



EVALUATION OF PROXIMATE, MINERAL AND PROTEIN PROFILE OF FIVE ACCESSIONS OF *Oryza sativa* (L.) (RICE)



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Received: June 16, 2016 Accepted: September 02, 2016

Abstract: Study on proximate, mineral and protein profile of five accessions of *Oryza sativa* (rice) was carried out. Samples comprising of three local cultivars from Bassa, Idah and Ibaji, and two improved cultivars (F2 and F4) from National Cereals Research Institute (NCRI), Badeggi in Niger State, Nigeia were used for the study. The rice samples were analyzed for proximate components using Association of Official Analytical Chemists, while the mineral element contents were determined using Atomic Absorption Spectrophotometer (AAS). Six proximate parameters (Moisture, Ash, Crude fibre, Fat, Protein and Carbohydrate) and four mineral components (Magnesium, Calcium, Iron and Phosphorus) were analyzed in triplicate for the rice samples. The total seed protein of the rice samples was resolved on 12% SDS-PAGE. The results revealed significant difference for the six proximate and four elemental components of the five rice cultivars which indicated genetic diversity among the cultivars for the proximate and elemental compositions. Sixteen consistent bands and similarity coefficient range of 0.77 to 0.97 were recorded which indicate level of genetic diversity among the five samples in terms of protein profile. Results showed that rice sample from Ibaji could be harnessed for future breeding programme because of its outstanding proximate and mineral compositions.

Keywords: Genetic diversity, mineral, *Oryza sativa*, protein profile, proximate, SDS-PAGE

Introduction

Rice (*Oryza sativa*) has been considered the best stable food among all cereals consumed by over 3 billion people, constituting over half the world's population (Odeogu *et al.*, 2007; Folorunsho *et al.*, 2016; Hashmi and Tianlin, 2016; Rahaman, 2016). The observation of Oyewole *et al.* (2010) that average Nigerian consumes 21kg of rice annually, representing 9% of the total cereal consumption makes research into the crop's nutritional composition a necessity. In Nigeria, demand for rice since mid-1970 has increased at a much faster rate than any other African country (Food and Agriculture Organization, 2001). Rice according to Sanni *et al.* (2005) is grown in all the ecological zones of Nigeria, with different varieties possessing adapted traits to each ecological zone. The prevalent types of rice production systems in Nigeria are the rain-fed upland, rain-fed lowland and irrigated lowland (Singh and Mowa, 1997). Fashola *et al.* (2007) reported that agriculture (especially rice cultivation) should have been the mainstay of the Nigerian economy beyond oil if properly harnessed. According to Erenstein *et al.* (2003), rice has been substantially produced in Nigeria to meet local consumption before the oil boom of 1970s that brought in huge foreign exchange, which diverted the incentive to increase domestic production of rice. This led to acute shortage of rice and increasing demand of foreign rice in the 1990s. In the bid to address the demand-supply gap, government at various times came up with different policies and programmes on rice production in Nigeria (Ogundele *et al.*, 2004). However, in spite all these programmes local rice production has not kept up with its domestic consumption demands (Sotelo *et al.*, 1990). Oyewole *et al.* (2010) reported that despite the huge potentials for rice production in Nigeria, imported rice is usually viewed as superior to local rice to the extent that the former often enjoyed government subsidy.

Rice (*Oryza sativa*) production in Kogi state concentrates more around the riverine areas of the state. These riverine areas in Kogi state include Ibaji, Idah and Bassa. Rice production been the main mandate of Agricultural Transformation Agenda (ATA) of Kogi state should be assessed from the nutritional perspective to aid the

