

NUTRITIONAL AND PHYSICOCHEMICAL CHARACTERIZATION OF SELECTED DROUGHT TOLERANT NIGERIAN LOCAL RICE CULTIVARS

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Abstract

Drought-tolerant rice cultivars with inherent good quality traits are important in ensuring adequate and nutritious food for humans, particularly in sub-Saharan African regions like Nigeria. Thus, for the attainment of effective nutrition security in a climate-changing world, it is expedient to scientifically characterize, identify, and develop good quality rice cultivars with intrinsic drought tolerance potentials. In this study, four potentially drought-tolerant rice cultivars were collected from local rice farmers in some Northern parts of Nigeria. They were first subjected to preliminary drought tolerance validation, then physicochemical, proximate, mineral, and transgene diagnostic analyses. The proximate analysis results showed that the cultivars have good protein content particularly “Nassarawa-Lafia” ($10.20 \pm 0.00\%$) while “Jigawa-Mafa” has the highest fiber and magnesium content of $1.81 \pm 0.01\%$ and $31.80 \pm 0.04\%$ respectively. Mineral content analysis results also revealed cultivars like “Jigawa-Mafa” and “Nassarawa-Lafia” as high potassium enriched up to $118.72 \pm 0.41\text{mg}/100\text{g}$ and $117.06 \pm 0.91\text{mg}/100\text{g}$. The molecular diagnostics showed no transgene presence in all the samples. These findings highlight the good nutritional qualities of the cultivars and their potential health benefits. This calls for a more in-depth search of the expansive local genetic pool for crops with promising abilities for cultivation and subsequent breeding programs to address the increasing food and nutrition challenges.

Keywords: cultivars, drought, nutrition, quality, rice

Introduction

The influence of unfavorable environmental conditions on plants leads to various biotic and abiotic stresses that affect their growth, development, and yields (Yadav et al. 2020). Drought is one of such condition and it adversely reduces rice productivity by causing abscission and senescence in the plant. This in turn impacts negatively on food and nutrition security as rice is a major staple food across the globe (Ndjiondjop et al. 2018).

Rice (*Oryza sativa* L.), is highly valued as one of the world's most important food crops for its rich dietary and calorific values (Sen et al. 2020). It is a rich staple food of choice for a large section of the global population especially in developing countries like Nigeria, due to its pleasant taste and ease of cooking. It supplies the body with a high amount of energy and digestible carbohydrates. With its protein quality and appreciable amounts of minerals and vitamins that play key body functional and regulatory roles, rice meals are adequate food for

meeting and sustaining significant human nutritional needs (Mohidem et al. 2022). However, its high-water demand makes it very susceptible to drought, which is a major abiotic threat to plants in sub-Saharan Africa. Rice production has increasingly been exposed to unpredictable and devastating losses caused by drought stress due to the prevailing climate change conditions (Dar et al. 2020). Cultivation of some modern and improved rice cultivars with good quality potential has most times ended in huge losses because of the effect of unexpected drought conditions (Giri et al. 2020). Also, cultivars with good drought tolerance but little quality traits, on the other hand, are short of the 'adequate and nutritious' purpose of food consumption. The quality of rice is generally taken from two major aspects, viz; the physical quality which is mostly based on the appearance, and the second aspect, the nutritional content (Custodio et al. 2019). The physical outlook is a major determinant factor in the preliminary assessment of rice quality and represents the first quality evaluation of rice especially from the appearance point of view. This focal point of quality evaluation is a measure of the grain color, aroma, size, shape, and breakability (Custodio et al. 2019). The overall quality of rice is a key factor in its acceptability, economic significance, nutritional, and health benefits.

