International Journal of Genetics
ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 10, Issue 3, 2018, pp.-365-369.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000226

Research Article

GENETIC CONFIRMATION OF MUNGBEAN GENOTYPES (*Vigna radiata* L. WILCZEK) USING MOLECULAR MARKERS

NATH ANAMIKA[1*], MALOO S.R.[1], JAIN DEVENDRA[2], NATH SRIKANTA[3], CHAKMA ASHIM[3] AND VERMA RAJANI[1]

[1]Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, 313001, Rajasthan
[2]Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, 313001,
[3]Department of Agriculture, O/o the Supdt. Of Agriculture, Govt. of Tripura, Gandacherra, 799284, Tripura
*Corresponding Author: Email- anamikanath22@gmail.com

Received: March 31, 2018; Revised: April 03, 2018; Accepted: April 05, 2018; Published: April 15, 2018

Abstract- The present study was undertaken to identify the genetic variation present among different mungbean genotypes. Out of 25 RAPD primers only 17 were amplified. A total of 104 amplified bands, 91 polymorphic, 13 monomorphic bands and 88 % polymorphism. A total of 112 amplified bands were obtained from 18 ISSR primers, out of which 88 polymorphic. The average number of bands per primer was 6.22 and average numbers of polymorphic bands per primer 4.89. The RAPD and ISSR data were evaluated to obtain a combined similarity matrix and cluster tree analysis. The similarity coefficient values lay between 0.46-0.68 and cluster tree analysis showed that the eight genotypes could be divided into 4 clusters. The genotype BM-4 was grouped in separate VI cluster and PDM-139 was grouped on cluster IIA. In the light of RAPD and ISSR study the parents of the cross BM-4 x PDM-139 noticed for their genetic diversity. Here validation of these data compared with phenotypic character in the field and revealed that cross BM-4 x PDM-139 turned out to be the most promising on the basis of its high *per se* performance and also for their high genetic diversity.

Key words- Mungbean, RAPD Marker, ISSR Markers, and yield components.

Citation: Nath Anamika, *et al.*, (2018) Genetic Confirmation of Mungbean genotypes (*Vigna radiata* L. Wilczek) using Molecular Markers**.** International Journal of Genetics, ISSN: 0975- 2862 & E-ISSN: 0975-9158, Volume 10, Issue 3, pp.-365-369. DOI: http://dx.doi.org/10.9735/0975-2862.10.3.365-369

Introduction

India is the largest producer and consumer of pulses in the world accounting 33 per cent of the area and 25 per cent of the global out-put. Green gram [*Vigna radiata* (L.) Wilczek) is the most important legume crop in India after chickpea and pigeonpea. It belongs to family *Leguminaceae*, subfamily *Papillionaceae* [1]and its chromosome number is 2n = 2 x = 22. India is the primary green gram producer and contributes to about 75 per cent of the world pulses production. Green gram is extensively grown in India under varying soil types and climatic conditions and it improves soil fertility by fixing atmospheric nitrogen. It is a small herbaceous annual drought tolerant crop and suitable for dry land farming and predominantly used as intercrop with other crops. It contains about 24 per cent protein of high digestibility and quantity which is about two third of the protein content of soybean, twice that of wheat and thrice that of rice [2,3]. Besides being a rich source of protein, green gram enriches soil fertility through atmospheric nitrogen fixation with the help of rhizobium bacteria in nodules and humus thus, plays a crucial role in furthering sustainable agriculture.

For any successful breeding programme to improve grain yield and component characters, it is essential to know precisely the genetic architecture of these characters under prevailing conditions. Morphological markers used for diversity studies on different crop cultivars or crop varieties are not adequate, cause these markers are subject to environmental influences, whereas the DNA based molecular markers have proven better. In contrast to the morphological markers, molecular markers, are now available in plant system involves improvement in the efficiency of conventional plant breeding by carrying out indirect selection through QTL, RAPD and ISSR techniques that provide a new alternative for cultivar identification [4-7]. Ever since thermo stable DNA polymerase was introduced in 1988, the use of PCR [8,9] in research has increased tremendously. The DNA markers become the marker of choice for the study of crop genetic diversity, especially those based on DNA sequence variations which are increasingly being utilized in crops for construction of genetic maps and marker-assisted selection studies. Application of molecular markers to plant breeding has established the need for information on variation in DNA sequence even in those crops in which little classical genetic and cytogenetic information is available. The present investigation was carried out to analyse relatedness and diversity among eight mungbean parents and find out the best hybrid.