ongoing improvement programme (Oyewole *et al.*, 2010). Therefore, there is need to compare the nutritional quality of the improved rice varieties with indigenous varieties with the obvious intention of not losing the genetic integrity of the already existing varieties. The preservation of genetic integrity can be achieved by assessing the level of genetic diversity using molecular markers like seed protein profiling. The use of molecular markers for assessing genetic diversity according to Woldesenbet *et al.* (2015) has received a great deal of attention in recent years. The study of genetic diversity in crops is essential for high productivity and crop improvement (Bharathi *et al.*, 2014; Sarwar *et al.*, 2015). Traditional landraces of crops as well as related wild species are important source of genetic diversity for breeders and therefore form the backbone of agricultural production (Odongo *et al.*, 2015; Sharma *et al.*, 2014). Akbar *et al.* (2012) and Sadia *et al.* (2009) reported that SDS-PAGE is considered to be a dependable method for evaluating genetic diversity in plants because seed storage proteins are independent of environmental fluctuations. This study will furnish rice farmers and consumers with the best rice accessions for cultivation and consumption in Kogi state. There is dearth of information on proximate, minerals and protein profile of rice accessions in Kogi state. Therefore, this study aims at evaluating the proximate, minerals and protein profile of different rice cultivars from Kogi State and Badeggi in Niger state with the objective of furnishing the farmers and consumers information on the best cultivar (s) for cultivation and consumption in the state.

Materials and Methods

Determination of proximate composition of the rice samples

Rice samples comprising of three local cultivars from Bassa, Idah and Ibaji, and two improved cultivars (F2 and F4) from National Cereals Research Institute (NCRI), Badeggi in Niger State, Nigeia were considered for the study. These samples were ground into powder and approximately 30 g of the ground rice was sieved with 2 mm mesh were taken from each rice variety for analyses in triplicates. Data were recorded for six different seed

Evaluation of Nutritional Composition of Five Accessions of Rice

quality traits (crude protein, crude fat, fibre, mineral ash, moisture and carbohydrate contents). Proximate analysis was carried out according to the Association of Official Analytical Chemists (AOAC, 1990).

The crude protein contents of the seeds were determined by using 0.25 g of each sample and measuring the nitrogen released using Kjeldah method was carried out according to the Association of Official Analytical Chemists (AOAC, 1990). Then percentage of Nitrogen obtained was multiplied by a factor 5.4. Crude fat/oil content was determined by extracting 3g of each sample with Petroleum Benzene on Soxhlet extraction unit. Fibre content in seed samples was measured as crude fibre and was determined by the loss in mass upon ignition of the dried residue remaining after 1 g of the sample was digested with dilute tetraoxosulphate VI acids and potassium hydroxide. Moisture content was determined by taking 2 g of samples and putting it in an oven at 103°C for seventeen hours and then, taken out of the oven, cooled in desiccator and weighed. The carbohydrate content of the samples were calculated by the difference carbohydrate = 100 – (Protein + Fat + Mineral + Moisture). All proximate compositions were analyzed and calculated on dry weight basis (dwb). Data pooled on each proximate attribute (in triplicate) were subjected to Analysis of Variance (ANOVA) and means with significant differences were separated using the Duncan Multiple Range Test (DMRT) statistical methods and hierarchical cluster analysis.

Determination of proximate composition of the rice samples

The elements determined in this study were magnesium (Mg), calcium (Ca), iron (Fe) and phosphorus (P). The four (4) elements considered in this study were determined using Atomic Absorption Spectrophotometric method described by Bamigboye *et al.* (2010) by drying 10g of each sample at 105°C for 48 h. 10 ml of 6 N HCl was therefore added to the samples and the solution filtered into measuring flasks and filled up to 50 ml with distilled water. The stock solutions were used for analysing the magnesium (Mg), calcium (Ca), iron (Fe) and phosphorus (P).

Protein finger printing using SDS gel electrophoresis of seed proteins

Powdered samples were dried in an oven before homogenizing with an extraction buffer containing 0.05M Tris-HCl (pH 7.4) 4°C. Bromophenol blue was added to the sample buffer as a tracking dye to watch the movement of proteins in the gel. The homogenate was centrifuged at

10,000 r.p.m for 15minutes at 4°C and the supernatant was used for electrophoresis. The 12% SDS-gel was used for the run following the Discontinuous Electrophoretic method of Leammli (1970). The Sigma^(R)Maker used to trace the bands contains 13 proteins ranging from 6,500 to 205,000 kbs.

