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Evaluation of *Chokuwa* Paddy (Semi glutinous) Landraces of Assam for Nutritive Values and Genetic Diversity Based on Seed Protein Profile by SDS-PAGE

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*Chokuwa*s are a group of unique and indigenous landraces of paddy cultivated in certain pockets of Upper Assam as Kharif paddy. Analysis of nutritive value for this lesser known and little explored paddy group shows that protein content varied from 7.20% to 10.86%, carbohydrate from 68.05% to 81.52% and lipid content from 1.90% to 3.57% with calorific value in the range of 320.87 Kcal/100gm to 376.83 Kcal/100gm. On the other hand dietary fibre varied from 0.92% to 1.40% while total mineral in the form of ash content varied from 0.90% to 1.43%. *Chokuwa*s are rare, endemic with limited number of landraces but molecular analysis based on seed protein profile shows that considerable genetic diversity exists. Dendrogram based on seed protein electrophoretic profile showed three distinct clusters. Notably, five different landraces with same ethnic name "*Soru Chokuwa*" collected from five different localities have been found to be all different from each other in terms of nutritional parameters as well as genetically. Three protein bands with size 20.3, 13.4 and 11.6 Kd were consistently found in all landraces and can be considered as markers.

Key Words: *Chokuwa* rice, Nutritional Components, Seed Protein Profile, SDS-PAGE

Introduction

Re-emphasis on indigenous landraces of crop species, particularly paddy is gaining momentum with the realization that many of them are rich in nutritive and nutraceutical value(Loying *et al.*, 2008, 2010); harbour precious genes for resistance to biotic and abiotic stress (Toledo and Burlingame, 2006) and in view of alarming gene erosion (Swaminathan, 2011). North East India is widely recognized as a secondary centre of origin for paddy. Among them the indigenous paddy landraces of Assam are unique in many ways. The International Rice Research Institute, Manila has a separate and special collection labeled as "Assam Rice Collection" which reflect the importance attached to the paddy landraces of Assam for the valuable traits they possess. Among them a group of landraces called "*Chokuwa*" are unique in many ways and least explored. *Chokuwa*'s are basically kharif paddy and appears to be endemic since their cultivation is restricted to few pockets of Upper Assam. In terms of taste and cooking quality they are intermediate between glutinous rice (*Bora Rice*) and non-glutinous rice. The difference between Bora (glutinous) and *Chokuwa* are in relative proportion of amylose content, while Bora is characterised by trace, *Chokuwa* contain 15-20% amylose (Sarma and Barooah, 2005) However, like glutinous rice they are exclusively used for preparation of various delicacies, rice beer etc and not used for regular consumption. Paddy cultivation in Assam is heavily dependant on kharif paddy (Sali) with 68% area under cultivation accounting for 73% total paddy production (Barthakur,1992) and *Chokuwa*'s have little contribution to overall paddy production and food security. Hence they remain neglected by farmers, policy planners, Govt. Departments and even researchers and hence they are facing the danger of gene erosion. Earlier studies have shown that many indigenous land races are rich in nutritive values, particularly protein, eg. deep water paddy (Loying *et al*., 2010) as well as dietary antioxidants (Loying *et al*., 2008); unique flavor and aroma, eg. Joha (Dutta Roy 2010). The delicacies prepared with *Chokuwa* are highly valued but *Chokuwa*s remain by and large unexplored in terms of genetic diversity, agro-technology, nutritive value etc. The foremost requirement and pre-requisite for conservation programme is study of nutritive values and genetic diversity and other major qualities and traits. The present study attempts to contribute in that direction.

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Materials and Methods

17 indigenous landraces of *Chokuwa* paddy namely *Kajoli Chokuwa*, *Ronga Chokuwa*, *Bor Chokuwa*, *Soru Chokuwa* (5 Nos.) *Bora Chakuwa*, *Nepali Chokuwa*, (3 Nos), *Khesheng Chokuwa*, *Maigum Chokuwa*, *Aam pakhi Chokuwa*, *Gorundra pakhi Chokuwa, and Boga pakhi Chokuwa*. The prefix "Bor" stand for bigger size and "Soru" stand for small size. Five landraces with the same ethnic name *Soru Chokuwa* were collected from five different localities with distinct morphological differences. Similarly three landraces with same ethnic name *Nepali Chokuwa* were collected from different areas. All samples were collected from village area of Lakhimpur, Sonitpur, Dhemaji and Jorhat District of Upper Assam.

