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Biochemical characterization of two high protein rice cultivars from Assam rice collections

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ABSTRACT

Two high protein rice cultivars viz. ARC 10063 and ARC 10075 from traditional Assam Rice Collections were found to contain about 15 – 16.42% crude protein (Nx5.95) on dry weight basis. The cultivars were analyzed for soluble protein, free amino acids, tryptophan, methionine and lysine contents. Total free amino acid content was found to be higher in the cultivars having higher grain protein content. However, among the three individual amino acids, only lysine was found be higher in the high grain protein containing cultivars. SDS-PAGE of grain proteins showed that except one band which was significantly thicker in the high protein cultivars ARC 10063 and ARC 10075, others were similar between high and low protein containing cultivars. ARC 10063 and ARC 10075 also had an additional slow moving globulin band. Moreover, in addition to two additional bands, one glutelin polypeptide was found to be highly expressed in the high protein cultivars.

Key words: *rice, high protein, globulins, glutelin, Assam rice collection*

Cereals are a major source of dietary protein for humans. When foods are taken in combination (cereals, legume seeds, meat, etc.) compositions of individual dietary components are of little real importance in relation to nutritional requirements. However, in areas where a single cereal may account for a major part of the total protein intake, the nutritional quality (i.e. content of essential amino acids) of the protein as well as the amount may be important. Rice is typically a low protein (5.8-7.7%) cereal crop (Champagne *et. al.,* 2004) as a result in areas where rice is the major staple food, people occasionally suffer from protein malnutrition.

The level of protein in the mature rice grain has been found to be consistently higher in some cultivars than in others. In an effort to identify rice cultivars with higher grain protein content some traditional cultivars of rice from the eastern Indian state of Assam were screened and two cultivars viz. ARC 10063 and ARC 10075 having significantly higher grain protein content were obtained. In this paper we report the results on biochemical analysis of these cultivars along with a low protein cultivar viz. ARC 1069 of the same stock for comparison grown under identical conditions.

MATERIALS AND METHODS

Rice cultivars ARC 10063 and ARC 10075 were screened out for grain protein content from a collection of 300 Assam Rice Collections collected from the Rice Gene Bank of Central Rice Research Institute, Cuttack. Grain characteristics of the cultivars have been given in Table 1. The cultivars were grown in the wet seasons of 2007 and 2008 with standard package of agronomic practices (NPK @ 80:40:40 kg ha^{-1}) and crude protein content of the grain was determined by the microkjeldahl method of FAO (1970). Soluble protein content was determined by Folin Ciocalteau reagent (Lowry *et al.* 1951). Lysine, tryptophan and methionine contents were determined by the methods of Beckwith *et al.* (1975), Subramanian *et al*. (1970) and Horn et al. (1946), respectively. Albumin, globulin, prolamin and glutelin fractions were extracted by the method of Pascual *et al*. (1981), Sugimoto *et al.* (1986) and Villareal and Julliano (1978), respectively. For SDS-PAGE, soluble proteins were extracted with 50mM Na-phosphate buffer, pH 7.0 containing, 1% PVP and 1mM PMSF.

Table1. Characteristics of the three collections

Variety	Seed weight (mg)*	Grain type
ARC10063	21.41± 0.078	Long bold
ARC10075	19.86± 0.136	Long bold
ARC10069	17.12± 0.205	Short bold

** figures are mean±sd weight of 10 seeds*

RESULTS AND DISCUSSION

Rice is typically a low protein (5.8 – 7.7%) cereal grain (Champagne *et. al.,* 2004). However, brown rice of ARC 10063 and ARC 10075 showed 16.41 and 15.27% crude protein content on dry weight basis while 11069 showed only 10.96% (Nx5.95) [Table 2]. Similar results were observed when the cultivars were grown in the previous year. These values were significantly higher (almost double) than the value usually found in rice. A range of 7 to 11% protein was observed by Webb *et al.* (1968) in 4,380 varieties of rice *(Oryza sativa* L.) from 49 countries. Juliano *et al*. (1968) also reported a mean of 10.6 ± 1.6% protein in 7,760 varieties in the world rice collection at the International Rice Research Institute but found at least 13.5% in several varieties. Recently, a group of scientists reported the development of a variety of hybrid rice with double the protein content of normal rice. The product showed a protein content of 12.4 % which is 18 percent and 28 percent higher than those of the parents (Ahmed *et al.* 2008). Soluble protein varied from 1.1 to 1.5% with low protein containing ARC 1069 having the lowest (0.91%) [Table 2].

