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STUDY OF PHENOTYPIC AND GENETIC DIVERSITY IN CLUSTER BEAN(CYAMOPSISTETRAGONOLOBA[L.]TAUB) GENOTYPES BY RAPD & ISSR MARKERS

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Abstract

Molecular markers are one of the most important tools for evaluating genetic diversity and genotypic identification was used in a plant breeding program. These are the major source for genetic predictability for breeding and crop improvement in Cluster bean (Cyamopsistetragonoaloba [L.]Taub). The present study deals with the genetic diversity in 22 genotypes of Cluster bean seeds collected from different states in India. The different genotypes were characterized for morphological traits and molecular analysis were performed using seven Randomly Amplified Polymorphic DNA (RAPD) and four Inter Simple Sequence Repeat (ISSR) molecular markers. Total 31 Amplicon pattern was obtained by RAPD primers. Among the four ISSR primers, UBC-819 produced the highest number of banding pattern. Word's Elucidean-Distances tree was constructed with RAPD and ISSR data. The genetic diversity and phenotypic distance was analyzed by words elucidation distance was genotypically 0.4 – 0.92 and phenotypic distance range from 0-300 was observed.

Keywords: Molecular markers, Cluster bean, RAPD, ISSR, Polymorphism

Introduction

Cluster bean [Cyamopsistetragonoloba[L.]Taub.)belongs to the fabaceae family grown as a commercial crop in semi-arid regions of India. It is drought tolerant, hardy and deep-rooted summer annual leguminous herb, highly suitable to arid and a semi-arid parts of the world. It requires less cost for the crop cultivation. The cluster beanis commercially important as a source for industrial gum. The guar endosperm [30-35%] is a prosperous resource of high-quality galactomannan (Das and Arora et al. 1978). Gum produced by guar has extensive applications in mining, cosmetic, petroleum, textile paper, oil, pharmaceuticals, explosives, and in food industries. It is also used to cure diabetics as well as to reduce the effects of high cholesterol effects (Kumar et al. 2013; Punia et al 2009). In conventional breeding, molecular markers play a major role to find the dissimilar parents for breeding aspects. RAPD and ISSR primer sequences were conserved in the crop genome, hence it helps for easily observe the polymorphism in the crop plants for breeding (Casiva et al.2002; Cottrell et al.1997; Williams et al. 1990; Nadeem et al.,2018).

Developing hybrids which can tolerate major biotic and abiotic factors in the cluster bean is most essential to provide sufficient food for today's growing population. The environmental factors may change morphological and physiological characters but genetic characters may not get change hence, molecular markers data are more require for classical breeders (Brijmohan singh bhau et al.,2016; Z.A Bhat et al.,2010; Rabbani et al. 2008) .Lack of complete genome sequence availability of cluster bean it is very difficult for breeders to develop new varieties. Selectable characters can be obtained through transgenic approaches but breeders can develop all desirable traits by using breeding aspects. In the past, new hybrids were developed based on phenotypic characters, but in the present generation, breeders are developing new hybrids by considering genetic diversity data (Nadeem et al., 2018; Jonah et al. 2011) .Moreover breeders had to wait for months to screen any trait in plants but nowadays they are screening specific traits in plants within a few days by using these molecular markers to save the time (Peleman and Vander vort 2003; Getu Hailu and Yirgashewa Asfere 2020).

Molecular markers act as a tag to indicate where the genes are located throughout the genome. After considering this marker data QTL analysis will be done to know the gene location for a specific trait on which chromosome. Hence molecular markers data are more essential for the crop development (Getu hailu and yirgashewa Asfere 2020).There is a great need for molecular markers to study this genetic diversity. Therefore, in present study we used RAPD and ISSR markers to analyze the genetic diversity in the cluster bean.

Material and Methods

Field design:

A total of 22 genotypes were selected for the present study and these genotypes were collected from all over the countryand the experiments were conducted at the agricultural research station, Rekulakunta, Anantapuram, Andhra Pradesh, India. Seeds were sown in the field (Red soil) and space between plants is 15cm and 30cm width, five plants were selected from each genotype for analysis. Seeds were sown in June 2014 and phenotypic data were collected in October 2014.

Shoot length

After the end of the crop period, plants were harvested and plant heights were carefully recorded in centimeters (cm). Measurements were taken on five biological replicates for each genotype and average was calculated..

Root length:

After harvesting the crop, the plants were over irrigatedand soil was removed carefully from the rootsand root lengths were recorded in centimeters (cm). For each genotype five biological replicates were maintained and taken the average.

100 seeds weight/plant:

After harvesting, the fruits were dried and processed to collect the seeds form five random plants in each genotype andseeds were weighed by using standard weighing balance (Shimadzu) and values were recorded and averaged.

Shoot and root fresh weight

The shoot and root fresh weights were recorded immediately by using the standard weighing balance.

