Hindawi
Journal of Food Quality
Volume 2018, Article ID 5134569, 12 pages
https://doi.org/10.1155/2018/5134569

Research Article

Quality and Grain Yield Attributes of Rwandan Rice (*Oryza sativa* L.) Cultivars Grown in a Biotron Applying Two NPK Levels

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Received 28 February 2018; Accepted 20 May 2018; Published 24 June 2018

Academic Editor: Eduardo Puértolas

High-yielding rice cultivars with good processing quality and rich in nutrition suitable to a changing climate are of particular importance for future rice-based food production. Here, seven Rwandan rice cultivars were grown in a climate chamber of the biotron facility at the Swedish University of Agricultural Sciences, to be evaluated for their grain yield, nutritional composition, and dough mixing properties. Two different levels of inorganic fertilizer were applied weekly from the seedling stage until flowering. Significant differences for grain yield and quality attributes were found between cultivars. Jyambere showed significantly the highest yield while Ingwizabukungu, Nemeyubutaka, and Jyambere were high in mineral elements content. Ndamirabahinzi and Mpembuke had the highest levels of TPC and TAC. Generally, the lower fertilizer dose resulted in a better performance of the cultivars for both yield and quality attributes. Significantly higher content of Fe, Ca, and Ba was found in grains from the moderate fertilizer dose, whereas K, Na, P, S, Zn, Cd, and Pb increased in grains from the higher fertilizer dose. The cultivar Ndamirabahinzi showed less variability of evaluated characters across fertilizer doses. The results from this study may be used for rice breeding of cultivars with high yield and good grain quality.

1. Introduction

Rice (*Oryza sativa* L.) is one among the leading cereal staple foods together with wheat and maize [1]. Grain yield has been steadily increasing since the 1940s due to breeding efforts focusing on high-yielding cultivars and on improvements in crop husbandry [2, 3]. Quality of rice is of increasing interest. Four traits are recognised as the most important for rice quality: grain appearance, milling properties, eating and cooking qualities (ECQs), and nutritional composition [4]. Grain appearance refers to length, width, and chalkiness of grains. ECQs are mainly influenced by starch, amylose content, gel consistency, and gelatinization temperature [5]. However, protein content and composition in the rice grain also contribute to ECQs, besides being an important part of the nutritional composition of the grain [6–9]. Furthermore, the storage protein content and composition determine the flour quality and its dough mixing properties, for example, the dough development time, peak time, peak height, and loaf volume [10–12]. Phytochemical compounds such as phenolic acids, flavonoids, and tannins are potential antioxidants. Polyphenols contribute to plant protection and have human health promoting properties, including being anticarcinogenic and having antimicrobial effects, and also they are known for their reduction of cardiovascular diseases [13, 14]. Correlations have been reported among content of specific mineral elements and ECQs for rice [15]. Until now, physical appearance and ECQs, both contributing to the commercial value of the product through consumer evaluations, have received a higher attention as compared to other quality traits [16].

Table 1: Characteristics of 7 Rwandan rice cultivars (ISAR 2010).

ID	Name/code	Popular name	Characteristics						
			Type	Plant height	Flag leaf	Panicle exertion	Tillering ability	Lifespan (days)	Potential yield ($t{\cdot}ha^{-1}$)
1	N/A	Ingwizabukungu	*indica*	Intermediate	Intermediate	N/A	N/A	N/A	N/A
2	WAT 1395-B-24-2	Intsindagirabigega	*indica*	Intermediate	Intermediate	Well exerted	Medium	120–150	8.0
3	WITA 4	Jyambere	*indica*	Intermediate	Intermediate	Moderate Well exerted	Medium	152	10.9
4	WAB923-B-6-AL1	Mpembuke	*indica*	Intermediate	Intermediate	Moderate Well exerted	Medium	170	8.3
5	WAB 569-35-1-1-1- HB	Ndamirabahinzi	*indica*	Intermediate	Intermediate	Well exerted	Medium	143	7.6
6	WAB 880-1-38-20-28- P1-HB	Nemeyubutaka	*indica*	Intermediate	Erect	Well exerted	Medium	152	9.3
7	Zong geng	"Kigoli"	*japonica*	Tall	Intermediate	Moderate well exerted	Medium	180	6.0

NA: unavailable information.

Quality traits vary largely among rice cultivars, thereby indicating the presence of a strong genetic component in their determination [17–20]. Rice grain quality traits are also influenced by environmental factors such as the soil status, fertilizer applications, and climate variations [21]. Nitrogen applications appear to be positively correlated with protein content [22–24], while negatively with amylose content [9]. Potassium fertilization increases grain protein content without affecting gelatinization temperature and amylose content [25]. Significant genotype × environment interactions have been noted for protein content [25], heavy metals [26], and mineral elements [27].

The increasing world population calls for an enhanced food production, but adverse environmental and climate conditions may lead to great difficulties in achieving this goal. Hence, there is an increasing need to breed nutritional, high-yielding, and high-quality genotypes, adapted to stressful environments of various types. For success, relationships among traits such as grain yield, quality, nutrient content, and stress adaptation must be studied in detail. Previous research investigated the relationship between morphological traits and grain quality [27], between mineral elements and other quality traits [28, 29], and between grain yield and physiological grain traits [30]. However, a full understanding of the possibility to produce high-yielding rice of good quality at stressful conditions, in particular drought, is still lacking.

The aim of this study was to characterize the variation in grain yield (and its components) and nutritional composition in a selection of Rwandan rice cultivars. The second aim of this study was therefore to understand the interplay between grain yield components and nutritional quality traits in these cultivars, thereby creating the basis for the breeding of high yielding and nutritionally beneficial cultivars for rice production in Rwanda.

