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Starch and protein profile of hill rice cultivars of Assam

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ABSTRACT

Seventeen traditional hill rice cultivars of Assam were evaluated for their starch and protein profile and compared with Ranjit, a popular sali variety of plains. On dry weight basis, moisture contents ranged from 7.49 to 10.1%, crude fat from 3.14 to 5.27%, crude protein from 9.27 to 11.42%, starch content from 70.31 to 78.13%, amylose from 18.61 to 23.39%, amylopectin from 76.61 to 81.39%, total soluble protein content from 4.5 to 6.4% and lysine content from 2.94 to 3.88% of total protein. The ratio of four soluble protein fractions viz., albumin, globulin, prolamin and glutelin was found as 12:15:7:66. Sakbothung recorded highest protein content while Ranjit showed the highest starch content. Bijor recorded the highest lysine content. SDS-PAGE of total soluble protein and the three fractions, albumin, globulin and glutelin did not reveal any major variation among the cultivars. Association among the rice cultivars revealed by Unweighted Pair Group Method with Arithmatic Averages (UPGMA) analysis based on SDS-PAGE of total protein and the three fractions resulted in four clusters showing greater homology between rice cultivars. The close genetic similarity among the cultivars emphasizes the exploitation of large germplasm collections for use in rice improvement programme.

Key words : *hill rice,Assam, starch, protein profile*

North east India, including Assam, is endowed with exceptionally rich rice diversity. Variations in ecological conditions, ethnic diversity, diverse cultural practices and different quality preferences contribute to the diversity present in different types of rice, locally known as *ahu, sali, boro, bora, joha, lahi* etc. (Pathak, 2001). In Assam, rice is grown in all the six agro-climatic zones. The Hill zone constitutes 20% of the geographical area of the state with 1.24 lakh ha area and production of 1.74 lakh tonnes of rice. The farmers of hill region of Northeast India follow a traditional method of cultivation called "*jhum*" or shifting cultivation. A large number of traditional rice cultivars are grown in the hill region of Assam. These cultivars are neither described nor evaluated for their nutritional composition.

In India and abroad, already a large number of rice germplasm have been analyzed for biochemical composition of the germplasm are mainly from the plain zones. No systematic efforts have so far been made for the biochemical characterization of hill rice germplasm. Therefore, an attempt was made to study the starch, protein profile and the most limiting amino acid content of the hill rice cultivars of Assam to supplement the rice varietal improvement programme.

MATERIALS AND METHODS

The hill rice cultivars were procured from the Regional Agricultural Research Station, Assam Agricultural University, Jorhat. The grains were oven dried at 80°C (± 2°C) and converted into fine powder with the help of an electric grinder and kept in a desiccator for analysis. Moisture content was determined by following the method of AOAC (1970). The starch content was estimated by the method as described by Chopra and Konwar (1976). Amylose content was determined by the method described by Swawbhagya and Bhattacharya (1979). The amylopectin content was determined by subtracting the percentage of the amylose from 100 on moisture free basis. Total nitrogen was estimated as per Kjeldahl modified method by Scales and Harrison (1920) and converted to protein values by multiplying nitrogen percentage with the factor 5.95. Crude fat was determined from oven dried sample using a Soxhlet

apparatus (AOAC, 1970). Lysine content of the rice cultivars was estimated as mentioned by Sadasivam and Manickam (1996).

Total soluble protein was extracted from 0.2 gm of defatted sample, soaking overnight at 4°C in 1 ml of 50 mM Tris-Cl buffer (pH 7.6) containing 6 mM β-mercaptoethanol and subsequently grinding and homogenizing in a precooled pestle and mortar. The suspension was centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant so obtained was used for protein estimation. Albumin, globulin, prolamin and glutelin were extracted by modified Osborne's method (Juliano and Boulter, 1976). Total soluble protein and the four fractions were quantified by method of Lowry's *et al.* (1951). The protein samples after quantification were used for SDS-PAGE using the method of Laemmli (1970). Standard broad range protein molecular weight marker was used to determine the molecular weight of polypeptides.

Ignoring the intensity, bands were scored as present (1) or absent for each protein profile. An agglomerative method of clustering genotype was employed utilizing the unweighted pair group method with arithmetic averages (UPGMA) by using similarity matrix as input data (Sokal and Sneath, 1963).