Shoot and root dry weight

After taking the fresh weights of shoots and roots were immediately placed in a hot air oven at 68° C for 48 hours for dry weights and values were recorded.

The number of pods per plant:

The number of pods per plant were carefully counted from each genotype.

SPAD Readings:

Total Chlorophyll measurement was done by using SPAD meter (SPA D-502, Konica Minolta, Japan.) SCMRreadings were collectedduring9.00-11.00am fromthe2ndfullyexpandedleaffromthetopofthe main stem described by NageswaraRao et al. (2001) and care was taken for the SPAD meter sensor fully covered the leaf lamina by avoiding the interference of veins and midribs. Top 3 leaves were taken for SCMRmeasurementsfromfiveplantsin each genotype at60Days after sowing.

Specific Leaf Area (SLA) and dry weight:

SLA was measured according to Sebahattin and Necdet 2007, and the leaf samples were oven-dried at 80°C for 48 h to determine the leaf dry weight. SLA was calculated using the following formula.

SLA =(Leaf area (cm^2))/(Leaf dry weight(g))

DNA extraction

DNA was extracted using CTAB method according to Doyle and Doyle (1990). The isolated DNA was purified through RNase treatment. DNA pellet was dissolved in TE buffer and tested on agarose gel electrophoresis and quantified by nanodrop (NanoDropND-1000 Version 3.1.1). 50ng of DNA sample were taken for 25 µlof PCR reaction volume.

PCR reaction by RAPD and ISSR primers

Polymerase chain reaction (PCR) was performed in 25 µl reaction volume containing the Taq enzyme (1U), 2.5 mMMgCl2, 5mMdNTPs, 10picomoles of primer and 50ng of template DNA. The annealing temperature for each primer was determined by gradient PCR. The program was carried out with an initial denaturation (94ºC) for 4 min followed by 35 cycles of 1 min at 94ºC, 1 min at 37ºC and 1 min at 72ºC. A final extension was carried out at 72ºC for 10 min. Out of the 15 ordered primers, 7 primers were used for diversity studies, which produced a clear and unambiguous banding pattern. The PCR program was similar to above except annealing temperature at 47ºC for 1 min. The RAPD and ISSR primers were custom synthesized by Eurofins Genomics Pvt. Ltd, Bangalore, India.

The PCR amplified products were mixed with bromophenol blue dye and electrophoresed on 2% agarose gel forRAPDand1.2% for ISSR markers in 0.5XTBE buffer under room temperature at a constant voltage of 100 V along with a marker (100 bp DNA ladder). Gels were stained with EthBr and gel image was captured on UV gel documentation(SynGeneVersion08-3d.3.SynopticLimited2000–2008). PCR reaction and agarose gel electrophoresis were repeated three times for marker development and checked the consistency of the bands before scoring for polymorphisms between cluster bean genotypes. The primers sequences, annealing temperature, number of genotypes amplified per primers, PIC values and percentage of polymorphism of rapid and ISSR markers were mentioned in Table 1.

Data analysis

Amplified bands were scored visually for each primer. Bands were analyzed by scoring 1 of the present band and scoring 0 to the bands that did not appear to be the same size. The results of these values are further analyzed in the similarity using UPGMA online software for phylogenetic tree compilation. Phenotypic clustering analysis was drawn by Nytsispc software.

Results:

22 cluster bean genotypes were sowed in the field as per mentioned above and grown plants for the present study was shown in Fig.5.

Phenotypic Clustering

Phenotypic clustering based on morphological traits was drawn using Nytsispc software it showed between genotypes and traits. Morphological traits separate into two main groups. Group-I contains four traits those are seed weight/100 (SWWPP), SPAD, SLA, and a total number of pods.Group-IIhasseventraits those are plant height, root fresh weight, shoot fresh weight, root length, number of leaves, and root dry weights. From the phenotypic dendrogram (Fig.1)it is clear that most of the lines/selection acrossIndiawereintermingled along with the lines/selection.

