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Studies on isolation, rheological properties and diversity analysis of guar gum

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The isolation method, rheological properties and diversity analysis of guar gum were studied. Further cetyl trimethyl ammonium bromide (CTAB) method of DNA isolation procedure was standardised to yield good quality of DNA from cluster bean. A PCR optimisation with appropriate concentration as well as temperature and time adjustment was done. Decamer primer used to analyse hybridization study with DNA. The products were run on agarose gel and photographed. The random amplified polymorphic DNA (RAPD) marker was used to study the fifteen genotypes of cluster bean. A total 20 primers were screened out of which 10 have good results. The amplification of genomic DNA of 15 genotype, using RAPD primers yielded 494 fragments that could be scored, of which 55 were polymorphic in all crosses. Per cent of polymorphism ranged from 20.51-100%. Similarity matrix obtained through NTSYS-pc software revealed that wild variety *NSG-369* shows maximum diversity. The rheological properties of guar gum found to contain galactomannan for its gummy characteristics. Finally it can be concluded that there is no correlation between the diversity of the variety and its galactomannan content but wild one variety shows great diversity than the other varieties.

Keywords: Cluster bean, DNA, marker, primers, rheological properties, galactomannan.

Guar (*Cyamposis tetragonoloba* L. Taub) is a widely grown crop but not on commercial basis on large scale. Further it is an important self pollinated, multipurpose and restorative leguminous vegetable crop. Gillet (1958) alienated the genus into three races, namely, *C. tetragonoloba, C. senegalensis* and *C. serrata.* The haploid and diploid chromosome number of all the three genus species of *Cyamopsis* were reported to be n=7 and n=14. The centre of origin guar is in the Tropical Africa. The crop is mainly grown in the dry habitats of Rajasthan, Haryana, Gujarat and Punjab. The green and tender pods of guar are used as vegetable in many parts of India. Guar has assumed great significance due to presence of good quality of galactomannan as gum (80%) in the endosperm (35-40%) which is the chief product used in many industries (Kumar and Singh 2002). Guar gum is odourless yellowish white powder. It is a natural high molecular weight hydrocolloidal polysaccharide composed of

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galactan and mannan units combined through glycosidic linkage and described as galactomannan. Further it is mainly used in paints, pharmaceuticals, cosmetics, textiles, jewellery, food products, oil drilling, explosives and insulations (Bangali 2006). Guar gum is commonly used as stabilizer in many food products like ice cream, fruit beverages, chocolate milk and milk products, cake topping etc. The other stabilizer used are starches, dextrin and protein derivatives which stabilizes and thicken the food products by combining with water to increase the viscosity and firm gel (Rodge *et al.,* 2006). India leads the list of the major guar producing countries of the world contributing to around 75-80% of the world's total production. The high morphological diversity is found in guar. Therefore the present investigation attempts to analyze the genetic diversity in the cultivated guar using allozyme polymorphism and it comparison with morphological diversity and rheological properties of guar gum.

There are different types of DNA markers which have been developed viz., amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), sequence tagged sites (STS), allele specific associated primers (ASAP), expressed sequence tag markers (EST), single strand conformation polymorphism (SSCP), restriction landmark genomic scanning (RLGS), variable number of tandem repeats (VNTRs), hyper variable regions (HVRs), random amplified polymorphic DNA (RAPD), sequence characterised amplified regions for amplification of specific band (SCAR), randomly amplified microsatellite polymorphisms (RAMPO), cleaved amplified polymorphic sequences (CAPs). RAPD markers are DNA fragments from PCR amplification for random segments of genomic DNA with single primer of arbitrary nucleotide sequence. Pham *et al*. (2008) reported 83% polymorphism using RAPD as molecular marker in assessing genetic diversity of sesame (*Sesamum indicum* L.) cultivars in Vietnam and Cambodia. RAPD polymorphism is detected by using oligonucleotide usually 10 bases long of random sequence as primer in Polymerase chain reaction (PCR) reaction. In a strain that has in its genomic DNA sequence complementary to primer oligonucleotide, PCR product will be detected in gel. While those strains that do not have complimentary sequence no product will be detected. Some of these bands are present in some and absent in other strains. These bands constitute RAPD loci. Thus RAPD generated by specific oligonucleotides are used in PCR reaction.

