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GENETIC DIVERSITY STUDY OF CLUSTER BEAN (*Cyamopsis tetragonoloba* (L.) Taub) LANDRACES USING RAPD AND ISSR MARKERS

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ABSTRACT:
The main source of genetic variability for breeding and crop improvement in cluster bean is landraces. Genetic diversity in 29 landraces and 19 commercial varieties of cluster bean belonging to Gujarat, Rajasthan, Haryana and Delhi regions of India were analyzed in the present study using 13 RAPD (Randomly Amplified Polymorphic DNA) and 7 ISSR (Inter Simple Sequence Repeat) markers. Amplification with RAPD primers produced a total of 118 bands, out of which 103 were polymorphic. Out of the 13 primers used, OPQ-09 produced the highest number of bands (12). The average percentage polymorphism for RAPD markers was 87.63. Seven ISSR markers produced 64 bands out of which 50 were polymorphic. Among the ISSR primers used UBC-868 produced highest number of bands (13); the average percentage polymorphism for ISSR markers was 77.82. UPGMA tree was constructed using Jaccard's similarity for RAPD, ISSR and RAPD+ISSR data. RAPD and RAPD+ISSR showed separate clustering of landraces from commercial varieties. AMOVA showed the presence of higher variance within the populations than between the populations. The results obtained from the present study can be used for selecting accessions for breeding and crop improvement in cluster bean.

Key words: Cluster bean, Guar, *Cyamopsis tetragonoloba*, RAPD, ISSR, PCR, Molecular Markers, Landrace.

[I]INTRODUCTION

Cluster bean (*Cyamopsis tetragonoloba* (L) Taub.) is an important crop grown mainly semi-arid regions of India, Pakistan and USA. It was traditionally used as forage, green manure and vegetable [4]. Now cluster bean is an industrial crop due to the applications of galactomannan

obtained from the endosperm of its seeds in various industrial processes. Emphasis on molecular biology of the crop has increased because of two recent studies, the cloning of β-mannan synthase gene from developing guar endosperm [2] and analysis of ESTs for studying galactomannan synthesis [13].

We lack an overview of the available genetic diversity in guar which is essential for any crop improvement programme. Conventionally guar accessions have been characterized on the basis of their phenotypes [11], but the limitation of this approach is that the phenotype is influenced by the environmental factors during the developing stage of plant.

C. tetragonoloba mainly being a cultivated crop, is not found in wild conditions [8]. It is considered to be originated in North Western India by trans domestication of *C. senegalensis*. Because of this reason the available landraces from North Western India are considered as the main source of genetic variability for guar breeding programs. Apart from variability, landraces are also known for adaptations to the selective environments in the region of cultivation [1]. Hence, it is essential to systematically study the genetic diversity present in landraces of guar through molecular markers.

Molecular markers reveal natural variation at the DNA sequence level. These markers can be used to study the genetic diversity [10] because of its power to show variation at molecular level. Randomly Amplified Polymorphic DNA (RAPD) markers, introduced in the year 1990, have been widely used because of their low cost and requirement of very less DNA samples [21]. There are only two preliminary RAPD studies on some commercially grown genotypes of cluster bean [15, 18] but there is no information on the genetic variability in the landraces of cluster bean. Further, Inter Simple Sequence Repeat (ISSR) marker, which have a greater reproducibility in comparison to RAPD markers [20], have not yet been tested in cluster bean. Hence the present study was carried out to assess the genetic diversity in landraces and commercially grown varieties of guar using RAPD and ISSR markers.

[II]MATERIALS AND METHODS

2.1 Plant material

Accessions of guar landraces were kindly provided by National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Twenty nine accessions were selected based on their place of origin as previously described [4]. The regions of origin of selected accessions are diagrammatically represented in Fig. 1.

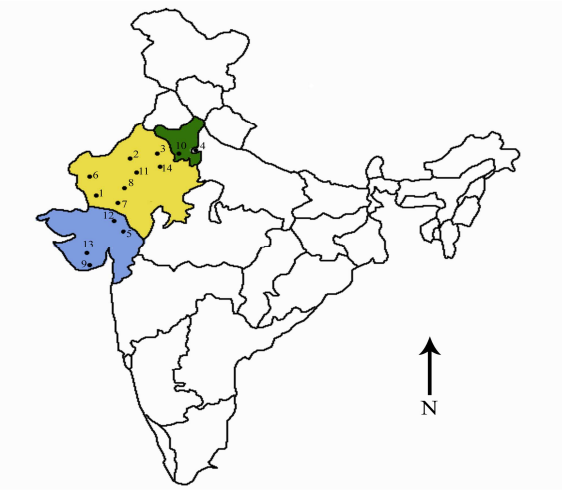

Fig 1. Diagrammatic representation of locations of collection of landraces as stated by Dwivedi et al. 1995 used in the study ; 1-Barmer, 2- Bikaner, 3- Churu, 4- Delhi, 5- Himmatnagar, 6- Jaisalmer, 7- Jalore, 8- Jodhpur, 9- Kachchh, 10- Mahendragarh, 11- Nagaur, 12- Palanpur, 13- Rajkot, 14- Sikar.

