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Genetic Diversity Clusters in Germplasm of Cluster Bean (*Cyamopsis tetragonoloba* L., Taub), an Important Food and an Industrial Legume Crop

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ABSTRACT

Genetic diversity in cluster bean (*Cyamopsis tetragonoloba* L.; *Fabaceae*) genotypes was studied using Inter-Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD) to derive conclusions about diversity analysis in groups of accessions of a germplasm. The two methods, individually as well as cumulatively revealed the range of diversity in profiles among 104 genotypes collected from different geographical regions of India. A simulated clustering of the collected plant genotypes was divided into defined affinity groups using Structure program and the cluster analysis of molecular markers data revealed six broad sub-clusters. These results were validated with a Principal Coordinate analysis. The combined data was more informative than either of the individual method data. The diversity range was found to be wide and the presence of six broad clusters suggests the existence of many genetic lineages that can constitute useful starting points for the use of germplasm diversity in the selection and improvement of the cluster bean crop.

Keywords: Diversity, ISSR, NJ, PCA, RAPD.

INTRODUCTION

Cluster bean or guar is a drought-tolerant, annual, self pollinated, diploid (2x= 14) legume crop (Ayyangar and Krishnaswami, 1933). It has been cultivated in India and Pakistan for many centuries. Guar has many facets used for: (i) Within a crop rotation cycle; (ii) Human and animal nutrition, and (iii) As a source of galactomannan, an edible gum and categorized as an industrial crop. In fact, much more than its use as a food crop, it is best valued for the gelling agent galactomannan (guar gum) most significantly used in several different industries. Over 75% of world production of cluster bean is from India (Punia *et al*., 2009a). However, due to the increasing demand for the plant, it is now also introduced into newer areas for cultivation. In India, though the plant is cultivated in several states, Rajasthan, Haryana, Punjab and Gujarat however, account for over 80% of the total production of cluster bean in the country. Elsewhere in the world, cluster bean is cultivated in Pakistan, Sudan, and USA. In its native range in the country, the plant does not seemingly grow in the wild states. Under cultivation, the cluster bean crop season is ~16 weeks of basically a warm weather plus moderate rainfall duration. The cluster bean however, is also well adapted to cultivation in semi-arid to arid climate zones in India (Dwivedi *et al*., 1999; Henry and Mathur 2005; Pathak *et al*., 2010). Basic information of genetic and phenotypic variability, heritability, genetic

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advance, correlation and direct path effects of yield related traits (Patil, 2014b), genetic components of variance (genotypic, phenotypic and error variance) and genotypes×sowing dates interaction (Patil, 2014a) were established which could facilitate the improvement of varieties and help to select a suitable breeding procedure for semi arid tract of Deccan plateau region belonging to Western Ghats of Maharashtra, India. Despite the economic importance of cluster bean, studies on genetic diversity analysis have been limited. Fifteen genotypes of cluster bean were examined for genetic diversity using three RAPD primers (Ajit *et al*., 2013) while Sharma *et al*. (2014) analyzed 35 genotypes with 20 RAPD and 10 ISSR primers that revealed ~90 and 97% polymorphic bands respectively. Prior to these, Punia *et al*. (2009a) and Pathak *et al*. (2010) analyzed 34 and 32 genotypes with 10 and 5 RAPD primers respectively. Since these earlier studies on cluster bean have been carried out with limited genotypes, they will not reflect a true extent of genetic diversity. In the present study, a wide diversity was reported by analysis of 104 genotypes of cluster beans using ISSR and RAPD profiles. Furthermore, in order to estimate the number of sub-populations among the collected *C. tetragonoloba* germplasm, population structure was simulated using model-based clustering method implemented in the program Structure and six distinct lineages were determined supporting the assessment of a wide range of genetic diversity among these genotypes.

MATERIALS AND METHODS

Plant Material

A total of 104 genotypes of cluster beans were collected from different geographical regions of India for the assessment of molecular genetic diversity which have been listed in Table 1.

Isolation and Purification of Genomic DNA

Total genomic DNA from the young leaves was isolated following the Thompson and Murray (1980) method using Cetyl Trimethyl Ammonium Bromide (CTAB).

Quantification of DNA

The quality and quantity of isolated DNA samples were checked by measuring absorbance at 260 and 280 nm using a nano-drop spectrophotometer (ND 1000). Additionally DNA samples were electrophoresed on 0.8% (w/v) agarose gel at 75V for 3-5 hours in TBE (0.5X) buffer pH 8.3 to check for the size of the isolated DNA.

ISSR Primers and PCR Conditions

A number of ISSR primers were available. Therefore, pilot experiments were carried out with small sets of cluster bean DNAs and as many as 50 ISSR primers were screened for consistent, well banded, reproducible profiles in triplicate experiments. Additionally, in these pilot reactions, the annealing temperatures for the ISSR primers were also optimized and the final reactions were always carried out at these optimized temperatures. Only those primers resulted in discrete profiles of separated bands, which were selected for analysis of full set of cluster bean genotypes (Table 2). Typical ISSR reaction consisted of 50 ng DNA, 10 pmoles primers, 1X PCR buffer (10 mM Tris pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin), 200 µM each dNTP and 0.75 U of *Taq* DNA Polymerase (Bangalore Genei, Bangalore, India) in a final volume of 25 µl; while the PCR parameters included pre-denaturation at

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Table 1. The cluster bean genotypes collected from different locations in the country. All samples were collected or procured as seed packets. The seeds were planted in a well spaced out of the field plot in the botanical garden of the Institute and fresh healthy leaf tissue were harvested for the DNA isolation.

