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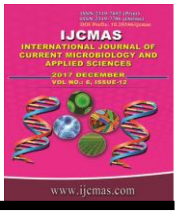

Original Research Article

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An Insight into Total Soluble Proteins across Rice (*Oryza sativa* L.) Germplasm Accessions

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ABSTRACT

Cereals are the staple foods of mankind and they provide the major portion of the energy, protein and other nutrients in the diets. Protein is an essential component required for growth, antibodies production and immunity in human beings. Screening and evaluation of protein content in 150 rice germplasm accessions was undertaken to identify protein rich germplasm. The germplasm accessions comprised of landraces from different parts of the country and world, CHIR lines, IG lines, ARB lines and RPHP lines. The results of the study revealed that total soluble protein content ranged from 7.54 g/100g to 14.54 g/100g of sample. Among 150 accessions, eight lines had recorded significantly higher protein content (>10.50 g/100g), 48 lines had registered moderate content (9.01 to 10.50 g/100g) and 94 lines had registered low protein content (< 9.00 g/100g). Cluster analysis based on available protein content revealed that, 150 accessions were grouped into two major clusters at the similarity coefficient of 2.24. Cluster I is comprised of three genotypes *viz.*, RG1 (Mapillaisamba), RG7 (Kudaivazhai) and RG110 (Norungan) which recorded higher protein content (14.54 to 12.38g/100g) and these are landraces of Tamil Nadu. Cluster II comprised of 147 accessions that branched into three sub clusters. Sub-cluster 1 had genotypes with high protein content in the range of 10.70 to 10.96 g/100g, while sub cluster II consisted of 48 genotypes with moderate protein content in the range of 9.16 to 10.25g/100g. Finally, 94 genotypes with low protein content (7.54 to 8.71g/100g) fell in sub cluster 3. The high protein genotypes identified in this study can be potentially utilized for mapping of key genomic regions associated with protein content in rice and in biofortification programmes.

Keywords

Rice, Total soluble proteins, Landraces, Germplasm, Clustering.

Article Info

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Introduction

Among cereals, rice is one of the staple foods for more than half of the world's population and it has defined the culture, diets and economies of billions of people. In Asia, it has a very long history of cultivation and is deeply rooted in the daily lives of people (Narayanan *et al.*, 2000). Cereals based food habits had created the situation of under nutrition and it is one of the ever increasing issues in the developing world most vulnerable to the protein deficiency. Only twenty per cent of the people in the world have access to nutritious diet. Protein energy malnutrition approximately affects 230 million of children in developing countries (WHO Bulletin, 1993).

In India, more than 50 per cent of children received insufficient calories everyday to meet their potential growth and development requirements (Mahendra *et al.*, 2004). Sub-Saharan Africa and South Asia are expected to remain "Hot Spots" of child malnutrition in 2020 (Andersen *et al.*, 1999).

Studies on children recovering from protein energy malnutrition demonstrated that essential amino acids like lysine and tryptophan were important in improving nitrogen retention (Pellett and Ghosh, 2004). The processing of rice grain in terms of milling removes the bran layer from the endosperm. This implies not only the loss of a nutritionally valuable rice component in human diet but also a reduction of the quantity of rice available for human nutrition by around 10 to 15 per cent. The loss of protein from brown rice during milling ranged from 11.0 to 26% at milled rice recoveries of 86-90% and it was found that high protein rice offered greater resistance to milling by standard methods. Glutelin (alkali or acid soluble proteins) account for 80% or more of the total seed protein and thus the principal protein of whole grain (brown and milled rice), while albumin and globulin were the major proteins in rice bran (Cagampang *et al.*, 1966; Juliano, 1972; Villareal and Juliano, 1978). Refined milling of successive layers of brown rice has revealed that the second outermost 5% fraction had the highest level of protein (Hogan *et al.*, 1964).

Milled rice is the staple food of tropical Asia and it contributes 40 to 80 per cent of the calories and 40 per cent of the protein requirement (Shobarani *et al.*, 2006). Compared to other protein sources, rice protein is deficient in lysine, the sulphur containing amino acid and tryptophan (FAO, 1957), but the lysine content of rice protein (3.5 to 4.0 percent) is highest among cereal proteins. A positive correlation of tryptophan with lysine content (r = 0.076) and a strong negative correlation with total protein content (r = - 0.923) was recorded by Banerjee *et.al* (2011).

Environment affects the protein content of cereals and in rice, the grain protein accumulation of a variety exhibited a range of about 6% on dry weight basis (IRRI, 1963). The average protein content of milled rice is relatively low (about 7% at 14% moisture) whereas brown or dehulled rice contains about 8% protein. Increasing the protein content of rice would mean an increased supply of protein in rice based diets. Rice represents one of the largest *ex-situ* germplasm collections in the world which includes many land races breeding lines, donors and their wild relatives.

