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Morphological and Molecular Diversity Analysis in Cluster Bean [*Cyamopsis tetragonoloba*(L.) Taub].

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Abstract
A study was conducted on thirty-four genotypes of cluster bean to find out the morphological and molecular diversity. Fifteen morphological characters revealed 0.19 average diversity (Manhattan's) with the range from 0.06 to 0.41. RAPD generated a total of 77 amplicons of which only 25 were polymorphic whereas, ISSRs generated 52 amplicons detecting no polymorphisms. RAPD based (Jaccard's) dissimilarity coefficient ranged from 0.01 to 0.24 with the mean genetic diversity of 0.10. Polymorphism information content (PIC) for RAPD, with an average of 0.05, ranged from 0.0 to 0.26. The dissimilarity matrix generated for morphological traits was positively correlated with RAPD dissimilarity matrix (r = 0.29). Vegetable types (M-83 and HVG2-30) were more diverse with each other and from other genotypes Varieties developed at Jhansi and Punjab were closer to Gujrat varieties, indicating their origin from Gujrat material. AMOVA showed higher diversity within (83%) than among (17%) populations.

Keywords
AMOVA, Cluster bean, Dissimilarity Coefficient, Genetic diversity, RAPD, ISSR.

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INTRODUCTION:
An indutrial crop, guar, also known as cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] (2n = 14), a member of family fabaceae, is a major crop of arid and semi-arid regions (Kumar *et al*, 2015). Cluster bean crop comes up reasonably well in warm weather and moderate rainfall prevalent in the Thar desert of Indian subcontinent (India and Pakistan). It is also grown on a small scale in a number of countires including Australia, Bangladesh, Myanmar, USA, South Africa, Brazil, Congo, Sri Lanka (Kuravadi *et al*, 2013; Boghra *et al,* 2015). This multipurpose legume is mainly used for industrial purpose (50-55%), human consumption and cattle feed (30-40%), medicinal use (5%) as well as for soil improvement and other miscellaneous purposes (Boghara *et al*,2016).
Cluster bean is predominantly autogamous and bears beans or pods in clusters, hence named as cluster bean. The light-grey, pink, white or black coloured seed consists of three parts, germ (43-47%), endosperm (35-42%) and the husk (14-17%) (Goldstein and Alter, 1959). Cluster bean gum has diversified uses in textile, ore-/metal-refining, coalmining, paper, petroleum drilling, cosmetic and pharmaceuticals, explosives, purification of potash,

tobacco and food industries (Punia *et al*, 2009; Kuravedi *et al*, 2013).

The studies on phenotypic, genetic and molecular diversity are essential to determine the genetic distance among genotypes and to identify groups with similar genetic backgrounds for conserving, evaluating and utilizing germplasm for hybridization (Shabanimofrad *et al*, 2013). Several DNA marker systems are used in assessing genetic diversity of plants (Karp *et al*, 1997), the most commonly used marker systems are random amplified polymorphic DNA (RAPD) (Williams *et al*, 1990), restriction fragment length polymorphism (RFLP) (Soller and Beckmann, 1983), amplified fragment length polymorphism (AFLP) (Seehalak *et al*, 2006), inter simple sequence repeats (ISSRs) (Zietkiewicz *et al*, 1994) and microsatellites or simple sequence repeats (SSRs) (Becker and Heun, 1994).

Among all the molecular markers, random markers like RAPDs and ISSRs are most widely used because they are inexpensive, quick, simple and do not require sequence information (Williams *et al*, 1990; Zietkiewicz *et al*, 1994). RAPD being a multi locus marker (Karp *et al*, 1997) with the simplest and fastest detection technology has been used for diversity analysis in many crop species (Demeke *et al*, 1992; Agarwal *et al*, 2008) including legumes (Kaga *et al*, 1996; Weder, 2002; Punia *et al,* 2009; Kuravadi *et al,*2014; Sharma *et al*, 2014; Tanwar *et al*, 2017). Similarly, ISSRs have successfully been used in genetic diversity analysis of black gram and chick pea plants (Souframanien and Gopalakrishna, 2004; Rao *et al*, 2007) and both types of marker have been useful for identifying relationships at the cultivar and species level (Rao *et al*, 2007; Sharma *et al*, 2008). Though, with the advent of recent methods in molecular biology, different molecular markers have been applied to the study of molecular diversity in cluster bean like RAPD (Punia *et al*, 2009; Pathak *et al*, 2010), rDNA (Pathak *et al*, 2011) and ISSR (Sharma *et al*, 2014). Information regarding the extent and pattern of genetic variation in cluster bean is limited (Sharma *et al*, 2014). Thus due to availability of limited molecular and genomic resources like molecular markers, the pace of cluster bean breeding has been hindered and slow (Kuravadi *et al*, 2014). The high variability manifested at morphological level (Dwiwedi *et al*, 1999; Henry and Mathur, 2005; Kumar et al, 2016) has scarcely been studied in relation to variation at DNA level in guar germplasm which can offer a clear picture of diversity. Consequently, biotechnological approaches have not been fully utilized for the improvement of this important commercial crop (Randhawa and Verma, 2014).

Therefore, in present study, attempts have been made to study phenotypic and genotypic divergence in guar for detection of relationships among genotypes using a collective approach of morphological traits and molecular markers to accelerate the future crop improvement program of guar.

MATERIALS AND METHODS:

Plant material and field evaluation:

Thirty-four varieties of cluster bean developed by different institutions located in Rajasthan, Gujarat, Haryana, Punjab and Uttar Pradesh states of India were procured for study. Seeds were sown in field of Central Arid Zone Research Institute, Jodhpur. The experiment was conducted for three consecutive years in randomized block design (RBD) with three replications. The genotypes were sown in two rows of 4 m length following spacing of 45 x 15 cm. The crop was raised with optimum management practices under rain fed conditions. Fresh leaves were collected for DNA extraction.

