www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(9): 2811-2817

www.thepharmajournal.com
Received: 02-06-2022
Accepted: 07-07-2022

VB Gore
PG Student, Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

PJ Rathod
Assistant Professor. Biochemistry College of Agriculture, Department of Biochemistry & Biotechnology Junagadh Agricultural University, Junagadh, Gujarat, India

AG Vala
Assistant Professor, (PMBB) College of Agriculture, Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India

D Kumar
Ph.D., Student Department of Biotechnology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

Corresponding Author:
VB Gore
PG Student, Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

Analysis of protein profiling through SDS-PAGE of spreading types varieties of groundnut grown in India

VB Gore, PJ Rathod, AG Vala and D Kumar

Abstract
Groundnut seed protein SDS-PAGE profiling of spreading varieties were studied with sequential extraction of in defeated powder with water, NaCl, phosphate buffer and alcohol for protein fraction *viz.*, albumin, globulin, glutelin and prolamin respectively. The results of albumin% and globulin% content found to be in range of 16.2 to 20.43% and 72.05-78.5% respectively. The globulin protein fraction content was higher proportion then other three fractions. Glutelin% and prolamin% was found to be very lower in all varieties with the mean of 2.17% and 2.57% respectively. Ten varieties of speeding types were subjected to profiling through SDS-PAGE analysis. The results indicated considerable variation in genetic make-up through total number of bands and MW-Rf values for respective protein fractions. Albumin and globulin had the highest MW-Rf values in bands collectively (20–23), whereas glutelin and prolamin had the lowest MW-Rf values bands with ranged between 6-10. The qualitative and quantitative differences were revealed that seed protein profiles can be the best solution for correcting same protein in varietal identification with MW-RF in SDS PAGE. Correlation matrix between protein fractionation indicated that globulin was negatively correlated with prolamin and glutelin fraction.

Keywords: Groundnut, spreading albumin, globulin, prolamin, glutelin, SDS-PAGE

Introduction
The Groundnut (*Arachis hypogaea* L.) is an annual legume and is also known as peanut, earthnut, goober pea, jack nut, pygmy nut, monkey-nut, manila nut and ground bean. Groundnut is said to have originated in South America. Groundnut is an important element of India's oilseed economy. It is an important oilseed crop in India which occupies the first position in terms of area and second position in terms of production after soybean. According to the 1st advance estimates, groundnut production estimate (Kharif) was 82.54 lakh tones for 2021-22. (Anon., 2022) [2]. the area, production and productivity of groundnut in India during 2020- 21 were 4.8 Mha, 6.25 Mt and 1.3 Mt/ha respectively (FAO-STAT 2021) [5]. The importance of peanuts both as an object of human food and as a feedstuff is becoming generally recognized. Defatted peanut flour (DPF), a protein-rich (47–55%) and underutilized by-product of the peanut industry, is produced in huge quantities after the extraction of peanut oil. (Regena and Chen, 2010) [12] The seeds are a great source of vitamins B and include 40–50% oil, 20–30% proteins, and other nutrients. Small-seeded cultivars are utilized for oil, whereas large-seeded species are used for roasting and confections. The protein-rich groundnut meal can be fed to animals or consumed by humans. For the purpose of characterizing both farmed and wild species of groundnut, many researchers utilized seed protein electrophoresis [9-10]. The examination of seed storage proteins offers information on the evolutionary connection of the accession as well as aids in the identification and characterization of variation in crop varieties, cultivars, and their wild counterparts. Because of their features, including nutritional value, contribution to food texture, solubility, etc., peanut proteins play a significant role in many food items. Due to its features, including water and oil absorption, gel formation, foaming, and emulsification, large quantities of by-products from the peanut industry may be used to produce a respectably high quantity of protein. Seed storage proteins of legumes contain a low concentration of sulfur-containing amino acids and plant breeders have to consider this problem in any improvement programs (Summerfield and Roberts, 1985) [16]. In groundnut, about 8770 of the total seed protein consists of a globulin fraction (Basha and Pancholy 1981) [3] Peanut seed proteins contain three known classes of storage proteins including globulins (94–95%), albumins (2–3%), and prolamins (1–2%) [14]. Each species was varying in genetic constitution therefore our hypothesis was based on that every variety must had change in constituent as well as the nutritional quality not in terms of quantity but in

quality. As we all aware of that groundnut protein are superior in terms of leguminous crops. It has both benefits oils as well as protein therefore to undermine this relation and keeping in mind that distinguish variety have differences in nutritional value of groundnuts. Researchers must consider protein content as well as with their quality analysis by protein profiling through electrophoresis (SDS-PAGE). One more benefit was to understand the seed storage protein variation among the different types of varieties. It can also be used to identify the genetic variation in groundnut.

