LR-5145
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RESEARCH ARTICLE Legume Research- An International Journal

Comparative Assessment of Nutritional, Industrial and Agronomic Valuable Traits of Underutilized Guar Genotypes

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10.18805/LR-5145

ABSTRACT

Background: Guar (*Cyamopsis tetragonoloba*), also known as cluster bean, is an annual legume with a high gum (galactomannan) content that is widely used in food, biopharmaceutical and other non-food industries. Despite the fact that several studies have been conducted to develop genomic resources for guar, information on its nutritional and other important biochemical profile is largely unknown.

Method: In the present study, we investigated the extent of genetic variability of nutritional characteristics and gum content among different indigenous guar genotypes, as well as the correlation between them. A total of 40 guar genotypes were morphologically and biochemically characterized in order to be classified into nutritional and industrial usage groups.

Result: Genotypes RGC-1055, RGR-7, RGC-1033, RGC-1002, RGC-1031, RGC-1038 and RGC-1055 were found to be superior for galactomannan, whereas genotypes HG-2-20, HG-258, HG-832, RGC-936, RGC-1066 and Pusa Navbahar were found to contain important quantitative traits such as a greater number of pods/cluster and clusters/plant, which is directly related to higher yield. Furthermore, correlation and PCA (principal component analysis) revealed that chlorophyll content is positively correlated with protein (0.2%) and gum content (0.16%). The data generated from this study could help breeders and biotechnologists for developing novel potential hybrids with desirable morphological, nutritional and industrial properties. Furthermore, enhanced crop quality may be achieved in the future by genetically modifying the nutritional and industrially significant targeted genes from the selected superior guar genotype using CRISPR/Cas9 precision gene editing technology.

Key words: Galactomannan, Guar, Micronutrients, PCA, Pearson correlation, Protein content, Quality traits.

INTRODUCTION

Guar (*Cyamopsis tetragonoloba*, 2n=14) is an annual legume crop and is classified under family Leguminaceae and subfamily paplionaceae (Pathak *et al.* 2011). It is an important crop of arid and semi-arid regions (Kumar *et al.* 2015) and is exceptionally adapted to the drought prone environment due to its deep-rooted system and short maturity period (Dhaka *et al.*, 2019). It is an economically important pulse crop of India (Manjunatha *et al.*, 2018) which alone accounts for 75-82% of the world's clusterbean production followed by Pakistan (10-12%). Rajasthan, which is known as the industrial hub of guar gum processing and export, accounts for approximately 75% of India's total guar production. Guar gum is primarily imported by Canada, United States, United Kingdom, China, Russia and South American countries as well as European countries (NRAA, 2014).

Galactomannan, commonly known as gum, is an odorless, whitish, or yellowish (Thombare *et al.* 2016), water-soluble heteropolysaccharide found in the endosperm of guar seeds is known for its pharmacological properties including anti-cancerous, anti-diabetic, anti-ulcer, anti-coagulant, anti-microbial, anti-inflammatory (Sharma *et al.* 2011). Owing to its high galactomannan content, this crop has emerged as one of the most important commercial crops. It is also rich in protein, iron (Fe) and contains significant amounts of other micronutrients/trace elements such as zinc (Zn) and copper (Cu) (Sharma *et al.*, 2017; Akçura *et al.*, 2020). However, the nutritional quality of this important food crop has been affected by the presence of various anti-nutritional factors such as tannins, phytic acid and polyphenols (Rusydi and Azrina, 2012; Badr *et al.*, 2014). Therefore, in order to remove anti-nutritional compounds different processing methods have been

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How to cite this article: Sharma, S., Tyagi, A., Ramakrishna, G., Saxena, S., Mithra, A., Mehla, H.R., Golui, D., Sharma , R. and Gaikwad, K. (2024). Comparative Assessment of Nutritional, Industrial and Agronomic Valuable Traits of Underutilized Guar Genotypes. Legume Research. 1-11. doi: 10.18805/LR-5145.

Submitted: 29-03-2023 **Accepted:** 30-08-2024 **Online**: 25-01-2025

executed in clusterbean (Sharma *et al*., 2017). Earlier studies have observed variability in many morphological, biochemical and productive parameters in different guar genotypes (Sultan *et al.* 2012 a; Kumar *et al.* 2013; Naik *et al.* 2013 a; Sharma *et al.* 2014; Ansari *et al.* 2017; Sefatullah *et al.* 2017; Wankhade *et al.* 2017; Muthuselvi *et al.* 2017; Gresta *et al*. 2018; Ramanjaneyulu *et al.* 2017, 2018; Reddy *et al.* 2018; L. Ashwini *et al.* 2019; Santonoceto *et al.* 2019; Kgasudi *et al.* 2020). Furthermore, molecular studies such as genome size estimation, identification of genomic SSRs, miRNAs associated with galactomannan biosynthesis, SNPs-INDELs, chloroplast genome analysis and transcriptome analysis, have been carried out to improve the industrial profile and genomic database of guar (Tyagi *et al.* 2018, 2019; Tribhuvan *et al.* 2019; Sahu *et al*., 2020; Kaila *et al*., 2017; Rawal et sl., 2017; Sharma *et al*., 2021; Hu *et al*., 2019; Chaudhury *et al*., 2019). Recently, the chromosome scale genome assembly of clusterbean has been completed that could help to understand molecular basis of the galactomannan biosysnthesis and other important traits (Grigoreva *et al*., 2019; Gaikwad *et al*., 2023). However, there is a lack of information on the complete characterization of micronutrient profiling, particularly regarding iron (Fe) and zinc (Zn) of guar genotypes (Gresta *et al.* 2018). Further, due to low genetic diversity a very few reports have tried to explore the diversity among indigenous guar genotypes with respect to above mentioned traits. The present work was designed to identify the genetic variability for Fe, Zn, protein and galactomannan content in different guar genotypes of Indian origin. In addition, we have also highlighted the correlation between galactomannan and other nutritional components such as Fe, Zn, protein content which could be useful for farmers in future breeding program.