RESULTS AND DISCUSSION

The moisture content of cereals is not an absolutely fixed property. The range of moisture content of 17 hill rice cultivars was found to vary from 7.49 to 10.1% (Table 1). The moisture content was found to be lower than the values for Assam rice reported by different workers (Bhagabati, 2000 and Deka, 2003). However, Dutta and Baruah (1978) explained that the moisture content is dependent upon many factors such as variety, proportionate amount of chemical constituent of the grains, time of harvesting, processing, storage conditions, environmental factors etc.

Rice protein is nutritionally superior over most of the other cereals, but most part of it (7-8%) is lost during milling, as protein is mostly distributed in the bran and periphery of the endosperm (Juliano *et al.*, 1964). The range of crude protein content in the present investigation was found to be 9.27-11.42% (Table 1). Some wider and higher range of protein content was recorded by Kandali and Borah (1992). Protein content in the hill rice cultivars was found almost similar with the glutinous, non-glutinous, scented and *boro* rice varieties of Assam (Kandali *et al.*, 1995; Bhagabati, 2000; Deka, 2003; Ahmed 2003). Juliano (1977) recorded lipid content of 2.4-3.9% in brown rice, 17.5-21.5% in bran and 0.3-0.6% in polished rice. The crude fat contents in the hill rice cultivars (3.14-5.27%) were found higher than the check Ranjit (2.99%) and other glutinous, *boro* and scented rice varieties of Assam (Bhagabati, 2000; Deka, 2003; Ahmed, 2003). Though limiting in cereal proteins, lysine content is found to be highest in oats and rice amongst the cereals. In the present investigation, the hill rice cultivars recorded 2.94 to 3.88% lysine which is comparable to the normal lysine contents (3.2-3.9%) in brown rice (Sikka *et al.*, 1989). Juliano *et al.* (2006) reported that the lysine content of rice protein varied at most by 0.5% at any protein level and described a negative correlation between lysine content and protein content of brown and milled rice in varieties with protein contents below 10%.

The starch content of hill rice cultivars ranged from 70.31 to 78.13% and was found to be lower than the glutinous and non-glutinous rice varieties of Assam (Dutta and Baruah, 1978) but higher than those of *boro* and scented rice varieties (Deka, 2003 and Ahmed, 2003). The amylose content of the hill rice cultivars was found to range from 18.61 to 23.39% (Table 1). Allahgholipour *et al.* (2006) studied 167 land races of rice from 23 different rice growing countries. They separated those varieties into four groups based on the amylose content. The four amylose content groups were waxy rice (0-8%), low amylose content (8.1-16%), intermediate amylose content (16.1-24%) and high amylose content (>24.1). Thus the cultivars under study may be considered as intermediate amylose containing rice cultivars. Such rice remains fluffy and soft on cooking and is mostly preferred over other varieties. The amylopectin content of the hill rice cultivars varied from 76.61 to 81.39%.

The total soluble protein content of the hill rice cultivars (4.5-6.4%) was found comparable with those of glutinous, non-glutinous and aromatic rice varieties of Assam (Bhagabati, 2000; Ahmed, 2003), but found to be lower than the *boro* rice varieties (Deka, 2003). The ratio of albumin: globulin: prolamin: glutelin in the

Table1. Moisture context, crude protein, crude fat, starch, amylose, amylopectin, total soluble protein content and lysine content of hill rice of Assam.