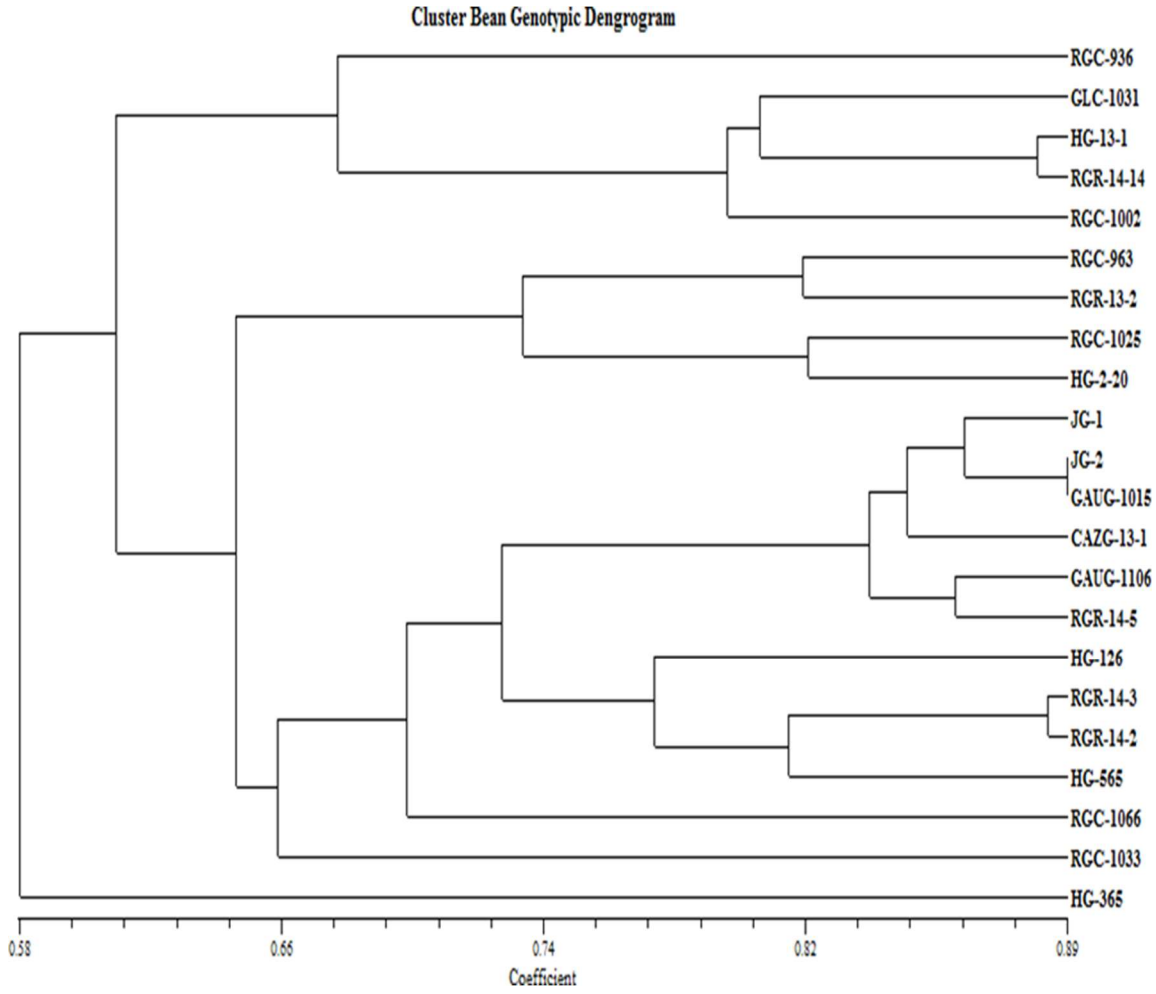


Fig.1.Dendogram on the basis of combined (RAPD +ISSR) molecular markers data of 22 cluster bean varieties

PCR Analysis by RAPD and ISSR

All 22 accessions were used for RAPD analysis, out of the 20 RAPD primers, 7 primers were able to show polymorphism and reproducibility. The RAPD primers amplified the genome ranged from 3 to 5 bands (Table. 2) with an average number of bands per primer being 3 as well as ISSR primers were amplified from 5 to 8 bands (Table. 2) the average number of bands per primer is 4. OPF-3 was produced least number of bands and OPA-4, OPT-7 and OPQ-9 primers produced a larger number of bands in RAPD and ISSR-846 produced the least number and ISSR-819 primer produced the largest number of bands. A total of 21 genotypes got amplification with the RAPD primer OPF-3 with the polymorphic index content (PIC) value of 0.23. Similarly, 21 genotypes showed amplification with ISSR-819 with the PIC value of 0.16 and 20 genotypes showed amplification with ISSR-846 & ISSR-876 primers with the PIC values of 0.28 and 0.35. The present study RAPD (7 Primers) and ISSR (4 Primers) ampliconpattern in 22 genotypes with different primers were shown in Fig. 3 &4. The value in terms of genetic distance combined by RAPD and ISSR markers ranged from 0.44 to 0.92 (Fig.1).