The grains were manually dehusked, grounded to fine powder and dried in oven at 60°C till constant weight was recorded for biochemical analysis. Moisture data were recorded and chemical analysis was carried out using dried sample. Crude protein was estimated by working out the total nitrogen by microkjeldahl method (AOAC, 1970). Total Carbohydrate was estimated by anthrone method as outlined by Clegg (1956) with prior digestion of the dry matter using 2.5N HCl for 30 minutes in a hot water bath. For total soluble sugar the sample was extracted with warm 80% ethanol. The mixture was centrifuged to obtain the supernatant and the residue washed twice with warm 80% ethanol. Subsequently ethanol was removed by evaporation and the extract was redissolved with distilled water. From this soluble sugar was estimated by anthrone method. Lipid content was estimated by extracting the sample in Soxhlet apparatus (AOAC, 1970) using petroleum ether (40º-60ºC) and the amount of lipid was determined after removal of petroleum ether by fractional distillation. Dietary fiber was estimated as per the protocol of Sadasivam and Manickam (1996). Total mineral (ash content) was determined by ashing the sample in Muffle furnace at 630ºC for three hours, (AOAC, 1970).Calorific value was computed by using the formula of Sherman (1952)

Seed protein profile were worked out by SDS-PAGE method outlined by Laemmli (1970). Manually dehusked grains (300 mg) were extracted with ice-cold Tris-buffer 0.2 M (pH :7.5). The sample was homogenized and centrifuged at 12000 rpm for 8 minutes at 4ºC. The supernatant was collected and sample weight to extract volume was adjusted to 1:5 ratio. Seed proteins were resolved in two phase acrylamide gel-stacking gel 4%, separating gel 13.5%. Protein bands were visualized by staining with Coomassie Brilliant Blue R-250 stain. 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 races and molecular weight of individual band was deduced from standard curve prepared with size marker. Gel scoring was done in terms of presence or absence of particular protein band for a landrace to prepare the frequency distribution table. From this table similarity index (SI matrix) were generated using Nei and Li's co-efficient (1979). The SI matrix was used to generate the dendrogram by unweighted pair group method with arithmetic averages (UPGMA) using the software NTSYS pc.V2.02j.

Results and Discussion

The present work tries to explore the nutritive value of *Chokuwa* rice which has remained little explored despite their potential. Moisture content among the sample varied within the limited range of 2.60% to 4.15% with a mean of 3.35%. It has been found that protein content varied in the range of 7.20% to 10.86% with a mean of 9.12% (Table 1). It is high in carbohydrate as total carbohydrate varied in the range of 68.05% to 81.52% with a mean of 73.65%. Four landraces had total carbohydrate of 80.00% or nearly so. Total soluble sugar was found in the range of 0.81% to 1.78% with a mean of 1.20%. Unlike crude protein and total carbohydrate it is low in lipid content. Total lipid varied from 1.90% to 3.57% with a mean of 2.37%. Compared to other constituents dietary fibre contents were low and occurred in the range of 0.92% to 1.40% with a mean value of 1.17%. Mineral content (Ash content) were very impressive and varied from 0.90% to 1.43% with a mean value of 1.17%. Because of high carbohydrate content calorific value is high and varied from 320.87 Kcal/100gm in *Bor Chokuwa* to 376.83 Kcal/100gm in *Soru Chokuwa-4* with a mean value of 352.48 Kcal/100gm (Table 1).

From nutritional view point the most promising aspect of *Chokuwa* paddy is its high protein content although considerable variation exist among the land races. 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). The present study is comparable with this as it is 7.20% to 10.86% with the mean of 9.12%. For carbohydrate the mean value in the present

Table 1. Nutritional components of *Chokuwa* (Semi glutinous) paddy landraces of Assam. The values are mean of three replication ± Standard Error of Mean.

