



Variation in Crude Protein Content among Recombinant Inbred Lines of Rice (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2024/v16i31397

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/114394>

Original Research Article

Received: 08/01/2024

Accepted: 12/03/2024

Published: 15/03/2024

ABSTRACT

Rice, *Oryza sativa* L., is the world's most important staple crop, feeding more than half of the world's population, protein plays important role in human nutrition. Protein is the second most abundant storage matter in rice grain. Protein, crucial for growth, antibody production, and immunity, ranks as the second most abundant storage component in rice grains. Various factors, including grain protein content, amino acid composition, and fat content, influence the nutritional quality of rice, with grain protein content being particularly significant. This study conducted screening and evaluation of crude protein content across 200 recombinant inbred lines of rice, derived from an inter-specific cross between BPT5204 and HPR14 parents. Results indicated a range of total crude protein content from 14.99 (mg/g) to 28.11 (mg/g) among samples. Grouping analysis based on available crude protein content categorized the lines into three major groups: approximately 3% (7 lines) exhibited significantly higher protein content (>25mg/g), 59% (119 lines)

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displayed moderate content (20 mg/g to 25 mg/g), and 38% (76 lines) showed low protein content (<20 mg/g). The identification of high protein content genotypes in this study holds potential for mapping key genomic regions associated with protein content in rice.

Keywords: Rice; crude protein; RILs.

1. INTRODUCTION

Rice is the most well-known cereal staple food which serves as major carbohydrates for more than half of the world population [1]. With an alarming increase in the population throughout the world, the demand of rice will continue to increase in near future. Therefore, rice breeder across the world aim at increasing the productivity. A better understanding of the relationship between grain yield and its component traits becomes necessary for making an efficient selection for the development of new varieties with improved economically important traits [2]. Protein is the second most abundant storage matter in rice grain. Endosperm, averaging 8 per cent of dry seed weight with varietal differences ranging from 4.3 per cent to 18.2 per cent [3]. Several components influencing nutritional quality of rice such as grain protein content (GPC), amino acid content and fat content has been reported. Among them GPC has been considered as main component. Protein plays important role in human nutrition. Protein content in rice varies from 4.5 to 19.3 percent in *Oryza sativa* species and 10.2–15.9 percent in *Oryza glaberrima* species (Lin et al. 2008). Rice accounts for 21, 14 and 2 percent of global energy, protein and fat supply, respectively. The amino acid profile of rice shows that it is high in glutamic and aspartic acid, while lysine and threonine are the limiting amino acid. Supplemented rice protein can be compared with casein, milk protein [4]. In Asia, rice accounts for 715 kcal/capita/day. Rice provides 20 percent of the world's dietary energy supply, while wheat supplies 19 percent and maize 5 percent [5]. Although protein energy malnutrition is more common in low income countries, children's from higher income countries are also affected, including children's from large urban areas in low socioeconomic neighborhoods. Protein deficiency, amino acid imbalance and malnutrition can lead to variety of ailments including mental retardation and cause specific health disorders and also to affect the growth and development in humans [6]. Enhancement of total protein in rice is of immense importance for supply of protein in rice eating population. Breeding for high yield rice and high protein mainly focused on production than the nutritional

enhancement to feed the large rice eating population [7,8]. The primary objective of this investigation was to evaluate rice protein content variability in recombinant inbred lines of rice for subsequent genetic enhancement of rice.

2. MATERIAL AND METHODS

2.1 Plant Materials

The materials which were utilized in the present study consisted of 200 recombinant inbred lines of rice derived by pedigree method by crossing Samba Mahsuri (BPT-5204) and HPR-14.

2.2 Experimental Design

The study was undertaken at during Summer and Kharif seasons of the year 2020 in the experimental plots at A block of College of Agriculture, V C Farm, Mandya, which is located at an altitude of 697 m above mean sea level (MSL) and 76°50'01.7"E latitude and 12°34'25.4"N longitudes. Care was taken to raise the crop by adopting package of practice, UASB including regular irrigation to raise a good crop growth for both the seasons. 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 (Lowry et al. 1951).

2.3 Extraction of Protein

2.3.1 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 15000 rpm for 15 minutes at 4°C. 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" [9].

