Pak. J. Bot., 51(2): 487-491, 2019. DOI: 10.30848/PJB2019-2(27)

GENETIC VARIABILITY STUDY OF ELITE GUAR (*CYAMOPSIS TETRAGONOLOBA* L.) GERMPLASM AS REVEALED BY SDS-PAGE TECHNIQUE

SOHAIL AHMAD JAN[1*], ZABTA KHAN SHINWARI[2,3], MALIK ASHIQ RABBANI[4*], ALI TALHA KHALIL[2,3] AND AZHAR HUSSAIN SHAH[1]

[1]Department of Biotechnology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan
[2]Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan
[3]Qarshi University, Lahore, Pakistan
[4]Plant Genetic Resources Program, Bio-Resources Conservation Institute, National Agricultural Research Centre, Islamabad, Pakistan
[*]Corresponding author's email: sjan.parc@gmail.com; rabbani316@yahoo.com

Abstract

Biochemical characterization of plant species through SDS-PAGE method plays a key role in seed storage protein based variations. Limited reports are available about the protein profiling of elite *Cyamopsis tetragonoloba* L. (Guar) germplasm. In present study we have characterized the seed storage protein profiles of elite accessions of guar. The 24 guar germplasm were characterized though standard SDS-PAGE protocol with minor modifications. These genotypes were analyzed with novel 2D and 3D methods to visualize diverged genotypes from the close angle. Significant variability was observed in protein profile of all tested genotypes. The polypeptide band sizes varied from 10-180kDa. Maximum (100%) polymorphic banding patterns were observed among genotypes. The genetic similarity values ranged from 20 to 100% among genotypes. The accessions 28952 and 31682 indicated the highest genetic similarity value (100%). While the lowest similarity value (20%) was recorded between accessions 31731 and 31764. All these genotypes were classified into 7 diverse groups and the last 3 were highly diverged as compared to others. The 2D and 3D protein structure models identified 3 unique accessions like 31764, 31731 and 31761. The resulted novel genotypes will be useful for further improvement of this important plant species.

Key words: Genetic variability, SDS-PAGE, Novel genotypes, 2D and 3D structure.

Introduction

Guar (*Cyamopsis tetragonoloba* L.) is one of the important minor crops of Pakistan that belongs to family Fabaceae (Whistler & Hymowitz, 1979; Sultan *et al.*, 2012; Sultan *et al.*, 2013). Guar is considered very important from industrial perspective due to the presence of gums (Hymowitz & Matlock, 1963). Guar gum is used for the treatment of many lethal diseases including cancer, osteoarthritis, and many more (Shyale *et al.*, 2006). Pakistan is among the major supplier of guar and account for the about 15% of total guar produced in the world. The rest of guar production countries are USA, Sudan, Australia and India (Jackson & Doughton, 1982). Morpho-biochemical and molecular evaluation of crop species is important to screen best genotypes among enormous populations (Pervaiz *et al.*, 2010; Rabbani *et al.*, 2010; Shinwari *et al.*, 2011; Jan *et al.*, 2016[a,b]; Jan *et al.*, 2017[a]; Qadir *et al.*, 2017; Shinwari *et al.*, 2018; Akbar *et al.*, 2019).

The Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) is an efficient, reliable and time economical method to characterize a large number of plants germplasm. It provides the complete protein profile and taxonomic relationship among large plant genotypes and also been devoid of any environmental effect (Das & Mukherjee, 1995). This method has been useful for seed storage protein based variability among different plant species. Same method was followed previously for characterizing different accessions of *Eruca sativa*. Elite protein profile was reported for different genotypes that sizes ranged from 15-220 kDa. Moreover, small and medium size protein bands were also reported (Shinwari *et al.*, 2013). Similarly, Zada *et al.*, (2013) found 31 different protein bands in 94 Ethiopian mustard germplasm by SDS-PAGE method and found 50-100% similarity coefficient values among all tested germplasm. Recently, Jan *et al.*, (2017[b]) reported maximum level of seed protein based variability among brown sarson sub-species of *Brassica rapa* genotypes. The total seed storage protein profile varied among different plant genotypes. There are insufficient reports on the protein profile of the important guar germplasm. Therefore, in present study we have characterized elite guar germplasm through SDS-PAGE method.