The biodiversity among these collections offers a valuable resource for various essential nutrients *viz.*, zinc, iron, proteins and vitamins. Screening and exploitation of these resources is the only source for genetic improvement of protein and other essential micronutrients in an improved cultivar which opens up a new wizard for the elimination of malnutrition (Khush and Virk, 2002). Enriching rice with protein either through conventional or marker assisted introgression holds a great promise for sustainable food based solutions (Graham *et al.*, 2001). The primary objective of the present investigation was to assess the variability of rice protein content in an association mapping panel for their subsequent utilization in the genetic enhancement of rice.

Materials and Methods

Genetic materials

The materials which were utilized in the present study consisted of 150 germplasm accessions collected from different sources such as Paddy Breeding Station (PBS), Department of rice germplasm stock, International Rice Research Institute (IRRI) germplasm maintained at Paddy Breeding Station, Harvest Plus lines from Directorate of Rice Research, CHIR and ARB lines from West Bengal and Karnataka. It includes 50 landraces (traditional varieties) from Tamil Nadu, 52 lines from IRRI germplasm, four Improved Chinsurah lines from West Bengal, two IRRI varieties, five landraces from different parts of our country other than Tamil Nadu, 22 RPHP lines from Harvest Plus project, four ARB lines from Karnataka, one variety from Kerala (Pattambi), one improved line from Andhra Pradesh, three IRRI cultures, one variety from West Bengal and five improved varieties from Tamil Nadu.

Experimental design

The study was undertaken at Department of Rice, Tamil Nadu Agricultural University, Coimbatore during *Kharif*, 2015. Crop was raised under irrigated condition in an area of 26 cents with the recommended fertilizer dosage of 150:60:60 kg/ha of NPK. Once it attained physiological maturity, plants were harvested; seeds cleaned and dried up to the moisture level of 13%.

Three samples per accession were evaluated for total soluble protein content. The dried seeds were dehusked using hand palm dehusker, ground evenly and sieved up to the talcum powder size with pestle and mortar. The powdered samples of unpolished rice were used for protein analysis using modified Lowry's method (Hartree, 1972).

Extraction of protein

Sample preparation

Powdered samples were subjected to extraction of protein by 0.1M phosphate buffer with pH of 7.4. One gram of sample from each entry was macerated with 50 ml of phosphate buffer using Pestle and Mortar and centrifuged at 4000 rpm for 20 minutes. The supernatants were collected for protein estimation by discarding the pellet. The above steps were performed for each entry separately until a clear extract was obtained. The extract was stored in deep freezer until further analysis.

Protocol

Total protein was estimated by modified Lowry's method given by Hartree, 1972. Determination of protein concentration by ultraviolet absorption depends on the presence of aromatic amino acids in the proteins. Although different proteins will have different amino acid compositions and thus different molar absorptivities, this method can be very accurate when comparing different solutions of the same protein (UK assays, 2017).

Extracted samples of 0.2ml were taken into test tube and the volume was made up to 1 ml with distilled water. To it, 4.5 ml of alkaline $CuSO_4$ reagent was added and incubated at room temperature for 10 minutes followed by 0.5 ml of Folin's phenol reagent. The contents were mixed well and the absorbance was measured at 650 nm after 15 minutes in a spectrophotometer. From the standard graph, the amount of protein in the given unknown solution was calculated.

Results and Discussion

Protein energy malnutrition affects 25% of children where their dietary intake is mainly on rice and staple crops have low levels of essential amino acids (Gearing, 2015). Rice is the principal source of carbohydrate and protein for the people in South East Asia (Cagampang *et al.*, 1966) though it has the lowest protein level of 7.3g/100g in brown rice among the cereals (Souci, Fuchmann and Kraut, 1986). Based on the mean true digestibility of egg, milk, cheese, meat and fish protein of 95 percent, the relative digestibility of milled rice is 93 percent (WHO, 1985) Recommended Dietary Allowance for protein per day is 0.8 gram per kilogram of bodyweight (Harvard Men's Health Watch, 2015). Though other sources of food rich in protein like egg, meat, milk, fish and pulses are present, their affordability across the section of society differs. The low income groups of people still depend on rice based diet. Protein energy and micronutrient malnutrition are widespread among rural and poor population. Hence, to meet out the required threshold level of protein in their rice based diet, finding a good source of rice protein from the available gene pool of rice is essential.

Total protein content

Among 150 germplasm accessions taken for our study, the protein content ranged from 7.54 g/100g in RG 96 (RP BIO 226) to 14.70 g/100g in RG1 (Mapillaisamba) with the coefficient of variation of 0.4% (Table 1). Fifty six lines were found to record significantly higher protein content than the grand mean of 8.88 g/100g. About 62.67% (94 accessions) had low protein content (< 9.00 g/100g) and 5.33 % (eight accessions) recorded higher protein content of more than 10.50 g/100g of sample. About, 32.0% of accessions recorded moderate level of protein content with range of 9.01 to 10.50 g/100g, (Fig. 1 and Table 2). It could be realized that in general most of the genotypes descend under the category of low protein. However, there is a handful of material representing 5.0% of the collections with high protein that gives the ray of hope that a significant variation exists among the rice germplasm materials taken for our investigation.