Data analysis

To avoid ambiguity in the data, only clear and consistent protein bands between 6,500 and 205,000 kbs were considered for data recording. Bands clearly visible in at least one genotype were scored (1) for present, 0 for absent and entered into a data matrix. Similarity Matrix (coefficient) proposed by Nei and Li (1979) was used to calculate the degree of similarity (S_{ab}), between two cultivars a and b according to the formula:

$$S_{ab} = 2N_{ab} / (N_a + N_b)$$

Where: N_{ab} = number of bands common to both species a and b; N_a = number of bands in species a; N_b = number of bands in species b. Hierarchical clustering (dendrogram) was constructed using Unweighted Pair Group Method with arithmetic Average (UPGMA) with SPSS v 20 window software.

Results and Discussion

The significant difference observed for the proximate and elemental components of the five rice cultivars as shown in Tables 1 and 2 indicates the presence of genetic diversity among the rice varieties for nutritional improvement. The presence of genetic diversity among plants of the same species according to Alege *et al.* (2014) can be used to design improvement strategy in the crop. The rice samples contained moisture contents ranging from 2.91% to 4.50% (Table 1) which is lower than the 3.67% to 18.00% and 11.2 to 11.9% moisture contents reported by Oko and Ugwu (2011) and Kariyawasam *et al.* (2016), respectively. Rice sample from Bassa contained the highest percentage moisture content ($4.50 \pm 0.10\%$) while Ibaji sample had the lowest percentage moisture content ($2.91 \pm 0.11\%$) (Table 1). Ebuechi and Oyewole (2007) reported that high moisture content of rice affects its storage potentials. Therefore, rice samples considered in this study have higher storage potentials compared to the ones reported by Oko and Ugwu (2011) and Kariyawasam *et al.* (2016) in their studies. From the result, rice samples from Ibaji will have longer shelf life compared to other rice varieties considered in this study.

Table 1: Proximate composition of the five rice (*Oryza sativa*) accessions studied

Accession Name	Moisture (%)	Ash (%)	Crude Fibre (%)	Fat (%)	Protein (%)	Carbohydrate (%)
BASSA	4.50 ± 0.10^d	1.23 ± 0.25^c	2.30 ± 0.20^b	1.58 ± 0.20^a	7.81 ± 0.78^d	82.69 ± 0.58^b
IDAH	3.50 ± 0.10^b	1.45 ± 0.50^d	2.61 ± 0.10^d	1.79 ± 0.10^b	6.20 ± 0.65^b	84.46 ± 0.35^e
IBAJI	2.91 ± 0.11^a	1.58 ± 0.25^c	2.67 ± 0.10^c	2.55 ± 0.50^d	8.08 ± 0.65^c	82.22 ± 0.21^a
F2	4.06 ± 0.60^c	0.90 ± 0.00^a	2.08 ± 0.20^a	2.83 ± 0.30^c	6.00 ± 0.85^a	84.14 ± 0.35^d
F4	3.55 ± 0.50^b	1.05 ± 0.26^b	2.52 ± 0.20^c	2.39 ± 0.10^c	7.26 ± 0.00^c	83.23 ± 0.88^c
LSD Value	0.074	0.022	0.014	0.231	0.052	0.084

Values are means \pm Standard Deviation of triplicate determination. Means with the same letters in the same column are not significantly different at 5% level of significance.