Materials and Methods

Seed Material

The experiment material comprised of ten spreading type groundnut varieties used for the present study were obtained from main oilseeds research station, Junagadh Agricultural University, Junagadh.

Soluble Protein extraction in seed kernel

Protein fractionation were performed as shown in Flow chart for performing the extraction for four soluble protein fractions in groundnut *viz*., Albumin, Globulin, Glutelin and Prolamin present in fig.5.

Each protein fraction was read by using folin lowry methods [7] and calculate the loading value for PAGE analysis. Defatted groundnut seed powder (500mg) was taken in 15 ml centrifuge tube in that 5ml distilled water was added centrifuged at 10,000 rpm for 6 min. supernatant was collected and used as assay for the estimation of albumin% and in remaining pellet 5ml NaCl (5.0M) was added and then for 6 min centrifuge at 10,000 rpm, supernatant was collected to estimate globulin% and pellet were used for successively extraction of glutelin% protein fraction by adding 0.2 M phosphate buffer (pH 8.0) 5 ml added centrifuged for 6 min at 10,000 rpm, from the obtained supernatant glutelin was estimated and in remaining pellet 5 ml of 70% alcohol centrifuge at 10,000 rpm for 6 min. Supernatant collect used as assay for estimation of prolamin%. The absorbance was then measured against a blank at 660nm.The graph was plotted versus concentration using measurements from reference samples, and a standard curve (BSA) was created to determine the concentration of unknown protein samples using appropriate formula.

$$\text{Total soluble protein (\%)} = \frac{\text{GF} \times \text{OD} \times \text{Total Volume} \times 100 \times 10^{-6}}{\text{Sample Aliquot} \times \text{Wt. of sample (g)}}$$

SDS- PAGE analysis:

Total protein samples from ten groundnut varieties were analyzed using SDS-PAGE (12% separating gel with 5% stacking gel) according to the method of Laemmli [6] with the Mini-PROTEIN ®II Dual Slab Cell System (BIO-RAD, Hercules, CA). Proteins samples (100µl) from each sample were loaded onto SDS-PAGE gels. Low-range protein markers (BSA) were used as molecular mass standard. Electrode buffer was filled into the electrophoresis tank once the sample was loaded. The power supply was connected to the electrophoresis machine. The current was turned on for 20 minutes, allowing 50mA to flow until the sample passed through the stacking gel. After that, the current was increased to 60mA until the tracking dye was 0.5cm to 1cm above the bottom. The power was turn off when the tracking dye reached the end of the running gel following complete molecular separation. The gel was gently lifted from the area between the plates and immersed in a tray of bromophenol blue staining solution (0.125% Coomassie blue R-250 in 40% methanol and 10% acetic acid). The tray was stored overnight after being shaken repeatedly to ensure consistent staining. Distaining solution (40% methanol and 10% acetic acid) was used to remove the stain from the gel. The technique was repeated until the background was completely colorless. The relative mobility of the various protein bands was manually noted. The band intensity was assessed visually by placing gel over transilluminator and recorded as faint and dark bands and as absence or presence of specific bands. The Rf of resolved protein band were calculated as follows. GEL ANALYZER (19.1) software was used for data analysis of Rf value in individual protein fraction for bands. The resulted similarity matrix was used to make dendrogram was using Past software.

$$Rf = \frac{\text{Distance migrated by protein band (cm)}}{\text{Distance migrated by tracking dye from the top of the separation gel (cm)}}$$

Results and Discussion

Protein fraction content

The result pertaining to seed protein fractions of ten varieties was found to be significant. The globulin content was found to be highest amongst the all four-protein fraction.

The data for albumin extracted from seed kernel of spreading type of groundnuts were found to be significant. As showed in Table 2 and Figure 1 it was in the range of 16.2 to 20.43%. The maximum Albumin content was found to be in variety GJG-17 (20.43%) and it was at par with GG-12 (20.05%) whereas minimum albumin content observed in variety GG-11 (16.2%). These results are in agreement with Afify *et al*. (2011) [1], they studied the effect of γ-radiation on total protein solubility, albumin, globulin also with Youle and Huang (1981). In comparison among the groundnut varieties indicated that higher globulin protein% was found to be in GG-11 (76.95%) variety and it was at par with GG-HPS-1 (76.9%) and GAUG-10 (76.52%) and lower percentage was recorded in M-13 (71.43%). Comparative chemical extraction of glutelin for spreading type's varieties from protein fraction was ranged from 1.46 to 3.2%. The higher value for glutelin% was observed in JVR-700 (3.2%) variety and it was at par with GG-HPS-1 (2.70%). whereas lower percent for was observed in GAUG -10 variety (1.46%). This variation may be due to their solubility and differentially expression of genes for glutelin in types of varieties. Analysis of spreading type's varieties for the prolamin content was also found to be significant with the ranges from 2.06% to 3.83%. The higher value for prolamin was% reported in variety GG-12 (3.83%) and lower value for prolamin was found in GG-11 variety (2.06%). This variation among the varieties shown differently due to less solubility and extraction method. The data showed significant deviations among the varieties of spreading types. These results are in agreement with Sebei *et al.* (2013) [13]. They studied peanut seed proteins with classes of storage proteins and found that globulin, prolamin and glutelin content was in ranged between 94–95%, 1-2%. and 0.5-1%. From the correlation matrix it can be stated that the globulin and albumin were negatively correlated and giving the significant correlation at 1% level for spreading type varieties of groundnut seed. The correlation study between protein fractions (table 2) showed that globulin was found to be negatively corelated with glutelin and prolamin with

