

African Journal of Plant Science

Full Length Research Paper

Grain morphological characterization and protein content of sixty-eight local rice (*Oryza sativa* L) cultivars from Cameroon

Marlyn Njimeni Tchuisse^{1,2}, Eddy Leonard Magaptche Ngonkeu^{3,4}, Dorothy K. Malaa³, Honoré Tekeu⁴, Thomas Mballa⁴, Joseph Hubert Yamdeu Galani⁵, Akindeh Nji² and Thaddée Boudjeko^{1,2}*

¹Laboratory of Phytoprotection and Plant Valorisation, Biotechnology Centre, Yaoundé, Cameroon.
 ²Department of Biochemistry, Faculty of Science, University of Yaoundé I, P. O. Box 812, Yaoundé, Cameroon.
 ³Institute of Agricultural Research for Development (IRAD), P. O. Box 2123, Yaoundé, Cameroon.
 ⁴Department of Plant Physiology, Faculty of Science, University of Yaoundé I, P. O. Box 812, Yaoundé, Cameroon.
 ⁵School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK.

Received 27 October, 2019; Accepted 18 November, 2019

Rice (*Oryza sativa* L.) cultivated in Cameroon is appreciated by consumers for its nutritive quality and good taste. Diversity of 68 local rice cultivars was investigated via grain morphology and protein content characterization. The size and shape of grains were determined and used with yield parameters to classify the cultivars and perform Principal Component Analysis (PCA). Total protein content and glutelin content of eight selected cultivars (CMRGNd, CMRGDn, CMRGTī, CMRTBa, CMRDWb, CMRDTc3, CMRDTx5 and CMRDTx6) were evaluated by Bradford assay and correlation analysis of all the parameters studied was performed. Long size grains (42) were predominant over extra-long (16), medium (9) and short (1) grains. Slender shaped grains (36) were distinguished as well as medium (28) and bold (4) grains. The 68 cultivars were grouped into four clusters independent of their origins. PCA revealed three principal components accounting for 74.4% of total variation. Highest total protein content was observed in CMRGNd (14.3%) and highest glutelin content in CMRGDn (10.1 mgEqvBSA/g DW). Pearson correlation of the different variables revealed no significant correlation between total protein and glutelin contents with the agro-morphological parameters evaluated in this study. This suggests that none of these parameters could be descriptor for protein content. Positive correlation between grain length and yield (r = 0.7) suggests grain length as yield descriptor.

Key words: Rice, diversity, grain morphology, protein, glutelin.

INTRODUCTION

Rice (*Oryza sativa* L.) is the second most consumed cereal in Cameroon after maize and is presently a staple food for both rural and urban populations. However, the national production estimated at about 360000 tons in 2017 (FAO, 2019), remains far below consumption that is

estimated at about 635,000 tons (USDA, 2017). The deficit between demand and production results from several factors among which a non-effective distribution of high impact technologies and the low competitiveness of local rice. Domestic rice though, is dominated by

imported rice and fails to compete both quantity and quality-wise with imported rice.

However, rice produced in Cameroon is appreciated by many; 90% of the national produce is exported to Nigeria in paddy form. While trying to identify the physicochemical characteristics that determine the consumption preferences of local and imported rice in the North-west region, Fon and Fonchi (2016) obtained 83.9% of respondents confirming that locally produced rice tastes better than imported rice. Consumer demand for fine rice varieties is high due to good nutritional quality, palatability, aroma and taste. But quality can as well be considered from the view point of size, shape and appearance of grain (Cruz and Khush, 2000). IRRI classifies brown rice grain length into extra-long (>7.50 mm); long (6.61-7.50 mm); medium (5.51-6.60 mm); and short (≤5.50 mm). IRRI's Standard Evaluation System (SES) equally classifies shape as slender (length-width ratio >3.0, medium (ratio 2.1-3.0), bold (ratio 1.1-2.0) and round (ratio <1.1) (IRRI, 1996).

Scientific studies are indispensable to effectively demonstrate the quality of the local rice. In this light, Odenigbo et al. (2014) studied the gelatinization properties and amylose content of some local rice varieties and provided information on the physical, gelatinization, cooking and textural properties of TOX 3145, an improved rice variety cultivated in Cameroon. These studies focused on the cooking and eating quality and did not report the nutritional quality of the local rice. Protein content and composition are crucial to rice grain quality and nutritional value (Lin et al., 2005) but the genetic base of rice seed proteins in Cameroon's locally cultivated rice is relatively narrow.

