Estimation of Total Protein and Protein Classes of Promising EMS Samba Mahsuri Mutants

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Abstract—**Rice is the staple food of half of the world. Rice is easily digestible, rich source of starch followed by proteins and lipids as major components. Rice proteins are having great nutritional value and show many health benefits. Rice has significant amount of protein which is available in mainly four fractions such as Globulins, Albumins, Glutelins and Prolamins. Rice protein is high in Sulphur containing aminoacids. Rice protein is the major factor for determining texture, pasting capacity and sensory characteristics there by determines the cooking and eating qualities of rice. The yield of utilizable protein is higher in rice. The proportion of protein classes varies among genotypes and even among brown rice, milled rice and rice bran. The present study can be used to know the total protein content and amount of protein classes among Samba Mahsuri and 97 mutants due to its novelty to screen quality and its wider applications in food industry.**

Index Terms—**Albumins, Globulins, glutelins, prolamins, mutants, pasting, sensory, texture.**

I   INTRODUCTION

Rice is the most important cereal crop of the world's population. Rice stands as a valuable reservoir of nutritional (robust profile by Tian et.al,2024) and nutraceutical elements beneficial to human health (Juliano, 1985).Rice grain is easily digestible. It is a rich source of starch followed by proteins and lipids. Additionally, it also contains minerals, vitamins and simple sugars in small amounts. Rice potein are potential resources for human nutrition. (Day.L.et.al). The total protein present in rice is 4.3 to 20.2% (Chattopadhyay et al., 2012; Karina et al., 2013). Proteins in rice grain are present in storage organelles called as protein bodies (PBs) that are located in outer coatings (bran) of the starchy endosperm and the germ (Amagliani, et.al). PB are of two types namely type I (PB-I) and type II (PB-II). PB-I has a lamellar structure, is spherical in shape and is rich in prolamins. Whereas PB-II has a crystalline structure, displays an irregular shape and contains predominantly glutelin (Bechtel and Juliano, 1980). Endosperm storage proteins comprise 60-65 % of PB-II, 20-25 % of PB-I and 10-15 % albumin and globulin in the cytoplasm (Ogawa et al. 1987).

The biological value of rice protein was higher than of most other cereal proteins. The lysine content of rice protein is higher than other staple cereals (Ju et.al). Rice protein is rich in glutamine and aspargine like other cereal proteins. Rice protein is hypoallergic (Amagliani et.al) (absence of gluten), digestible and nutritional (Eggum et.al), hence the consumption is recommended to infants and gluten allergic people (Gopika Jayaprakash et.al 2022.). Rice protein has many properties such as buffering capacity, foaming activity, emulsifying nature and binding capacity (oil and water), surface hydrophobicity at present importance of rice protein extraction is increasing due to its multiple industrial applications (Sachini Senrathna et.al). The nutritional profile of rice protein varies by genotype, environmental factors and fertilizers application (Nitrogen fertilizer can increase the protein content).

Rice proteins are categorized into albumin (water-soluble), globulin (salt-soluble), glutelin (alkali/acid-soluble), and prolamin (alcohol-soluble) according to their solubility (Osborne 1924). Their availability varied among the varieties).Albumin and globulin exist in the outer layers of the brown rice amounting to 4-22 % and 5-13 % of total protein respectively (Hoogenkamp, 2017). Glutelin (oryzenin) and prolamin constitute 80% and 20-25 % of rice endosperm protein respectively. Apart from solubility the protein classes differ in many properties such as aminoacid composition. More number of aminoacids were observed in albumins followed by glutelins,

globulins and prolamins while the proportion of essential aminoacid was highest in albumins followed by globulins, glutelins and prolamins (G.NIHARIKA 2019). Generally, glutelins are higher in rice protein, followed by globulins, followed by albumins and finally prolamins.

Albumins,are water soluble, heat liable, denatures at 75.7^0& 73^0C and undergoes coagulation. This fraction accounts for 2-6% in total seed protein and 4-8% of the endosperm. Globulins—are salt soluble, most abundant in storage protein (15-36%), rich

in cysteine and methionine. Glutelins are high molecular weight proteins, present in higher amounts and soluble in alkali, accounts for about 75-81% in brown rice and 79-88% in endosperm. Prolamins are mostly reserve proteins, soluble in alcohol mostly contain nonessential aminoacids, present in minor quantity.