correlation coefficient of -0.207, -0.934 and on the other hand, albumin negative correlated with globulin.

SDS page analysis

The pattern of seed storage protein of groundnut showed differences in banding pattern among the varieties on the basis of MW-Rf value. These banding pattern of SDS- PAGE had been applied for the detection of polymorphism in difference varieties of groundnut. It can be a protein marker for the identification of groundnut variety. To explore variability among the seed kernel protein through SDS-PAGE in different type of groundnut varieties shown the following results.

The number of bands was found in the ten spreading type varieties was with range from 11-16, 12 to 21, 4-7 and 2-6 for the albumin, globulin, glutelin and prolamin fraction. In detected 20 bands for albumin, 8 bands (with Rf of 0.012, 0.233, 0.351, 0.404, 0.547, 0.761, 0.817, 0.991) were found to be monomorphic whereas, remaining 12 bands were polymorphic. These specific protein bands migrating to the same distance gave some evidence of homology in molecular structure and function. Among the varieties, the maximum protein bands (16) were found to be in GG-11 and JVR-700 variety whereas minimum bands (11) were observed in GJG-17 and GG-13 variety of spreading type. Protein band with MW-Rf of 0.047 was present in variety GG-11 and JVR-700 out of ten spreading varieties of groundnut. Varieties *viz*., M-13 and JVR-700 were not resolved for the protein band with MW-Rf value of 0.404. The maximum and minimum globulin protein bands were found to be in GG-11(21) variety and M-13(12) variety respectively. The variations were due to differences in size and number of polypeptides. Monomorphic band with MW-Rf value 0.035, 0.266, 0.463, 0.559, 0.590, 0.674, 0.825 and 0.974 were commonly found in all the ten spreading type varieties of groundnut whereas remaining 15 were polymorphic in nature. Band with MW-Rf value of 0.067 only resolved in GG-11, GG-12, GTG-17, GTG-19, GG-HPS-14 and GG-41 varieties for globulin fraction. Out of ten varieties of spreading type band of MW-Rf value of 0.092 of globulin only present in G-13 and GG-5, GJG-17 varieties. Muniappan *et al.* (2016) [8] they studied four commonly cultivated groundnut varieties such as ICGV00351, TMV-7, CO-4, CO-6 and TG-374 for quantitative and qualitative analysis of seed protein by SDS-PAGE. Their results revealed that the variation in total number of bands and MW-Rf values. The maximum number of MW-Rf value was recorded in TG-374 and ICGV00351, and minimum MW-Rf values was 11 found to be in TMV-7 and CO-6. With the total 8 bands found in SDS-PAGE analysis for the glutelin protein fraction in ten spreading type varieties the maximum protein bands (7) were found to be in GG-13 variety whereas minimum bands (4) were observed in GJG-19, M-13, JVR-700 and GAUG-10 variety of spreading type. The monomorphic protein band with the MW-Rf value of 0.489 was commonly noticed in the all spreading type variety of groundnut whereas remaining 7 bands were polymorphic. Whereas for prolamin fraction total 7 bands were found in ten spreading type varieties. The monomorphic protein bands with the MW-Rf value of 0.303 and 0.741 were commonly noticed in the all ten-spreading type variety of groundnut. Four protein bands were polymorphic in appearance *viz*., MW-Rf value 0.164, 0.522, 0.625 and 0.911 in ten spreading type varieties. The maximum protein bands (6) were found to be variety in GG-13 and KAUSHAL whereas minimum bands (2) were observed in JVR-700 variety of spreading type. Protein band with MW-Rf value 0.164 only shown by verity GG-11, GG-12, GAUG-10, M-13, and JVR-700. Similar findings for protein bands in groundnut seed were also observed by Singh *et al.* (2018) [15] Similar findings for protein bands in groundnut seed were also observed by senakoon *et al.* (2015) [14] they examined the seed prolamins obtained by alcohol extraction and subsequent acetone precipitation analyzed by SDS–PAGE. Similar results for protein bands in groundnut seed were also observed by Chandran *et al.* (2002) [4] they studied seventy Indian released groundnut cultivars for seed storage protein on SDS-PAGE they found total thirty-six different bands and polymorphism was observed for the banding pattern. Rao *et al*. (2013) [11] findings same as above data. Correlation data explained that globulin was negatively correlated with the prolamin and glutelin while for it exactly reverse. Albumin negative correlated with globulin.

