Kaur et al., Vegetos 2016, 29:3
DOI: 10.5958/2229-4473.2016.00067.7

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

Assessment of Genetic Diversity in Mungbean Genotypes using ISSR Markers

Gunnjeet Kaur, Arunabh Joshi, Devendra Jain*, Deepak Rajpurohit and Divya Vyas

Abstract

Molecular assessment of genetic diversity was done for 23 mungbean (*Vigna radiata* (L.) Wilczek) genotypes using ISSR marker system. Twenty ISSR primers were used in the study out of which thirteen primers enabled DNA amplification in all genotypes. A total of 75 amplified and reproducible amplicons were obtained from 13 primers, out of which 60 were found polymorphic. The total number of amplified bands varied between 2 (UBC-813) and 11 (UBC-810) with an average of 6.5 per primer. The overall size of PCR amplified products ranged between 200 to 2500 bp. PIC values ranged from 0.07 to 0.35 with an average value of 0.208 across all genotypes. Four unique bands were detected in four genotypes with 3 (UBC-818, UBC-820 and UBC-826) out of 13 ISSR primers. The genotype IC-393407 gave maximum number of distinct bands. The similarity indices between the 23 genotypes ranged from 0.38 to 0.94. The extent of diversity among genotypes was also estimated in relation to their source and a set of genotypes with narrow genetic bases developed from various region were identified. Based on a dendrogram generated through UPGMA method and PCA, most of the genotypes could be divided into two main clusters. Cluster I included twenty genotypes, while cluster II included three genotypes. The genotype GM-9925 lay apart from all the two clusters. A minimum similarity co-efficient of 0.38 was observed between genotypes GM-9925 and EC-398885 thereby indicating maximum genetic divergence. The Mantel statistical analysis (r = 0.92) also supported cluster analysis.

Keywords

Dendrogram; ISSR; Mantel test; Similarity coefficient

Introduction

Mungbean contributes to about 14% of total pulses cultivation area and 7% of total pulses production in India. Maharashtra, Rajasthan, Madhya Pradesh, Bihar, Punjab and Andhra Pradesh are the leading producers of mungbean. Pulses are grown in nearly 25.4 million hectare area in the country with production status of nearly 19.66 million tones, on an average productivity level of 770 kg/ha [1]. As of the 2012-13 cultivation statistics, green gram was grown on 2.75 million hectares with a production status of 1.19 million tonnes and yield of 436 kg/ha [1]. In Rajasthan, it was grown on 796.9 million hectares with a production status of 241.2 million tonnes and yield of 303 kg/ha [1].

***Corresponding author:** Devendra Jain, Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India, Tel: 91 294 247 1302; E-mail: devendrajain@mpuat.ac.in

Received: June 14, 2016 **Accepted:** July 19, 2016 **Published:** July 25, 2016

Mungbean belongs to the family *Fabaceae (Leguminoceae)*. The roots of this legume has the ability to fix atmospheric nitrogen @30-50 kg/ha. It is a diploid with the chromosome number, 2n= 22 [2]. It is grown primarily as an intercrop with wheat, maize, potato, etc during *kharif* and as monocultures during rabi and zaid. On account of its short duration, photo-insensitivity and dense crop canopy, it assumes special significance in crop intensification, diversification, and conservation of natural resources as well as sustainability of the production system. Mungbean is an excellent source of high quality protein [3]. Its seed contains 24.2% protein, 1.3% fat and 60.4% carbohydrate. The seeds are also rich in Vitamin C, Vitamin B, folate and mineral nutrients eg. calcium, magnesium, phosphorous, potassium etc. Although germplasm collection within India is quite large, much diversity has not been reported on the basis of morphological characters. Therefore, an urgent need to identify genetic divergence based on molecular analysis is an essential pre-requisite for further utilization in crop improvement programmes.