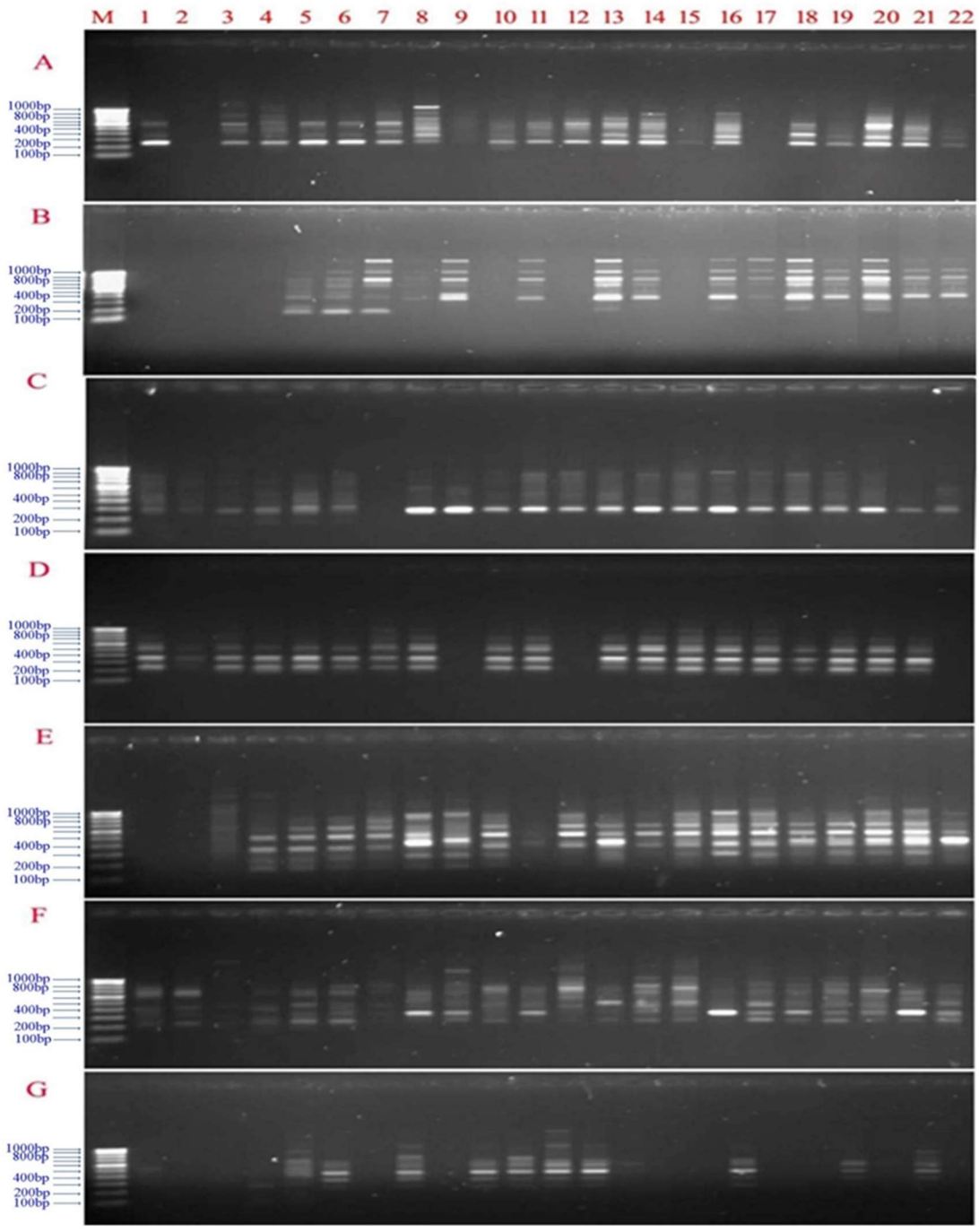


Fig. 3.PCR products profile of 22 Cluster bean varieties using RAPD markers. (A: OPA-4, B: OPA-7A, C: 0PF-3, D: OPG-14, E: 0PT-7, F: OPP-3, G: OPQ-9) M: 100bp ladder, Lane 1-22are as follow varieties 1 - RGC-936; 2 - RGC-963; 3 - RGC-1025; 4 - JG-1; 5 - JG-2; 6 - GLC-1031; 7-HG- 365; 8 - RGC-1002; 9 - RGC-1066; 10 - CAZG-13-1; 11 - HG-2-20; 12-RGR-13-2;13 - HG-13-1; 14 - RGR-14-14; 15 - GAUG-1106; 16 - HG-126; 17- RGR-14-3; 18 -RGC-1033; 19- GAUG-1015; 20- HG-565; 21 - RGR-14-5 and 22 - RGR-14-2.