Sample no.	Genotype	Collected from	Sample no.	Genotype	Collected from
Ct1	325806	CAZRI, Jodhpur	Ct53	311442	CAZRI, Jodhpur
Ct2	329036	CAZRI, Jodhpur	Ct54	415162	CAZRI, Jodhpur
Ct3	329030	CAZRI, Jodhpur	Ct55	323992	CAZRI, Jodhpur
Ct4	370478	CAZRI, Jodhpur	Ct56	373438	CAZRI, Jodhpur
Ct5	373557	CAZRI, Jodhpur	Ct57	311393	CAZRI, Jodhpur
Ct6	415342	CAZRI, Jodhpur	Ct58	373467	CAZRI, Jodhpur
Ct7	402298	CAZRI, Jodhpur	Ct59	415145	CAZRI, Jodhpur
Ct8	421810	CAZRI, Jodhpur	Ct60	329062	CAZRI, Jodhpur
Ct9	258101	CAZRI, Jodhpur	Ct61	311405	CAZRI, Jodhpur
Ct10	329030 LT	CAZRI, Jodhpur	Ct62	311449	CAZRI, Jodhpur
Ct11	329030 SPS Trial	CAZRI, Jodhpur	Ct63	311444	CAZRI, Jodhpur
Ct12	311406	CAZRI, Jodhpur	Ct64	325743	CAZRI, Jodhpur
Ct13	370509	CAZRI, Jodhpur	Ct65	415157	CAZRI, Jodhpur
Ct14	369789	CAZRI, Jodhpur	Ct66	311414	CAZRI, Jodhpur
Ct15	415166	CAZRI, Jodhpur	Ct67	370502	CAZRI, Jodhpur
Ct16	311433 LT	CAZRI, Jodhpur	Ct68	369868	CAZRI, Jodhpur
Ct17	Selection-1	Lucknow	Ct69	415153	CAZRI, Jodhpur
Ct18	RGC-1033	CAZRI, Jodhpur	Ct70	311392	CAZRI, Jodhpur
Ct19	HG-563	Lucknow	Ct71	370468	CAZRI, Jodhpur
Ct20	RGC-197	CAZRI, Jodhpur	Ct72	373480	CAZRI, Jodhpur
Ct21	RGC-1055	CAZRI, Jodhpur	Ct73	324023	CAZRI, Jodhpur
Ct22	Pusa Navbahar	Lucknow	Ct74	RGC-1002 PAS	CAZRI, Jodhpur
Ct23	RGC-471	CAZRI, Jodhpur	Ct75	RGC-1066	RARI, Jaipur
Ct24	RGC-986	RARI, Jaipur	Ct76	Sharda	Lucknow
Ct25	RGC-1038	CAZRI, Jodhpur	Ct77	Raipur hybrid	Lucknow
Ct26	RGC-1003	RARI, Jaipur	Ct78	RGC-1038	RARI, Jaipur
Ct27	Jaisalmer local	SKRAU, Bikaner	Ct79	RGC-936	CAZRI, Jodhpur
Ct28	RGC-1066	CAZRI, Jodhpur	Ct80	Gst 13-1	SV University, Tirupati
Ct29	RGC-1017	RARI, Jaipur	Ct81	Gst 13-19 PAS	SV University, Tirupati
Ct30	RGC-1002	CAZRI, Jodhpur	Ct82	Gst 13-13	SV University, Tirupati
Ct31	Gst 13-11	SV University, Tirupati	Ct83	Gst 13-18	SV University, Tirupati
Ct32	Gst 13-10	SV University, Tirupati	Ct84	Gst 13-17	SV University, Tirupati
Ct33	Gst 13-9	SV University, Tirupati	Ct85	Gst 13-16	SV University, Tirupati
Ct34	Gst 13-8	SV University, Tirupati	Ct86	Gst 13-15	SV University, Tirupati
Ct35	Gst 13-7	SV University, Tirupati	Ct87	Gst 13-14	SV University, Tirupati
Ct36	Gst 13-6	SV University, Tirupati	Ct88	Gst 13-12	SV University, Tirupati
Ct37	Gst 13-5	SV University, Tirupati	Ct89	324008	CAZRI, Jodhpur
Ct38	Gst 13-4	SV University, Tirupati	Ct90	370490	CAZRI, Jodhpur
Ct39	Gst 13-3	SV University, Tirupati	Ct91	420332	CAZRI, Jodhpur
Ct40	Gst 13-2	SV University, Tirupati	Ct92	415112	CAZRI, Jodhpur
Ct41	Lucknow local	Lucknow	Ct93	311422	CAZRI, Jodhpur
Ct42	Gst 13-19	SV University, Tirupati	Ct94	311407	CAZRI, Jodhpur
Ct43	Desi Anupama	Lucknow	Ct95	402294	CAZRI, Jodhpur
Ct44	Bhusawal local	Lucknow	Ct96	311438	CAZRI, Jodhpur
Ct45	RGC-936	RARI, Jaipur	Ct97	373427	CAZRI, Jodhpur
Ct46	RGC-1031	RARI, Jaipur	Ct98	415148	CAZRI, Jodhpur
Ct47	Swati-55	Lucknow	Ct99	415131	CAZRI, Jodhpur
Ct48	Raipur local	Lucknow	Ct100	311428	CAZRI, Jodhpur
Ct49	Kanpur local	Lucknow	Ct101	415111	CAZRI, Jodhpur
Ct50	311401	CAZRI, Jodhpur	Ct102	415102	CAZRI, Jodhpur
Ct51	415160	CAZRI, Jodhpur	Ct103	Surat local	Surat, Gujarat
Ct52	369861	CAZRI, Jodhpur	Ct104	Omaxe parth hybrid	Jabalpur, Madhya Pradesh
			OG-1	Outgroup (*Clitorea ternatea*)	Lucknow

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Table 2. The different primers, their sequences and annealing temperatures used for ISSR and RAPD methods are listed. The primers were selected for amplifying all the *C. tetragonoloba* genotype DNAs after screening in pilot reactions as described.