Materials and methods

Plant material

A sixteen genotypes of guar (RGC-986, RGC-1002, CAZG-06-03, HSG 04-875, NSG-369, RGr-07-36, GAUG-013, CAZG-08-2, HG-24, SRG-1058, RGr-07-21, CAUG-501, RGC-08-01, CAUG-08-01, HG-100 and CAUG-309) were collected from All India Co-ordinated Research Project on Arid Legumes, Marathwada Krishi Vidyapeeth, Parbhani. The seeds of guar were washed with teepol followed by three washing of sterile water and treatment of 0.01% mercuric chloride to avoid contamination and subsequently washed with sterile distilled water, germinated on wetted paper blotters under laminar air flow. These blotters were folded, tied and arranged on germination tray. The trays were incubated at temperature (37^0C) for the period (7 days). The germinated seeds of guar (1-2 cm) length from shoot portion were used for DNA extraction.

Isolation of genomic DNA and purification

The protocol of cetyl trimethyl ammonium bromide (CTAB) DNA extraction method of Saghai-Maroof (1984) was standardised. A 2 g of leaf tissue was ground to fine powder in liquid nitrogen (-196^0C). The 20 ml of 2% DNA extraction buffer in β mercaptoethanol (0.2%) was added, mixed and incubated at temperature (65^0C) for 30 min. The mixture was

centrifuged (10,000 rpm) at temperature (4^0C) for 10 min. The supernatant was mixed with equal volume of chloroform: isoamyl alcohol mixture, mixed and recentrifuged. To the collected supernatant 0.7 volume of chilled isopropanol was added and DNA was allowed to precipitated at temperature (20^0C) overnight. The DNA was pelleted by centrifugation (10000 rpm) for 10 min at room temperature. Further the pellet was washed with 500 µl of 70% ethanol. After short centrifugation, supernatant was discarded. The pellets were air dried in laminar air flow and dissolved in appropriate amount of TE buffer. DNase and RNase-A was added at a final concentration of 20µg/ml and incubated (37^0C) for 1 h. The equal volume of phenol: Chloroform (1:1) mixture was added and centrifuged (10000 rpm) for 10 min. The aqueous phase was collected in fresh tube and above step was repeated till no white interface was seen. The sodium acetate (0.1 volume) and ice cold absolute ethanol (2 volume) was added, mixed and kept for temperature (-20^0C) for 1 h. The DNA was pelleted by centrifugation (10,000 rpm) for 10 min at room temperature and pellet was washed with 100 µl of 70% ethanol. After short centrifugation, supernatant was discarded. The washing procedure was repeated twice and repelleted by centrifugation (10000 rpm). The pellets were air dried in laminar air flow and dissolved in appropriate amount of TE buffer and stored at 20^0C.

DNA quantification

The DNA concentration thus estimated and rechecked quantitatively by running on 0.8% agarose gel.

Dilution of DNA samples

The DNA samples were diluted with appropriate quantity of sterilized distilled water to yield a working concentration of 25mg/µl and stored at temperature (4^0C) until PCR amplifications.

Primer screening

The thirty random primers from OPA, OPB series (Bio Serve Biotechnologies, Hyderabad, India) were selected using DNA from three varieties (RGC-986, HG-100, GAUG-013) and seven primers from different series were screened on the basis of reproductive and scrorable amplifications for the analysis of all the sixteen genotypes of cluster bean (Table 1).

Table 1 Primers selected for RAPD analysis of the selected guar genotypes

Primer	Primer sequence (5′-3′)
OPA-10	GTGATCGCAG
OPA-9	GGGTAACGCC
OPD--16	AGGGCGTAAG
OPA-4	AATCGGGCTG
OPM-7	CCGTGACTCA
OPM-16	AGCCAGCGAA
OPM-12	TCGGCGATAG
OPD-11	GGTCTACACC
OPA-13	CAGCACCCAC
OPM-20	AGGTCTTGGG

PCR optimization and RAPD amplification

PCR optimization for RAPD analysis

The concentration of genomic DNA (25, 50, 75 ng/25µl reaction mixture) $MgCl_2$ (1.5, 2.5 and 3.5 mM) and *Taq* DNA polymerase (0.5 and 0.6 U) were tried to obtain an optimum reaction mixture. Te different concentration of the primers (5, 10, 15, 20 and 25 pm/µl) was also tried for the complete amplification of the genomic DNA. The optimization of primer annealing the different temperature (34, 35, 36 and 37^0C) was tried.