In India, so far, three high protein (≥10%) rice varieties- CR Dhan 310 (10.3%), CR Dhan 411 (9.8%) and CR Dhan 311 (10.1%) protein in milled rice were released through AICRIP biofortification trial wherein FT-NIR standardized with kjeldahl method (Kjeldahl et.al) is being used for estimation. One high protein rice- BRRI Dhan 84 (9.7%) was released in Bangladesh. (Sanjeevarao et.al) Mutational breeding is advantageous over traditional in terms of cost, time and efficiency. Induced mutations by physical agents(alpha,beta,gamma,x-rays,neutrons etc) and chemical agents (ethyl methyl sulphate,nitrosurea,sodium azide etc) show higher efficiency than other methods (insertions by T-DNA, transposons, RNAi ,TALEN-genome editing and CRISPR/cas genome editing).Among chemical mutagens Ethyl Methane Sulphonate is frequently used which can create point mutations 2-10 /mb by alkylation of specific base leads to single nucleotide change. Hence EMS was used to create mutations to produce mutants with novel traits.

Therefore, 97 promising SM mutants were screened for total protein and its categories in polished rice grain in comparison with its wild type.

II MATERIALS AND METHODS

Samba Mahsuri ethyl methane sulphonate (EMS) mutant lines were developed jointly by IIRR and CCMB Based on the morphological and yield parameters, 97 promising mutants were selected for evaluating total

protein content and protein classes.

This study was conducted in two wet seasons (2022 and 2023) in the experimental fields of ICAR-Indian Institute of Rice Research, Hyderabad.The promising lines among samba Mahsuri mutants were cultivated and multiplied in field following standard package of practices.

There are several methods for the estimation of protein by using different methods by Braspaiboon S. et.al alkaline and enzymatic extraction and studied on solubility of proteins present in rice. Cagampang, et.al also studied extraction and composition of rice proteins. Protein estimated by extraction buffers is superior to traditional analytical methods.

Estimation of protein done by Lowry et al. (1951) method and classes estimated by extraction with buffer method For the total proteins- 50mM Tris-HCl (pH 7.5), 2% SDS, 0.6% 2-mercaptoethanol, 20% glycerol, 4M urea (Lang et al. 2013), albumins- 500μL of 10mM Tris HCl solution (pH 7.5) and 1mM EDTA, globulins- 10mM trisHCl (pH 7.5), 1mM EDTA & 0.5 mL NaCl, prolamins- Isopropanol 60% (Santos et al. 2013)and glutelins- 50mmol TrisHCl (pH 6.8), 4% SDS, 5% 2-mercaptoethanol, 20% Glycerol, 8M Urea (Kawakatsu et.al) buffers were used for extraction. The extracted total protein as well as each category were separately precipitated with ethanol, centrifuged, pellet was washed with water, resolubilized in alkali and protein content was estimated. Total rice protein varies from 4.5 to 15.9% with four different buffers and reported high yield of total protein content from 50mM Tris-HCl (pH 7.5), 2% SDS, 0.6% 2-mercaptoethanol, 20% glycerol, 4M urea buffer (Lang et al. 2013) by adding 500ul of 10 mM Tris HCl solution (pH7.5) and 1 mM EDTA .extraction buffer method (Santos et.al., 2013) . Sadaiah et.al compared buffer method with NaOH & Kjeldahl method and suggested extraction buffer method followed by estimation is best method for total protein estimation.

III RESULTS

Rice varieties like Samba Masuri, Swarna, IR 64, Pusa Basmati 1121, etc. are popular among consumers and farmers. Among the non-aromatic rice with medium slender grain type, Samba Mahsuri is highly popular in Southern and Eastern parts of India. It holds highest acreage in the states of Andhra Pradesh, Telangana, Tamilnadu and Karnataka. It shows best cooking

quality with good yield. Many breeding programmes aimed to reach similar cooking quality by using Samba mahsuri as one of the parents or subjecting it to mutagenesis to get better yield with same cooking quality. Considering the above, EMS Samba Mahsuri mutants were developed and the best mutant lines were screened for total protein content and protein categories.