Primer	Sequence (5' – 3')	Annealing Temperature (°C) [c]	Numbers of bands (T= Total; P= Polymorphic, M= Monomorphic)			Proportion of P bands (%)
			T	P	M	
808 [a]	*AGAGAGAGAGAGAGAGC*	51 [c]	9	9	0	100
809 [a]	*AGAGAGAGAGAGAGAGG*	46 [c]	8	8	0	100
823 [a]	*TCTCTCTCTCTCTCTCC*	52 [c]	12	11	1	92
824 [a]	*TCTCTCTCTCTCTCTCG*	51 [c]	5	5	0	100
825 [a]	*ACACACACACACACACT*	42 [c]	9	9	0	100
855 [a]	*ACACACACACACACACYT*	54 [c]	15	15	0	100
866 [a]	*CTCCTCCTCCTCCTCCTC*	51 [c]	11	10	1	91
868 [a]	*GAAGAAGAAGAAGAAGAA*	46 [c]	9	9	0	100
880 [a]	*GGAGAGGAGAGGAGA*	42 [c]	14	14	0	100
888 [a]	*(B)(D)(B)CACACACACACACA*	51 [c]	10	9	1	90
			102	**99**	**3**	**97.3**
OP-F02 [b]	*GAGGATCCCT*	35	16	16	0	100
OP-F04 [b]	*GGTGATCAGG*	35	16	16	0	100
OP-F05 [b]	*CCGAATTCCC*	35	14	11	3	79
OP-F09 [b]	*CCAAGCTTCC*	35	11	6	5	55
OP-F18 [b]	*TTCCCGGGTT*	35	17	17	0	100
OP-G04 [b]	*AGCGTGTCTG*	35	14	13	1	93
OP-G13 [b]	*CTCTCCGCCA*	35	14	10	4	71
OP-W04 [b]	*CAGAAGCGGA*	35	12	12	0	100
OP-W10 [b]	*TCGCATCCCT*	35	11	9	2	82
OP-W15 [b]	*ACACCGGAAC*	35	14	13	1	93
			139	**123**	**16**	**87.3**

[a] ISSR Primers; [b] RAPD primers, [c] Annealing temperatures for ISSR primers were optimized separately in pilot experiments while those for the RAPD primers were always set at 35°C. The total numbers of bands and proportion of polymorphic bands for ISSR and RAPD primers respectively are indicated in bold fonts.

94°C for 5 minutes, followed by 45 cycles of denaturation at 94°C for 45 seconds, annealing at optimized temperature for 45 seconds and extension at 72°C for 2 minutes. The final cycle allowed an additional extension at 72°C for 5 minutes.

RAPD Primers and PCR Conditions

Pilot experiments were carried out with small sets of cluster bean DNAs and as many as 60 RAPD primers were screened (20 primers from each of the three kits, F, G and W; QIAGEN OPERON primers) for consistent, well banded, reproducible profile in triplicate experiments. The typical RAPD reaction consisted of 50 ng DNA, 10pmoles primers, 1X PCR buffer (10 mM Tris pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin), 200 µM each dNTP and 0.75 U of *Taq* DNA Polymerase (Bangalore Genei, Bangalore, India) in a final volume of 25 µl; while the PCR parameters included pre-denaturation at 94°C for 3 minutes, followed by 45 cycles of denaturation at 94°C for 1 minute, annealing at 35°C for 1 minute and extension at 72°C for 1.5 minutes. The final cycle allowed an additional extension at 72°C for 5 minutes.

Agarose Gel Electrophoresis

The PCR products were electrophoresed on agarose gels 1.2% (w/v) in 0.5X TBE buffer, pH 8.3 and containing ethidium bromide (0.5 µg ml^{-1}) at a constant 75 volt. After electrophoresis, the gels were viewed on UV transilluminator and images were saved using the Alpha Imager gel documentation system. The low range DNA ruler was used as the DNA size marker for comparing the profiles among the different genotypes of cluster bean.

Band Scoring and Data Analysis

Clear and well separated bands were coded in a binary form by denoting '0' and '1' for absence and presence of bands, respectively in each genotype and for each primer. For a given method bands from all primers were scored cumulatively. Additionally a cumulative data matrix was generated by combining RAPD and ISSR data. The band data were analyzed for pair wise distance using the Jaccard coefficient in Free Tree Package (Pavlicek *et al.*, 1999). Likewise, the 1,000 replicate bootstrap tree was also generated using the same package by the NJ method. The tree files were viewed, annotated and printed using the Tree View program (Page, 2001). For an estimation of the number of sub-populations among the collected *C. tetragonoloba* germplasm, population structure simulation analysis was carried out using model-based clustering method implemented in the Program Structure, version 2.3.4 (Pritchard *et al.*, 2000). The membership of each genotype was tested for the range of genetic clusters from K= 2 to 10 with admixture model and without prior information on their origin. Three independent runs were assessed for each labeled K and each run consisted of 30,000 burn-in period and 100,000 iterations. The optimal value of K was determined by examination of the ΔK statistic and L (K) according to Evanno *et al.* (2005) using the program Structure Harvester (Earl and von Holdt, 2012). The Principle Coordinate plot was generated using the program GenAlEx, ver. 6.5 (Peakall and Smouse, 2012).