Rice Cultivar	Moisture (%)	Crude protein (%)	Crude fat (%)	Lysine (g 100 g^{-1} of protein)	Starch (%)	Amylose (%)	Amylopectin (%)	Total soluble protein (%)	Protein fraction (% of extracted protein)			
									Albumin	Globulin	Prolamin	Glutelin
Bairing Gurmu	10.10	9.88	4.99	3.30	76.65	23.39	76.61	4.70	12.30	16.27	6.94	64.48
Vandana	9.47	10.84	3.50	3.03	75.35	21.64	78.36	4.65	11.50	15.67	6.35	66.47
Joradhan	7.49	10.67	5.15	3.49	73.71	21.89	78.11	4.50	12.72	15.10	6.56	65.60
Miren	8.30	11.06	5.06	3.01	76.41	21.06	78.94	5.35	13.43	16.40	7.90	62.25
Laldhan	9.34	10.15	3.30	3.62	74.55	22.33	77.67	6.20	13.27	14.42	7.88	64.42
Maibee	8.87	11.15	5.04	3.11	71.65	22.67	77.33	4.80	9.66	13.60	8.67	68.04
Maichukik	9.45	10.06	4.97	3.56	76.74	22.55	77.45	5.20	11.91	17.38	8.2	62.50
Dimrou	8.96	10.75	5.18	3.06	75.51	20.88	79.12	5.60	13.17	16.67	7.4	62.75
IRAT-141	9.29	9.8	5.19	3.82	78.13	21.26	78.74	4.70	12.17	16.37	8.58	62.87
Bairing	8.96	11.02	5.10	3.15	76.27	18.61	81.39	4.55	11.47	16.09	5.03	67.40
Sakbothung	8.64	11.42	4.82	2.94	72.81	21.38	78.62	6.40	11.15	18.13	6.97	65.73
Buarcha	8.65	10.49	4.37	3.09	71.59	21.21	78.79	4.60	11.50	15.08	5.95	67.46
Sakcharap	8.29	11.19	4.76	3.05	70.31	21.45	78.55	4.95	12.60	13.44	6.72	67.23
Vijoy	9.05	10.75	5.27	3.24	75.39	20.68	79.32	4.60	12.47	14.28	7.85	65.39
Bijor	8.50	9.27	4.75	3.88	75.30	23.13	76.87	4.50	13.77	14.37	5.99	65.86
Pakai	7.70	11.06	4.84	3.11	71.15	20.07	79.93	6.25	10.30	14.14	8.08	67.68
Konchom	8.69	10.75	3.14	3.09	71.87	21.57	78.43	5.60	9.6	13.83	7.31	69.16
Ranjit	9.40	11.02	2.99	3.25	78.19	22.82	77.18	6.60	12.89	15.22	6.74	63.58
Mean	8.84 (8.81)	10.63 (10.61)	4.58 (4.67)	3.27 (3.27)	74.53 (74.32)	21.59 (21.52)	78.41 (78.48)	5.21 (5.13)	11.99 (11.94)	15.39 (15.37)	7.17 (7.20)	65.49 (65.61)
S.Ed.(±)	0.347	0.157	0.138	0.091	0.436	0.453	0.453	0.325	0.279	0.200	0.191	0.207
CD 0.05	0.703	0.319	0.280	0.184	0.884	0.919	0.919	0.660	0.567	0.406	0.388	0.420
CD 0.01	0.942	0.428	0.376	0.246	1.185	1.233	1.233	0.885	0.760	0.545	0.521	0.563

Data in parentheses represent the mean of 17 hill rice cultivars

hill rice cultivars was found as 12:15:7:66. Thus glutelin was the major storage protein, while prolamin accounted for the lowest. The high level of albumin and glutelin in the rice cultivars might play an important role in increasing biological value as the level of lysine and methionine is generally more in these two fractions of rice protein.

SDS-PAGE of total soluble protein resulted in polypeptide bands with molecular weight (MW) ranging from 14 kD to 97 kD (Fig. 1a). All the bands were monomorphic. There was a good agreement of the polypeptide composition of rice protein observed in the present study with those reported earlier (Santra *et al.*, 2000; Baishya *et al.* 2003). Variations in number of subunits and molecular weights may be emerging from the systems employed for electrophoresis and the protein constitution of different cultivars of rice (Padhye and Salunkhe, 1979). Yupsanis *et al.* (1992) recommended SDS-PAGE of albumin and globulin as a more suitable method to distinguish closely related varieties and the results obtained could be used by rice breeders in order to characterize varieties. SDS-PAGE for albumin fraction of rice protein showed 14 polypeptide bands of which 11 were monomorphic (Fig. 1b). Four major bands with MW~14, 60, 66 and 97 kD were observed in all the cultivars. Two bands with MW 49 and 54.5 kD were found absent in Bairung Gurmu and Vandana. Another band with MW 27 kD was found in all the cultivars except for Bairung Gurmu. SDS-PAGE of globulin fraction of rice protein revealed three major monomorphic bands with MW14, 17 and 23 kD, but with no qualitative differences (Fig.1c). However there were qualitative differences in the remaining three monomorphic bands. Two of these with MW 49 and 54.5 kD were prominent only in Laldhan and Maibee. Two polypeptides with MW 60 and 90 kD were absent in the cultivars Vandana, Joradhan and Miren. Sakbothung did not have the polypeptides with MW 54.5, 66 and 97 kD, but was distinguished by the presence of a unique polypeptide band with MW 45 kD. SDS-PAGE analysis of glutelin fraction of rice protein showed six bands with MW ranging from ~14 to 82 kD (Fig. 1d). The band with MW 82 kD was polymorphic and was absent in Dimrou and IRAT-141. Chandi and Sogi (2007) reported glutelin fraction of rice protein resolving into subunits ranging from 22-88 kD.