2.3.2 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” [10]. “Extracted samples of 0.2 ml were taken into test tube and the volume was made up to 1 ml with distilled water. To it, 4.5 ml of alkaline CuSO₄ 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” [9].

3. RESULTS AND DISCUSSION

Rice is a major protein source for most of the Asian rice growing countries. Rice protein is superior in lysine content than wheat, corn and sorghum (Hegested, 1974) and has more balanced amino-acid profile. High-protein rice has the potential to enhance human nutrition in poor rural families where rice serves as the staple food [11]. Therefore, in the improvement of rice storage protein, the main target has been to improve the quality and nutritional quality of the protein in rice. Rice grain contains a large amount of storage protein which can be classified into protein prolamin, glutelin, albumin, and globulin based on their solubility properties. With an aim of breeding rice cultivar with high protein content, Hittalmani [12] identified high protein (14 %) local landrace HPR 14 from Karnataka. HPR- 14 was crossed with BPT 5204 (high yielder, low protein content, fine grain). To address the prevalent issue of protein deficiency

among low-income groups relying heavily on rice-based diets, it's crucial to explore viable sources of protein within rice varieties. Despite alternative protein-rich foods like eggs, meat, milk, fish, and pulses being available, their affordability varies across different sections of society. Particularly in rural and impoverished areas, where rice serves as a staple, protein deficiency remains a significant concern. Considering the recommended daily protein intake of 0.8 grams per kilogram of body weight [13]. Based on the mean true digestibility of proteins sources like eggs, milk, cheese, meat, and fish protein of 95 *per cent*, as well as the relative digestibility of milled rice protein at 93 *per cent* [14], there's a clear need to enhance the protein content of rice varieties. 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.

3.1 Total Protein Content

Among 200 RILs of rice taken for the present study, the protein content ranged from 14.99 mg/g in BH-RIL-00623 to 28.11 mg/g in BH-RIL-00317 with a coefficient of variation of 10.058 mg/g (Table 1). Seven lines were found to have significantly higher protein content than the grand mean of 20.646 mg/g. About 38 % (76 RILs) of RILs had low protein content (<15.00 mg/g) and 3.0 % (8 RILs) recorded higher protein content of more than 25.00 mg/g of sample. About, 59.00 % (119 RILs) of samples recorded moderate level of protein content with range of 20.00 to 25.00 mg/g, (Fig. 1 and Table 2). It could be realized that in general most of the genotypes categorised under moderate protein content. However, there is a handful of material representing 3.00 % of the RILs with high protein that gives the ray of hope that a significant variation exists among the rice recombinant inbred lines taken for our investigation.

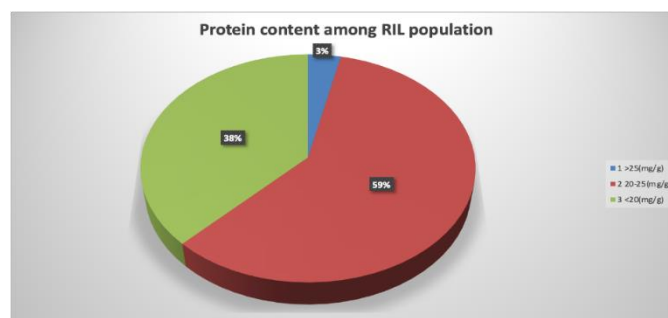


Fig. 1. Pie chart representation of protein content among 200 recombinant inbred lines of rice

Conclusion

Table 1. Total available crude protein content (mg/gram) in 200 recombinant inbred lines of rice

Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)
BPT 5204	C1	17.393	BPT 5204	C1	17.393	BPT5204	C1	17.393	BPT5204	C1	17.393
HPR-14	C2	23.966	HPR-14	C2	23.966	HPR-14	C2	23.966	HPR-14	C2	23.966
1	BH-RIL-00001	18.263	51	BH-RIL-00248	20.874	101	BH-RIL-00522	15.136	151	BH-RIL-00829	22.437
2	BH-RIL-00009	18.369	52	BH-RIL-00249	19.956	102	BH-RIL-00523	17.205	152	BH-RIL-00830	20.286
3	BH-RIL-00022	18.534	53	BH-RIL-00260	20.474	103	BH-RIL-00529	15.841	153	BH-RIL-00836	21.65
4	BH-RIL-00023	18.604	54	BH-RIL-00262	20.345	104	BH-RIL-00530	18.252	154	BH-RIL-00793	22.355
5	BH-RIL-00024	19.251	55	BH-RIL-00263	20.827	105	BH-RIL-00540	16.229	155	BH-RIL-00843	21.861
6	BH-RIL-00028	19.968	56	BH-RIL-00264	20.38	106	BH-RIL-00573	18.957	156	BH-RIL-00845	21.955
7	BH-RIL-00029	19.451	57	BH-RIL-00269	20.862	107	BH-RIL-00576	18.381	157	BH-RIL-00852	21.744
8	BH-RIL-00030	17.417	58	BH-RIL-00272	19.827	108	BH-RIL-00594	17.182	158	BH-RIL-00856	22.343
9	BH-RIL-00031	17.323	59	BH-RIL-00282	20.674	109	BH-RIL-00595	17.57	159	BH-RIL-00859	19.545
10	BH-RIL-00032	16.653	60	BH-RIL-00287	20.286	110	BH-RIL-00595	16.711	160	BH-RIL-00861	19.909
11	BH-RIL-00037	18.546	61	BH-RIL-00298	21.626	111	BH-RIL-00610	17.57	161	BH-RIL-00865	19.592
12	BH-RIL-00038	18.687	62	BH-RIL-00302	23.249	112	BH-RIL-00618	17.605	162	BH-RIL-00866	21.073
13	BH-RIL-00039	18.604	63	BH-RIL-00306	23.096	113	BH-RIL-00623	14.995	163	BH-RIL-00867	18.393
14	BH-RIL-00041	18.075	64	BH-RIL-00317	28.116	114	BH-RIL-00633	17.981	164	BH-RIL-00869	21.414
15	BH-RIL-00042	19.18	65	BH-RIL-00332	22.778	115	BH-RIL-00636	16.617	165	BH-RIL-00877	19.557
16	BH-RIL-00045	17.84	66	BH-RIL-00333	22.285	116	BH-RIL-00638	18.04	166	BH-RIL-00881	20.074
17	BH-RIL-00047	17.981	67	BH-RIL-00334	26.541	117	BH-RIL-00640	16.888	167	BH-RIL-00884	22.014
18	BH-RIL-00048	16.794	68	BH-RIL-00339	27.505	118	BH-RIL-00641	16.182	168	BH-RIL-00896	20.58
19	BH-RIL-00049	18.934	69	BH-RIL-00344	20.968	119	BH-RIL-00656	15.971	169	BH-RIL-00914	21.814
20	BH-RIL-00053	19.239	70	BH-RIL-00345	19.063	120	BH-RIL-00662	19.075	170	BH-RIL-00923	21.026
21	BH-RIL-00061	20.474	71	BH-RIL-00347	20.521	121	BH-RIL-00669	20.932	171	BH-RIL-00947	20.227
22	BH-RIL-00065	20.074	72	BH-RIL-00348	23.919	122	BH-RIL-00670	23.049	172	BH-RIL-00950	18.51
23	BH-RIL-00066	22.543	73	BH-RIL-00349	19.11	123	BH-RIL-00673	24.33	173	BH-RIL-00976	17.746
24	BH-RIL-00071	20.474	74	BH-RIL-00350	21.556	124	BH-RIL-00677	22.943	174	BH-RIL-01004	19.157
25	BH-RIL-00072	21.638	75	BH-RIL-00352	19.463	125	BH-RIL-00680	21.885	175	BH-RIL-01005	20.862
26	BH-RIL-00082	19.204	76	BH-RIL-00353	20.509	126	BH-RIL-00682	20.497	176	BH-RIL-01009	22.238
27	BH-RIL-00083	21.003	77	BH-RIL-00357	19.51	127	BH-RIL-00684	19.992	177	BH-RIL-01017	20.486
28	BH-RIL-00095	20.791	78	BH-RIL-00358	20.392	128	BH-RIL-00692	20.18	178	BH-RIL-01034	20.145
29	BH-RIL-00132	19.016	79	BH-RIL-00361	18.275	129	BH-RIL-00694	22.802	179	BH-RIL-01044	21.297

Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)
BPT 5204	C1	17.393	BPT 5204	C1	17.393	BPT5204	C1	17.393	BPT5204	C1	17.393
HPR-14	C2	23.966	HPR-14	C2	23.966	HPR-14	C2	23.966	HPR-14	C2	23.966
30	BH-RIL-00136	20.098	80	BH-RIL-00362	20.333	130	BH-RIL-00701	19.298	180	BH-RIL-01048	20.756
31	BH-RIL-00139	19.486	81	BH-RIL-00364	20.297	131	BH-RIL-00706	20.603	181	BH-RIL-00554	23.061
32	BH-RIL-00142	19.157	82	BH-RIL-00368	23.19	132	BH-RIL-00719	19.486	182	BH-RIL-01061	23.648
33	BH-RIL-00152	19.98	83	BH-RIL-00369	23.072	133	BH-RIL-00722	22.014	183	BH-RIL-01067	22.026
34	BH-RIL-00153	20.533	84	BH-RIL-00370	21.461	134	BH-RIL-00722	22.578	184	BH-RIL-01068	24.471
35	BH-RIL-00155	19.804	85	BH-RIL-00386	20.909	135	BH-RIL-00727	22.002	185	BH-RIL-01073	22.026
36	BH-RIL-00180	21.226	86	BH-RIL-00421	25.483	136	BH-RIL-00731	22.814	186	BH-RIL-01078	24.401
37	BH-RIL-00190	17.675	87	BH-RIL-00439	22.578	137	BH-RIL-00749	20.874	187	BH-RIL-01089	23.743
38	BH-RIL-00196	19.286	88	BH-RIL-00440	22.966	138	BH-RIL-00762	21.767	188	BH-RIL-01101	26.894
39	BH-RIL-00200	17.887	89	BH-RIL-00441	22.355	139	BH-RIL-00764	18.51	189	BH-RIL-01107	25.577
40	BH-RIL-00203	18.063	90	BH-RIL-00442	19.933	140	BH-RIL-00770	18.463	190	BH-RIL-01108	22.343
41	BH-RIL-00205	22.332	91	BH-RIL-00443	23.26	141	BH-RIL-00771	22.038	191	BH-RIL-01110	22.778
42	BH-RIL-00208	22.437	92	BH-RIL-00030	21.356	142	BH-RIL-00773	21.238	192	BH-RIL-01112	21.567
43	BH-RIL-00209	22.296	93	BH-RIL-00453	22.802	143	BH-RIL-00791	21.121	193	BH-RIL-01116	21.156
44	BH-RIL-00214	20.885	94	BH-RIL-00456	23.084	144	BH-RIL-00793	21.273	194	BH-RIL-01117	22.014
45	BH-RIL-00215	20.956	95	BH-RIL-00465	25.483	145	BH-RIL-00796	20.932	195	BH-RIL-01118	22.637
46	BH-RIL-00216	19.933	96	BH-RIL-00466	22.626	146	BH-RIL-00800	22.249	196	BH-RIL-01120	22.496
47	BH-RIL-00225	20.638	97	BH-RIL-00501	21.732	147	BH-RIL-00805	22.296	197	BH-RIL-00876	20.979
48	BH-RIL-00230	19.604	98	BH-RIL-00503	23.554	148	BH-RIL-00810	19.463	198	BH-RIL-01025	20.004
49	BH-RIL-00231	21.779	99	BH-RIL-00508	23.178	149	BH-RIL-00826	22.943	199	BH-RIL-01054	23.249
50	BH-RIL-00242	19.122	100	BH-RIL-00509	21.179	150	BH-RIL-00827	20.78	200	BH-RIL-01125	21.379
Mean				20.646							
C. V %				10.058							
C. D (0.05 %)				0.046							
S. Em				0.017							
SE (d)				0.024							
Protein Range lowest				14.99 (mg/g)							
Protein Range highest				28.11 (mg/g)							

Table 2. Grouping of Recombinant inbred lines based on mean crude protein content