In Nigeria, over the past years, there has been a remarkable increase in rice production due to new Government policies that banned importation to encourage local production. Over five million metric tons of local rice is now produced in the country annually (Statista 2022). Regardless of this boom in production, there is also an astronomical rise in the cost of rice and Nigerians still crave for imported rice with higher qualities. This is majorly because of the problem of the production-to-consumption imbalance of rice, compounded by the cultivation of cultivars that fall short of adequate nutrition qualities and stress survival abilities. As a result of this, smuggling and racketeering of foreign rice from other countries like Thailand, China, and India becomes a bubbling side business.

Thus, this great demand for high-quality, drought-tolerant Nigerian rice cultivars requires rapid and more natural approaches in rice research programs (Danbaba et al. 2020). This is quite necessary for cultivation and subsequent breeding programs to meet the increasing demands of an expanding population size in Nigeria. Although different studies have been carried out on drought tolerance and nutritional analysis of some rice cultivars in Nigeria separately, not much has been reported for combined drought tolerance, physicochemical quality traits, and transgene diagnostic analyses.

This study is therefore devised to evaluate key nutritional and physicochemical characteristics of selected drought-tolerant indigenous rice cultivars devoid of genetic modification.

Materials and Methods

Preliminary Drought Tolerance Investigation

To confirm their drought tolerance using the preliminary seedling validation method, 50g of each rice cultivar seed specimens collected from major rice-growing states in the Northern part of Nigeria were taken to the Biotechnology Advanced Research Center in Sheda Science and Technology Complex. Lab codes (Samples 1-4) were assigned to them. Sample 1 denotes "Nassarawa-Lafia", Sample 2; "Niger-Jerusalem" while Sample 3 and 4 denote "Kebbi-Jirani", and "Jigawa-Mafa" cultivars respectively. These cultivars are local drought-tolerant varieties of rice cultivated by the local farmers in Kebbi, Nassarawa, Jigawa, and Niger states of Nigeria, hence their names. Polyethylene glycol (PEG-6000) was used at a high concentration of 20% on the seedlings to verify their tolerance to drought by creating a high level of potential water dearth according to the method previously described by Susilawati et al. (2022). Twenty grams (20 g) of the collected rice seed specimens from each of the cultivars were pulverized and subjected to physicochemical, mineral content, and transgene detection analyses using proximate, atomic absorption spectrophotometry (AAS) and molecular diagnostic analyses.

Physical Analysis

Samples from the four cultivars were subjected to the various physical analyses as follows:

Chalkiness index

The chalkiness indices were determined using twenty (20) dehulled rice grains from each sample on a lightbox and visually examining their chalkiness appearance. The percentage chalkiness of each sample was calculated by taking the average of the 20 values. The method as previously described by Dela-Cruz (2000) was used to express the chalkiness levels as; 0 (no chalkiness), 1 (less than 10% chalkiness), 5 (10 - 20% chalkiness), and 9 (more than 20% chalkiness).

Color

The visual method was used to physically identify the grain colors as shown in Figure 2 while the Lovibond system was used to corroborate it. Lovibond color system for visually determining and comparing colors using the Lovibond comparator scale according to the method previously described by Hadi et al. (2021).

Aroma

Following the method previously described by AOAC (2013) using pulverized samples in 1.7% KOH solution and incubated at 60°C for 10 min., the aroma evaluation was carried out.

Grain Dimension (Length and Width)

Grain dimensions were determined using the general Vernier Caliper by randomly taking 20 grains from each sample and measuring their lengths and widths according to the method previously described by Ilia et al. (2023).

Grain kernel weight

This was determined using 1000 kernels from each sample and weighing separately to determine bulk Kernels' weight according to the method previously described by Singh et al. (2003).

Breakability

The breakability ratio was determined using the method previously described by Khosravi et al. (2011) which involved calculating the percentage of the broken grains after subjecting them to a Unimax 1010 shaker (Heidolph, Germany) at moderate speed.

Proximate Composition Analysis

The rice samples were properly sorted to remove all extraneous matters, then dehulled, pulverized, sieved, and weighed to collect 100 g of fine powdered samples for each cultivar. The proximate composition analytical process was used for evaluating moisture, crude proteins, crude fiber, crude lipid, crude ash, and carbohydrate contents in triplicates to determine the nutritional contents of the four different cultivar samples.