The PCR based ISSR marker system allows DNA amplification at regions located between two closely spaced, oppositely oriented SSR sequences, resulting in a reproducible pattern of genomic fragments. Thus, ISSR markers show high level of polymorphism, bearing high potential for genetic diversity evaluation at inter and intra-specific levels [4]. Since knowledge of genetic diversity is essential for crop improvement programmes, molecular marker based analyses are in rapidly using in many related research areas [5]. The ISSR technique is useful in studies on genetic diversity, phylogenetic studies, gene tagging and genome mapping in a wide range of legume crops. In the present the genetic diversity of 23 genotypes of green gram were estimated using ISSR.

Material and Method

Plant material and DNA isolation

In the present investigation seeds of 23 genotypes of green gram were procured from Agriculture Research Station (ARS), Durgapura, Jobner Agriculture University, Jaipur. Source details of the materials used are given in Table 1. Genomic DNA was isolated and purified from young leaves of 21-28 day old seedlings [6] and stored at -20^{0}C.

ISSR-PCR amplification

A total of 20 primers identified by the University of British Columbia (UBC) were synthesized from Bangalore Genei Pvt. Ltd., Bangalore, India and used for ISSR-PCR optimization trials.Thirteen primers, which gave the best amplification results with the sample DNA, were selected for final ISSR-PCR analysis. PCR amplification was carried out in a 20-µL reaction volume containing 200 µM of dNTP mix, 1U of *Taq* polymerase, 1.5 mM $MgCl_2$, 1X reaction buffer, 0.5 µM of primer and double distilled water with 25 ng genomic DNA. The amplification was performed in a Eppendrof Master cycler, with reaction conditions programmed as initial predenaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 42.9-60°C for 1 min, and extension at 72°C for 2 min with a hold temperature of 4 °C. A final extension was done for 10 min at 72°C. Amplicons were separated by electrophoresis on 1.2% agarose gels stained with ethidium bromide run at constant voltage

doi:10.5958/2229-4473.2016.00067.7

Table 1: The origin, latitude, longitude and city of 23 genotypes.

S.No.	Origin	Latitude	Longitude	Genotype	City
1	Delhi	28.38	77.12	1. PUSA-672 2. IC- 393407	IARI NBPGR
2	Maharashtra	20.00	76.00	1. AKM- 962	PKV, Akola
3	Utrakhand	30.15	79.15	1. UPM-02-18 2. GBPAU	GBPAU, Pantnagar GBPAU, Pantnagar
4	Punjab	30.4	75.5	1. ML-729 2. MG- 331	PAU, Ludhiana Gurdaspur, Punjab
5	Taiwan	23.50	121.00	1. EC-398885 2.PRATEEKSHA-NEPAL	AVRDC, Taiwan AVRDC, Taiwan
6	Haryana	30.30	74.60	1. ASHA	HAU, Hisar
7	Gujarat	23.00	80.00	1.GM- 9925	S.K. Nagar
8	Rajasthan	27.00	74.60	1. RMG-62 2. RMG- 353	RAU, Durgapura RAU, Durgapura
9	U.P.	27.40	80.00	1. IPM- 02-01 2. IPM-02-3 3. IPM-02-14 5. IPOI-1539 6. PDM-288 7. MEHA (IPM -99-125) 8. SAMRAT (PDM-139) 9. HUM-1 10. HUM-12	IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur IIPR, Kanpur BHU, Varanasi BHU, Varanasi

(3V/cm of gel) till bromophenol blue/loading dye migrated to other end of the gel [7]. The gel was visualized on a UV-transilluminator and photographed using gel documentation system (Alpha Innotech Corporation). Only clear, reproducible and unambiguous bands were scored. The size of the amplified bands of ISSR were observed based on its migration and confirmation relative to standard molecular size markers (100 bp DNA ladder and 1 kb DNA ladder obtained from Bangalore Genei Pvt. Ltd., Bangalore, India).