Morphological traits:

Observations were recorded on individual plant basis for the following morphological characters: plant height (PTHT), nodes per plant (NP), number of branches per plant (BP), number of clusters on main stem (CM) and branches (CB), cluster length (CL, cm), pods per cluster (PC), pod length (PL, cm), pods per plant (PP), seeds per pod (SPP), 100 seed weight (100 SW, gm) yield per plant (YP, gm)and total yield per plot (TY, gm) while flower initiation (FI), 50% flowering (50% F) recorded on plot basis. Data were recorded on individual five plants per plot selected randomly and averaged over three years for analysis.

DNA Extraction and PCR Amplification:

Genomic DNA was extracted from fresh leaves using cetyltrimethyl ammonium bromide (CTAB) method described by Doyle and Doyle (1990) was treated with RNAase also. Assessed on 0.8% agarose gel and quantified using UV/VIS spectrophotometer (Thermo scientific UV-VIS). The quantified DNA was diluted to 25 ng μl^{-1} concentration for PCR amplification.

Fourteen RAPD primers of OPA, OPB, OPC and OPN series (Operon Technologies) and ten ISSR primers synthesized from Life Technologies India Pvt Ltd. were used for diversity analysis. The PCR was carried out in 25µl reaction volume consisting of 1x assay buffer, one-unit Taq DNA polymerase (Banglore Genei Pvt. Ltd., India), 200 µM of each dNTPs (Banglore Genei Pvt. Ltd., India), 10 pM primers and 50 ng template DNA. PCR amplifications were

performed in a CGI-96 thermal cycler (Corbett Research, Australia) with following cycling conditions - initial denaturing at 94 °C for 5 min; 44 cycles x [94 ºC for 1 min, 37ºC (RAPD) / 42ºC (ISSR) for 1 min, 72 ºC for 2 min] followed by final extension at 72 ºC for 5 min.

Agarose gel electrophoresis:

The PCR product was electrophoretically separated on 1.2% and 2% agarose gel for RAPD and ISSR respectively in 1x TAE buffer, containing ethidium bromide (10 mM). GeneRuler™ DNA ladder mix (Fermentas International Inc.) was used as size marker. After electrophoresis the gel was carefully taken out of the casting tray and photograph was taken on a gel documentation system (Syngene gene genius, bio imagine system) Well resolved fragments obtained through amplification were considered and scored manually. The scoring of fragments was done on the basis of their presence ('1') or absence ('0') in the gel and missing data was denoted by ('9').

Data analysis:

SIMQUAL program was used to calculate the Jaccard's similarity coefficient for pairwise comparisons based on the proportion of shared bands produced by the primers. The dendrogram was generated from similarity matrix data by cluster analysis using unweighted pair group method for arithmetic mean (UPGMA). Polymorphic information content (PIC) that provides a measure of the degree of polymorphism was calculated following Botstein *et al.* (1980) using Power Marker Version 3.25 (Liu and Muse, 2005) software. Pairwise population comparisons were performed with analysis of molecular variance (AMOVA) using Genalex 6.5 (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION:

On the basis of field performance of thirty-four cluster bean varieties for morphological traits the obtained mean, standard deviation, range and coefficient of variation has been summarized in Table-1. The results showed maximum coefficient of variation value for clusters on branch (44.68%) followed by yield per plant (35.03%) indicating higher level of variation for these traits. Varieties RGC-1003, HG-884, and HG-2-30 had maximum number of clusters per plant (17.47), number of pods per plant (62.87) and yield per plant (24.36g), respectively. All of the genotypes studied represent same flowering group with days to flower initiation ranging between 27 to 32.67 d.

In order to determine similarities among genotypes morphological data were put to Manhattan's (Morphological) coefficient analysis (Table-6) which ranged from 59 – 94 %. Highest dissimilarity was observed between RGC-986 and FS-277 (0.41) while lowest between HG2-30 and HG 563 and between HG2-20 and HG-884 (0.06). In respect to morphological data (Table-5) average 19% diversity was detected among the genotypes studied. Haryana genotypes with 19% diversity remained the most diverse material followed by Rajasthan (18%) and Punjab (17%). The Punjab material was most diverse from both Haryana and Rajasthan (20%) but closely related to genotypes from Gujrat and Jhansi. Since, natural distribution of guar is in drier areas of Gujarat and Rajasthan the Jhansi and Punjab material could have been collections from Gujarat. The low and comparable diversity within and between regions could be because of common origin of the base genetic material which is expected for a crop of region specific adaptation.

UPGMA cluster analysis (Figure 3) based on Manhattan distance using morphological matrix grouped the thirty-four varieties into one main cluster leaving aside three genotypes namely M-83, RGC-1003 and HG 2-30. The two vegetable types M-83 and HVG 2-30 were more diverse with each other and from other genotypes studied, signifying their diverse origin which is obvious from their being vegetable types that are non-hairy and bearing long fleshy pods and are grown on a large region covering diverse ecozones. Grouping of four genotypes of single stem RGC-197, AG- 112, FS-277, and RGC-1066 in a cluster is expected due to morphological similarity. All genotypes from Jhansi grouped together in a cluster. The results suggested that the genotypes within a cluster might have same ancestral relationship and/or morphological similarity. Similar results were also reported in cluster bean by Singh *et al.* (2003) and Pathak *et al.* (2009).

DNA profiles developed for thirty-four varieties of cluster bean using RAPD revealed genetic diversity whereas no polymorphic bands were generated through ISSR primers. The ISSRs failed to detect diversity in cluster bean, because of conserved nature of SSRs revealing low polymorphism (Kumar et al, 2016). This was also substantiated in our study of SSRs (data not given). All the fourteen RAPD primers used in the study successfully amplified producing polymorphic bands for five primers, rest (9) being nonpolymorphic. Total amplicons generated were 77 (5.5 per primer) of which only 25 were polymorphic (Table 2) The size of amplicons generated varied from 250-2000bp. Maximum number of amplicons (10) were produced by primer OPC-2 (Figure 1) while primers OPA-8 and OPB-8

produced least (2). PIC values for RAPD markers, with an average of 0.05, ranged from 0.0 to 0.26 (OPA-9). In case of ISSRs (Figure-2), ten primers generated 52 amplicons. The size of amplicons generated varied from 450- 1800bp. Maximum number of amplicons were produced by primer ISSR-6 (8) (Table-3).