RAPD amplification

A master mix was prepared in sterile distilled water. Twenty five ng of genomic DNA from different samples were taken into individual 0.5 ml PCR tubes and the master mix was added to them to a final concentration of 25 µl. Amplifications were performed in a thermal cycler (Biometra) using the cyclic parameters (Table 2).

Table 2 RAPD amplification using cyclic parameters

Steps	Temperature (^{0}C)	Time
Initial denaturation	94	4 min
Denaturation	94	1 min
Annealing	35	1 min
Primer extension	72	2 min
40 cycles		
Final Extension	72	10 min
Holding	4	Variable

Agarose gel electrophoresis

The amplified products were resolved on 1.5% Agrose gel in 1 x TAE buffer at 100V for 210 min. The gel was strained in ethidium bromide. After electrophoresis the gel was carefully taken out of the casting tray and photograph was taken on gel documentation system (Alphamager™ 2200).

Scoring and data analysis

Each amplifications product was considered as RAPD marker and was scored across all samples. Molecular weights of bands were estimated by using 1kb DNA ladder (MBI, Fermentas, UK) as standards. All amplifications were repeated at least twice and only reproducible bands were considered for analysis. The data was used for similarity based analysis using the program NTSYS-Pc (Version 2.02) developed by Rohlf (2000). Dice's similarity coefficients (F′) were calculated using the program SIMQUAL. Similarity coefficients were used to construct UPGMA (un-weighted pair group method with average) to generate dendrogram. The polymorphic percentage of the obtained bands was calculated by using following formula,

Polymorphic (%) = (Number of polymorphic bands/total bands) x 100

Isolation and purification of galactomannan from clusterbean

The isolation and purification of galactomannan from clusterbean was done by wet processing method (Rodge *et al.,* 2006). The seeds were dehusked using alkaline solution (2% NaOH), heated (98^{0}C) for 10 min and neutralized with 0.1 N HCL solution. The dehusked were thoroughly washed with tap water and dried. The gum splits were pulverized to fine powder (size 100 mesh). The crude gum thus obtained was further purified by isopropanol.

Rheological character of clusterbean gum

The viscosity of cluster bean gum was determined by using Roto-viscometer (Hakke RV 200 Model, Germany). Further the share stress at different share rate and apparent viscosity of cluster bean were determined.

Results and discussion

The agarose gel electrophoresis showed good quality of DNA. The quantity of DNA was checked by comparing DNA samples with known amount of lambda DNA. The quantity of all samples was found to be in the range of 70-200 ng/µl. The samples were used as stocks and working samples of 30 ng/µl were used.

Genetic diversity analysis using RAPD marker

After isolation of genomic DNA from all 15 genotypes lines of guar DNA samples was subjected to PCR using RAPD primers. A total of nine primers generated 494 amplicons with an average of 54.6 amplicons per primers. The 55 amplicons were found polymorphic with polymorphism (20.51%) and the average number of polymorphic bands per primer was 6.1 (Table 3). To estimate the genetic similarity of guar germplasm a similarity matrix has been showed which is obtained by Jaccard's similarity coefficient (Table 4). The biochemical and molecular markers were used in germplasm characterization and diversity analysis as they were phenotypic independent and focused on genetic variations controlled by genes Capo-chichi *et al.,* 2003).

The primer OPM-20 was least informative as generated only monomorphic bands. The average size of fragments was between 100-6000 bp (Figure

1). The primer OPA-12 was most informative as it generated highest number of polymorphic amplicons (7) (Figure 7). The number of DNA amplified fragments per primer ranged from 1 (OPM-20) to 7 (OPA-12) (Figure 2, 3, 4, 5, 6, 8, 9, 10). The biochemical markers viz., proteins and ioszymes may also be biased, since only small portion of the genome is represented and exhibit low level of polymorphism (Wilkes 1983).