Data analysis

Amplified bands generated from ISSR -PCR amplification were scored based on the presence (1) or absence (0) of bands for each primer and were used to calculate a genetic similarity matrix employing the SMC using NTSYS-pc version 2.1 [8]. Cluster analysis was performed on both morphological and molecular data using the unweighted pair group method using arithmetic means algorithm (UPGMA), from which dendrograms depicting similarity amongst the genotypes were drawn and plotted using NTSYS-pc. The cophenetic correlation was calculated to find the degree of association between the original similarity matrix and the tree matrix in both morphological and molecular analyses. Mantel test [9] was performed for these genotypes by calculating the correlation between data sets in NTSYS-pc. Using the same software, PCA was also calculated to identify any genetic association amongst the genotypes.

Polymorphism information content (PIC)

To measure the polymorphism information of ISSR marker system the PIC was calculated according to following formula: PIC = 1-Σpi^2 where, N= total number of allele detected for a locus of a marker, Pi= frequency of the 1st allele [15].

Results and Discussion

Thirteen out of the twenty ISSR primers used in the present investigation, thirteen showed amplification in all genotypes. A total of 75 amplified bands were obtained from them, out of which 60 were found polymorphic. The total number of amplified bands varied between the second (UBC-813) and eleventh (UBC-810) with an average of 6.5 bands per primer (Table 2). The polymorphism percentage ranged from 37.5% (UBC-836) to 100% for six primers (UBC-813, UBC-815, UBC-818, UBC-820, UBC-826 and UBC-878) used. Average polymorphism across all the 23 genotypes of *V. radiata* L. was found to be 80%. The overall size of PCR amplified products ranged between 250 to 2500 bp. The PCR amplification using ISSR primers resulted in reproducible amplification products. The number of potential ISSR markers depends on the genotypes and the frequency of microsatellites, which tends to change with species and the SSR motifs that are targeted [10]. Minimum polymorphic frequency ranged between 0.00-0.38 found with UBC-836 where as maximum between 0.08-0.47 with UBC-810. The average PIC was 0.208 ranging from 0.07 to 0.35. The lowest and highest PIC values were recorded for primers UBC-836 and UBC-810, respectively (Table 2). Similar results were shown by Das et al. [11] who used ten primers that amplified a total number of 353 bands under 93 loci across five genotypes of mungbean with an average of 9.3 loci / primer, exhibiting an overall polymorphism of 52.7%. Singh et al. [12] also studied ISSR markers that were used to study DNA polymorphism in elite mungbean genotypes. They found that percentage polymorphism had ranged from 25% (UBC844) to 85% (UBC846, UBC864, UBC895), with an average value of 58.3% across all the genotypes. Tantasawat et al [13] measured genetic diversity and relatedness in 17 mungbean (Vigna radiata (L.) Wilczek) and 5 blackgram (Vigna mungo (L.) Hepper) genotypes by ISSR analysis.. The 18 ISSR primers had produced 341 scorable fragments of which 309 fragments were found to be polymorphic (90.6%). Percentage polymorphism ranged from 66.7% (ISSR 856) to 100% (ISSR 811, 836, 885), with an average value of 89.51%, across all the genotypes.

Four unique bands were detected in three genotypes *viz.,* IC-393407, PUSA-672 and GM-9925 with 3 ISSR primers (UBC-818, UBC-820 and UBC-826). The genotype IC-393407 gave maximum number of distinct bands *i.e.,* 2. The size of these unique bands ranged from 300-950 bp (Table 2). Amplification profiling of 23 genotypeswith two ISSR primers viz., UBC-810 and UBC-822 is shown in Figure 1.

doi:10.5958/2229-4473.2016.00067.7

Table 2: Characteristics of DNA profiles generated in *V. radiata* using 13 ISSR primers.