Since, only RAPD generated polymorphic bands further relationship and grouping analysis were based on it. Jaccard's dissimilarity coefficient (Table 6) ranged from 0.01 to 0.24 with the mean genetic diversity of 0.10. Highest dissimilarity was observed between genotypes of Rajasthan and Haryana (M-83 and HG-119)., while it was lowest (0.01) between genotypes of rajasthan (RGC-1017 and RGC-936) and between BG-1 and BG-3 (Jhansi).

The RAPD profiles generated using fourteen primers revealed low average diversity (10%) among all the genotypes compared to morphological data. The dissimilarity matrix generated for morphological traits was positively correlated with RAPD dissimilarity matrix (r = 0.29). The genotypes belonging to Rajasthan, Punjab and Haryana remained most diverse following the trend of morphological markers with average within location dissimilarity coefficient of 0.10, 0.09 and 0.09 respectively (Table 5). The diversity between regions was maximum for Haryana and Jhansi (13%) whereas Jhansi – Punjab were most similar (94%). Morever varieties developed at Jhansi and Punjab were closer to Gujrat varieties, indicating their origin from Gujrat material. In present study the genotypes (BG-1, BG-2 and BG-3) released from Jhansi are closer to each other, similar results for genotypes BG-1 and BG-2 were obtained by Kumar *et al*. (2015). The two vegetable types remained most diverse from other groups both for morphological and RAPD markers. A wide geographical spread and adaptation of vegetable types might be causal factor for diversification. Greater morphophysiological diversity estimates compared to genetic diversity (RAPD) indicates oligo genic nature of these traits that is explained by implicating fewer genes. AMOVA (Table-4) was used to partition the diversity within and among populations. The diversity within population was higher (83%) than among population (17%).

UPGMA cluster analysis based on Jacard's coefficient (Figure 4) for genetic diversity grouped the genotypes in different clusters mainly including genotypes of common origin. However, all major clusters consisted of a combination of genotypes from different areas of their development. The narrow genetic base and implication of same genetic material for breeding purpose has been evident from the grouping pattern (Pabal *et al*, 2013; Boghara *et al*, 2016). Genotypes GG-1 and GG-2, both released by Gujrat were distributed in different clusters confirming their diverse origin. As GG-1 is a mutant of kutch-8 local genotypes and was obtained by 10 KR gamma rays treatment whereas GG-2 is hybrid of HG-74 and P2-1 Telio dwarf. Similar results were revealed by Kumar et al, 2016, 2016. Genotypes released from Jhansi (BG-1, BG-2 and BG-3) are closer to each other shows the same cluster might have the same ancestor (Pathak *et al*. (2010, 2011).

Table 1: Mean, standard deviation, Range and Coefficient of variation in thirty-four varieties of cluster bean

Trait	FI	50% F	PTHT	NP	BP	CM	CB	CL	PC	PP	PL	SPP	100 SW	YP	TY
Mean	28.92	31.56	106.85	16.13	12.27	8.45	10.88	7.40	5.64	47.47	5.77	7.64	4.05	9.79	408.54
SD	1.96	2.50	12.32	4.47	3.84	1.39	4.86	1.10	0.91	12.15	0.22	0.35	0.35	3.43	78.82
Min	27.00	28.33	86.22	0.20	2.68	6.71	0.19	4.95	4.36	5.87	5.41	6.96	3.25	5.93	237.86
Max	32.67	36.33	133.76	20.23	23.42	13.20	17.47	9.53	7.86	62.87	6.27	8.36	4.49	24.36	653.67
CV%	6.79	7.93	11.53	27.70	31.28	16.47	44.68	14.83	16.12	25.60	3.80	4.64	8.56	35.03	19.29

Here, SD = standard deviation, CV% = coefficient of variation, FI= Flower initiation, 50%F= 50% flowering, PTHT= Plant height (cm), BP = Branch per plant, NP= Nodes per plant, CM= clusters on main branch, CB = clusters on branch, CL= cluster length (cm), PC= Pods per cluster, PP = Pods per plant, PL= Pod length (cm), SPP= Seeds per pod, 100 SW =100 seeds weight, YP=Yield per plant(gm), TY= Total yield per plot.

S.No	Primer	Sequences	Total number of bands	Polymorphic bands	% Polimorphism	Size range (bp)
1	ISSR 5	CCA+(GTG)4	2	0	0	900-1031
2	ISSR 6	GCGA+(CA)6	8	0	0	450-1400
3	ISSR 7	CGC+(GA)6	5	0	0	500-850
4	ISSR 10	G4+(TG)5T	7	0	0	500-1200
5	ISSR 11	CCC+(GT)6	5	0	0	750-1200
6	ISSR 12	GCAA+(GACA)3	4	0	0	750-1500
7	ISSR 15	CA(GA)7G	7	0	0	400-1200
8	ISSR 16	(GACA)4	3	0	0	450-950
9	ISSR 21	(GCCT)8	6	0	0	800-1800
10	ISSR 25	(GACAC)4	5	0	0	450-1100

Table 2: Summary of PCR amplification of RAPD primers used for genetic diversity analysis in cluster bean