The earlier workers Kennedy and Burlingame (2003), Cao *et al.*, (2009), Silveira *et al.*, (2010) proposed a classification for the protein contents as high (≥12%), medium (11.9–9%), and low (≤8.9%). Based on the range of protein content present in our association mapping panel, classification of high (>10.5g/100g), moderate (9.01-10.5g/100g) and low levels of proteins (<9.0g/100g) is followed. Heda and Reddy (1984) also considered values >10% as high protein content analyzed in their F_1 to F_3 progenies of six crosses. Studies have shown that protein content in 1622 milled rice samples from 24 countries ranged from 4 to 14 percent and mean protein ranged from 6.3 to 9.2 percent and the overall mean was 7.8 percent (Juliano and Villareal, 1993) at 12% moisture.

Kennedy and Burlingame (2003) analyzed the protein contents of 2,869 genotypes of rice (2,674 *O. sativa* and 195 *O. glaberrima*), and found 8.8% as the mean for *O. sativa*, ranging from 4.5 to 15.9%. The mean protein content of 8.88% in our present analysis comprising of *O. sativa* germplasm and the highest protein estimation of 14.70 g/100g is in almost close correspondence with their results.

The crude protein content of ten Philippine rice cultivars evaluated by Riza *et al.*, (2004) showed a range of 6.3% (PR-27423-MS6) to 9.1% (PR-31595-PSC101). Wide variation of as low as 2.8% to 9.9% for protein concentration in milled rice germplasm lines of Chhattisgarh have been reported by Chandel *et al.*, (2005). Cao *et al.*, (2009) found a wide variation in the levels of storage protein content (7.38–15.41%) in Chinese varieties of *O. sativa* rice.

A wide variability of 6.19 to 10.75% with a mean value of 8.07% for protein content was observed by Banerjee *et al.,* (2010) in brown rice of 46 genotypes including cultivated *indica* and *japonica* cultivars, germplasm accessions, advanced breeding lines and wild rice genotypes estimated by modified micro Kjeldahl method. Silveira *et al.,* (2010) found a range of 4.4– 20.2% for storage protein contents of 550 accessions, in the rice Core Collection of Embrapa, with an average of 10.31%.

Protein content of milled grain among 258 land races of rice belonging to the extra-early group (95 days) maintained at IGKV, Raipur, Chhattisgarh ranged from 4.91% -12.08 % with the mean of 6.63 ***%.*** Out of total 258 lines analyzed, fifty two were found with < 6.0% protein**,** 202 landraces were found to possess protein levels between 6.0 - 9.0% whereas, four lines were found with >9.0% of grain protein (Banerjee *et al.,,* 2011). According to Totok *et al.,* (2011), genotype x location interaction influenced grain protein content of 10 upland rice genotypes. They could identify three genotypes namely UNRAM 4E, UNRAM 9E, UNRAM 17E to possess stable protein content of > 8.0% protein in their milled rice by Kjeldahl's method. Mohanty *et al.,* (2011) reported 16.41 and 15.27% of crude protein in brown rice of ARC 10063 and ARC 10075 rice accessions respectively on dry weight basis.

They observed the total free amino acid content to be higher in these accessions and lysine content was positively correlated with the grain protein content in contrary to the view of Juliano *et al.,* (1964) and Cagampang *et al.,* (1966). Hanumantrao (2013) estimated protein content in brown rice of 58 rice germplasm lines and varieties by modified Micro kjeldahl method. The protein content varied from 6.09 to 11.2% estimated in the varieties CGR-436 and Danteshwari. By exploiting ARC 10075 as a donor, CR Dhan 310 (IET 24780) rice variety was developed with high protein content of 11% and rich in threonine and lysine (NRRI Annual Report 2014–2015).

Santos *et al.,* (2013) analyzed twenty nine accessions of the wild rice species *Oryza glumaepatula*, collected from five Brazilian states and two commercial cultivars for storage protein profile and amino acid content. Total protein levels ranged from 14.94% (wild genotype BGA14280) to 9.07% (BGA14179). The control cultivars BRS Bonança and Primavera, together with the wild accessions BGA14210, BGA14232, BGA14233, and BGA14179, showed the lowest levels of total protein. Seven accessions possessed high protein with the range of 13.98 to 14.94%. The second group had nine accessions with a range of 12.3 to 13.35%. Thus, out of 29 genotypes evaluated, 16 had high total protein content and 13 had medium protein content. A scrutiny of above findings on the estimation of protein content by different workers reveals that the low protein category ranged from 2.8 to 7.38% and the other extreme ranged from 9.07 to 15.9% in genotypes belonging to *O. sativa*. The lowest value of 7.54g/100g recorded in this study material is above the range mentioned by earlier workers.

Diversity of germplasm for total soluble protein content

Cluster analysis was performed to construct a dendrogram depicting the diverse sources of the genotypes in terms of geographic location, (Fig. 2) in order to compare with the three groupings given in Table 2 and also with the protein content classification adopted by earlier workers.