Table 2. Levels of different biochemical parameters in grains of three different rice cultivars

Biochemical Parameters	ARC10063	ARC10075	ARC10069
Crude protein content (%)	16.41+0.05	15.27+0.61	10.96+0.54
Soluble protein content (%)	1.15+0.07	1.55+0.07	0.91+0.02
Free amino acid content (%)	0.42+0.007	0.26+0.01	0.17+0.03
Methionine content (%)	0.050+0.007	0.082+0.01	0.065+0.006
Lysine content(%)	0.075+0.004	0.049+0.0014	0.043+0.0035
Tryptophan content (%)	0.068+0.0028	0.063+0.0056	0.078+0.013

* *Results are mean± sd of three replicates*

Total free amino acid content of the three cultivars varied from 0.17 (ARC 1069) to 0.42% (ARC 10063) [Table 2]. ARC 10063 which had the highest crude protein content also showed highest free amino acid content followed by ARC 10075 (0.26%). Amino acids are building blocks of proteins so an increased amount of protein content was also expected to be reflected in the amino acid content. A higher level of these amino acids will contribute to a faster and greater accumulation of protein in the so-called protein bodies (Perez *et al.,* 1973). Studies at the International Rice Research Institute (IRRI) showed that the developing grains of lines with high percentages of protein had higher amounts of free amino acids than their low protein counterparts (Cagampang *et al.* 1971; Cruz *et al.* 1970). The results observed here also suggest the same trend.

However, the contents of three individual amino acids followed somewhat different trend [Table 2]. In the case of methionine and tryptophan no correlation could be observed with the grain protein content. Methionine content of the three cultivars varied from 0.05 (ARC 10063) to 0.0825% (ARC 10075) [Table 2]. The three cultivars had a tryptophan content of 0.063 (ARC 10075) to 0.078 (ARC 1069) [Table 2]. Tryptophan being a limiting amino acid in rice assumes significance. The three cultivars had a lysine content of 0.043 (ARC 1069) to 0.075 (ARC 10063) [Table 2]. Although lysine content was reported to be negatively correlated with the grain protein content (Juliano *et al*., 1964; Cagampang *et al*., 1966) in the present study lysine content was found to be higher in the cultivars having higher grain protein content.

Differences in protein content between the high and low protein containing cultivars were also evident upon SDS-polyacrylamide electrophoresis. Interestingly, the two high protein cultivars showed a protein band (positioning below 43kD marker) which was significantly thicker as compared to that in the low protein cultivar ARC 1069 (1).

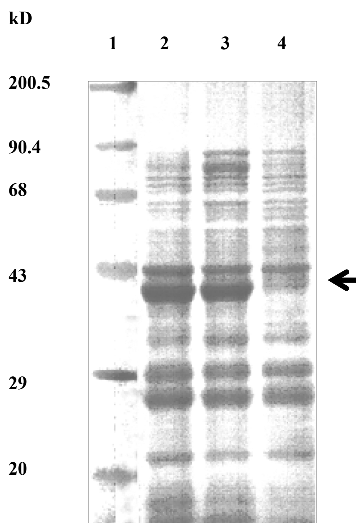

Fig. 1. SDS-PAGE of total soluble proteins extracted from the grains of different cultivars. Lanes 1, 2, 3 and 4 denote Marker, ARC 10063, ARC 10075 and ARC 10069, respectively.

