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Variation in the protein and galactomannan content in guar seeds of the different genotypes

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Abstract: Investigations were carried out at College of Agriculture, Acharya N G Ranga Agricultural University, Rajendranagar, Hyderabad to screen guar genotypes. A set of 22 guar diverse genotypes were used for the study. The study was intended to identify the environmental conditions responsible for realization of higher seed and gum yields and to determine the protein and galactomannan content in seeds among genotypes, during the different seasons i.e. *Kharif*, *Rabi* and summer. Guar seed quality traits i.e. galactomanman content, percent endosperm, viscousity, total carbohydrate and protein contents were not significantly influenced by the environment. The values for these seed quality characters were followed almost a similar trend across the three seasons of sowing with inconsistent differences.

Keywords: Carbohydrate, protein, SPAD, galactomannan, guar (*Cyamopsis tetragonoloba*)

Introduction

Guar or cluster bean is believed to have originated in Africa but is been grown throughout southern Asia since ancient times as a vegetable and fodder crop. Guar has been cultivated in India and Pakistan for ages for use of its tender pods as fresh vegetables and other parts of the plants to be used as cattle feed. The major world supplier of guar seed are India, Pakistan and United States. Guar is a crop of semi arid–sub tropical areas spread over the North and North West of India and East and South East of Pakistan. Guar is grown in arid zones of Rajasthan, some parts of Gujarat, Harayana, Madhya Pradesh, Jodhpur City in the North Western state of Rajasthan. The endosperm of guar seeds (guar beans; *Cyamopsis tetragonoloba*) is the source of a soluble hydrophilic polysaccharide gum that is used as an emulsifier, thickener and stabilizer in a wide range of foods and is part of the seed total dietary fibre (TDF). Guar seed (*Cyamopsis tetragonoloba*) composed of the hull (30 -33%), endosperm (27 -30%) and germ (43 - 47%). The germ and hull of the guar seeds are known as guar meal, which rich in protein, hence used for the cattle feed. The endosperm is commercially important part in the guar seed, as it is converted into powder gum. It contains 41% of the dry weight and acetone insoluble solids of the seed,3 - 11% of the nitrogen and phosphorus. At least 75% of acetone insoluble solids of the endosperm are galactomannose and 12% being accounted for as pentosan, protein, pectin, phytin, ash and dilute acid insoluble residue.The guar crop has acquired an economic importance after the discovery of the gummy substance (Galactomannan) in its endosperm . Guar seeds are a rich source of mucilage or gum which forms a viscous gel in cold water and used as an emulsifier, thickener, stabilizer in a wide range of food and industrial application.

Materials and Methods

The investigations were taken up during three consecutive seasons of 2008-09, namely, *Summer* 2007-08, *Kharif* ' 2008 and *Rabi* ' 2008-09.

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Protein content in Guar seeds was estimated as per the method developed by Lowry *et al.* (1951). 50 mg of Bovine serum albumin was dissolved in distilled water and the final volume was made upto 50 ml in a volumetric flask. From this, 10 ml of stock solution was taken into another standard flask and volume was made upto 50 ml. From the working standard, different concentrations were prepared. 500 mg of seed sample was weighed and ground with pestle and mortar with 5 ml of 10 per cent Trichloro Acetic Acid (TCA). Then it was washed with 5 ml of cold TCA and kept in ice for 15 min. The material was centrifuged at 3500 rpm for 15 minutes and the supernatant was discarded and the precipitate was dissolved in 4 ml of 2N NaOH. It was allowed to stand for overnight. Then it was centrifuged at 3500 rpm for 15 minutes, the supernatant was collected and finally the aliquot was made upto 10 ml.0.1 ml of sample extract was pipetted out to which 5 ml of reagent-C was added. The contents were mixed well and allowed to stand for 10 minutes. Afterwards 0.5 ml of reagent-D was added, mixed well and incubated for 30 minutes at room temperature in dark. The colour intensity was then measured at 660 nm with colorimeter. From the standard curve, concentration of protein content in per cent was estimated for the different genotypes.