Material and Methods

Final experimental trial comprising 8 parents along with 28 F_1s was evaluated during *kharif,* 2014 in randomized block design with three replications at RCA college farm, MPUAT, UDAIPUR. From 21 no of green gram genotypes, eight diverse genotypes were selected as parents for 8 x 8 diallel crossing programme, these are IPM-99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045 [Table-1].

Molecular analysis using RAPD and ISSR markers was done exclusively for the parental material only [10]. Molecular marker analysis was done for the parental material to see the diversity present among the parental material. DNA extracted from different green gram cultivars were compared using RAPD and ISSR methodology. The leaves were harvested after 21 days and DNA was isolated with the help of [11] given protocol. DNA was extracted from young leaves around 3–4 weeks old using CTAB method. The amplified samples were separated on

agarose gel electrophoresis (1.2%).

Table-1 *Experimental material and their pedigree*

Parent	Pedigree	Source
IPM 99-125	PM 3 x APM 36	IIPR, Kanpur
BM 4	MUTANT of T44	ARS, Badnapur
ML 131	ML 1 x ML 23	ARS, Durgapura
IPM 02-03	IPM 99-125 x Pusa bold 2	IIPR, Kanpur
PDM 139	ML 20/19 x ML 5	IIPR, Kanpur
RMG 1035	RMG 492 x ML 818	ARS, Durgapura
RMG 344	MOONG SEL.1 x J 45	ARS, Durgapura
RMG-1045	RMG-62 x KM 2170	ARS, Durgapura

The details of the technique of DNA isolation, RAPD and ISSR are as given below: The DNA content in 20 µl of the reaction mixture was 50 ng. The reaction contained 10X reaction buffer, 200 µM each of dNTPs, 0.5 µM of each primer and 1 unit of Taq DNA polymerase [Table-2].

Table-2 *PCR reaction mixture content*

Components	Final concentration	Single tube/20 (µl)
DNA template	50ng	2.00 µl
Master Mixture		
(i) dNTP MIX	200µM	1.6 µl
(ii) Taq polymerase	1 U	0.33µl
(iii) Reaction buffer (10x)	1X	2.00 µl
(iv) Primer	0.5 µM	1.00µl
(vi) dd H_2O		12.07µl

Submerged gel electrophoresis unit was used for fractionating amplified PCR products on 1.2% agarose gel. The gel was prepared in 1X TAE buffer containing 0.5 µg/ml of ethidium bromides. Bands were designated on the basis of their molecular size ranging between 100-1000 bp. Electrophoresis was carried out at 100 V for 3 hr in 1X TAE electrophoresis buffer. The details of operon code sequence of the primer and G:C contents are given in [Table-3].

Table-3 *PCR reaction cycle*

Cycle	Denaturation		Annealing		Extension	
First cycle	94°C	5 min	-	-	-	-
2-35 Cycle	94°C	1 min	Tm (Pr)	45 sec	72 °C	2 min
Last cycle	-	-	-	-	72°C	10min

Gel was viewed under UV transilluminator and photographed by gel documentation system. Presences of amplified product were scored as 1 and its absence as 0 for all genotypes and primer combinations. These data matrices were then entered into NTSYS-PC [12]. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the genotypes using computer program NTSYSpc version 2.02.

Result and Discussion

The RAPD and ISSR techniques are more informative for estimating the extent of genetic diversity and relationships between green gram varieties. So far, very little attention has been given to varietal improvement of legumes [13]. The present study aimed to analyze the extent of genetic diversity, using a total of 25 RAPD and 25 ISSR primers, respectively, to generate DNA fingerprints of eight parents of *V. radiata* L. with a view to detect polymorphism and access to information on diversity among these genotypes. The present investigation was carried out to analyse relatedness and diversity among eight parents *viz.* IPM 99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045 [Table-4]. 757 to 1518 ng/µl amount of DNA was isolated from different genotypes of green gram. The genotype RMG-344 yielded the lowest amount of DNA (757ng/µl) and IPM 02-03 yielded the highest amount of DNA (1518 ng/µl). Whereas the lowest amount of DNA (757ng/µl) was obtained from genotype RMG-344. The ratio of absorbance (A260/A280) ranged from 1.70 to 1.89 revealing that the DNA obtained was free from contaminants like polysaccharides, protein and RNA. The quality of DNA as also checked by gel electrophoresis revealed a single discrete band in all genotypes showing that genomic DNA was intact and had high molecular weight, free from any mechanical or enzymatic degradation, free from RNA contamination and was of high quality.