S.No.	Primer	Sequences	Total number of bands	Polymorphic bands	Size range (bp)	% Polimorphism	Major Allele Frquency	Allele No	Availability	Genetic Diversity	PIC
1	OPA 4	AATCGGGCTG	9	7	400-1400	77.78	0.80	1.78	1.00	0.27	0.21
2	OPA 5	AGGGGTCTTG	2	0	250-700	0.00	1.00	1.00	1.00	0.00	0.00
3	OPA-7	GAAACGGGTG	4	0	500-1100	0.00	1.00	1.00	1.00	0.00	0.00
4	OPA-8	GTGACGTAGG	2	0	500-650	0.00	1.00	1.00	1.00	0.00	0.00
5	OPA 9	GGGTAACGCC	7	6	350-650	85.71	0.77	1.86	1.00	0.33	0.26
6	OPA 13	CAGCACCCAC	7	4	250-1100	57.14	0.89	1.57	1.00	0.15	0.12
7	OPB-7	GGTGACGCAG	5	0	450-1100	0.00	1.00	1.00	1.00	0.00	0.00
8	OPB-8	GTCCACACGG	2	0	450-550	0.00	1.00	1.00	1.00	0.00	0.00
9	OPC 2	GTGAGGCGTC	10	4	250-2000	40.00	0.95	1.40	1.00	0.08	0.07
10	OPC 6	GAACGGACTC	6	0	900-2000	0.00	1.00	1.00	1.00	0.00	0.00
11	OPC 8	TGGACCGGTG	6	0	350-1031	0.00	1.00	1.00	1.00	0.00	0.00
12	OPC 11	AAAGCTGCGG	3	0	1150-2000	0.00	1.00	1.00	1.00	0.00	0.00
13	OPC 20	ACTTCGCCAC	7	4	450-2000	57.14	0.94	1.43	1.00	0.10	0.09
14	OPN 12	CACAGACACC	7	0	450-1180	0.00	1.00	1.00	1.00	0.00	0.00
	Mean		5.5	1.79	250-2000	22.70	0.95	1.22	1	0.07	0.05

Table 3: Summary of PCR amplification of ISSR primer used for genetic diversity analysis in cluster bean.

	Degree of freedom	Sum of Squares	Mean Squares	Estimated Variance	%
Among Populations	4	28.44	7.11	0.67	17%
Within Populations	29	94.85	3.27	3.27	83%
Total	33	123.29		3.94	100%

Table 4: Analysis of molecular variance (AMOVA) in cluster bean.

WITHIN			**BETWEEN**		
Population	**Morphological based**	**RAPD based**	**Population**	**Morphological based**	**RAPD based**
OVERALL	0.19	0.1	HR	0.19	0.10
HARYANA	0.19	0.09	HG	0.17	0.11
RAJASTHAN	0.18	0.10	HJ	0.18	0.13
GUJRAT	0.16	0.08	HP	0.2	0.12
JHANSI	0.14	0.05	RG	0.16	0.09
PUNJAB	0.17	0.09	RJ	0.18	0.11
SS	0.18	0.12	RP	0.2	0.12
VEG TYPE	0.24	0.23	GJ	0.12	0.08
			GP	0.17	0.09
			JP	0.14	0.06
Here, H= Haryana, R= Rajasthan, G= Gujrat, J= Jhansi, P= Punjab, SS= Single Stem, VEG type= M-83 and HVG 2-30.			SS	0.21	0.11
			VEG TYPE	0.26	0.13

Table 5: Average dissimilarity coefficient based on morphological and RAPD analysis in Cluster bean.

Table 6: Morphological (Manhattan) and RAPD based (Jaccard's) dissimilarity coefficient