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of forty guar genotypes (IC-329639, IC-421826, HG-16, HG-870, RGR-7, HG-14, HG-100, RG-1002, IC415163, RGC-1017, RGC-1031, BG-2, IC-373480, HG-2-20, Pusa Navbahar, HG-884, RGM-112, IC-325811, HG-832, RGC-936, IC-324032, IC-421837, HG-11-1, HG-258, RGR-13-1, M-83, IC-421839, RGC-1066, IC-415102, IC-421840, IC-421825, RGC-1038, IC-421821, HG-75, RGC-1033, HG-563, HG-11, IC-370478, RGC-1055, HG-119) were obtained from Central Arid Zone Research Institute (CAZRI), Jodhpur, India. The four seeds/pot were grown in 12-inch cemented pots under net house conditions at ICAR-National Institute for Plant Biotechnology, New Delhi, India during the 2020-21 kharif season (Fig 1). After one week, the seedlings were thinned to make three plants per pot of each genotype. Morphological data was collected and seeds were collected in three biological replicates after maturation for biochemical analysis.

Morphological traits

Various morphological traits such as plant height (cm), 100 seed weight, days after flowering (DAF), number of pod/cluster and number of cluster/ plant were recorded in triplicates for each guar genotype.

Estimation of protein content

The crude protein content of samples from various guar genotypes was determined using the Kjeldahl method (Stuart, 1936).

Fig 1: Fourty guar genotypes grown under net house conditions at ICAR-National Institute for Plant Biotechnology, New Delhi, India during the 2020-21 *kharif* season.

The percentage of total nitrogen was calculated by the following formula:

N% = (T-B)*N*1.4 divided by Weight of sample (g).

Where,

T= volume of the hydrochloric acid required for sample solution.

B= Blank.

N= Normality of the digested sample.

1.4= atomic weight of Nitrogen.

Weight of sample=0.5 g.

Crude protein was estimated by multiplying the percentage nitrogen with standard conversion factor 6.25. (*i.e.*, % crude protein (CP) = 6.25×N%)

Estimation of Fe and Zn content

Total Fe and Zn content among various guar genotypes were estimated using ICP-MS (Ikem et la., 2023). Standard preparation was done by diluting the available stock multi-elemental standard solution (1000 mg L^{-1}) in 0.5% (v/v) nitric acid using ICP-MS (PerkinElmer NexION 300) (Fig 2). (Ikem *et al.*, 2023).

Extraction and evaluation of galactomannan content

Gum extraction was carried out as per the manufacturer's protocol using K-GALM kit (MEGAZYME KIT). Initially guar seeds of various genotypes were pre-soaked in distilled water for overnight to get the soft imbibed seeds and then the outer layer (Hull) was removed.

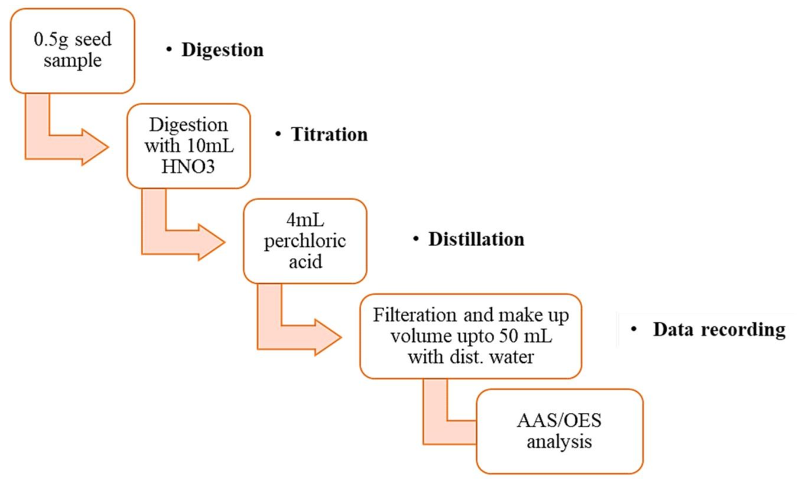
Statistical analysis

Multivariate analysis based on PCoA (Principal coordinate analysis) and PCA (Principal component analysis) as well as cluster analysis based on dendrogram was performed in 40 guar genotypes to assess the genetic diversity based on quantitative and qualitative traits such as plant height, maturity (DAF), pod/cluster, cluster/pod, chlorophyll content protein, 100 seed weight, Fe, Zn and galactomannan content. Pearson correlation analysis was performed to estimate the significant correlation within and between morphological traits, nutritionally important traits like protein, iron, zinc and industrially important trait *i.e.*, galactomannan content. All statistical analysis has been performed using Microsoft excel and Past software.