Crude Protein Content Determination

The crude protein contents of the rice samples were determined using the digestion and distillation method as previously outlined by Oko et al. (2012), in which the crude protein contents of samples are derived by measuring their total nitrogen contents and multiplying by a definite factor (6.25).

Moisture Content Determination

Moisture content was measured using the Standard Official Methods of Analysis as previously described by AOAC (1990) which involved drying the samples to a constant weight at 100°C and calculating the moisture after the weight losses of the dried rice samples.

Fiber Content Determination

The crude fiber content was determined using the protocols previously described by AOAC (1990), which involves hydrolyzing with petroleum ether.

Lipid Content Determination

The total crude lipid content in the rice sample was determined using Soxhlet extraction for 4 hours with methanol and ethanol according to the method previously described by Eromosele and Eromosele (1994).

Carbohydrate Content Determination

The percentage carbohydrate content was deduced by calculating the difference between the sum of other constituents and 100 as previously described by Onyeike et al. (1995).

Mineral Contents Determination

The Potassium, magnesium, Iron, Zinc, and Calcium contents of the four different cultivar samples were determined using flame Atomic Absorption Spectrophotometry (AAS) protocols according to the method previously described by Kouassi et al. (2013). Two grams (2 g) of pulverized sample from each cultivar was used for the analysis. This involved sample digestion using a combined mixture of tetraoxonitrate (v) acid and perchloric acid in a 4:1, v/v ratio and solubilization with distilled water prior to measurements with AAS.

Molecular Analyses

The molecular diagnostic analysis for the presence of transgene was carried out by extracting the DNA of the samples from the four different cultivars and amplifying them with the common rice transgene primers (Cry 1Ab and VIP3) along with positive and negative controls. Extraction of DNA from the leaves of the specimen samples was carried out with Zymo Research Plant DNA extraction kit according to the manufacturer's protocol (Zymo Research 2016) using 150 mg of leaf samples from each cultivar. The PCR amplification was carried out for transgene detection by VIP3/Cry 1Ab primer amplification according to the method described by Safaei et al. (2019).

Statistical Analysis

The samples were analyzed in triplicates and the generated data was evaluated using descriptive (means and standard deviation) and inferential statistics (one-way Analysis of Variance) using SPSS software (Version 20, SPSS Inc. Chicago, USA). The results were analyzed by applying the Duncan testing algorithm and expressed as mean $\pm$ SD and variations considered statistically significant when $P < 0.05$.

Results and discussions

The drought tolerance preliminary validation results of the selected rice cultivars' seedlings with PEG-6000 were shown in Figure 1 while the statistical analysis using tables are shown in Table 1 (A, B, and C). The grain physical quality analyses were shown in Figure 2 and Table 2 while the proximate composition analyses were depicted in Table 3. Mineral content and molecular analyses results were presented in Table 4 and Figure 3 respectively.

Table 1.A: Drought Tolerance Preliminary Validation Indices 1

S/N	Germination Percentage (%)		Plant Height (mm)		Root Length (mm)	
	Control	Drought	Control	Drought	Control	Drought
Sample 1	100.00 $\pm$ 0.00b	85.00 $\pm$ 1.00a	13.00 $\pm$ 0.50a	11.00 $\pm$ 0.00a	12.30 $\pm$ 0.10b	17.00 $\pm$.00d
Sample 2	100.00 $\pm$ 0.00b	96.00 $\pm$ 1.00c	14.00 $\pm$ 1.00ab	14.07 $\pm$ 0.06c	13.70 $\pm$ 0.00d	16.70 $\pm$ 0.17c
Sample 3	95.00 $\pm$ 1.00a	92.00 $\pm$ 2.00b	15.00 $\pm$.00b	14.70 $\pm$ 0.20d	10.27 $\pm$ 0.06a	14.03 $\pm$ 0.06a
Sample 4	100.00 $\pm$ 0.00b	98.00 $\pm$ 0.00c	14.73 $\pm$ 0.35b	13.70 $\pm$ 0.10b	13.30 $\pm$ 0.10c	15.30 $\pm$ 0.10b

*Each value is the mean $\pm$ standard deviation of three replicates. Means in the same column followed by the same letter are not significantly different at $p \geq 0.05$.