Protein profiles of 10 groundnut varieties were scored as matrix comparison pot 0 and 1 for missing and appearing protein bands to analyze for the distance similarity using PAST data analysis software. Then the data were used to make a dendrogram (fig.6 to10) the analysis dendrogram classified 10 groundnut varieties into 3 groups for albumin fraction. Group I comprised the most similar accessions containing 2 varieties from GG-13 and GG-11 Group second has one variety. Group third is consisting of remaining 7. Globulin proteins of varieties GG-HPS-1 and GG-41 while variety GG-13 form separate group. In case of glutelin fraction three group showed in analysis of dendrogram. Group 1 comprised the only one variety GG-11. Varieties JVR-700, M-13 and GAUG-10 showed similarity was investigated for the glutelin and varieties GG-13 and GJG-17 also in same group. prolamin fraction similarity dendrogram showed two major group in 1 group three varieties while other 7 varieties in that GG-13, GJG-17, GJG-19 and GG-HPS-1 showed similarity in banding pattern for the prolamin protein.

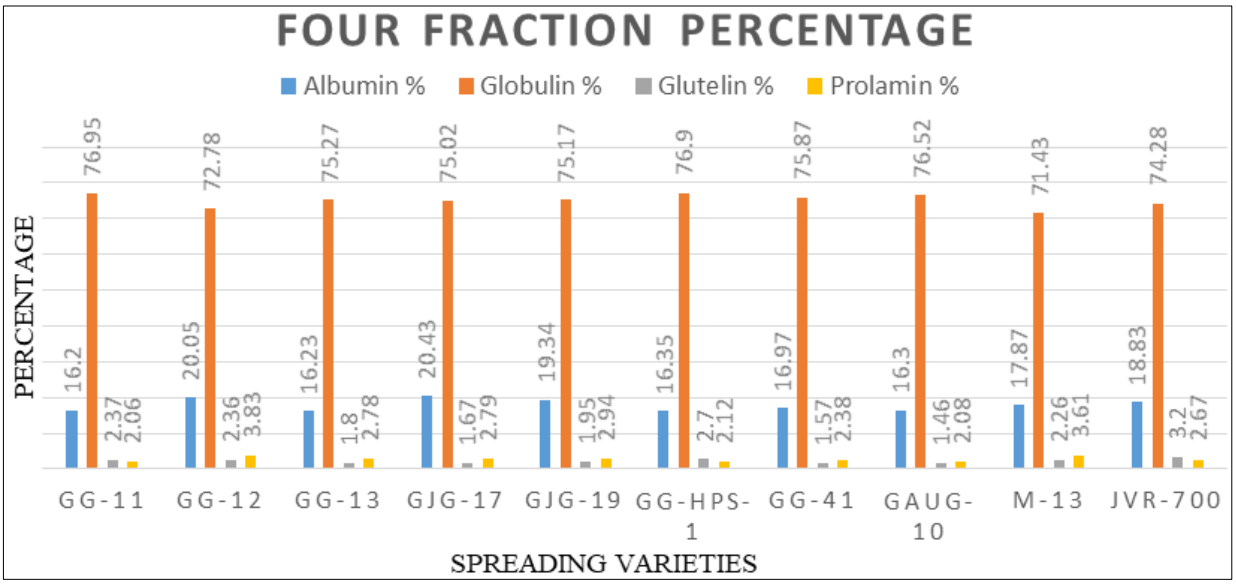
Table 1: Correlation matrix of total soluble protein and protein fractions for spreading varieties of groundnut seed

	Albumin%	Globulin%	Glutelin %	Prolamin %
Albumin %	1			
Globulin %	-0.55374	1		
Glutelin %	0.110346	-0.20708	1	
Prolamin %	0.655556	-0.93442	0.11336	1

Table 2: Protein content in seeds of spreading groundnut varieties

Sr. No.	Name of varieties	Albumin %	Globulin %	Glutelin %	Prolamin %
1	GG-11	16.2	76.95	2.37	2.06
2	GG-12	20.05	72.78	2.36	3.83
3	GG-13	16.23	75.27	1.8	2.78
4	GJG-17	20.43	75.02	1.67	2.79
6	GG-HPS-1	16.35	76.9	2.7	2.12
5	GJG-19	19.34	75.17	1.95	2.94
6	GG-HPS-1	16.35	76.9	2.7	2.12
7	GG-41	16.97	75.87	1.57	2.38
8	GAUG-10	16.3	76.52	1.46	2.08
9	M-13	17.87	71.43	2.26	3.61
10	JVR-700	18.83	74.28	3.2	2.67
Mean		17.86	75.02	2.13	2.73
S.Em		0.3	0.7	0.13	0.144
C.D. (0.05)		0.87	2.01	0.31	0.35
C.V.%		2.37	1.32	8.29	7.91