2. Material and Methods

2.1. Plant Material. Production characteristics for the seven rice cultivars used in this study, obtained from the College of Agriculture, Animal Science and Veterinary Medicine of the University of Rwanda, are presented in Table 1. The rice cultivars have been released by the Agricultural Research Institute of Rwanda (ISAR), now known as the Rwanda Agricultural Board (RAB). The cultivar Zong geng commonly called "Kigoli" was introduced from China in the 1960s [31], while Intsindagirabigega was introduced in 2002 from WARDA (currently, Africa Rice Centre) and released in 2004. The remaining cultivars were released in 2010. The cultivars were selected because they were the most cultivated in Rwanda and their medium water requirements were as described by ISAR [32].

2.2. Growing Conditions in the Climate Chamber and Experimental Setup. To allow proper comparison of characters among the rice cultivars, the impact of environmental effects was minimized through cultivation in a controlled environment. Rice plants were grown in a climate chamber in the biotron facility of the Swedish University of Agricultural Sciences at Alnarp, Sweden. The day/night temperature was set to 30°C and 25°C, respectively, according to Wopereis [33], with 11 hours of light and 13 hours of darkness [34, 35]. Light intensity of 350 PAR $\mu mol{\cdot}s^{-1}$ was chosen following Hubbart et al. [36]. The atmospheric relative humidity was 70% according to Hirai et al. [37].

2.2.1. Soil Potting and Sowing. Pots with the size $6 \times 10 \times 12$ cm were filled with soil. The soil composition was ($g{\cdot}m^{-3}$) 180, 90, 195, 260, 1000, 2000, 6, 3.5, 2.5, 1.5, 0.5, and 3 for N, P, K, Mg, S, Ca, Fe, Mn, Cu, Zn, B, and Mo, respectively. Pots were placed into big plastic trays capable to hold water. Potted soil was gently sprinkled with tap water before sowing. Two seeds per pot were directly sown into the wet soil at 1 to 2 cm depth. After emergence, the seedlings were trimmed to one seedling per pot. The soil was regularly watered with tap water from soil surface until the seedlings were three weeks old. After three weeks, water was regularly added into the plastic trays, and plant roots had access to water through holes in the bottom of the pots.

2.2.2. Fertilizer Application. The plastic trays were arranged into two compartments, one for moderate fertilizer dose and the other for high fertilizer dose. Each compartment contained five replicates per cultivar. The quantity of nitrogen to be applied per plant was calculated based on the fertilizer rate recommended by Manzoor et al. [38]. Universal blue water-soluble fertilizer 18-11-18 NPK was used as the source of nutrients. Fertilizer solution was gently sprinkled on the soil surface. The fertilization started three weeks after sowing and was weekly applied until flowering. The fertilizer solution was applied at two different doses, and each plant was given either 0.127 $g{\cdot}plant^{-1}$ (0.023 g·N, moderate dose) or 0.255 $g{\cdot}plant^{-1}$ (0.046 g·N, high dose).

2.3. Grain Yield Attributes. The number of tillers $plant^{-1}$, number of fertile tillers $plant^{-1}$, spike length, and number of spikelets $spike^{-1}$ were measured at harvesting time following the rice standard evaluation system [39]. The grains were threshed by hand, and grain yield $plant^{-1}$ was measured using a precision balance.

2.4. Nutritional Content of Rice Grains

2.4.1. Sample Preparation. Rice grains harvested per cultivar and per fertilizer dose were pooled and stored at −20°C. Samples were freeze-dried for 48 hours. Dry samples were grinded to fine flour using IKA- WERKE grinder type A_{10} (Skafte MedLab, Germany). The flour was kept at −20°C until further analyses.

2.4.2. Determination of Mineral Elements and Heavy Metal Content. A mixture of 500 mg lyophilised flour sample and 10 ml of HNO_3 in two replicates was combusted at 185°C for 17 minutes. The volume of cooled mixture was adjusted to 100 ml by adding water. The analyses of minerals and heavy metal content were made using an ICP-OES, Optima 8300, and PerkinElmer [40] at Lund University following methods described in [41, 42].

2.4.3. Total Phenolic Content. Total phenolic content was determined following Singleton et al. [43] with minor modifications. Total phenolic compounds were extracted from lyophilised flour sample in triplicate using 70% ethanol, 1% HCl, and sonication for 1 hour. The extract was centrifuged at 8000 $g{\cdot}min^{-1}$ for 10 min, and the supernatant was recuperated into a new tube. Sixty-microliters of extract and 60 μl of Folin–Ciocalteu reagent were added to 250 μl of water. The samples were left to react for 6 min before adding 600 μl of 7% Na_2CO_3 per sample. The mixture was then left for 75 minutes at room temperature. The optic density was determined using a Thermo Scientific Multiskan Go spectrophotometer at 650 nm. Gallic acid was used as standard. Total phenolic content in rice samples was expressed as Gallic equivalent per 100 g of dry sample.

2.4.4. Determination of Total Antioxidant Capacity. The total antioxidant capacity was determined following the method of Pérez-Jiménez and Saura-Calixto [44] with slight modifications. 50 mg of flour was measured from each sample in triplicate. The extraction was done in two steps. In the first step, 1 ml of 50% methanol, pH 2, was added to the flour sample. The mixture was shaken at 1000 $g{\cdot}m^{-1}$ for 1 hour at room temperature and then centrifuged at 8000 $g{\cdot}min^{-1}$ for 10 minutes. The supernatant was recuperated in a new tube. 70% acetone was added to the pellet and then shaken and centrifuged as described above. The supernatant (second extract) was added to the first extract. For the assay, a reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3{\cdot}6H_2O$ in a 10 : 1 : 1 ratio. $FeSO_4$ $7H_2O$ was used as standard solution. About 200 μl of fresh reagent was added to 20 μl of sample extract or standard solution in a 96-well plate. The plate was heated at 37°C for 30 minutes in a microwave oven, and the absorbance was measured using the Thermo Scientific Multiskan Go spectrophotometer at 593 nm. Total antioxidant capacity was expressed in μmol Fe^{2+} equivalent per gram of the sample's dry weight.