RESULTS AND DISCUSSION

Guar genotypes revealed significant variations in their morphological attributes

A total of six morphological quantitative traits were assessed for 40 guar genotypes and the data showed considerable variation among varieties and IC lines for most of the traits *viz.* plant height (5.6±0.31 cm to 53.0± 0.27 cm), chlorophyll content (26.2±0.2 to 5.4±0.2), pods/ cluster (1-2 to 7-9), Clusters/ plant (1-2 to 6-7), days after flowering (56 to 86). The results revealed huge variation among indigenous under-utilized guar genotypes. RGC-936 was found to be early maturing genotype (56 DAF) with maximum pod/cluster (7-9), while HG-832 was observed to have maximum clusters/plant was observed, although pods/ cluster were same as for RGC-936. IC lines were found to be shorter (IC-421826) and late maturing genotypes (IC-324032 and IC-421837). Chlorophyll content was found to be highest in (M-83 85.4±0.2) and lowest in BG-2 (26.2±0.2) genotypes. 100 seeds weight ranged between 1.39 and 3.19 g among different genotypes as shown in (Table 1). Most of the genotypes (28) showed pink colour flower trait, whereas only 11 genotypes exhibited white flower colour. These results were consistent with what is expected in a natural setting. Further, our morphological data grouped HG-16, HG-870, RG-1002,



(a) Digestion and micronutrient analysis, (b) Digestion unit of Kjeldhal, (c)Titration unit, (d) Distillation unit, (e) Data recording.

Fig 2: A flowchart of samlpe preparation for micronutrient analysis using AAS/OES in guar.

BG-2, HG-2-20, HG-884, HG-832, HG-258, RGC-1066, HG-75, HG-563, HG-11, HG-119 into same clusters as observed previously (Sultan *et al.* 2012b; Kumar *et al.* 2013; Boghara *et al.* 2016). Besides, it also observed that guar varieties from arid regions like Rajasthan and Haryana were clustered into same group, while the IC-lines were grouped separately as shown in the previous study (Kumar and Ram, 2015).

Principal component analysis of morphological characters

Principal component analysis (PCA) for morphological parameters such as plant height, chlorophyll content, pods/ cluster, clusters/ plant, days after flowering and 100 seeds weight grouped the 40 guar genotypes into six co-ordinates based on scoring (Table 2.) However, the clustering of PCA

Table 1: Phenotypic variation in 40 guar genotypes for the important traits *viz.*, plant height, chlorophyll content, pod/cluster, cluster/ pod, days after flowering (DAF) and 100 seed weight.

Genotypes	Plant height (cm)	Chlorophyll content (SPAD)	Pod/cluster	Cluster/plant	DAF	100 Seed weight (g)
IC-329639	8.5 ± 0.29	46.8 ± 0.2	2 to 3	3 to 4	68	1.93
IC-421826	5.6 ± 0.31	42 ± 0.1	3 to 4	2 to 3	73	2.20
HG-16	31.3 ± 0.33	31.3 ± 0.3	1 to 2	2 to 3	58	2.52
HG-870	30.4 ± 0.35	31.8 ± 0.4	4 to 5	3 to 4	65	2.12
RGR-7	53.0 ± 0.27	45.4 ± 0.2	2 to 3	2 to 3	49	3.19
HG-14	29.2 ± 0.44	66.5 ± 0.3	2 to 3	2 to 3	60	2.85
HG-100	13.5 ± 0.29	54.4 ± 0.2	2 to 3	1 to 2	63	2.00
RG-1002	35.4 ± 0.23	47.7 ± 0.3	2 to 4	2 to 5	61	2.34
IC415163	6.5 ± 0.29	63.4 ± 0.2	2 to 3	3 to 4	72	2.10
RGC-1017	33.9 ± 0.43	65.7 ± 0.1	3 to 5	2 to 3	58	2.30
RGC-1033	58.0 ± 0.09	50.6 ± 0.2	2 to 3	3 to 4	72	2.50
BG-2	44.7 ± 0.24	26.2 ± 0.2	2 to 3	2 to 3	71	2.20
IC-373480	10.2 ± 0.42	53.5 ± 0.3	2 to 3	3 to 4	68	2.29
HG-2-20	25.5 ± 0.32	54.1 ± 0.5	5 to 6	3 to 4	56	2.62
Pusa Navbahar	51.6 ± 0.23	65.3 ± 0.2	2 to 3	3 to 4	64	3.43
HG-884	23.8 ± 0.17	54.8 ± 0.4	3 to 4	2 to 3	61	2.13
RGM-112	43.9 ± 0.12	45.5 ± 0.3	2 to 3	2 to 3	57	2.86
IC-325811	19.3 ± 0.35	51.1 ± 0.5	2 to 3	3 to 4	76	2.14
HG-832	28.4 ± 0.32	50.5 ± 0.3	6 to 7	6 to 7	63	2.50
RGC-936	19.3 ± 0.35	68.3 ± 0.2	7 to 9	2 to 3	59	2.60
IC-324032	7.3 ± 0.35	53.4 ± 0.2	2 to 3	2 to 3	86	2.48
IC-421837	10.2 ± 0.44	66.4 ± 0.2	2 to 3	2 to 3	86	2.13
HG-11-1	33.5 ± 0.26	62.5 ± 0.5	2 to 3	4 to 5	60	2.26
HG-258	29.7 ± 0.33	55.6 ± 0.2	4 to 7	3 to 4	58	2.86
RGR-13-1	51.4 ± 0.23	52.5 ± 0.3	2 to 3	2 to 3	56	2.83
M-83	26.8 ± 0.15	85.4 ± 0.2	1 to 2	2 to 3	64	2.20
IC-421839	5.5 ± 0.29	61.2 ± 0.5	2 to 3	2 to 3	70	2.00
RGC-1066	31.3 ± 0.33	36.5 ± 0.2	5 to 8	2 to 3	63	2.67
IC-415102	8.3 ± 0.33	54.2 ± 0.2	1 to 2	2 to 3	70	3.00
IC-421840	10.8 ± 0.44	64.6 ± 0.3	2 to 3	2 to 3	82	2.65
IC-421825	12.1 ± 0.19	48.4 ± 0.3	3 to 4	3 to 4	75	3.10
RGC-1038	45.0 ± 0.15	72.5 ± 0.3	1 to 2	2 to 3	58	2.32
IC-421821	8.3 ± 0.35	51.3 ± 0.2	2 to 3	2 to 3	75	2.00
HG-75	29.5 ± 0.29	51.1 ± 0.2	2 to 3	2 to3	79	2.68
RGC-1031	43+ 0.12	70.1+ 0.2	1 to 2	2 to 4	56	2.69
HG-563	33.3 ± 0.33	46.1 ± 0.5	2 to 3	4 to 5	59	2.68
HG-11	24.2 ± 0.42	42.6 ± 0.4	2 to 3	2 to 3	61	2.23
IC-370478	11.3 ± 0.35	60.8 ± 0.2	2 to 3	2 to 3	82	2.52
RGC-1055	51.1 ± 0.38	38.5 ± 0.3	2 to 4	3 to 4	59	2.89
HG-119	27.3 ± 0.33	50.8 ± 0.1	1 to 3	1 to 2	65	2.23