Table 3 RAPD primers and polymorphic amplicons generated

Primer code	Total number of amplicons	Number of polymorphic amplicons	Polymorphism (%)
OPA-10	57	7	12.28
OPA-09	32	5	15.62
OPD-16	60	4	6.66
OPA-04	41	5	12.19
OPM-07	73	7	9.58
OPA-16	41	5	12.19
OPA-12	77	7	9.09
OPD-11	35	3	8.57
OPA-13	79	12	15.18
OPM-20	76	8	10.60
Total	494	55	101.36

Table 4 Average Jaccard's similarity coefficient values of each germplasm line obtained by RAPD markers

Guar genotypes	Similarity coefficient value
RGC 986	0.678
RGC 1002	0.687
CAZG 06-03	0.690
HSG 04-875	0.701
NSG 369	0.513
RGr 07-36	0.676
CAUG 013	0.596
CAZG 08-2	0.598
HG 24	0.655
SRG 1058	0.725
RGr 07-21	0.642
CAUG 501	0.717
CAUG 08-1	0.704
HG100	0.678
CAUG 309	0.636
Total	0.651

The data obtained by RAPD markers was analyzed by NTSYS-Pc and dendrogram was generated by using Jaccard's similarity coefficient. Dendrogram is presented in Figure 11. Jaccard's (1908) method was used to calculate the data set of genotypes of cluster bean with reproducible bands. The maximum similarity was observed between HG-24 and HG-100. These two germplasm were found highly similar with similarity coefficient 0.667 (Table 5).