Table.1 Total available protein content (g/100g) in 150 rice germplasm accessions

S.No	RG No	Protein Content	S.No	RG No	Protein content	S.No	RG No	Protein content	S.No	RG No	Protein content
1	RG1	14.54**	24	RG36	9.16**	47	RG65	8.22	70	RG99	8.22
2	RG2	8.29	25	RG37	8.17	48	RG66	8.23	71	RG100	9.66**
3	RG3	9.82 **	26	RG39	9.66**	49	RG67	8.20	72	RG101	8.21
4	RG4	9.91**	27	RG41	8.24	50	RG68	9.66**	73	RG102	9.66**
5	RG5	9.67**	28	RG42	8.19	51	RG69	8.25	74	RG103	8.22
6	RG6	9.68**	29	RG43	9.66**	52	RG70	8.25	75	RG104	9.67**
7	RG7	13.22**	30	RG44	8.26	53	RG71	8.22	76	RG105	8.24
8	RG8	9.87**	31	RG45	8.23	54	RG72	8.23	77	RG106	9.68**
9	RG9	9.70**	32	RG46	9.66**	55	RG74	8.21	78	RG107	8.23
10	RG12	10.96**	33	RG48	8.26	56	RG76	9.65**	79	RG108	9.85**
11	RG14	9.98**	34	RG50	8.23	57	RG77	9.63**	80	RG109	8.22
12	RG15	8.71	35	RG51	8.23	58	RG80	8.22	81	RG110	12.38**
13	RG17	10.70**	36	RG52	8.22	59	RG81	8.22	82	RG112	8.22
14	RG18	7.91	37	RG53	8.23	60	RG82	8.17	83	RG113	8.22
15	RG20	9.54**	38	RG54	9.66**	61	RG83	8.17	84	RG114	9.56**
16	RG22	10.25**	39	RG55	9.67**	62	RG85	8.23	85	RG115	8.21
17	RG25	8.68	40	RG56	8.23	63	RG86	8.2	86	RG116	8.23
18	RG26	8.16	41	RG57	8.21	64	RG89	8.21	87	RG117	9.67**
19	RG31	9.66**	42	RG58	8.24	65	RG91	8.21	88	RG118	8.2
20	RG32	8.61	43	RG59	8.21	66	RG92	8.23	89	RG120	8.21
21	RG33	8.21	44	RG60	9.66**	67	RG95	7.55	90	RG121	10.8**
22	RG34	8.18	45	RG62	10.88**	68	RG96	7.54	91	RG122	8.23
23	RG35	8.22	46	RG63	8.22	69	RG98	8.22	92	RG123	9.65**

*Significant at1% level ** Significant at 5% level

Table.1 Contd…

S.No	Accession No	Protein content	S.No	Accession No	Protein content	S.No	Accession No	Protein content	S.No	Accession No	Protein content
93	RG124	8.22	107	RG142	8.21	121	RG159	9.78**	135	RG175	9.77**
94	RG126	9.66**	108	RG143	9.77**	122	RG160	8.17	136	RG176	9.77**
95	RG127	9.66**	109	RG145	9.65**	123	RG161	8.13	137	RG178	9.77**
96	RG128	9.66**	110	RG146	8.22	124	RG162	9.75**	138	RG180	8.40
97	RG129	8.41	111	RG147	8.22	125	RG163	8.22	139	RG181	8.22
98	RG130	8.24	112	RG148	9.66**	126	RG164	8.22	140	RG182	9.80**
99	RG131	8.21	113	RG149	8.22	127	RG165	8.58	141	RG183	8.38
100	RG132	8.21	114	RG150	8.24	128	RG166	8.23	142	RG184	9.61**
101	RG133	9.16**	115	RG151	8.41	129	RG168	9.80**	143	RG185	9.64**
102	RG134	8.22	116	RG152	8.18	130	RG169	8.13	144	RG186	8.38
103	RG135	8.42	117	RG154	8.38	131	RG170	8.29	145	RG187	9.55**
104	RG136	9.56**	118	RG156	8.17	132	RG172	8.12	146	RG188	9.67**
105	RG137	8.34	119	RG157	8.18	133	RG173	8.22	147	RG189	8.43
106	RG141	8.22	120	RG158	8.19	134	RG174	8.19	148	RG190	9.82**
Grand mean											8.88
Range											7.54 - 14.54
SE (d)											0.032
CD (0.05%)											0.062
CD (0.01%)											0.081
CV%											0.44
*Significant at 1% level ** Significant at 5% level											