Table-4 *Quality and quantity of total genomic DNA of V. radiata L. isolated and purified by CTAB method*

Genotypes	Parents' Name	Concentration (ng/ µl)	Ratio 260/280
P1	IPM 99-125	1420	1.81
P2	BM-4	968	1.77
P3	ML-131	1250	1.79
P4	IPM 02-03	1518	1.89
P5	PDM-139	1251	1.80
P6	RMG-1035	1012	1.81
P7	RMG-344	757	1.74
P8	RMG-1045	998	1.82

RAPD has been used extensively for classification of varieties, identification of cultivars and diversity estimation in various crops such as green gram [14]. Similarly, ISSR markers are useful in detecting polymorphism among accessions by generating a large number of markers that target multiple microsatellite loci distributed across the genome [15].

Optimization of PCR Conditions for RAPD and ISSR Analysis

PCR amplification conditions such as concentration of template DNA, primers, concentration of $MgCl_2$, *Taq* DNA polymerase and annealing temperature were optimized for RAPD and ISSR primers. Reproducible and clear banding patterns were obtained in a reaction mixture of 20 µl containing 50 ng of template DNA, 2 µl of 10 X *Taq* DNA polymerase buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.30 µM of primer and 1 U of *Taq* DNA polymerase, at an annealing temperature of 37°C (RAPD) and 42.9°-67° C (ISSR) for PCR amplification. Similarly, optimization of the concentration of template DNA, $MgCl_2$, *Taq* polymerase and of primers were found similar [16]. Out of 25 RAPD primers only 17 were amplified. A total of 104 amplified bands were obtained of which 91 were polymorphic and 13 monomorphic that showed 88 % polymorphism [Table-5]. The total number of amplified bands varied between 5 and 8.

Table-5 *Details of the RAPD and ISSR primers used for amplification of DNA in green gram*

DNA primers	RAPD	ISSR
Total number of primers	25	25
Number of primers which showed amplification	17	18
Number of primer which showed polymorphism	17	18
Total number of monomorphic bands	13	21
Total number of polymorphic bands	91	88
Total number of bands	104	109
Total number of amplicon produced	391	563

The average number of bands per primer was found to be 6.12 and average numbers of polymorphic bands per primer were 5.35. The polymorphism amongst all genotypes of *V. radiata* L. was 88% and the overall size of PCR amplified products ranged between 100 bp to 2500 bp. The per cent polymorphism ranged from as low as 60 % (OPA-15 and OPB-06) to as high as 100 % (OPA-09, OPA-10, OPA-08, OPB-03, OPB-07, OPE-03, and OPA-16). A total of 112 amplified bands were obtained from the 18 ISSR primers, out of which 88 were polymorphic [17-19]. The total number of amplified bands varied between 5 and 8 [Table-7]. Average polymorphism across all the genotypes of *V. radiata* L. was found to be 79%. Overall size of PCR amplified products ranged between 100 bp to 2000 bp [20-22].

Similarity Matrix for Combined RAPD and ISSR Markers

Perusal of the combined RAPD and ISSR similarity matrix data revealed that the

values for different genotypes ranged from 0.42-0.68 [Table-6]. The average similarity value across the genotypes was found out to be 0.55, indicating that there is sufficient genetic diversity among the genotypes. The genotypes that exhibited the highest similarity matrix values (0.68) are RMG-1035 and RMG-44; RMG-1035 and RMG-1045. However, BM-4 and IPM 02-03 were found to be genetically diverse with a minimum similarity value of 0.42 [23-25].