The total carbohydrate content was estimated as per the method developed by Dubois *et al.* (1956). The sample was powdered and made moisture free. Further, 500 mg of sample was weighed and acid hydrolysis was carried out (0.5g of sample + 2 ml of 72% H_2SO_4) in 250 ml of dry conical flask. It was stirred with a glass rod upto 10 minutes in chilled water or ice. The volume was then made up to 25 ml by adding 23 ml of distilled water. This solution was kept in a water bath at 90-95°C for 4 hours. After this, the solution was filtered through coarse filter paper in a volumetric flask and the volume was made upto 100 ml. 1ml of the filtrate was taken and the volume was made up to 100 ml. one ml of the resultant solution thus obtained from each sample was taken in a test tube and 1 ml of distilled water was taken to prepare blank. Further, 0.2 ml of phenol was added to the blank and sample test tubes. Then 5 ml of concentrated H_2SO_4 was added and shaked using vortex mixer. Later, it was allowed to cool and the optical density observations were recorded at 480 nm on spectrophotometer. 100 µg of glucose was dissolved in 1 ml of distilled water or 0.01 g of glucose was dissolved in 100 ml of distilled water. From this working standard different concentrations were prepared. From the standard curve, concentration of total carbohydrates was estimated for the different samples in per cent.

For estimation of endosperm percentage the procedure given by Das *et al.* (1977) was used. 30 gm of guar seeds was taken and subjected to wet processing (2% NaOH) with vigorous boiling at 98°C for 5 minutes. The solution was sieved through coarse sieve to remove excess NaOH. The leachate was discarded and wet de husked seeds were acidified slightly for 10 minutes in 0.1 N Hcl and washed with water. The de husked seeds were then air dried for 2-3 days. Later, the dried de husked seeds were pulverized to get endosperm splits and germ meal. Further, the germ meal was discarded using 1 mm sieve.

Weight of the pure endosperm splits was recorded and the endosperm percentage was calculated.

For estimation of gum content also, the procedure given by Das *et al.* (1977) was used. Endosperm splits obtained from the above procedure were soaked in distilled water in 1:5 proportions and kept for 4-5 hours. The soaked splits were then ground in a blender to get viscous solution of thick consistency and it was kept overnight. Later, the thick solution was disturbed using glass rod and then 50-100 ml of isopropanol was added leading to precipitation of gum on the top. Further, excess isopropanol was removed from the lumps (gum) with the help of strainer and the lumps were then vacuum dried. Dried lumps were further powdered in a blender and the gum content was calculated.

Results and Discussion

Chlorophyll content (SPAD)

Chlorophyll content of 22 guar genotypes at 40 and 60 DAS are presented in Table 6. A significant difference in chlorophyll content was observed between genotypes growing season and their interactions.

Chlorophyll content was high in summer season(59.20)) followed by *Kharif* (56.96) and *Rabi* seasons (28.48) both at 40 and 60 DA chlorophyll content values were almost similar at 40 and 60 DAS. Whereas, RGM 115 recorded highest chlorophyll content followed by RGM 114 in all different growing seasons compared to other genotypes.

Galactomannan content in the seed of guar genotypes as affected by growing seasons is presented in Table 3. There was no significant variation in galactomannan content between growing season of guar genotypes and interaction effect.

Viscosity of guar genotypes (centi pascals second^{-1})

Viscosity of guar genotypes is presented in Table 4. There was no significant difference in viscosity between different growing seasons. The differences among

Table 1: Influence of growing seasons and genotype on total carbohydrate (%).

S.No.	Total carbohydrate content (%)				
	Genotype	Summer	Kharif	Rabi	Mean
1	RGC 1017	32.20	32.43	33.18	32.60
2	RGC 1024	32.50	33.78	33.24	33.17
3	RGC 1026	32.27	34.37	32.32	32.99
4	RGC 1027	34.48	37.31	36.28	36.02
5	RGC 1028	31.73	32.80	33.34	32.62
6	RGC 1033	36.33	38.96	36.87	37.39
7	RGC 986	37.94	38.14	39.43	38.50
8	RGC 1038	35.84	37.77	36.89	36.83
9	RGC 1047	31.45	33.45	31.61	32.17
10	RGC 1076	34.71	36.48	34.92	35.37
11	RGC 1077	37.33	38.63	39.21	38.39
12	RGC 1078	32.18	33.17	34.42	33.26
13	RGC 1079	30.08	32.16	31.86	31.37
14	RGC 936	30.12	32.49	30.60	31.07
15	RGM 111	39.12	39.33	39.27	39.24
16	RGM 112	30.75	32.62	31.55	31.64
17	RGM 114	34.37	35.28	36.18	35.28
18	RGM 115	34.06	35.02	34.84	34.64
19	RGM 116	38.53	38.59	38.68	38.60
20	GAUG 011	32.41	34.08	33.08	33.19
21	GAUG 001	30.21	32.53	31.70	31.48
22	HGS 884	29.36	32.35	30.00	30.57
	Mean	33.54	35.08	34.52	
		Season (S)	Genotype (G)	Interaction (SxG)	
	SEm±	0.05	0.13	0.22	
	CD(0.05)	Ns	0.25	0.43	

