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Assessment of molecular diversity of greengram [*Vigna radiata* (L.) Wilzek] through RAPD

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ABSTRACT

A total twenty three genotypes of green gram (*Vigna radiata*) were subjected to Randomly amplified polymorphic DNA (RAPD) analysis for molecular characterization. A total of 25 randomly selected decamers were screened, out of which only 15 generated 126 amplification products from which 117 bands were found polymorphic, the average polymorphism being 93.48%. The total number of amplified bands varied between 2 (primer OPP-09) to 17 (primer OPA-1) with an average of 9.5 bands per primer. The overall size of PCR amplified products ranged between 200 bp to 2900 bp. The average Polymorphism Information Content(PIC) was 0.32 ranging from 0.17 to 0.46. Primer OPA-01 and OPP-06 detected two unique bands ranged between 250 bp to 2500 bp in two genotypes (PUSA-672 and HUM-12). Jaccard's similarity coefficient values ranged from 0.28-0.90 with an average of 0.59. Based on dendrogram generated through UPGMA method and PCA, most of the genotypes got divided into four main clusters. Genotype EC-398885 lay far apart and thus showed maximum genetic distance. The assessment of genetic diversity is a prerequisite and important step for the improvement of any legume crop. Thus, present results of the present study could be further extrapolated to other green gram accessions in *Vigna* germplasm.

Key words: Diversity analysis, PCA, Polymorphism, RAPD, Similarity coefficient, UPGMA.

INTRODUCTION

Green gram (*Vigna radiata*) also known as mungbean belongs to the family *Fabaceae* (*Leguminoceae*). It is a self pollinated crop with genome size 579 Mbp and is diploid (2n) with 22 chromosomes (Arumuganathan and Earle, 1991). Since it is a legume, it possess the ability to fix atmospheric nitrogen (30-50 kg/ha). It is a fast growing crop with a short life span, photo-insensitive and has a dense crop canopy, these qualities gives it a special significance in crop intensification, diversification and conservation of natural resources as well as sustainability of production system. It has strategic position in Southeast Asian countries for nutritional security and as a sustainable crop. As of the 2012-13 cultivation statistics in India, green gram was grown on 2.75 million hectares with a production status of 1.19 million tonnes and yield of 436 kg/ha (Economic Survey, 2012-2013). Green gram is rich in easily digestible good quality protein (25.9%) and lysine (504 mg/g) for both human and animals (Saini *et al.*, 2010). The major constraints in achieving high yield of this crop are lack of genetic variability, poor harvesting index and susceptibility to biotic and abiotic stresses. The major factor out of these remains the lack of genetic variability and non availability of suitable ideotypes for various cropping plans (Singh *et al.*, 2013). Although the germplasm collection from India is very large, much diversity has not been reported in morphological characters. Therefore, there is an urgent need to identify genetic divergence based on morpho-molecular basis for utilization in breeding programmes. There are numerous techniques available for assessing the genetic variability and relatedness among crop germplasm.

Several studies have shown that DNA markers have many advantages over morphological markers (Bernatzky *et al*.1989 and Gepts *et al*.1993). Estimation of the genetic variation in green gram has been carried out using various molecular markers that includes RAPD (Santalla *et al.* 1998, Afzal *et al.* 2004, Lavanya *et al.* 2008, Saini *et al.* 2010, Undal *et al.* 2011, Sony *et al.* 2012 and Bhuyan *et al.* 2014), AFLP (Bhat *et al.* 2005), ISSR (Reddy *et al.* 2008) and SSR (Gwag *et al.* 2010, Gena *et al.* 2015). For simple, efficient and economic way of cultivar identification and diversity analysis RAPD-PCR based DNA finger printing has widely used (Gherardi *et al.*, 1998). RAPD markers have the advantage that they are random and do not require any prior sequence information for implementation. RAPDs are generated by PCR amplification using single, short, synthetic, random oligonucleotide as a primers that acts both as forward as well as reverse primer (Yadav *et al.*, 2014).

RAPD analysis can be mostly used to reveal genetic relationship between genotypes of a species (Bhuyan *et al*, 2014). DNA markers such as RAPD provides a direct

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measure of genetic diversity and go beyond diversity based on agronomic traits or geographic origin thus helping breeders to better management of germplasm and develop more efficient strategies for crop improvement. The success of using RAPD markers to characterize green gram is very well reported, however not much documented in the indigenous green gram genotypes. The objective of the present study has been to characterize the genetic relationships among twenty three genotypes of green gram with respect to RAPD markers.