between coordinate 1 and coordinate 2 divided the genotypes based on the highest percent of Eigen values (Euclidean similarity index) (as 59.67% to 30.13%) (Fig 3)

PCA has grouped the 40 genotypes into 4 clusters from 0 to 3 (Fig 4). Cluster 0 has 14 genotypes followed by Cluster 1 containing 13 genotypes, Cluster 2 containing 7 genotypes and Cluster 3 containing 5 genotypes, respectively. Coordinate 1 (Plant height) consisted of highest Eigen value in RGR-7 (31.7). Apart from that, coordinate 2 (chlorophyll content) consisted of highest values in RGR-13-1 (31.5). In addition, coordinate 3 (pod/cluster) had highest value in RGC-1031 (18.1), followed by Co-ordinate 4 (cluster/plant) having a highest value in RGC-936, Co-ordinate 5 (days after flowering) had highest value in HG-832 (2.2) and co-ordinate 6 (100 seed weight) had highest value in IC-415102 (0.79), respecively.

Table 2: Principal Component Analysis (PCA) scoring based on 6 morphological parameters in guar genotypes *viz*., plant height, chlorophyll content, pod/cluster, cluster/pod, days after flowering (DAF) and 100 seed weight.

Genotype list	PC1 (Plant height) cm	PC2 (Chlorophyll content) SPAD	PC3 (DAF)	PC4 (Pod /cluster)	PC5 (Cluster /pod)	PC6 (100 seed weight) g
IC-329639	-15.8971	-9.76037	-5.91215	-1.14232	0.337553	-0.29011
IC-421826	-19.4804	-15.602	-2.88297	-0.45751	-1.01748	-0.01255
HG-16	11.60889	-19.8671	-6.48555	-2.84665	-0.21751	0.124558
HG-870	7.994046	-20.5138	-0.71979	1.14922	0.289827	-0.39166
RGR-7	31.7829	-1.385	-5.12318	-1.27537	0.132145	0.476014
HG-14	1.849223	13.91985	-3.64742	-0.72184	0.148246	0.342641
HG-100	-10.9957	-0.84959	-7.98295	-1.60274	-0.57874	-0.28297
RG-1002	10.83754	-3.61325	-1.36415	0.82924	1.5567	-0.2384
IC415163	-22.5976	5.606443	-2.17778	-0.17215	1.142604	-0.15696
RGC-1017	7.018018	14.13315	-3.75123	1.051279	-0.67352	-0.32109
RGC-1031	26.2516	1.426434	18.09074	0.51741	-0.34069	-0.35641
BG-2	19.61111	-24.4419	10.41625	-0.80841	-0.9852	-0.41735
IC-373480	-15.7192	-2.93423	-4.84452	-0.95023	0.289138	0.030516
HG-2-20	2.607826	1.685272	-9.72073	1.784459	0.01258	0.102967
Pusa Navbahar	20.66149	15.83613	9.042633	0.13031	-0.18844	0.636019
HG-884	-1.03111	1.457066	-5.6705	-0.14408	-0.1589	-0.31315
RGM-112	20.46545	-3.83075	-1.55782	-0.97186	0.103983	0.235607
IC-325811	-10.1737	-4.88307	5.98179	0.186796	0.966474	-0.25423
HG-832	3.232816	-2.32671	-2.49259	4.38758	2.210253	-0.11096
RGC-936	-6.98933	14.12046	-8.94745	4.445223	-2.14541	0.022488
IC-324032	-25.3198	-5.90917	10.32032	0.213579	0.009821	0.20438
IC-421837	-25.3429	7.26718	12.22396	0.542661	-0.07062	-0.21644
HG-11-1	6.525997	10.6965	-2.14481	0.098999	1.945856	-0.29992
HG-258	5.30601	3.548975	-6.18568	2.980126	-0.48388	0.249002
RGR-13-1	26.17354	4.362076	0.988679	-1.108	-0.90848	0.101791
M-83	-5.69415	31.48558	0.135585	-1.05657	0.445875	-0.30791
IC-421839	-22.2848	3.567468	-4.50277	-1.19235	-0.63054	-0.22239
RGC-1066	8.648424	-15.5462	-2.12394	3.476522	-1.835	0.049573
IC-415102	-18.3515	-2.80394	-3.67098	-2.14099	-0.24192	0.793006
IC-421840	-22.876	6.141815	8.73732	0.263617	-0.00587	0.31227
IC-421825	-15.6925	-8.56467	1.919842	0.816869	0.667696	0.772837
RGC-1038	15.59557	22.62446	1.388409	-1.65738	-0.53376	-0.35638
IC-421821	-19.7196	-6.327	0.62709	-0.97775	-0.71358	-0.24495
HG-75	-2.18044	-3.6299	12.8738	0.271124	-0.12611	0.155377
RGC-1031	15.0667	20.22385	-1.37443	-1.88388	-0.46927	0.050488
HG-563	10.05176	-5.23292	-4.04663	-0.29535	2.023678	0.16943
HG-11	1.787418	-10.3893	-6.11062	-1.28964	0.285357	-0.15341
IC-370478	-21.6607	2.507342	8.728359	0.195173	0.003526	0.186393
RGC-1055	27.57896	-9.79528	2.712711	0.526764	0.519893	0.152292
HG-119	1.351364	-2.40398	-0.74687	-1.17189	-0.76629	-0.22041