RESULTS

The total genomic DNAs were prepared from leaf tissues as described. The DNA was found to be high molecular weight (as determined by agarose gel electrophoresis, data not shown), and had spectrophotometer ratios A260/A280 in the range of 1.82-2.17 determined by Nano-Drop 1000 spectrophotometer. Between three and five DNAs were randomly selected to set up pilot reaction for screening ISSR and RAPD

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primers as described. On the basis of the pilot experiments, primers that resulted in distinct well separated bands in case of all the genotype clustered were selected for further analysis and these primers have been listed in Table 2. In the above pilot experiment DNA from out-group taxon (Table 1) was also processed under similar conditions to that of cluster beans.

ISSR-PCR ANALYSIS

ISSR method resolved a total of 102 bands for all the selected primers (Table 2), from which 99 bands were polymorphic (~97%). The 10 primers individually resolved between five (with primer 824) and 15 (with primer 855) bands across all the genotypes. Seven of the ten primers revealed 100% polymorphic bands and only the primers 823, 866 and 888 revealed mono-morphic bands as well (Table 2). Band data obtained in case of all the ISSR primers for the cluster bean genotypes were input as the binary matrix of "1" and "0" as described. From this binary matrix pair wise, Jaccard distances were computed. The range of distances between pairs of cluster bean genotypes were found to be 0 (Ct67 and Ct69) to 0.90 (Ct64 and Ct104) with an average distance across all the pairs of 0.55 (Table 3). The NJ dendrogram resolved all the cluster bean genotypes into broad sub-clusters with the out-group taxa clearly separated from the cluster bean genotypes (Figure 1-a).

RAPD-PCR Analysis

RAPD method resolved a total of 139 bands for all the selected primers (Table 2), from which 123 bands were polymorphic (~87%). The 10 primers individually resolved between 11 (with primer OP-F09 and OP-W10) and 17 (with primer OP-F18) bands across all the genotypes. Unlike ISSR primers, only four (OP-F02, OP-F04, OP-F18 and OP-W04) of the ten primers revealed 100% polymorphic bands while the remaining primers revealed monomorphic bands as well (Table 2). Band data obtained in case of all the ISSR primers for the cluster bean genotypes were input as the binary matrix of "1" and "0" as described. From this binary matrix pair wise, Jaccard distances were computed. The distances between pairs of cluster bean genotypes were found to be in the range 0.024 (Ct87 and Ct91) to 0.68 (Ct57 and Ct104) with an average distance across all the pairs of 0.41 (Table 3). Similarly in this case, the NJ dendrogram resolved all the cluster bean genotypes into broad sub-clusters with the out-group taxa clearly separated from the cluster bean genotypes (Figure 1-b).

Cumulative Band Data

In order to better resolve the affinities of genotypes to each other the ISSR and RAPD data were considered cumulatively. Here the least (0.023) and highest (0.75) pair wise distances were found to be between genotypes Ct67, Ct69, Ct64 and Ct104

Table 3. Pairwise Jaccard distances were computed as described for the band data of each method separately as well as for the combined data of the two methods. The ISSR method revealed a wider range of distances while the RAPD method revealed the smallest range.

Method	Jaccard Distances			Δ Range of distances
	Minimum	Maximum	Average	
ISSR	0.00	0.90	0.55	0.90
RAPD	0.024	0.68	0.41	0.56
ISSR + RAPD	0.023	0.75	0.47	0.63

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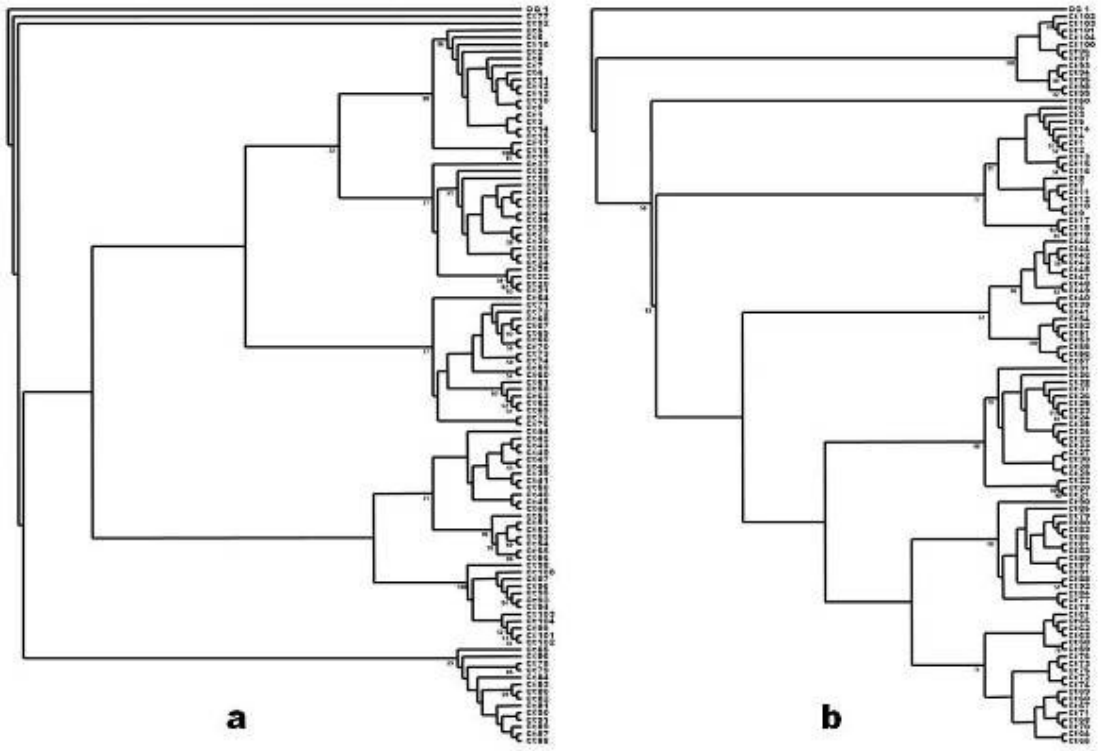