S. No.	Name of the cultivar	Moisture (%)	Crude protein(%)	Carbohydrate (%)	Total soluble sugar(%)	Lipid(%)	Dietary fibre (%)	Total mineral (%)	Calorific value (Kcal/100gm)
1	Kajoli	3.20±0.13	10.21±0.58	71.32±0.62	0.82±0.10	3.57±0.14	0.99±0.04	1.15±0.09	358.25±0.66
2	Ronga	3.00±0.13	10.86±0.77	68.39±0.51	1.21±0.16	2.71±0.08	1.16±0.16	0.90±0.04	341.39±0.66
3	Maigum	3.43±0.09	10.47±0.57	69.53±0.55	1.25±0.17	2.95±0.04	1.14±0.07	1.31±0.04	346.55±0.60
4	Bor	4.15±0.10	7.33±0.48	68.05±0.56	0.81±0.12	2.15±0.16	1.34±0.06	1.12±0.08	320.87±0.57
5	Soru-1	2.80±0.13	10.11±0.57	73.39±0.63	1.65±0.16	2.52±0.10	1.21±0.03	1.09±0.05	356.68±0.68
6	Soru-2	3.15±0.06	7.75±0.55	76.12±0.57	0.97±0.05	2.61±0.13	1.33±0.04	1.09±0.05	358.97±0.56
7	Aampakhi	3.63±0.06	8.49±0.66	73.23±0.61	0.96±0.06	2.33±0.10	1.15±0.04	1.26±0.05	347.85±0.61
8	Nepali-1	3.32±0.05	9.63±0.68	70.38±0.61	1.41±0.05	2.33±0.08	1.22±0.06	1.35±0.06	341.01±0.54
9	Soru-3	3.0±0.13	9.16±0.59	71.61±0.64	1.12±0.06	1.90±0.11	1.33±0.08	0.99±0.05	340.18±0.59
10	Bora	3.72±0.06	7.99±0.60	68.36±0.57	1.78±0.06	2.46±0.08	1.32±0.07	1.09±0.08	327.54±0.59
11	Gorundrapakhi	3.12±0.06	7.72±0.58	79.62±0.87	1.11±0.06	2.45±0.09	0.92±0.07	1.43±0.05	371.41±0.63
12	Nepali-2	2.85±0.05	9.77±0.57	79.17±0.72	0.97±0.04	2.32±0.09	1.19±0.07	1.18±0.06	376.64±0.65
13	Khesheng	4.23±0.05	7.55±0.51	77.44±0.59	1.3±0.08	2.03±0.06	1.15±0.06	1.20±0.05	358.23±0.55
14	Soru-4	3.18±0.08	10.81±0.60	79.01±0.47	1.36±0.06	1.95±0.05	1.40±0.04	1.07±0.09	376.83±0.58
15	Soru-5	3.56±0.08	9.55±0.57	75.69±0.40	1.69±0.05	1.93±0.06	0.95±0.08	1.23±0.06	358.33±0.62
16	Nepali-3	2.60±0.05	10.50±0.56	72.57±0.76	1.26±0.05	2.19±0.06	1.17±0.13	1.23±0.07	351.99±0.68
17	Boga pakhi	4.1±0.09	7.20±0.59	81.52±0.43	0.96±0.05	1.99±0.07	0.98±0.05	1.22±0.06	359.39±0.60
Mean		3.35	9.12	73.65	1.21	2.37	1.17	1.17	352.48
CD at 5%		0.33	1.73	1.77	0.31	0.27	0.07	0.19	1.77
CD at 1%		0.48	2.33	2.39	0.42	0.36	0.09	0.25	2.38

study (73.65%) has been found to be comparable to 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). Ash content, total soluble sugar, lipid and dietary fibre values in the present study have been found to be comparable with other rice in general (Juliano *et al*.,1964).