Biochemical characterization of guar genotypes

The biochemical results of 40 cluster bean genotypes revealed significant genotype variability for the traits studied in this study. Total protein content and galactomannan content are presented in Table 3. The total protein content varied from 11.8±0.10% (RCG-1055) to 20±0.38% (IC-329639) with mean of 22±0.4%. Genotypes with total protein content higher than 25% were categorized as high protein group, between 25 and 15% as medium protein group and those with less than 15% as low protein group (Table 3). Pathak *et al.* (2011) also reported RGC-1038 variety to be the highest in terms of protein content among tested genotypes. Naik *et al.* (2013) reported highest protein values of 27.73% and 34.27% in RGC-1028 and RGC-986, respectively. Müftüoðlu *et al.* (2019), also reported the yield and protein content range in edible guar genotypes

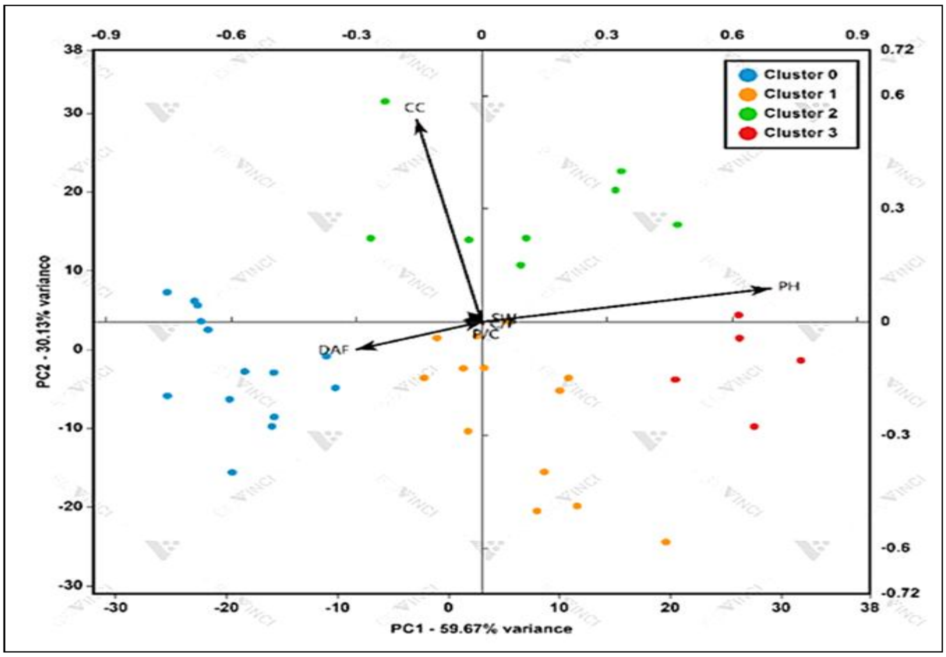


Fig 3: Principal Component Analysis (PCA) of six morphological parameters including plant height, chlorophyll content, pod/cluster, cluster/pod, days after flowering (DAF) and 100 seed weight in 40 guar genotypes.