Table-6 *Similarity matrix of green gram genotypes*

	IPM-99-125	BM-4	ML-131	IPM-02-03	PDM-139	RMG-1035	RMG-344	RMG-1045
IPM-99-125	1.00							
BM-4	0.45	1.00						
ML-131	0.56	0.44	1.00					
IPM-02-03	0.48	0.42	0.49	1.00				
PDM-139	0.44	0.47	0.52	0.45	1.00			
RMG-1035	0.48	0.48	0.58	0.46	0.60	1.00		
RMG-344	0.50	0.47	0.59	0.47	0.60	0.68	1.00	
RMG-1045	0.48	0.47	0.49	0.44	0.59	0.68	0.62	1.00

Table-7 *List of RAPD and ISSR primers*

Sl No	ISSR Primer	Sequence (5'-3')	Total No of bands (a)	Total no. of polymorphic bands (b)	Polymorphism % (b/a X 100)	Range of band size
			ISSR Primer			
1	ISSR-01	(GGC)5AT	8	8	100	100-1500
2	ISSR-02	(AAG)5GC	7	4	57	200-2000
3	ISSR-03	(AAG)5TC	NA	NA	NA	
4	ISSR-04	(AAG)5CC	5	3	60	100-700
5	ISSR-05	(AGC)5CA	7	6	86	200-2000
6	ISSR-06	(AGC)5CG	NA	NA	NA	
7	ISSR-07	(GGC)5TA	8	6	75	100-1500
8	ISSR-08	(AGC)5GA	8	7	88	100-1000
9	ISSR-09	(AAG)5CG	5	3	60	100-700
10	ISSR-33	(AG)8AT	NA	NA	NA	
11	UBC-810	(GA)8T	7	4	57	300-1000
12	UBC-811	(GA)8C	7	6	86	300-1000
13	UBC-813	(CT)8T	NA	NA	NA	
14	UBC-817	(CA)8A	5	5	100	200-600
15	UBC-818	(CA)8G	6	6	100	200-1000
16	UBC-820	(GT)8T	5	5	100	100-700
17	UBC-822	(TC)8A	7	5	71	100-1500
18	UBC-824	(TC)8G	NA	NA	NA	
19	UBC-836	(AG)8YA	5	4	80	300-900
20	UBC-840	(GA)8YT	NA	NA	NA	
21	UBC-845	(CT)8RG	7	3	43	200-600
22	UBC-848	(CA)8RG	5	4	80	300-1000
23	UBC-854	(TC)8RG	6	6	100	200-1500
24	UBC-873	(GATA)4	NA	NA	NA	
25	UBC-878	(GGC)5AT	4	3	75	500-2000
			RAPD Primer			
1	OPA-02	TGCCGAGCTG	7	6	86	200-1000
2	OPA-05	AGGGGTCTTG	6	5	83	300-2000
3	OPA-07	GAAACGGGTG	7	6	86	300-1000
4	OPA-08	GTGACGTAGG	7	7	100	400-2000
5	OPF-19	CCTCTAGACC	6	4	67	200-1500
6	OPP-03	CTGATACGCC	5	4	80	300-1500
7	OPB-06	TGCTCTGCCC	5	3	60	100-900
8	OPA-10	GTGATCGCAG	6	6	100	200-1000
9	OPP-10	TCCCGCCTAC	8	8	100	200-1500
10	OPA-11	CAATCGCCGT	6	5	83	400-1500
11	OPA-14	TCTGTGCTGG	NA	NA	NA	-
12	OPA-15	TTCCGAACCC	5	3	60	400-1000
13	OPC-01	TTCGAGCCAG	NA	NA	NA	-
14	OPB-03	CATCCCCCTG	6	6	100	100-1500
15	OPA-09	GGGTAACGCC	7	7	100	200-2500
16	OPB-07	GGTGACGCAG	6	6	100	300-1000
17	OPC-05	GATGACCGCC	NA	NA	NA	-
18	OPE-03	CCAGATGCAC	5	5	100	400-1500
19	OPA-16	AGCCAGCGAA	6	6	100	400-2000
20	OPC-06	GAACGGACTC	NA	NA	NA	-
21	OPB-02	TGATCCCTGG	6	4	67	400-2000
22	OPB-04	GGACTGGAGT	NA	NA	NA	-
23	OPB-05	TGCGCCCTTC	NA	NA	NA	-
24	OPB-08	GTCCACACGG	NA	NA	NA	-
25	OPB-10	CTGCTGGGAC	NA	NA	NA	-

