.) of South Brazil. *Euphytica*, 120: 257-263.

Mohan, V.R. and Janardhanan, K. 1995. Chemical analysis and nutritional assessment of lesser known pulses of the genus *Mucuna*. *Food Chemistry,* 52: 275-280.

Nei, M. and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci USA*, 76: 5269-5273.

Padmesh, P., Reji, J.V., Jinish Dhar, M. and Seeni, S. 2006. Estimation of genetic diversity in varieties of *Mucuna pruriens* using RAPD. *Biologia Plantarum,* 50: 367-372.

Parab, G.V., Krishnan, S., Janarthanan M.K., Sivaprakash, K.R. and Parida, A. 2008. ISSR and RAPD markers assessed genetic variation of *Aerides maculosum* – an epiphytic orchid from Goa, India. *J Plant Biochem Biotech*, 17: 107 – 109.

Pawel, S., Jerzy, A.P. and Dariusz, Z. 2009. RAPD Markers Reveal Genetic Diversity in *Salix purpurea* L. *Crop Sci,* 49: 857-863.

Ranade, S.A., Rana, T.S., Srivastava, A.P. and Nair, K.N. 2006. Molecular differentiation in *Murraya* Koenig ex L. species in India inferred through ITS, RAPD and DAMD analysis. *Curr Sci*, 90: 1253 – 1258.

Ratnaparkhe, M.B., Gupta, V.S., Venmurthy, M.R. and Ranjekar, P.K. 1995. Genetic fingerprinting of pigeonpea (*Cajanus cajan* (L.) Millsp.) and its wild relatives using random amplified polymorphic DNA markers. *Theor. and Appl. Genet,* 91: 893-898.

Saini, M., Singh, S., Hussain, Z. and Yadav, A. 2010. RAPD analysis in mungbean [*Vigna radiata* (L.) Wilczek]: I. Assessment of genetic diversity. *Indian J. Biotech*, 9: 137 –146.

Sambrook, J. and Russell, D.W. 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual,* vol. I. 2nd edition. Cold Spring Harbor Laboratory Press.

Siddhuraju, P. and Becker, K. 2005. Nutritional and antinutritional composition *in vitro* amino acid digestibility, starch digestibility and predicted glycemic index of differentially processed *Mucuna* beans (*Mucuna pruriens* var. *utilis*): An under-utilized legume. *Food Chem*, 91:275-286.

Siddhuraju, P., Becker, K. and Makker, P.S. 2000. Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilised tropical legume, *Mucuna pruriens* var. *utilis*. *J. Agricul. and Food Chem,* 48: 6048 – 6050.

Singh, A.K., Singh, M., Singh, R., Kumar, S. and Kalloo, G. 2006. Genetic diversity within the genus *Solanum* (Solanaceae) as revealed by RAPD markers. *Curr Sci*, 90: 711-716.

Singh, N., Singh, M., Kumar, S., Kumar, R., Singh, V., Prasanna, H.C. and Mathura, R. 2007. RAPD markers for hybrid seed purity testing in tomato (*Solanum lycopersicum* L.). *Curr Sci,* 93: 462 – 463.

Sivaprakash, K.R., Prasanth, S.R., Mohanty, B.P. and Parida, A. 2004. Genetic diversity of black gram landraces as evaluated by AFLP markers. *Curr Sci*, 86: 1411-1415.

Vadivel, V., and Pugalenthi, M. 2008. Effect of various processing methods on the levels of antinutritional constituents and protein digestibility of *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) baker ex burck (Velvet bean) seeds. *J. Food Biochem*, 32: 795-812.

Wolfe, A.D. and Liston, A. 1998. Contributions of PCR-based methods to plant systematic and evolutionary biology. *In plant molecular systematic*. 2: 55 - 63.