The highest ash content was observed in rice samples from Ibaji ($1.45 \pm 0.50\%$) (Table 1) while F2 (improved sample from Badeggi) had the least ash content of $0.90 \pm 0.00\%$. The ash content of a food sample according to Oko and Ugwu (2011) gives an idea of the mineral elements present in the sample. Among the five rice varieties, Ibaji rice had the highest fibre content ($2.67 \pm 0.10\%$) while F₂ (improved sample) had the least fibre content ($2.08 \pm 0.20\%$) (Table 1). The fibre content range from 2.08% to

2.67% was higher than what Oko and Ugwu (2011) and Kariyawasam *et al.* (2016) reported in their studies. The percentage fat content of ranged from $1.58 \pm 0.20\%$ to $2.83 \pm 0.30\%$. This is similar to the report of Oko and Ugwu (2011) and Kariyawasam *et al.* (2016) on rice samples from Abakaliki and Sri Lankan, respectively. The fat content observed in this study was higher than the 1.23% to 2.21% reported by Odeogu *et al.* (2007). The values for percentage crude protein were in the range of

6.00 ± 0.85% to 8.08 ± 0.65% and this was higher than what was reported by Oko and Ugwu (2011). It was similar to the report of Odeogu *et al.* (2007) and lower than what was observed by Kariyawasam *et al.* (2016) on Sri Lankan rice landraces. The carbohydrate content was very high compared to other proximate components studies. The values of carbohydrate obtained were higher than what was reported by Odeogu *et al.* (2007) and Kariyawasam *et al.* (2016) and was similar to the values reported by Oko and Ugwu (2011).

Rice sample from Ibaji had low moisture, high ash, crude fibre and protein contents, but produced relatively low carbohydrate contents. Thus, it may be a very good parental stock during rice improvement programmed. Sample from Idah had the highest carbohydrate content but contained other inferior proximate qualities which therefore make the sample a good genetic source for carbohydrate improvement.

The result obtained on elemental composition in Table 2 revealed that rice sample from Ibaji had the highest values for all the four elemental compositions considered in this study (magnesium calcium, iron and phosphorus) while Idah rice sample was inferior for magnesium (88.37 ± 0.55 mg/g), calcium (251.00 ± 0.00 mg/g) and phosphorus content (104.19 ± 0.60 mg/g). This finding showed that rice sample from Ibaji is superior for elemental composition and could be selected for hybridization during programme for improving elemental compositions in rice. It is therefore clear from this study that Idah sample although superior for carbohydrate content but is not a good parental source of elemental composition for rice improvement protocol. The result showing absence and presence of bands in Table 3 revealed that bands numbers 3, 4, 5, 6, 9, 10, 12, 13, 14 and 16 are common to the five rice accessions while no band is unique to any of the accessions. The presence of these common bands strongly suggests that the genes coding for the protein bands are fixed in the studied rice accessions while lack of unique band (band peculiar to an accession) among the studied rice samples indicates a common gene expression.

Table 2: Mineral composition of the five rice (*Oryza sativa*) accessions studied

Accession Names	Magnesium (mg/g)	Calcium (mg/g)	Iron (mg/g)	Phosphorus (mg/g)
BASSA	109.67 ± 0.33 ^d	295.50 ± 0.50 ^d	21.60 ± 0.20 ^c	193.21 ± 0.09 ^d
IDAH	88.37 ± 0.55 ^a	251.00 ± 0.00 ^a	19.64 ± 0.14 ^b	104.38 ± 0.12 ^a
IBAJI	121.6 ± 0.20 ^c	324.00 ± 1.00 ^c	26.75 ± 0.25 ^c	206.19 ± 0.60 ^c
F2	98.32 ± 0.95 ^c	256.33 ± 0.58 ^b	13.86 ± 0.60 ^a	170.57 ± 0.35 ^b
F4	93.43 ± 0.13 ^b	282.00 ± 1.00 ^c	24.28 ± 0.70 ^d	181.62 ± 0.22 ^c
LSD Value	0.154	0.587	0.132	0.99

Values are means ± Standard Deviation of triplicate determination. Means with the same letters within the same column are not significantly different at 5% level of significance.