ANOVA analysis indicates that the variation in protein content and the various categories significantly varied among the 97 SM mutants and wild type (Table 2).

Among 97 promising mutant lines, the total protein content ranged from 1.9% to 13.01% Ti-128 has shown highest total protein content 13.01% followed by Ti-60 (12.76), Ti-49 (11.4) %), Ti-3 (10.9%) and Ti-40 (10.57%). Samba Mahsuri has shown 11.3% of total protein. Among the protein categories, globulins were at the highest concentration followed by glutelins, albumins and prolamins. Globulins content ranged from 1.4% (TI-60) to 10.3% (BB-134) in mutants. Glutelins ranged from 0.08% (SM-70) to 8% (Ti-56); Albumin content ranged from 0.37% (TI-41) to 3.12% (TI-48). Prolamins ranged from 0.0076% (TI-27) to 0.26% (TI-4 & TI-10) (Table.2).

Samba Mahsuri has shown 0.86% of albumins ,3.55% 0f globulins ,0.04% of prolamins and 2.45% glutelins.69 mutants showed higher albumin content , 52 mutants noted higher globulin content and 64 mutants has shown higher prolamin content and finally 25 mutants has shown higher glutelin content (Table2).They are considered promising regarding each class.A few mutants has shown higher percentage in three distinct protein classes viz TI-6,TI-56,TI-45,TI-113,TI-40,TI-15 ; A few mutants has shown higher percentages in two distinct protein classes viz TI-14,TI-27,TI-35,TI-60,TI-128,TI-132,TI-9,TI-20.

These mutants found to be more promising among 97 and recorded higher amounts in different protein classes compared to Samba Mahsuri. (Table.2).

Mutants with higher albumin content which contains a greater number of essential aminoacids followed by globulin content followed by glutelin content and followed by prolamin content is the promising mutant line. Albumin is the source of aminoacids to other tissues of the body and it is influenced by protein proportion in the diet.

Multiple correlation analysis (Figure) indicates that albumins showed significant positive (P 0.01) correlation with globulins, significant negative (P 0.05) correlation with prolamins. Globulins showed significant positive correlation (P 0.05) with glutelins (P 0.05) and significant negative correlation (P 0.05) with prolamins. The total protein content or Protein (Ex) showed positive significant correlation (P 0.05) with prolamins and with Glutelins(P0.001).

IV DISCUSSION

Proteins in rice grain are distributed both in the aleurone layer and also in the endosperm. Proteins are only source of aminoacids in particular, essential aminoacids. Almost all the rice protein is digestible and less immunogenic. Brown rice contains more protein than polished rice.

Rice grain protein content is a quality trait controlled by multiple genetic factors with a complex genetic basis. There are large differences in the protein content among the different varieties. Chen et al. determined the grain protein content and reported its range from 44.06 to 106.71 mg/g in 2014 and 32.64 to 80.08 mg/g in 2015. Similarly, Yang et al. 2019 used near infrared spectroscopy (NIR) to measure the protein content for two-years and showed that the protein content of rice varied from 5.33% to 14.83%, and the protein content of most varieties was distributed between 7.5% and 11.5%. Liu et al. 2005 measured the protein content of in japonica rice varieties collected from different regions in China and reported the range from 6.45% to 11.1%, with an average of 8.26%. Webb et al. analyzed the protein content of approximately 4,000 rice varieties from different countries (57) and found that the protein content ranged from 5.3% to 13.6%.

Rice protein is a typical quantitative trait, phenotypic differences in protein content among genotypes due to effects of the environment. Ecological factors, such as temperature, light, and carbon dioxide concentration, and environmental factors, such as cultivation, affect the protein content of rice. The interaction between the protein content genotype and the environment is large, and the heritability is relatively small. Some studies have shown that the heritability of the phenotype of protein content in rice is only 13.0% and 37.2% (Hillerislambers D et.al) Some reports reveal the heritability can reach 58.8% (Tsuzuki E et.al). The protein content of the rice grain is sensitive to temperature changes during the grain-filling stage. High temperatures during the filling stage usually led to an increase in grain protein content, decrease in

amylose content and taste value, and a decrease in grain quality (Sanadya.A et.al. 2023).