Figure 1. NJ trees for ISSR **(a)** and RAPD **(b)** primer data. The band data for the two methods were analyzed by FREETREE and NJ trees were computed on the basis of pair wise Jaccard distances. The generated tree files were viewed and annotated by TREE View program. The OTU names in the trees are the *Cyamopsis tetragonoloba* genotypes and out-group as in Table 1. The lengths of the branches in each tree are scaled according to the Jaccard distances.

respectively, while the average distance across all the pairs was 0.47 (Table 3). The NJ dendrogram, after a 1,000 replicate bootstrap is shown in Figure 3-a. The out-group taxa were well separated from the rest of the cluster bean genotypes which were in turn grouped into six clusters marked with colored bars.

Model-based Structure Simulation Analysis

The observation that the combined ISSR and RAPD data resolved an NJ tree with all genotypes divided in six broad clusters suggests that these clusters represent many genetic lineages. The NJ tree however, does not reveal genetic admixtures, if any, among the genotypes and in order to assess this, a simulation was carried out using model-based clustering in the Structure program where data were analyzed for membership of each genotype in two to ten possible clusters allowing admixtures. This analysis of simulation with each of the ten values of *K* revealed that the simulation of six population clusters best resolved the data in terms of least deviations in probabilities of the estimates and the highest delta *K* values (Figures 2-a and -b). On this basis the extent of membership of each genotype in the six population clusters was plotted by assigning a different color to the population. This plot has been given in Figure 3-b along with the NJ tree (Figure 3-a) for the combined PCR profile data for comparison. Colored bars representing the total clusters in both the NJ tree as well as the structure plot are used to

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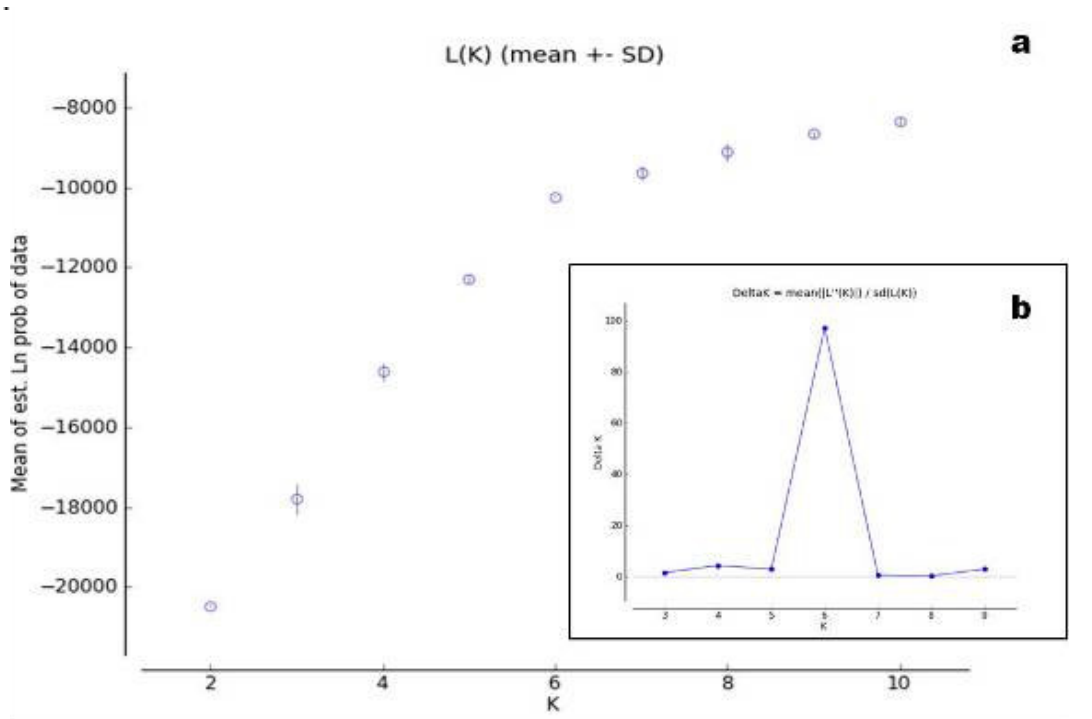

Figure 2. (a) Plot of *K* values with mean of estimated log probability. The small vertical bars in the plot at each K value indicate the deviation range of estimated log probability means in different replicates. **(b)** Plot of *K* values with ΔK generated for simulation analysis using Structure program and Structure Harvester as described. In both plots, for *K* value 6, least deviation in log probability and maximum ΔK indicate that this value of *K* represents the best possible numbers of clusters that the genotypes can be grouped under the conditions of the simulation carried out.

show congruence of genotypes by both methods. The six clusters identified in the present study are validated by a Principle Component Analysis where the plot of genotypes is consistent with the grouping in the Structure simulation clusters (Figure 4). The genotype groupings in the present study however, do not show any relationship to the provenance of the genotypes. Primarily the clusters data indicate that there are as many as six genetic lineages among the tested genotypes in the present study. Such a distribution of genotypes in the collection is useful to identify potentially contrasting genotypes for agro-morphological traits as well as their utilization for the subsequent cluster bean improvement program.