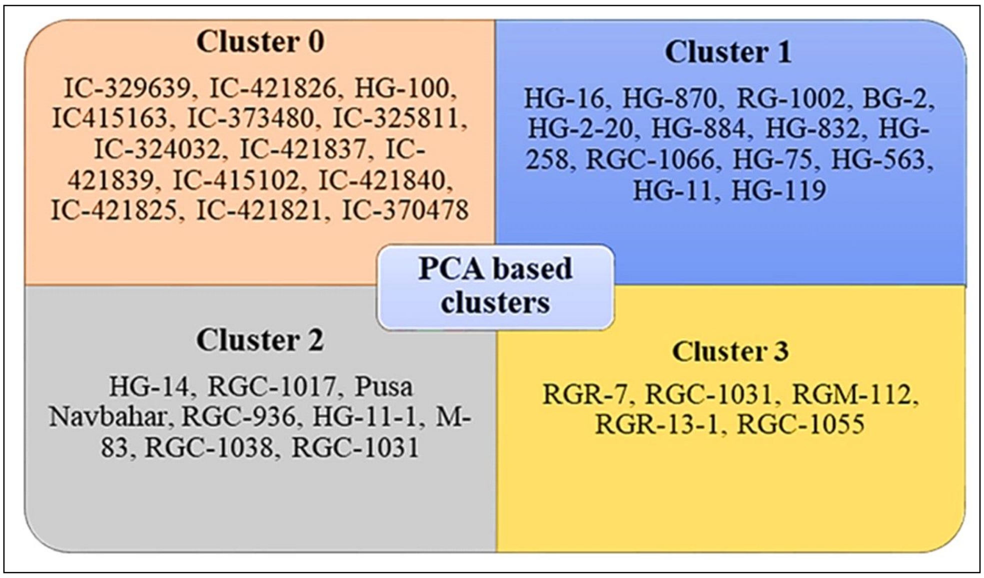


Fig 4: An illustration showing the distribution of 40 guar genotypes into four clusters (Cluster 0, Cluster 1, Cluster 2 and Cluster 3) using principal component analysis (PCA).

with 4.38-17.22% crude protein and 65.40-75.25% digestible protein. Also, they confirmed green pod yields had significant positive correlations with the digestible protein content. Similarly, H.W. Ashwini *et al.* (2019) highlighted comparable to RGC-1033 (35.07%), RGC-1038 (33.40%) and HG-870 (33.10%), the genotype RGC-1002 had a noticeably greater proportion of crude protein (35.80%). Nonetheless, the genotypes RGC-986 (25.43%) had the lowest crude protein content.

In contrast, galactomannan content ranged from 7.67% to 30.93% in our study. The highest content of galactomannans were seen in RGC-1055 (30.93 ± 0.06%), whereas lowest galactomannan content was found in HG-100 (7.67±0.36%) (Table 3). Similar results were reported by Naik *et al.* (2013). However, H. W. Ashwini *et al.* (2019) reported that the genotype HG-870 recorded the highest percentage of gum (35.23%) in comparison to RGC 986 (34.73.%) and RGC-1002 (33.47%). This variation might

Table 3: Total protein content, galactomannan content and micronutrient content data (Fe and Zn) of 40 guar genotypes.

Genotype	Protein Content	S.D	Fe Content	S.D	Zn Content	S.D	Galacto mannan	S.D
IC-329639	28.57	±0.38	410.67	±9.24	57.33	±4.04	25.22	±0.24
IC-421826	18.99	±0.18	343.67	±10.12	68.33	±3.21	18.67	±1.23
HG-16	19.96	±0.93	570.33	±8.14	42.67	±4.73	17.54	±0.73
HG-870	20.81	±0.27	338.00	±8.72	45.00	±2.65	16.40	±0.55
RGR-7	20.00	±0.12	253.00	±23.58	33.33	±3.21	28.03	±0.51
HG-14	25.20	±0.29	297.33	±10.69	41.00	±4.36	21.56	±0.20
HG-100	16.43	±0.73	322.33	±9.87	42.33	±5.69	7.67	±0.36
RG-1002	25.01	±1.26	467.33	±9.87	41.67	±2.08	27.66	±0.84
IC425163	24.99	±1.23	329.00	±9.54	54.00	±3.46	24.65	±0.35
RGC-1017	17.01	±0.15	427.00	±9.54	43.00	±3.46	22.19	±1.03
RGC-1033	23.31	±0.90	331.00	±9.54	38.67	±2.08	27.05	±3.35
BG-2	24.78	±0.20	304.67	±9.24	53.33	±3.21	19.1	±2.01
IC-373480	26.29	±0.39	281.33	±8.08	35.00	±3.46	13.27	±0.30
HG-2-20	21.66	±0.48	397.67	±6.66	41.00	±5.00	15.71	±0.02
Pusa Navbahar	23.65	±0.91	678.33	±10.79	62.67	±3.79	23.26	±2.34
HG-884	20.37	±0.89	282.00	±8.72	42.00	±2.65	22.67	±0.23
RGM-112	23.95	±0.50	320.67	±10.12	39.67	±3.79	22.27	±3.07
IC-325811	22.40	±0.30	389.67	±10.21	44.33	±1.53	25.36	±0.34
HG-832	25.79	±0.10	318.00	±10.58	48.67	±3.79	22.66	±0.16
RGC-936	23.63	±0.55	288.67	±10.21	38.67	±3.79	23.85	±0.32
IC-324032	13.21	±0.58	281.67	±8.39	37.67	±3.06	23.02	±0.14
IC-421837	25.15	±0.22	387.67	±9.29	44.33	±3.21	20.32	±0.08
HG-11-1	19.88	±0.20	613.33	±8.96	53.67	±2.89	16.35	±0.18
HG-258	26.17	±0.81	189.67	±9.29	50.67	±2.08	15.63	±0.07
RGR-13-1	22.01	±0.01	359.33	±8.96	45.67	±3.79	19.08	±0.93
M-83	22.33	±0.05	346.67	±10.12	47.00	±2.65	19.34	±0.26
IC-421839	21.03	±0.59	343.67	±10.12	48.00	±4.36	23.19	±0.07
RGC-1066	22.01	±0.00	287.33	±9.87	35.33	±2.31	18.95	±3.17
IC-415102	23.12	±0.17	869.00	±10.44	34.33	±0.58	23.88	±0.7
IC-421840	19.83	±0.38	267.67	±10.12	43.33	±4.04	19.07	±0.71
IC-421825	13.00	±0.13	318.00	±8.66	43.00	±3.46	23.53	±0.34
RGC-1038	26.25	±0.21	437.00	±10.44	37.67	±3.79	27.66	±4.12
IC-421821	24.00	±1.41	658.00	±8.66	44.33	±1.53	20.72	±0.05
HG-75	18.71	±0.72	373.33	±8.96	44.33±	4.04	21.77	±0.22
RGC-1031	25.17	±0.01	264.67	±23.35	42.00	±4.36	27.05	±3.35
HG-563	16.42	±0.25	346.33	±19.40	51.33	±3.51	12.88	±0.15
HG-11	20.66	±6.87	250.33	±10.97	34.33	±2.31	11.82	±0.12
IC-370478	20.66	±0.06	447.33	±10.79	43.00	±2.65	22.65	±0.39
RGC-1055	11.94	±0.10	230.00	±9.54	42.00	±3.00	30.93	±0.06
HG-119	27.81	±0.27	313.67	±9.24	44.33	±3.21	19.19	±0.21