Seeds of 19 commercial varieties of *C. tetragonoloba*, which are commonly grown in Gujarat, Rajasthan and Haryana, were obtained from Central Arid Zone Research Institute (CAZRI), Jodhpur, Rajasthan. The details of the accessions are given in Supplementary Table 1. Plants were grown in field conditions in Indian Institute of Technology Roorkee, India. Young leaves were taken from 3 weeks old plants for DNA extraction.

2.1 DNA extraction

DNA was extracted from approximately 0.5g leaves collected from field grown plants using

CTAB method as described previously [3] with some modifications. Approximately 0.5g leaves were ground to fine powder in liquid nitrogen using sterile, pre-chilled pestle and mortar. The pulverized leaf powder was transferred to a 2 ml microfuge tube containing 1ml of pre-warmed DNA extraction buffer (5% CTAB, 100mM Tris HCl, 20mM EDTA, 1.4mM NaCl, 0.2mM DTT). The contents were mixed well, followed by incubation at 65^{o}C for 1 hour. The tubes were then kept at room temperature for 10 min. Equal volume of Chloroform : Isoamyl alcohol (24:1) was added and mixed gently. The tubes were centrifuged at 8000 RPM for 10 min at room temperature to separate the phases. The upper aqueous layer was pipetted out carefully into a fresh tube. The DNA was precipitated by adding equal volume of ice cold iso-propanol and kept at 4^{o}C for 2 hours. The precipitated DNA was pelletted by centrifugation at 8000 RPM for 10 min. The pellet was washed with 70% ethanol, air dried and dissolved in TE buffer. RNA was removed by treatment with 2µl of RNaseA (10mg/ml) and incubation at 37^{o}C in a water bath for 1 hour followed by treatment of chloroform:isoamyl alcohol (24:1). After centrifugation the supernatant was transferred to another tube. DNA was precipitated using 100% ethanol, followed by centrifugation at 8000 RPM for 10 min. Supernatant was discarded and pellet was air dried. Finally DNA was dissolved in TE buffer. The quality and quantity of the extracted DNA was checked by gel electrophoresis on 0.8% agarose and diluted to approximately 50ng/µl. Diluted DNA samples were stored at -20^{o}C until use.

2.3 DNA amplification conditions and gel electrophoresis

All 48 accessions, along with a negative control without DNA, were used for RAPD analysis. Out of the 20 primers initially screened 13 decamer RAPD primers (Supplementary Table 2) were selected on the basis of polymorphism and reproducibility. Polymerase chain reaction (PCR) was performed in 20µl reaction volume containing 1U Taq DNA polymerase (Biotools, Spain), 2.5 mM $MgCl_2$ (Biotools, Spain), 5 mM dNTPs, 1µL primer and 50ng of template DNA. Amplification was carried out in a Mastercycler gradient programmable thermal cycler (Eppendorf). The annealing temperature for each primer was determined by gradient PCR. The PCR was programmed with initial denaturation step at 94^{o}C for 4 min, followed by 35 cycles of 1 min at 94^{o}C, 1 min at annealing temperature (Supplementary Table 2) and 1 min at 72^{o}C. A final extension was carried out at 72^{o}C for 10 min and a hold temperature of 4^{o}C at the end.

ISSR primers were custom synthesized by Ocimum Biosciences, Hyderabad and used for screening polymorphism. Out of 15 primers initially screened, 7 primers which produced clear and unambiguous bands (Supplementary Table 2) were used for diversity study. PCR was performed in 20µl reaction volume containing 1U Taq DNA polymerase (Biotools, Spain), 2.5 mM $MgCl_2$, 5 mM dNTPs, 1µL primer and 50ng of template DNA. Amplification was performed in Mastercycler gradient programmable thermal cycler (Eppendorf). The annealing temperature for each primer was determined by gradient PCR. The PCR was programmed with initial denaturation step at 94^{o}C for 4 min, followed by 30 cycles of 1 min at 94^{o}C, 1 min at annealing temperature (Supplementary Table 2) and 1 min at 72^{o}C. A final extension was carried out at 72^{o}C for 10 min and hold temperature of 4^{o}C at the end.

PCR amplified products were electrophoresed on 1.5% (w/v) agarose gels stained with ethidium bromide in 1x TBE buffer. The gel was run at 5v/cm for 2.5 to 3 hours. A 100 bp DNA ladder was used to determine the approximate size of the fragments.

2.4 Data analysis

The RAPD and ISSR bands were scored according to their positions. The binary data was recorded

using '1' for presence of band and '0' for the absence of band at a particular position. A similarity matrix was computed for RAPD, ISSR and RAPD+ISSR combined data using Jaccard's coefficient by the software package PAST [7]. A dendrogram was obtained by Unweighted Pair-Group Average (UPGMA) method using the similarity matrix by Jaccard's similarity coefficient [9] to determine the relatedness of 48 genotypes under study. Bootstrapping was done with 1000 repetitions to evaluate the clusters formed. Principle component analysis (PCA) of the landrace data was done using PAST to reveal the genetic relations among the landraces according to their geographic origin. Population Genetic Analysis (POPGENE Ver 1.32) software was used to determine the values of Nei's genetic diversity [14], Shannon's information index, total genetic diversity among populations (Ht), genetic diversity within population (Hs) and mean coefficient of gene differentiation (Gst) for two populations, i.e. landraces and commercial varieties. RAPD and ISSR data was also subjected to Analysis of Molecular Variance (AMOVA) at individual and population level, using GenALEx software [16]. Correlation was determined between molecular and geographical data [4] by performing Mantel tests between their Euclidian distance matrices using the software package PAST.