The high temperature during the mature stage leads to abnormal rice quality, shape, and color, due to a decrease in enzyme activity, respiratory consumption of assimilation products, at grain filling stage (Tsukaguchi T, lida Y.2008 and Jin Z et.al). Light, is another key factor that affects protein synthesis. It affects the increase in essential aminoacids such as lysine and threonine, in rice harvested after low-light treatment at the filling stage; however, cooking quality decreased (Liang CG et.al, 2015). Some studies have shown that the atmospheric concentration of CO2 has an important effect on rice quality. Goufo et al. 2014 demonstrated that elevated carbon dioxide levels result in reduced protein content in plants (inhibition of nitrate assimilation).

Moisture and fertilizer are the two most important factors in cultivation management. Soil moisture status has a significant impact on yield and grain quality (Dingkuhn M, Le Gal PY. 1996). Rice is generally grown under submerged conditions. The protein content is the most affected by water management (Cheng W et.al) Nitrogen is crucial for plant growth and development. As nitrogen is still the main component of proteins, applying nitrogen fertilizer can also significantly affect the quality of grains (Upadhyay R et.al). The application of nitrogen fertilizer at different stages of panicle differentiation, heading, flowering, and grain filling could significantly increase the content of grain storage protein (Chen Y et.al.). In addition to nitrogen, potassium is another fertilizer necessary for rice production. Some studies have shown that the application of potassium fertilizer increased gel consistency and grain protein content but had no significant effect on gelatinization temperature and amylose content (Bahmaniar MA,Ranjbar GA,2007). Salinity is another important factor that significantly influences crop quality. Siscar-Lee et al. found that salt-tolerant and salt-sensitive rice varieties grown in saline-alkali soil had higher storage protein content than those grown in normal soil. While testing the varieties in experimental fields, a specific amount of nitrogen fertilizer used is in a different concentration compared to generally used by farmer's fields (generally the applied nitrogen will be higher). Hence, the mega varieties will generally note protein content of around 6% in experimental fields and this value is lesser than the high protein varieties identified and released through different experimental trials. Due to continuous raise in the price of fertilizer, the cost of cultivation has been increasing. Hence, the rice varieties having higher nitrogen assimilation efficiency along with higher percolation into grain are required.

V CONCLUSION

- In recent studies mutant lines promising for protein content and protein classes were identified.
- Ti-128 (13.01%), Ti-60 (12.76%), Ti-49 (11.4) %), Ti-3 (10.9%) and Ti-40 (10.57%) were found promising for total protein content.
- None of the mutant has shown higher amounts in all protein classes. Ti-8, SM-70, SM-71 shown highest albumin content; BB-134, Ti-53, Ti-49, Ti-26, Ti-27 highest Globulin content; Ti-10, Ti-4, Ti-5 for Prolamins and Ti-56, Ti-55, Ti-22, Ti-113 shown high Glutelin content.
- TI-6, TI-56, TI-45, TI-113, TI-40, TI-15, TI-60, SM-70, TI-49 and TI-128 etc. mutant lines has shown desirable total protein content and protein classes.
- This material can be considered as nutritionally good among the Samba Mahsuri mutants.
- Further study is required to know the relationship between rice protein, cooking, and eating quality is required to provide a scientific basis for breeders to select and cultivate varieties with superior tastes with high nutritive values as well as good cooking and eating qualities

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Parameter		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Protein	Genotype	97	923.5	9.521	4.304	2.42E-12***
	Residuals	98	216.8	2.212		
Protein (Ex)	Genotype	97	128.8	1.3281	54.21	<2E-16***
	Residuals	98	2.4	0.0245		
Albumins	Genotype	97	72.13	0.7436	40.5	<2E-16***
	Residuals	98	1.8	0.0184		
Globulins	Genotype	97	475	4.897	76.62	<2E-16***
	Residuals	98	6.3	0.064		
Prolamins	Genotype	97	0.5998	0.006184	36.26	<2E-16***
	Residuals	98	0.0167	0.000171		
Glutelins	Genotype	97	254.39	2.6226	203	<2E-16***
	Residuals	98	1.27	0.0129		