Table.2 Grouping of germplasm lines based on mean protein content

Protein content g/100g	Individuals	Number of Accessions	Percentage of individuals	*Grouping
>10.51	RG1,RG7,RG12,RG17,RG62,RG110,RG121,RG191	8	5.33	High
9 to 10.50	RG3,RG4,RG5,RG6,RG8,RG9,RG14,RG20,RG22,RG31, RG36,RG39,RG43,RG46,RG54,RG55,RG60,RG68,RG76,RG77, RG100,RG102,RG104,RG106,RG108,RG114,RG117,RG123, RG126,RG127,RG128,RG133,RG136,RG143,RG145,RG148, RG159,RG162,RG168,RG175,RG176,RG178,RG182,RG184, RG185,RG187,RG188,RG190	48	32.00	Moderate
< 9.00	RG2,RG15,RG18,RG25,RG26,RG32,RG33,RG34,RG35,RG37, RG41,RG42,RG44,RG45,RG48,RG50,RG51,RG52,RG53,RG56, RG57,RG58,RG59,RG63,RG65,RG66,RG67,RG69,RG70,RG71, RG72,RG74,RG80,RG81,RG82,RG83,RG85,RG86,RG89,RG91, RG92,RG95,RG96,RG98,RG99,RG101,RG103,RG105,RG107, RG109,RG112,RG113,RG115,RG116,RG118,RG120,RG122, RG124,RG129,RG130,RG131,RG132,RG134,RG135,RG137, RG141,RG142,RG146,RG147,RG149,RG150,RG151,RG152, RG154,RG156,RG157,RG158,RG160,RG161,RG163,RG164, RG165,RG166,RG169,RG170,RG172,RG173,RG174,RG180, RG181,RG183,RG186,RG189,RG192	94	62.67	Low

*Grouping
>10.50g/100g protein- High
9.01 to 10.50g/100g protein- Moderate
< 9.00g/100g protein- Low

Table.3 Clustering and grouping pattern of rice germplasm using UPGMA method

Main cluster	Sub cluster	Accessions	No. of Accessions	Cluster mean range(g/100g)	*Grouping
I	1	RG1	1	14.54	High
	2	RG7,RG110	2	13.22 to 12.38	High
II	1	RG12,RG62,RG17,RG191,RG121	5	10.70 to10.96	High
	2	RG3,RG4,RG5,RG6,RG8,RG9,RG14,RG20, RG22,RG31,RG36,RG39,RG43,RG46,RG54, RG55,RG60,RG68,RG76,RG77,RG100,RG102, RG104,RG106,RG108,RG114,RG117,RG123, RG126,RG127,RG128,RG133,RG136,RG143, RG145,RG148,RG159,RG162,RG168,RG175, RG176,RG178,RG182,RG184,RG185,RG187, RG188,RG190	48	9.16 to 10.25	Moderate
	3	RG2,RG15,RG18,RG25,RG26,RG32,RG33,RG34, RG35,RG37,RG41,RG42,RG44,RG45,RG48,RG50, RG51,RG52,RG53,RG56,RG57,RG58,RG59,RG63, RG65,RG66,RG67,RG69,RG70,RG71,RG72,RG74, RG80,RG81,RG82,RG83,RG85,RG86,RG89,RG91, RG92,RG95,RG96,RG98,RG99,RG101,RG103, RG105,RG107,RG109,RG112,RG113,RG115,RG116, RG118,RG120,RG122,RG124,RG129,RG130,RG131, RG132,RG134,RG135,RG137,RG141,RG142,RG146, RG147,RG149,RG150,RG151,RG152,RG154,RG156, RG157,RG158,RG160,RG161,RG163,RG164,RG165, RG166,RG169,RG170,RG172,RG173,RG174, RG180,RG181,RG183,RG186,RG192	94	7.54 to 8.71	Low

*Grouping >10.51g/100g- High protein; 9.01 to 10.50g/100g- Moderate protein; < 9.00 –Low protein

Annexure.1 Details of rice genotypes falling under high and moderate levels of total soluble protein content

S.No	Main Cluster	Sub cluster	Group	RG.No.	Genotype	Parentage	Origin
1	I	1	High	RG1	Mapillaisamba	Landrace	Tamil Nadu, India
2	I	2	High	RG7	Kudai vazhai	Landrace	Tamil Nadu, India
3	I	2	High	RG110	Norungan	Landrace	Tamil Nadu, India
4	II	1	High	RG12	Vellai chithiraikar	Landrace	Tamil Nadu, India
5	II	1	High	RG 62	Purpleputtu	Landrace	Tamil Nadu, India
6	II	1	High	RG17	Sivapuchithiraikar	Landrace	Tamil Nadu, India
7	II	1	High	RG191	IG 15(EC 728910- 117901)	Sze guen zim	China
8	II	1	High	RG121	IG 74(EC 728622- 117517)	Kinandang Patong::IRGC23364-1	IRRI, Philippines
9	II	2	Moderate	RG3	Senkar	Landrace	Tamil Nadu, India
10	II	2	Moderate	RG4	Murugankar	Landrace	Tamil Nadu, India
11	II	2	Moderate	RG5	CHIR 6	Improved chinsurah	West Bengal
12	II	2	Moderate	RG6	CHIR 5	Improved chinsurah	West Bengal
13	II	2	Moderate	RG8	CHIR 8	Improved chinsurah	West Bengal
14	II	2	Moderate	RG9	Kuruvai kalanjiyam	Landrace	Tamil Nadu, India
15	II	2	Moderate	RG14	Jothi	Variety	Kerala,India
16	II	2	Moderate	RG20	Kalvalai	Landrace	Tamil Nadu, India
17	II	2	Moderate	RG22	IR 36	IR1561-228-L2/IR1737/ /CR94-13	IRRI, Philippines
18	II	2	Moderate	RG31	Chinthamani	Landrace	Tamil Nadu, India
19	II	2	Moderate	RG36	Kattikar	Landrace	Tamil Nadu, India
20	II	2	Moderate	RG39	Kaatu ponni	Landrace	Tamil Nadu, India
21	II	2	Moderate	RG43	RPHP 129	Kamad	Jammu and Kashmir
22	II	2	Moderate	RG46	IG 4 (EC 729639- 121695)	TD2: : IRGC 9148-1	IRRI, Philippines
23	II	2	Moderate	RG54	PTB 19	Variety	Kerala, India
24	II	2	Moderate	RG55	IG 67(EC 729050- 120988)	IR77384-12-35-3-12-1-B::IRGC117299-1	IRRI, Philippines
25	II	2	Moderate	RG60	Rama kuruvaikar	Landrace	Tamil Nadu, India
26	II	2	Moderate	RG68	IG 63(EC 728711- 117674)	Caawa/Fortuna	IRRI, Philippines
27	II	2	Moderate	RG76	Matta kuruvai	Landrace	Tamil Nadu, India