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
		Manhatton's dissimilarity coefficient																																
1	Jaccard's dissimilarity coefficient	0.19	0.16	0.19	0.16	0.20	0.18	0.18	0.17	0.17	0.29	0.17	0.25	0.21	0.28	0.15	0.23	0.20	0.17	0.20	0.16	0.19	0.19	0.21	0.29	0.16	0.23	0.25	0.19	0.18	0.18	0.21	0.21	0.21
2		0.07	0.08	0.19	0.12	0.09	0.23	0.17	0.08	0.09	0.35	0.11	0.21	0.10	0.36	0.14	0.15	0.10	0.12	0.15	0.10	0.23	0.10	0.14	0.34	0.17	0.14	0.20	0.15	0.17	0.15	0.18	0.19	0.20
3		0.06	0.10	0.16	0.12	0.08	0.21	0.17	0.06	0.06	0.32	0.12	0.23	0.14	0.33	0.13	0.16	0.12	0.08	0.12	0.08	0.22	0.09	0.13	0.34	0.14	0.17	0.22	0.16	0.18	0.17	0.20	0.20	0.20
4		0.07	0.09	0.04	0.15	0.14	0.11	0.09	0.12	0.18	0.35	0.17	0.27	0.16	0.36	0.13	0.25	0.17	0.18	0.15	0.15	0.17	0.15	0.19	0.34	0.13	0.16	0.23	0.14	0.18	0.14	0.23	0.14	0.17
5		0.06	0.10	0.03	0.07	0.11	0.13	0.14	0.10	0.13	0.30	0.15	0.19	0.09	0.30	0.07	0.18	0.09	0.13	0.10	0.10	0.13	0.08	0.10	0.28	0.10	0.12	0.23	0.14	0.14	0.14	0.23	0.15	0.17
6		0.06	0.07	0.06	0.10	0.06	0.21	0.17	0.06	0.12	0.33	0.16	0.27	0.12	0.34	0.15	0.21	0.11	0.12	0.10	0.10	0.22	0.09	0.11	0.33	0.16	0.15	0.21	0.20	0.23	0.21	0.27	0.20	0.23
7		0.14	0.15	0.11	0.12	0.08	0.11	0.08	0.19	0.20	0.36	0.16	0.25	0.18	0.34	0.11	0.26	0.16	0.19	0.18	0.16	0.10	0.18	0.18	0.30	0.11	0.14	0.22	0.14	0.14	0.11	0.22	0.10	0.12
8		0.09	0.13	0.06	0.07	0.03	0.09	0.06	0.15	0.17	0.36	0.14	0.24	0.14	0.37	0.11	0.25	0.14	0.18	0.17	0.15	0.15	0.16	0.18	0.29	0.15	0.12	0.19	0.12	0.14	0.12	0.20	0.13	0.17
9		0.11	0.10	0.11	0.12	0.08	0.08	0.05	0.11	0.10	0.32	0.13	0.23	0.11	0.32	0.12	0.18	0.09	0.10	0.09	0.08	0.20	0.08	0.11	0.32	0.14	0.13	0.20	0.16	0.19	0.18	0.22	0.17	0.18
10		0.09	0.10	0.06	0.07	0.06	0.06	0.11	0.09	0.08	0.33	0.09	0.23	0.16	0.32	0.12	0.16	0.13	0.10	0.12	0.08	0.21	0.09	0.16	0.34	0.14	0.17	0.23	0.15	0.16	0.16	0.18	0.20	0.18
11		0.13	0.14	0.10	0.14	0.10	0.10	0.04	0.10	0.07	0.10	0.33	0.31	0.36	0.24	0.29	0.35	0.35	0.34	0.34	0.31	0.34	0.32	0.35	0.24	0.33	0.36	0.26	0.36	0.32	0.34	0.32	0.34	0.34
12		0.10	0.08	0.07	0.08	0.07	0.07	0.07	0.10	0.04	0.04	0.05	0.22	0.18	0.34	0.12	0.18	0.14	0.14	0.15	0.10	0.18	0.15	0.21	0.34	0.15	0.16	0.21	0.13	0.13	0.12	0.17	0.18	0.13
13		0.12	0.05	0.11	0.10	0.11	0.12	0.14	0.12	0.14	0.11	0.13	0.10	0.19	0.41	0.18	0.10	0.18	0.19	0.24	0.21	0.23	0.19	0.22	0.28	0.22	0.23	0.27	0.21	0.19	0.21	0.15	0.24	0.24
14		0.09	0.07	0.08	0.10	0.08	0.06	0.11	0.09	0.08	0.08	0.10	0.07	0.09	0.35	0.11	0.17	0.08	0.15	0.14	0.14	0.18	0.11	0.11	0.28	0.13	0.11	0.20	0.14	0.17	0.17	0.23	0.17	0.20
15		0.11	0.10	0.11	0.12	0.11	0.06	0.11	0.14	0.05	0.06	0.09	0.04	0.14	0.06	0.29	0.40	0.36	0.34	0.33	0.32	0.35	0.35	0.35	0.28	0.30	0.34	0.37	0.35	0.33	0.34	0.36	0.34	0.33
16		0.07	0.06	0.07	0.08	0.07	0.04	0.10	0.10	0.04	0.04	0.08	0.03	0.10	0.04	0.04	0.19	0.12	0.13	0.13	0.10	0.15	0.10	0.15	0.29	0.11	0.12	0.21	0.12	0.12	0.12	0.20	0.14	0.15
17		0.10	0.11	0.10	0.09	0.10	0.07	0.10	0.10	0.10	0.10	0.14	0.11	0.15	0.13	0.10	0.08	0.15	0.13	0.19	0.14	0.25	0.15	0.18	0.30	0.20	0.22	0.24	0.19	0.21	0.20	0.14	0.25	0.24
18		0.11	0.12	0.11	0.12	0.11	0.11	0.11	0.14	0.05	0.08	0.09	0.07	0.16	0.08	0.05	0.07	0.15	0.10	0.10	0.10	0.15	0.07	0.08	0.27	0.12	0.09	0.18	0.12	0.16	0.15	0.19	0.16	0.17
19		0.13	0.14	0.12	0.11	0.12	0.12	0.09	0.12	0.07	0.10	0.11	0.08	0.18	0.10	0.07	0.08	0.14	0.01	0.10	0.07	0.19	0.07	0.12	0.30	0.13	0.15	0.23	0.14	0.16	0.17	0.18	0.19	0.18
20		0.10	0.11	0.10	0.09	0.10	0.10	0.10	0.10	0.07	0.07	0.11	0.06	0.15	0.10	0.07	0.06	0.11	0.07	0.06	0.07	0.16	0.07	0.09	0.31	0.13	0.15	0.25	0.19	0.20	0.21	0.25	0.21	0.20
21		0.09	0.10	0.08	0.10	0.08	0.09	0.11	0.11	0.06	0.06	0.10	0.04	0.14	0.08	0.06	0.04	0.13	0.06	0.07	0.01	0.18	0.07	0.13	0.32	0.13	0.15	0.21	0.15	0.16	0.15	0.20	0.18	0.17
22		0.14	0.10	0.14	0.15	0.14	0.11	0.11	0.16	0.06	0.11	0.10	0.07	0.14	0.11	0.08	0.07	0.15	0.08	0.10	0.07	0.06	0.17	0.14	0.25	0.11	0.18	0.25	0.16	0.15	0.14	0.22	0.14	0.14
23		0.06	0.07	0.09	0.10	0.09	0.09	0.14	0.11	0.08	0.06	0.12	0.07	0.12	0.09	0.08	0.04	0.13	0.08	0.10	0.07	0.06	0.09	0.08	0.29	0.12	0.12	0.22	0.14	0.16	0.16	0.20	0.17	0.18
24		0.07	0.03	0.10	0.11	0.10	0.07	0.15	0.13	0.10	0.10	0.14	0.08	0.07	0.07	0.10	0.06	0.14	0.12	0.14	0.11	0.10	0.10	0.04	0.24	0.13	0.15	0.23	0.18	0.22	0.22	0.23	0.17	0.21
25		0.12	0.11	0.14	0.13	0.17	0.17	0.24	0.20	0.19	0.17	0.23	0.18	0.12	0.17	0.19	0.16	0.19	0.19	0.21	0.16	0.14	0.17	0.12	0.11	0.26	0.31	0.23	0.29	0.30	0.31	0.27	0.26	0.32
26		0.11	0.13	0.11	0.12	0.14	0.08	0.13	0.16	0.08	0.08	0.12	0.07	0.16	0.08	0.03	0.07	0.10	0.08	0.09	0.10	0.08	0.11	0.08	0.10	0.17	0.12	0.21	0.11	0.11	0.13	0.20	0.14	0.15
27		0.11	0.10	0.11	0.10	0.11	0.11	0.11	0.14	0.05	0.11	0.12	0.07	0.14	0.11	0.08	0.07	0.07	0.11	0.12	0.07	0.06	0.08	0.11	0.13	0.14	0.08	0.16	0.11	0.12	0.13	0.22	0.14	0.15
28		0.12	0.14	0.09	0.11	0.09	0.12	0.07	0.12	0.04	0.09	0.08	0.05	0.15	0.12	0.09	0.08	0.11	0.09	0.11	0.11	0.09	0.09	0.10	0.11	0.18	0.07	0.07	0.19	0.19	0.20	0.22	0.21	0.24
29		0.13	0.11	0.15	0.14	0.15	0.15	0.12	0.17	0.07	0.15	0.13	0.11	0.15	0.15	0.12	0.11	0.11	0.09	0.11	0.14	0.12	0.10	0.10	0.11	0.15	0.09	0.07	0.05	0.09	0.07	0.13	0.13	0.13
30		0.14	0.12	0.13	0.15	0.13	0.14	0.11	0.16	0.05	0.13	0.12	0.09	0.16	0.13	0.11	0.09	0.12	0.08	0.09	0.12	0.11	0.08	0.08	0.10	0.17	0.08	0.08	0.04	0.01	0.07	0.18	0.16	0.13
31		0.15	0.14	0.14	0.13	0.14	0.15	0.12	0.17	0.07	0.14	0.13	0.11	0.17	0.12	0.09	0.11	0.11	0.07	0.08	0.11	0.09	0.09	0.15	0.16	0.18	0.09	0.04	0.08	0.05	0.07	0.14	0.10	0.10
32		0.16	0.12	0.13	0.12	0.13	0.16	0.11	0.16	0.08	0.13	0.12	0.09	0.14	0.13	0.11	0.12	0.12	0.08	0.09	0.14	0.13	0.11	0.13	0.15	0.19	0.11	0.08	0.07	0.04	0.05	0.04	0.17	0.17
33		0.13	0.11	0.12	0.14	0.12	0.10	0.09	0.15	0.04	0.10	0.08	0.05	0.15	0.10	0.07	0.06	0.14	0.04	0.05	0.06	0.04	0.04	0.10	0.11	0.18	0.09	0.07	0.08	0.08	0.07	0.05	0.09	0.08
34		0.16	0.12	0.15	0.14	0.15	0.13	0.15	0.18	0.12	0.10	0.14	0.08	0.13	0.15	0.10	0.11	0.14	0.15	0.16	0.11	0.10	0.07	0.13	0.14	0.19	0.13	0.10	0.14	0.14	0.15	0.14	0.12	0.11