Table 1.B: Drought Tolerance Preliminary Validation Indices 2

S/N	Plant Length (mm)		Fresh Weight (g)		Root-shoot Ratio	
	Control	Drought	Control	Drought	Control	Drought
Sample 1	25.30±0.20a	28.00±0.00a	0.09±0.00a	0.07±0.00a	0.92±0.010c	1.55±0.02c
Sample 2	27.57±0.21b	30.70±0.10d	0.17±0.00d	0.17±0.01d	0.98±0.01d	1.99±0.03d
Sample 3	25.30±0.00a	28.70±0.00b	0.12±0.00b	0.11±0.00b	0.69±0.01a	0.95±0.00a
Sample 4	28.00±0.00c	29.00±0.00c	0.14±0.00c	0.12±0.00c	0.90±0.00b	1.12±0.01b

*Each value is the mean ± standard deviation of three replicates. Means in the same column followed by the same letter are not significantly different at $p \geq 0.05$

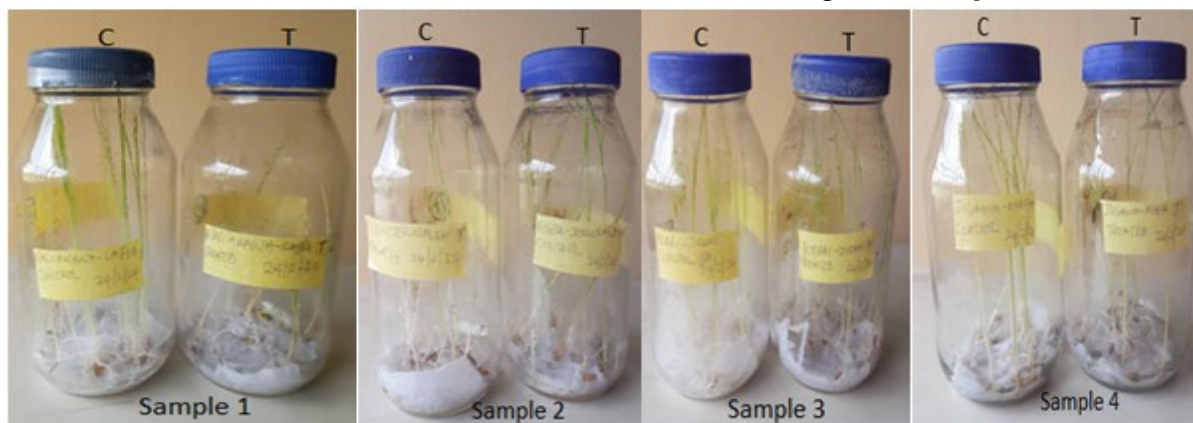
Table 1.C: Summary of Drought Tolerance Preliminary Validation Indices

Samples	PSI (%)	RLI (%)	PLI (%)	DTI (%)
Sample 1	84.00±0.01a	138.20±0.02d	110.70±0.02b	85.00±0.01c
Sample 2	100.00±0.00d	121.90 ±0.01b	111.20±0.01c	87.50±0.02d
Sample 3	98.01±0.01c	135.90±0.02c	113.40±0.03d	84.60±0.01b
Sample 4	93.40 ±0.01b	115.00 ±0.01a	103.70±0.03a	83.30±0.02a

*Each value is the mean ± standard deviation of three replicates. Means in the same column followed by the same letter are not significantly different at $p \geq 0.05$.