Materials and Methods

Plant materials: All the experiments were performed in the Evaluation Lab of Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. The fresh and mature seeds of guar germplasm were collected from the Gene bank of PGRI, NARC, Islamabad, Pakistan. The list of elite accessions is given in Table 1.

Grinding of seeds and protein extraction: Five seeds of each genotype were ground properly with the help of mortar and pestle. The 0.01 ground materials were transferred to each 1.5ml centrifuge tube with addition of 600-700 µl protein extraction buffer (Tris (0.6g), Sodium dodecyle sulphate (0.23g), urea (30g), mercaptoethanol (1ml), and bromophenol blue dye (little bit), distilled water (70ml), pH (8). The samples were than mixed with vertexes for 2-3 minutes and stored overnight in refrigerator at -20^{o}C.

Gel electrophoresis: The separation and stacking gels, Ammonium Per Sulphate (APS), staining and de-staining solutions were prepared following protocols of Jan *et al.,* (2016[c]) with minor modification. The samples were centrifuged at 13,000rpm for 10 minutes and 10µl upper clear layer of each sample was loaded into each well along with addition of protein ladder and allowed to run on electrophoresis at 125V for 2-3 hours. The movement of all protein bands were observed and stopped until reached at the bottom of plate. The gels were carefully removed and added in staining and de-staining solution following method of Jan *et al.,* (2016[c]).

Data analysis

The clear protein bands were marked as present (1) or absent (0). All the data was arranged in MS Excel sheet and dendrogram was constructed through NTSYS pc version 2.1 software (Rohlf, 2000) following method of Sneath & Sokal (1973). The 2D and 3D structure of proteins was established from Dice similarities values through NTSYS pc version 2.1 software (Rohlf, 2000).

Table 1. List of elite guar germplasm used in study.

Sr. No.	Accession	Source
1.	28924	NARC, Pakistan
2.	28948	NARC, Pakistan
3.	28952	NARC, Pakistan
4.	31603	NARC, Pakistan
5.	31673	NARC, Pakistan
6.	31596	NARC, Pakistan
7.	31665	NARC, Pakistan
8.	31682	NARC, Pakistan
9.	31710	NARC, Pakistan
10.	31711	NARC, Pakistan
11.	31714	NARC, Pakistan
12.	31731	NARC, Pakistan
13.	31733	NARC, Pakistan
14.	31737	NARC, Pakistan
15.	31756	NARC, Pakistan
16.	31761	NARC, Pakistan
17.	31762	NARC, Pakistan
18.	31763	NARC, Pakistan
19.	31764	NARC, Pakistan
20.	31806	NARC, Pakistan
21.	31812	NARC, Pakistan
22.	31826	NARC, Pakistan
23.	31836	NARC, Pakistan
24.	31869	NARC, Pakistan

Results

Variation in polypeptides banding patterns among guar genotypes: A total of 24 guar genotypes were screened for the presence or absence of both major and minor bands through SDS-PAGE methods. Significant variability in protein band sizes was observed in all tested genotypes. All types of bands (small, medium and large) were recorded. The 4 major bands were common in almost all genotypes. The high level of variability was noted for minor bands. Maximum polymorphic banding patterns were recorded as compared to monomorphic. These results showed that all genotypes exhibited maximum variability with one another. The polypeptide band sizes varied from 10-180 kDa (Fig. 1a, b).

Genetic similarity among guar germplasm: The Dice coefficients values showed close similarities or dissimilarities exist among accessions. In present study the genetic similarity values among genotypes ranged from 20 to 100%. The genotypes 28952 and 31682 showed 100% similarity with one another followed by 99% in accessions 31665 and 31714. The least similarity coefficient value of 20% was recorded between genotypes 31731 and 31764 followed by 22% in 31714 and 31731 (Table 2). The other genotypes also showed low to moderate or high level of similarities with one another.