Crude protein content (mg/g)	Individuals	Number of Recombinant inbred lines	Per centage of individuals	*Classification
>25 (mg/g)	BH-RIL-00317, BH-RIL-00339, BH-RIL-01101, BH-RIL-00334, BH-RIL-01107, BH-RIL-00421, BH-RIL-00465	7	3 %	High
20 to 25(mg/g)	BH-RIL-01025, BH-RIL-00065, BH-RIL-00881, BH-RIL-00136, BH-RIL-01034, BH-RIL-00692, BH-RIL-00947, BH-RIL-00287, BH-RIL-00830, BH-RIL-00364, BH-RIL-00362, BH-RIL-00262, BH-RIL-00264, BH-RIL-00358, BH-RIL-00071, BH-RIL-00260, BH-RIL-00061, BH-RIL-01017, BH-RIL-00682, BH-RIL-00353, BH-RIL-00347, BH-RIL-00153, BH-RIL-00896, BH-RIL-00706, BH-RIL-00225, BH-RIL-00282, BH-RIL-01048, BH-RIL-00827, BH-RIL-00095, BH-RIL-00263, BH-RIL-01005, BH-RIL-00269, BH-RIL-00248, BH-RIL-00749, BH-RIL-00214, BH-RIL-00386, BH-RIL-00669, BH-RIL-00796, BH-RIL-00215, BH-RIL-00344, BH-RIL-00876, BH-RIL-00083, BH-RIL-00923, BH-RIL-00866, BH-RIL-00791, BH-RIL-01116, BH-RIL-00509, BH-RIL-00180, BH-RIL-00773, BH-RIL-00793, BH-RIL-01044, BH-RIL-00030, BH-RIL-01125, BH-RIL-00869, BH-RIL-00370, BH-RIL-00350, BH-RIL-01112, BH-RIL-00298, BH-RIL-00072, BH-RIL-00836, BH-RIL-00501, BH-RIL-00852, BH-RIL-00762, BH-RIL-00231, BH-RIL-00914, BH-RIL-00843, BH-RIL-00680, BH-RIL-00845, BH-RIL-00727, BH-RIL-00884, BH-RIL-00722, BH-RIL-01117, BH-RIL-01073, BH-RIL-01067, BH-RIL-00771, BH-RIL-01009, BH-RIL-00800, BH-RIL-00333, BH-RIL-00209, BH-RIL-00805, BH-RIL-00205, BH-RIL-00856, BH-RIL-01108, BH-RIL-00441, BH-RIL-00793, BH-RIL-00208, BH-RIL-00829, BH-RIL-01120, BH-RIL-00066, BH-RIL-00722, BH-RIL-00439, BH-RIL-00466, BH-RIL-01118, BH-RIL-00332, BH-RIL-01110, BH-RIL-00694, BH-RIL-00453, BH-RIL-00731, BH-RIL-00677, BH-RIL-00826, BH-RIL-00440, BH-RIL-00670, BH-RIL-00554, BH-RIL-00369, BH-RIL-00456, BH-RIL-00306, BH-RIL-00508, BH-RIL-00368, BH-RIL-00302, BH-RIL-01054, BH-RIL-00443, BH-RIL-00503, BH-RIL-01061, BH-RIL-01089, BH-RIL-00348, HPR-14,	119	59 %	Moderate

Crude protein content (mg/g)	Individuals	Number of Recombinant inbred lines	Per centage of individuals	*Classification
<20 (mg/g)	BH-RIL-00673, BH-RIL-01078, BH-RIL-01068 BH-RIL-00623, BH-RIL-00522, BH-RIL-00529, BH-RIL-00656, BH-RIL-00641, BH-RIL-00540, BH-RIL-00636, BH-RIL-00032, BH-RIL-00595, BH-RIL-00048, BH-RIL-00640, BH-RIL-00594, BH-RIL-00523, BH-RIL-00031, BPT-5204, BH-RIL-00030, BH-RIL-00595, BH-RIL-00610, BH-RIL-00618, BH-RIL-00190, BH-RIL-00976, BH-RIL-00045, BH-RIL-00200, BH-RIL-00633, BH-RIL-00047, BH-RIL-00638, BH-RIL-00203, BH-RIL-00041, BH-RIL-00530, BH-RIL-00001, BH-RIL-00361, BH-RIL-00009, BH-RIL-00576, BH-RIL-00867, BH-RIL-00770, BH-RIL-00764, BH-RIL-00950, BH-RIL-00022, BH-RIL-00037, BH-RIL-00039, BH-RIL-00023, BH-RIL-00038, BH-RIL-00049, BH-RIL-00573, BH-RIL-00132, BH-RIL-00345, BH-RIL-00662, BH-RIL-00349, BH-RIL-00242, BH-RIL-01004, BH-RIL-00142, BH-RIL-00042, BH-RIL-00082, BH-RIL-00053, BH-RIL-00024, BH-RIL-00196, BH-RIL-00701, BH-RIL-00029, BH-RIL-00352, BH-RIL-00810, BH-RIL-00139, BH-RIL-00719, BH-RIL-00357, BH-RIL-00859, BH-RIL-00877, BH-RIL-00865, BH-RIL-00230, BH-RIL-00155, BH-RIL-00272, BH-RIL-00861, BH-RIL-00216, BH-RIL-00442, BH-RIL-00249, BH-RIL-00028, BH-RIL-00152	76	38 %	Low