MATERIALS AND METHODS

Plant materials and DNA isolation: In the present investigation seeds of twenty three genotypes of green gram were procured from Dr. O.P. Kedar, Senior Scientist, Agriculture Research Station (ARS), Durgapura, Jobner Agriculture University, Jaipur. Source details of the materials used are given in Table 1. Laboratory studies were undertaken at the Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Udaipur. Genomic DNA was isolated and purified from young leaves of 21-28 days fromthe 23 genotypes (Doyle and Doyle, 1987) and stored at -20^{0}C.

RAPD analysis (Srivastava *et al.*, 2013): A total of twenty five RAPD primers (Operon Inc.) were used for PCR amplification. PCR (Eppendorf Mastercycler, Germany) reaction was performed in 20 µl reaction mixture containing 25 ng of template DNA, 200 µM of dNTP mix, 1U of *Taq* polymerase, 1X of reaction buffer and 2µl of 5µM of primer. The annealing temperature of 37^{0}C was found to be optimum for generating clear and reproducible bands for RAPD primers. The amplification reaction with pre-denaturation for 4 minutes at 94^o C followed by 44 amplification cycles then 1 minute denaturation at 94^{o}C followed by 1 minute annealing at 37^{o}C and 2 minutes extension at 72^{o}C. Final post extension was carried out for 10 minutes at 72^{o}C with a hold temperature of 4^{0}C at the end that resulted in clear and reproducible bands.

Table 1: Source of the 23 *V. radiata* L. genotypes used in research

Genotype	Source
PUSA-672	IARI, New Delhi
AKM- 962	PKV, Akola
UPM-02-18	Pantnagar
ML-729	PAU, Ludhiana
EC-398885	AVRDC, Taiwan
IPM- 02-01	IIPR, Kanpur
IPM-02-3	IIPR, Kanpur
IPM-02-14	IIPR, Kanpur
IPOI-1539	IIPR, Kanpur
RMG-62	RAU, Durgapura
PDM-288	IIPR, Kanpur
RMG- 353	RAU, Durgapura
PRATEEKSHA-NEPAL	AVRDC, Taiwan
MEHA(IPM -99-125)	IIPR, Kanpur
PANT	GBPAU, Pantnagar
ASHA	HAU, Hisar
MG- 331	Gurdaspur, Punjab
GM- 9925	S.K. Nagar, Gujarat
IC- 393407	NBPGR, New Delhi
DRA-24	IIPR, Kanpur
SAMRAT(PDM-139)	IIPR, Kanpur
HUM-1	BHU, Varanasi
HUM-12	BHU, Varanasi

Agarose Gel Electrophoresis (Sambrook *et al.*, 1989): The above obtained PCR products were mixed with 2 µl of loading dye (Bromophenol blue) and were loaded on 1.2 per cent agarose gel prepared in 1X TAE buffer containing ethidium bromide (10 mg/ml) concentration of 3µl/100ml. Electrophoresis was carried out at a constant voltage (3V/ cm of gel). The gel was visualized on a UV-transilluminator and photographed using gel documentation system (Alpha DigiDoc, Germany). Only clear, reproducible and unambiguous bands were scored. The size of the amplified bands of RAPD were observed based on its migration and confirmation relative to standard molecular size markers (100 bp DNA ladder and 1 kb DNA ladder from Bangalore Genei Pvt. Ltd., Bangalore, India).

Data analysis (Jaccard, 1908): Clear and well marked RAPD amplified bands were coded in a binary form by denoting '0' and '1' intended for absence and presence of bands, respectively in each genotype and this data was used as input for further calculations. The SIMQUAL sub-programme was used to calculate the Jaccard's coefficient of similarity. Similarity matrices based on these indices were calculated and used to construct the UPGMA (un-weighted pair group method with arithmetic average) dendrograms to elucidate the diversity among the studied genotypes (Sneath and Sokal 1973). A two dimensional and three dimensional principal component analysis (PCA) was constructed to provide another means of testing the relationship among the cultivars using the EIGEN programme NTSYS-pc version 2.02 (Rohlf,2004).