Fig 1: Protein% in 10 spreading type varieties



M	GG 11	GG 12	GG 13	GJG 17	GJG 19	GG HPS1	GG 41	GAUG 10	M 13	JVR-700

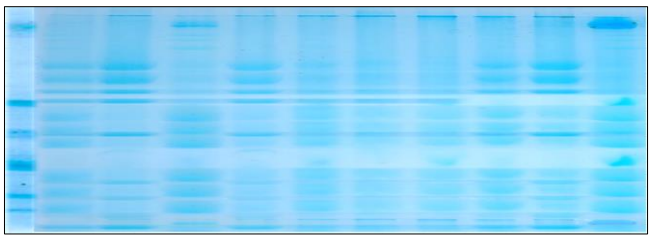


Fig 2: Albumin SDS PAGE analysis

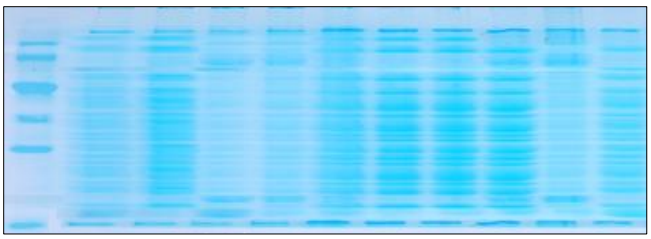


Fig 3 Globulin SDS PAGE analysis

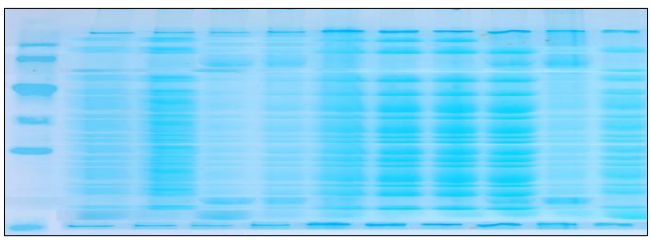


Fig 4: Glutelin SDS PAGE analysis

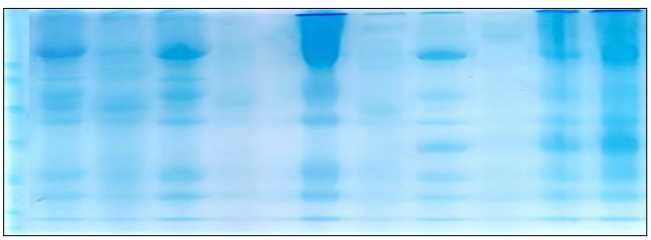


Fig 5: Prolamin SDS PAGE analysis

Table 3: MW-Rf value on different albumin protein fraction bands of spreading type groundnut varieties based on SDS-Page

Band number	MW-Rf	GG-11	GG-13	GG-132	GJG-17	GJG-19	GG-HPS-1	GG-41	GAUG-10	M-13	JVR-700
1	0.012	1	1	1	1	1	1	1	1	1	1
2	0.047	1	0	0	0	0	0	0	0	0	1
3	0.102	1	0	1	0	0	0	0	0	0	0
4	0.125	1	0	1	0	0	0	0	0	0	0
5	0.161	1	0	1	0	0	0	0	0	0	1
6	0.233	1	1	1	1	1	1	1	1	1	1
7	0.310	1	1	0	1	1	1	1	1	1	1
8	0.351	1	1	1	1	1	1	1	1	1	1
9	0.404	1	1	1	1	1	1	1	1	0	0
10	0.469	1	0	1	1	1	1	1	1	1	1
11	0.506	0	0	0	0	0	1	0	1	0	0
12	0.547	1	1	1	1	1	1	1	1	1	1
13	0.600	1	1	1	0	1	0	1	1	0	1
14	0.670	0	0	0	1	0	0	1	0	0	1
15	0.723	0	0	0	0	0	1	1	1	1	1
16	0.761	1	1	1	1	1	1	1	1	1	1
17	0.817	1	1	1	1	1	1	1	1	1	1
18	0.906	1	0	1	0	1	1	1	1	1	1
19	0.942	0	1	1	1	1	1	1	1	1	1
20	0.991	1	1	1	1	1	1	1	1	1	1

Table 4: MW-Rf value of different globulin protein fraction bands of spreading type groundnut varieties based on SDS-Page