Polymorphic Information Content (PIC) for dominant marker systems was calculated [19] as $PIC_i = 2f_i\ (1- f_i)$; where PIC_i is polymorphic information content of marker 'i', f_i is frequency of the amplified allele (band present), and $(1-f_i)$ is frequency of the null allele (band absent). PIC was averaged for all the fragments for each primer.

Resolving power (Rp) of each primer was calculated [17] as $Rp = \sum I_{b,}$ where, I_b is the informativeness of the bands scored which can be calculated as $Ib = 1-(2*|0.5-p|)$ where 'p' is the proportion of 48 genotypes containing bands.

[III]RESULTS

A set of 13 RAPD and 7 ISSR primers (Supplementary Table 2) were used for analysis of genetic diversity among 29 landraces and 19 commercial varieties of cluster bean. The total number of bands, number of polymorphic bands, percentage of polymorphic bands, polymorphic information content (PIC) and resolving power (Rp) obtained for each primer are shown in Table 1

Among the RAPD primers used OPN-01 has the highest resolving power where as OPX-12 has the lowest. Similarly, among ISSR primers UBC-868 has the highest resolving power and UBC-854 has the lowest.

3.1 RAPD analysis

RAPD PCR amplification produced a total of 118 bands, out of which 103 were polymorphic and 15 were monomorphic. Out of the 13 primers used OPQ-09 produced highest number of bands (12) with 100% polymorphism, whereas the primer OPX-12 produced only 5 bands.

[Table-1]

Marker	Total number of bands	Polymorphic bands	Monomorphic bands	% polymorphism	PIC	RP
RAPD						
OPA 1	7	5	2	71.42	0.143	**1.208**
OPD 12	6	6	0	100.00	0.419	**3.625**
OPM 2	9	5	4	55.55	0.141	2.000
OPM 12	8	8	0	100.00	0.199	2.000
OPM 15	11	7	4	63.64	0.189	3.083
OPN 1	11	10	1	90.90	0.363	**6.333**
OPN 2	10	10	0	100.00	0.252	**3.500**
OPN 3	10	10	0	100.00	0.371	5.417
OPN 4	10	9	1	90.00	0.199	2.542
OPN 5	10	9	1	90.00	0.186	2.500
OPQ-09	12	12	0	100.00	0.296	4.875
OPU 15	9	7	2	77.78	0.227	3.208
OPX 12	5	5	0	100.00	0.151	0.917
ISSR						
UBC808	10	7	3	70.00	0.179	2.208
UBC818	12	9	3	75.00	0.258	4.500
UBC820	6	6	0	100.00	0.266	2.250
UBC854	3	2	1	66.67	0.174	0.625
UBC856	9	6	3	66.67	0.081	1.083
UBC868	13	11	2	84.62	0.258	4.708
UBC879	11	9	2	81.82	0.273	4.208

Table 1. Details of all the primers (13 RAPD and 7 ISSR) used and their percentage polymorphism, polymorphic information content and resolving power.

The accessions of cluster bean distinguished into two major clusters at 75% similarity and a third

cluster at lower similarity in the dendrogram constructed using RAPD band data (Supplementary Fig. 1); the first major cluster consists only of landraces and has four sub-clusters. The second and third major clusters have only commercial genotypes and the second cluster could be clearly differentiated into two sub-clusters. Three landraces namely IC-116953, IC-116958 and IC-116595 did not fall into any cluster. PCA analysis (Supplementary Fig. 2) of landraces showed clustering of landraces into a single major group containing the landraces from central Rajasthan and two loosely bound groups.

The observed number of alleles, effective number of alleles, Nei's genetic diversity, Shannon's information index for landraces and commercial varieties using 13 RAPD markers were found to be 1.872±0.335, 1.589±.351, 0.333±0.170, and 0.490±0.230, respectively. The value of total genotypic diversity among population (Ht) was 0.333±0.029, whereas diversity within population (Hs) was found to be 0.283±0.026. Mean coefficient of gene differentiation (Gst) value 0.148 indicated 86.2% of genetic diversity present within the population. The estimated gene flow in the population was 2.857 (Table 2).

[Table-II]

		Observed number of alleles	Effective number of alleles	Nei's gene diversity	Shannon's Information index	Ht	Hs	Gst	Estimate of gene flow
RAPD	**Mean**	1.8729	1.589	0.3339	0.4908	0.3333	0.283	0.148	2.857
	St. Dev	0.3345	0.351	0.1709	0.2309	0.0295	0.026		
ISSR	**Mean**	1.7812	1.4627	0.267	0.3988	0.2639	0.253	0.041	11.549
	St. Dev	0.4167	0.3844	0.1939	0.2681	0.0378	0.035		
RAPD + ISSR	**Mean**	1.8407	1.5446	0.3103	0.4584	0.3089	0.272	0.116	3.787
	St. Dev	0.367	0.3671	0.1817	0.2478	0.0333	0.029		

Table 2. A compartive list of geneitic variability factors across the acessions using RAPD, ISSR and RAPD+ISSR primers.