RAPD and ISSR Markers Based Combined Cluster Tree Analysis

The average linkages between *V. radiata* L. genotypes were used for constructing a tree depicting the phylogenetic relationship among eight *V. radiata* L. genotypes. The RAPD and ISSR data were evaluated to obtain a combined similarity matrix [Table-7]. The similarity coefficient values lay between 0.46-0.68 [26, 27]. The RAPD and ISSR cluster tree analysis showed that the eight genotypes could be divided into 4 clusters [Fig-2]. Cluster I included two genotypes *viz.,* IPM 99-125 and ML-131 that were similar to each other at a coefficient of 0.56. Cluster II included two sub clusters, sub cluster II A included genotype PDM-139 and II B divided in two sub clusters, genotypes RMG-1035 and RMG-344 included in sub clusters IIB 1 at similarity coefficient 0.68, while sub clusters IIB 2 has only one genotype RMG-1045. Cluster III and cluster IV included with each other at similarity coefficient 0.46 [19,21,28-31].

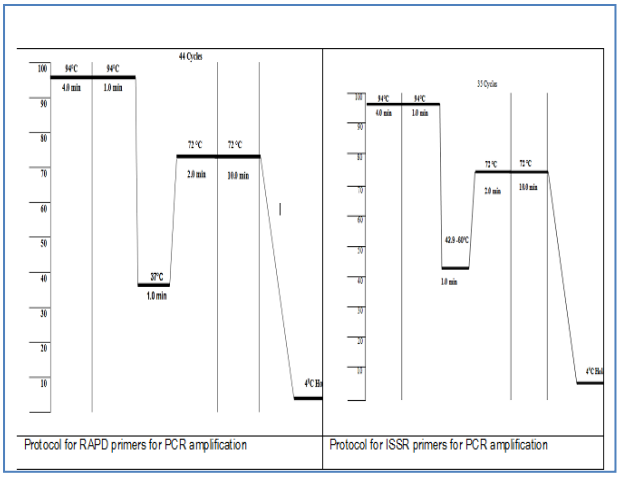

Fig-1 Protocol used for PCR amplification (standardize protocol at institution laboratory)

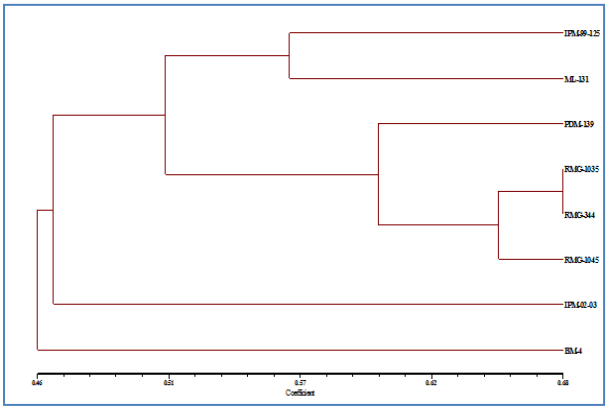

Fig-2 Dendrogram of greengram genotypes using RAPD and ISSR markers

Conclusion

Molecular analysis through RAPD and ISSR markers revealed that cross BM-4 x PDM-139 turned out to be the most promising on the basis of its high *per se* performance both for seed yield and its components. The parent BM-4 was grouped in cluster II A while PDM-139 in cluster IV thereby confirming that there was concurrence between the results obtained by molecular (RAPD and ISSR) and morphological markers along with their known pedigree. Therefore, this study suggested that this cross can be gainfully utilized for plant breeding programme.

Application of research: This research finding will help to know the diversity present among the selected green gram genotypes, can choose the best parents for cross combination, as well as F_1 hybrid, which can be used for further breeding programme.