Band number	MW-Rf	GG-11	GG-12	GG-13	GJG-17	GJG-19	GG-HPS-1	GG-41	GAUG-10	M-13	JVR-700
1	0.035	1	1	1	1	1	1	1	1	1	1
2	0.067	1	1	0	1	1	1	1	0	0	0
3	0.092	0	0	1	1	0	0	0	0	0	0
4	0.126	1	1	0	1	1	1	1	1	1	1
5	0.227	1	0	1	1	1	1	1	1	1	1
6	0.266	1	1	1	1	1	1	1	1	1	1
7	0.338	1	1	1	0	1	1	1	1	0	1
8	0.385	0	1	0	0	0	0	0	0	0	0
9	0.422	1	0	1	1	1	1	1	1	0	1
10	0.463	1	1	1	1	1	1	1	1	1	1
11	0.52	1	0	0	0	1	1	1	1	0	1
12	0.559	1	1	1	1	1	1	1	1	1	1
13	0.59	1	1	1	1	1	1	1	1	1	1
14	0.637	1	1	1	0	1	1	1	1	0	0
15	0.674	1	1	1	1	1	1	1	1	1	1
16	0.704	1	1	1	1	1	1	1	1	0	1
17	0.765	1	1	1	1	1	1	1	1	0	1
18	0.8	1	1	1	0	0	0	0	0	0	0
19	0.825	1	1	1	1	1	1	1	1	1	1
20	0.85	1	1	0	0	0	1	1	1	0	1
21	0.889	1	1	1	1	1	0	0	1	1	1
22	0.923	1	1	0	1	1	1	1	1	1	1
23	0.974	1	1	1	1	1	1	1	1	1	1

Table 5: MW-Rf value of different glutelin protein fraction bands of spreading type groundnut varieties based on SDS-Page

Band number	MW-Rf	GG-11	GG-13	GG-132	GJG-17	GJG-19	GG-HPS-1	GG-41	GAUG-10	M-13	JVR-700
1	0.052	1	1	1	1	0	1	0	0	0	0
2	0.193	0	0	0	0	0	0	0	0	0	0
3	0.257	1	1	1	1	0	0	1	0	0	0
4	0.321	0	1	1	1	0	1	1	1	1	1
5	0.374	0	0	0	0	0	0	0	0	0	0
6	0.489	1	1	1	1	1	1	1	1	1	1
7	0.593	1	0	0	0	0	0	0	0	0	0
8	0.762	0	1	0	0	1	1	1	1	1	1
9	0.845	0	1	1	1	1	1	1	1	1	1
10	0.937	1	1	1	1	1	1	0	0	0	0

Table 6: MW-Rf value of different prolamin protein fraction bands of spreading type groundnut varieties based on SDS-PAGE

Band number	MW-Rf	GG-11	GG-13	GG-132	GJG-17	GJG-19	GG-HPS-1	GG-41	GAUG-10	M-13	JVR-700
1	0.164	1	1	0	0	0	0	0	1	1	1
2	0.303	1	1	1	1	1	1	1	1	1	0
3	0.381	0	0	0	0	0	0	0	0	0	0
4	0.522	1	1	1	1	1	1	0	0	0	0
5	0.625	0	1	0	0	0	0	0	0	0	0
6	0.741	1	1	1	1	1	1	1	1	1	1
7	0.911	1	1	1	1	1	1	1	1	0	0