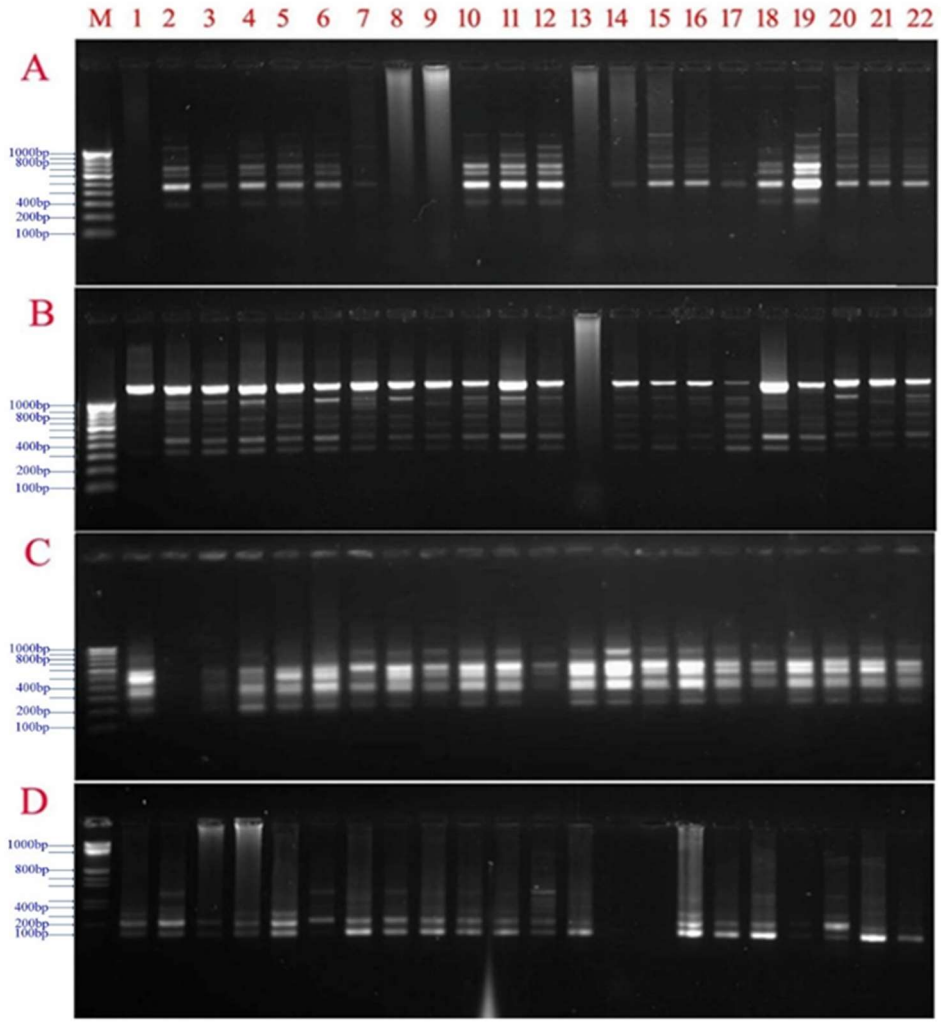


Fig. 4.PCR products profile of 22 Cluster bean varieties using ISSR markers (A: ISSR- 817B: ISSR-819 C: ISSR-846 D: ISSR-876).M: 100bp ladder,Lane 1-22are as follow varieties 1 - RGC-936; 2- RGC-963; 3 - RGC-1025; 4 - JG-1; 5 - JG-2; 6-GLC-1031; 7-HG-365; 8 - RGC-1002; 9- RGC-1066; 10 - CAZG-13-1; 11 - HG-2-20; 12-RGR-13-2; 13 - HG-13-1; 14 - RGR-14- 14; 15 - GAUG-1106; 16 - HG-126; 17- RGR-14-3; 18 -RGC-1033; 19 -GAUG-1015; 20-HG- 565; 21 - RGR-14-5 and 22 - RGR-14-2.

S.NO	Primers code	Primer sequence	$T_m$of theprimer (^{0}C)	Number of amplicons	PIC	No. of genotypes amplified	Percentage of Polymorphism
1	OPA-4	AATCGGGCTG	37	5	0.36	18	92
2	OPA-7A	AGCCCCCAAG	37	5	0.48	14	100
3	0PF-3	CCTGATCACC	37	3	0.26	21	85.7
4	OPG-14	GGATGAGACC	37	4	0.23	19	77