Table 2: Influence of growing seasons and genotype on protein content (%) in seed in guar

S.No.	Protein content (%)				
	Genotype	Summer	Kharif	Rabi	Mean
1	RGC 1017	31.49	32.56	33.16	32.40
2	RGC 1024	29.97	32.35	30.42	30.91
3	RGC 1026	28.65	29.37	28.42	28.81
4	RGC 1027	31.33	32.35	32.76	32.15
5	RGC 1028	32.00	34.27	33.10	33.12
6	RGC 1033	31.78	32.13	32.21	32.04
7	RGC 986	33.97	34.27	35.35	34.53
8	RGC 1038	31.57	32.27	33.24	32.36
9	RGC 1047	30.90	33.41	28.02	30.77
10	RGC 1076	30.52	31.07	32.28	31.29
11	RGC 1077	28.95	31.28	29.36	29.86
12	RGC 1078	31.09	32.56	32.93	32.19
13	RGC 1079	29.90	32.56	30.19	30.88
14	RGC 936	29.39	32.35	30.83	30.86
15	RGM 111	25.98	28.07	26.02	26.69
16	RGM 112	27.54	29.16	28.15	28.28
17	RGM 114	31.02	31.71	32.20	31.64
18	RGM 115	28.66	29.16	28.76	28.86
19	RGM 116	27.58	27.73	28.48	27.93
20	GAUG 011	29.67	30.45	31.04	30.39
21	GAUG 001	27.84	30.65	28.83	29.11
22	HGS 884	29.02	30.85	30.25	30.04
	Mean	29.95	31.39	30.73	
		Season (S)	Genotype (G)	Interaction (SxG)	
	SEm±	0.13	0.36	0.63	
	CD(0.05)	Ns	0.71	1.23	

genotypes were significant. Interaction effects were also significant. RGM 114 recorded highest viscosity followed by RGC 1026 in all the seasons.

Endosperm per cent of guar genotypes (%)

Endosperm per cent of 22 guar genotypes is presented Table 5. There was no significant difference in per cent of endosperm content of guar genotypes among the seasons, however significant variability recorded among the genotypes and their interactions.

In *Kharif* season GAVG 011 recorded highest endosperm per cent (34.82%) followed by RGM 112(34.17%) and RGC 1033 (33.75). In summer season GAUG 001 also recorded highest endosperm per cent (33.60%) followed by RGC 1026 (32.89%) and RGC 1038 (32.77%), whereas in *Rabi* season RGC 1038 recorded highest endosperm per cent (35.26%) followed by RGC 1078 (34.47%) and GAUG 011 (33.83%).

Total carbohydrate content (%)

The total carbohydrate content of guar genotypes as influenced by seasons is presented in Table 1. There is no significant variation in total carbohydrate content due to guar genotypes among seasons. However, among

Table 3: Influence of growing seasons and genotypes on Galactomannan in seed (%).

S.No.	Galactomannan (%)				
	Genotype	Summer	Kharif	Rabi	Mean
1	RGC 1017	30.02	31.07	30.58	30.56
2	RGC 1024	31.86	31.73	32.13	31.91
3	RGC 1026	32.11	30.79	31.45	31.45
4	RGC 1027	30.28	29.57	29.67	29.84
5	RGC 1028	31.55	30.02	30.45	30.67
6	RGC 1033	32.15	31.72	31.29	31.72
7	RGC 986	30.15	29.69	30.64	30.16
8	RGC 1038	29.86	30.70	29.51	30.02
9	RGC 1047	30.15	31.82	30.46	30.81
10	RGC 1076	31.08	30.66	30.14	30.63
11	RGC 1077	30.53	30.26	31.21	30.67
12	RGC 1078	31.86	32.03	32.07	31.99
13	RGC 1079	30.49	30.83	30.15	30.49
14	RGC 936	31.57	30.04	32.12	31.24
15	RGM 111	30.06	29.84	30.87	30.26
16	RGM 112	32.33	31.59	31.43	31.78
17	RGM 114	31.53	31.09	29.47	30.70
18	RGM 115	30.08	29.10	30.51	29.90
19	RGM 116	29.44	29.43	30.47	29.78
20	GAUG 011	28.45	32.89	31.58	30.97
21	GAUG 001	30.62	31.76	30.54	30.97
22	HGS 884	30.12	29.67	30.27	30.02
	Mean	30.74	29.38	30.77	
		Season (S)	Genotype (G)	Interaction (SxG)	
	SEm±	3.71	10.05	17.41	
	CD(0.05)	7.30	NS	NS	

Table 4: Influence of growing seasons and genotypes on viscosity (centi pascals per second).