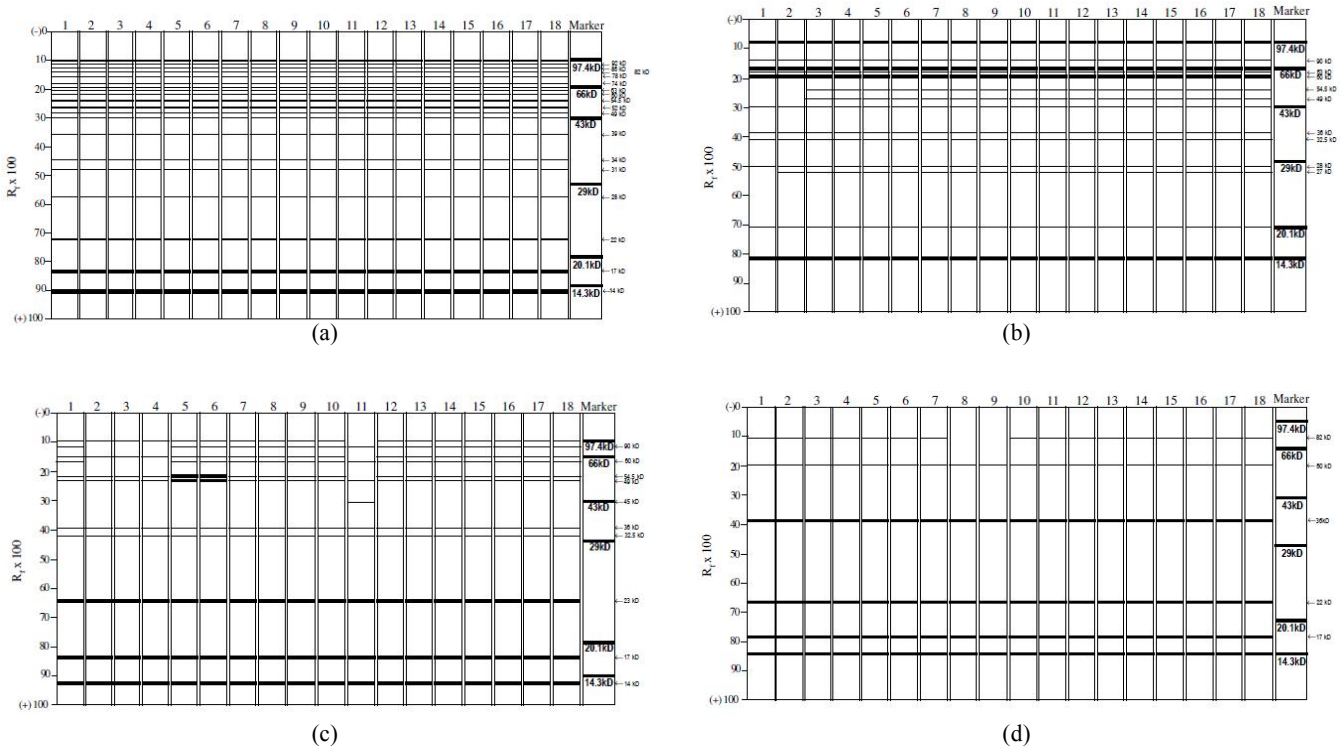

Fig.1 Electrophoretic banding pattern of SDS-PAGE of (a) total protein, (b) Albumin, (c) globulin & (d)glutelin of hill rice cultivars of Assam.

The similarity coefficient for 18 rice cultivars based on SDS-PAGE analysis of total soluble protein and the three fractions *viz.*, albumin, globulin and glutelin varied from 0.840 to 1.000 (Table 2). The similarity coefficient analysis revealed that most of the rice cultivars under study were almost same in their protein profile. Dendrogram constructed based on protein data resulted in four clusters (Fig.2). Cluster 1 has two sub-groups-1a and 1b. Sub-group 1a consists of the cultivars

