Oryza Vol. 47. No.2, 2010 (136-141)

Nutritive value and characterization of Joha rice cultivars of Assam through seed protein electrophoresis

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ABSTRACT

Chemical analysis for nutritive value of 14 Joha rice cultivars revealed that crude protein content varied from 7.03 to 8.45%; total carbohydrate from 65.75 to 80.88% while lipid content varied from 2.03 to 2.88% with the exception of Naga Joha that had 3.73% lipid content. However, crude fibre and ash content showed limited variability, the ranges being 0.25 to 0.75% and 1.0 to 1.66% respectively. Calorific value varied from 315.31 to 381.36 Kcal/100 gm. SDS-PAGE analysis revealed a total 16 seed protein ranging in size from 97.4 to <14.0Kd. The lowest number of 5 protein bands were observed in Keteki Joha while highest number of 13 protein bands were observed in Biri Joha. Proteins with molecular weight 25.0, 22.6 and 15.7 Kd appear to be protein molecular marker since they were found in all the cultivars. Dendrogram analysis revealed five cluster and Keteki Joha has been found to be odd type distantly related to others.

Key words: *scented rice, Assam, Joha, nutritive value, seed protein profile*

Scented rice is a major foreign exchange earner for many rice growing countries because of their ever increasing demand in the international market although their share to global rice production is very small. Scented rices are highly valued in local as well as international market because of their unique and pleasant aroma, palatability and cooking qualities. (Khush, 2000). Scented rice are broadly grouped into three different classes – long, slender Basmati type of India, Jasmine type of Thailand and medium and small grain type. Because of their low productivity, scented rice are of limited cultivation and primarily meant for export market or domestic consumption mainly on festive occasions. International market for scented rice is mainly dominated by Basmati and Jasmine types. However, in recent times medium and short grain type scented rice cultivars from Assam are witnessing demand in the international market, particularly Middle East and Europe (Anon, 2007) which is a new phenomenon. The indigenous medium and short grain type scented rice cultivars of Assam are locally known by the collective name "Joha". These are cultivated as winter paddy and fetch the highest price in local market which is nearly double the price of general non-scented types. Because of their low productivity and cultivation in limited area, primarily meant for domestic consumption; they attract little attention from researchers. Hence information about them is limited (Borthakur, 1995). Information about basic nutritive value is of paramount importance while evaluating paddy cultivars, particularly when it is finding appreciation in international market. Rice is not only the most important food crop but it has the highest number of intraspecific variability with over 50,000 cultivars. Their characterization is a necessity and an enormous task. Traditionally they are characterized by morphological and biochemical traits which are influenced by environmental and geographical factors and also by cultivation practices. Hence molecular markers are gaining more acceptability because of their reliability and reproducibility. Compared to other molecular techniques, seed protein profile involve relatively simple and less expensive procedure and have been successfully employed to elucidate genetic variability and phylogenetic relationship in many plant species studied (Naik and Kole, 2002). The present investigation was intended to work out the basic nutritive values of 14 indigenous Joha cultivars of Assam. Moreover, with the growing importance of intellectual property right (IPR), patenting of

bioresources like species/cultivar is gaining momentum is a pre-requisite for this characterization of the cultivars considering this, the present study was carried out for molecular characterization based on electrophoretic profile of seed protein.

MATERIAL AND METHODS

Fourteen scented rice cultivars used in the study are – Babuli, Biri, Boga, Gabharu, Givinda, Hung, Kola, Keteki, Kharika, Kunkuni-1, Khutki, Naga, Tulsi and Kunkuni-2. Among these Babuli, Hung, Keteki and Naga Joha had medium to long awn. Among them, the awn of Hung Joha is the longest and most prominent ('Hung' in Assamese language mean long slender structure). The grains of Kunkuni-2 were very small while others had small to medium sized grains. The grains were collected from farmers from Mangalodoi of Central Assam on the north bank of Brahmaputra river. The grains were manually dehusked and grounded to fine powder using mortar and pestle following which they were dried in oven at 60 ^{o}C till constant weight was recorded. Chemical analyis was carried on dry weight basis. Crude protein was estimated by working out the total nitrogen in the sample by Microkjeldahl method (AOAC, 1965).Total carbohydrate was estimated by anthrone method as described by Clegg (1956). Lipid content was estimated by extracting the sample with petroleum ether in a Soxhlet apparatus for 8 hours and then the amount of lipid was determined after removal of petroleum ether. (AOAC, 1970). Crude fibre was estimated as per the method outlined by Sadasivam and Manickam (1996). Ash content was determined by ashing the sample in muffle furnace at 600 ^{o}C for three hours and then the weight of ash was recorded. Calorific value was computed using the formula of Sherman (1952).