The rice grain is composed of 12% water, 75-80% starch and 7% protein with a full complement of amino acids (Verma and Srivastav, 2017). Its high protein digestibility and excellent biological value make it an important part of consumers' daily nutrient intake. Yang et al. (2011) reported the digestibility of rice protein as being a major factor that influences cholesterol metabolism through the inhibition of cholesterol absorption. Rice protein hydrolysates are equally known to possess antioxidative and blood pressure regulating properties (Zhao et al., 2012; Phongthai et al., 2017). These physiological functions make rice suitable to prevent life style-related diseases such as malnutrition, obesity and high blood pressure which are adult risk factors. Besides, the enhancement of rice seed storage proteins to improve rice nutritive value has lately and gradually become an important target for rice quality breeding (Jiang et al., 2014). Data on characterization of grain and proteins of local Cameroon rice cultivars, which

will be very useful in upscaling the potential of its production, is scanty. This study therefore explored Cameroon's locally cultivated rice to determine the quality of the cultivars cropped and provide additional rice diversity information.

MATERIALS AND METHODS

Establishment of an inventory of local rice cultivars

Sixty-eight cultivars from three agro-ecological zones of the country were used in this study (Table 1): sudano-sahelian zone (Garoua), humid forest with bimodal rainfall zone (Yaoundé) and western highlands zone (Dschang and Tonga). The cultivars collected in Yaoundé had recently been introduced from Benin while those from Garoua, Dschang and Tonga had been cultivated for long by local farmers over several campaigns.

Grain morphological characterization of cultivars

The morphology of grains was studied by measuring their dimensions (length and width) and determining the length/width ratio, which were used to characterise them following IRRI's standard evaluation system for rice (2013). They classify brown rice grain as extra-long (more than 7.5 mm), long (6.6 to 7.5 mm), medium (5.51 to 6.6 mm) and short (5.5mm or less) for size; and slender (over 3.0), medium (2.1 to 3.0), bold (1.1 to 2.0) and round (less than 1.1) for shape (length/width ratio) (IRRI, 1996).

Measurement of grain dimensions

Paddy samples were dehulled with a Satake rice dehuller machine (Satake, USA) and cleaned to eliminate dirt and husks. Length and width of three representative paddy and kernel grains from each sample were determined using a Vernier calliper. The grain shapes were then determined using the following equation:

Length to width ratio = Average grain length (mm)/Average grain width (mm).

Protein content evaluation

Samples were ground with a blender and sieved through a 5 mm mesh sieve to obtain fine powder. The powder was then stored at room temperature (25° C) in airtight bottles untill analysis.

Total protein extraction

Total proteins were extracted by mixing 0.1 g of rice powder with 1 mL of Tris urea buffer (0.05 M Tris-HCL, 5 M Urea, 2 % SDS, 1 % β -mercaptoethanol, and pH 8.0) and centrifuging at 10,000 rpm for 15 min (Tanaka et al., 2016). The supernatant was collected and the pellet rinsed with another 1 mL of Tris urea buffer. The new supernatant was collected into the previous and the proteins were quantified by Bradford assay (Bradford, 1976).

*Corresponding author. E-mail: boudjeko@yahoo.com.

Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u>

Table 1. Rice cultivars used in this study.