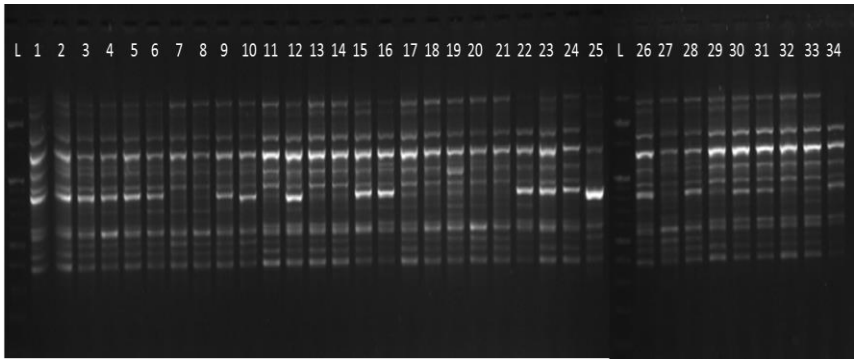

Figure 1: RAPD Profile of cluster bean using OPC-2, (L= Ladder), 1-34 are genotypes.

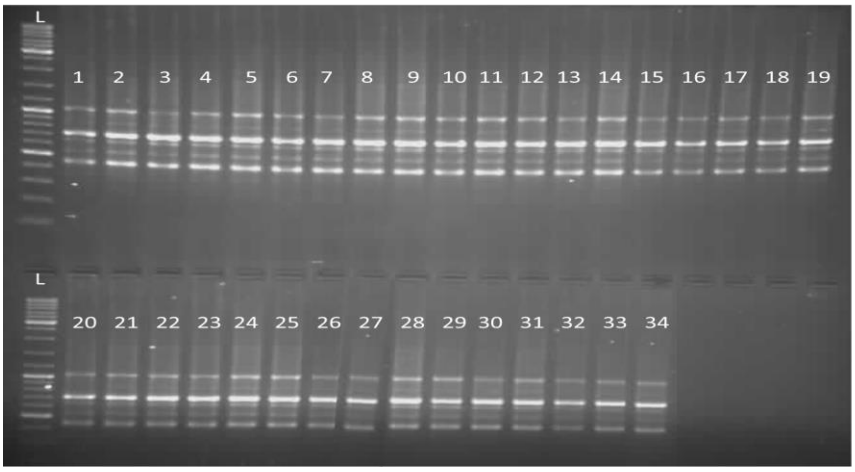

Figure 2: ISSR Profile of cluster bean using primer ISSR-25, (L= Ladder).