Seed protein profile was analysed by SDS-PAGE method as outlined by Laemmli (1970). The seeds were manually dehusked and the kernels were washed with distilled water following which the surface water was removed with a blotting paper. About 150 mg kernels were grounded in a pre-cooled morter and pestle with ice cold 0.3M Tris buffer pH 8.0. The sample was homogenized and centrifuged in a refrigerated centrifuge at 8000 rpm for 8 minutes at 4 ^{o}C. The sample weight to extract volume was adjusted to 1:5 ratio. The protein extract was electrophoresed in a 13.5% polyacrylamide gel following which the bands were visualized using Coomassie Brilliant Blue R-250. Standard protein molecular weight marker (PMW-M, Bangalore Genei) was co-electrophoresed to determine the molecular weight of the proteins in the samples. The data for biochemical analysis were subjected to one way analysis of variance and CD at 1% and 5% were computed. The protein profile was recorded in terms of presence or absence of the individual proteins. From the protein profile similarity indices were generated using Nei and Li co-efficient (Nei and Li, 1979). The similarity matrix was used to generate the dendrogram by UPGMA using the software NTSYS p.c V2.02j.

RESULTS AND DISCUSSION

Crude protein content of the 14 cultivars exhibited considerable variation in the range of 6.12% in Biri Joha to 8.43% in Gabharu Joha. However, as many as 5 cultivars had crude protein of 8% or above which is the upper level in the present study. The variation among the cultivars for protein content has been found to be significant. Like crude protein, total carbohydrate also exhibited significant variation in the range of 65.75% in Boga Joha to 80.88% in Tulsi Joha. Lipid contents were found to be consistently low and unlike crude protein and total carbohydrate exhibited limited variability. Lipid content varied from 2.03% in Gabharu Joha to 2.88% in Babuli Joha. Naga Joha was an exception with 3.73% which is much higher than the highest values among the rest. Statistically the variation in lipid contents has been found to be significant. Crude fibre contents was found to be lowest of all the components. Crude fibre content varied from 0.25 in Kunkuni-1 to 0.75% recorded in as many as five cultivars namely – Hung, Kola, Keteki, Kharika and Naga Joha. Total minerals in the form of ash content have been found to be low with little variation. The range of variation for ash content was 1.0 to 1.66%; the lowest value was recorded for four cultivars viz. Babuli, Biri, Tulsi and Kunkuni-2. Highest value of 1.66% was recorde in two cultivars viz. Boga and Kharika Joha while as many as 7 cultivars had identical value of 1.33 indicating that variability for ash content is limited. Calorific values varied from 315.31 Kcal/ 100 gm in Kunkuni-2 to 381.36 Kcal/100 gm in Babuli Joha and the variation among the cultivars has been found to be significant (Table 1).

Table 1. Biochemical composition for the major nutritional parameters and calorific values for the fourteen indigenous cultivars of scented rice (Joha)