Table.1 : Two-way ANOVA of total protein & total protein(e) and Protein classes in Samba Mahsuri EMS mutants Significant at P 0.001 (***), P 0.01 (**) and P 0.05 (*)

S.No.	Mutant	Protein%	Protein (Ex)%	Albumin%	Globulin%	Prolamin%	Glutelin%
							Mutants Protein & Classes
1	93R	7.87 ±1.19	0.86±0.05	1.46±0.07	3.05±0.17	0.03±0.01	0.87±0.01
2	BB111	5.94±0.04	1.59±0.08	2.18±0.12	6.28±0.02	0.07±0.00	1.81±0.18
3	BB114	4.84±0.93	3.09±0.11	1.09±0.01	3.54±0.02	0.04±0.01	1.50±0.08
4	BB116	5.98±0.07	2.25±0.07	2.13±0.24	5.48±0.15	0.02±0.01	2.25±0.02
5	BB129	8.05±1.63	3.15±0.02	1.67±0.30	3.86±0.07	0.02±0.01	3.32±0.13
6	BB130	5.39±0.50	3.28±0.16	1.54±0.18	4.95±0.37	0.02±0.00	3.32±0.08
7	BB131	5.27±1.22	2.45±0.20	2.52±0.25	4.64±0.29	0.03±0.01	2.85±0.12
8	BB134	5.76±0.88	3.09±0.02	1.74±0.18	10.30±0.23	0.02±0.00	1.73±0.03
9	BB32P1	6.51±0.57	2.50±0.07	1.92±0.09	1.46±0.09	0.05±0.01	1.52±0.05
10	BB32P2	5.44±0.74	1.36±0.10	1.81±0.10	3.64±0.01	0.05±0.00	0.84±0.01
11	BB36P1	4.01±0.33	2.19±0.07	0.80±0.01	4.48±0.28	0.03±0.01	1.16±0.14
12	BB36P2	3.91±0.38	2.43±0.12	0.57±0.04	5.17±0.02	0.16±0.04	1.63±0.00
13	BB36P3	10.12±4.07	1.92±0.09	2.32±0.05	3.61±0.47	0.03±0.01	1.52±0.04
14	BB36P4	5.40±0.23	1.55±0.18	1.77±0.13	5.72±0.96	0.03±0.00	1.68±0.01
15	BB38	4.83±0.02	0.95±0.01	1.14±0.05	4.48±0.09	0.01±0.00	2.07±0.03
16	BB42	7.76±1.73	1.79±0.30	0.94±0.01	3.15±0.20	0.02±0.01	1.72±0.07
17	BB49	4.89±0.53	1.77±0.09	1.77±0.09	6.18±0.33	0.02±0.00	1.47±0.12
18	BB61	5.95±0.16	1.45±0.10	1.26±0.01	3.64±0.05	0.03±0.01	1.95±0.11
19	BB72	5.80±0.95	1.84±0.08	1.16±0.01	4.37±0.15	0.09±0.01	1.43±0.07
20	BB93	5.73±0.35	2.63±0.08	1.43±0.04	4.43±0.04	0.05±0.00	1.18±0.15
21	BPT	11.30±1.03	0.43±0.02	0.86±0.05	3.55±0.09	0.04±0.00	2.45±0.05
22	SB166	5.74±1.00	1.66±0.04	0.62±0.15	2.52±0.09	0.05±0.02	1.75±0.21
23	SB17	8.42±3.71	2.03±0.03	0.62±0.13	4.02±0.15	0.06±0.01	1.24±0.01
24	SB170	4.03±0.31	1.12±0.07	0.75±0.12	2.28±0.04	0.14±0.04	0.75±0.04
25	SB18	4.83±0.20	1.25±0.82	0.80±0.14	2.32±0.01	0.03±0.00	1.85±0.04
26	SB20	4.00±1.73	1.09±0.09	0.71±0.19	2.42±0.03	0.06±0.01	1.26±0.04
27	SB22	6.64±2.04	0.93±0.02	1.04±0.09	3.93±0.23	0.04±0.00	2.75±0.05
28	SB23	5.12±0.67	1.47±0.09	0.56±0.02	2.52±0.12	0.07±0.02	0.95±0.01
29	SB52	6.44±1.64	1.77±0.04	1.76±0.41	4.56±0.01	0.04±0.00	0.98±0.11
30	SB6	5.42±1.53	2.73±0.13	1.21±0.00	2.09±0.02	0.07±0.02	1.22±0.00
31	SB62	3.20±0.13	2.75±0.09	0.89±0.05	3.02±0.11	0.11±0.02	1.44±0.03
32	SB8	8.78±1.99	0.94±0.03	0.95±0.24	4.26±0.15	0.08±0.03	1.12±0.07
33	SB87	7.67±4.67	1.58±0.03	0.75±0.01	3.27±0.08	0.06±0.01	1.10±0.05