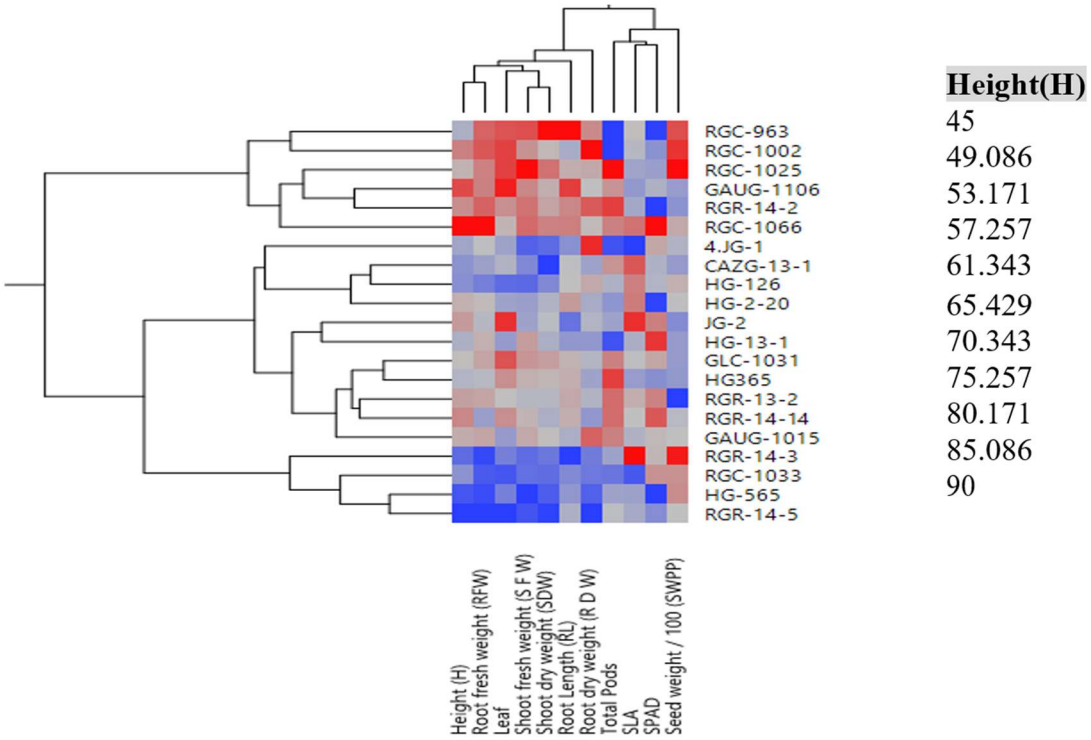
5	0PT-7	GGCAGGCTGT	37	5	0.36	18	92
6	OPP-3	CTGATACGCC	37	4	0.23	20	77
7	OPQ-9	TCCCACGCAA	37	5	0.35	10	89
8	ISSR-817	CACACACACACACACAA	45	7	0.33	16	84
9	ISSR-819	GTGTGTGTGTGTGTGTA	45	8	0.16	21	68
10	ISSR-846	CACACACACACACACART	45	5	0.28	20	79
11	ISSR-876	GATAGATAGACAGACA	45	6	0.35	20	92

Table 2.Genotypic polymorphism in the 22 cluster bean varieties using RAPD &ISSR Primers.

Tm : Melting Temperature , PIC : Polymorphic Information Content

This cluster analysis (Word's Method Euclidian Distance) based on a total of 58ampliconsgeneratedfrom11markersrevealed (Fig.1)that22varietieswere divided into 2 major clusters,cluster1 and cluster 2. Cluster 1 contains a single variety, HG-365. While, Cluster-II contains remaining 21 varieties, of which further fall into Sub-clusters IIa and IIb. Sub cluster-II a, contains sixteen varieties, RGC-963, RGR-13-2, RGC-1025, HG-2-20, JG-1, JG-2, GAUG-1015, CAZG-13-1, GA UG-1106, RGR-14-5, HG-126, RGR-14-3, RGR-14-2, HG-565, RGC-1066 and RGC-1033. While, Sub-cluster IIb has five variety RGC-936, GLC-1031, HG-13-1, RGR-14-14, and RGC-1022. Based on this result, it is confirming that HG-365, is much dissimilar than other varieties, which means it may contain new genetic components.

Morphological and physiological analyses of all genotypes were observed, RGC-1066 & RGC-963 genotypes showed high chlorophyll values. The total number of pods werehighinRGC-1066genotype.Morphological dendrogram construction was shown in Fig.2.The genotypes groups were decided after drawn the dendrogram for 22 genotypes based on the banding pattern of RAPD and ISSR markers Fig.1



Discussion

Genetic diversity is one of the great aspects of the sustainability of plant populations (Wang et al.2007) and the genetic structure of the plant populations reflects the interactions of many different processes such as the long term evolutionary history of the species, mutation genetic drift mating system, gene flow and selections (Nirajetal.2012; Schaaletal.1998; Slatkin,1987). In crop improvement strategy, the analysis of genetic relationship in crop species for the crosses between two diverse varieties is compulsory (Sharma et al.2012). Molecular marker technology is most essential for studying germplasm characterization, purity testing, phylogenetic analysis, genetic diversity studies, population structure analysis, dense and comprehensive genetic maps construction, marker-associated traits and gene function identification studies (Niraj et al. 2012; Raina et al. 2001; Zietkiewicz et al. 1994; Wang et al. 2009;Ramesh et al.,2020). Hence, genetic diversity is one of the most important aspects in crop improvement programme or development of new varieties by breeding aspects. Therefore genetic diversity should be studied in each crop using molecular markers (Bhandari et al., 2017).