[Table-III]

Primers	Source of variance	Estimated variance	Percentage (%)	φ_{pt}	P(rand >= data)
RAPD	**Among Pops**	4.594	27%	0.271	0.001
	Within Pops	12.355	73%		
ISSR	**Among Pops**	0.608	8%	0.082	0.001
	Within Pops	6.783	92%		
RAPD + ISSR	**Among Pops**	5.202	21%	0.214	0.001
	Within Pops	19.138	79%		

Table 3. Details of Analysis of Molecular Variance (AMOVA) based on RAPD, ISSR and RAPD+ISSR marker data within and among landraces and commercial varities (level of significance based on 999 iteration steps)

AMOVA helps in understanding RAPD variation among and within the populations. Percentage of molecular variance was found to be 27% among populations and 73% variance was attributed to variance within the population. Table 3 shows the φ_{pt} value, estimated variance and percentage variance among and within the populations.

3.2 ISSR Analysis

The primers $(AG)_8$, $(CA)_8$ and $(AC)_8YA$ produced more bands when compared to $(GT)_8C$ and $(TC)_8RG$ primers. The primers $(GAA)_6$ and $(CTTCA)_3$ produced 13 and 11 bands, respectively. The primer $(GA)_8YC$ produced unclear bands; the primer $(AT)_8C$ did not produce any band. The 7 primers used produced a total of 64 bands out of which 50 were polymorphic and 14 were monomorphic. Three unique bands were observed in accession number IC116752 with 2 bands in primer UBC-856 at about 320bp and 350bp and a single band with primer UBC-868 at about 275bp.

The dendrogram (Supplementary Fig. 3) from ISSR data showed one major cluster at 75% similarity and five minor clusters at lower level

of similarity. The major cluster possesses six sub-clusters. The dendrogram did not differentiate between landraces and commercial varieties. The PCA analysis (Supplementary Fig. 4) of the landraces formed a loose group. The accessions IC116953, IC116595 and IC116682 were distant from the group.

The observed number of alleles, effective number of alleles, Nei's genetic diversity, Shannon's information index for landraces and commercial varieties using 7 ISSR markers were found to be 1.7812±0.4167, 1.4627±0.3844, 0.267±0.1939, and 0.3988±0.2681, respectively. The value of total genotype diversity among population (Ht) was 0.2639±0.0378 whereas diversity within population (Hs) was found to be 0.253±0.035. Mean coefficient of gene differentiation (Gst) value was 0.041 and the estimated gene flow in the population was 11.549. AMOVA was used to analyze variation among and within the populations. Molecular variances were 8% and 92% among and within the population, respectively (Table 3).

3.3 RAPD and ISSR combined analysis

The accessions of cluster bean distinguished into two major clusters at 75% similarity and 3 minor clusters with lower similarity in the dendrogram constructed using the combined data of RAPD and ISSR (Fig 2) the 1st major cluster has only landraces and 5 sub-clusters. The second major cluster had only commercial genotypes and it consisted of 3 sub-clusters. PCA analysis (Supplementary Fig 5) of landraces showed clustering of landraces into a two major groups. A tightly linked group containing the landraces from Jodhpur, Jalore, Churu and Palanpur, and one loosely bound group with accessions from Barmer, Mahendragarh, Nagour, Bikaner and Rajkot.

The observed number of alleles, effective number of alleles, Nei's genetic diversity, Shannon's information index for landraces and commercial varieties using 13 RAPD and 7 ISSR markers were found to be 1.8407±0.367, 1.5446±0.3671, 0.3130±0.1817, and 0.4584±0.2478, respectively.

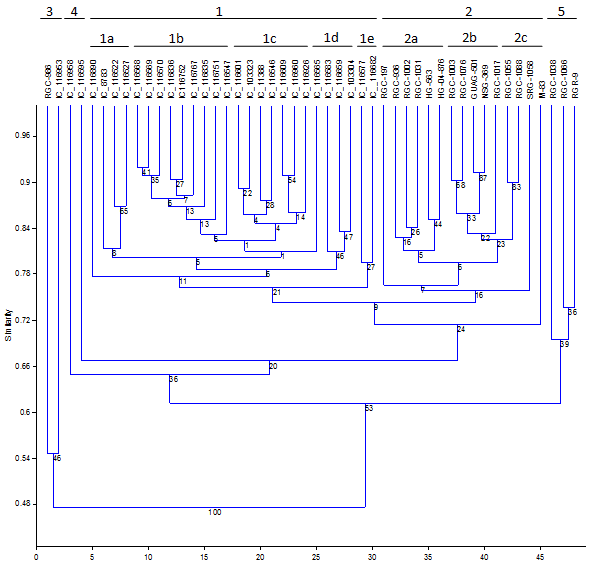

Fig. 2. Dendrogram generated by UPGMA method, showing relationship among 48 accessions of cluster bean based on genetic profile from RAPD+ISSR data. The numbers at the forks show the confidence limits for the grouping of those accessions in the branch, based on 1,000 cycles of bootstrap analysis

The value of total genotype diversity among population (Ht) was 0.3089±0.0333 whereas diversity within population (Hs) was found to be 0.272±0.029. Mean coefficient of gene differentiation (Gst) value was 0.116 and the estimated gene flow in the population was found to be 3.787. AMOVA was used to analyze variation among and within the populations. Molecular variance among populations was found to be 21% and that within the population was 79% indicating higher variation within the population (Table 3).