Polymorphism Information Content (PIC) (Smith *et al.*, 1997): To measure the polymorphism information of RAPD marker system. The PIC was calculated according to following formula:

$$PIC = \sum_{i=1}^{n} 1 - pi^2$$

Where, N= total number of allele detected for a locus of a marker
Pi= frequency of the 1st allele

RESULTS AND DISSCUSION

The objective of the present study is the molecular characterization of the genotypes of 23 green gram genotypes (*Vigna radiata*) through RAPD markers. Quality of analyzed DNA was pure as indicated by the ratio of A_{260}/A_{280} nm which ranged from 1.7 to 2.1. The A_{260}/A_{280} nm ratio also indicated

that the analyzed DNA was free from contaminants like polysaccharides, protein and RNA.

RAPD markers having a GC contect of 60 percent or more were used for the identification and determination of the genetic relationship. In the present study, fifteen RAPD primers, among the 25 primers from OPA/E/F/C/K/P series were amplified presented in Table 2.

RAPD analysis (Figure 1) showed a high percent of polymorphism (93.48%). Out of 126 amplified bands obtained 117 were polymorphic. The total number of

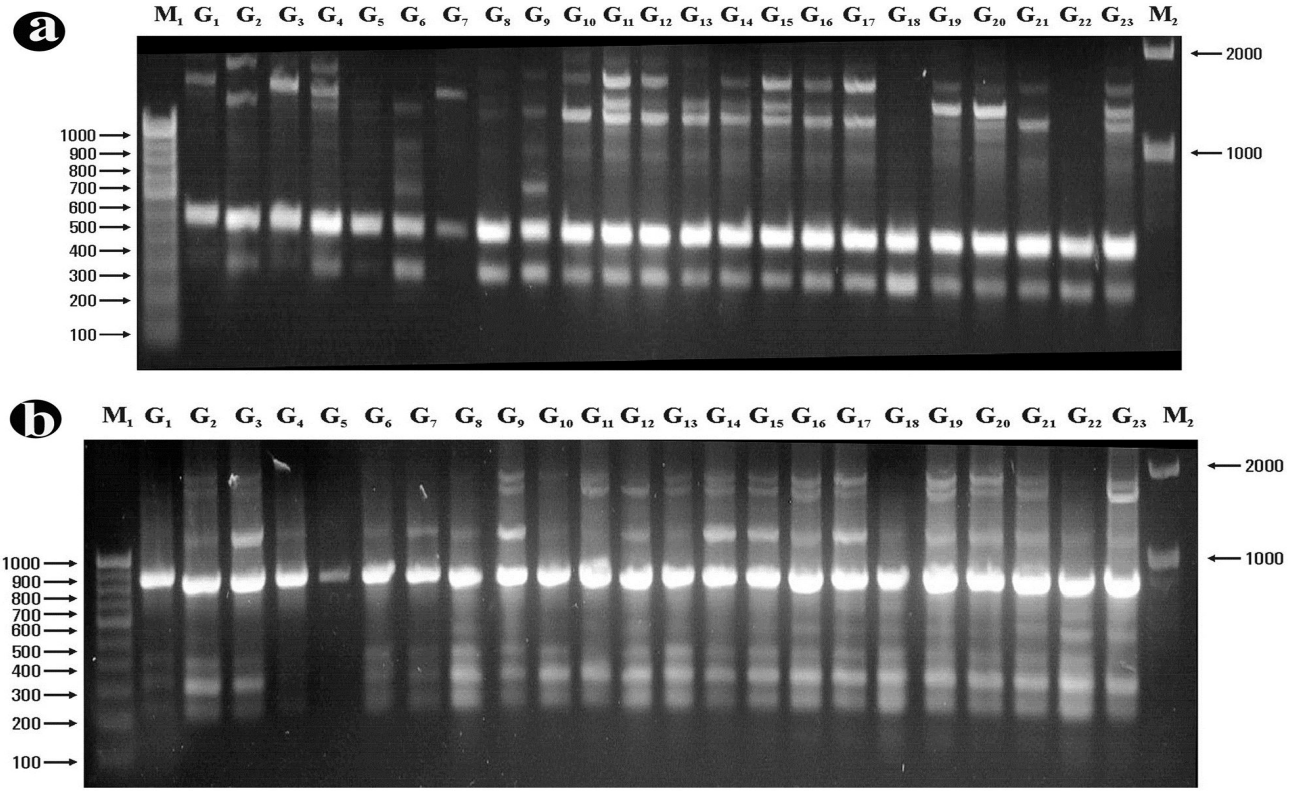

Fig 1: RAPD profiles of *Vigna radiata* L. genotypes generated through (a) OPP04 (b) OPP05. G1-G23 represents G1-PUSA672; G2-AKM962; G3-UPM02-18; G4-ML-729; G5-EC-398885; G6-IPM-02-1; G7- IPM-02-03; G8-IPM02-14; G9-IPOI-1539; G10-RMG-62; G11-PDM-288; G12-RMG-353; G13-PRETEEKHSA-NEPAL; G14-MEHA; G15-PANT; 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