DISCUSSION

The ISSR and RAPD markers, individually or together with other markers, are most widely used for identifying relationships at the cultivar and species level as well as for estimating genetic diversity in plant germplasm. These markers have been used individually as well as together and have generated many useful data about the plant genotypes studied. Though these markers were first described 20 and 24 years ago (ISSR - Zietkiewicz *et al*., 1994; RAPD - Williams *et al*., 1990; Welsh and McClelland, 1990), they are still being used with considerable success. Recent reports of the application of these markers included the

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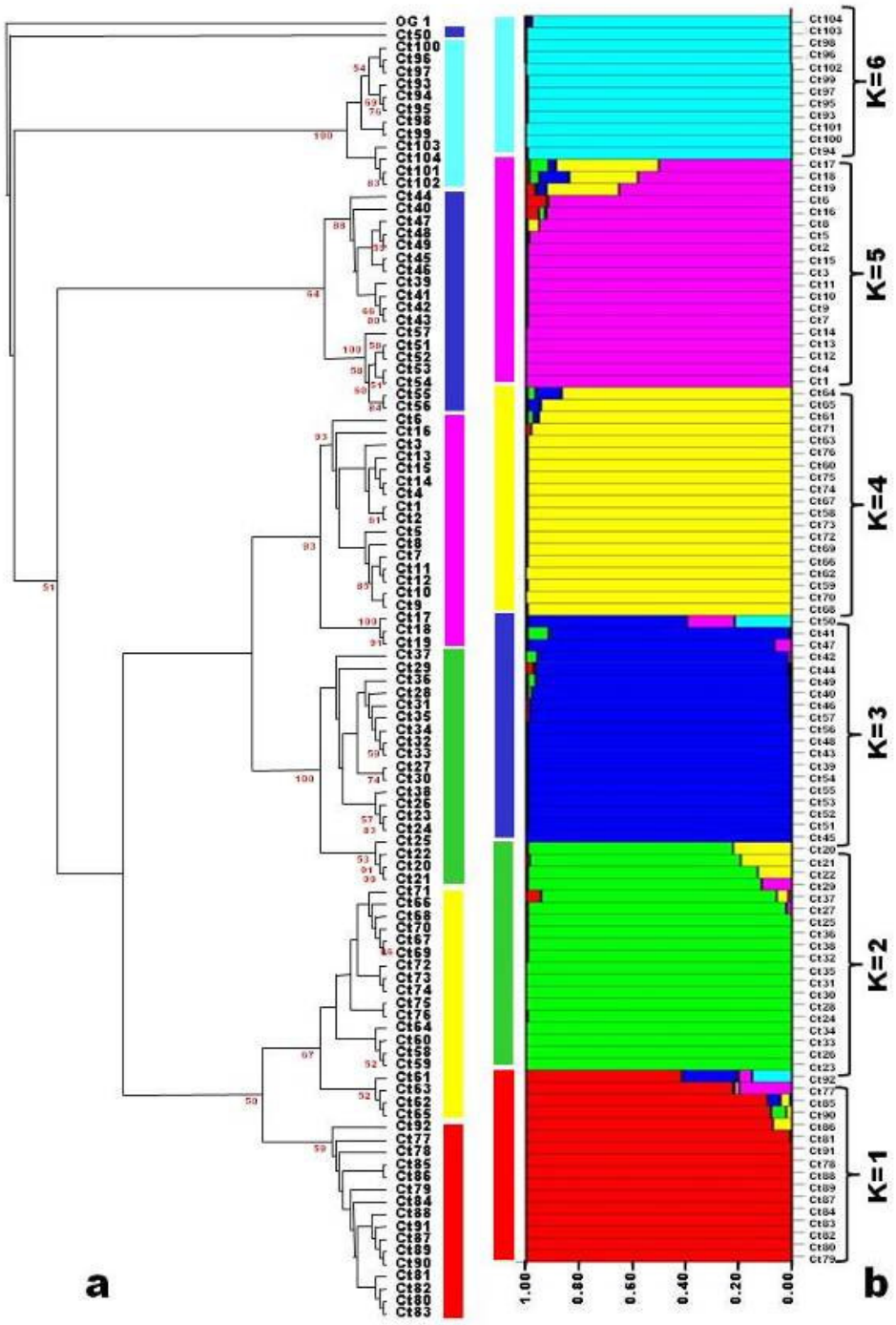

Figure 3. (a) The NJ tree from the combined band data of both ISSR and RAPD primers. The tree was generated after a 1000 replicate bootstrap and only 50% or greater bootstrap supports are indicated for clarity (small numbers before the nodes). The tree indicates resolution of all the *Cyamopsis tetragonoloba* genotypes into six broad clusters. The genotypes are identified by numbers as in Table 1. **(b)** The *K* plot of all genotypes in a model of six clusters (designated as K= 1 to K =6) is depicted in this figure. The genotypes are identified by numbers as in Table 1. The cluster distribution of the genotypes is color coded with six colors to further depict admixtures, if any. Similar color bars corresponding to the total clusters in both NJ tree as well as the *K* plot have been provided such that the color coding enables easy identification of the clusters congruent in both plots.