3.4 Correlation between molecular and geographical data in landraces

The Mantle test revealed a significant correlation between the molecular data and the geographic data. The correlation values for RAPD, ISSR and RAPD+ISSR data were R=0.5252(P=0.0066), R=0.3144(P=0.0266) and R=0.5303 (P=0.0076), respectively. This indicates that molecular

variation corresponds to differences in geographic distribution of landraces.

[IV]DISCUSSION AND CONCULSION

Guar crop has multiple uses as food, fodder and green manure. The galactomannan from seeds of guar have various industrial applications. The crop is self-pollinated and there are no reports on successful wide hybridization for trait transfer in this crop. Hence, landraces are the main source of genetic variability in guar. Our study of genetic polymorphism compared 29 landraces originating from various regions of India and 19 commercial genotypes at molecular level. PCR using 13 RAPD primers produced a total of 118 bands, whereas 7 ISSR primers produced 64 bands. Both the markers revealed genetic variability among the accessions studied. The average level of polymorphism revealed by RAPD was 87.63 which is higher than 77.82 for ISSR. Similarly higher polymorphism for RAPD primers as compared to ISSR primers was reported previously while assessing genetic variation in rice bean [12]. PIC and Rp values of markers reveal the discriminating power of markers. PIC values ranged between 0.141(OPM-02) to 0.419(OPD-12) for RAPD and 0.081(UBC-856) to 0.273(UBC-879) for ISSR markers. Rp of a marker gives a moderate idea about the number of genotypes that could be resolved by that marker [17]. The values of resolving powers for RAPD markers were between 0.917(OPX-12) and 6.333(OPN-1) while that of ISSR markers ranged between 0.625(UBC-854) and 4.708(UBC-868) indicating better resolving capability of RAPD over ISSR. However, in cowpea (*Vigna unguiculata* (L.) Walp.) landraces, more polymorphic loci were detected with ISSR than with RAPD fingerprinting [5].

Shannon's Information Index is a commonly used index to characterize gene diversity in a population; the value of the Shannon's information index was similar for RAPD and RAPD+ISSR data but low for ISSR data. The values of total genetic diversity among population (Ht) also showed similar pattern as that of Shannon's information index. Nei's measure of the average gene diversity per locus within population (Hs) was similar for RAPD (0.283±0.026), ISSR (0.253±0.035) and RAPD+ISSR (0.275±0.029) data showing that both the markers revealed similar level of genetic differences among the genotypes.

AMOVA analysis showed higher polymorphism within the population (73% for RAPD, 92% for ISSR and 79% for RAPD+ISSR combined data). AMOVA results also showed that RAPD was more efficient in detecting the variation between the populations.

Cluster analysis was done on RAPD, ISSR and RAPD+ISSR data. The results based on RAPD and RAPD+ISSR data grouped landraces and commercial varieties separately showing the presence of distinguishable genetic difference existing between them. The accession IC116958 formed an out-group singlet with RAPD data. Similar results with singlet out-group in dendrogram were reported by Punia et al. (2009). The dendrogram from ISSR data produced cluster with commercial varieties along with the landraces. The difference in the clustering pattern can be partially attributed to the number of amplified loci analyzed (118 for RAPD and 64 for ISSR). Similar difference in clustering pattern between RAPD and ISSR markers was observed in genotypes of *Jatropha curcas* [6].

PCA analysis of the landraces showed that the accessions belonging to central Rajasthan grouped together indicating genetic similarity. The Mantle test between the molecular data and the geographic data of the landraces showed significant correlation. The results of our study support the previous observation on the origin of this crop in North Western India by trans domestication of *C. senegalensis* [8] as the molecular variation observed corresponds to geographic distribution of landraces.

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Supplementary Figures and Tables:

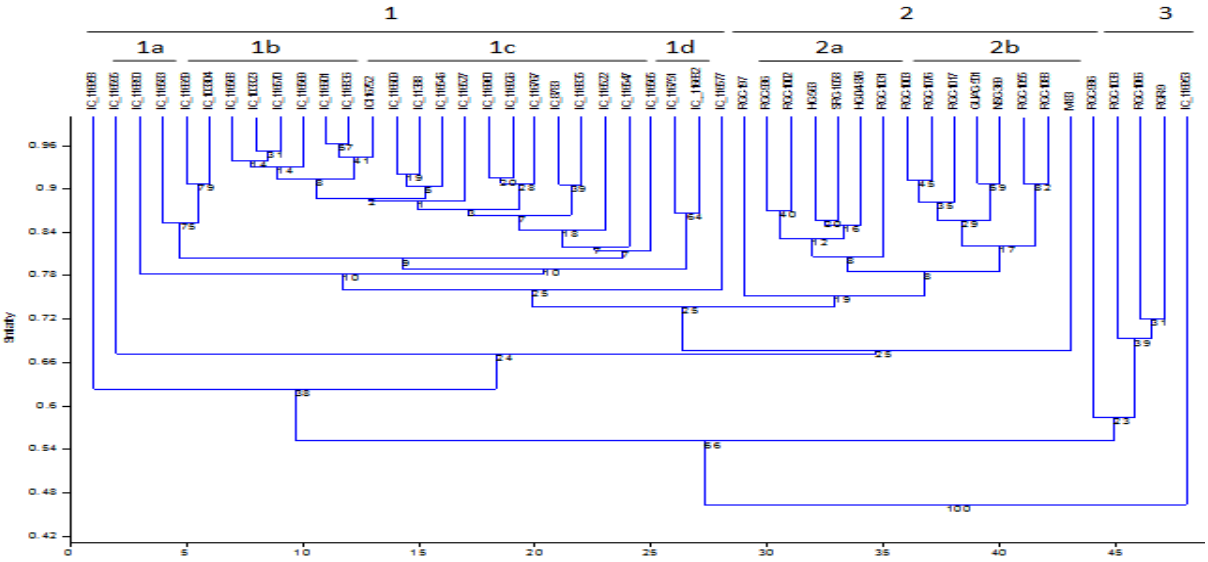

Fig. 1. Dendrogram generated by UPGMA method, showing relationship between 48 accessions of cluster bean based on genetic profile from RAPD data. The numbers at the forks shows the confidence limits for the grouping of those accessions in the branch, based on 1,000 cycles of bootstrap analysis

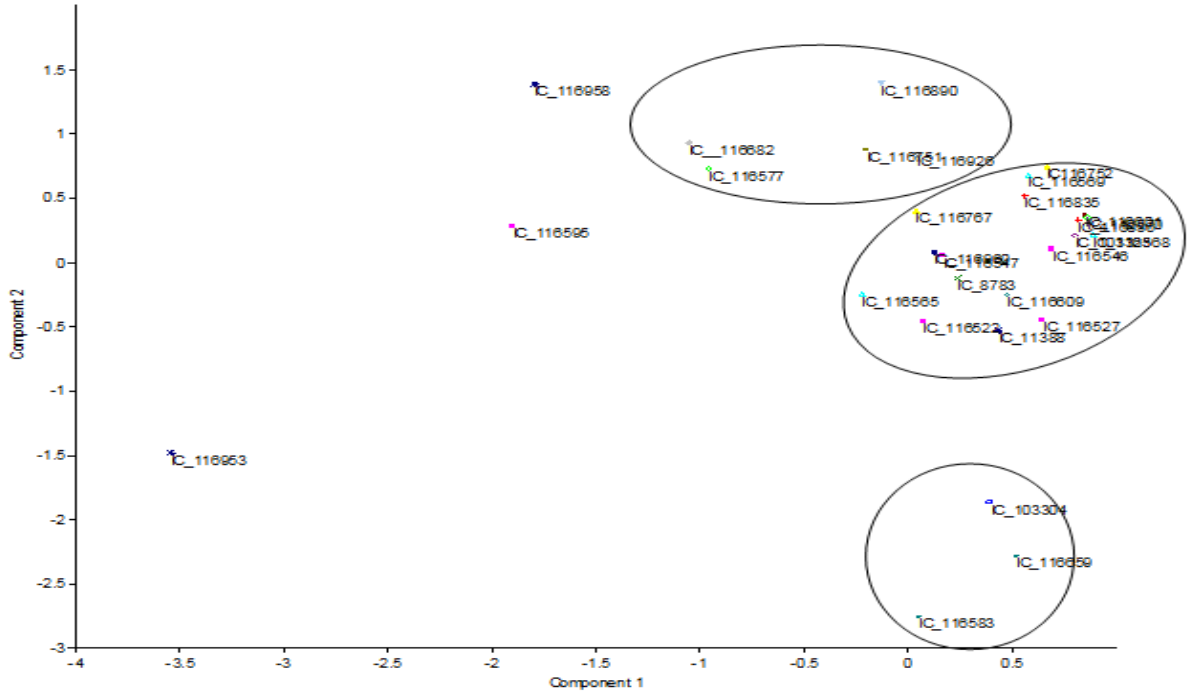

Fig. 2. Two-dimensional plot of principle component analysis (PCA) of cluster bean landraces using RAPD analysis. The circles indicate the group of accessions which are similar to each other in the PCA analysis.