Table 2: DNA amplification profile and polymorphism generated in *V.radiata* L. using 15 RAPD primers.

Primer Code	Sequence 5' to 3'	Molecular weight range (bp)	Total number of bands amplified (x)	Polymorphic bands		PIC
				Number	Frequency(%)	
OPA-01	CAGGCCCTTC	2500-200	**17**	17	100	0.31
OPA-02	TGCCGAGCTG	2200-700	08	08	100	0.32
OPC-08	TGGACCGGTG	2500-300	11	11	100	0.38
OPE-03	CCAGATGCAC	1700-300	09	09	100	0.24
OPF-17	AACCCGGGAA	1500-400	06	04	66.66	**0.17**
OPF-19	CCTCTAGACC	2800-200	11	11	100	0.37
OPK-06	CACCTTTCCC	2100-700	07	07	100	0.34
OPK-07	AGCGAGCAAG	2900-200	10	10	100	0.38
OPP-04	GTGTCTCAGG	1800-300	10	08	80	0.35
OPP-05	CCCCGGTAAC	1800-250	09	08	88.88	0.30
OPP-06	GTGGGCTGAC	1800-250	12	08	66.66	0.21
OPP-07	GTCCATGCCA	1800-500	03	03	100	0.20
OPP-08	ACATCGCCCA	700-500	03	03	100	0.42
OPP-09	GTGGTCCGCA	900-600	**02**	02	100	**0.46**
OPP-10	TCCCGCCTAC	1800-300	08	08	100	0.44
Total			**126**	**117**	**93.48**	**0.32**

amplified bands varied between 2 (primer OPP-09) and 17 (primer OPA-01) with an average of 9.5 bands per primer. The overall size of PCR amplified products ranged between 200 bp to 2900 bp. The percent polymorphism ranged from as low as 66.66 (OPF-17 and OPP-06) to as high as 100 (OPA-01, OPA-02, OPC-08, OPE-03, OPF-19, OPK-06, OPP-07, OPP-08, OPP-09 and OPP-10). A similar finding has also been reported by Datta *et al.*, (2012).

The average PIC was 0.32 and values ranged 0.17 to 0.46. The lowest and the highest PIC value were recorded for primer OPP-17 and OPP-09 respectively. Reddy *et al.*, (2008) reported that accessions with most distinct DNA profiles are likely to contain the greatest number of novel alleles. It is these accessions that are likely to uncover the largest number of unique and potential agronomically useful alleles. Similarly two unique bands were detected in two genotypes *viz.*, PUSA-672 and HUM-12 with 2 RAPD primers (OPA-01 and OPP-06). The size of these unique bands ranged from 250-2500 bp.

Genetic relationship and cluster tree analysis: Jaccard's similarity coefficient values ranged from 0.28-0.90 with an average of 0.59 (Table 3). The average similarity across all the genotypes was found out to be 0.59 showing that genotype were moderately diverse genetically. Maximum similarity value of 0.90 was observed between genotypes MEHA and ASHA and MG-331 and ASHA. Similarly minimum similarity value of 0.28 was observed between genotypes RMG-353 and EC-398885 and these genotypes were highly diverse at genetic level. Similar results were also shown by Saini *et al.*, (2010).