| Quala | | Origin | Overla (dava) | | | 1000 GWt at |
|-----------|----------------------------|------------|---------------|------------|--------------|--------------|
| Code | variety name | (locality) | Cycle (days) | N° tillers | riela (Kg/h) | 14% moisture |
| CMRYOr1 | B1 ORYLUX 1 | Yaoundé | 119 | 12.4 | 7689.2504 | 24.0262 |
| CMRYOr3 | B2 ORYLUX 3 | Yaoundé | 119 | 15 | 7471.7740 | 22.3744 |
| CMRYOr4 | B3 ORYLUX 4 | Yaoundé | 119 | 12.6 | 10327.8066 | 23.1682 |
| CMRYOr5 | B4 ORYLUX 5 | Yaoundé | 131 | 14.2 | 7580.3924 | 22.7685 |
| CMRYOr6 | B5 ORYLUX 6 | Yaoundé | 105 | 24.8 | 4664.6814 | 22.8311 |
| CMRYAr1 | B6 ARICA 1 | Yaoundé | 133 | 20.4 | 6465.9251 | 23.7566 |
| CMRYAr2 | B7 ARICA 2 | Yaoundé | 111 | 7 | 8819.1999 | 23.2846 |
| CMRYAr3 | B8 ARICA 3 | Yaoundé | 111 | 11.6 | 9065.1237 | 23.9182 |
| CMRYAr4 | B9 ARICA 4 | Yaoundé | 105 | 6.4 | 2884.3836 | 20.3011 |
| CMRYAr7 | B12 ARICA 7 | Yaoundé | 112 | 5.6 | 3441.2896 | 22.5624 |
| CMRYAr8 | B13 ARICA 8 | Yaoundé | 133 | 13.2 | 6669.0641 | 22.2713 |
| CMRYAr9 | B 14 ARICA 9 | Yaoundé | 119 | 8.4 | 6628.4652 | 22.8311 |
| CMRYAr10 | B15 ARICA 10 | Yaoundé | 111 | 15.8 | 4886.5829 | 20.1414 |
| CMRYAr11 | B16 ARICA 11 | Yaoundé | 111 | 12 | 4977.8014 | 22.98023 |
| CMRYAc1 | B17 ARC-39-155-L-2 | Yaoundé | 119 | 11.6 | 5857.4306 | 24.6205 |
| CMRYAc2 | B18 ARC-37-16-1-5-G | Yaoundé | 119 | 19 | 7798.2232 | 23.3664 |
| CMRYAc3 | B19 ARC-36-2-P-2 | Yaoundé | 119 | 11 | 6583.9304 | 23.7080 |
| CMRYAc4 | B20 ARC-39-135-VL-5 | Yaoundé | 112 | 13.6 | 6495.7222 | 23.6442 |
| CMRYAc5 | B21 ARC-39-130-EP-4 | Yaoundé | 112 | 13.4 | 3063.6420 | 22.0008 |
| CMRYAc6 | B22 ARC-39-145-EP-3 | Yaoundé | 112 | 8.2 | 6588.3679 | 22.1286 |
| CMRYFI | B24 FL478-1-53 | Yaoundé | 112 | 6.4 | 5275.0568 | 23.5972 |
| CMRYGc | B25 GOLD COAST FINGO | Yaoundé | 119 | 9.8 | 6364.5332 | 21.7439 |
| CMRYIr1 | B26 IR4630-22-2 | Yaoundé | 133 | 24.4 | 5741.6468 | 22.3521 |
| CMRYNI23 | B28 NERICA-L-23 | Yaoundé | 111 | 11.6 | 6944.9369 | 22.6136 |
| CMRYNI24 | B29 NERICA-L-24 | Yaoundé | 111 | 10.4 | 6391.1240 | 22.3101 |
| CMRYNI27 | B30 NERICA-L-27 | Yaoundé | 112 | 10.8 | 4976.9713 | 23.1682 |
| CMRYNI9 | B31 NERICA-L-9 | Yaoundé | 119 | 12.8 | 7184.6219 | 23.0962 |
| CMRYIr2 | B32 IR64-SUBI | Yaoundé | 112 | 5.8 | 3630.5247 | 22.1446 |
| CMRDTx1 | D13 TOX 3145-34-2-3 | Dschang | 119 | 8.6 | 7111.4402 | 25.9163 |
| CMRDTx2 | D14 TOX 3145-34-2-3-1 | Dschang | 119 | 20.6 | 12621.0426 | 24.6890 |
| CMRDTx3 | D15 TOX 3440-151-2-3 | Dschang | 113 | 13.2 | 6427.3875 | 23.3579 |
| CMRDTx4 | D16 TOX 3440-151-2-3 | Dschang | 107 | 9 | 7598.6249 | 26.0926 |
| CMRDTx5 | D17 TOX 3887-6-2-3 | Dschang | 107 | 9.2 | 4657.1479 | 25.6578 |
| CMRDTx6 | D18 TOX 40094-4-3 | Dschang | 115 | 16.8 | 15755.5428 | 24,7880 |
| CMRDTc1 | D20 TOC 2N 14-2 | Dschang | 114 | 14.4 | 4221.6066 | 26.1110 |
| CMRDIr2 | D26 IR 7167-33-2-3 | Dschang | 77 | 11 | 7978.0822 | 27.2030 |
| CMRDIr3 | D27 IR 155-79-135-3 | Dschang | 114 | 13.2 | 5847.0000 | 24.1402 |
| CMRDWt | D28 WAT 311. WAS 7083-5-11 | Dschang | 114 | 10 | 5450.6507 | 24.2009 |
| CMRDIt306 | D30 ITA 306 | Dschang | 110 | 13.4 | 7513.4600 | 24.8605 |
| CMRDFk60 | D31 FKR 60 | Dschang | 114 | 10.6 | 6315.0885 | 23.3981 |
| CMRDWb | D32 WAB | Dschang | 114 | 15.8 | 13848.4079 | 22.9255 |
| CMRDIb23 | D35 IB 23 | Dschang | 114 | 8.8 | 6346.2671 | 22.5624 |
| CMRDRv5 | D36 RV 5 | Dschang | 112 | 9.2 | 5068.1764 | 24.3010 |
| CMRTM16 | T2 M 16 | Tonga | 115 | 13.2 | 8320,7283 | 23.3981 |
| CMRTNI56 | T3 NERICA-I -56 | Tonga | 115 | 13.8 | 7618 5760 | 25 5708 |
| CMRTNI59 | T4 NERICA-L-59 | Tonga | 115 | 10 | 8176.6444 | 24,2481 |
| CMRTBa | T9 Bankou | Tonga | 115 | 9.2 | 5393.0761 | 23.8159 |
| CMRGIr46b | G14 IR 46 | Garoua | 114 | 10.4 | 7644.5798 | 23,5520 |
| CMRGNe3 | G3 NERICA 3 | Garoua | 110 | 3.2 | 1809.9551 | 23,2877 |
| CMRGNI42 | G19 NERICA-L-42 | Garoua | 114 | 13.8 | 7383.2482 | 20.7660 |