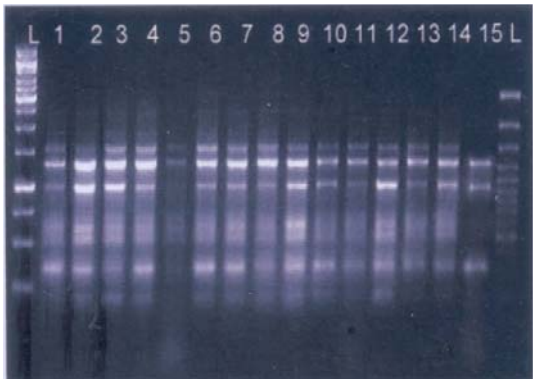

Figure 1: RAPD profile of Guar germplasm lines generated with primer OPA-10

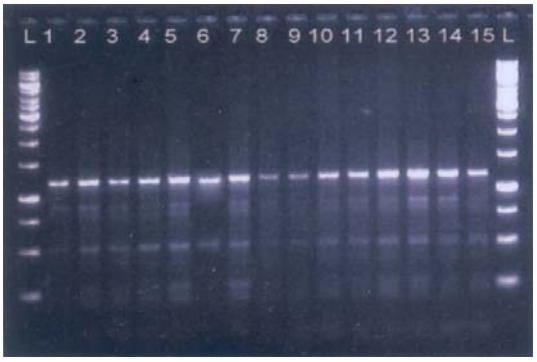

Figure 2. RAPD profile of Guar germplasm lines generated with primer OPA-9

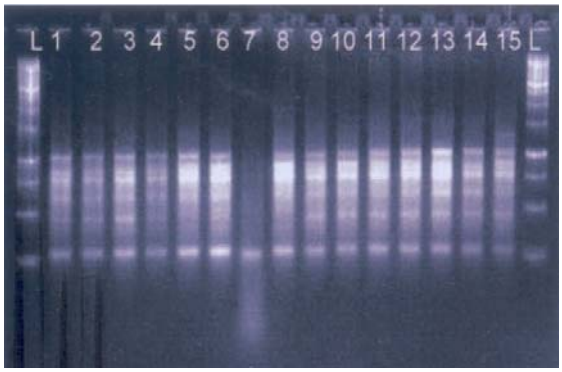

Figure 3. RAPD profile of Guar germplasm lines generated with primer OPD-16

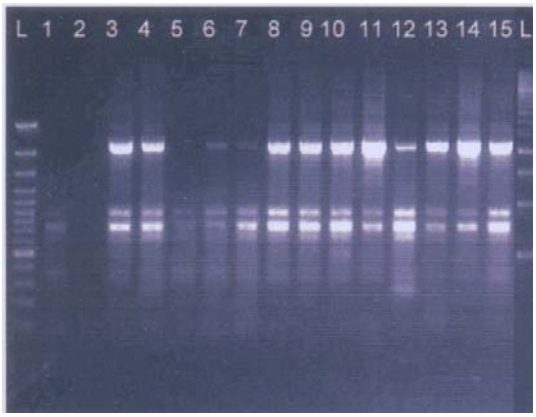

Figure 4. RAPD profile of Guar germplasm lines generated with primer OPA-4

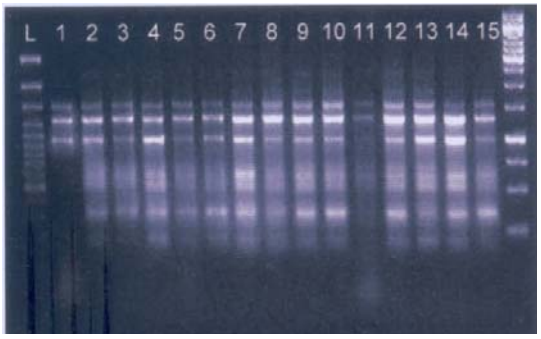

Figure 5. RAPD profile of Guar germplasm lines generated with primer OPM-07

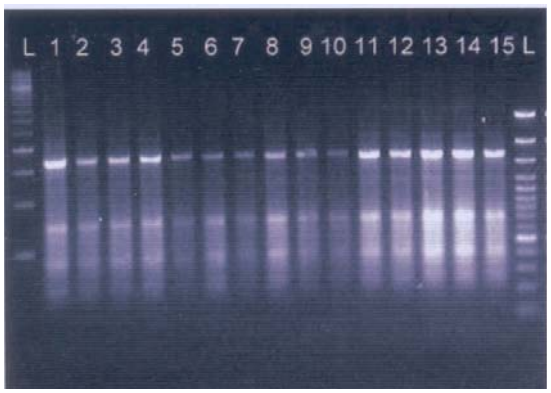

Figure 6. RAPD profile of Guar germplasm lines generated with primer OPM-16

Cluster analysis of RAPD markers

The dendrogram was generated by UPGMA cluster analysis based on Jaccard's similarity coefficient. The cluster analysis revealed that the average genetic similarity was 66.51%. On the basis of bootstrapping of similarity coefficient the germplasms were divided into four major clusters naming cluster A, B, C and D. The cluster A and B showed least similarity highly diverse germplasm (NSG-369). The level of genetic polymorphism was 50.6% and dendrogram revealed that considerable amount of genetic diversity.