2.4.5. Amount and Size Distribution of Polymeric and Monomeric Protein. Amount and size distribution of polymeric and monomeric proteins was evaluated by size-exclusion high-performance liquid chromatography (SE-HPLC) according to Johansson et al. [45]. Proteins were extracted from rice sample flour in triplicates. Available proteins were first extracted in a buffer including 0.5% SDS + 0.05 M NaH_2PO_4 (pH 6.9). A mixture of 20 mg flour sample and 1.4 ml buffer was shaken at 2000 $g{\cdot}m^{-1}$ for 5 minutes and then centrifuged at 10000 $g{\cdot}m^{-1}$ for 30 minutes. The supernatant was transferred into a new vial. A total of 1.4 ml of buffer was added to the pellet remaining from the first extraction and SDS-non-extractable proteins were extracted by ultrasonication for 45 seconds and then centrifuged as described above. The second extract was transferred to a new different vial. The percentage of total polymeric proteins and polymeric proteins that are present in an unextractable form (%UPP) was determined according to Gupta et al. [46], and percentage solubility of rice flour proteins was calculated as proposed by Oszvald et al. [11].

2.5. Statistical Analysis. Statistical analysis was carried out using the Minitab 16 software. The analysis of variance was done by general linear model (GLM) analyses, whereas Tukey's method was used for mean comparisons. In order to determine the relationships among characteristics as well as similarities and differences among analysed cultivars, principal components analysis was applied.

3. Results and Discussion

3.1. Results. Two of the cultivars, that is, Intsindagirabigega and Zong geng, failed to flower at the higher fertilizer dose and did not produce grains for nutritional analysis. Therefore, data from the cultivation at moderate fertilizer

Table 2: Mean mineral element content (mg·kg^{-1} dry weight) (10^2) in Rwandan rice cultivars.

Cultivar	Al	B	Ba	Ca	Cu
Ingwizabukungu	0.12 ± 0.002a	0.060 ± 0.002a	0.010 ± 0.0002a	8.6 ± 0.005ab	0.12 ± 0.010a
Intsindagirabigega*	0.12 ± 0.001	0.001 ± 0.000	0.005 ± 0.0000	3.5 ± 0.000	0.06 ± 0.015
Jyambere	0.12 ± 0.001a	0.030 ± 0.001a	0.020 ± 0.0010a	14.8 ± 0.040a	0.07 ± 0.026a
Mpembuke	0.14 ± 0.007a	0.050 ± 0.002a	0.004 ± 0.0005a	5.7 ± 0.006b	0.08 ± 0.006a
Ndamirabahinzi	0.12 ± 0.001a	0.060 ± 0.006a	0.007 ± 0.0004a	5.2 ± 0.006b	0.08 ± 0.001a
Nemeyubutaka	0.14 ± 0.020a	0.040 ± 0.007a	0.020 ± 0.0006a	10.0 ± 0.012ab	0.10 ± 0.008a
Zong geng*	0.13 ± 0.007	0.009 ± 0.001	0.010 ± 0.0000	6.5 ± 0.001	0.09 ± 0.001
Fertilizer dose					
High	0.13 ± 0.009a	0.05 ± 0.006a	0.006 ± 0.0004b	7.1 ± 1.960b	0.09 ± 0.012a
Moderate	0.13 ± 0.003a	0.05 ± 0.007a	0.020 ± 0.0041a	11.0 ± 0.490a	0.10 ± 0.003a
Cultivar	*Fe*	*K*	*Mg*	*Mn*	*Mo*
Ingwizabukungu	0.18 ± 0.002a	48.2 ± 3.75ab	18.0 ± 0.16a	0.51 ± 0.012b	0.022 ± 0.002a
Intsindagirabigega*	0.18 ± 0.015	32.7 ± 0.79	10.8 ± 0.19	0.15 ± 0.003	0.005 ± 0.005
Jyambere	0.19 ± 0.001a	69.8 ± 9.93a	18.1 ± 0.43a	0.41 ± 0.021c	0.007 ± 0.004a
Mpembuke	0.17 ± 0.026ab	39.2 ± 1.79b	13.5 ± 0.48b	0.17 ± 0.026d	0.008 ± 0.004a
Ndamirabahinzi	0.12 ± 0.006b	47.9 ± 3.41ab	13.4 ± 0.14b	0.14 ± 0.018d	0.012 ± 0.004a
Nemeyubutaka	0.20 ± 0.020a	57.0 ± 2.04ab	19.2 ± 0.63a	0.75 ± 0.016a	0.012 ± 0.004a
Zong geng*	0.17 ± 0.004	42.6 ± 0.65	26.8 ± 0.13	0.24 ± 0.002	0.015 ± 0.001
Fertilizer dose					
High	0.15 ± 0.011b	59.5 ± 0.50a	16.5 ± 0.78a	0.38 ± 0.07a	0.01 ± 0.002a
Moderate	0.19 ± 0.009a	45.4 ± 0.20b	16.4 ± 0.87a	0.41 ± 0.06a	0.01 ± 0.002a
Cultivar	*Na*	*P*	*S*	*Zn*	
Ingwizabukungu	0.37 ± 0.0661a	49.3 ± 1.94ab	16.5 ± 0.47ab	0.46 ± 0.010a	
Intsindagirabigega*	0.17 ± 0.0005	34.1 ± 0.31	11.6 ± 0.01	0.37 ± 0.012	
Jyambere	0.41 ± 0.1520a	50.9 ± 5.03a	19.8 ± 2.97a	0.38 ± 0.034ab	
Mpembuke	0.28 ± 0.0591a	38.2 ± 1.34c	12.2 ± 0.61b	0.31 ± 0.094b	
Ndamirabahinzi	0.45 ± 0.0321a	39.4 ± 0.60bc	13.8 ± 0.16ab	0.33 ± 0.008b	
Nemeyubutaka	0.36 ± 0.0232a	50.3 ± 1.04ab	15.3 ± 0.60ab	0.41 ± 0.007a	
Zong geng*	0.20 ± 0.0012	29.4 ± 0.65	10.4 ± 0.07	0.32 ± 0.004	
Fertilizer dose					
High	0.48 ± 0.03a	48.7 ± 0.26a	17.0 ± 0.14a	0.39 ± 0.022a	
Moderate	0.26 ± 0.02b	42.5 ± 0.18b	14.0 ± 0.52b	0.36 ± 0.014b	