Key:

1. **G%** = $N/n \times 100$ (where n is the number of germinated seeds at the end of experiment, N is the total number of the seeds)
2. **Drought Tolerance Index (DTI)** = $\frac{\text{G\% under drought}}{\text{G\% under control}} \times 100$
3. **Plant Height Stress Index (PHSI in %)** = $\frac{\text{Plant height of plant under drought stress}}{\text{Plant height of control plants}} \times 100$
4. **Root Length Drought Stress Index (RLSI in %)** = $\frac{\text{Root length of stressed plant}}{\text{Root length of control plants}} \times 100$

**Figure 1.** Preliminary Drought Validation of The Cultivars using 20% PEG-6000: C= Control, T= Treatment

Compared with the controls, the treated cultivars exhibited substantial resilience to the subjected drought stress treatment as shown in Figure 1 and Table 1(A, B, and C) which is a validation of their drought-tolerance ability.

Table 2. Physical Analyses

Samples	Color	Chalkiness	Aroma	Length (mm)	Width (mm)	Weight (g)	Breakability (%)
Sample 1	L-B	0	0	5.52±0.11a	2.24±0.04a	22.10±0.22b	6.16±0.06a
Sample 2	W	1	0	6.4±0.32b	2.41±0.02a	30.35±0.12d	9.48±0.10b
Sample 3	W	1	1	6.60±0.82b	2.27±0.01b	26.78±0.05c	11.13±0.02c
Sample 4	L-B	0	1	5.82±0.17a	2.53±0.02c	20.39±0.21a	13.48±0.12d

*Each value is the mean ± standard deviation of three replicates. Means in the same column followed by the same letter are not significantly different at $p \geq 0.05$; L-B = Light Brown; W= White; 0= none; 1= less than 10%

The four selected cultivars showed varying physical characteristics. “Niger-Jerusalem” and “Kebbi-Jirani” cultivars are somewhat whitish in color while “Nassarawa-Lafia” and “Jigawa-Mafa” are light-brownish in color respectively, as shown in Figure 2 below. The analysis of variance showed significant differences in the measured physical parameters at $P < 0.05$ among some of the cultivars as depicted with letters in the same column as shown in Table 2. From their Length and width values, “Niger-Jerusalem” and “Kebbi-Jirani” cultivars can be described as long grains while “Nassarawa-Lafia” and “Jigawa-Mafa” belong to the medium grain category in agreement with Nádvorníková et al. (2018). The breakability indices of the four cultivars ranging between 6.20 to 13.3% are a measure of their resistance to physical pressure and indication of good evacuation, nutritional, cooking, and storage qualities in line with Ashizume et al. (2005).

**Figure 2.** Physical Appearance Analysis of the Samples**Table 3.** Proximate Analyses

Samples	Moisture (%)	Ash (%)	Fibre (%)	Lipid (%)	Protein (%)	Carbohydrate (%)
Sample 1	10.10±0.04a	1.10±0.02b	0.44±0.01c	1.38±0.02c	10.20±0.00a	76.89±0.04a
Sample 2	11.01±0.02b	0.82±0.02a	0.18±0.01a	1.35±0.02c	7.44±0.00a	79.20±0.05d
Sample 3	11.10±0.05c	0.82±0.04a	0.20±0.00b	1.18±0.00a	8.04±0.00a	77.05±0.02b
Sample 4	11.58±0.01d	1.39±0.00c	1.81±0.01d	1.27±0.02b	8.13±0.00a	77.43±0.02c

*Each value is the mean ± standard deviation of three replicates. Means in the same column followed by the same letter are not significantly different at $p \geq 0.05$