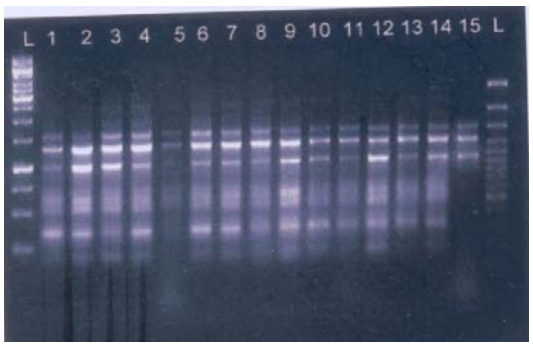

Figure 7. RAPD profile of Guar germplasm lines generated with primer OPM-12

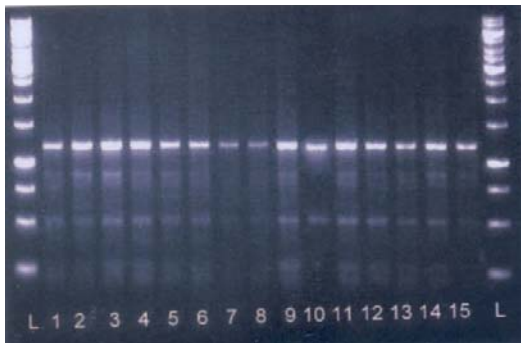

Figure 8. RAPD profile of Guar germplasm lines generated with primer OPD-11

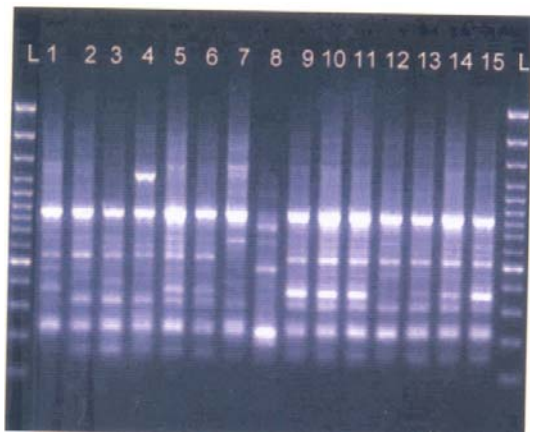

Figure 9. RAPD profile of Guar germplasm lines generated with primer OPA-13

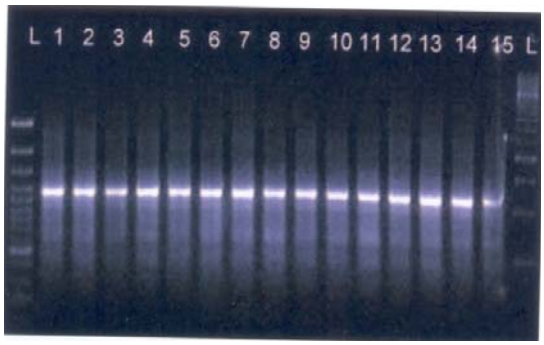

Figure 10. RAPD profile of Guar germplasm lines generated with primer OPM-20

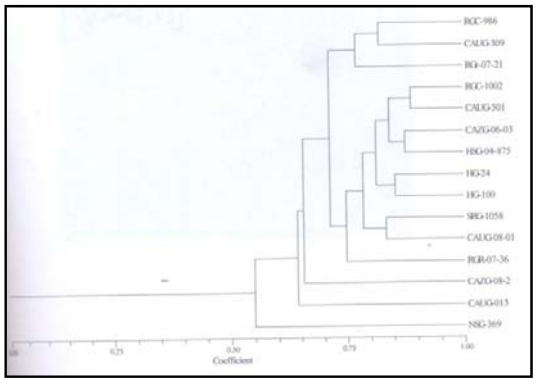

Figure 11. Dendrogram generated by RAPD markers

Table 5 Average Jaccard's similarity coefficient value at each germplasm line obtained by RAPD markers

	RGC 986	RGC 1002	CAZG 06-03	HSG 04-875	NSG 369	RGr 07-36	CAUG 013	CAZG 08-2	HG 24	SRG 1058	RGr 07-21	CAUG 501	CAUG 08-1	HG100	CAUG 309
RGC 986	1														
RGC 1002	0.722	1													
CAZG 06-03	0.750	0.833	1												
HSG 04-875	0.697	0.833	0.875	1											
NSG 369	0.581	0.553	0.514	0.514	1										
RGr 07-36	0.733	0.722	0.75	0.806	0.633	1									
CAUG 013	0.576	0.676	0.6	0.679	0.581	0.677	1								
CAZG 08-2	0.655	0.611	0.677	0.733	0.500	0.714	0.600	1							
HG 24	0.676	0.811	0.794	0.794	0.459	0.676	0.629	0.606	1						
SRG 1058	0.800	0.778	0.758	0.813	0.594	0.800	0.742	0.667	0.788	1					
RGr 07-21	0.821	0.649	0.719	0.618	0.548	0.700	0.545	0.621	0.647	0.710	1				
CAUG 501	0.758	0.889	0.824	0.879	0.528	0.758	0.706	0.688	0.800	0.818	0.767	1			
CAUG 08-1	0.828	0.75	0.727	0.781	0.563	0.767	0.710	0.690	0.706	0.833	0.793	0.844	1		
HG100	0.758	0.838	0.824	0.824	0.528	0.706	0.657	0.588	0.853	0.818	0.781	0.829	0.844	1	
CAUG 309	0.821	0.649	0.719	0.667	0.600	0.700	0.545	0.621	0.6	0.767	0.724	0.676	0.733	0.727	1