Name of the cultivar	Crude protein (%)	Total carbo-hydrate (%)	Lipid (%)	Crude fibre (%)	Total Ash (%)	Calorific value kcal/100gm
Boga	8.09	65.75	2.43	0.66	1.66	317.23
Keteki	7.34	70.12	2.66	0.75	1.33	375.06
Kharika	7.69	68.43	2.33	0.75	1.66	325.45
Khutki	7.03	67.51	2.33	0.55	1.49	319.13
Gabharu	8.43	66.75	2.03	0.33	1.33	319.01
Naga	7.39	70.50	3.73	0.75	1.33	345.13
Biri	6.12	70.74	2.13	0.43	1.00	326.61
Kola	8.35	72.62	2.06	0.75	1.33	342.42
Gobinda	7.09	75.08	2.80	0.50	1.33	353.88
Tulsi	8.38	80.88	2.66	0.50	1.00	380.98
Hung	7.63	72.31	2.16	0.75	1.33	339.20
Babuli	8.26	80.59	2.88	0.66	1.00	381.36
Kunkuni I	7.43	65.85	2.46	0.25	1.33	315.31
Kunkuni II	7.06	66.56	2.33	0.33	1.33	315.45
CD (P=0.05)	0.432	1.877	0.183	0.141	0.062	2.50
CD at 1%	0.583	2.530	0.247	0.187	0.083	3.36

Considerable polymorphism was observed with regard to protein profile. A total of 16 bands ranging in size from 97.4 Kd to < 14.0 Kd were recorded covering all the cultivars. For convenience proteins in the size range of 97.4 to 43.2 Kd were considered as high molecular weight (HMW) proteins; those in the size range of 37.0 to 23.2 Kd were considered as medium molecular weight (MMW) proteins while those smaller than MMW were considered as low molecular weight (LMW) proteins (Fig 1a,b). Highest number of protein bands were observed in Biri Joha with 13 bands while lowest number of 5 bands were observed in Keteki Joha. Frequency distribution showed that proteins with molecular weight 25.0, 22.6 and 15.7 Kd were prominently present in all the cultivars and hence can be considered as protein marker for Joha category cultivars (Table 2).

The protein band 62.0 Kd was also present in all the cultivars except Keteki. Dendrogram analysis revealed five clusters among the 14 cultivars in the present study indicating considerable genetic diversity (Fig. 2). Keteki Joha has been found to be distantly related to all others based on SI values in the range of 28.57% to 45.45% and forms a separate cluster alone (Table 3). Dendrogram further reveal that Kunkuni -1, Kunkuni –2 and Hung are identical at molecular level. Likewise, Govinda and Tulsi have identical profile and hence not different at molecular level.

In international market scented rice is highly valued for its traits which are attractive for consumers, like pleasant aroma, palatability and cooking qualities. However nutritive values are equally important or rather more important although they receive lesser attention. Quality traits mostly studied for scented rice are length breadth ratio of kernel, gelling temperature, gel consistency, amylose content, volume expansion upon cooking, softness, palatability etc. Out of these amylose content is based on chemical analysis while few others are physical characteristics, but some others are based on visual impression and perception and cannot be quantified with numerical values while nutritive values have universal significance, quality traits preference varies from country to country and also from one ethnic group to other. Information about nutritive values of scented rice is limited. Tsuzuki *et al.* (1979) evaluated 41 Japanese native scented rice cultivars and reported that with the exception of 2 cultivars for the rest, protein content varied from 7.19 to 9.62%. In the present study protein content have been found to vary from 7.03 to 8.43% with the

Table 2. Frequency distribution of seed protein in fourteen scented paddy land races. (T–1 Total for a particular land race; T–2 Total for a particular protein)

(Kd)	Boga	Keteki	Kharika	Khutki	Gabharu	Naga	Biri	Kola	Govinda	Tulsi	Hung	Babuli	Kunkuni 1	Kunkuni 2	T- 2
97.4							P								1
66.0			P												1
62.0	P		P	P	P	P	P	P	P	P	P	P	P	P	13
48.0					P		P								2
44.5				P	P		P								3
43.2	P		P		P		P	P	P	P	P	P	P	P	11
37.0			P	P	P	P	P	P	P	P	P	P	P	P	12
32.6	P		P	P	P	P	P		P	P	P	P	P	P	12
25.0	P	P	P	P	P	P	P	P	P	P	P	P	P	P	14
23.2									P		P	P	P	P	5
22.6	P	P	P	P	P	P	P	P	P	P	P	P	P	P	14
21.0	P		P	P	P		P	P	P	P	P		P	P	11
15.7	P	P	P	P	P	P	P	P	P	P	P	P	P	P	14
14.3	P			P	P	P	P								5
14.0		P	P					P	P	P	P		P	P	8
<14.0	P	P	P	P	P	P	P								7
T - 1	9	5	11	10	12	8	13	9	9	9	10	8	10	10	

