Grain Yield and Quality Traits of Rice (*Oryza sativa* L.) Cultivars under Intermittent Drought and Contrasting Temperatures

Physiological, Biochemical and Molecular Characterisation

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Cover: Rice plants surviving drought treatment during tillering and reproductive stages.
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Abstract

Rice (*Oryza sativa*) has evolved in a semi-aquatic tropical environment and therefore thrives better in a water intensive system and optimal temperature between 25 and 30°C. With the future climate change, frequent droughts and heat stress are predicted to happen simultaneously in field but little is known about their combined effects on rice productivity and quality. This thesis investigated the effects of high temperature combined with recurring drought at different stages of plant development and identified quantitative trait loci associated with drought tolerance. Drought sensitivity, growth characteristics, grain yield and its component traits, and grain quality traits were also evaluated in seven rice cultivars grown in Rwanda.

The results showed that grain yield was strongly influenced by the number of panicles per plant and spikelet fertility. A combination of high temperature and repeated drought at seedling and tillering stages dramatically limited panicle development, whereas spikelet fertility was negatively affected by drought at the reproductive stage. Grain yield was reduced by 30% to 100% as a result of drought and temperature stress as compared with well-watered control conditions.

Quantitative trait loci (QTL) for number of panicles per plant were found on chromosomes 1, 4, and 8 whereas QTL for spikelet fertility were located on chromosomes 1, 5 and 9. Four QTL each were found for grain yield after drought at seedling stage (located on chromosomes 1, 4, 7, 9) and for grain yield after drought at tillering stage (located on chromosomes 6, 8, 11, and 12). These QTL could be introgressed into elite cultivars for improvement of their adaptation to intermittent drought.

The cultivars displayed genetic diversity in their tolerance to stresses and quality characteristics. 'Intsindagirabigega' and 'Jyambere' were adapted to high temperature site conditions. 'Intsindagirabigega' had the highest amylose content. 'Ndamirabahinzi' and 'Mpembuke' were rich in total phenolic and total antioxidant capacity, and displayed a beneficial stress memory. 'Jyambere' had high total protein content and high content in mineral elements together with 'Nemeyubutaka' and 'Ingwizabukungu'.

The results in this thesis indicate potential for developing resilient, high-yielding and nutritionally rich rice cultivars that can be grown in stressful environments.

Keywords: Oryza sativa, cultivar, drought, quality, quantitative trait loci, Rwanda, temperature, yield

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Avkastning och Kvalitativa Egenskaper hos Rissorter (*Oryza sativa* L.) Odlade under Återkommande Torkperioder och Vid Olika Temperaturer. Fysiologisk, Biokemisk och Molekylär Karakterisering.

Sammanfattning

Ris (*Oryza sativa*) utvecklades i semi-akvatiska, tropiska miljöer och trivs följaktligen bäst i vattenintensiva system med en temperatur är mellan 25 och 30^{o}C. Den nuvarande klimatförändringen förväntas leda till frekventa perioder av torka och höga temperaturer, men vi vet mycket lite om hur dessa faktorer i kombination påverkar produktivitet och kvalitet hos ris. I denna avhandling undersöktes effekterna av hög temperatur i kombination med återkommande torka på risplantan under dess olika utvecklingsstadier. Vidare identifierades ’quatititative trait loci’ kopplade till torktolerans. Torkkänslighet, tillväxtparametrar, avkastning och egenskaper associerade med avkastning, samt kvalitetsegenskaper hos riskornet utvärderades för sju rissorter som odlas i Rwanda.

Resultaten visar att avkastningen kraftigt påverkades av antalet vippor per planta och småaxens förmåga att sätta frö. Kombinationen hög temperatur och upprepade torkperioder på fröplantsstadiet och på det stadium då plantan sätter rotskott begränsade utvecklingen av vippor medan småaxens förmåga att sätta frön påverkades negativt av torka under reproduktionsstadiet. I jämförelse med välvattnade plantor sjönk avkastningen med 30 till 100% för plantor utsatta för torka och temperaturstress.

Quantitative trait loci (QTL) för antal vippor per planta identifierades på kromosom 1, 4 och 8, medan QTLs för småaxens förmåga att sätta frö var lokaliserade på kromosom 1, 5 och 9. Fyra QTLs korrelerade med avkastning vid torka på fröplantestadiet (lokaliserade på kromosom 1, 4, 7, 9) och fyra med avkastning vid torkstress under det stadium då plantan sätter rotskott (lokaliserade på kromosom 6, 8, 11, 12). Dessa QTLs skulle kunna föras in i elitsorter för att öka avkastningen vid upprepade perioder av torka under de vegetativa och reproduktiva utvecklingsstadierna.

Det fanns genetiska skillnader mellan de studerade sorterna vad gäller stresstolerans och kvalitativa egenskaper. "Intsindagirabigega" och "Jyambere" var mer anpassade för odling i hög temperatur. "Intsindagirabigega" hade det högsta amylosinnehållet. "Ndamirabahinzi" och "Mpembuke" hade högt fenolinnehåll och hög antioxidativ kapacitet, och uppvisade ett fördelaktigt stressminne. "Jyambere", "Nemeyubutaka" och "Ingwizabukungu" innehöll höga halter protein och mineralämnen.

Resultaten från denna avhandling visar på möjligheter att utveckla motståndskraftiga, högavkastande och näringsrika sorter som kan odlas i miljöer där ris utsätts för olika typer av stress.