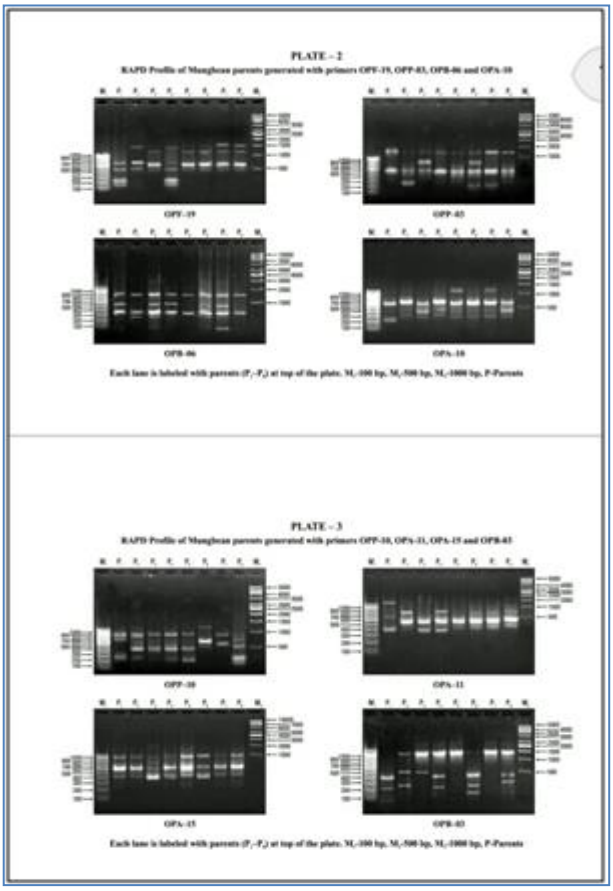

Fig-3 PCR profiles of mungbean genotypes using RAPD markers

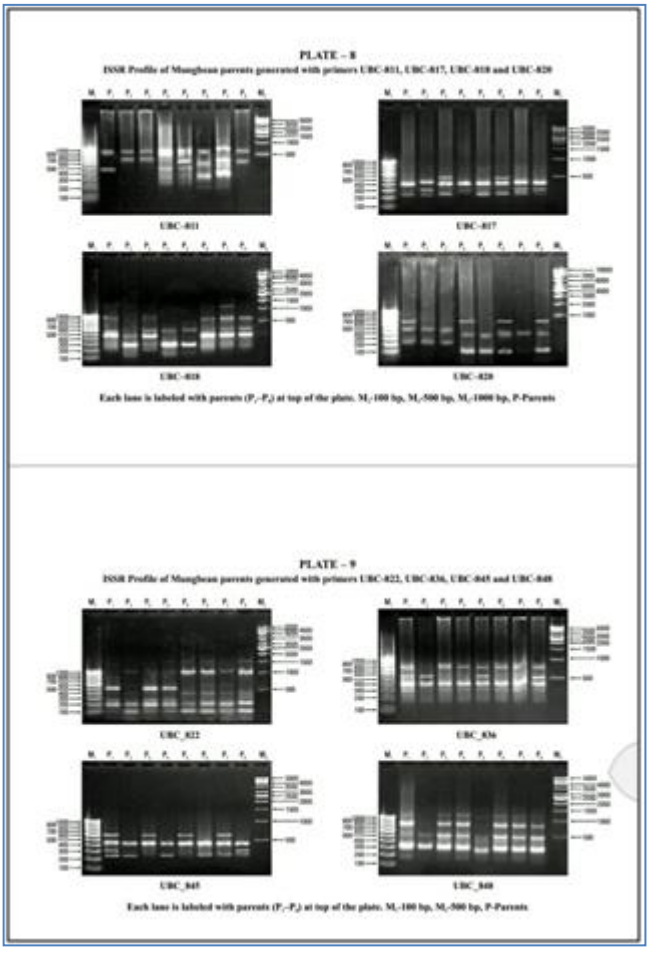

Fig-4 PCR profiles of mungbean genotypes using ISSR markers

Abbreviations

RAPD-Random Amplified Polymorphic DNA,
ISSR- Inter Simple Sequence Repeat,
PCR-Polymerase chain reaction
CTAB- Hexadecyltrimetyl ammonium bromide

Acknowledgement / Funding: The Author thankful to department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, 313001, Rajasthan. The authors would like to acknowledge Dr S. R. Maloo, Former Dean, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, 313001, Rajasthan for help in conducting the research work successfully.