Means and standard errors per cultivar are the average at both fertilizer doses; means and standard errors per dose are the average for all cultivars. Means followed by the same letter within a column are not significantly different between cultivars or between doses according to Tukey's test at $P \leq 0.05$. *Mean data only for moderate fertilizer dose.

dose of Intsindagirabigega and Zong geng are included in tables to contribute with options for comparison.

3.1.1. Mineral Content. Mean values for each of the minerals analysed, for each of the cultivars, and at each of the fertilizer doses are available in the supplementary data (Table S1). Significant differences were found among both cultivars and between plants grown at different fertilizer doses for mineral content and composition (Table 2). Three cultivars were shown in this study to be more mineral dense than the other evaluated cultivars: Ingwizabukungu being high in Fe, Mg, P, and Zn, Jyambere being high in Ca, Fe, K, Mg, P, and S, and Nemeyubutaka being high in Fe, Mg, Mn, P, and Zn. The moderate fertilizer dose resulted in higher contents of Fe, Ca, and Ba in rice grains than the high fertilizer dose, while contents of Zn, K, P, Na, and S were significantly higher in grains of plants fertilized with the high dose (Table 2).

3.1.2. Heavy Metal Content. Mean values for each of the heavy metals analysed, for each of the cultivars, and at each of the fertilizer doses are available in the supplementary data (Table S2). Significant differences were recorded among cultivars and fertilizer doses for heavy metal content in the grains (Table 3). Low contents of heavy metals were found in the cultivars Mpembuke (especially of Cd and Cr) and Ndamirabahinzi (especially of As and Co), while high contents were found in the cultivars Ingwizabukungu (Co and Cr) and Jyambere (As and Cd). The high fertilizer dose resulted in higher contents of Cd and Pb in the rice grains than the moderate fertilizer dose (Table 3).

3.1.3. Bioactive Compounds in Grains. Mean values for total phenolic content (TPC) and total antioxidant capacity (TAC), for each of the cultivars, and at each of the fertilizer doses are available in the supplementary data (Table S3). Significant differences were observed between cultivars for TPC and TAC (Table 4). A strong positive correlation ($P \leq 0.001$) was found between TPC and TAC. The cultivar Ndamirabahinzi had the highest TPC and TAC.

Table 3: Heavy metal content (mg·kg^{-1} dry weight) (10) in Rwandan rice cultivars.

Cultivar	As	Cd	Co	Cr
Ingwizabukungu	0.006 ± 0.0001bc	0.013 ± 0.0011ab	0.003 ± 0.0001a	0.060 ± 0.006a
Intsindagirabigega*	0.008 ± 0.0000	0.005 ± 0.0000	0.001 ± 0.0000	0.038 ± 0.004
Jyambere	0.010 ± 0.0001a	0.017 ± 0.0020a	0.002 ± 0.0000ab	0.034 ± 0.002ab
Mpembuke	0.005 ± 0.0004bc	0.003 ± 0.0004c	0.002 ± 0.0004ab	0.021 ± 0.001b
Ndamirabahinzi	0.004 ± 0.0000c	0.005 ± 0.0008bc	0.001 ± 0.0000b	0.030 ± 0.004ab
Nemeyubutaka	0.008 ± 0.0005b	0.012 ± 0.0011ab	0.002 ± 0.0001ab	0.040 ± 0.002ab
Zong geng*	0.010 ± 0.0000	0.003 ± 0.0000	0.001 ± 0.0000	0.023 ± 0.001
Fertilizer dose				
High	0.006 ± 0.0003a	0.012 ± 0.0012a	0.002 ± 0.0001a	0.035 ± 0.0002a
Moderate	0.007 ± 0.0003a	0.008 ± 0.0006b	0.002 ± 0.0001a	0.039 ± 0.0003a
Cultivar	*Ni*	*Pb*	*Se*	
Ingwizabukungu	0.18 ± 0.021a	0.016 ± 0.0017a	0.003 ± 0.0003a	
Intsindagirabigega*	0.20 ± 0.002	0.008 ± 0.0002	0.002 ± 0.0000	
Jyambere	0.17 ± 0.016a	0.016 ± 0.0028a	0.002 ± 0.0000a	
Mpembuke	0.14 ± 0.025a	0.007 ± 0.0009a	0.002 ± 0.0001a	
Ndamirabahinzi	0.17 ± 0.022a	0.009 ± 0.0008a	0.002 ± 0.0002a	
Nemeyubutaka	0.12 ± 0.012a	0.012 ± 0.0016a	0.002 ± 0.0001a	
Zong geng*	0.11 ± 0.002	0.006 ± 0.0001	0.002 ± 0.0000	
Fertilizer dose				
Moderate	0.16 ± 0.010a	0.008 ± 0.0011b	0.002 ± 0.0000a	
High	0.15 ± 0.010a	0.015 ± 0.0006a	0.002 ± 0.00001a	

Means and standard errors per cultivar are the average at both fertilizer doses; means and standard errors per dose are the average for all cultivars. Means followed by the same letter within a column are not significantly different between cultivars or between doses according to Tukey's test at $P \le 0.05$. *Mean data only for moderate fertilizer dose

Table 4: Total phenolic content (TPC) and total antioxidant capacity (TAC) in Rwandan rice cultivars.