Primer Code	Squence	Annealing Temp (°C)	Molecular weight range (bp)	Total number of bands amplified (x)	Number of polymorphic bands (y) (%polymorphism)	Unique band	Frequency of polymorphic bands	PIC
UBC-810	$(GA)_8T$	42.9	1800-200	**11**	10(90.90)	**0**	**0.08-0.47**	**0.35**
UBC-813	$(CT)_8T$	43.3	1000-400	**02**	2(100)	0	0.15-0.28	0.22
UBC-815	$(CT)_8G$	44.9	2200-900	03	3(100)	0	0.08-0.34	0.19
UBC-817	$(CA)_8A$	52	1000-400	04	2(50)	0	0.00-0.49	0.14
UBC-818	$(CA)_8G$	52	700-350	04	4(100)	1	0.08-0.28	0.13
UBC-820	$(GT)_8T$	50	1000-450	06	6(100)	2	0.08-0.42	0.24
UBC-822	$(TC)_8A$	45	2500-300	08	7(87.5)	0	0.00-0.47	0.23
UBC-826	$(AC)_8C$	52	1400-300	07	7(100)	1	0.08-0.49	0.34
UBC-836	$(AG)_8YA$	43.3	1600-250	08	3(37.5)	**0**	**0.00-0.38**	**0.07**
UBC-840	$(GA)_8YT$	45	1100-300	05	3(60)	0	0.00-0.22	0.12
UBC-848	$(CA)_8RG$	55.5	1500-250	07	4(57.14)	0	0.00-0.42	0.17
UBC-873	$(GACA)_4$	45	2100-600	07	6(85.71)	0	0.00-0.42	0.25
UBC-878	$(GGAT)_4$	60	900-400	03	3(100)	0	0.22-0.34	0.26
Total				**75**	**60(80%)**	**4**		**0.208**

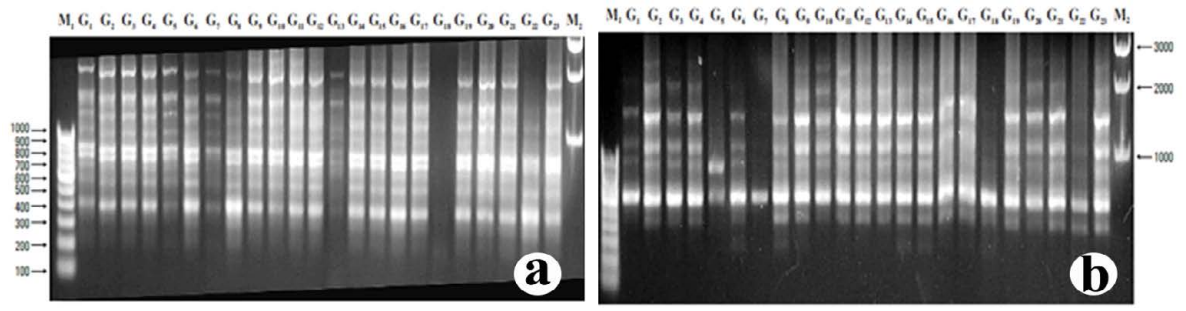

Figure 1: ISSR profiles of *Vigna radiata* genotypes generated through (a) UBC-810 (b) UBC-822. G1-G23 represents G1-PUSA672; G2-AKM962; G3-UPM02-18; G4-ML-729; G5-EC-398885; G6-IPM-02-1; G7- IPM-02-03; G8-IPM02-14; G9-IPOI-1539; G10-RMG-62; G11-PDM-288; G12-RMG-353; G13-PRETEEKHSA-NEPAL; G14-MEHA; G15-PANT; G16-ASHA;G17-MG331;G18-GM9925, G19-IC-393407; G20-DRA-24; G21-SAMRAT; G22-HUM-1 and G23-HUM-12. M1-100 bp DNA Ladder and M2- 1000 bp DNA Ladder.

Similarity Matrices based on ISSR markers

ISSR similarity matrix values of 23 *V. radiata* L. genotypes revealed a close relationship amongst them. The similarity indices between genotypes ranged from 0.38 to 0.94 *i.e.* 38-94% or the genetic diversity ranged from 6 to 62% (Table 3). The average similarity across all the 23 genotypes was found out to be 0.66, showing that all genotypes were moderately similar to each other. Maximum similarity value of 0.94 was observed between genotypes SAMRAT and DRA-24 , HUM-12 and DRA-24 ,MG-331 and ASHA , ASHA and RMG-232,DRA-24 and PDM-288 ,HUM-12 and PDM-288 followed by RMG-62 and IPOI-1539, SAMRAT and RMG-62, PANT and RMG-535 and ASHA and PANT, MG331 and PANT with similarity coefficient of 0.93. Genotypes GM-9925 and EC-398885 were found to be genetically diverse with a minimum similarity value of 0.38 followed by GM-9925 and AKM-962, GM-9925 and IPM99-125, MEHA and IPM 99-125 with a similarity value of 0.53.