Polymorphism by RAPD analysis may arise due to the deletion or substitutional addition of the base within the priming site sequence (Williametal.1990). Nagaoka and Ogihara (1997) have also reported that the ISSR primers produced several times more information than the RAPD markers in wheat and groundnut (Raina et al.2001), Bacopamonnieri (Niraj et al.2012) and Jatropha (Gupta et al. 2008). RAPD also successfully utilized for the identification of medicinal, herbal medicinal components of plants (Niraj et al. 2012; Shakoor et al., 2022; Shindeetal. 2007; Boomibalagan et al.,2021) , ornamental plants (De Benedetti et al. 2001) and in endemic species of Rhabdosciadium

aucheri Boiss(Fatemeh Kazemeini et al.,2020). During the environmental adaptations, isolated individual species tend to accumulate genetic variations (Sarwat et al. 2008). In the present study, genetic variation analysis was done by 7 RAPD and 4 ISSR markers which revealed significant variation between the 22 cluster bean varieties. The highest PIC values like 0.48,0.36 &0.35 were observed with RAPD Primers of OPA-7A, OPA-4, OPT-7, and OPQ-9 similarly 0.35 and 0.33 were observed with ISSR primers of ISSR-875 and ISSR-817. By using RAPD and ISSR genetic banding pattern cluster analysis was done according to Niraj et al.2012. Similar results have been reported in earlier studies in Portuguese Tronchuda cabbage and GalegaKale, Indian mustard, Bacopamonnieri and mustard (Dias et al. 1993; Gupta et al. 1991; Niraj et al. 2012; Rabbani et al. 1998). Selecting the parental lines to develop the guar populations for genomic mapping and breeding purposes, genetic variations obtained from RAPD and ISSR markers information is most useful (Reddy et al. 2002; Sharma et al. 2014).

In the present study, 77-100% of polymorphism was observed with RAPD primers and 68-92% polymorphism was observed with the ISSR primers and a positive correlation was observed between all genotypes in morphological studies (Table 1). Based on morphological characters, 18 cluster bean genotypes were selected for genetic diversity studies by using 37 decamers, and the number of bands ranged from 4 to 22 with an average of 10.29 bands per primer was obtained (Punia et al. 2009). Ajit et al. (2013) were selected 15 genotypes for cluster bean genetic diversity studies with 3 RAPD primers and 78.94% polymorphism was observed.

Similarly, genetic diversity investigation was carried out in 19 cluster bean genotypes by using 13 RAPD and 7 ISSR primers and the average percentage of polymorphism by RAPD is 87.63% and minimum is 55 and the maximum is 100%, whereas by using ISSR primers 66.6% to 100 % of polymorphism were observed with 75% average polymorphism (Nagesh et al. 2013). Sandeep et al. (2014) were also studied genetic variability in 56 genotypes and high levels of polymorphism were observed with 25 RAPD primers. Genetic variability studies were done not only by molecular markers but biochemical traits also one of the methods. Manivannan et al. (2017) and Manivannanet al.(2013) was studied genetic variability in 42 Indian cluster bean genotypes by analyzing the crude protein, crude fat, crude fiber, ash, carbohydrate, and gum content and yield parameters of cluster bean. Kishan et al. (2012) were studied the molecular characterization of bacterial leaf blight by using 40 primers and galactomannan content by 120 RAPD primers in 16 cluster bean genotypes.

RAPD markers have been used extensively for the genetic diversity studies in other crops like pigeon pea (Ratnaparkhe et al. 1995), soybean (Barakat 2004), Among Ber (Ziziphus spp.) (Devanshi et al. 2007) cowpea (Xavier et al. 2005), wheat (Cenkci et al. 2008), Wheat (Nagaoka et al. 1997) Maize (Antonio et al. 2004), Vigna germplasm (Seehalak et al. 2006),Buteamonosperma (Vaishali et al. 2008), Vignaangularis (Yee et al. 1999), and Aromatic rice (Zakiyah et al.2019).Recently, this study was conducted in greater yam (Rao et al.,2020),60.98 % of polymorphism in castor (Kim et al.,2021),89.90% of polymorphism in 24 potato genotypes (Somana et al., 2021), 0.19 and 0.84% polymorphism in 10 potato varieties

(Amardeep sing et al., 2021) , high yielding rice varieties (Epe et al.,2021), and in six tomato line (El-Mansy et al., 2021).

Recently, Similar genetic polymorphism was studied extensively by ISSR primers with 74.53% in castor (Kim et al.,2021), molecular characterization in six tomato lines (El-Mansy et al., 2021), screening and genetic polymorphism in 29 Jordanian tomato varieties (Brake et al., 2021), genetic polymorphism in 23 finger millets genotypes by using 15 ISSR primers (Venkatesan et al., 2021), genetic polymorphism in greater yam and in Nitraria sibirica pall with 7 primers (Rao et al.,2020; Khozyaykina and Banalev 2021)

RAPD and ISSR markers are used for extensive diversity studies in several crops like Wide Pistachio Germplasm (Kafkas et al. 2006), Wild Tea (Lai et al. 2001), Chilean Nothofagus species (Mattioni et al. 2002), Peanut (Raina et al. 2001), Black Gram (Souframanien et al. 2004), Cashew germplasm (Thimmappaiah et al. 2009), common bean (Akram et al. 2012), rice bean (Muthusamy 2008).