Cultivar	TPC (GAE.100 g^{-1} DW) (10^2)	TAC (μmol Fe^{2+} g^{-1} DW) (10^2)
Ingwizabukungu	1.8 ± 0.06c	0.024 ± 0.000c
Intsindagirabigega*	2.2 ± 0.15	0.059 ± 0.000
Jyambere	2.0 ± 0.17c	0.040 ± 0.007c
Mpembuke	3.6 ± 0.21b	0.327 ± 0.008b
Ndamirabahinzi	5.8 ± 0.35a	0.561 ± 0.007a
Nemeyubutaka	1.9 ± 0.09c	0.084 ± 0.003c
Zong geng*	2.4 ± 0.16	0.118 ± 0.002
Fertilizer dose		
High	3.0 ± 0.45a	0.208 ± 0.010a
Moderate	3.0 ± 0.28a	0.206 ± 0.008a

Means and standard errors per cultivar are the average at both fertilizer doses; mean and standard error per dose are the average for all cultivars. Means followed by the same letter within a column are not significantly different between cultivars or between doses according to Tukey's test at $P \le 0.05$. *Mean data only for moderate fertilizer dose.

3.1.4. Amount and Size Distribution of Polymeric and Monomeric Protein. Figure 1 shows representative SE-HPLC chromatograms from the cultivar Mpembuke, which are subdivided into five fractions based on retention time. Protein fractions eluting fast (elution time < 17 minutes; peak 1, 2, and 3) were designated as high molecular weight proteins while those with slow elution (elution time > 17 minutes; peak 4 and 5) were designated as low molecular weight proteins. Mean values for the different analysed protein fractions (see Materials and Methods for description), for each of the cultivars, and at each of the fertilizer doses are available in the supplementary data (Table S4). Significant differences were found among the cultivars for solubility, %UPP, and total extractable proteins, whereas percentage of polymeric proteins was not found to differ significantly (Table 5). High protein solubility was recorded for Jyambere and Mpembuke, while Ingwizabukungu, Ndamirabahinzi, and Nemeyubutaka showed high %UPP. In all cultivars, the percentage of total extractable proteins was higher for the slow-eluting fraction (peak 4 and 5). This suggests that the largest proportion (>50%) of proteins in these cultivars is of low molecular weight.

3.1.5. Yield-Related Traits and Grain Yield. Mean values for yield and related traits, for each of the cultivars, and at each of the fertilizer doses are available in the supplementary data (Table S5). Significant differences were recorded among the cultivars for all characters except fertile tillers plant^{-1} (Table 6). The cultivar "Jyambere" had the highest yield among the cultivars and also showed a high number of tillers

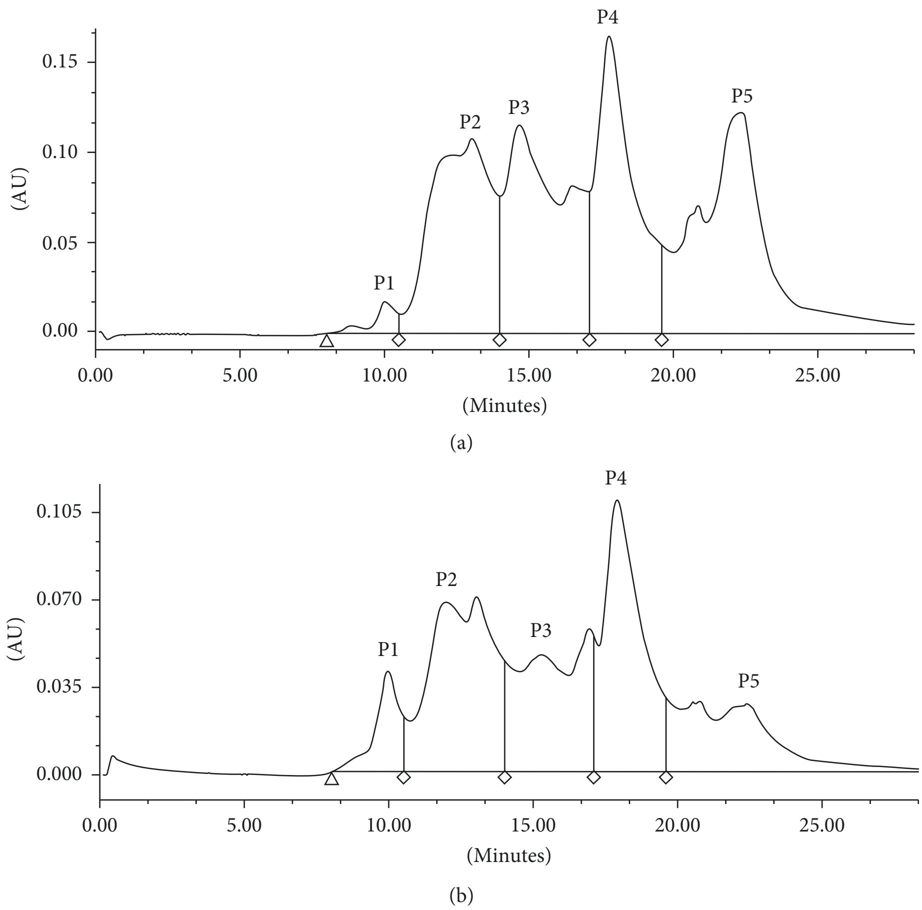

Figure 1: The SE-HPLC profile of the protein content in Mpembuke grain: first extraction (a) and second extraction (b). P1–P5 shows the different peaks referred to in Table 6 and in the text.

plant^{-1} and fertile tillers, long spikes, and the highest number of spikelets spike^{-1}. The cultivar "Zong geng" had tall plants (139 cm on average) but a low number of tillers plant^{-1}. Significant differences in characters were also observed between the two fertilizer doses, except for the number of tillers plant^{-1} and spike length (Table 6). The high fertilizer dose (0.255 g·plant^{-1}) resulted in taller plants as compared to the low fertilizer dose (0.127 g·plant^{-1}). A higher number of productive tillers plant^{-1}, higher number of spikelets spike^{-1}, and higher grain yield were noted at the moderate dose. Extrapolated to yield ha^{-1}, the grain yield varied between 5.2 for Mpembuke and Ingwizabukungu and 14.5 t·ha $^{-1}$ for Jyambere. However, the results in the biotron may largely differ from the grain yield in the field because there are many environmental factors interacting with the treatments under studies and may cause great yield variations in the field.