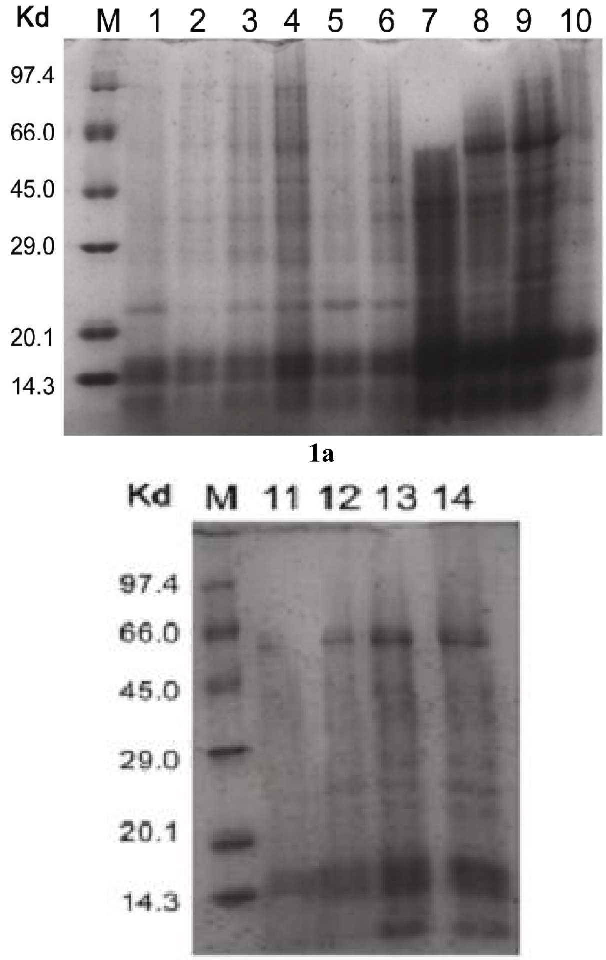

Fig 1a, 1b. Seed protein of 14 indigenous land races of scented paddy resolved in 14 % polyacrylamide gel. Lane M- Marker, 1- Boga, 2-Keteki, 3-Kharika, 4-Khutki, 5-Gabharu, 6-Naga, 7-Biri, 8-Kola, 9-Govinda, 10-Tulsi, 11-Hung, 12-Babuli , 13-Kunkuni 1, 14-Kunkuni 2.

exception of Biri Joha with 6.12% and as many as five contained over 8.0% protein. Dikshit *et al.* (1992) working with 26 indigenous scented rice of Orissa found considerable variation and the values recorded were 9.0 to 10.0%.

The findings of the present study are in corroboration with the above findings and in general terms protein content in Joha rice cultivars can be considered to be of medium level. Medhi *et al* (2004) working with 40 indigenous scented rice cultivars of Assam reported that starch content varied from 65.81 to 81.89% which is nearly equivalent to that of total carbohydrate. In the present study total carbohydrate varied from 65.75 to 80.88% which is comparable to

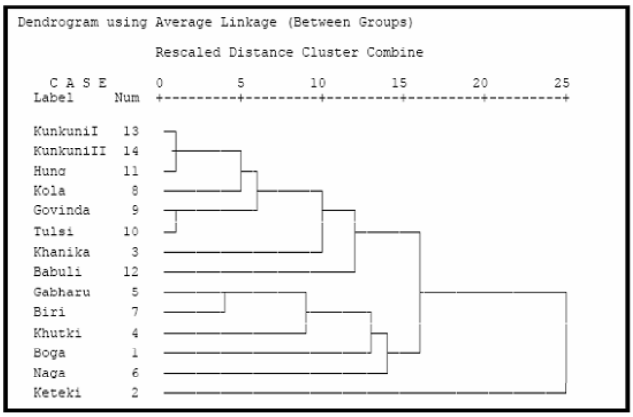

Fig. 2. Dendrogram analysis based on seed protein profile showing the inter relationship of the fourteen scented paddy land races.