Similar findings are reported by Das et al. [11] in mungbean cultivars. The value of Jacaard's similarity coefficient ranged from 0.566 to 0.793. Singh et al. [12] found similar results in 30 mungbean genotypes through Dice analysis, the similarity coefficient ranging from 0.65 to 0.85 with an average of 0.69.

According to Jaccard's similarity coefficient genetic similarity between groups of genotypes of common geographical origin has also been evaluated (Table 4). Genotypes from Rajasthan state showed highest range of similarity 0.90. This revealed high diversity amongst genotypes of Rajasthan origin. Similarity coefficient values of 0.84 have been observed in genotypes from Punjab state. A maximum of 10 genotypes have also been evolved from Uttar Pradesh showing similarity (range 0.62 to 0.94) with a mean value of 0.78 which are moderate mean values of similarity coefficient. Lowest similarity coefficient of 0.64 was found between genotypes of Taiwan. A maximum of 45 numbers of paired combinations was found in genotypes grouped under genotypes of Uttar Pradesh of origin (Table 4).

ISSR Marker based cluster tree analysis

The ISSR data based derivation of similarity matrix shown in Table 3 reveal the similarity values lay between 0.60-0.95. The 23 genotypes could be divided into two major clusters at a similarity coefficient of 0.74 (Figure 2). Cluster I was the main one that included twenty genotypes *viz.,* PUSA-672, UPM-02-18, PDM-288, IPOI-1539, RMG-62, RMG-353, DRA-24, HUM-12, SAMRAT, PRATEEKSHA-NEPAL, ASHA, MG-331, PANT, IC-393407, ML-729,IPM-02-01, MEHA , HUM-1, AKM-962 and IPM-02-14 at a similarity coefficient of 0.72. It could be further divided into six sub-clusters. Subcluster I included two genotypes UPM-02-18 and PDM-288 are similar to each other at a similarity coefficient of 0.90. Subcluster II included

doi:10.5958/2229-4473.2016.00067.7

Table 3: Jaccard's similarity coefficient values for ISSR pattern as generated by agarose gel electrophoresis.