34	SM48	4.34±0.60	0.81±0.05	1.26±0.01	3.60±0.13	0.04±0.00	3.27±0.01
35	SM70	2.08±0.77	1.05±0.14	2.64±0.09	6.92±0.92	0.05±0.01	0.08±0.01
36	SM71	3.45±0.35	1.53±0.05	2.56±0.20	2.60±0.00	0.10±0.02	1.08±0.18
37	SM92	2.76±0.30	1.64±0.01	1.16±0.10	3.40±0.15	0.06±0.01	1.29±0.02
38	Ti10	7.63±0.58	2.72±0.03	0.63±0.05	3.17±0.22	0.26±0.01	1.35±0.12
39	Ti11	5.90±1.07	1.99±0.15	0.90±0.02	4.25±0.28	0.06±0.01	1.98±0.13
40	Ti112	9.26±0.40	1.40±0.04	1.29±0.15	4.91±0.02	0.07±0.01	2.56±0.12
41	Ti113	6.16±0.55	1.23±0.01	1.28±0.21	3.65±0.04	0.08±0.00	3.41±0.01
42	Ti114	3.80±1.16	2.38±0.01	1.51±0.13	3.12±0.07	0.05±0.01	2.19±0.01
43	Ti12	6.03±1.85	1.67±0.25	0.74±0.02	2.09±0.07	0.05±0.00	1.43±0.09
44	Ti125	5.51±0.25	2.30±0.03	0.99±0.08	6.14±0.57	0.14±0.01	3.31±0.04
45	Ti128	13.01±3.05	2.07±0.01	1.23±0.12	2.83±0.24	0.10±0.01	1.83±0.04
46	Ti13	6.95±0.62	2.25±0.20	0.65±0.02	3.18±0.04	0.02±0.00	1.82±0.14
47	Ti132	9.40±1.53	1.96±0.01	0.47±0.01	4.51±0.26	0.20±0.01	1.23±0.07
48	Ti139	3.73±1.83	1.94±0.09	1.02±0.13	2.92±0.20	0.03±0.01	3.35±0.26
49	Ti14	7.41±1.85	1.80±0.11	0.96±0.02	3.00±0.00	0.02±0.01	2.78±0.01
50	Ti15	7.55±1.92	1.07±0.02	0.92±0.01	4.92±0.07	0.10±0.00	1.52±0.18
51	Ti16	4.27±1.68	1.71±0.35	0.73±0.09	3.64±0.05	0.14±0.00	1.44±0.05
52	Ti166	7.40±0.51	1.06±0.12	2.91±0.03	6.60±0.41	0.07±0.01	4.25±0.24
53	Ti167	9.15±4.12	0.91±0.01	0.70±0.00	6.62±0.35	0.10±0.01	2.22±0.03
54	Ti17	3.58±0.33	1.59±0.08	0.58±0.10	3.72±0.09	0.08±0.01	3.78±0.03
55	Ti170	8.33±1.08	1.24±0.07	1.04±0.09	3.31±0.07	0.19±0.01	2.15±0.09
56	Ti18	8.59±2.25	2.86±0.36	0.52±0.03	3.78±0.02	0.06±0.03	1.92±0.08
57	Ti19	7.21±4.02	1.33±0.05	1.56±0.05	3.55±0.01	0.04±0.01	1.61±0.21
58	Ti20	6.26±0.80	1.51±0.68	0.67±0.00	3.64±0.01	0.10±0.00	2.52±0.01
59	Ti21	7.00±0.42	0.39±0.02	2.90±0.02	3.41±0.01	0.12±0.02	0.72±0.03
60	Ti22	6.56±0.30	2.28±0.11	1.30±0.04	2.55±0.16	0.14±0.00	4.07±0.01
61	Ti23	3.02±0.83	1.04±0.07	1.26±0.04	2.02±0.33	0.03±0.00	0.31±0.04
62	Ti25	6.92±1.83	2.66±0.12	0.57±0.13	4.30±0.40	0.10±0.02	3.88±0.10
63	Ti26	5.75±0.34	2.38±0.03	2.86±0.22	7.32±0.29	0.02±0.00	3.09±0.00
64	Ti27	8.80±1.77	0.30±0.03	0.48±0.09	7.38±0.16	0.01±0.00	2.42±0.14
65	Ti28	6.83±0.85	1.42±0.21	1.19±0.14	5.55±0.09	0.01±0.00	2.60±0.02
66	Ti29	6.87±3.10	0.59±0.09	1.09±0.02	3.15±0.12	0.02±0.00	0.98±0.01
67	Ti3	10.91±1.20	1.78±0.26	1.10±0.02	5.78±0.40	0.09±0.01	1.22±0.23