| CMRGR48 | G22 R48 | Garoua | 110 | 10.6 | 7432.5723 | 24.5873 |
|---------|-----------------------|--------|-----|------|------------|----------|
| CMRGTï | G25 TAÏTCHINGA | Garoua | 127 | 15 | 11072.6567 | 22.8612 |
| CMGNI60 | G27 NERICA-L-60 | Garoua | 115 | 15.2 | 6509.0001 | 22.8928 |
| CMRGRw | G28 RWISI | Garoua | 115 | 21.8 | 5410.8288 | 24.1886 |
| CMRGNe3 | G18 NERICA 3 | Garoua | 110 | 6.8 | 3767.1202 | 23.70808 |
| CMRGRw2 | G32 RWISI (2) | Garoua | 110 | 11.2 | 4010.5932 | 23.0137 |
| CMRGDj | G33 DJOUNGA | Garoua | 110 | 12 | 6513.3394 | 23.1225 |
| CMRGNd | G35 NDOUNGOURI SAMORI | Garoua | 115 | 12.6 | 11291.5231 | 23.6932 |
| CMRGLa | G36 LASSIRI | Garoua | 110 | 9.8 | 5700.4089 | 23.4834 |
| CMRGDn | G38 DOUNGOURI SANTA | Garoua | 107 | 10.6 | 4580.6372 | 24.2481 |
| CMRGTx | G41 TOX | Garoua | 93 | 17.8 | 8873.4296 | 24.3932 |
| CMRG13 | G13 Variété M | Garoua | 114 | 14.6 | 10558.2449 | 21.1733 |

Table 1. Contd.

B, Benin; D, Dschang; G, Garoua; T, Tonga.

Glutelin extraction

Glutelins were extracted with 0.2 N acetic acid after elimination of albumins, globulins and prolamins with 35 mM KPi buffer (pH 7.6) (Nasri and Triki, 2007; Tanaka et al., 2016). One millilitre of inorganic potassium phosphate (KPi) buffer was added to 0.1 g of sample in the Eppendorf tube and mixed. The mixture was then placed on a rotating wheel at 34 rotations per minute (rpm) for 2 h 30 min for homogenization, and extraction was performed at 10,000 rpm for 15 min and the supernatant discarded. The pellet was then mixed with 1 mL of 0.2 N acetic acid and homogenized on the rotating wheel (34 rpm for 2 h 30 min) before centrifugation (10,000 rpm for 15 min). The supernatant was then collected into a new Eppendorf tube and quantified by Bradford assay. Glutelin content was expressed as milligrams equivalent of Bovine Serum Albumin per dry weight (mgEqvBSA/g DW).

Protein and glutelin data were analysed with SPSS software version 20. One way ANOVA test and Pearson correlation analysis were performed. The differences were considered as significant if P < 0.05, and highly significant if P < 0.01.

Statistical analyses

The data collected were subjected to multivariate analyses. The construction of the phylogenetic tree was done using software R. The Ward D method was used for calculating Euclidean distance. Principal component analysis (PCA) was performed using the SPAD V5 software.

RESULTS

Grain dimensions

Kernel sizes ranged from extra-long to short (Figure 1), with 16 extra-long cultivars, 42 long cultivars, 9 medium cultivars and 1 short cultivar. Kernel shapes were slender (36 cultivars), medium (28) and bold (4). It appeared that majority of the varieties from each of the localities were of long size (Yaoundé-56.6%, Dschang-57.9%, Garoua-60% and Tonga-75%), and slender shape (Yaoundé-66.7%, Dschang-52.6%, Garoua-53.3% and Tonga-50%).