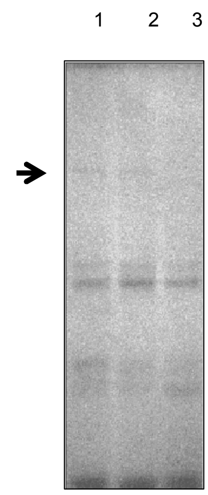

Fig. 2. SDS-PAGE of globulins extracted from the grains of different cultivars. Lanes 1, 2 and 3 denote ARC 10063, ARC 10075 and ARC 10069, respectively.

As expected highest yield was obtained with glutelins (88.30 to 90.63%) followed by albumins (4.63 to 5.91%), globulins (2.60 to 3.44%) and prolamin (2.00 to 2.78%) (Table 3).

(the slowest moving band and a band below 68kD marker (Fig 3).

Milled rice has an amino acid score of 65, which is much higher than 41of wheat flour and 32 of corn grits (Resource Council/ Science and Technology Agency, 1982; Ohtsubo, 1995) suggesting that quality

Table 3. Amount (%) of various protein fractions extracted from grains of different rice cultivars.

Variety	Albumin%	Globulin%	Glutelin%	Prolamin%	Total
ARC-10063	0.132+ 0.012 (5.91)	0.074+ 0.005 (3.31)	1.98+ 0.125 (88.63)	0.048+ 0.01 (2.15)	2.234
ARC-10075	0.097+ 0.011 (4.63)	0.072+ 0.007 (3.44)	1.88+ 0.105 (89.82)	0.044+ 0.010 (2.10)	2.093
ARC-10069	0.092+ 0.008 (4.64)	0.058+ 0.0.01 (2.92)	1.79+ 0.19 (90.22)	0.044+ 0.010 (2.22)	1.984

* *Figures are mean ± sd of three replicates. Figures in parentheses indicate percentage of that fraction*

Globulins and glutelin profiles were remarkably dissimilar. In case of globulin, a total of six bands were observed on the gel. Interestingly, the slowest moving band was visible only in ARC 10063 and ARC 10075 but not in the low protein containing cultivar ARC 1069 (Fig 2). In case of glutelins nine bands were seen on the gel. There were qualitative as well as quantitative differences among the cultivars. One particular band having molecular wt. of less than 29kD was found to be highly expressed in the high protein cultivars, ARC 10063 and ARC 10075. Moreover, some bands were found to be exclusively expressed in high protein cultivar

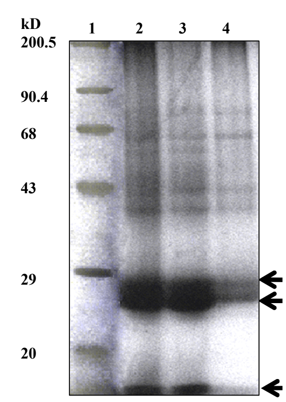

Fig. 3. SDS-PAGE of glutelins extracted from the grains of different cultivars. Lanes 1 2, 3 and 4 denote ARC 10063, ARC 10075 and ARC 10069, respectively.

of rice protein is superior to that of wheat flour and corn. However, protein content of milled rice Asian rice (*Oryza sativa* L) varieties is as low as 8.1% on dry matter basis although it is 9.5% in corn grits and 10.5% in first grade medium wheat flour (Resource Council/ Science and Technology Agency, 1982). These facts highlight the importance of development of high protein rice. In spite of its importance, progress in breeding for high grain protein content in rice has been slow and difficult. The first limitation is that genetic variation for protein content is small compared with variation due to differences in growing environments. The second limitation is that there is a strong negative correlation between grain protein content and grain yield; cultivars with high grain protein content tend to be low yielder. Thus, the two cultivars identified in this study can serve as suitable breeding material for transferring this character to high yielding popular rice cultivars.

In high grain protein containing cultivars even though there were extra bands in globulin fraction, higher grain protein content in these cultivars might be because of higher expression of a particular glutelin polypeptide (since glutelin constitute about 80% of the rice seed storage proteins). The thicker band in the SDS-PAGE protein profile of total soluble proteins might be altogether a different polypeptide since its MW was different from that of the glutelin subunit.

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