be due to the fact that seeds were taken at different developmental stages for analysis as well as from different geographical regions.

One important aspect of this study was the micro-nutrient analysis including Fe and Zn content in guar genotypes which has not been reported so far. Fe and Zn are two of the most important essential micronutrients whose deficiency has been referred to as "Hidden hunger" by World Health Organization (WHO). The Fe content in guar genotypes ranged from 189 to 869 ppm in our study with an average Fe content of 373.3±0.4 ppm. Maximum Fe content was recorded in IC-415102 (869±10.44 ppm) whereas minimum Fe content was noted in genotypes HG-258 (189±9.29 ppm) (Table 3). However, the Zn content ranged from 34 to 68 ppm with an average value of 44.4± 6.0 ppm. Maximum Zn content was found in genotype IC-421826 (68.33±3.21 ppm), whereas minimum Zn content was observed in IC-415102 (34.33±2.31 ppm) (Table 3). A summary of the average values of all the morpho-biochemical traits in the present study of different guar genotypes have been given in Table 4.

Our results revealed that IC-415102 and IC-421826 genotypes have highest Fe and Zn content which means that these genotypes are of nutritional significance. Genotypes with moderate to high protein, iron and zinc content (as IC-421826, HG-11-1 and Pusa Navbahar) can be used as food crops, while genotypes with high gum content mentioned above can be further improved to be used for industrial purposes. Interestingly, some varieties (Pusa Navbahar, HG-832 and RGC-936) were found to be rich in all the traits in the present study. These genotypes might be of considerable interest for plant breeders and biotechnologists to develop novel guar varieties with desirable nutritional and gum profiles.

Correlation, PCA analysis between morphological and other biochemical factors

Pearson correlation analysis (p value < 0.05) between various morphological and biochemical parameters demonstrated significant variation (Fig 5a). Between morpho-biochemical parameters, seed weight was found to have significant positive correlation with plant height (0.47 cm) followed by chlorophyll content which correlated positively with protein content (0.2%) and gum content (0.16%). Among morphological parameters clusters/plant was found to have positive correlation with pods/cluster (0.41). However, negative correlation was observed between plant height and DAF (-0.61). After analyzing biochemical parameters, it was observed that all traits are positively correlated with each other except gum and Zn (-0.09). Correlation between galactomannan and protein content (0.08%) was also positive but not significant. However, no correlation was observed between seed weight and chlorophyll content as shown in Fig 5b.

Principal component analysis based on variance-covariance matrix demonstrated that maximum variance was observed in PC1 *i.e.*, protein (97.06%) followed by PC2 iron (1.46%) and minimum in PC09 *i.e.* clusters/plant (0.0042%) followed by PC10 *i.e.* seed weight (0.00044%) as shown in Fig 5c. Scoring of genotypes based on all morpho-biochemical traits have been shown in Table 4.

Genetic diversity was analyzed using neighbour-joining (NJ) clustering method for various morpho-biochemical parameters and classified 40 guar genotypes based on euclidean similarity index (ESI) into two subgroups A and B as shown in Fig 6. Further, Subgroup A was divided into five clusters (I to V) while subgroup B into three clusters (VI-VIII). The four nutritionally important guar genotypes rich in Fe and Zn content was found in the same cluster IV which was significantly correlated with our biochemical data showing highest Fe content in IC-415102 (869±10.44 ppm) followed by Pusa Navbahar (678.33±10.79 ppm), HG-11-1 (613.33±8.96 ppm) and HG-16 (570.33±8.14 ppm) whereas IC-421826 with highest Fe content (68.33 ± 3.21 ppm) was found to be located in cluster III. Here, Cluster I was found to be galactomannan rich genotypes (IC-421825 and IC-324032) whereas cluster VI with single genotype IC-32969 was found to be rich in all three nutritional traits viz protein, Fe and Zn.

Table 4: Summary of average values of all morpho-biochemical parameters of 40 guar genotypes under this study.

	Protein	Iron (ppm)	Zinc (ppm)	Gum (%)	Plant height (cm)	Chlorophyll (SPAD)	DAF	Pod/ cluster	Cluster/ plant	Seed weight (g)
Min	11.94	189.67	33.33	7.67	5.5	26.2	49	2	2	1.93
Max	28.57	869	68.33	30.93	58	85.4	86	9	7	3.43
Mean	21.804	373.3835	44.47475	21.0455	26.7975	53.485	65.95	3.65	3.15	2.481
Std. error	0.624533	21.48995	1.186269	0.77437	2.435247	1.915576	1.429071	0.2570793	0.1700302	0.05748668
Variance	15.60165	18472.72	56.28935	23.98597	237.2172	146.7772	81.68974	2.64359	1.15641	0.1321887
Stand. dev	3.949892	135.9144	7.502623	4.897548	15.40186	12.11516	9.038238	1.625291	1.075365	0.3635777
Coeff. var	18.11545	36.40074	16.8694	23.27124	57.47497	22.65152	13.70468	44.54553	34.13858	14.65448