Cluster and PCA analysis

Cultivars were grouped into four main clusters (Figure 2). Cluster 1 had six cultivars relatively low yielding (1810 to 3767 t/h) with varying sizes and shapes from extra-long and slender to long and medium. Cluster 2 had 34 cultivars with relatively high yields (6315 to 9065 t/h) and mainly slender and medium shaped grains, as well as many extra-long and long size grains with a few medium size and one short size grain cultivar. Cluster 3 had 8 cultivars with the highest yields (4752 to 15755 t/h) and mainly extra-long size and medium shape grains. Cluster 4 had 20 cultivars with low yields (4221,6 to 5858,2 t/h), mainly slender and medium in shape with one bold grain cultivar and many long size grain cultivars.

PCA analysis demonstrated three components with Eigen value greater than 1 (Table 2): PC1, PC2 and PC3 which all contributed to 74.41 % of total variation. PC1 involved grain dimensions, PC2 involved agronomy parameters while PC3 involved yield components (Figure 3). Grain dimensions (paddy and kernel length and length/width ratio) which are associated to PC1 demonstrated a better distinction of the cultivars than other variables such as paddy and kernel width, number of tillers, thousand grain weight, cycle and yield.

Eight cultivars were selected for protein evaluation; four which were taught to be traditional and four improved cultivars which had PCA values similar to those of the traditional for comparison. Table 3 shows the eight selected varieties with their localities of origin and ecologies, and Figure 4 shows images of the paddy and kernels of these cultivars.

Total protein extraction and quantification

Protein contents were appreciably high, greater than the reported range of 7 to 10% (Figure 5). Highest protein



Figure 1. Cameroon agro-ecological zones.



Figure 2. Kernel size (A) and shape (B) of cultivars collected.

| | Eigen value | Variance (%) | Cumulative (%) |
|-----|-------------|--------------|----------------|
| PC1 | 4.6922 | 46.92 | 46.92 |
| PC2 | 1.4182 | 14.18 | 61.10 |
| PC3 | 1.3303 | 13.30 | 74.41 |
| | | | |

 Table 2. Principal Components for ten quantitative characters in 68 rice cultivars.



Figure 3. Dendrogram presenting the association between sixty-eight local rice varieties based on agromorphological parameters.

| N° | Code | Variety | Origin | Ecology | | | | | |
|-----------------------|-----------------------|-----------------------|---------|-------------------|--|--|--|--|--|
| Traditional varieties | | | | | | | | | |
| 1 | CMRGNd | G35 NDOUNGOURI SAMORI | Garoua | Rain fed upland | | | | | |
| 2 | CMRGDn | G38 DOUNGOURI SANTA | Garoua | Rain fed upland | | | | | |
| 3 | CMRTBa | T6 BANKOU | Tonga | Rain fed upland | | | | | |
| 4 | CMRGTI G25 TAÏTCHINGA | | Garoua | Irrigated lowland | | | | | |
| | | | | | | | | | |
| Impro | ved varieties | | | | | | | | |
| 5 | CMRDTx5 | D17 TOX 3887-6-2-3 | Dschang | Irrigate lowland | | | | | |
| 6 | CMRDTc3 | D22 TOC 3317-9-1 | Dschang | Irrigated lowland | | | | | |
| 7 | CMRDWb | D32 WAB | Dschang | Irrigated lowland | | | | | |
| 8 | CMRDTx6 | D18 TOX 40094-4-3 | Dschang | Irrigated lowland | | | | | |

Table 3. Origin and ecology of the rice varieties selected.



Figure 4. Principal component illustration of 68 local rice cultivars.



Figure 5. Samples selected for protein analysis.



Figure 6. Total protein content of eight local rice cultivars.

Table 4. Glutelin contents (GC) and percentages (G %) of eight local rice varieties.

| Variety | CMRGNd | CMRGDn | CMRGTï | CMRTBa | CMRDWb | CMRDTc3 | CMRDTx5 | CMRDTx6 |
|---------------------|--------|--------|--------|--------|--------|---------|---------|---------|
| GC (mg BSAeqv/g DW) | 4.43 | 10.1 | 7.7 | 6.4 | 8.8 | 8.1 | 6.5 | 2.5 |
| G % | 30.9 | 77.7 | 62.1 | 53.8 | 73.3 | 63.8 | 80.2 | 29.1 |

content was obtained in CMRGNd (14.3%), followed by CMRGDn (13%) which was not significantly different from CMRGTī (12.2%). There was no significant difference between the protein contents of CMRTBa, CMRDWb, CMRDTc3 and CMRDTx6. The lowest protein content was exhibited by CMRDTx5 (8.%) which was the only variety with a protein content that fell within the reported range of 7-10%.