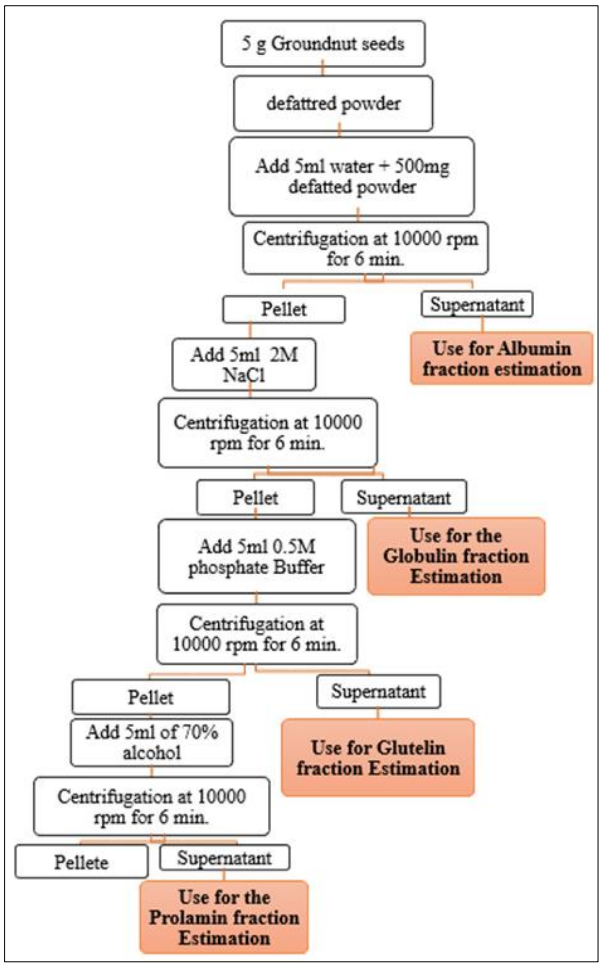


Fig 6: Flow chart of sequential extraction four protein fraction in groundnut

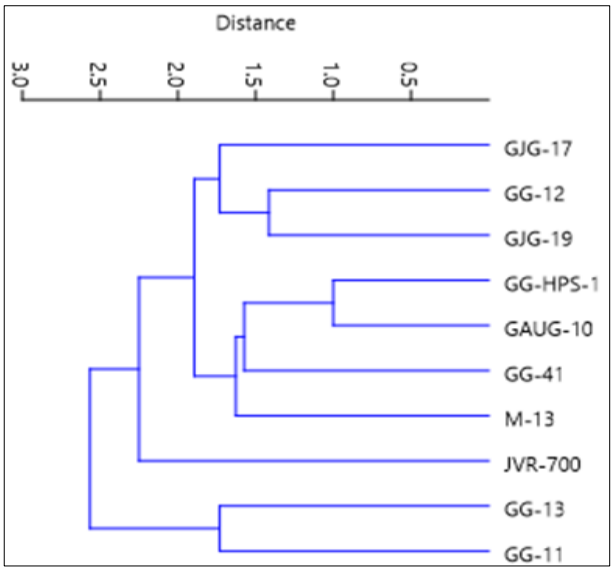


Fig 7: Dendrogram generated using albumin protein of 10 groundnut varieties through SDS-PAGE profile

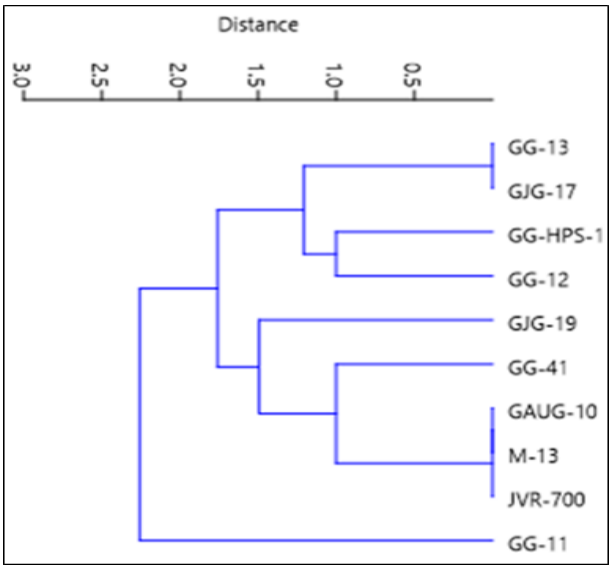


Fig.8: Dendrogram generated using glutelin protein of 10 groundnut varieties through SDS-PAGE profile

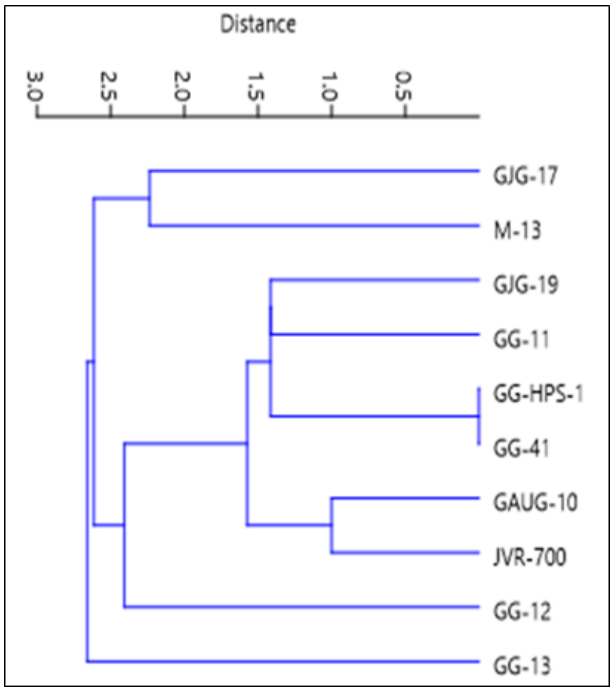


Fig 9: Dendrogram generated using globulin protein of 10 groundnut varieties through SDS-PAGE profile