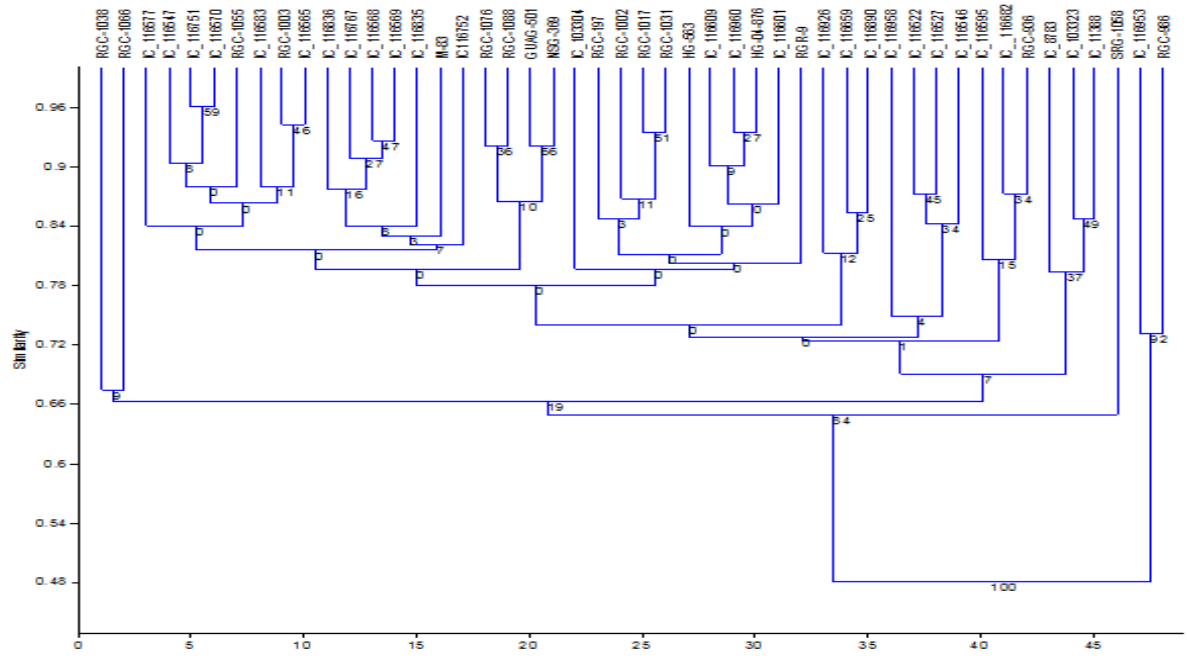

Fig. 3. Dendrogram generated by UPGMA method, showing relationship among 48 accessions of cluster bean based on genetic profile from ISSR data. The numbers at the forks show the confidence limits for the grouping of those accessions in the branch, based on 1,000 cycles of bootstrap analysis

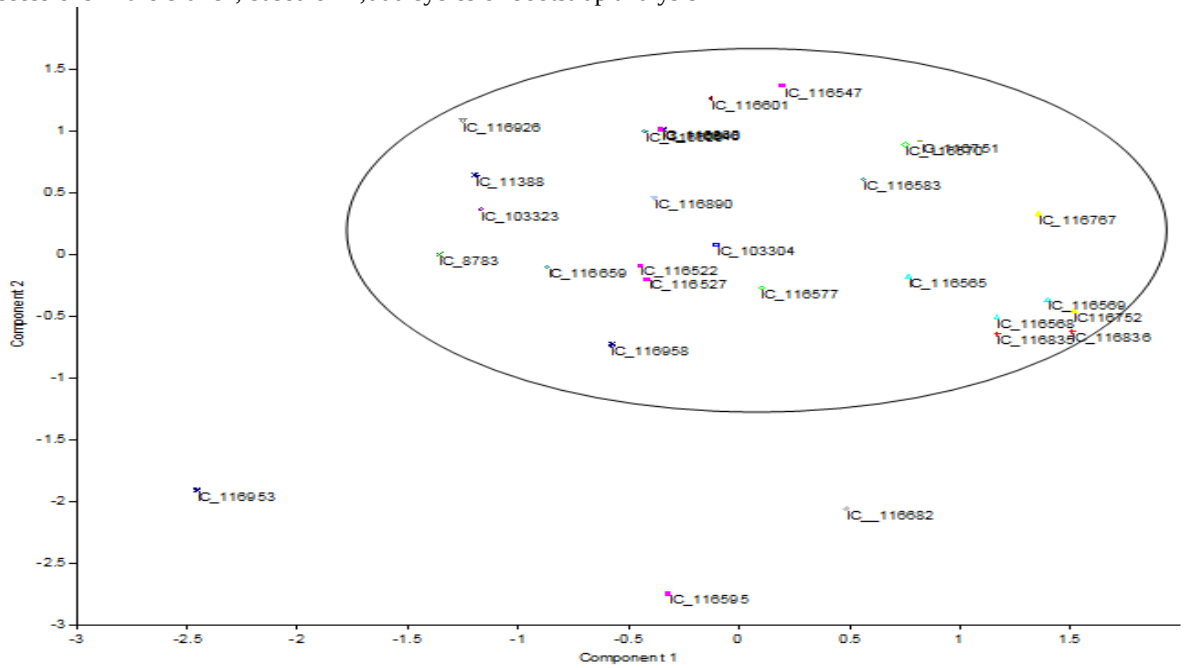

Fig. 4. Two-dimensional plot of principal component analysis (PCA) of cluster bean landraces using ISSR and analysis. The circles indicate the group of accessions which are similar to each other in the PCA analysis.