Glutelin extraction and quantification

Glutelin content ranged from 2.5 to 10.1 mgEqvBSA/g DW (Figure 6) and from 29.1 to 80.2% of total proteins (Table 4). Highest glutelin content was exhibited by CMRGDn (10.1 mgEqvBSA/g DW) followed by CMRDWb (8.8 mgEqvBSA/g DW), CMRDTc3 (8.1 mgEqvBSA/g DW) and CMRGTī (7.7 mgEqvBSA/g DW) which were not significantly different from each other. CMRDTx5 and CMRTBa had glutelin contents of 6.5 and 6.4 mgEqvBSA/g DW respectively, followed by CMRGNd (4.43 mgEqvBSA/g DW). Lowest glutelin content was observed in CMRDTx6 (0.25 mgEqvBSA/g DW).

Cluster analysis of the eight varieties using total protein and glutelin contents (Figure 7) presented two main clusters: A and B. cluster A branches into several sub clusters while cluster B branches to two varieties: CMRGNd and CMRDTx6. Cluster A branches at the highest level into cluster C which is linked to the variety CMRDTx5. Cluster C branches into cluster D and CMRGDn and cluster D gives cluster E and CMRTBa. Finally, cluster E gives CMRDWb and cluster F which contains the varieties CMRGTï and CMRDTc3.

Correlation results

A highly significant negative correlation (-0.863**) was observed between cycle duration and grain width, while cycle duration and grain length/width ratio showed a significant highly positive correlation (0.812*). Number of tillers presented a highly significant positive correlation with yield (0.921**), a significant negative correlation with grain width (-0.742*) and a significant positive correlation with grain length/width ratio (0.731*). Yield demonstrated a significant positive correlation with grain length (0.740*)



Figure 7. Glutelin content of eight local rice cultivars.

 Table 5. Pearson correlation of variables involved in the study.

| | Cycle | N° of tillers | Yield | Grain length (L) | Grain width (W) | Grain L/W ratio | Protein content | Glutelin content |
|------------------|----------|------------------|---------|---------------------|--------------------|--------------------|-----------------|---------------------|
| Cycle | - | | | | | | | |
| N° of tillers | 0.631 | - | | | | | | |
| Yield | 0.552 | 0.921** | - | | | | | |
| Grain length (L) | 0.602 | 0.593 | 0.740* | - | | | | |
| Grain width (W) | -0.863** | -0.742* | -0.744* | -0.872** | - | | | |
| Grain L/W ratio | 0.812* | 0.731* | 0.759* | 0.927** | -0.989** | - | | |
| Protein content | 0.256 | 0.136 | 0.219 | 0.335 | -0.117 | 0.185 | - | |
| Glutelin content | -0.226 | -0.344 | -0.511 | -0.182 | 0.239 | -0.177 | 0.091 | - |

**Correlation is significant at the 0.01 level (2-tailed). *correlation is significant at the 0.05 level (2-tailed).

and length/width ratio (0.759*), and a significant negative correlation with grain width (-0.744*). Grain length demonstrated a highly significant negative correlation with grain width and a highly significant positive correlation with grain length/width ratio (-0.872** and 0.927** respectively) (Table 5). Grain width showed a highly significant negative correlation (-0.989**) with grain length/width ration but protein and glutelin content neither had significant correlation with either of the other variables nor with each other. These two put aside, grain width particularly demonstrated negative correlation with all other variables (Figure 8).

DISCUSSION

The size and shape categories distinguished in this study

are in accordance with IRRI's Standard Evaluation System (SES) for rice (IRRI, 1996). Except the aroma, fine rice is generally appreciated for its long and slender grains. The predominance of long and slender rice grains (42 and 36 respectively out of 68) within the varieties studied is therefore indicative of the potential of these varieties for breeding programs and processing into fine rice for commercial purposes and represents a great exploitable potential for Cameroonian rice.

Cluster analysis with agro-morphological parameters yielded four clusters, with cultivars from different localities distributed within the same clusters, and cultivars from the same localities distributed in different clusters. This translates the variability that exists between varieties from the same environment and the similarity between varieties from different environments, which indicates that the decisive factors controlling these agro-morphological



Figure 8. Dendrogram presenting the relationship between eight local rice varieties based on protein and glutelin content.

traits lie in the cropping systems and/or the rice genome. The high yielding cultivars were grouped together in cluster 3 and were mainly cultivars with slender shape, long size grains and high tiller number. Malaa et al. (2017) demonstrated farmers' preference for long grains (generally appreciated on market) and high tiller number of plants, which are associated to high yields. The high yields observed may thus be explained by the long size of the grains and the higher number of plant tillers. These high yields may as well be attributed to the genetic makeup of the cultivars, their environment and cropping conditions. In the same sense, low yields observed in cultivars grouped in cluster 1 may be explained by their low tiller number, genetic constitution and unfavourable environmental and cropping conditions. PCA analysis presented grain dimensions as the major contributors of the total variation, thereby presenting paddy length, width and length/width ratio as well as kernel length, width and length/width ratio as the major determinants of phenotypic diversity. This is in accordance with Rai et al. (2013) results on landraces of aromatic indica rice, which showed that grain length and width among other morphological characters are the major determinants of phenotypic diversity.