3.1.6. Principal Components Analysis between Grain Yield, Its Components, and Nutritional Content. The PCA showed that PC1 (explaining 32% of the variation) values increased and PC2 (explaining 19.9% of the variation) values decreased with the increased fertilizer dose for all evaluated cultivars (Figure 2), with the largest change for Jyambere and the least change for Ndamirabahinzi. Thus, the cultivar Ndamirabahinzi showed the highest stability for all evaluated characters combined over the fertilizer doses applied. Furthermore, the high values of yield components (including grain yield) and Fe attributed to the cultivar Jyambere (Tables 2 and 3) could mainly be annotated to the moderate fertilizer dose (these parameters are found with positive PC2 values, as is Jyambere with the moderate fertilizer dose). Similarly, the high values of Ca, K, Na, P, S, and Cd of the same cultivar (Tables 3 and 4) could mainly be annotated to the high fertilizer dose (Figure 2). The cultivars Ingwizabukungu and Nemeyubutaka with moderate fertilizer dose were shown by the PCA to combine in the best way high yield with high Fe and Zn content, although showing low levels of bioactive components (Figure 2).

3.2. Discussion. To our knowledge, our study is the first to characterize the Rwandan rice cultivars for the combination of their grain yield attributes their nutritional value of minerals and bioactive compounds as well as their dough mixing properties. So far, efforts in rice production in Rwanda have been focused on improving the productivity level and postharvest processing [47]. This study clearly grouped Rwandan-grown cultivars into two nutritionally distinct clusters: a group of bioactive compound-rich

Table 5: Amount and size distribution of polymeric and monomeric protein in Rwandan rice cultivars.

Cultivar	Polymeric protein (%)	Solubility (%)	%UPP
Ingwizabukungu	7.1 ± 2.8a	50.2 ± 2.4c	66.3 ± 0.04ab
Intsindagirabigega*	4.9 ± 0.0	56.5 ± 1.1	57.9 ± 0.01
Jyambere	3.1 ± 0.3a	61.9 ± 1.6a	56.8 ± 0.04b
Mpembuke	3.3 ± 0.2a	64.0 ± 2.1a	44.0 ± 0.02c
Ndamirabahinzi	4.2 ± 1.0a	59.7 ± 3.0ab	69.2 ± 0.04a
Nemeyubutaka	4.3 ± 0.4a	53.9 ± 2.4bc	67.2 ± 0.02ab
Zong geng*	4.9 ± 0.0	57.3 ± 3.9	61.6 ± 0.04
Fertilizer dose			
High	3.5 ± 0.2a	59.1 ± 1.6a	63.2 ± 0.03a
Moderate	5.4 ± 1.2a	56.7 ± 2.2a	58.2 ± 0.04a

Cultivar	*% total extractable proteins*				
	Peak1	*Peak2*	*Peak3*	*Peak4*	*Peak5*
Ingwizabukungu	6.9 ± 2.7a	16.2 ± 0.8bc	16.8 ± 0.4b	27.9 ± 1.2a	32.1 ± 2.0b
Intsindagirabigega*	4.8 ± 0.2	24.7 ± 0.6	20.8 ± 0.2	26.2 ± 0.8	23.5 ± 0.5
Jyambere	3.0 ± 0.3a	20.5 ± 1.4ab	18.1 ± 1.2b	25.8 ± 0.2ab	32.6 ± 2.8b
Mpembuke	3.2 ± 0.2a	23.1 ± 0.8a	23.2 ± 0.9a	23.2 ± 0.2bc	27.2 ± 3.4b
Ndamirabahinzi	4.1 ± 0.9a	15.6 ± 2.4c	17.2 ± 1.5b	22.0 ± 1.4c	41.1 ± 3.5a
Nemeyubutaka	4.2 ± 0.4a	19.8 ± 1.0abc	17.8 ± 0.4b	24.4 ± 0.6abc	33.7 ± 2.0ab
Zong geng*	4.8 ± 0.1	16.5 ± 0.6	15.4 ± 1.3	19.3 ± 0.5	44.0 ± 4.2
Fertilizer dose					
High	3.4 ± 0.2a	18.5 ± 1.1a	18.0 ± 0.9a	24.9 ± 0.9a	35.3 ± 1.2a
Moderate	5.2 ± 1.1a	19.7 ± 1.1a	19.3 ± 0.7a	24.5 ± 0.8a	31.4 ± 2.5a

Mean and standard error per cultivar are the average of both fertilizer doses; mean and standard error per dose are the average of all cultivars Means followed by the same letter within a column are not significantly different between cultivars or between doses according to Tukey's test at $P \leq 0.05$. *Mean data only for moderate fertilizer dose.

Table 6: Yield and yield component variation between rice cultivars and fertilizer doses.