S.No.	Viscosity (centi pascals per second)				
	Genotype	Summer	Kharif	Rabi	Mean
1	RGC 1017	282.87	284.93	284.44	284.08
2	RGC 1024	267.14	268.85	268.48	268.16
3	RGC 1026	310.55	310.96	311.51	311.01
4	RGC 1027	241.67	243.93	242.25	242.62
5	RGC 1028	207.84	210.83	209.66	209.44
6	RGC 1033	255.61	257.50	256.93	256.68
7	RGC 986	249.85	252.56	250.48	250.96
8	RGC 1038	250.41	252.83	251.15	251.47
9	RGC 1047	234.45	234.53	235.10	234.69
10	RGC 1076	250.02	251.70	250.29	250.67
11	RGC 1077	218.05	219.90	218.89	218.95
12	RGC 1078	289.66	234.60	233.37	252.54
13	RGC 1079	260.38	261.83	261.57	261.26
14	RGC 936	262.94	265.16	263.64	263.91
15	RGM 111	261.09	263.43	261.99	262.17
16	RGM 112	250.63	251.20	250.88	250.90
17	RGM 114	320.77	323.36	322.43	322.19
18	RGM 115	270.63	272.00	271.19	271.27
19	RGM 116	229.45	230.67	230.58	230.23
20	GAUG 011	263.85	265.40	264.48	264.58
21	GAUG 001	209.25	211.13	209.88	210.09
22	HGS 884	232.04	232.85	232.37	232.42
	Mean	255.42	254.55	253.71	
		Season (S)	Genotype (G)	Interaction (SxG)	
	SEm±	1.07	2.89	5.00	
	CD(0.05)	NS	5.66	9.79	

Table 5: Influence of growing seasons and genotypes on per cent of endosperm in guar

S.No.	Per cent of endosperm				
	Genotype	Summer	Kharif	Rabi	Mean
1	RGC 1017	30.63	33.35	32.08	32.02
2	RGC 1024	32.57	33.72	32.72	33.00
3	RGC 1026	32.89	33.06	33.34	33.10
4	RGC 1027	31.29	31.69	31.71	31.56
5	RGC 1028	30.16	32.24	30.78	31.06
6	RGC 1033	30.03	33.75	31.17	31.65
7	RGC 986	31.10	32.02	31.55	31.56
8	RGC 1038	32.77	32.80	35.26	33.61
9	RGC 1047	31.91	32.89	32.40	32.40
10	RGC 1076	30.69	32.25	31.10	31.35
11	RGC 1077	29.58	32.55	31.40	31.18
12	RGC 1078	32.36	33.97	34.47	33.60
13	RGC 1079	30.03	32.40	32.12	31.51
14	RGC 936	30.87	33.41	31.21	31.83
15	RGM 111	29.54	32.24	29.91	30.56
16	RGM 112	32.11	33.73	33.59	33.14
17	RGM 114	30.58	34.17	31.45	32.07
18	RGM 115	28.25	32.22	29.75	30.07
19	RGM 116	30.78	31.72	32.55	31.68
20	GAUG 011	33.60	34.82	33.83	34.08
21	GAUG 001	32.48	33.73	33.46	33.23
22	HGS 884	29.57	31.89	31.15	30.87
	Mean	31.08	32.94	32.14	
		Season (S)	Genotype (G)	Interaction (SxG)	
	SEm±	0.07	0.18	0.32	
	CD(0.05)	Ns	0.36	0.62	

genotypes and interaction effect between genotypes and seasons were significant.

RGM 111 recorded highest total carbohydrate content both in *Kharif* (39.33%) and summer season (39.12%) followed by RGC1033 (38.96%) and RGM 116 (38.59%) in *Kharif* season, and RGM 116 (38.53%) and RGC 986 (37.94%) in summer season. However in *Rabi* season RGC 986 recorded highest total carbohydrate content (39.43%) followed by RGM 111 (39.27%) and RGC 1077 (39.21%).

Protein content of guar genotypes (%)

Protein content of 22 guar genotypes is presented in Table 2. There are no significant differences in protein content among seasons. However significant variability was observed in protein content among guar genotypes. The interaction effect between genotypes and seasons were also significant.