The analysis of variance of the proximate parameters showed significant differences at $P < 0.05$ among some of the cultivars as depicted with letters in the same column as shown in Table 3. The high carbohydrate content across the four cultivars is an affirmation of their primary role as a major energy source and mirrors other research findings like that of Oko and Onyekwere (2010) and Fukagawa and Ziska (2019). The crude fiber content which ranged between 0.44% to 1.81% showed variations across the 4 cultivars with “Niger-Jerusalem” having the least value

while “Jigawa-Mafa” had the highest value. Dietary fiber plays a regulatory role in carbohydrate absorption via hydrolytic enzyme action inhibition which helps to reduce glycemic index and type 2 diabetes risk factors (Makki et al. 2018, Mao et al. 2021). Fiber contents of food enhance their laxative effect in the gut and decrease the incidence of constipation. Good fiber content in the “Jigawa-Mafa” cultivar is a good indication of its potential healthy bowel and metabolic functions.

The results of crude protein contents of the four cultivars (7.44% to 10.20%) are quite indicative that they are important candidates for nutrition security particularly the “Nassarawa-Lafia” cultivar. Protein which plays a crucial role in the growth, development, and replenishment of worn-out tissues was notably high in some cultivars affirming the nutritional quality of the cultivars. Rice protein is of high biological value because of its hypo allergenicity and balanced profile of amino acids like threonine, leucine, and phenylalanine (Jayaprakash et al. 2022). It also has a high lysine content in comparison to most other cereals (Eggum et al. 1993).

The amounts of crude lipids present in the various rice cultivars were relatively low, in slight contrast with Arowora et al. (2021) but comparable to the results of Obembe et al. (2022). These variations could be attributed to genetic and edaphic factors. Nonetheless, the lipid content of rice is almost void of cholesterol (Silver 2017) due to their predominant constituents of unsaturated fatty acids like linoleic acid and palmitic acids which are known as essential fatty acids (Juliano and Goddard 1986). The moisture contents of the cultivars which ranged between 10.01% to 11.10% were on the lower ebb and good for long-term storage, in agreement with Oko et al. (2012). Moisture contents significantly affect the shelf-life of grains as well as their milling characteristics and taste (Ebuehi and Oyewole 2007, Zheng and Lan 2007, Yasothai 2020). While the ash content of rice is relatively small compared to lipid, protein, and carbohydrate, it is quite a significant factor in evaluating the mineral content and quality of rice. The four cultivars have good ash contents ranging from 0.81% to 1.39%.

Table 4. Mineral Content Analyses

Samples	Potassium (mg/kg)	Magnesium (mg/kg)	Iron(mg/kg)	Calcium(mg/kg)	Zinc(mg/kg)
Sample 1	118.72±0.41d	24.76±0.28c	2.22±0.04b	15.14±0.03a	1.51±0.03c
Sample 2	113.65±0.20b	23.09±0.01b	5.23±0.02d	18.16±0.13c	1.35±0.03b
Sample 3	110.10±0.26 a	22.64±0.13a	4.63±0.04c	19.20±0.01d	1.10±0.00a
Sample 4	117.06±0.91c	31.80±0.04d	2.15±0.01a	14.40±0.06b	1.98±0.05d

*Each value is the mean ± standard deviation of three replicates. Means in the same column followed by the same letter are not significantly different at $p \geq 0.05$

The calcium, zinc magnesium, and iron contents of rice are of very important health value (Weyh et al. 2022). The four cultivars have good ash contents ranging from 0.81% to 1.39%. The iron and calcium contents of “Niger-Jerusalem” and “Kebi-Jirani” cultivars were considerably higher than the other two, in line with Priya et al. (2019), probably due to genetic and edaphic factors. “Jigawa-Mafa” cultivar on the other hand exhibited the highest magnesium content followed by “Nassarawa-Lafia” which makes them good for people predisposed to metabolic disorders. Magnesium is an important mineral that plays a vital role in the regulation of blood pressure and sodium balance in the body. It helps in lowering the risk of metabolic syndrome; a critical indicator of cardiovascular disease like heart attacks (Kass et al. 2012). The varying levels of five essential minerals analyzed depicted desirable values which are very crucial for improving micronutrient activities like growth, blood pressure, metabolism and the general wellbeing of human life.

