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Nutritive value and seed protein profile of deep-water rice cultivars of Assam

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ABSTRACT

Nutritive value of ten indigenous land races of deep water rice cultivars from Assam were analysed for major nutritional components. Crude protein varied from 9.63 to 13.22%; total carbohydrate from 69.68 to 85.43%; lipid from 2.33 to 3.80%; crude fibre from 0.45 to 0.90%, while ash content varied from 1.13 to 2.00%. Calorific value varied from 345.41 to 405.78 Kcal.100gm^{-1}. SDS-PAGE analysis of seed protein revealed considerable polymorphism with a total 22 bands ranging in size from 97.40 to <13.2 Kd. Two land races, viz. Negheri and Bawla had the lowest number of 9 protein bands each but with different profiles. Highest number of 15 bands were observed in the land race Do-Kokua. Three proteins with molecular weight 26.7, 17.0 and 15.7 Kd were common to all the land races. Dendrogram analysis revealed that the land race Bawla is markedly different from the rest.

Key words : *deep water rice, Assam, Bao dhan, nutritive value, seed protein profile*

Deep water paddy is a nature's marvel and its cultivation represent ancient technical know how to co-exist with hostile nature by many ethnic communities living in flood prone river basins of some Asian and African countries. Deep water paddy cultivation is prevalent in Bangladesh, India, Thailand, Myanmar, Vietnam, Indonesia of Asia; Guinea, Mali, Gambia, Niger, Nigeria and Sierra Leone of Africa and Ecuador of Latin America (Grist, 1984). Deep-water paddy is the economic life line for many ethnic communities and in countries like Bangladesh 19% of the population are dependant on it. Although not traded in international market they provide nutrition to millions of poor people in the third world countries and hence information about basic nutritional parameters of deep water paddy is of paramount importance. However, information on this aspect is very scanty and fragmentary.

In Assam, the north eastern state of India, deep water paddy is being cultivated since time immemorial by several ethnic communities living in the Brahmaputra river basin. About 0.48 m. hectare land are under deep water cultivation (Sarma and Borgohain, 1998) and these land races are collectively known as "Bao dhan". Although not properly enumerated, it is known that more than 70 land races of Bao dhan exist. Information about and works on deep water paddy land races of Assam are very limited (Haloi, 1987; Sarma and Borgohain, 1998; Baruah et. al 2006). The present study was undertaken to work out the basic nutritional parameters of ten deep water rice land races of Assam and their molecular characterization through electrophoretic profile of seed protein.

MATERIAL AND METHODS

Ten indigenous land races of Bao dhan namely Negheri, Jool, Bam-Kokua, Do-Kokua, Kolioi, Adolia, Ranga, Gotha, Bawla and Holodhar collected from flood prone villages of Bokakhat and Dergaon sub-division of Upper Assam were taken for the present study. Among them Adolia, Ranga and Holodhar had white kernel while the rest seven had red kernel with Negheri being the darkest red of all. Bawla had the unusual characteristics of two prominent bracts flanking the grain along the whole length which gives a wing like appearance.

The grains were manually dehusked, grounded to fine powder and dried in oven at 60^0 C till constant

weight was recorded for biochemical analysis. Chemical analysis was carried out on dry weight basis. Crude protein was estimated by working out the total nitrogen by microkjeldahl method (AOAC, 1965). Total carbohydrate was estimated by anthrone method as outlined by Clegg (1956). Lipid content was estimated by extracting the sample with petroleum ether in Soxhlet apparatus and 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 600^0 C for 3 hours. Calorific value was computed by using the formula of Sherman (1952).

Molecular analysis for seed protein was carried out by SDS-PAGE technique outlined by Laemmli (1970). Manually dehusked grains were washed with distilled water and blotted dry. About 150 mg kernels were grounded with ice cold 0.3 M Tris (pH 6.5). The sample was homogenized and centrifuged at 8000 rpm for 8 minutes at 4^0 C. The supernatant was collected and sample weight to extract volume was adjusted to 1:5 ratio. The proteins were resolved in 14% polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. Protein molecular weight marker (PMW-M, Bangalore Genei) was co-electrophoresed to determine the molecular weight of the individual protein. Total number of protein bands were counted for each land race and recorded in terms of presence or absence of a particular protein band with respect to a reference sample. Similarity indices (SI matrix) were generated using Nei and Li's co-efficient (1979) from the base data. The SI matrix was used to generate the dendrogram by unweighted pair group method with arithmatic averages (UPGMA) using the software NTSYS pc. V2.02j.