In *Kharif* season RGC 986 and RGC 1028 recorded highest protein content (34.27%) followed by RGC 104 (33.41%) and RGC 1079 (32.56%). In summer season also RGC 986 recorded highest protein content (33.97%) followed by RGC 1033 (31.78%) and RGC 1027 (31.33%). RGC 986 also recorded highest protein content (35.35%) in *Rabi* reason followed by RGC 1038 (33.24) and RGC 1028 (33.10%).

RGM 115 recorded highest SPAD values followed by RGM114 in all the three seasons, both at 40 and 60 DAS. These varieties also recorded high net assimilation rates, which could have increased the dry matter accumulation as well as dry matter partitioning. In the present study there was no significant variation in galactomannan content between 22 guar genotypes and the gum contents in guar genotypes ranged from 28.47% to 32.89%. Dabas (1982) reported that gum contents in guar varieties ranged from 15.92 to 31.81% in their study. Dwivedi *et al* (1999) reported that gum content varied from 21.77-34.38%. Further the collections made from Rajasthan had exhibited more diversity for gum content due to climate geographical conditions different from that in the other states studied highest crude gum content and crude gum yield recorded with RGM112 followed by RGC1003 and GAVG 9703 (Kalyani, 2006). Highest viscosity was observed in the guar genotypes RGM114 with 320 to 323 c.pa.s^{-1} in all the three seasons RGC 988 recorded highest viscosity of 323.36 c.pa.s^{-1} which was reported by Raghu Prakash (2006). AUG 011 recorded higher endosperm percent in all the three seasons. The endosperm percent of 22 guar genotypes ranged from 29-35%.The endosperm percent range was 31.16-35.36 in the guar genotypes studied by Raghu prakash (2006).

Table 6: Influence of different growing seasons and genotypes on chlorophyll content (SPAD) at different stages in guar

S. No.	60 DAS				
	Genotype	Summer	Kharif	Rabi	Mean
1	RGC 1017	52.69	50.19	25.10	42.66
2	RGC 1024	48.52	46.54	23.27	39.44
3	RGC 1026	54.25	51.87	25.94	44.02
4	RGC 1027	51.08	48.52	24.26	41.29
5	RGC 1028	54.09	50.86	25.43	43.46
6	RGC 1033	64.56	62.37	31.19	52.71
7	RGC 986	51.11	54.27	27.14	44.17
8	RGC 1038	61.11	63.13	31.57	51.94
9	RGC 1047	51.47	48.05	24.03	41.18
10	RGC 1076	55.51	58.37	29.19	47.69
11	RGC 1077	65.19	62.53	31.27	53.00
12	RGC 1078	53.14	57.13	28.57	46.28
13	RGC 1079	54.94	50.94	25.47	43.78
14	RGC 936	62.11	59.33	29.67	50.37
15	RGM 111	55.94	53.83	26.92	45.56
16	RGM 112	61.88	58.83	29.42	50.04
17	RGM 114	71.98	69.21	34.61	58.60
18	RGM 115	76.61	74.62	37.31	62.85
19	RGM 116	61.54	58.66	29.33	49.84
20	GAUG 011	51.42	47.59	23.80	40.94
21	GAUG 001	65.69	62.90	31.45	53.35
22	HGS 884	64.68	63.30	31.65	53.21
	Mean	58.61	56.96	28.48	
		Season (S)	Genotype (G)	Inter-action (SxG)	
	SEm±	0.30	0.82	1.41	
	CD(0.05)	0.59	1.60	2.77	

The total carbohydrate content of guar genotypes ranged from 30.07 to 39.34 %. RGM 111 recorded higher total carbohydrate content values in all the three seasons. Raghu Prakash (2006) reported that highest carbohydrates content was recorded in the genotypes ABKG-73 (44.29%) and lowest for the genotype RGC-2021 (32.16%) . Highest protein content was observed in guar genotype RGC 986 in all the three seasons and the protein content of the 22 guar genotypes varied from 25.98 to 35.35%.

Highest crude protein was recorded in RGC 1088 (Raghu Prakash 2006) and RGC 1033 (Kalyani, 2006). The crude protein content in *guar* genotypes varied from 22.43 to 29.24% (Dwivedi *et al*., 1999) and 27.43 to 34.27% (Raghu Prakash, 2006).

Seed quality parameter i.e. viscosity, percentage galactomaman, endosperm percentage, seed carbohydrate and protein had little influence by environmental factors. In the present study, seasons had no concomitant influence on all the biochemical seed quality parameters as the most of the differences are non significant.

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