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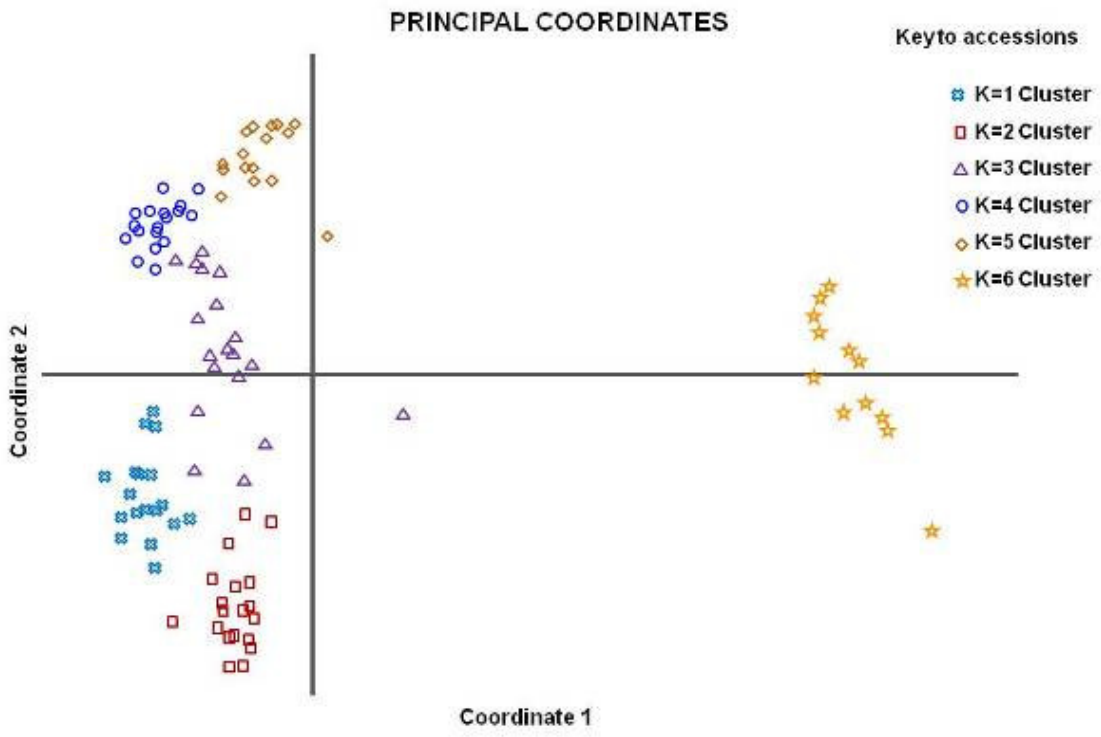

Figure 4. Principle Coordinate Analysis plot for 104 genotypes of cluster bean considered as belonging to the six clusters (K= 1 to K= 6) according to the Structure simulation. Consistent with the simulation, very little admixture is found among the genotypes. The genotypes in a given cluster share the same symbol as shown in the figure key.

assessment of genetic diversity in plants like sugarcane (Khaled *et al*., 2015); castor (Kallamadi *et al*., 2015); and *Aconitum* germplasm (Zhao *et al*., 2015). In a plant like *Harpagophytum*, Muzila *et al*. (2014) have used RAPD and ISSR markers to provide evidence of introgression. These two markers have also been used to assess genetic structure of olive populations (Linos *et al*., 2014) also for divergence analysis and conservation management in a medicinal plant named *Justicia adhatoda* L. (Kumar *et al*., 2014). Similarly Vaishali *et al*. (2014) have compared genetic diversity analysis of *Butea monosperma* from different agro-ecological regions of India by ISSR and RAPD marker systems. These markers have also been used in case of *Moringa oleifera* Lam. (Saini *et al*., 2013) cultivars from India where the authors have resolved considerable genetic diversity among the cultivars. In another study, Jayabalan *et al.* (2013) have assessed genetic diversity in *Solanum trilobatum* L., an important medicinal plant from South India and showed that the UPGMA cluster analysis grouped all Tamil Nadu accessions in one cluster relative to accessions from other states. All of these recent reports continue to support the utility of both marker systems to resolve genetic diversity in plants. The two markers have also been employed in several legumes in the recent times for studies on diversity, population structure as well as for germplasm characterization (Cowpea – Anatala *et al*., 2014; Gajera *et al*., 2014; Khan *et al*., 2015; Pigeonpea - Yadav *et al*., 2014; Chickpea - Singh *et al*., 2014; Mung bean and other Vigna species – Das *et al*., 2014; Singh *et al*., 2014; Faba bean -

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Ammar *et al*., 2015; Cluster bean - Sharma *et al*., 2014; winged bean – Chen *et al*., 2015; horsegram – Sharma *et al*., 2015; lentils – Idrissi *et al*., 2015; tree legume – Li and Geng, 2015). The profusion of these recent studies suggests that many leguminous crops are now the subjects of investigation with molecular markers.