The protein contents of the varieties studied (8.1-14.3%) were higher than those reported by Juliano and Villareal (1993); Khush (1997) (7-10%). This might be because of adequate environmental conditions and cropping system. Buresova et al. (2010) reported that water supply, handling, application of fertilizer (soil nitrogen availability), environmental stress (such as salinity and alkalinity, temperatures and diseases), location of growing areas, growing conditions and time tend to increase grain protein content. Hence, these factors might have been adequate for the different cultivars studied. The higher protein content of the traditional varieties as compared to the improved varieties demonstrates their nutritional quality. Guo et al. (2007) reported that rice populations cropped in upland conditions had higher protein contents than those grown in lowland conditions. This explains the higher protein contents observed in CMRGNd and CMRGDn, which are upland varieties. CMRGNd, which showed highest protein content (14.3%), can be exploited to develop rice of better nutritional and technological quality. Rice protein is a major factor in determining texture (e.g. stickiness),

pasting capacity, and sensory characteristics of rice. High protein content makes eating texture harder. This implies CMRGNd, CMRGDn and CMRGTï, which that demonstrated high protein content, would be adequate for cooking hard texture rice. In addition, these varieties shall be beneficial to farmers as they would provide increased returns due to less wastage during milling, which is associated to high storage proteins, as was reported by Leesawatwong et al. (2005). CMRTBa had a red pericarp, which is indicative of the presence of phenolic compounds which account for antioxidant property. Bhat and Riar (2017) obtained total protein contents of 7.24-8.85% with pigmented traditional cultivars from India. CMRTBa, which is equally a pigmented and traditional cultivar demonstrated a higher protein content (11.9%) and hence constitutes good genetic resource for breeding medicinal rice or developing a nutraceutical.

Glutelin is the most abundant and most nutritious of the four storage protein types present in rice appeared to be of significant differential content in the varieties selected. The quantities obtained were in accordance with the values reported by Kawakatsu et al. (2008), that is 60-80% of total proteins except for CMRTBa (53.8 %) and CMRDTx6 (29.1 %). CMRGDn, which showed the highest glutelin content (10.1 mgBSAEqv/g DW) is of particular interest for the development of a rice variety with better nutritional quality.

Classification of the eight selected varieties by cluster analysis based on total protein and glutelin content grouped together CMRGNd (traditional) and CMRDTx6 (improved) which are both characterized by high yields, with that of CMRGTx6 slightly higher. However, CMRGNd had higher protein and glutelin contents than CMRGTx6. This classification aligns with the one based on agro-morphology in which both varieties were grouped in the same cluster. CMRGTï (traditional) and CMRDTc3 (improved) were equally grouped in the same cluster; which is explained by their similar high protein and glutelin contents, the protein content of CMRGTi being slightly higher than that of CMRGTc3. Besides, CMRGTï presented a far greater yield than CMRTc3. With these two traditional varieties showing better traits than their improved counterparts, the quality of Cameroon's local and native varieties is clearly unveiled.

Positive correlation observed between cycle duration and grain length/width ratio might imply that slender grains are associated to a long cycle. Likewise, bold grains would be associated to a short cycle. In the same way, positive correlation observed between number of tillers, yield and grain length/width ratio would imply that we should expect high yield and slender grains from a variety that presents numerous tillers and low yield with bold grains from one that presents fewer tillers. Positive correlation observed between yield, grain length and grain length/width ratio may indicate that a variety characterised by long grains should normally be a high yielding one as opposed to one with short grains. A variety with slender grains should equally yield more than a variety with bold grains. Also, positive correlation between grain length and grain length/width ratio may indicate that long grains will generally be slender while short grains would be bold. Negative correlations observed between grain width and cycle; grain width and number of tillers may imply that a short cycle plant will yield short grains and long cycle plants will yield long grains. A variety with numerous tillers will provide long grains and a variety with few tillers will provide short grains.

Conclusion

The major findings suggest that Cameroon possesses high diversity in rice germplasms, most of which are improved varieties though a few native varieties still exist. The predominance of long, slender kernels and the high protein and glutelin contents of the varieties studied indicate the good quality of Cameroon's locally cultivated rice, especially those thought to be traditional. However, there is need to investigate the genetic make-up of these four thought traditional cultivars and confirm or not their nativity as well as provide relevant information for breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge the institutional and material support from the Biotechnology Centre of the University of Yaounde I. The authors are also grateful to M. Tene Tayo Paul Martial for his excellent technical assistance.