Genotypes	PUSA-672	AKM-962	UPM-02-18	ML-729	EC-398885	IPM- 02-01	IPM -02-3	IPM-02-14	IPOI-1539	RMG-62	PDM-288	RMG- 353	P. NEPAL	MEHA	PANT	ASHA	MG- 331	GM- 9925	IC-393407	DRA-24	SAMRAT	HUM-1	HUM-12
PUSA-672	1.0																						
AKM- 962	0.72	1.0																					
UPM-02-18	0.82	0.78	1.0																				
ML-729	0.77	0.76	0.89	1.0																			
EC-398885	0.70	0.54	0.65	0.69	1.00																		
IPM- 02-01	0.78	0.76	0.78	0.86	0.70	1.00																	
IPM-02-3	0.72	0.65	0.70	0.73	0.76	0.76	1.00																
IPM-02-14	0.68	0.68	0.70	0.73	0.64	0.74	0.72	1.00															
IPOI-1539	0.78	0.73	0.88	0.85	0.64	0.78	0.64	0.74	1.00														
RMG-62	0.77	0.74	0.89	0.86	0.65	0.78	0.68	0.76	0.93	1.00													
PDM-288	0.78	0.76	0.90	0.82	0.62	0.77	0.69	0.73	0.86	0.85	1.00												
RMG- 353	0.76	0.73	0.85	0.85	0.62	0.78	0.66	0.78	0.92	0.90	0.86	1.00											
P. NEPAL	0.76	0.76	0.84	0.81	0.62	0.78	0.68	0.74	0.89	0.89	0.85	0.92	1.00										
MEHA	0.70	0.77	0.70	0.70	0.54	0.72	0.53	0.69	0.81	0.77	0.74	0.82	0.80	1.00									
PANT	0.72	0.74	0.82	0.82	0.62	0.82	0.66	0.76	0.90	0.88	0.86	0.90	0.93	0.84	1.00								
ASHA	0.76	0.80	0.84	0.84	0.60	0.84	0.70	0.76	0.89	0.89	0.85	0.92	0.94	0.80	0.93	1.00							
MG- 331	0.70	0.76	0.84	0.84	0.58	0.78	0.68	0.72	0.89	0.86	0.85	0.89	0.92	0.80	0.93	0.94	1.00						
GM- 9925	0.56	0.53	0.56	0.56	0.38	0.58	0.53	0.62	0.58	0.64	0.60	0.61	0.69	0.54	0.62	0.66	0.64	1.00					
IC- 393407	0.70	0.69	0.78	0.76	0.56	0.73	0.62	0.70	0.81	0.81	0.77	0.84	0.86	0.77	0.85	0.89	0.86	0.61	1.00				
DRA-24	0.73	0.73	0.88	0.85	0.61	0.76	0.69	0.76	0.92	0.93	0.86	0.94	0.92	0.77	0.90	0.92	0.92	0.64	0.86	1.00			
SAMRAT	0.78	0.72	0.85	0.85	0.66	0.77	0.69	0.76	0.89	0.93	0.86	0.92	0.90	0.78	0.89	0.90	0.88	0.65	0.85	0.94	1.00		
HUM-1	0.66	0.65	0.70	0.73	0.73	0.73	0.70	0.72	0.77	0.76	0.72	0.80	0.78	0.64	0.77	0.84	0.78	0.69	0.73	0.80	0.80	1.00	
HUM-12	0.73	0.76	0.88	0.82	0.60	0.76	0.66	0.76	0.92	0.90	0.89	0.94	0.92	0.77	0.90	0.92	0.89	0.61	0.84	0.94	0.92	0.80	1.00

Table 4: Jaccard's similarity coefficient among 23 genotypes implies genotypes to variety of origins.

Origin	No of genotypes	Paried combination	Similarity Coefficient range
Delhi	2	1	0.70
Maharastra	1	-	-
Utrakand	2	1	0.82
Punjab	2	1	0.84
Taiwan	2	1	0.62
Rajasthan	2	1	0.90
Haryana	1	-	-
Gujarat	1	-	-
U.P.	10	45	0.62-0.94

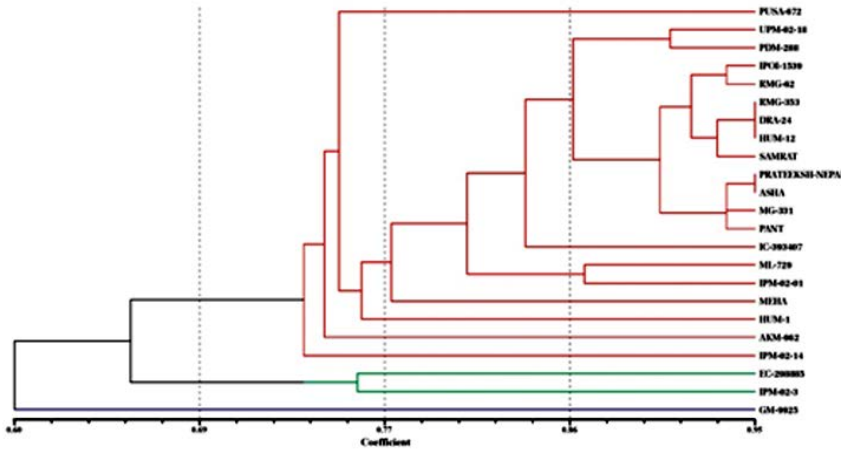

Figure 2: Dendrogram constructed with UPGMA clustering method among 23 genotypes of *V. radiata* L. using ISSR markers.