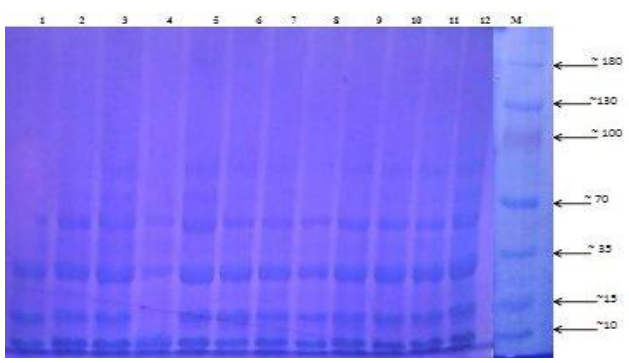

Fig. 1a. Electrophoretic banding patterns of elite guar germplasm accessions via SDS-PAGE of total seed storage proteins. M represents molecular size marker, while numbers from 1-12 represent genotypes 28924, 28948, 31665, 31731, 31826, 31737, 28952, 31596, 31682, 31710, 31711 and 31714, respectively.

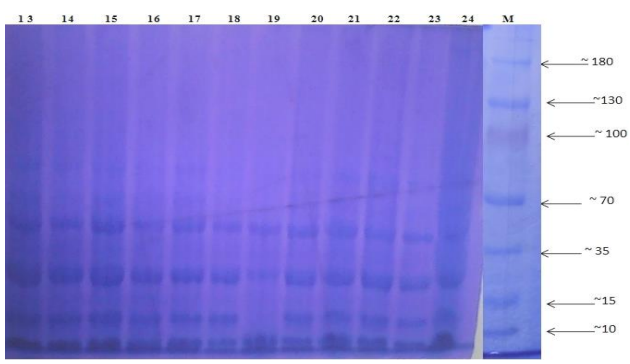

Fig. 1b. Electrophoretic banding patterns of elite guar germplasm accessions via SDS-PAGE of total seed storage proteins. M represents molecular size marker, while numbers from 13-24 represent genotypes 31733, 31836, 31756, 31761, 31762, 31763, 31764, 31869, 31812, 31806, 31673 and 31603, respectively.

Phylogenetic relationships and 2D and 3D structure of elite guar germplasm: The phylogenetic tree was constructed on the basis of Dice coefficient values among genotypes. All the genotypes were classified into 7 different groups. The group I, II, III and IV contained 7, 2, 4 and 8 genotypes, respectively. The last three groups (V-VII) included one genotype each (Fig. 2). These last 3 groups included highly diverged genotypes (31731, 31761 and 31764). The 2D and 3D models confirmed the results of clustering analysis. The 2D model showed that genotypes 31731, 31761, 31764 and 31812 were highly diverged. However, some genotypes were present outside border and these were considered as outliers (Fig. 3). The 3D structure further characterized all genotypes from the close angle. The 3D model showed that accessions 31764, 31731 and 31761 were unique genotypes as compared to others (Fig. 4).

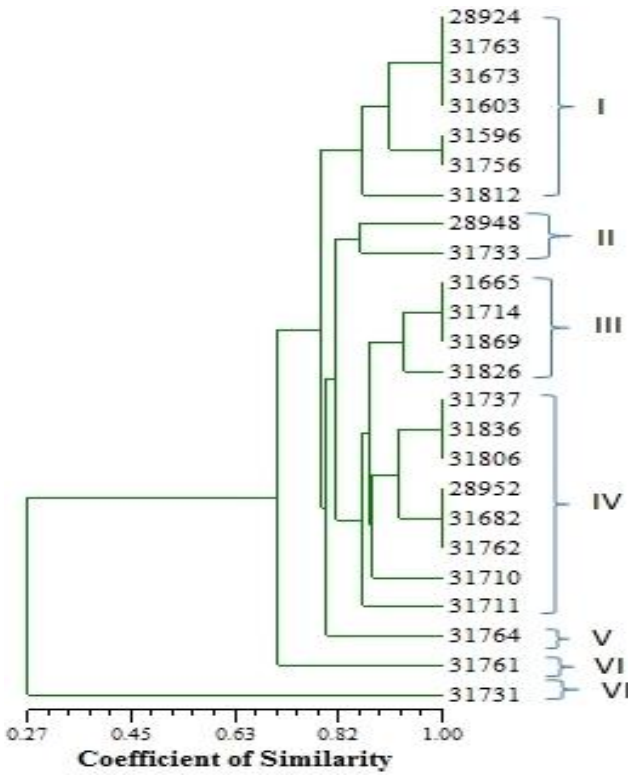

Fig. 2. Phylogenetic relationships among 24 elite guar germplasm accessions.