RESULTS AND DISCUSSION

Crude protein content showed considerable variability and varied from 9.63 to 13.22% with a mean of 11.08% (Table 1). As many as 5 land races had crude protein 11% and above. Total carbohydrate varied in the range of 69.68 to 85.43%, indicating that like crude protein, total carbohydrate also exhibited significant variation. The mean for the ten land races was 77.62% and four land races had total carbohydrate of 81% and above. Unlike crude protein and total carbohydrate, lipid content varied within a narrow range of 2.33 to 3.80% with a mean of 3.1%, It was found that all the seven land races with red kernel had lipid content of 3% and above while the three with white kernel had less than 3% lipid content. Among all the constituents, crude fibre occurred in lowest proportion and varied from 0.45% to 0.90% with a mean value of 0.60%. The variations of lipid content among the land races was found to be non significant. Total mineral in the form of ash content varied from 1.13 to 2.0% with a mean value of 1.60%. Calorific value varied from 345.41 Kcal 100 gm^{-1} in Jool bao to 405.78 Kcal 100 gm^{-1} in Holodhar bao with a mean value of 381.88 Kcal 100 gm^{-1} (Table 1).

Table1. Nutritive value of deep water rice cultivars of Assam.

Name of the cultivar	Crude protein (%)	Total carbo-hydrate (%)	Lipid (%)	Crude fibre (%)	Total Ash (%)	Calorific value kcal $100gm^{-1}$
Negheri	12.08	71.37	3.80	0.75	1.67	368.00
Jool	9.63	69.68	3.13	0.90	2.00	345.41
Bam-kokua	12.35	76.03	3.47	0.75	1.67	384.75
Do-kokua	13.22	74.58	3.37	0.66	1.53	381.53
Kolioi	10.73	74.06	3.30	0.60	1.69	368.86
Adolia	9.63	77.64	2.73	0.50	1.83	373.65
Ranga	11.51	83.17	2.33	0.55	1.67	399.69
Gotha	11.12	82.68	3.23	0.70	1.56	396.17
Bawla	10.37	81.56	3.03	0.64	1.27	394.99
Holodhar	9.94	85.43	2.70	0.45	1.13	405.78
Mean	11.08	77.62	3.11	0.60	1.60	381.88
CD at 5%	0.300	1.610	0.210	0.181	0.179	2.380

Among the ten land races a total 22 protein bands were observed ranging in size from 97.4 Kd to < 13.2 Kd. Negheri and Bawla bao had lowest number of 9 bands each; however, the profiles were different. Highest number of 15 bands were found in Do-Kokua followed by Jool bao with 14 bands. The high molecular weight proteins (97.40 to 52.2 Kd) were found mostly in the land races – Bam-Kokua, Do-Kokua, Jool, Kolioi and Gotha. Other proteins were evenly distributed. Among the cultivars Kolioi and Gotha had identical protein profile, each with 12 protein bands (Table-2). Hence they are not different at molecular level with Similarity Index (SI) value of 100%. Adolia and Holodhar bao had 14 bands each and SI value of 100% (Table-3). Among the landraces Ranga appear to be quite different from the rest because it has no high molecular weight protein. Furtermore, it has two proteins of 25.2 Kd and 20.0 Kd that are unique since they were not found in the other land races. Another such unique protein of 33.8 Kd was found in Adolia and Holodhar which are found to be identical land races at molecular level. These unique proteins can be considered as marker protein for the respective land races. Despite the variability, three proteins viz. 26.7, 17.0 and 15.7 Kd were found to be present in all the cultivars and hence can be considered as protein molecular marker for the species (Fig-1).