Seed proteins resolved in polyacrylamide gel revealed a total 14 proteins in the size range of >108 Kd to 11.6 Kd indicating wide variation from very high molecular weight to very low molecular weight protein. Highest number of 10 protein bands were observed in land races *Nepali Chokuwa-2*, *Khesheng* and *Soru Chokuwa-4* with identical profile. Lowest number of 5 protein bands were observed in *Maigum Chokuwa*. The protein bands with molecular weight 20.3, 13.4 and 11.6 Kd were consistently found in all the landraces and appear to be protein molecular marker for *Chokuwa*s. Apart from qualitative difference there were quantitative difference in terms of band thickness and corresponding pixel intensity (Fig. 3). Most prominent band was the one with molecular weight 13.4 Kd. Pixel intensity analysis show that this band is thickest in *Nepali Chokuwa-1* and lowest in *Bora Chokuwa*.

Seed protein profile has been recognized as a molecular tool to characterize a species at interspecific and intraspecific level by International Seed Testing Association (ISTA, 1996). Seed protein profile remain unchanged in seed of different stage of maturity, unaffected by environmental factors, geographical factors etc. (Ladizinsky and Hymowitz 1979, Naik and Kole, 2002) and hence considered to be protein fingerprint. A number of workers have used seed protein profile for molecular characterization as well as genetic diversity study in paddy and many other crops which shows the effectiveness and reliability of this technique. Loying *et al.* (2010) working with deep water paddy rice land races indigenous to Assam found a total 22 protein bands while Dutta Roy *et al.* (2010) working with scented rice (Joha) landraces of Assam recorded a total 16 protein bands. This indicate that depending upon ecotype as well as different paddy type, seed protein profile are highly variable which is reflective of high degree of genetic variability in paddy. In both the studies the marker proteins were three each and in medium to low

molecular weight size range of 26.7 to 15.7 Kd. In the present study a total 14 protein bands were observed and there were three markers in the medium to low molecular weight category. SI matrix and dendrogram analysis showed that there are three major clusters with considerable genetic diversity. Two landraces, *Aampakhi Chokuwa* and *Soru Chokuwa (2)* have been found to be distantly related to the rest as they do not belong to any of the three clusters.

The findings ofthe present study appear to be in conformity with the earlier reports. Among all crop species paddy exhibit highest genetic diversity. Using DNA based markers like RAPD, high degree of genetic diversity was also demonstrated for other group of paddy like glutinous rice (Sarma and Barooah, 2005). Based on seed protein Handique *et al.* (2007) demonstrated significant intraspecific variation among the cultivars of *Vigna umbelleta* (Rice bean) although it is a self pollinated crop. Similar varietal differences together with phylogenetic relationship has been demonstrated for ground nut (Chandran *et al.,* 2002), country bean (Sharma *et al.*, 2010). The most significant aspect of the present study is that the landrace with ethnic name Soru Chokuwa was collected from five different localities. Although same name, they showed some differences in seed morphology. Biochemical analysis revealed that composition of nutritional parameters were different. Seed protein profile showed that phylogenetically they