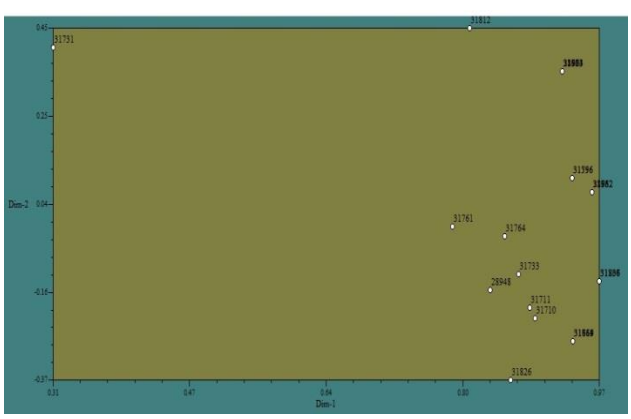

Fig.3. 2D representation of 24 elite guar germplasm accessions.

Table 2. Genetic similarity matrix among 24 elite guar accessions.

Acc #	28924	28948	31665	31731	31826	31737	28952	31596	31682	31710	31711	31714	31733	31836	31756	31761	31762	31763	31764	31869	31812	31806	31673	31603
28924	1.00																							
28948	0.73	1.00																						
31665	0.77	0.86	1.00																					
31731	0.33	0.29	0.22	1.00																				
31826	0.67	0.77	0.93	0.25	1.00																			
31737	0.83	0.77	0.93	0.25	0.86	1.00																		
28952	0.91	0.83	0.86	0.29	0.77	0.92	1.00																	
31596	0.91	0.67	0.86	0.29	0.77	0.92	0.83	1.00																
31682	0.91	0.83	0.86	0.29	0.77	0.92	1.00	0.83	1.00															
31710	0.73	0.67	0.86	0.29	0.92	0.92	0.83	0.83	0.83	1.00														
31711	0.73	0.83	0.86	0.29	0.77	0.92	0.83	0.83	0.83	0.83	1.00													
31714	0.77	0.86	0.99	0.22	0.93	0.93	0.86	0.86	0.86	0.86	0.86	1.00												
31733	0.77	0.86	0.88	0.23	0.80	0.80	0.86	0.71	0.86	0.71	0.71	0.88	1.00											
31836	0.83	0.77	0.93	0.25	0.86	0.99	0.92	0.92	0.92	0.92	0.92	0.93	0.80	1.00										
31756	0.91	0.67	0.86	0.29	0.77	0.92	0.83	0.98	0.83	0.83	0.83	0.86	0.71	0.92	1.00									
31761	0.73	0.50	0.71	0.29	0.77	0.77	0.67	0.83	0.67	0.83	0.67	0.71	0.71	0.77	0.83	1.00								
31762	0.91	0.83	0.86	0.29	0.77	0.92	0.97	0.83	0.97	0.83	0.83	0.86	0.86	0.92	0.83	0.67	1.00							
31763	0.98	0.73	0.77	0.33	0.67	0.83	0.91	0.91	0.91	0.73	0.73	0.77	0.77	0.83	0.91	0.73	0.91	1.00						
31764	0.80	0.73	0.77	0.20	0.67	0.83	0.91	0.73	0.91	0.73	0.73	0.77	0.77	0.83	0.73	0.55	0.91	0.80	1.00					
31869	0.77	0.86	0.97	0.23	0.93	0.93	0.86	0.86	0.86	0.86	0.86	0.98	0.88	0.93	0.86	0.71	0.86	0.77	0.77	1.00				
31812	0.89	0.60	0.67	0.40	0.55	0.73	0.80	0.80	0.80	0.60	0.60	0.67	0.67	0.73	0.80	0.60	0.80	0.89	0.67	0.67	1.00			
31806	0.83	0.77	0.93	0.25	0.86	0.97	0.92	0.92	0.92	0.92	0.92	0.93	0.80	0.98	0.92	0.77	0.92	0.83	0.83	0.93	0.73	1.00		
31673	0.98	0.73	0.77	0.33	0.67	0.83	0.91	0.91	0.91	0.73	0.73	0.77	0.77	0.83	0.91	0.73	0.91	0.97	0.80	0.77	0.89	0.83	1.00	
31603	0.97	0.73	0.77	0.33	0.67	0.83	0.91	0.91	0.91	0.73	0.73	0.77	0.77	0.83	0.91	0.73	0.91	0.96	0.80	0.77	0.89	0.83	0.98	1.00