28	II	2	Moderate	RG77	Karuthakar	Landrace	Tamil Nadu, India
29	II	2	Moderate	RG100	IG 7(EC 729598- 121648)	Vary manity:: IRGC69910-1	IRRI, Philippines
30	II	2	Moderate	RG102	Varakkal	Landrace	Tamil Nadu, India
31	II	2	Moderate	RG104	IG 53(EC 728752- 117719)	Carolina rinolda barsani	Uruguay
32	II	2	Moderate	RG106	Katta samba	Landrace	Tamil Nadu, India
33	II	2	Moderate	RG108	Red sirumani	Landrace	Tamil Nadu, India
34	II	2	Moderate	RG114	RPHP 159	Radhuni pagal	Bangladesh
35	II	2	Moderate	RG117	IG 65(EC 729024- 120958)	GODAHEENATI:: IRGC31393-1	IRRI, Philippines
36	II	2	Moderate	RG123	IG 2(EC 729808-121874)	BLUEBONNET 50::IRGC1181	IRRI, Philippines
37	II	2	Moderate	RG126	Kallimadayan	Landrace	Tamil Nadu, India
38	II	2	Moderate	RG127	IG 10(EC 729686- 121743)	Hasan seralirGC79564 -C1	IRRI, Philippines
39	II	2	Moderate	RG128	IG 75(EC 728587- 117420)	Aedal:: IRGC55441-1	IRRI, Philippines
40	II	2	Moderate	RG133	IG 42(EC 728798- 117774)	Kalubala vee	Srilanka
41	II	2	Moderate	RG136	IG 8(EC 729601- 121651)	Xi you zhan :: IRGC78574-1	IRRI,Philippines
42	II	2	Moderate	RG143	IG 46(IC 471826- 117647)	Baber	India
43	II	2	Moderate	RG145	IG 60(EC 728730- 117695)	Creole	Belize
44	II	2	Moderate	RG148	Chinna aduku nel	Landrace	Tamil Nadu, India
45	II	2	Moderate	RG159	Sembala	Landrace	Tamil Nadu, India
46	II	2	Moderate	RG162	IR 64	IR 5857-33-2-1 x IR 2061-465-1-5-5	IRRI, Philippines
47	II	2	Moderate	RG168	Haladichudi	Landrace	Odisha, India
48	II	2	Moderate	RG175	Vellai kudaivazhai	Landrace	Tamil Nadu, India
49	II	2	Moderate	RG176	Kodai	Landrace	Tamil Nadu, India
50	II	2	Moderate	RG178	IG 17(EC 728900- 117889)	Sigadis	Indonesia
51	II	2	Moderate	RG182	ARB 59	Variety	Karnataka, India
52	II	2	Moderate	RG184	IG 18(EC 728892- 117880)	Seratoes hari	Indonesia
53	II	2	Moderate	RG185	RPHP 36	Variety, TKM 9	Tamil Nadu, India
54	II	2	Moderate	RG187	Vadakathi samba	Landrace	Tamil Nadu, India
55	II	2	Moderate	RG188	RPHP 80	24(K)	Andhra Pradesh, India
56	II	2	Moderate	RG190	IG 26(IC0590943- 121899)	Basmati 370 ::IRGC 3750-1	IRRI, Philippines

Fig.1 Pie chart representation of protein content among 150 rice germplasm accessions