The level of genetic polymorphism was 50.6%. The dendrogram constructed based on that revealed considerable amount of genetic diversity (Sudhakar *et al.,* 2008). The second cluster contained B1a and B2b germplasm showed high percentage of similarity (65%). The cluster A showed average 53% similarity with cluster B. The germplasm of two varieties showed similarity in days of flowering, plant height and seeds per pod. Cluster C again divided into five sub-clusters (C1, C2, C3, C4 and C5). The C1 again divided into C1a and C1b which showed high percentage of similarity (88%). The RGC-1002 and CAUG-501 showed similarity nature and branching nature. The cluster C2 again divided into C2a and C2b. In that CAZG-06-03 and HSG-04-875 showed similarity in maturity time. The cluster C3 divided into cluster C3a and C3b and showed high percentage of similarity (84%). The cultivar HG-100

and HG-24 showed similarity in seeds per pod and days of flowering. The RAPD markers were used to determine genetic diversity in cluster bean from different agro ecological area. Further the RAPD marker showed 72.7% polymorphism and variation in genotype was not related to agro ecological differences (Punia *et al.,* 2009). The level of genetic diversity of cluster bean in tropical region was high with 65% similarity (Pathak *et al.,* 2009).

The cluster C4 again divided into C4a and C4b which showed high percentage of similarity (80%). The SRG-1058 and CAUG-08-01 showed similarity in harvesting time, plant height, pods per plant and seed per pod. The cluster C5 contains only one germplasm and showed 76% similarity to other C cluster germplasm. The cluster D divided into sub-clusters D1 and D2. The cluster D1 again divided into D1a and D1b, showed the high percentage of similarity (82%) and varieties RGC-986 and CAUG-309 showed similarity in harvesting, plant height and flowering time. The cluster D2 found to contain only one germplasm, showed 77% similarity with other cluster D germplasm. A molecular marker provides an opportunity to identify and isolate the genes relating to qualitative character by map based cloning (Singh and Ocampo 1997).

Isolation and purification of galactomannan

The galactomannan content of cluster bean varieties revealed that there was non-significant difference ($P > 0.05$) in the yield of galactomannan in all guar genotypes (Table 6).

Viscosity profile of 1% (w/v) galactomannan

The viscosity profile of galactomannan (1%) solution is from 2990-3491 m pas for all the genotypes of cluster bean. Further the genotype RGC-1002 resulted highest viscosity followed by HSG-04-875 whereas lowest showed by CAZG-08-2 (Table 7).

Table 6 Yield of galactomannan from guar genotypes

Guar genotypes	Gum content (%)
RGC-986	29.37
RGC-1002	30.58
CAZG 06-03	32.20
HSG 04-875	28.90
NSG 369	29.84
RGr 07-36	29.75
CAUG 013	30.88
CAZG 08-2	33.60
HG 24	32.67
SRG 1058	31.84
RGr 07-21	29.71
CAUG 501	32.58
RGC 08-01	33.79
CAUG 08-1	31.64
HG100	30.50
CAUG 309	31.11

Table 7 Viscosity profile of gum isolated from guar genotypes

Guar genotypes	Viscosity (m pas)
RGC-986	3262
RGC-1002	3291
CAZG 06-03	3491
HSG 04-875	3172
NSG 369	3361
RGr 07-36	3281
CAUG 013	3031
CAZG 08-2	2990
HG 24	3023
SRG 1058	3085
RGr 07-21	3328
CAUG 501	3262
RGC 08-01	3053
CAUG 08-1	3142
HG100	3242
CAUG 309	3062

Each value is average of three determinations.

Conclusions

RAPD marker method effectively classified the genotypes of cluster bean from different places. A grouping result from cluster analysis based on NTSYS-pc showed that cultivars selected had narrow genetic variation to considerable extent. The cultivars with DNA profile provided useful information for selection of parents to develop new cluster bean hybrids. The galactomannan isolated from cluster bean genotypes was found in the range of 30.6-33.2% on whole seed weight basis. The viscosity profile of galactomannan exhibits pseudo plastic behaviour.

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