From nutritional view point the most promising aspect of deep water paddy is its high protein content. In general protein content of rice vary from 6% to 14% with an overall mean of 9.5% (Gomez,1979) or 10.5% (Anon., 1967). Baruah *et. al.* (2006) working with ten indigenous land races of deep water paddy of Assam found crude protein in the range of 8.03 to 13.20% with a mean of 11.78%. This is comparable with the corresponding value in the present study, which was 11.08%. For total carbohydrate the mean value in the present study (77.62%) has been found to be little higher than that reported by Baruah *et.al.* (2006) which was 73.86%. Both the values are comparable with the range of carbohydrate content for paddy in general (Grist, 1984; Juliano *et al.*, 1964). Another promising aspect of deep water paddy is its relatively high lipid content. For paddy, in general the reported ranges of lipid content are – 2.37% to 3.13% (Juliano *et. al*, 1964); 2.0 to

Table 2. Frequency distribution of seed protein in deep water rice cultivars of Assam.

Protein(Kd)	Negheri	Bam-Kakua	Do-kakua	Jool	Kolioi	Gotha	Ranga	Adolia	Holodhar	Bawla	T-2
97.40	P	P	P	P	P	P	—	—	—	—	6
93.75	—	P	P	P	P	P	—	—	—	—	5
90.00	—	P	P	P	P	P	—	—	—	—	5
62.20	P	P	P	P	P	P	—	P	P	P	9
60.20	—	—	—	—	—	—	—	P	P	P	3
52.20	—	P	P	P	P	P	P	P	P	—	8
48.00	—	P	P	P	—	—	—	P	P	P	6
42.20	—	—	—	—	—	—	P	P	P	—	3
39.00	P	P	P	P	—	—	P	P	P	—	7
33.80	—	—	—	—	—	—	—	P	P	—	2
29.00	P	—	P	—	P	P	—	—	—	—	4
26.70	P	P	P	P	P	P	P	P	P	P	10
25.20	—	—	—	—	—	—	P	—	—	—	1
23.60	—	P	P	P	P	P	P	P	P	P	9
22.40	P	P	P	P	—	—	—	—	—	P	5
20.40	—	—	—	—	—	—	P	P	P	—	3
20.00	—	—	—	—	—	—	P	—	—	—	1
17.00	P	P	P	P	P	P	P	P	P	P	10
15.70	P	P	P	P	P	P	P	P	P	P	10
13.20	—	—	P	P	P	P	P	P	P	P	8
<13.20	P	P	P	P	P	P	P	P	P	-	9
<13.20	-	-	-	-	-	-	P	-	-	-	1
T-1	9	13	15	14	12	12	13	14	14	9	

T-1 Total for a particular landrace T-2 Total for a particular protein

Table 3. Similarity index matrix based on seed protein profile of deep water rice cultivars of Assam.

	Negheri	Bam-Kokua	Do-kokua	Jool	Kolioi	Gotha	Ranga	Adolia	Holodhar	Bawla
Negheri	100.00	57.14	60.00	53.33	50.00	50.00	29.41	35.29	35.29	38.46
Bam-kokua		100.00	86.66	92.85	66.66	66.66	36.84	50.00	50.00	46.66
Do-kokua			100.00	93.33	80.00	80.00	40.00	52.63	52.63	50.00
Jool				100.00	73.33	73.33	42.10	55.55	55.55	53.33
Kolioi					100.00	100.00	38.88	44.44	44.44	40.00
Gotha						100.00	38.88	44.44	44.44	40.00
Ranga							100.00	58.82	58.88	29.41
Adolia								100.00	100.00	53.33
Holodhar									100.00	53.33
Bawla										100.00

2.45% (Grist, 1984). Baruah *et al.* (2006) working with indigenous land races of deep water rice reported lipid content to be in the range of 2.42% to 4.64% with a mean of 3.43% which is slightly higher than that of the present finding. It is apparent that deep water rice contain higher lipid than that of average paddy. Ash content and crude fibre values in the present study have been found to be comparable with that of rice in general (Juliano *et. al*, 1964) as well as some other land races of deep water paddy (Baruah *et. al*, 2006)

Seed protein profile usually remains unaffected due to environmental factors, geographical locality or seasonal changes etc. and hence can be used as a reliable criterion to characterize a species or cultivars at molecular level (Naik and Kole, 2002). Gomathinayagam and Ramaswamy (1994) used seed protein profile to work out the phylogenetic relationship between cowpea and its related species. Seed protein profile can also be used to differentiate the cultivars within a species and to work out their relationship as has been shown for *Vigna umbellate* (Seyie, 2004). The major seed proteins for rice are reported to be 57, 39, 37, 23, 22, 16, 13 and 10 Kd (Tanaka and Ogawa, 1985). Sarma *et al.* (2006) reported 14 seed proteins for the rice cultivar Mahsuri; the major proteins being of 64.5, 54.0, 41.2 and 32.2 Kd size. In the present study, the variations among the deep water paddy land races have been reflected by the variation in the number of protein and their profile.