S.NO	Genotype name	Location	Seed characteristics	Shape of Seeds	Total days of crop
1	RGC-936	Rajasthan	Round, brown	Kidney square	85-90
2	RGC-963	Rajasthan	brown	Kidney square	85-95
3	RGC-1025	Rajasthan	Black	Elongated oval	85-90
4	JG-1	Gujarat	Brown	Kidney square	90-95
	JG-2	Gujarat	brown	Kidney square	90-95
6	GLC-1031	Gujarat	Light white	Round	85-95
7	HG- 365	Haryana	Milky white	Round bold	85-90
8	RGC-1002	Rajasthan	Gray	Round	80-90
9	RGC-1066	Rajasthan	Gray	Round	85-90
10	CAZG-13-1	Rajasthan	Brown	Round	85-90
11	HG-2-20	Haryana	Brown	Round bold	90-95
12	RGR-13-2	Rajasthan	Gray	Appressed square	80-90
13	HG-13-1	Haryana	gray	Round	90-95
14	RGR-14-14	Rajasthan	Light block	Round	85-90
15	GAUG-1106	Gujarat	Brown	Round	90-95
16	HG-126	Haryana	Brown	Round	85-95

17	RGR-14-3	Rajasthan	Gray	Round	85-90
18	RGC-1033	Rajasthan	Light gray	Round	85-95
19	GAUG-1015	Gujarat	Brown	Round	85-95
20	HG-565	Haryana	Brown	Round	85-90
21	RGR-14-5	Rajasthan	Brown	Round	85-90
22	RGR-14-2	Rajasthan	Brown	Round	80-90

Table 2Characteristic features of 22 cluster bean genotypes.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1.00																					
2	0.72	1.00																				
3	0.61	0.76	1.00																			
4	0.65	0.80	0.67	1.00																		
5	0.63	0.77	0.62	0.87	1.00																	
6	0.60	0.59	0.48	0.71	0.77	1.00																
7	0.34	0.55	0.51	0.56	0.64	0.55	1.00															
8	0.69	0.65	0.48	0.71	0.70	0.80	0.53	1.00														
9	0.48	0.71	0.52	0.65	0.70	0.60	0.57	0.56	1.00													
10	0.60	0.75	0.65	0.85	0.84	0.72	0.57	0.72	0.72	1.00												
11	0.71	0.81	0.82	0.79	0.74	0.65	0.55	0.55	0.64	0.72	1.00											
12	0.59	0.81	0.66	0.70	0.76	0.56	0.57	0.58	0.71	0.78	0.69	1.00										
13	0.69	0.48	0.47	0.55	0.63	0.78	0.50	0.78	0.54	0.54	0.66	0.44	1.00									
14	0.71	0.53	0.50	0.55	0.67	0.82	0.58	0.80	0.55	0.59	0.63	0.53	0.89	1.00								
15	0.57	0.71	0.61	0.84	0.80	0.73	0.58	0.80	0.68	0.84	0.69	0.67	0.54	0.59	1.00							
16	0.50	0.59	0.54	0.67	0.75	0.63	0.66	0.62	0.66	0.70	0.67	0.58	0.63	0.63	0.73	1.00						
17	0.56	0.66	0.60	0.75	0.82	0.74	0.66	0.68	0.80	0.77	0.74	0.62	0.66	0.76	0.82	0.75	1.00					
18	0.43	0.67	0.65	0.67	0.70	0.57	0.62	0.61	0.65	0.68	0.55	0.72	0.45	0.54	0.66	0.66	0.63	1.00				
19	0.63	0.77	0.58	0.85	0.89	0.70	0.55	0.70	0.70	0.84	0.70	0.73	0.56	0.59	0.84	0.72	0.75	0.66	1.00			
20	0.42	0.45	0.53	0.60	0.69	0.69	0.70	0.63	0.66	0.67	0.55	0.44	0.68	0.79	0.67	0.78	0.78	0.64	0.62	1.00		
21	0.59	0.64	0.56	0.82	0.83	0.64	0.60	0.81	0.64	0.83	0.61	0.65	0.66	0.64	0.86	0.72	0.72	0.69	0.86	0.84	1.00	
22	0.41	0.58	0.41	0.68	0.78	0.69	0.74	0.53	0.75	0.72	0.61	0.53	0.54	0.64	0.75	0.78	0.89	0.60	0.75	0.84	0.67	1.00

Table 1. Morphological correlation between 22 cluster bean genotypes

Conclusion

This RAPD and ISSR markers data is a highly useful technique for identifying the diversity and genetic variation analysis among different accessions of cluster bean. A positive correlation between 22 genotypic varieties concerning morpho-physiological characteristics using RAPD and ISSR analysis was designated in cluster bean lines. Based on RAPD and ISSR markers banding pattern indendrograms were separated into two clusters, where the first cluster contains a single genotype, second cluster contains two sub-clusters 21. When genetic diversity is more between the genotypes indicating that high genetic variability in the population. The genetic variability in a gene pool is considered as the major resource for the breeding program.

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