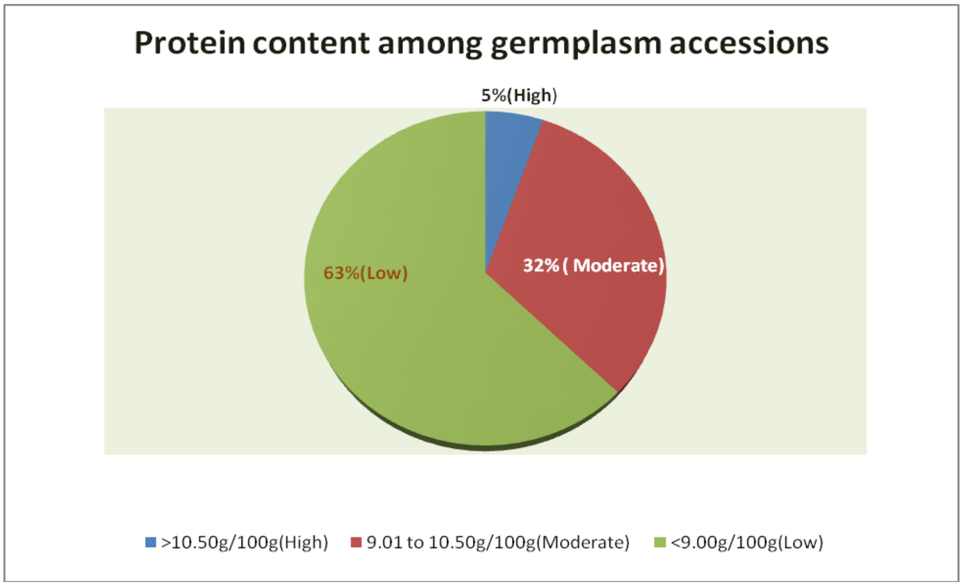
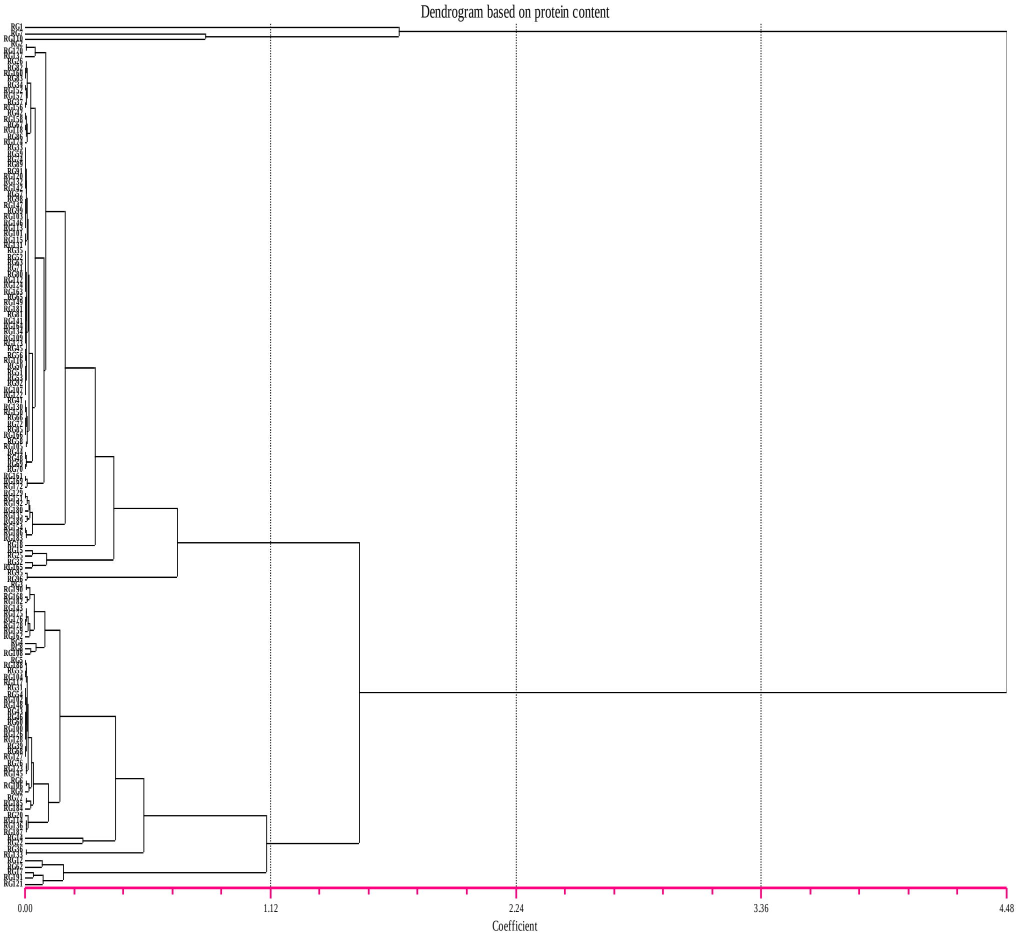


Fig.2 Clustering and grouping pattern of rice germplasm based on protein content



There was a formation of two major clusters at similarity coefficient of 2.24 (Table 3). Main Cluster I comprised of three genotypes namely RG1 (Mapillaisamba), RG7 (Kudaivazhai) and RG110 (Norungan) with very high protein content in the range of 12.38 to 14.54 g/100g rice sample. Thus it fitted perfectly with the classification of high protein content (> 12%) by earlier workers.