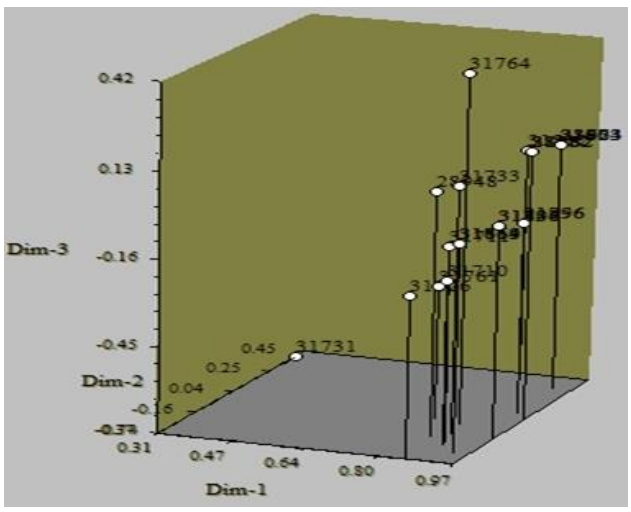

Fig.4. 3D representation of 24 elite guar germplasm accessions.

Discussion

Biochemical characterization plays key role in the identification of novel genotypes. Screening of seed storage protein profile through 2D and 3D structure method separate the elite genotypes from rest ones. In present study 24 elite genotypes of guar were used for SDS-PAGE based characterization. Maximum (100%) polymorphic major and minor bands were recorded. The sizes of these band patterns were ranged from 10-180 kDa. The coefficient of similarity values among these genotypes ranged from 20 to 100%. The genetic tree based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean) classified all genotypes into 7 different groups. Among these groups the last 3 groups were highly diverged from the remaining ones. The 2D and 3D structure model further justified these results and showed 3 unique genotypes (31764, 31731 and 31761). Our results were not in line with the findings of Zada *et al.*, (2013) who recorded 31 different protein bands in *Brassica carinata* germplasm. This deviation from our study might be due to genotypes difference and different SDS-PAGE protocol used. Our results showed similarities with the results of Jan *et al.*, (2016[a]) who recorded maximum polymorphic bands (93%) and genetic similarity values (47-100%) among elite *Brassica rapa* ecotypes and found 4 different groups through cluster analysis. Similarly, Shinwari *et al.,* (2013) recorded 17 different polymorphic and 1 monomorphic polypeptide bands among *Eruca sativa* accessions and noted moderate to high level of genetic similarity values (60-100%) among all tested genotypes. Khan *et al.,* (2014) recorded three elite clustered groups for 33 accessions of black gram by using similar method. Our findings showed deviation from the findings of Choudhary *et al.,* (2015) who noted 50% polymorphic and monomorphic bands in *Brassica napus* genotypes. Mottaghi *et al.,* (2015) used 2D and 3D method for some important Iranian *Achillea* by using principal coordinate analysis (PCoA) to select unique genotypes. They found maximum protein based variability among Iranian *Achillea* species through principal coordinate analysis (PCoA). The first 3 PCoA groups contributed maximum visibility of 83% than other component groups (Mottaghi *et al.*, 2015).

Conclusion

Maximum genetic variability was noted among twenty four elite genotypes of guar. Both the major and minor protein bands were recorded and all these bands were highly polymorphic. The genetic similarity values showed that maximum divergence existed among all tested genotypes. However the three novel genotypes i.e. 31764, 31731 and 31761 were highly diverse with both cluster and advanced 2D and 3D methods.

Acknowledgments

The authors are extremely thankful to the National Genebank of Pakistan, PGRI, NARC, Islamabad for providing guar germplasm. Laboratory facilities extending by Germplasm Laboratory of PGRI are also acknowledged for the conduct of SDS-PAGE analysis.

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(Received for publication 22 March 2018)