In case of cluster bean, the studies reported this far have not only used a limited number of genotypes but have also used only one marker type at a time (Punia *et al*., 2009b; Pathak *et al*., 2010; Ajit *et al*., 2013) and only Sharma *et al*. (2014) have used both markers. They have however, used limited genotypes and might have not represented a true extent of genetic diversity in cluster bean in the country. We have used a large number of genotypes collected from different locations and observed diversity amongst these with similarity coefficients ranging from the least 0.023 to the highest 0.75 when ISSR and RAPD data are considered together. Individually, the ISSR method revealed the most diversity. This result is in agreement with that reported earlier by Sharma *et al*. (2014) who also observed greater resolution power in the ISSR method relative to the RAPD method. However, considering that the ISSR method reveals polymorphism in the regions of genome rich in SSR while the RAPD profiles are from more dispersed regions of the genome, it is more useful to analyze the two methods in combination. In the present study a large number of genotypes were from CAZRI, Jodhpur followed by SV University, Tirupati, India (Table 1). However, the UPGMA dendrogram which revealed 5 broad clusters (Figure 1) does not show any specific grouping with reference to the provenance of the genotypes. Interestingly, both the Jodhpur and Tirupati genotypes are included among more than one cluster suggesting the genetic differences between them. A few genotypes, common to our study and those of Pathak *et al*. (2010) and Sharma *et al*. (2014), were reported as being separated broadly in two clusters (Pathak *et al*. 2010) while that of Sharma *et al*. (2014) had shown these genotypes to be mostly in one large cluster of more than 25 genotypes and 3-4 small clusters of just 1-2 genotypes each. Interestingly our study also reveals that these genotypes are distributed separately in the different sub-clusters. Thus the use of the two primers in our study has enabled a better distinction among these genotypes than those resolved by Pathak *et al.* (2010) and Sharma *et al*. (2014). Furthermore, even though a set of genotypes has been obtained from a single source collection, there is sufficient diversity among the genotypes which are grouped separately from each other. The geographical provenances (source collections) do not always correlate with genetic diversity. Earlier this was shown in case of Mung bean (Lavanya *et al*., 2008; Lavanya and Ranade, 2013) while the converse has been shown by Jayabalan *et al*. (2013) in case of a medicinal plant named *Solanum trilobatum*. In general, it can be stated that a majority of the studies using ISSR and RAPD markers have resolved the genotype collections tested in two or more discrete sub-clusters that may or may not reflect geographical provenance, but they are more due to the many genetic lineages among the collections.

The results of this study with a large number of cluster bean genotypes and using two methods suggests that: (i) The individual methods are useful for diversity analysis among the genotypes; (ii) When data from both methods are considered together it is more informative; (iii) The distribution of genotypes in clusters is not correlated to their provenance, and (iv) The broad clusters of genotypes in previous studies were better separated in the present study. Thus these results clearly suggest that the methods are not only useful in revealing diversity among the genotypes, but also suggest the existence of sufficient diversity among them to identify promising genotypes as an invaluable genetic resource for the improvement of cluster bean genotypes. Consistent with the increasing application of molecular markers, we find that the use of

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two markers together is more useful for the assessment of diversity in cluster bean as well, as was observed in several legumes stated above. The observed diversity is a useful range to select specific genotypes for agro-physiological traits such as drought resistance as well as for economic traits such as yields and gum quality and quantities amongst these genotypes for a sustained selection and breeding program in cluster bean.

Abbreviations

ISSR: Inter-Simple Sequence Repeat, NJ: Neighbor Joining, RAPD: Random Amplified Polymorphic DNA.

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تنوع ژنتیکی ژرم‌پلاسم لوبیا خوشه‌ای (*Cyamopsistetragonoloba* L.,Taub): محصولی با اهمیت غذایی و صنعتی

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چکیده

به منظور اطلاع از میزان تنوع ژنتیکی درون گروهی توده‌های یک ژرم‌پلاسم، تنوع ژنتیکی ژنوتیپ‌های لوبیا خوشه‌ای (*Cyamopsistetragonoloba* L.; Fabaceae) با استفاده از نشانگرهای ISSR و RAPD مورد مطالعه قرار گرفت. استفاده از دو روش مطالعه داده‌ها به صورت منفک و توأم، گستره‌ای از تنوع ژنتیکی بین مجموع ۱۰٤ ژنوتیپ جمع‌آوری شده از مناطق مختلف جغرافیایی هند، نشان داد. روش خوشه‌بندی شبیه‌سازی شده، ژنوتیپ‌های گیاهی جمع‌آوری شده را به گروه‌های خویشاوند مجزا تفکیک نمود. استفاده از برنامه Structure و تجزیه خوشه‌ای داده‌های حاصل از نشانگرهای مولکولی منجر به ایجاد شش زیرگروه بزرگ و مجزا شد. این نتایج با استفاده از تجزیه به مؤلفه‌های اصلی نیز مورد تأیید قرار گرفت. استفاده از مجموع داده‌ها در مقایسه با تجزیه داده‌ها به صورت مجزا، اطلاعات مفیدتری ارائه کرد. در این مطالعه بازه تنوع ژنتیکی مشاهده شده گسترده بود و ایجاد شش خوشه بزرگ، نشان دهنده وجود اجداد ژنتیکی زیادی می‌باشد که می‌تواند در استفاده از تنوع ژرم‌پلاسمی موجود و به منظور انتخاب و اصلاح گیاه لوبیا خوشه‌ای، نقطه شروع سودمندی باشد.

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