Table 3: Presence and absence of bands in the five rice (*Oryza sativa*) accessions

BANDS	BASSA	IDAH	IBAJI	F2	F4
1	+	+	0	+	+
2	0	+	0	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	0	0	0
8	+	+	0	+	+
9	+	+	+	+	+
10	+	+	+	+	+
11	+	+	0	+	+
12	+	+	+	+	+
13	+	+	+	+	+
14	+	+	+	+	+
15	0	+	0	0	+
16	+	+	+	+	+
Total	14	16	10	14	15

+ = Band present; - = Band absent

Table 4 showed that the highest similarity coefficient of 0.97 was observed between F2 and F4 rice samples while the least similarity coefficient of 0.77 was observed between Idah and Ibaji samples. The similarity coefficient range of 0.77 to 0.97 observed in this study indicates low level of genetic diversity among the five rice samples. This low genetic diversity reported in this study supported the finding of Galani *et al.* (2011) on ten elite rice genotypes from Sindh. The genetic diversity observed between Idah and Ibaji rice samples corroborates the earlier report of significant difference between the two samples for proximate and mineral attributes in this study.

Table 4: Similarity index for protein profile among the five rice (*Oryza sativa*) accessions studied

	BASSA	IDAH	IBAJI	F2	F4
BASSA	-				
IDAH	0.93	-			
IBAJI	0.83	0.77	-		
F2	0.93	0.93	0.83	-	
F4	0.89	0.96	0.80	0.97	-

The hierarchical cluster analysis for the proximate and mineral compositions put the five rice accessions into two major clusters (Fig. 1). The result obtained in this study can be exploited by rice producers and consumers in their choice of proximate and mineral compositions of the different cultivars. The fact that the five samples occupied two clusters is an indication that genetic diversity existed among the five rice accessions. The separation of Idah rice sample from other samples which had significantly higher carbohydrate content indicates that the sample may be a very good parental stock for rice improvement programme. Ibaji sample will be a good stock for improvement because of its excellent quantity of ash, crude fibre, protein and elemental contents. In addition, its low moisture content is a very good trait during storage.

Evaluation of Nutritional Composition of Five Accessions of Rice

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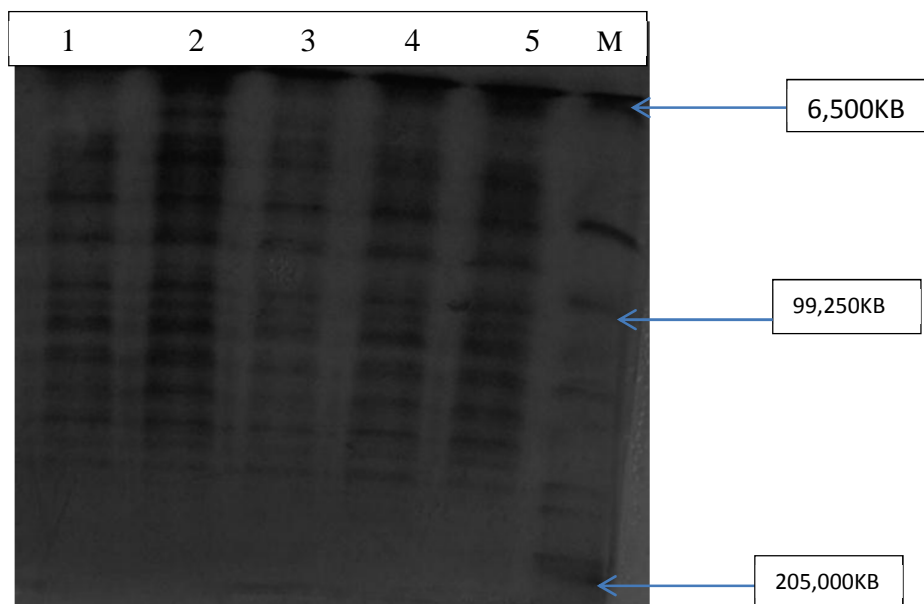


Plate 1: Protein Banding Patterns for the Five Rice Accessions Studied