68	Ti30	5.92±0.71	0.78±0.11	0.62±0.04	2.68±0.23	0.02±0.00	0.79±0.01
69	Ti33	5.73±1.07	0.26±0.03	1.17±0.01	4.55±0.10	0.05±0.02	1.55±0.03
70	Ti34	5.63±0.03	0.79±0.04	0.89±0.05	6.01±0.38	0.06±0.01	3.79±0.03
71	Ti35	4.85±1.87	1.17±0.16	0.92±0.02	3.25±0.09	0.02±0.00	1.37±0.24
72	Ti36	8.04±0.33	1.32±0.04	0.56±0.02	3.15±0.04	0.03±0.01	0.96±0.03
73	Ti37	4.66±0.91	1.19±0.16	0.84±0.02	2.85±0.02	0.03±0.00	0.26±0.02
74	Ti38	7.20±1.61	0.64±0.05	0.59±0.18	5.82±0.10	0.03±0.00	1.32±0.07
75	Ti4	6.98±0.49	2.28±0.13	0.99±0.02	3.09±0.11	0.25±0.05	2.60±0.04
76	Ti40	10.57±0.58	1.34±0.05	0.97±0.10	5.55±0.21	0.01±0.00	1.78±0.02
77	Ti41	10.17±0.32	0.73±0.11	0.37±0.07	2.33±0.29	0.02±0.01	0.85±0.01
78	Ti42	7.46±1.22	0.73±0.02	0.95±0.04	2.73±0.01	0.04±0.01	2.18±0.20
79	Ti43	6.12±0.30	1.21±0.07	0.60±0.03	2.46±0.00	0.02±0.00	2.35±0.10
80	Ti44	7.64±0.85	0.47±0.02	0.96±0.00	3.56±0.42	0.03±0.00	1.78±0.02
81	Ti45	7.26±1.29	1.47±0.03	1.20±0.31	3.56±0.29	0.04±0.00	2.98±0.20
82	Ti46	7.74±0.12	0.15±0.03	1.36±0.03	3.06±0.26	0.01±0.00	0.75±0.18
83	Ti47	6.22±1.36	2.17±0.16	1.66±0.35	2.28±0.02	0.07±0.01	1.37±0.20
84	Ti48	5.28±0.39	0.80±0.12	3.13±0.60	3.40±0.20	0.02±0.00	0.74±0.07
85	Ti49	11.40±0.25	1.99±0.23	0.62±0.20	6.27±0.08	0.05±0.00	0.99±0.08
86	Ti5	8.65±0.80	3.02±0.08	1.26±0.01	3.23±0.09	0.21±0.02	2.32±0.05
87	Ti50	5.84±0.71	1.76±0.01	1.53±0.01	3.18±0.23	0.04±0.00	0.25±0.07
88	Ti52	3.43±1.05	1.13±0.03	1.89±0.23	4.06±0.02	0.04±0.00	1.91±0.07
89	Ti53	5.49±2.03	3.87±0.16	1.33±0.02	7.96±0.79	0.05±0.01	3.12±0.29
90	Ti55	5.22±0.57	3.47±0.16	1.11±0.03	4.39±0.51	0.10±0.00	4.26±0.22
91	Ti56	9.47±0.78	3.75±0.00	1.33±0.10	3.75±0.10	0.07±0.01	8.04±0.10
92	Ti57	5.09±0.45	1.72±0.16	1.14±0.05	1.77±0.07	0.20±0.02	2.06±0.04
93	Ti6	7.30±0.33	1.73±0.03	1.16±0.01	3.56±0.16	0.12±0.00	2.60±0.11
94	Ti60	12.76±0.53	1.09±0.05	2.03±0.13	1.41±0.14	0.09±0.01	3.59±0.34
95	Ti62	8.40±2.15	0.95±0.01	1.57±0.09	7.02±0.38	0.07±0.02	1.22±0.23
96	Ti7	10.25±1.69	1.12±0.09	1.23±0.14	2.87±0.08	0.15±0.02	1.41±0.01
97	Ti8	5.14±0.22	1.12±0.04	0.67±0.04	2.02±0.03	0.19±0.01	0.70±0.12
98	Ti9	6.40±3.05	0.26±0.03	0.78±0.04	4.44±0.21	0.19±0.02	0.93±0.14