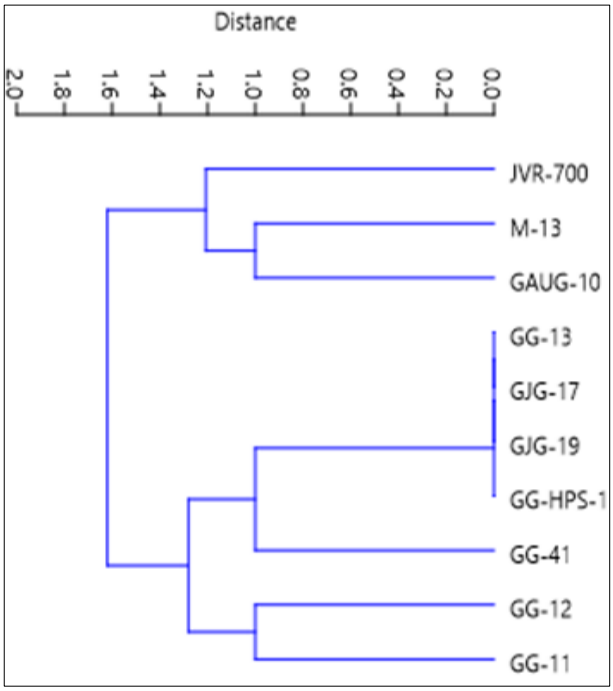


Fig 10: Dendrogram generated using prolamin protein of 10 groundnut varieties through SDS-PAGE profile

Conclusion

Resulted showed that salt soluble protein fraction globulin content in maximum and minimum prolamin and glutelin content amount while of These experimental results can be useful for plant breeders, food processors for deciding varying amount of protein as well as for the consumer too. This information generated from experiments can also be useful for the scientist working in groundnuts for analysis of biochemical constituents, and proteomics. However, heterogeneity in protein profiling in distinct types can be useful in identifying quality traits in groundnut genotypes from this study.

References

1. Afify AEMM, Rashed MM, Mahmoud EA, El-beltagi HS. Effect of gamma radiation on protein profile, protein fraction and solubilities of three oil seeds: soybean, peanut and sesame. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2011;39(2):90-98.
2. Anonymous, Groundnut Outlook – may 2022 Agricultural Market Intelligence Centre, TSAU https://pjtsau.edu.in/files/AgriMkt/2022/May/groundnut-May-2022.pdf accessed on 12 may, 2019.
3. Basha SMM, Pancholy SK, Polypeptide composition of arachin and non-arachin proteins from early bunch peanut (*Arachis hypogaea* L.) seed. Peanut Sci. 1981 Jul;8(2):82-8.
4. Chandran K, Rajgopal K, Radhakrishnan T Electrophoretic study on seed storage proteins of groundnut (*Arachis hypogaea* L.) cultivars of India. Indian Journal of Plant Genetic Resources. 2002;15(2):108-111.
5. FAOSTAT. Food and agriculture organization of the United Nations statistics division. Retrieved on April 18, 2021, from http://www.fao.org/faostat.
6. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970 Aug;227(5259):680-5.
7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 1951 Nov;193(1):265-75.
8. Muniappan V, Palanivel S, Parvathi S, Viswanathan MB Rajesh R. Analysis of seed proteins in groundnut cultivars (*Arachis hypogaea* L.). International Journal of Engineering Research and Applications. 2016;6(7):06-10.
9. Dawson R, McIntosh AD, Varietal and environmental differences in the proteins of the groundnut (*Arachis hypogaea*). Journal of the Science of Food and Agriculture. 1973;24(5):597-609.
10. Ory RL, Cherry JP, Protein from peanut cultivars (*Arachis hypogaea*) grown in different areas. V. Biochemical observations on electrophoretic pattens of proteins and enzymes. Journal of American Peanut Research and Education Association. 1972;4(1):32-40.
11. Rao PS, Bharathi M, Reddy KB. Identification of peanut (*Arachis hypogaea* L.) varieties through chemical tests and electrophoresis of soluble seed proteins. Legume Research-An International Journal. 2013;36(6):475-483.
12. Regena J, Chen ZX, Physico-functional properties of peanut meal flour as effected by processing methods. Journal of Food Biochemistry. 2010;34:229-243.
13. Sebei K, Gnouma A, Herchi W, Sakouhi F, Boukhchina S. Lipids, proteins, phenolic composition, antioxidant and antibacterial activities of seeds of peanuts (*Arachis hypogaea* L.) cultivated in Tunisia. Biological Research. 2013;46(3):257-263.
14. Senakoon W, Nuchadomrong S, Chiou RY, Senawong G, Jogloy S, Songsri P, *et al.* A Identification of peanut seed prolamins with an antifungal role by 2D-GE and drought treatment. Bioscience, Biotechnology, and Biochemistry. 2015;79(11):1771-1778.
15. Singh A, Raina SN, Rajpal VR, Singh AK. Seed protein fraction electrophoresis in peanut (*Arachis hypogaea* L.) accessions and wild species. Physiology and Molecular Biology of Plants. 2018;24(3):465-481.
16. Summerfield RJ, Roberts EH. Grain Legume Crops". Collins Pub. London, UK; c1985.