The 23 genotypes of *V. radiata* L. were grouped by subjecting the Jaccard's similarity values (Table 3) to UPGMA clustering which are divided in 4 major clusters at a similarity coefficient of 0.64 on dendrogram (Fig 2). Cluster I included three genotypes *viz.*, PUSA-672, UPM-02-18 and IPM-02-3 at similarity coefficient of 0.64. It was further divided into one sub-cluster that included two genetypes PUSA-672 and UPM-02-18 which were similar to each other at 0.71 similarity coefficient. Cluster II included 2 genotypes AKM-962 and ML-729 which were related to each other at 0.74 similarity coefficient. Cluster III was the main cluster having thirteen genotypes *viz.*, IPOI-1539, RMG-62, PRATEEKSHA NEPAL, DRA-24 , SAMRAT, PDM-288 , RMG-353 , MEHA , ASHA ,MG-331, PANT, HUM-12 and IC-393407 at similarity coefficient of 0.73 . Cluster III was further divided into three subclusters. Subcluster I contained two genotypes DRA-24 and SAMRAT which were similar to each other at similarity coefficient of 0.87. Subcluster II contained two genotypes PDM-288 and RMG-353 which were similar to each other at similarity coefficient of 0.85. Sub cluster III contained two genotypes MEHA and ASHA which were similar to each other at similarity coefficient of 0.90.

The cluster IV included four genotypes *viz.*, GM-9925, IPM-02-01, IPM-02-14 and HUM-1 at similarity coefficient of 0.65. It could be divided into one subcluster that contained GM-9925 and HUM-1 found related to each other at similarity coefficient of 0.80. Genotype EC-398885 fell apart from all the clusters. Similar results were obtained by Datta *et al.* (2012), the dendrogram revealed the genetic similarity among the twenty four varieties of green gram had ranged from 0.45 to 0.78. The RAPD cluster pattern included two main clusters, Cluster I comprising of sixteen varieties in five sub clusters (IA to IE) and cluster II comprises of four varieties.

Two and three dimension principal component analysis based on RAPD data (Fig. 3) showed similar clustering of 23 genotypes as evident from cluster tree analysis. Dice similarity coefficients ranged from 0.58 to 0.98. Most of the genotypes tended to cluster mainly into

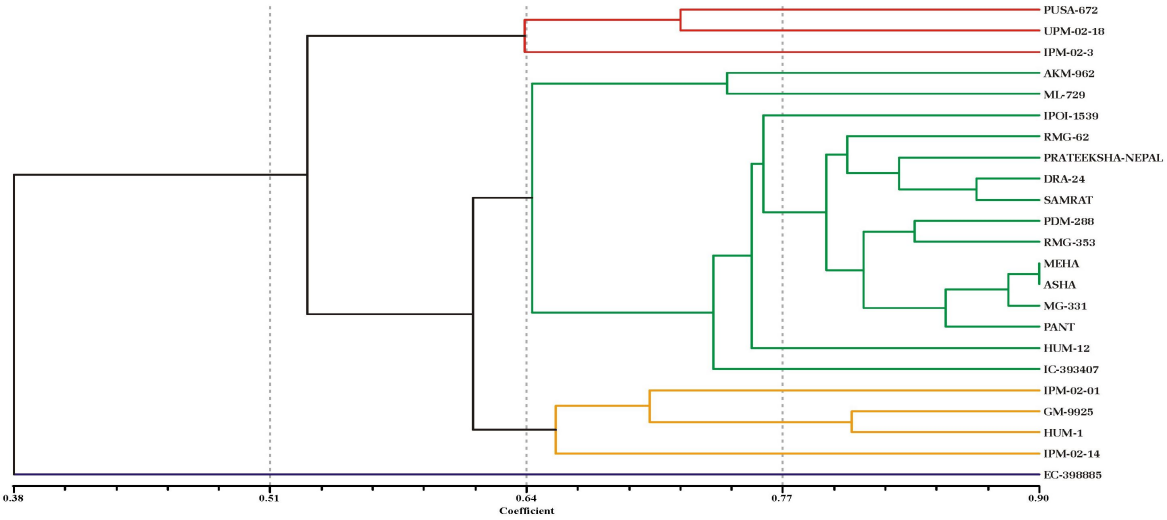

Fig 2: Dendrogram constructed with UPGMA clustering method among 23 genotypes of *V. radiata* L. using RAPD.