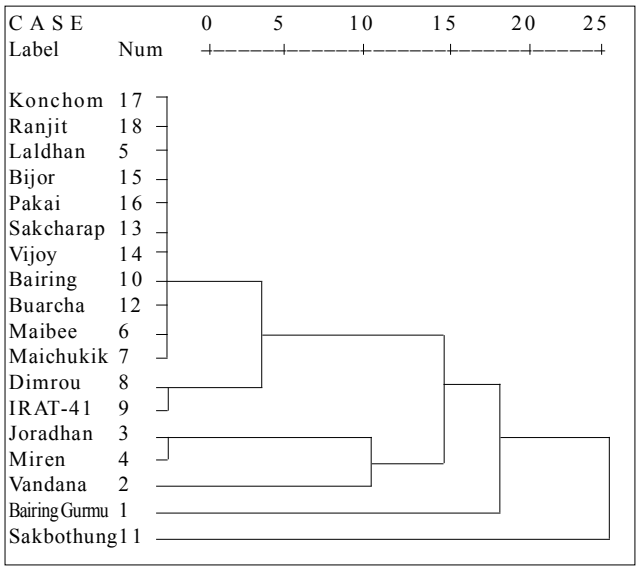

Fig. 2 Dendrogram of the rice cultivars constructed using unweighted pair group method with arithmatic average (UPGMA) based on Jaccard's similarity coefficients

Konchom, Ranjit, Laldhan, Bijor, Pakai, Sakcharap, Vijoy, Bairing, Buarcha, Maibee and Maichukik. Sub-group 1b includes Dimrou and IRAT-141. Cluster 2 has two sub-groups – 2a and 2b. While the sub-group 2a consists of the cultivars Joradhan and Miren, sub-group 2b has only one cultivar, Vandana. Cluster 3 consists of the cultivar Bairing Gurmu. Cluster 4 consists of the cultivar Sakbothung. The close genetic similarity among the cultivars emphasized the exploitation of large germplasm collections for use in rice improvement programme. Most of the hill rice cultivars used in the study were pigmented which might be due to the presence of anthocyanin. Anthocyanin-pigmented rice varieties are well known for its taste and health-improving properties including diverse physiological effects, protection against cytotoxicity, antineurodegenerative activity, antioxidative activity etc. (Chung and Shin, 2007).

Table 2. Similarity matrix for Jaccard's co-efficient of SDS-PAGE of total soluble protein, albumin, globulin and glutelin fractions of hill rice cultivars of Assam.

	Bairing Gurmu	Vandana	Joradhan	Miren	Laldhan	Maibee	Maichukik	Dimrou	IRAT -141	Bairing	Sakbothun	Buarcha	Sakcharap	Vijoy	Bijor	Pakai	Konchom	Ranjit
Bairing Gurmu	1.000																	
Vandana	.936	1.000																
Joradhan	.898	.957	1.000															
Miren	.898	.957	1.000	1.000														
Laldhan	.939	.918	.959	.959	1.000													
Maibee	.939	.918	.959	.959	1.000	1.000												
Maichukik	.939	.918	.959	.959	1.000	1.000	1.000											
Dimrou	.918	.898	.939	.939	.980	.980	.980	1.000										
IRAT-141	.918	.898	.939	.939	.980	.980	.980	1.000	1.000									
Bairing	.939	.918	.959	.959	1.000	1.000	1.000	.980	.980	1.000								
Sakbothung	.860	.840	.880	.880	.920	.920	.920	.900	.900	.920	1.000							
Buarcha	.939	.918	.959	.959	1.000	1.000	1.000	.980	.980	1.000	.920	1.000						
Sakcharap	.939	.918	.959	.959	1.000	1.000	1.000	.980	.980	1.000	.920	1.000	1.000					
Vijoy	.939	.918	.959	.959	1.000	1.000	1.000	.980	.980	1.000	.920	1.000	1.000	1.000				
Bijor	.939	.918	.959	.959	1.000	1.000	1.000	.980	.980	1.000	.920	1.000	1.000	1.000	1.000			
Pakai	.939	.918	.959	.959	1.000	1.000	1.000	.980	.980	1.000	.920	1.000	1.000	1.000	1.000	1.000		
Konchom	.939	.918	.959	.959	1.000	1.000	1.000	.980	.980	1.000	.920	1.000	1.000	1.000	1.000	1.000	1.000	
Ranjit	.939	.918	.959	.959	1.000	1.000	1.000	.980	.980	1.000	.920	1.000	1.000	1.000	1.000	1.000	1.000	1.000

The present study revealed that the hill rice cultivars were no way inferior in terms of starch, protein and lysine contents to the popular rice variety Ranjit. However, it is necessary to undertake a detailed study on the amino acid composition, mineral contents, vitamin contents and effect of parboiling on different nutritional components for the evaluation of the nutritional quality of these rice cultivars.