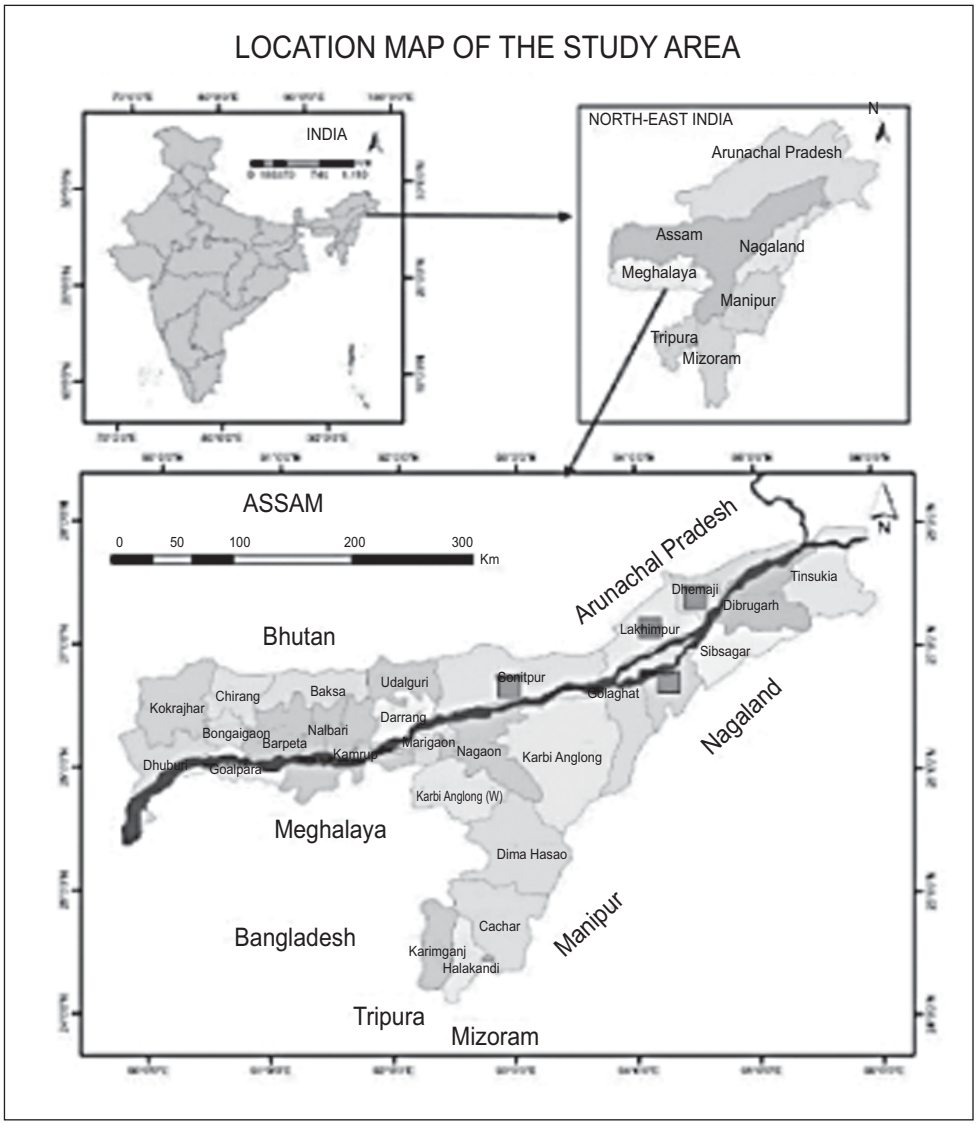

Fig 1. Location Map for sample collection.