doi:10.5958/2229-4473.2016.00067.7

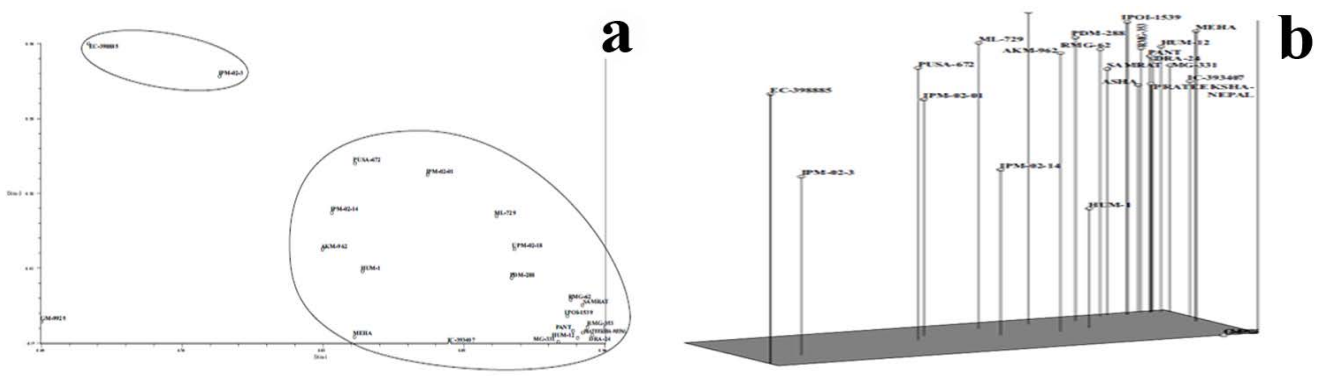

Figure 3: a. Two Dimensional Principal Component Analysis. **b:** Three Dimensional Principal Component Analysis based on Euclidean Cluster Analysis Using ISSR Markers.

two genotypes, IPOI-1539 and RMG-62 found similar to each other at a similarity coefficient of 0.93. Subcluster III included three genotypes *viz.,* RMG-353, DRA-24 and HUM-12 identified found at a similarity coefficient of 1.00. Subcluster IV included two genotypes, *viz.,* PRATEEKSHA-NEPAL and ASHA again found similar to each other at a similarity coefficient of 1.00. Sub-cluster V included two genotypes viz., MG-331 and PANT that were similar to each other at a similarity coefficient of 0.93. Finally subcluster VI included two genotypes, *viz.,* ML-729 and IPM-0201, at a similarity coefficient of 0.86. Cluster II included two genotypes EC-398885 and IPM-02-3 which were similar to each other at a similarity coefficient of 0.74. Genotype GM-9925 could be out grouped from both major clusters at a similarity coefficient of 0.60.

Similar results have been reported by Singh et al. [12]. The UPGMA distributed the 30 genotypes into five main clusters; clusters with Dice's analysis indicated similarity coefficient values ranging from 0.65 to 0.8. One genotype namely, ML-818 forms an out-group by not falling in any cluster. The variety PDM-139 used as standard check was grouped separately. The genetic variation amongst advanced lines of diverse crosses could be useful for selecting parents for crossing so as to yield populations required for breeding for yield and related agronomic traits.

Mantel test (Z) was done between cophentic matrix computed from marker based tree matrix and the original similarity data. This resulted in significant correlation (r=0.92) which revealed a good fit for the cluster analysis performed.