***Major Advisor of Research: Dr S. R. Maloo**
University: Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan 313001
Research project name: PhD Research work

Author Contributions: All author equally contributed

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Kannaiyan S. (1999) Bioresources Technology for Sustainable Agriculture, Associated Publishing Company, New Delhi, 422.
[2] Singh G., Kapoor S. and Singh K. (1988) *AVRDC*, VI, 290-296.
[3] Thirumaran A. S. and Seralathan M. A. (1988) Proceeding of the second international symposium. Taiwan, AVRDC, 470-485.
[4] Gunter L.E., Tuskan G.A. and Wullschleger S.D. (1996) *Current Science*, 36, 1017-1022.
[5] Lashermes P., Trouslot P., Anthony F., Combes M.C. and Charrier A. (1996) *Euphytica*, 87, 59-64.
[6] Bouchired N. (1997) 3rd International Symposium on Mineral Nutrition of Deciduous Fruit Tree, *Acta Horticulturae,* 448.
[7] Colombo C., Second G. and Charrier A. (2000) *Genetics and Molecular Biology*, 23, 189-199.
[8] Mullis K.B., Faloona F.A., Scharf S., Saiki R., Horn G. and Erlich H. (1986) *Cold* Spring *Harbor Symposium Quantitative Biology*, 51, 263 – 273.
[9] Mullis K.B. and Faloona F.A. (1987) *Methods of Enzymology*, 255, 335 – 50.
[10] Kanazin V., Marek L. and Shoemaker R. (1996) *Proc. Natl. Acad. Sci*., 93, 11746-11750.
[11] Doyle J. J. and Doyle J. L. (1987) *Phytochemical Bulletin*, 19,11-15.
[12] Rohlf F.J. (1993) NTSYS-PC. Numerical taxonomy and multivariate analysis system. Exeter Software, New York
[13] Nisar M., Ghafoor H., Ahmad K. M., Rashid A. S., Qureshi H. and Ali H. (2008) *Pakistan Journal of Botany,* 40(5), 2081-2088.
[14] Karuppanapandian T., Karuppudurai T., Sinha P.B., Haniya A.H. and Manoharan K. (2006) *African Journal of Biotechnology*, 5, 1214-1219.
[15] Reddy M.P., Sarla N. and Siddiq E.A. (2002) *Euphytica*, 128, 9-17.
[16] Khamassi K., Khoufi S., Chaabane R., Da Silva J. A. T. and Naceur M. B. (2011) *International Journal of Plant Breeding,* 12, 141-145.
[17] Das S. (Sur), Das S. S. and Ghosh P. (2014) *Advances in Applied Science Research*, 5, 93-97
[18] Datta S., Gangwar S., Kumar S., Gupta S., Rai R., Kaashyap M., Singh P., Chaturvedi S.K., Singh B.B. and Nadarajan N. (2012) *American Journal of Plant Sciences*, 3, 1085-1091.
[19] Saini M., Singh S. Hussain Z. and Sikka V.K. (2010) *Indian Journal of Biotechnology*, 09, 276-282.
[20] Singh R., Heusden A. W. V. and Yadav R. C. (2011) *African Journal of Biotechnology,* 12(47), 6574- 6582.
[21] Tantasawat P., Trongchuen J., Prajongjai T., Thongpae T., Petkhum C., Seehalak W. and Machikowa T. (2010) *African Journal of Biotechnology,* 9, 4152-4164.
[22] Undal V. S., Thakare P. V., Chaudhari U. S., Deshmukh V. P. and Gawande P. A. (2011) *Annals of Biological Research*, 2, 348-354.
[23] Chattopadhyay K., Ali M.N., Sarkar H.K., Mandal N. and Bhattacharyya S. (2005) *Indian Journal Genetics*, 65, 172-175.
[24] Datta J. and Lal N. (2011) *Advanced Biotech,* 11, 39-44.
[25] Singh M., Devi E. L., Aglawe S., Kousar N. and Behera C. (2013) *The Bioscan*., 8(4), 1393-1401.
[26] Pratap N., Shekhar R., Singh P. K. and Soni S. K. (2013) *The Bioscan*, 8(4), 1521-1528.
[27] Singh R. Heusden A. W. van and Yadav R. C. (2013) *African Journal of Biotechnology,* 12,6574-6582.
[28] Bhareti P., Singh D. P. and Khulbe R. K. (2012) *Journal of Food Legumes,* 25, 89-93.
[29] Dikshit H. K., Sharma T. R., Singh B. B. and Kumari J. (2009) *Journal of Genetics*, 88, 341-344.
[30] Lavanya G.R., Srivastava J. and Ranade S.A. (2008) *Journal of Genetics,* 87, 65–74.
[31] Singh S. K., Suresh B. G. Lavanya G. R., Lalit Arya Bhat K. V., Hussain Z. and Verma M. (2014) *Indian Journal of Agricultural Sciences*, 84, 534-539.