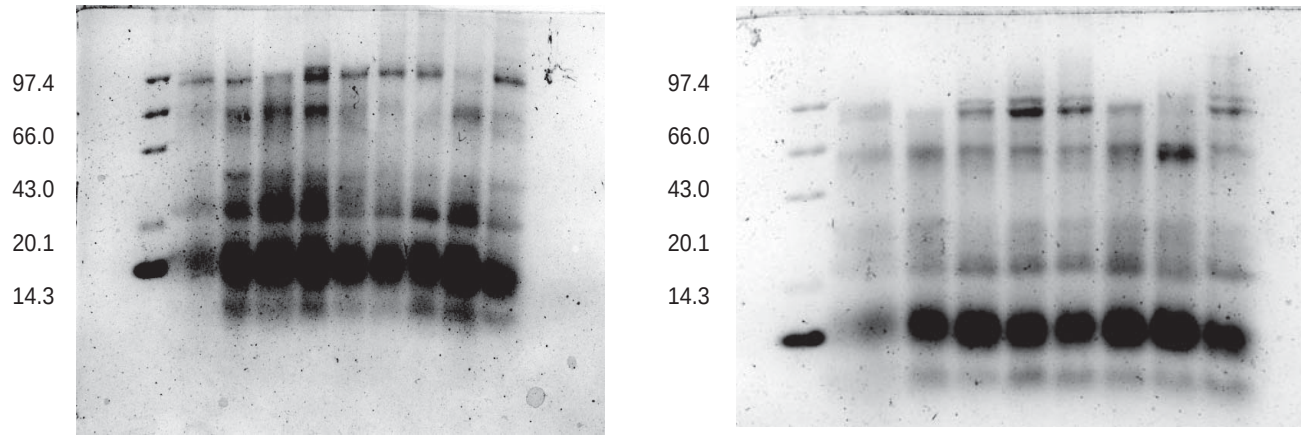

Fig. 2. Seed protein profile of 17 indigenous landraces of Chokuwa paddy (Semi glutinous) resolved in 13.5% polyacrylamide gel. M =Protein molecular weight marker; Lane 1 to 17- Individual landraces of Chokuwa.

Table 2. Frequency distribution of seed protein in *Chokuwa* paddy landraces of Assam

P=Presence of protein band; - Absent.

T-1 Total protein band for a landrace; T-2 Total landrace for a particular protein band.

S.No	Em (cm)	Mol. wt. (Kd)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	T-2
1	0.2	>108.0	-	-	-	P	-	P	P	P	P	-	-	-	-	-	-	-	P	6
2	0.7	>108.0	-	P	-	-	-	P	P	-	P	-	-	-	-	-	-	-	-	4
3	1.0	>108.0	P	P	-	-	-	P	P	-	P	-	-	-	-	-	-	-	P	6
4	1.4	>108.0	-	-	-	-	-	-	-	-	P	-	-	P	P	P	-	-	P	5
5	1.6	108.0	-	-	-	P	P	P	P	-	P	-	-	-	-	-	-	-	P	6
6	1.9	101.5	-	-	P	P	-	P	P	P	P	P	P	P	P	P	P	P	P	14
7	2.5	81.8	-	-	-	-	-	P	P	P	P	P	-	P	P	P	-	P	P	10
8	2.8	66.0	P	P	P	P	P	-	-	P	P	-	-	P	P	P	P	P	P	13
9	3.2	62.0	-	-	-	-	-	-	-	-	-	P	P	P	P	P	P	P	P	8
10	3.5	56.5	P	-	-	-	-	-	-	-	P	-	-	P	P	P	P	-	P	7
11	4.9	34.0	-	P	-	-	P	-	-	P	P	P	P	P	P	P	P	P	P	12
12	6.0	20.3	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	17
13	7.5	13.4	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	17
14	8.5	11.6	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	17
T-1			6	7	5	7	6	9	9	8	13	7	6	10	10	10	8	8	13	

Table 3. Similarity index matrix based on seed protein profile of *Chokuwa* paddy landraces of Assam

0	Cv-1	Cv-2	Cv-3	Cv-4	Cv-5	Cv-6	Cv-7	Cv-8	Cv-9	Cv-10	Cv-11	Cv-12	Cv-13	Cv-14	Cv-15	Cv-16	Cv-17
Cv-1	100	62.5	57.14	44.44	50	36.36	36.36	40	46.15	30	33.33	45.45	45.45	45.45	55.56	40	46.15
Cv-2		100	50	40	62.5	45.45	45.45	50	53.85	40	44.44	41.67	41.67	41.67	50	50	42.86
Cv-3			100	71.43	57.14	40	40	62.5	38.46	50	57.14	50	50	50	62.5	62.5	38.46
Cv-4				100	62.5	60	60	66.67	53.85	40	44.44	41.67	41.67	41.67	50	50	53.85
Cv-5					100	36.36	36.36	55.56	46.15	44.44	50	45.45	45.45	45.45	55.56	55.56	46.15
Cv-6						100	100	54.55	69.23	45.45	36.36	35.71	35.71	35.71	30.77	41.67	57.14
Cv-7							100	54.55	69.23	45.45	36.36	35.71	35.71	35.71	30.77	41.67	57.14
Cv-8								100	61.54	66.67	55.56	63.64	63.64	63.64	60	77.78	61.54
Cv-9									100	42.86	35.71	64.29	64.29	64.29	50	50	85.71
Cv-10										100	85.71	70	70	70	66.67	87.5	53.85
Cv-11											100	60	60	60	75	75	46.15
Cv-12												100	100	100	80	80	76.92
Cv-13													100	100	80	80	76.92
Cv-14														100	80	80	76.92
Cv-15															100	77.78	61.54
Cv-16																100	61.54
Cv-17																	100