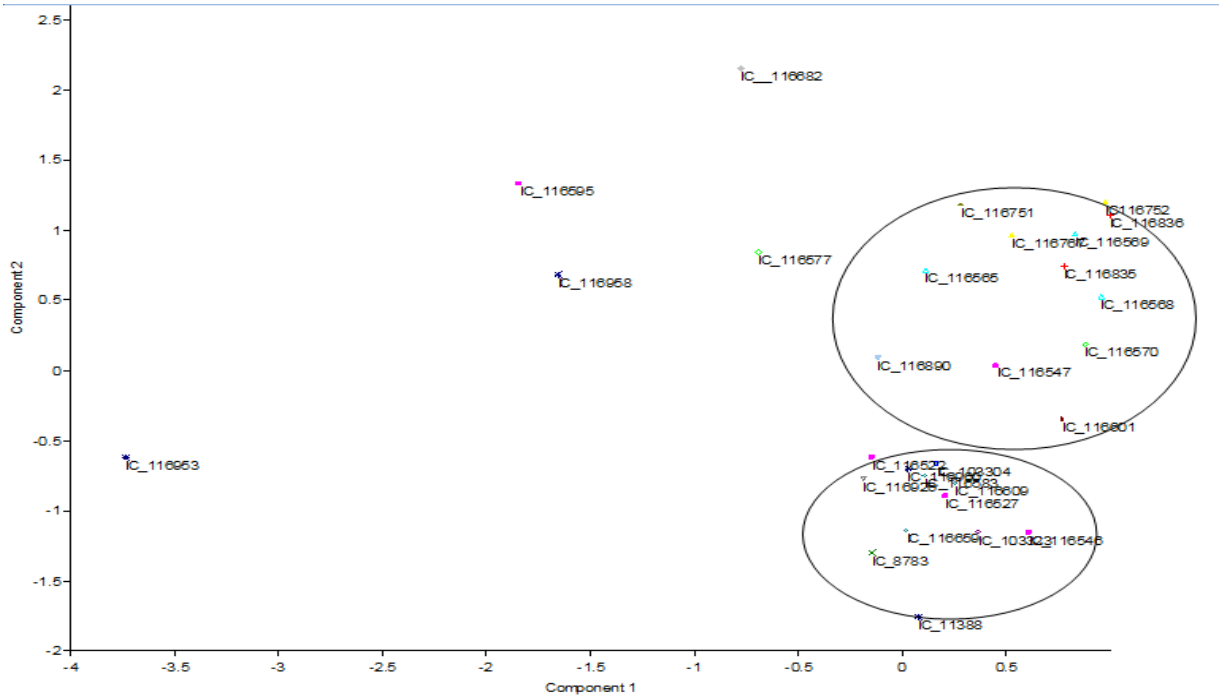

Fig. 5. Two-dimensional plot of principal component analysis (PCA) of cluster bean landraces using RAPD+ISSR analysis. The circles indicate the group of accessions which are similar to each other in the PCA analysis

Table 1. Details of cluster bean genotypes used for the study.

S.No	Accession Number	Location of collection
1	IC 8783	Delhi
2	IC 11388	Jalore
3	IC 103304	Kachchh
4	IC 103323	Himmat nagar
5	IC 116522	Jodhpur
6	IC 116527	Jodhpur
7	IC 116546	Jodhpur
8	IC 116547	Jodhpur
9	IC 116565	Barmer
10	IC 116568	Barmer
11	IC 116569	Barmer
12	IC 116570	Jaisalmer
13	IC 116577	Jaisalmer
14	IC 116583	Churu
15	IC 116595	Jodhpur
16	IC 116601	Bikaner
17	IC 116609	Churu
18	IC 116659	Churu
19	IC 116682	Sikar
20	IC 116751	Nagour
21	IC116752	Nagour
22	IC 116767	Nagour
23	IC 116835	Mahendra garh
24	IC 116836	Mahendra garh
25	IC 116890	Rajkot
26	IC 116926	Palanpur

27	IC 116953	-
28	IC 116958	-
29	IC 116960	-
30	M 83	Commercially grown genotypes of cluster bean
31	RGC 197	
32	RGC 936	
33	RGC 986	
34	RGC 1002	
35	RGC 1003	
36	RGC 1017	
37	RGC 1031	
38	RGC 1038	
39	RGC 1055	
40	RGC 1066	
41	RGC 1076	
42	RGC 1088	
43	RGR 9	
44	HG 563	
45	HG 04-876	
46	SRG 1058	
47	GAUG 501	
48	NSG369	

Table 2. Details of the RAPD and ISSR primers used in the present study.

Marker	**Sequence (5'-3')**	**Annealing temperature Tm(^{o}C)**
RAPD		
OPA 1	CAGGCCCTTC	40
OPD 12	CACCGTATCC	40
OPM 2	ACAACGCCTC	38.4
OPM 12	GGGACGTTGG	40
OPM 15	GACCTACCAC	38.4
OPN 1	CTCACGTTGG	37.9
OPN 2	ACCAGGGGCA	38.4
OPN 3	GGTACTCCCC	38.4
OPN 4	GACCGACCCA	39
OPN 5	ACTGAACGCC	38.4
OPQ-09	GGCTAACCGA	40
OPU 15	ACGGGCCAGT	39
OPX 12	TCGCCAGCCA	39
ISSR		
UBC808	AGAGAGAGAGAGAGAGC	55
UBC818	CACACACACACACACAG	56
UBC820	GTGTGTGTGTGTGTGTC	57
UBC854	TCTCTCTCTCTCTCTCRG	49
UBC856	ACACACACACACACACYA	57
UBC868	GAAGAAGAAGAAGAAGAA	44.5
UBC879	CTTCACTTCACTTCA	47

Y = pYrimidine (C, T)
R = puRine (A, G)