Table 3: Jaccard's similarity coefficient for RAPD profile 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	PRATEEKSHA NEPAL	MEHA	PANT	ASHA	MG- 331	GM- 9925	IC- 393407	DRA-24	SAMRAT	HUM-1	HUM-12
PUSA-672	1.00																						
AKM- 962	0.44	1.00																					
UPM-02-18	0.72	0.65	1.00																				
ML-729	0.52	0.74	0.64	1.00																			
EC-398885	0.58	0.41	0.42	0.55	1.00																		
IPM- 02-01	0.54	0.69	0.67	0.66	0.41	1.00																	
IPM-02-3	0.59	0.53	0.69	0.60	0.50	0.64	1.00																
IPM-02-14	0.36	0.57	0.52	0.51	0.33	0.66	0.57	1.00															
IPOI-1539	0.55	0.65	0.61	0.61	0.42	0.71	0.49	0.62	1.00														
RMG-62	0.56	0.71	0.61	0.65	0.38	0.70	0.43	0.58	0.80	1.00													
PDM-288	0.45	0.68	0.57	0.60	0.30	0.65	0.45	0.58	0.71	0.83	1.00												
RMG- 353	0.46	0.73	0.58	0.59	0.28	0.71	0.48	0.61	0.74	0.78	0.84	1.00											
PRATEEKSHA NEPAL	0.46	0.69	0.53	0.58	0.33	0.65	0.42	0.61	0.76	0.78	0.82	0.80	1.00										
MEHA	0.46	0.65	0.60	0.63	0.32	0.66	0.50	0.57	0.76	0.76	0.83	0.81	0.83	1.00									
PANT	0.43	0.62	0.57	0.57	0.33	0.65	0.53	0.61	0.69	0.67	0.78	0.73	0.76	0.88	1.00								
ASHA	0.49	0.67	0.57	0.62	0.31	0.68	0.46	0.59	0.77	0.76	0.81	0.86	0.83	0.90	0.85	1.00							
MG- 331	0.46	0.68	0.57	0.60	0.32	0.65	0.45	0.59	0.77	0.80	0.84	0.81	0.88	0.87	0.82	0.90	1.00						
GM- 9925	0.49	0.61	0.59	0.52	0.42	0.72	0.60	0.65	0.70	0.60	0.54	0.65	0.65	0.62	0.64	0.64	0.61	1.00					
IC- 393407	0.58	0.63	0.63	0.54	0.32	0.62	0.42	0.51	0.73	0.73	0.72	0.68	0.74	0.71	0.71	0.75	0.76	0.64	1.00				
DRA-24	0.51	0.74	0.63	0.69	0.36	0.73	0.50	0.61	0.80	0.80	0.83	0.76	0.86	0.81	0.76	0.83	0.88	0.65	0.80	1.00			
SAMRAT	0.52	0.74	0.61	0.65	0.35	0.76	0.48	0.61	0.80	0.82	0.76	0.76	0.80	0.78	0.69	0.78	0.81	0.65	0.73	0.87	1.00		
HUM-1	0.45	0.57	0.55	0.47	0.40	0.69	0.61	0.65	0.61	0.47	0.50	0.61	0.58	0.59	0.64	0.56	0.56	0.80	0.53	0.55	0.63	1.00	
HUM-12	0.48	0.60	0.54	0.57	0.30	0.65	0.44	0.60	0.73	0.66	0.73	0.73	0.80	0.76	0.72	0.78	0.81	0.58	0.75	0.79	0.77	0.60	1.00