. Pearson Table. 2

Fig 1
. correlation chart among Protein and Protein classes. Total Protein means Protein estimated by extraction buffer method.The x and y axis represent the two parameters that are being correlated respectively.
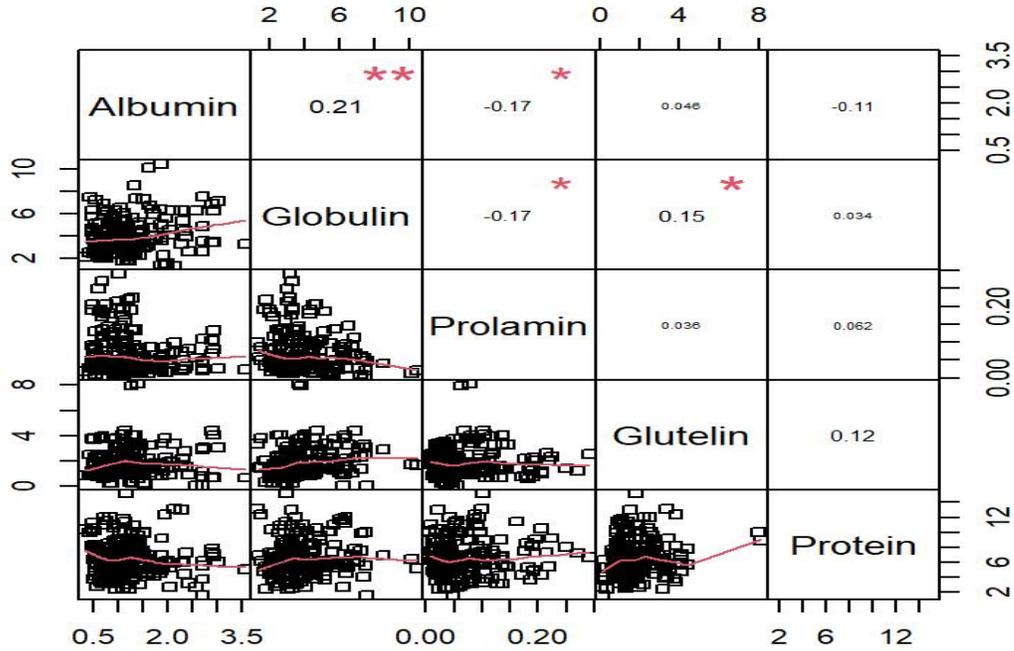
Fig .1. Pearson correlation chart among Protein and Protein classes. Total Protein means Protein estimated by extraction buffer method.The x and y axis represent the two parameters that are being correlated respectively.