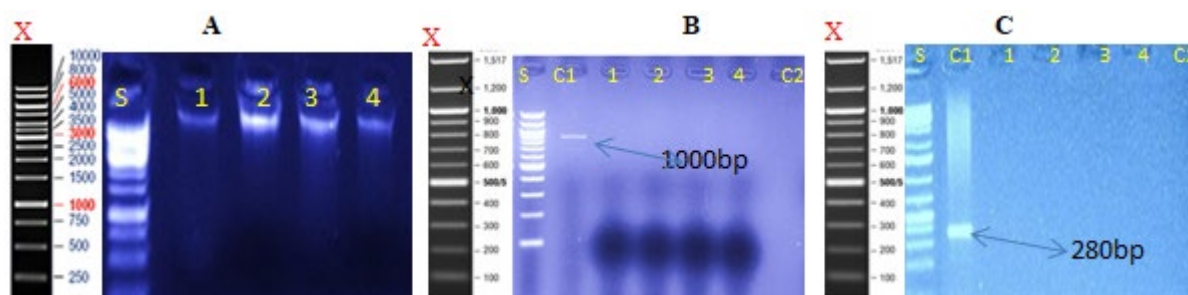


Figure 3. A. DNA Extraction, B. PCR Amplification using VIP 3 and, C. PCR Amplification using Cry Ab Primers

Key: X= Reference ladder chart, S= Step ladder, C1= +ve Control, C2= -ve control, 1-4 = sample 1 to sample 4.

The molecular diagnostic analysis conducted to check for transgene presence was to ascertain any sign of transgenic modification in the selected cultivars (Yang et al. 2010). Figure 3A showed the DNA extraction results of the samples while Figures 3B and 3C presented the molecular diagnostic analysis using PCR method for transgene detection. The results indicated the absence of any possible transgene, particularly the Cry 1Ab and Vip3 genes which are the most used transgenes in transgenic rice production (Xu et al. 2018).

The varying levels of five essential minerals analyzed depicted desirable values that are very crucial for improving micronutrient activities like growth, blood pressure, metabolism, and the general well-being of human life. Thus, the different nutritional contents of these selected four rice cultivars are not only culinary but medicinal as they play critical roles in reducing predisposing risk factors of many diseases thereby maintaining good health and preventing ailments. Rice meals enrich the body's nutritional value as they provide vitality and strength as well as help to regulate metabolic processes and remove toxic metabolites (Malabadi et al. 2022). Also, the hypoallergenic nature of rice protein which gives it an edge over other plant proteins and some animal sources is yet another value addition of rice cultivars with good protein contents.

Conclusions

The increasing demands for rice cultivars with good quality traits and high drought tolerance indices necessitate the need for more research focus in this area. The proximate analysis results showed that the cultivars have good nutritional qualities while the drought tolerance validation analyses reflected their substantial drought-tolerance abilities. The cultivars exhibited relatively high protein contents particularly “Nassarawa-Lafia” as well as good fiber and magnesium content as seen in the “Kebbi-Jirani” cultivar. Mineral content analysis results also revealed high potassium-containing cultivars like “Niger-Jerusalem” and “Nassarawa-Lafia”. The physicochemical and molecular analyses showed the natural physical qualities of the cultivars devoid of genetic modification. Thus, the cultivars demonstrated a blend of physicochemical and nutritional qualities which are key determinant factors in consumer acceptability, and nutritional and economic values of rice. These findings highlight the inherent good nutritional qualities of the cultivars, their potential health benefits, acceptability, and promising ability in helping to address food and nutrition challenges, especially in climate-changed and hunger-prone regions. This research therefore provides a foundation for more in-depth searching of our expansive local genetic pool for solutions to food and nutrition insecurity challenges. It also recommends future breeding approaches aimed at incorporating these cultivars into broader programs to enhance their availability, possible improvement for adoption among farmers, and commercialization.

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