REFERENCES

- Bhat FM, Riar CS (2017). Characterizing the traditional rice (*Oryza sativa* L.) cultivars on the basis of seed morphology and protein characteristics. Indian Journal of Plant Science 6:39-47.
- Bradford M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-254.
- Buresova I, Sedlackova I, Famera O, Lipavsky J (2010). Effect of growing conditions on starch and protein content in triticale grain and amylose content in starch. Plant, Soil Environment 56:99-104.
- Cruz ND, Khush GS (2000). Rice grain quality evaluation procedures. In. Singh RK, Singh US, Khush GS (Eds). Aromatic Rices, Oxford and IBH Publishing Co Pvt. Ltd, New Delhi, pp. 15-28.
- FAO (2019). Food and Agriculture Organization of the United Nations, FAOSTAT Database. Available at ; http://www.fao.org/faostat/en/#home.
- Fon DE, Fonchi D (2016). Rice consumption in Cameroon: A need for policy change. International Journal of Agricultural Policy and Research 4(11):242-248.
- Guo Y, Mu P, Liu J, Lu Y, Li Z (2007). QTL mapping and QXE interactions of grain cooking and nutrient qualities in rice under

upland and lowland environments. Journal of Genetics and Genomics 34:420-428.

- International Rice Research Institute (IRRI) (1996). Standard Evaluation System for Rice (SES). International Network for Genetic Evaluation of Rice. IRRI, Philippines.
- Jiang C, Cheng Z, Zhang C, Yu T, Zhong Q, Shen JQ, Huang X (2014). Proteomic analysis of seed storage proteins in wild rice species of the *Oryza* genus. Proteome Science 12:1-12.
- Juliano BO, Villareal CP (1993). Grain quality evaluation of world rices. International Rice Research Institute, Manila. The Philippines.
- Kawakatsu T, Yamamoto MP, Hirose S, Yano M, Takaiwa F (2008). Characterization of a new rice glutelin gene GluD-1 expressed in the starchy endosperm. Journal of Experimental Botany 59:4233-4245.
- Khush GS (1997). Origin, dispersal, cultivation and variation of rice. Plant Molecular Biology 35:25-34.
- Leesawatwong M, Jamjod S, Kuo J, Dell B, Rerkasem B (2005). Nitrogen fertilizer increases seed protein and milling quality of rice. Cereal Chemistry 82(5):588-593.
- Lin SK, Chang MC, Tsai YG, Lur HS (2005). Proteomic analysis of the expression of proteins related to rice quality during caryopsis development and the effect of high temperature on expression. Proteomics 5:2140-2156.
- Nasri N, Triki S (2007). Storage proteins from seeds of Pinus pinea L. Comptes Rendus Biologie 330:402-409.
- Malaa DK, Simo BH, Agboh-Noameshie AR, Jaff AB, Mouafo BI, Mfouapon A, Woin N (2017). Caractéristiques biophysiques préférées dans le riz par genre au Cameroun. International Journal of Innovation and Applied Studies 20:1099-1106.
- Odenigbo AM, Ngadi M, Ejebe C, Woin N, Ndindeng SA (2014). Physicochemical, Cooking Characteristics and Textural Properties of TOX 3145 Milled Rice. Journal of Food Research 3(2):82-90.
- Phongthai S, Homthawornchoo W, Rawdkuen S (2017). Preparation, properties and application of rice bran protein: A review. International Food Research Journal 24:25-34.
- Rai A, Debal D, Rajasri R, Chattopadhayay B (2013). Phenotypic characters of rice landraces reveal independent lineages of shortgrain aromatic indica rice. AoB Plants 5(32):1-9.

- Tanaka TK, Khan N, Yamaguchi S, Yamaguchi T, lida S (2016). Glutelin is partially degraded in globulin-less mutants of rice (*Oryza sativa* L.). Plant Production Science 19:401-410.
- USDA (2017). Quick stats. National Agricultural Statistics Service, US Department of Agriculture, Washington DC, USA.
- Verma DK, Srivastav PP (2017). Proximate composition, mineral content and fatty acids. Rice Science 24:21-31.
- Yang L, Chen J, Xu T, Qiu W, Zhang Y, Zhang L, Xu F, Liu H (2011). Rice protein extracted by different methods affects cholesterol metabolism in rats due to its lower digestibility. International Journal of Molecular Sciences 12:7594-7608.
- Zhao Q, Selomulya C, Wang S, Xiong H, Chen XD, Li W, Peng H, Xie J, Sun W, Zhou Q (2012). Enhancing the oxidative stability of food emulsions with rice dreg protein hydrolysate. Food Research International 48:876-884.