REFERENCES

Ahmed SA 2003. Studies of aroma in scented rice (*Oryza sativa* L.) of Assam. A Ph D thesis submitted to Assam Agricultural University, Jorhat

Allahgholipour M, Alia J, Alinia F, Nagamine T and Kojima Y 2006. Relationship between rice grain amylase and pasting properties for breeding better quality rice varieties. Plant Beed. 125 : 357-362

AOAC 1970. Official methods of analysis. 10th Ed. Association of Official Analytical Chemists, Washington, D.C.

Baishya S, Sachdev A, Johari RP and Mehta SL 2003. Studies on proteins and isozymes in aromatic and non-aromatic rice. Indian J. Plant Physiol. 8 : 323-328

Bhagabati RKM 2000. Biochemical and isozyme analysis of some glutinous and non-glutinous rice (*Oryza sativa* L.) varieties of Assam. M.Sc.(Agri.) Thesis, Assam Agricultural University, Jorhat.

Chandi GK and Sogi DS 2007. Biochemical characterization of rice protein fractions. International J. Food Sci. Technol. 42: 1357-1362

Chopra SL and Konwar JS 1976. In : Analytical Agricultural Chemistry, Kalyani Publ., Ludhiana.

Chung HS and Shin JC 2007. Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (*Oryza sativa* cv. *Heugjinjubyeo*). Food Chem. 104 : 1670-1677

Deka DK 2003. Biochemical evaluation of some boro rice (*Oryza sativa* L.) varieties grown in Assam. M.Sc.(Agri.) Thesis, Assam Agricultural University, Jorhat.

Dutta, L. and Baruah, J.N. 1978. Nutrient composition of glutinous and non-glutinous rice varieties grown in Assam. Indian J. Agric. Sci. 48: 610-613

Juliano BO 1977. Rice lipids, II. Riso 25 : 3

Juliano BO, Antonio AA and Esmama BV 2006. Effects of protein content on the distribution and properties of rice protein. J. Sci. Food Agric. 24 : 295-306

Juliano BO, Banitista GM, Dugay JC, and Reyes AC 1964. Studies on the physiological properties of rice. J. Agric. Food Chem 12 : 131-138

Juliano BO and Boulter D 1976. Extraction and composition of rice endosperm glutelin. Phytochemistry 15: 1601

Kandali R, Ahmed SA, Borah RC, Sarkar CR and Pathak AK 1995. Biochemical analysis of some varieties developed by the Assam Agricultural University, JAAS (Jpl. Vol.) pp. 42

Kandali R and Borah RC 1992. Screening of some local rice cultivars of Assam for crude protein and glutelin and correlation between protein and grain yield. Oryza 31:169-173

Laemmli UK 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 680-685

Lowry OH; Rosenbought NJ.; Ferr AL and Randall RJ 1951. Protein measurement with the Folin-phenols reagent. J. Biol. Chem. 193: 265-275

Padhye VW and Salunkhe DK 1979. Extraction and characterization of rice protein. Cereal Chem. 56: 389-393

Pathak PK 2001. Major cereal crops of Assam. In : Thakur, A.C.; Bhattacharya, H.; Sarma, D.K. (eds.). Agriculture in Assam. Directorate of Extension Education, Assam Agricultural University, Jorhat, India, pp. 53-77

Sadasivam S and Manickam A 1996. In Biochemical Methods, 2nd edition, New Age International Publishers : 45-46

Santra DK, Borah RC and Sen P 2000. Accumulation of storage proteins in developing grains of high and low protein rice varieties and their characterization. Oryza 37 : 198-201

Scales FM and Harrison AP 1920. Boric acid modification of the kjeldahl method for crop and soil. Analysis J. Indust. Eng. Chem. 12: 350

Sikka KC, Lodha ML and Mehta SL 1989. Nutritional quality of cereals and grain legume proteins. In Mehta SL, Lodha ML and Sane PV (eds.) Recent advances in Plant Biochemistry, ICAR publication : 303-340

Sokal RR and Sneath PHA 1963. Principles of numerical taxonomy. W. H. freeman, San Francisco, CA. USA.

Swawbhagya CM and Bhattacharya KR (1979). Simplified determination of amylose in millet rice. Starke 36: 159-163

Yupsanis T, Moustak M and Karakoli S 1992. Seed protein electrophoresis for variety identification in rice (*Oryza sativa* L.). J. Agron. Crop Sci. 168 : 95-99