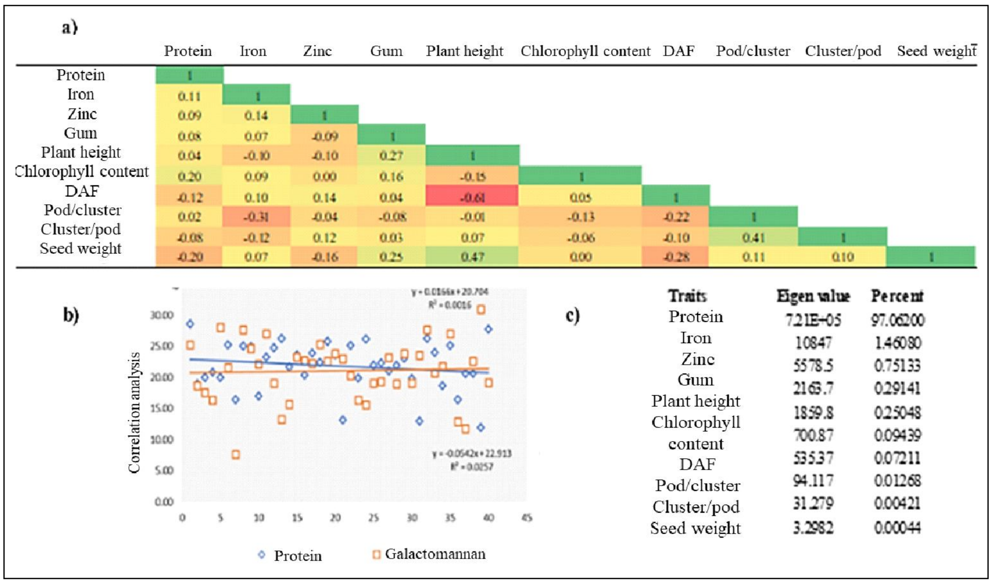


Fig 5: (a) Pearsons correlation analysis between various morphological and biochemical parameters among 40 genotypes, (b) Coorelation analysis between galactomannan and protein content in 40 genotypes, (c) Principal Component Analysis (PCA) based on varience-covarience matrixof morpho-biochemical parameters among 40 genotypes.

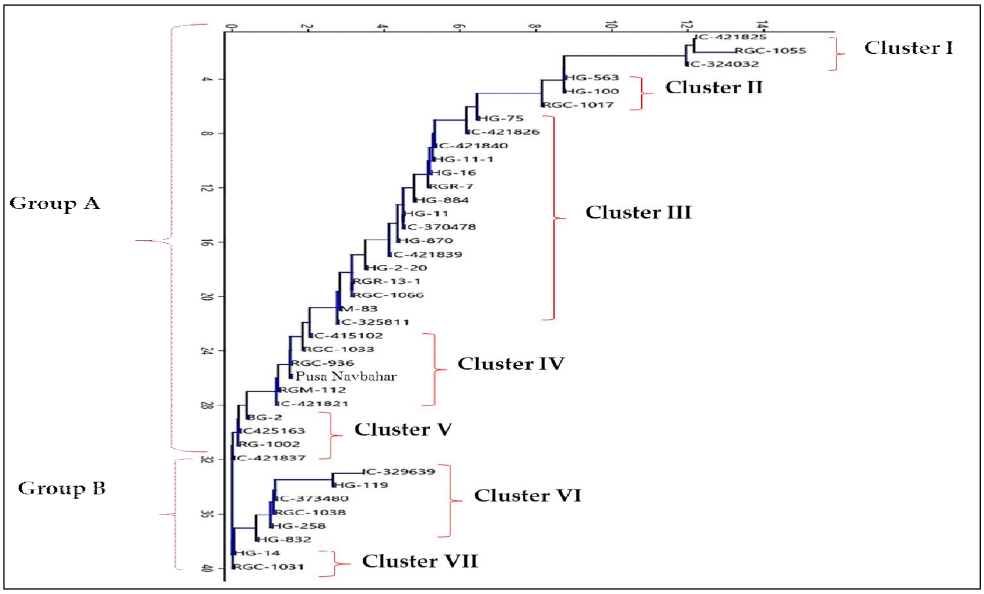


Fig 6: Dendrogram showing genetic diversity and classification of 40 guar genotypes based on morpho-biochemical parameters. These genotypes were divided into two major groups (A and B) and VII clusters based on their euclidean similarity index (ESI).

CONCLUSION

This study gives noval insights on the micro-nutrient variation in guar genotypes along with different morphological and biochemical parameters. Through this work, we have been able to categorize the different guar genotypes into edible and industrial usage groups and brought out the correlation among different parameters. The information generated from this work might contribute to the development of novel and improved guar varieties with enhanced gum and nutritional content using suitable genetic engineering and CRISPR/Cas9 gene editing technology. Furthermore, this research helps breeders to alleviate nutritional inadequacies in underdeveloped nations while also meeting the gum requirements of the industrial industry. As so, it can work like a miracle to mitigate the impact of common malnutrition (Protein, Fe and Zn deficiency).

ACKNOWLEDGEMENT

The authors acknowledge the ICAR-CRP on Genomics (NBFGR, Lucknow) for funding the project under which the work was carried out.

Conflict of interest

The authors declares no conflict of interest.

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