Cultivar	Plant height (cm)	Tillers plant^{-1}	Fertile tillers	Spike length (cm)	Spikelets spike^{-1}	Yield (g) plant^{-1}
Ingwizabukungu	108.4 ± 1.7d	3.4 ± 0.3ab	2.3 ± 0.1a	18.2 ± 0.8b	8.0 ± 0.5c	6.2 ± 0.5b
Intsindagirabigega	120.7 ± 5.0c	4.3 ± 0.4ab	3.7 ± 0.4*	24.8 ± 0.8*	11.3 ± 0.7*	14.0 ± 2.9*
Jyambere	117.1 ± 0.9c	4.4 ± 0.2ab	3.6 ± 0.2a	26.2 ± 0.5a	13.1 ± 0.5a	17.4 ± 1.3a
Mpembuke	132.0 ± 0.5abc	4.0 ± 0.1ab	2.7 ± 0.2a	20.5 ± 0.5b	7.6 ± 0.3c	6.2 ± 0.2b
Ndamirabahinzi	132.5 ± 1.3ab	5.2 ± 0.2a	3.0 ± 0.2a	27.7 ± 0.9a	10.1 ± 0.5b	7.4 ± 0.5b
Nemeyubutaka	122.0 ± 0.8bc	3.6 ± 0.2ab	2.6 ± 0.1a	19.3 ± 0.8b	8.7 ± 0.3c	9.3 ± 0.3b
Zong geng	139.0 ± 0.0a	3.3 ± 0.0b	2.0 ± 0.0*	20.0 ± 0.0*	13.0 ± 0.0*	12.7 ± 0.0*
Fertilizer dose						
High	127.0 ± 1.7a	4.4 ± 0.2a	2.1 ± 0.1b	24.6 ± 0.8a	8.0 ± 0.3b	6.3 ± 0.6b
Moderate	122.8 ± 1.3b	3.9 ± 0.1a	3.2 ± 0.1a	22.8 ± 0.6a	10.0 ± 0.3a	11.3 ± 0.8a

Means and standard errors per cultivar are the average at both fertilizer doses; means and standard errors per dose are the average for all cultivars. Means followed by the same letter within a column are not significantly different between cultivars or between doses according to Tukey's test at $P \leq 0.05$. *Mean data only for moderate fertilizer dose

cultivars formed by Ndamirabahinzi and Mpembuke, while Ingwizabukungu, Jyambere, and Nemeyubutaka were more preeminent in mineral elements. The cultivar Ingwizabukungu, Ndamirabahinzi, and Nemeyubutaka exhibited high %UPP, thus indicating high dough mixing strength.

The most mineral dense cultivars Ingwizabukungu, Jyambere, and Nemeyubutaka showed mineral levels similar to or higher than those reported by previous research [48–50]. Despite the relatively high mineral content in these cultivars, the content of Ca, Zn, and Fe in 100 g DW rice was below the content recommended as daily intake [51]. Mineral content in rice cultivars of the present study correlated positively with heavy metal content in these cultivars, meaning that mineral dense cultivars were also the most heavy metal dense cultivars. However, none of the evaluated cultivars showed levels above the maximum tolerable limit for humans [52]. Furthermore, the content of heavy metals in the rice grains was similar to/or lower than contents reported by previous research [25, 53–55].

In the present study, variation in phytochemical compounds was measured as content of TPC and TAC, being examples of quick and cheap methods being able to characterize such variations. To understand the full variation in phytochemicals in the evaluated rice cultivars, more sophisticated and expensive HPLC methods are a requirement. Our study confirmed the existence of a strong positive relation between phenolic compounds and antioxidant capacity, also reported by other researches [56, 57]. Bergman and Goffman [58] argued that phenolic compounds are the main factors of the antioxidant activity of rice grains. In fact,