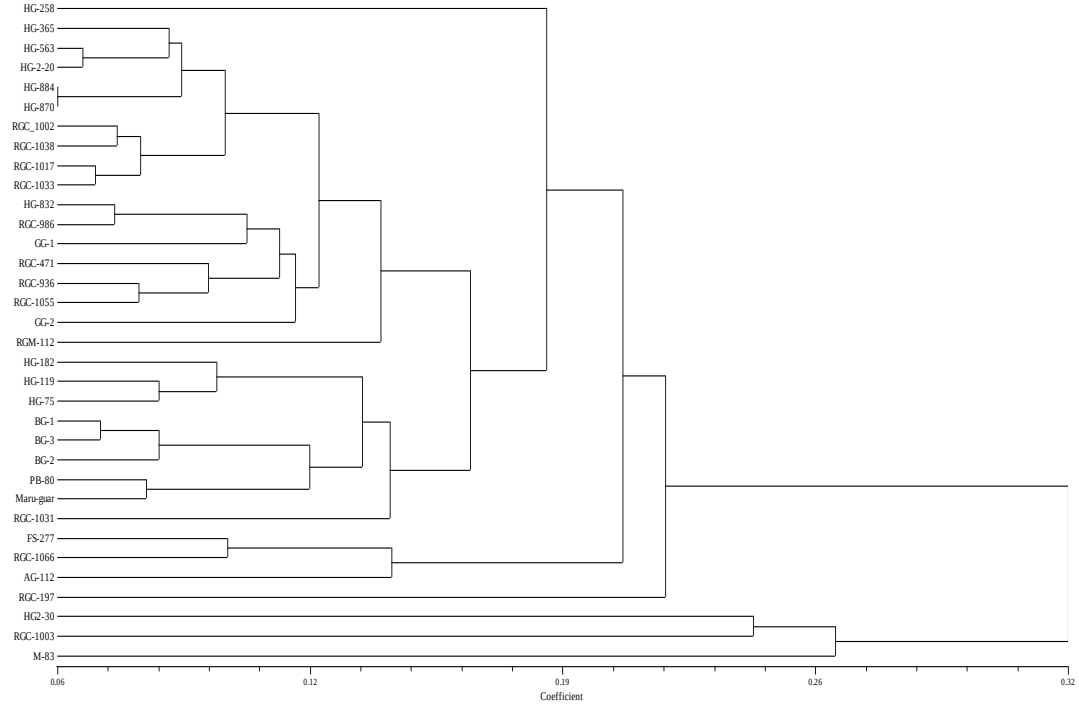

Figure 3: UPGMA dendrogram based on morphological (Manhattan) matrix data.

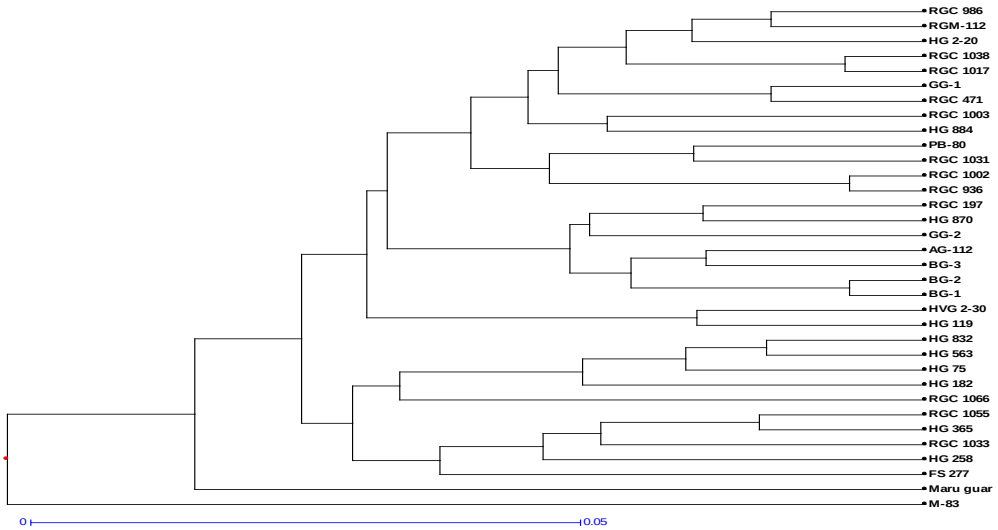

Figure 4: UPGMA dendrogram based on RAPD matrix data.

CONCLUSION:

Probing random DNA RAPD markers successfully revealed polymorphism, while ISSRs targeting repeat sequences generated non- polymorphic bands, indicating their conserved nature. Detection of low and same magnitude of diversity by morphological and RAPD markers with a positive correlation indicated low genetic base, expected for a crop evolved and adopted to a specific region. The varieties developed at distant places also aligned to region of adaptation (Thar Desert). The vegetable types having wider spread were diverse with a potential to add diversity for further genetic improvement of the crop.

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REFERENCES:

1. Agarwal, M., Srivastava, N. and Padh, H. 2008. Advances in Molecular Marker Techniques and Their Applications in Plant Sciences. *Plant Cell Rep*., 27: 617-631.
2. Becker, J. and Heun, M. 1994. Microsatellites: Allele Variation and Mapping. *Plant Mol. Bio*., 27(4): 835-845.
3. Boghara, M.C., Dhaduk, H.L., Kumar, S., Parekh, M.J., Patel, N.J., Sharma, R. 2016. Genetic divergence, path analysis and molecular diversity analysis in cluster bean (*Cyamopsis tetragonoloba* L. Taub.). Industrial Crop and Production 89:468–477.
4. Boghara, M.C., 2015. Assessment of genetic variability in cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes through morphological, biochemical and molecular markers. M.Sc. Thesis, Anand Agricultural University, Anand, India.
5. Botstein, D., White, R.L., Skolnick, M., Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment polymorphisms. *Am J Hum Genet* 32:314–331.
6. Demeke, T., Lynch, D. R., Kawchuk, L. M. G. C. and Armstrong, J. D. 1992. Genetic Diversity of Potato Determined by Random Amplified Polymorphic DNA Analysis. *Plant Cell Rep*., 15**:** 662-667.
7. Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. Focus 12,13–15.
8. Dwivedi, N.K., Bhandari, D.C., Bhatnagar, N., Dabas, B.S., Cahdel, K.P.S. 1999. Conservation of genetic diversiy of arid legumes. In: Management of Arid Ecosystem, Eds. A.S. Faroda, N.L. Joshi, S. Kathju and Amal Kar. Arid Zone Research Association and Scientific Publishers, Jodhpur pp 49-56.
9. Kaga, A., Tomooka, N., Egawa, Y., Hosaka, K. and Kamijima, O. 1996. Species Relationships in the Subgenus *Ceratotropis* (genus *Vigna*) as Revealed by RAPD Analysis. *Euphytica*, 88**:** 17-24.
10. Karp, A., Kresovichi, S., Bhat, K. V., Ayad, W. G. and Hodgkin, T. 1997. Molecular Tools in Plant Genetic Resources Conservation: A Guide to the Technologies. International Genetic Resources Institute, Rome, Italy, IPGR Technical Bull. No.2. 14.
11. Kumar, S., Parekh, M. J., Patel, C. B., Zala, H. N., Sharma, R., Kulkarni K. S., Fougat, R.S., Bhatt, R. K., Sakure, A.A. 2016. Development and validation of EST-derived SSR markers and diversity analysis in cluster bean (*Cyamopsis tetragonoloba*) Journal of

Plant Biochemistry and Biotechnology, 25(3): 263–269.