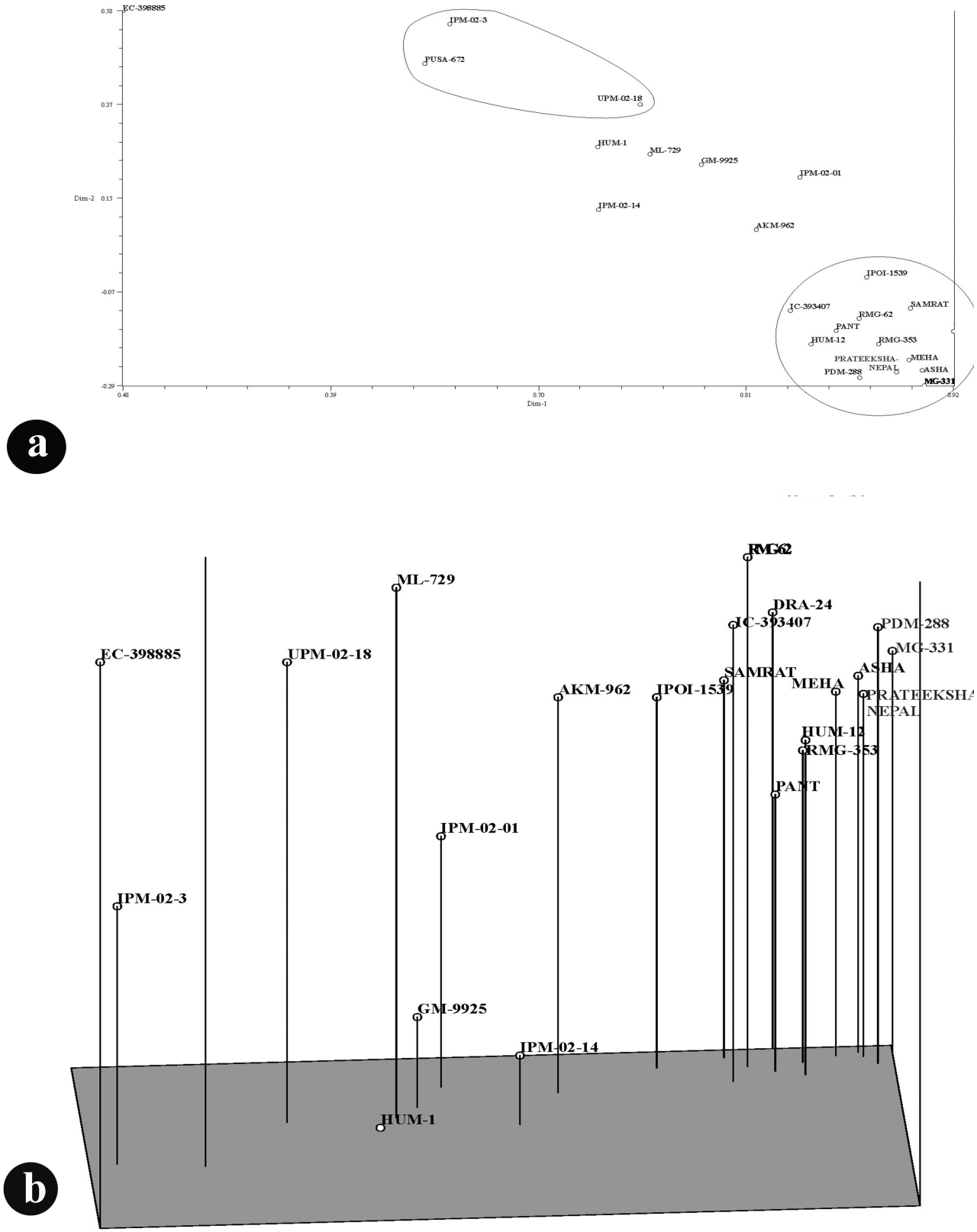

Fig 3: (a) Two Dimensional Principal Component Analysis (b) Three Dimensional Principal Component Analysis based on Euclidean Cluster Analysis Using RAPD Markers.

four clusters. Cluster I included three genotypes (PUSA-672, UPM-02-18 and IPM-02-3) while cluster II included two genotypes (AKM-962 and ML-729) that lay closer. Cluster III was the major one that included thirteen genotypes *viz*., IPOI-1 5 3 9, RM G -6 2 , P RAT E E K S HA N E PAL, D RA- 2 4 , SAMRAT, PDM-288, RMG-353, MEHA, ASHA, MG-331, PANT, HUM-12 and IC-393407. Cluster IV included four genotypes *viz.*, GM-9925, IPM-02-01, IPM-02-14 and HUM-1. Amongest all, the genotype EC-398885 was lying apart and showed maximum genetic distance. Similar results have been observed by Saini *et al.,* (2010).

In the present study, EC-398885 genotype is found to be most diverse among the 23 genotypes. This genotype had the lowest similarity (0.28) with the genotype RMG-353 indicating\ indicate that genotypes were highly diverse at genetic level and that EC-398885 genotype might be most frequently used in the green gram varietal development programme. RAPD studies have numerous advantages like wider applicability, accuracy and speed of estimation. Thus, RAPD analysis proved in the present to be applicable for genotyping, genetic diversity and relatedness evaluation. The analysis revealed narrow genetic base among green gram genotypes used in the study. In conclusion, the RAPD patterns obtained from our study can serve as a vital input to the conventional method of var ietal iden tification, future germplasm management and marker assisted selection to improve the efficiency of green gram germplasm improvement in future breeding that relies solely on morpho-molecular characterization. Such information may also be useful in selecting diverse parents and monitoring the genetic diversity periodically in the breeders' working collection of green gram.

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