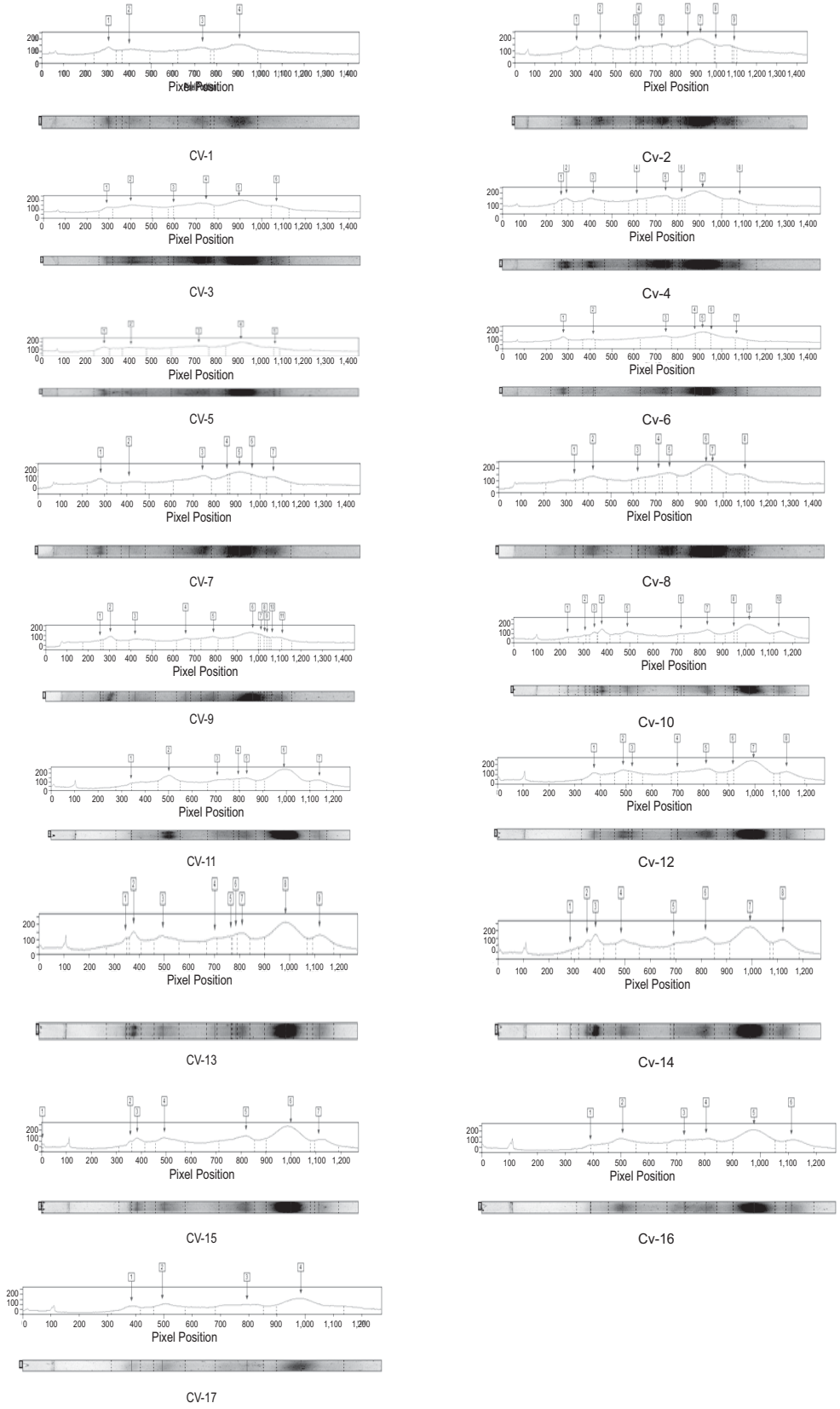

Fig. 3. Pixel intensity analysis for the 17 landraces of *Chokuwa* rice showing the relative proportion of individual proteins and comparision among the landraces.