12. Kumar, V., Ram, R.B., 2015. Genetic variability, correlation and path analysis for yield and yield attributing traits in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] genotypes. Int. J. Pure Appl. Biosci. 3, 143–149.
13. Kuravadi, A.N., Tiwari, P.B., Choudhary, M., Tripathi, S.K., Dhugga, K.S., 2013. Genetic diversity study of cluster bean (Cyamopsis tetragonoloba (L.) Taub) landraces using RAPD and ISSR markers. Int. J. Adv. Biotechnol. Res. 4, 460–471.
14. Kuravadi, A.N., Tiwari, P.B., Tanwar, U.K., Tripathi, S.K., Dhugga, K.S., Gill, K.S., Randhawa, G.S., 2014. Identification and characterization of EST-SSR markers in clusterbean (*Cyamopsis* spp.). Crop Sci. 54, 1097–1102.
15. Liu, K., Muse, S.V., 2005. PowerMarker: integrated analysis environment forgenetic marker data. Bioinformatics 21, 2128–2129.
16. Pabal, A., More, V., Kore, S., Vikhe, P., Ghorpade, B., 2013. Molecular characterization of cluster bean (*Cyamopsis tetragonoloba*) cultivars using PCR-based molecular markers. Int. J. Adv. Biotechnol. Res. 4, 158–166.
17. Pathak, R., Singh, M., Henry, A., 2009. Genetic divergence in cluster bean (Cyamopsis tetragonoloba) for seed yield and gum content under rainfedconditions. Indian J. Agric. Sci. 79, 559–561.
18. Pathak, R., Singh, S.K., Singh, M., Henry, A., 2010. Molecular assessment of genetic diversity in cluster bean (*Cyamopsis tetragonoloba*) genotypes. J. Genet, 89(2), 243-246.
19. Pathak, R., Singh, M., Henry, A., 2011. Genetic diversity and interrelationship among cluster bean genotypes for qualitative traits. Indian J. Agric. Sci. 81, 402–406.
20. Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-An update. *Bioinformatics* (*Oxford, England*) 28: 2537–2539.
21. Punia, A., Yadav, R., Arora, P., Chaudhary, A., 2009. Molecular and morphophysiological characterization of superior cluster bean (*Cyamopsis tetragonoloba*) varieties. J. Crop Sci. Biotech. 12, 143-148.
22. Randhawa, G.S., Verma, S., 2014. Guar: the green gold. Indo Global J. Pharm. Sci. 4,196.
23. Rao, L. S., Usha Rani, P., Deshmukh, P. S., Kumar, P. A. and Panguluri, S. K. 2007. RAPD and ISSR Fingerprinting in Cultivated Chickpea (*Cicer arietinum* L.) and Its Wild Progenitor *Cicer reticulatum* Ladizinsky. *Genetic Resources of Crop Evolution*, 54: 1235-1244.
24. Seehalak, W., Tomooka, N., Waranyuwat, A., Thipyapong, P., Laosuwan, P., Kaga, A. and Vaughan, D. A. 2006. Genetic Diversity of the *Vigna* germplasm from Thailand and Neighboring Regions Revealed by AFLP Analysis. *Genet. Resour. Crop Evol*., 53(5): 1043-1059.
25. Shabanimofrad, M., Rafii, M.Y., Megat Wahab, P.E., Biabani, A.R., Latif, M.A., 2013.Phenotypic: genotypic and genetic divergence found in 48 newly collected Malaysian accessions of Jatropha curcas L. Ind. Crop Prod. 42, 543–551
26. Sharma, K., Agrawal, V., Gupta, S., Kumar, R. and Prasad M. 2008. ISSR Marker- Assisted Selection of Male and Female Plants in a Promising Dioecious Crop: Jojoba (*Simmondsia chinensis*). *Plant Biotechnol. Rep*., **2:** 239-243.
27. Sharma, P., Sharma, V., Kumar, V., 2014. Genetic diversity analysis of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub) genotypes using RAPD and ISSR markers. J. Agric. Sci. Tech. 16, 433–443.
28. Singh, N.P., Choudhary, A.K., Chaudhary, S.P.S., 2003. Genetic divergence in clusterbean (*Cyamopsis tetragonoloba* L. Taub). Indian J. Agric. Sci. 73, 356–357.
29. Singh, J.V., Dahiya, B.S., 2004. "Advances in chemistry, biochemistry and industrial utilization of guar seed," in Guar, Indian Society of Forage Research, Hisar and Agricultural and Processed Food Products Export Development Authority (APEDA), New Delhi, India. pp. 197.
30. Soller, M. and Beckmann, J.S. (1983). Restriction fragment length polymorphism in genetic improvement. Theor. Appl. Genet., 67: 25-33.
31. Souframanien, J. and Gopalkrishna, T. 2004. A Comparative Analysis of Genetic Diversity in Black Gram Genotypes Using RAPD and ISSR Markers. *Theor. Appl. Genet.*, 109 (8): 1687-1693.
32. Tanwar, U.K., Pruthi, V., Randhawa, G.S., 2017. RNA-Seq of guar (*Cyamopsis tetragonoloba*, L. Taub.) leaves: de novo transcriptome assembly, functional annotation and development of genomic resources. Front. Plant Sci. 8https://doi.org/10.3389/fpls.2017.00091. 91.
33. Weder, J. K. P. 2002. Identification of Plant Food Raw Material by RAPD-PCR: *Legumes. J. Agr. Food. Chem.,* 50: 4456-4463.
34. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. 1990. DNA Polymorphisms Amplified by Arbitrary Primers Are Useful as Genetic Markers. Nucleic Acids Research, 18: 6531–6535.
35. Zietkiewicz, E., Fafalski, A. and Labuda, D. 1994. Genome Fingerprinting by Simple Sequence Repeat (SSR)-anchored Polymerase Chain Reaction Amplification. Genomics, 20: 176–183.