Principal component analysis based on ISSR markers

Two and three dimension principal component analysis based on ISSR data (Figure 3) showed similar clustering of 23 genotypes as evident from cluster tree analysis. Dice's similarity coefficients ranged from 0.69 to 0.96, indicative of an average degree of variation among the genotypes. As visible in the dendrogram, the genotypes that were closer were more similar than those that were lying apart. Similar observations were recorded with PCA as well. Most of the genotypes tended to associate mainly into two clusters. Cluster I was the major one that included seventeen genotypes *viz.* ,PUSA-672, UPM-02-18, PDM-288, IPOI-1539, RMG-62, RMG-353, DRA-24, HUM-12, SAMRAT, PRATEEKSHA-NEPAL, ASHA, MG331, PANT, IC-393407, ML-729, IPM-02-01, MEHA, HUM-1, AKM-962 and IPM-02-14 while cluster II included only 2 genotypes, *viz*., EC-398885 and IPM-02-3 that lay closer to each other. Genotype GM-9925 lay apart from both two clusters. Similar finding have been reported in Tantasawat et al. [13]

ISSR markers are easy, fast, inexpensive, accurate, reliable, and simultaneous in detection of polymorphisms at multiple loci in the genome using small quantities of DNA sample. These properties have made the ISSR markers useful for the genetic analysis of various plants [14-16]. Thus, this investigation may be useful for selecting the diverse parents and observing genetic diversity in breeding trails towards better genotype collection of mungbean.

Acknowledgement

Dr. Gunjeet Kaur thanks to UGC, New Delhi for Senior Research Fellowship under MANF. Authors are gratefully acknowledged the financial assistance from RKVY project "validation of important crop varieties through DNA fingerprinting".

References

1. Economic Survey (2012) Ministry of finance, department of economic affairs, economics division government of india, New Delhi, India.
2. Arumuganathan K, Earle ED (1991) Nuclear dna content of some important plant species. Plant Mol Biol Report 9: 208-218.
3. Saini M, Singh S, Hussain Z, Yadav A (2010) RAPD analysis in mungbean (*V. radiata* (L.) Wilczek): I. Assessment of genetic diversity. Indian J Biotechnol 9: 137-146.
4. Leian P, Bordallo P, Colova V (2005) Tracing the pedigree of cynthiana grape by dna microsatellite markers. Proc Fla State Hort Soc 118: 200-204.
5. Godwin ID, Aitken EAB, Smith LW (1997) Application of inter-simple sequence repeats (ISSR) markers to plant genetics. Electrophoresis 18: 1524-1528.
6. Doyle JJ, Doyle JL (1987) A rapid dna isolation procedure for small quantities of fresh leaf material. Phytochem Bull 19: 11-15.
7. Sambrook J, Fritschi EF, Maniatis T (1989) Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York, USA.
8. Rohlf FJ (2000) NTSYS-PC. Numerical taxonomy and multivariate analysis system. Exeter Software, New York, USA.
9. Mantel N (1967) The deduction of disease clustering and a generalized regression approach. Cancer Res 27: 209-220.
10. Despeiges A, Goubely C, Lenoir A, Cocherel S, Picard G, et al. (1995) Identification of the most represented repeat motif in *Arabidopsis thaliana* microsatellite loci. Theor Appl Genet 91: 160-168.
11. Das S, Das SS, Ghosh P (2014) Analysis of genetic diversity in some black gram cultivars using ISSR. Eur J Exp Biol 4: 30-34.
12. Singh R, Heusden AWV, Yadav RC (2011) A comparative genetic diversity analysis in mungbean (*Vigna radiata*) using inter-simple sequence repeat

(ISSR) and amplified fragment length polymorphism (AFLP). Afr J Biotechnol 12: 6574-6582.

13. Tantasawat P, Trongchuen J, Prajongjai T, Thongpae T, Petkhum C, et al. (2010) Variety identification and genetic relationships of mungbean and black gram in thailand based on morphological characters and ISSR analysis. Afr J Biotechnol 9: 4152-4164.

14. Reddy MP, Sarla N, Siddiq EA (2002) Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128: 9-17.

15. Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, et al. (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Z. mays* L.): comparisons with data from RFLPs and pedigree. Theoret and Appl Genet, 95: 163-173.

16. Singh A, Dikshit HK, Jain N, Singh D, Yadav RN (2013) Efficiency of SSR,ISSR and RAPD markers in molecular characterization of mungbean and other *Vigna* species. Indian J. Biotechnol 13: 81-88.

Author Affiliations

Top

Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India