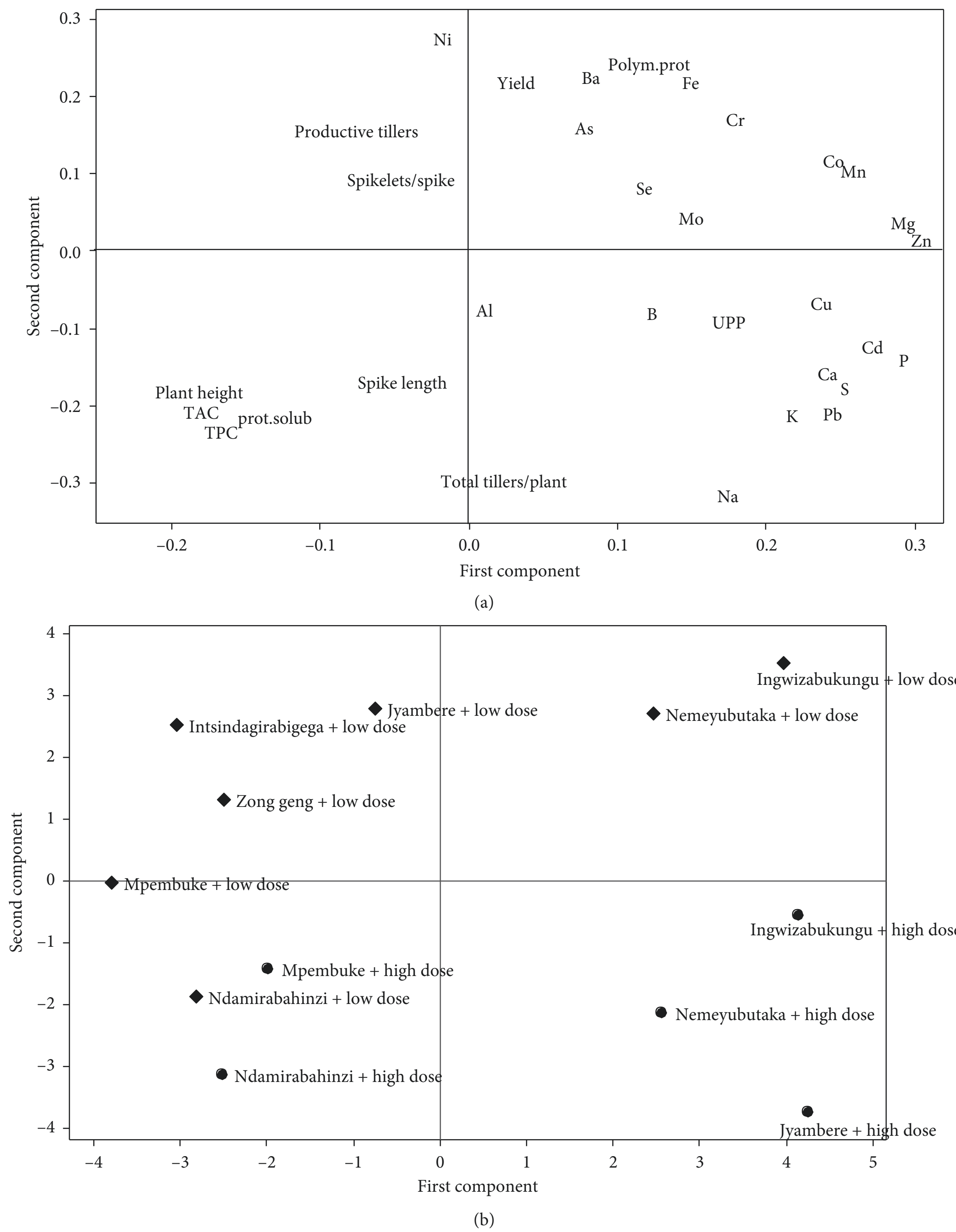

Figure 2: Loading (a) and score (b) plot from principal components analysis of grain yield, its components, mineral elements, total phenolic content, total antioxidant capacity, polymeric protein, protein solubility, and UPP. First principal component explained 32% of the variation while the second principal component explained 19.9% of the variation.

the chemical structure of both polyphenols [59] and their metal chelation potential [60] makes them powerful antioxidants. Moreover, some phenolic compounds play a role in stimulating antioxidant enzymes [61] or inducing antioxidant protein synthesis [62]. Shao et al. [63] hypothesized that the strong correlation between bioactive compounds may result from pleiotropy or genetic linkage between these traits. Phenolic compounds have been reported to play a role in plant defence and to have human health promoting benefits such as prevention of cardiovascular diseases and cancer [64]. The high content of TPC and TAC in the cultivar Ndamirabahinzi makes this cultivar a good candidate to be used in breeding for increased content of bioactive compounds in rice.

Rwandan cultivars exhibited a high proportion of low molecular weight proteins which is attributable to the evaluation of whole rice grain in this study. According to Van der Borght et al. [65], the fast eluting fraction contains α- and β-glutelin subunits, which are contributing to the mixing properties of the rice flour. The studied cultivars showed variation in solubility of the proteins and in the content of %UPP indicating differences in processing properties of the cultivars [11]. The slower eluting fraction in the SE-HPLC-based method is known to contain monomeric albumins, globulins, and prolamins, also being more soluble than the glutelin proteins [11]. The glutelins and prolamins are present in the endosperm of the rice seed while the albumins and globulins are dominating in the aleurone and the embryo, and as the latter are also richer in lysine, they are more nutritionally valuable than the glutelins and prolamins [66]. However, the aleurone and the embryo are removed by polishing. Thus, brown rice has the advantage over white rice for available nutrients in the grain especially those located in the aleurone and the embryo [67].

The moderate fertilizer dose was optimal for combining opportunities to produce high-grain yield and of nutritional quality for all cultivars, although the effect was more pronounced in some cultivars. Yu et al. [68] observed that grain content of N, P, and K increased with an increasing nitrogen supply up to 270 $kg{\cdot}hm^{-2}$ but decreased above this dose. Furthermore, excessive nitrogen fertilization was associated with a reduction in antioxidant capacity in wheat grains [69]. Nguyen and Niemeyer [70] reported a reduction of phenolic acids content and antioxidant capacity in basil at a high rate of nitrogen fertilizer. An increase in total phenolic content was observed with an increased dose of K fertilization but not with an increased dose of N fertilizer in Ziziphus jujube and apricot fruit [71, 72].

In the present study, the rice cultivation was carried out in a biotron, and despite mimicking the Rwandan climate, it is well known that results from a biotron are not fully comparable with those that will be obtained by field cultivation. Controlled growth in a climate chamber differs towards field cultivations in, for example, space in the soil for the roots, soil microbiota, and in abiotic and biotic stresses available in the field not present in the climate chamber. However, the present study presents the first characterization of Rwandan rice cultivars and their combined variation in grain yield components and nutritional quality. Thus, the results from this study may serve as a basis for selection and breeding of rice cultivars for increasing both grain yield and nutritional quality, and for further field selections within the material.

4. Conclusion

Potential uses in rice breeding for different purposes and end-uses varied among Rwandan rice cultivars. Generally, the low fertilizer dose was favourable for production of the majority of the rice cultivars in the biotron. Ndamirabahinzi may be included in crossbreeding for high phenolic content and antioxidant capacity, whereas Jyambere, Nemeyubutaka and Ingwizabukungu may be considered for the combined improvement of mineral element content and grain yield. Special care should be taken, however, to increase micronutrient content such as Zn and Fe in Rwandan rice cultivars.

Data Availability

The authors will make the raw data available upon request, which should be addressed to the corresponding author.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

Lund University is recognised for its collaboration in analysing mineral elements and heavy metals. The authors' sincere gratitude goes to Maria Luisa Prieto-Linde (SLU) for her technical assistance. This research was funded by the Swedish International Development Cooperation Agency (SIDA) through research program cooperation between University of Rwanda and SLU.

Supplementary Materials

This supplementary material presents the "raw" data as mean values of each of the characters analysed to allow interested readers the opportunity to compare data in between fertilizer levels for each of the cultivars separately. (*Supplementary Materials*)

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