that of earlier findings of Medhi *et al* Joha rice of Assam by Borthakur (1995) earlier reported lipid content to be in the range of 2.35% in Kon Joha to 3.20% in Prasad Bhog, respectively while crude fibre content varied from 1.29% in Kola Joha to 2.32% in Kon Joha. In the present study lipid content varied from 2.06 to 2.88% with the exception of Naga Joha with 3.73% and comparable to earlier findings. However, crude fibre contents were lower than that of earlier findings. It is well established that in general in milled rice nutritive values are significantly lower than the corresponding values for dehusked rice. The present findings are for dehusked rice.

Available evidences indicate that in paddy the cultivars vary with respect to the number of seed protein and more importantly protein profile. Among the seed protein those which are common to many cultivars can be considered as species specific and those which are unique for a cultivar can be considered as cultivar specific protein markers. The major seed protein for paddy are reported to be 57, 39, 37, 23, 22, 16, 13 and 10 Kd size (Yamagata *et al.,* 1982; Tanaka and Ogawa, 1985). Sarma *et al.* (2006) reported 14 proteins in the seed extract of Mashuri cultivar; the most prominents being of 64.5, 54.0, 41.2 and 32.2 Kd size. In the present study the number of seed protein varied from 5 to 13 in the size range of 97.4 to < 14.0 Kd which are agreeable with the earlier findings. Three protein bands with molecular weight 25.0, 22.6 and 15.7 Kd can be considered as protein marker for scented rice since they are consistently found in all the cultivars. The present

Table 3. Similarity index (SI) matrix for the fourteen scented rice land races based on seed protein profile

	Boga	Keteki	Kharika	Khutki	Gabharu	Naga	Biri	Kola	Govinda	Tulsi	Hung	Babuli	Kunkuni 1	Kunkuni 2
Boga	100.00	40.00	66.66	72.72	75.00	70.00	69.23	50.00	63.63	63.63	58.33	54.54	58.33	58.33
Keteki		100.00	45.45	36.36	30.76	44.44	28.57	40.00	40.00	40.00	36.36	30.00	36.36	36.36
Kharika			100.00	61.53	64.28	58.33	60.00	66.66	81.81	81.81	75.00	58.33	75.00	75.00
Khutki				100.00	83.33	80.00	76.92	46.15	58.33	58.33	53.84	50.00	53.84	53.84
Gabharu					100.00	66.66	92.30	50.00	61.53	61.53	57.14	53.84	57.14	57.14
Naga						100.00	61.53	41.66	54.54	54.54	50.00	60.00	50.00	50.00
Biri							100.00	46.46	57.14	57.14	53.33	50.00	53.33	53.33
Kola								100.00	80.00	80.00	90.00	70.00	90.00	90.00
Gobinda									100.00	100.00	90.00	70.00	90.00	90.00
Tulsi										100.00	90.00	70.00	90.00	90.00
Hung											100.00	80.00	100.00	100.00
Babuli												100.00	80.00	80.00
Kunkuni I													100.00	100.00
Kunkuni II														100.00

investigation showed that morphologically different cultivars may be identical at molecular level as has been found in case of Kunkuni–1, Kunkuni-2 and Hung Joha, while Kunkuni-1 had comparatively bigger grain with blackish colouration, Kunkuni-2 had much smaller but elongated grain with creamy colouration. However, their seed protein profile were identical. In the present study considerable polymorphism has been observed for seed protein which make it a reliable and strong criterion for characterization at molecular level and also to assess genetic diversity.

ACKNOWLEDGEMENT

The authors are grateful to Dr. L. Sahoo and Mr. Ganesh Thapa of Indian Institute of Technology, Guwahati for their help in the preparation of dendrogram.

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