Balindong et al. [15] reported that Protein constitutes 4 %–10 % of the milled rice grain and has very diverse properties, suggesting protein composition and not just protein content may contribute to rice grain eating quality.

Aiyswaraya et al. [9] reported that Screening and evaluation of protein content in 150 rice germplasm accessions was undertaken to identify protein rich germplasm. 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). 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.

Umadevi et al. [16] focused on assessing total protein and protein fractions. The protein fractions and total protein were isolated by specific extraction buffers from rice flour and quantified by Lowry method. The selected varieties for this study showed the range of total protein albumin, globulin, prolamin and glutelin from 4.95 % (Vibhava) to 6.52 % (BPT5204) and on par with Ravi (6.09 %); 0.03 % (Rasi) to 0.54 % (Vibhava); 0.02 % (Sampada) to 0.07 % (Vibhava); 0.28 % (Sampada) to 0.82 % (Rasi); and 0.16 % (Rasi) to 0.61 % (BPT5204) respectively. Among varieties total protein, albumin and globulin were statistically significant and prolamin and glutelin are found to be non-significant at p10.5g/100g), moderate (9.01-10.5g/100g) and low levels of proteins (10 % as high protein content analyzed in their F1 to F3 progenies of six crosses. Studies have shown that protein content in 1622 milled rice samples from 24 countries ranged from 4 to 14 per cent and mean protein ranged from 6.3 to 9.2 per cent and the overall mean was 7.8 per cent [17] at 12 % moisture.

Santos et al. [18] reported, twenty-nine accessions of the wild rice species *Oryza glumaepatula* were analyzed, sourced from five Brazilian states and two commercial cultivars, to assess their storage protein profile and amino acid content. The total protein levels varied across the accessions, ranging from 14.94% (for the wild genotype BGA14280) to 9.07% (for BGA14179). Notably, the control cultivars BRS

Bonança and Primavera, along with certain wild accessions (BGA14210, BGA14232, BGA14233, and BGA14179), exhibited the lowest total protein levels. Seven accessions demonstrated high protein content, falling within the range of 13.98% to 14.94%, while the second group comprised nine accessions with protein levels ranging from 12.3% to 13.35%. Consequently, out of the 29 evaluated genotypes, 16 displayed high total protein content, and 13 exhibited medium protein content. Upon comparing these findings with protein estimations from previous studies, it was observed that the low protein category ranged from 2.8% to 7.38%, while the higher end ranged from 9.07% to 15.9% in genotypes of *O. sativa*. The lowest value of 7.54g/100g recorded in this study our recombinant inbred lines of rice exceeded the range specified by earlier researchers.

The study has identified the desirable lines with significantly higher protein content (>25mg/g) namely BH-RIL-00317, BH-RIL-00339, BH-RIL-01101, BH-RIL-00334, BH-RIL-01107, BH-RIL-00421, BH-RIL-00465 and 76 lines had low protein content and 119 lines of RILs recorded moderate level of protein content. 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. [19]. Recent reviews, such as that by Mahender et al. [1], highlight the significance of nutritional traits in rice grains and the potential for enhancement using associated genes and Quantitative Trait Loci (QTLs) through advanced methodologies. Understanding the total protein accumulation in rice accessions provides valuable insights for breeders aiming to boost nutrient levels in rice grains [20].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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