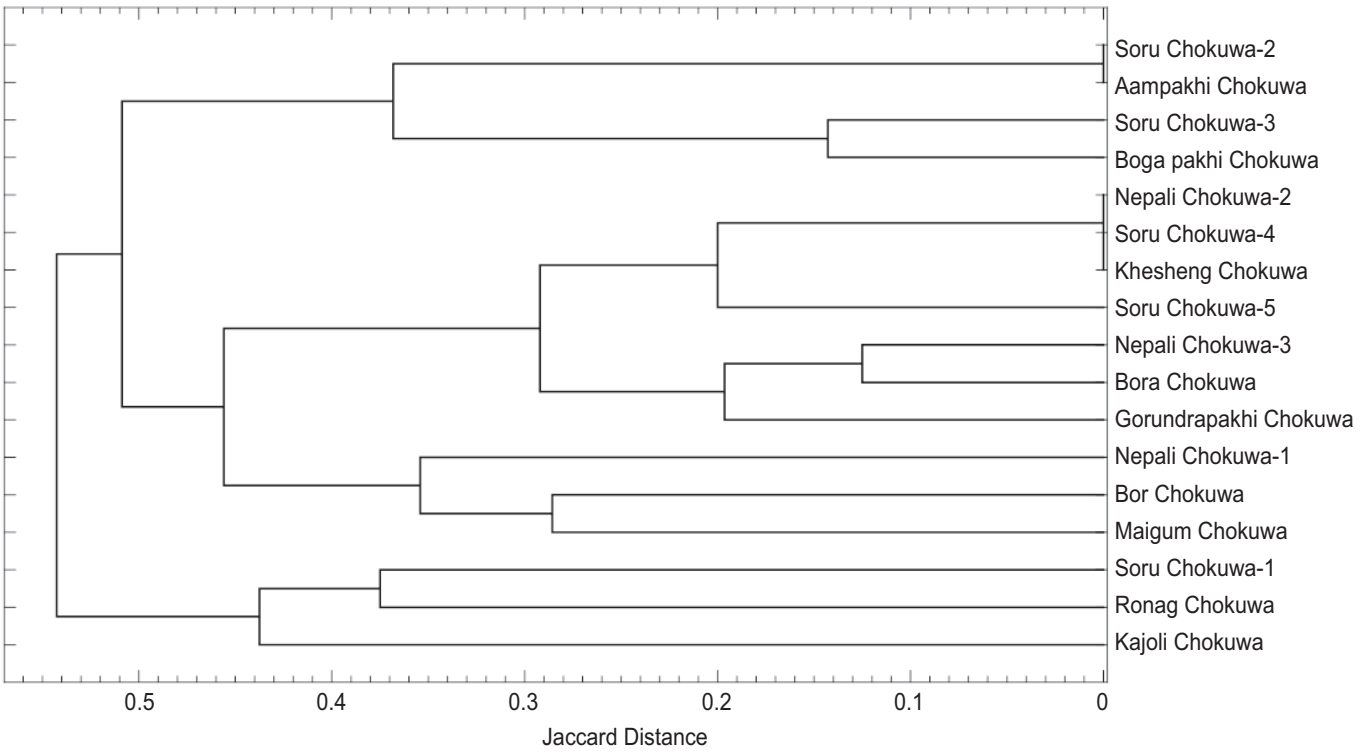

Fig. 4. Dendrogram analysis of *Chokuwa* paddy landraces of Assam based on seed protein profile

were completely different. This shows that these five land races are genetically different although ethnic name is same. Similar observation was made for three land races with the ethnic name *Nepali Chokuwa* collected from three different locations.

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