Major cluster II accommodated 147 genotypes branching into three sub clusters. Sub-cluster I with five accessions namely RG12 (Vellaichithiraikar), RG62 (Purpleputtu), RG17 (Sivapuchithiraikar), RG191 (IG15) and RG121 (IG74) were categorized as possessing high protein content in the range of 10.70 to10.96 g/100g. Sub-cluster 2 had 48 accessions (32.0%) with moderate protein content in the range of 12.01 to 19.01g/100g. Sub-cluster 3 had 94 genotypes representing 62.7% of the material with low protein content which ranged from 7.54 to 8.71 g/100g.

Cluster analysis grouped the 258 rice landraces into ten clusters (Banerjee *et al.*, 2011). The cluster IV consisted of maximum number of 36 rice landraces followed by cluster III containing 31 landraces, cluster VIII containing 30 landraces. Cluster VIII consisted of minimum number of 19 landraces.

A UPGMA dendrogram was derived through Nei's unbiased genetic similarity coefficients among 173 rice landraces based on their protein profile by Pervaiz *et al.*,(2011). Four distinct major clusters were derived at similarity value of 0.91. The first cluster consisted of four accessions with no or very low glutelin. The second cluster consisted of a single accession 6708 that has no *Wx* gene product and showing very low amylose contents, while alpha-2 and 3 of glutelin subunits. Third cluster consisted of nine accessions along with Kinmaze (Japonica standard) with no *Wx* gene product, only alpha-1 subunit of glutelin polypeptide and 32 kDa peptide were absent, while 'c' subunit of 13kDa prolamin was also missing. Fourth cluster consisted of majority of accessions numbering 160 accounting for 91% of total accessions including IR36 (Indica standard). No significant association of geographical location and variation in seed protein profile was observed. The landraces from different geographical zones were grouped together in same clusters.

The present study of the total available protein content in 150 accessions of whole grains using modified Lowry's method exhibited two-fold increase in protein content. Modified Lowry's method was considered as highly feasible method to estimate even the negligible amount of protein and this method provides the real picture of soluble protein level which directly depicts the bio-available level of protein content in the human body. In this study approximately 95% of the genotypes exhibited low to moderate level of protein.

It was well established from our experiment that high protein lines RG1 (Mapillaisamba), RG7 (Kudaivazhai) and RG110 (Norungan) grouped in cluster I belonged to local landraces of Tamil Nadu. Also out of five genotypes falling in subcluster 1 of main cluster II, except two exotic lines from IRRI (RG 121) and China (RG 191), three others *viz.*, RG 12 (Vellaichithiraikar), RG62 (Purple puttu) and RG17 (Sivappuchithiraikar) are landraces of Tamil Nadu. Landraces of rice are still the storehouse of wealth of genetic diversity for different traits in rice. These landraces are still on cultivation in certain pockets by farmers and marketed as specialty rices which are not affordable by low income groups. They are poor yielding and possess traits like tallness, lodging, photosensitivity, fertilizer non-responsive *etc*. which restrict their use as a commercial cultivar. Hence they can be recommended as promising parent material/ donor for breeding rice with enhanced protein level through hybridization and conventional selection as practiced by Heda and Reddy (1984) in six crosses from F_1 to F_3. The mean protein content of F_1 was higher than the protein content of respective high protein parent in five of the crosses. In F_2, the protein content was low in many of the segregants and the frequency of desirable segregants increased in F_3. The advent of modern tools aid in the identification of genes /QTLs for specific traits and hasten the conventional breeding through marker assisted selection or transgenic approaches in biofortification programmes.

The study has identified the desirable lines with high protein content namely RG1 (Mapillai Samba – 14.54 g/100g), RG7 (Kudaivazhai – 13.22 g/100g), RG12 (Vellaichithiraikar – 10.96 g/100g), RG17 (Sivapuchithiraikar – 10.70 g/100g), RG62 (Purple puttu- 10.88 g/100g), RG110 (Norungan – 12.38 g/100g), RG121 (IG74 – 10.80 g/100g) and RG191 (IG15 – 10.74 g/100g). Accessions from high and low protein content can be utilized in recombination breeding to map the genomic regions associated with protein content and also identify markers specific to the trait. Also, they can be used as donors in a recombination breeding programme with either high or moderate protein lines with acceptable phenotype to isolate the enhanced level of protein in recombinants with high yielding better plant types in the long run to achieve sustainability in rice production along with nutritional security.

The nutritional enhancement of rice grain especially protein is a primary objective of researchers to provide nutritional security for saving human community especially children. To meet these challenges, the role of plant breeders and biotechnologists together to explore efficient breeding strategies that integrate genomic technologies by using available germplasm resources to a new revolution in the field of plant breeding for complex traits has been emphasized by Perez-de-Castro *et al.,* (2012). The nutritional traits of importance in rice grains and their enhancement using associated genes and QTLs through advanced approaches has been recently reviewed by Mahender *et al.*, (2016).

The present knowledge on the total accumulation of protein in rice accessions would be helpful for breeders to enhance the level of nutrient accumulation in rice grains.

In near future, conventional breeding along with marker assisted selection or advanced genomic strategies will give fruitful results to achieve the targeted level of accumulation of proteins and other important nutrients in grains.

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