Dendrogram analysis revealed a total of 4 clusters. Bawla has been found to be quite different from the rest and forms a cluster alone. Likewise, Negheri has been found to be distinct from the rest and forms a separate cluster alone. Dendrogram further revealed that Adolia and Holodhar are identical at molecular level. Likewise, Kolioi and Gotha have no difference at molecular level (Fig-2).

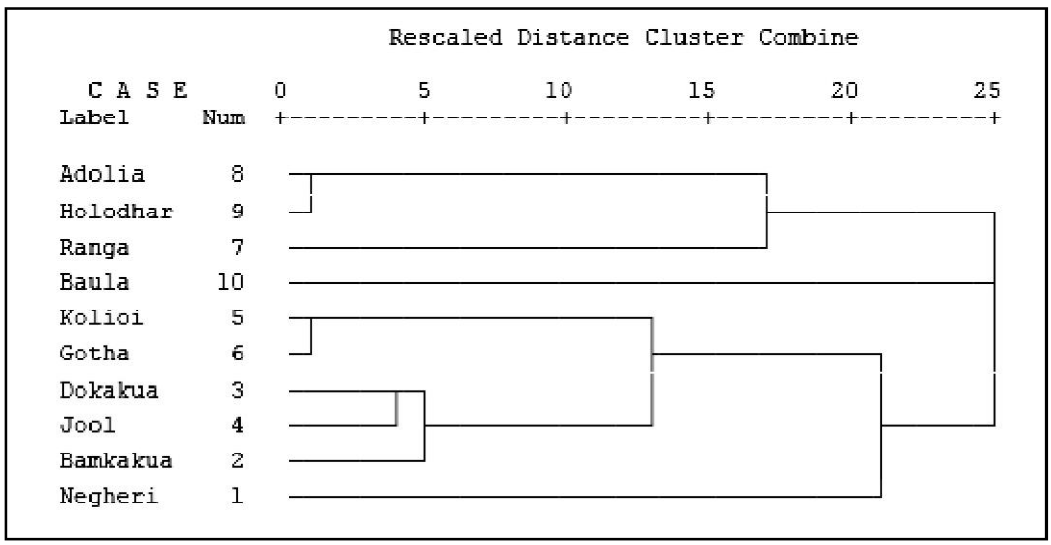

Fig 2. Dendrogram analysis of deep water rice cultivars of Assam based on seed protein profile.

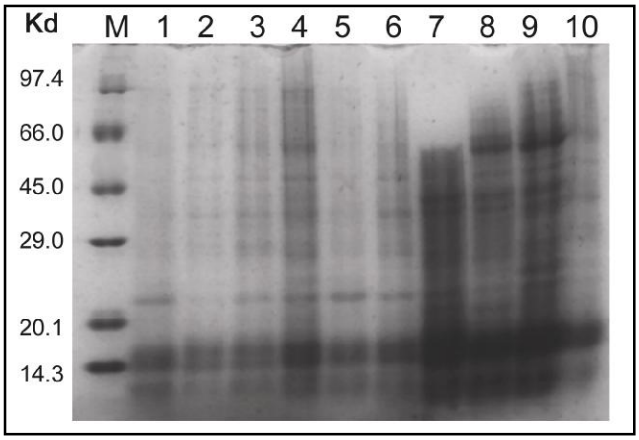

Fig 1. Seed protein of 10 indigenous land races of deep water paddy (Bao) resolved in 14 % polyacrylamide gel.

Lane M- Marker, 1- Negheri, 2-Bam-Kokua, 3-Do-kokua, 4-Jool, 5-Kolioi, 6-Gotha, 7-Ranga, 8-Adolia, 9-Holodhar, 10- Bawla

As a whole *Bao dhan* (deep water paddy) appears very promising in terms of nutritive value. Morover, the high degree of polymorphism of the seed protein make it conducive for molecular analysis and characterization for documentation. In view of the growing interest in trade circles (Anon., 2008) coupled with its promising nutritive values further study on deep water paddy needs resurgence.

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