Dedication

To my husband Celestin Habiyambere

Blessed are those who find wisdom, those who gain understanding
Proverbs 3:13

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Mukamuhirwa, A*., Hovmalm, H.P., Ortiz, R., Nyamangyoku, O., Johansson, E. (2018). Quality and grain yield attributes of Rwandan rice (*Oryza sativa* L.) grown in a biotron applying two NPK levels. *Journal of Food Quality*, 2018, doi.org/10.1155/2018/5134569

II Mukamuhirwa, A*., Hovmalm, H.P., Bolinsson, H., Ortiz, R., Nyamangyoku, O., Johansson, E. (2019). Concurrent drought and temperature stress in rice—a possible result of the predicted climate change: effects on yield attributes, eating characteristics, and health promoting compounds. *International Journal of Environmental Research and Public Health* 16, 1043; doi:10.3390/ijerph16061043

III Mukamuhirwa, A*., Hovmalm, H.P., Ortiz, R., Nyamangyoku, O., Prieto–Linde, M.L., Ekholm, A., Johansson, E. Effect of intermittent drought on grain yield and quality of rice (*Oryza sativa* L.) grown in Rwanda (submitted)

IV Mukamuhirwa, A*., Hovmalm, H.P., Ortiz, R., Nyamangyoku, O., Saripella, G.V., Chawade, A., Johansson, E. Quantitative trait loci for grain yield and its components under drought at different development stages of rice (*Oryza sativa* L.) (manuscript).

* Corresponding author.

The contribution of Alphonsine Mukamuhirwa to the papers included in this thesis was as follows:

I Planned and performed the experimental work, analysed the data and wrote the manuscript with the co-authors,

II Planned and performed the experimental work, analysed the data and wrote the manuscript with the co-authors,

III Planned and performed the experimental work, analysed the data and wrote the manuscript with the co-authors,

IV Planned and performed the experimental work, analysed the data and wrote the manuscript with the co-authors.

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Abbreviations

AmC	Amylose content
ATP	Adenosine triphosphate
CTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribonucleic acid
D0	No drought treatment
DR	Drought treatment during reproductive stage
DS	Drought treatment during seedling stage
DSR	Drought treatment during seedling and reproductive stage
DST	Drought treatment during seedling and tillering stage
DSTR	Drought treatment during seedling, tillering and reproductive stages
DT	Drought treatment during tillering stage
DTR	Drought treatment during tillering and reproductive stage
GAE	Gallic acid equivalent
GC	Gel consistency
GLM	General linear model
GT	Gelatinisation temperature
PCA	Principal component analysis
QTL	Quantitative trait loci
SE-HPLC	Size exclusion high performance liquid chromatography
SNP	Single nucleotide polymorphism
TAC	Total antioxidant capacity
TPC	Total phenolic content

1 Introduction

1.1 Taxonomy, agronomy and importance of rice

Rice belongs to the genus *Oryza* in the Gramineae family (which includes all cereal crops) and the genus comprises 22 species whose only two are cultivated (Ge *et al.*, 1999). The species currently cultivated worldwide, *Oryza sativa* evolved from *O. rufipogon* around 9000 years ago at a site close to the Yangtze valley of China and then split into the *Indica* and *Japonica* groups around 4000 years ago (New York University, 2011). The other cultivated species of rice, *O. glaberrima*, evolved in the Niger river basin from *O. barthii* 2000-3000 years ago and remained confined to West Africa (Linares, 2002).

Although rice grows in diverse environments, half of the land devoted to rice cultivation is irrigated, while lowland and upland rainfed systems occupy 25% and 13% of the cultivated area, respectively, and the remaining cultivation is in deep water ecosystems. Irrigated rice ecosystems are well managed and provide high yields (normally >5 t ha^{-1}), thus contributing 75% of total rice production, whereas rainfed ecosystems are vulnerable to submergence and frequent droughts and yield only 1-2.5 t ha^{-1} (Seck *et al.*, 2012).

Rice contributes 30% of total global cereal production and is a staple food for about half the world's population (Gnanamanickam, 2009). In particular, rice provides 35-59% of energy and 51.4-69.2% of protein in the diet of South Asian populations, where rice is produced and consumed to a higher extent than elsewhere (Juliano, 1993). The nutrient composition of rice is largely dependent on the cultivar, soil properties, fertilisation regime, and environmental conditions during the cropping period (Seck *et al.*, 2012). In general, rice grains contain 80% starch, 12% water, 7.5% protein, and 0.5% ash (Chandler, 1979). Moreover, rice grains are rich in vitamins such as B, E, riboflavin, thiamine and niacin, but have a low content of vitamins A, C and D (Juliano, 1993).

1.2 Effects of drought and extreme temperature stress on yield and quality of rice

Climate change is impacting the Earth's surface and atmosphere, causing a reduction in rainfall and elevated temperatures (Lamaoui *et al.*, 2018) as well as increasing unpredictability and frequency of extreme weather events (Wassmann *et al.*, 2009). Extreme weather events negatively affect plant physiology and productivity to a higher degree than gradual increases in drought and temperatures (Easterling *et al.*, 2000) because the stress severity goes abruptly beyond the physiological limits of plant adaptation (Gutschick & Bassirirad, 2003). Drought affects crop productivity when soil water content becomes insufficient to satisfy crop requirements and thus impede crop yield (Serraj et *al.*, 2009). Drought stress affects all plants although the magnitude of damage varies between species and between cultivars of the same species (Das & Uchimiya, 2002). Because of its phylogenetic origin as a semi-aquatic plant (Das and Uchimiya, 2002), the development and productivity of rice are highly water-demanding. On average, 2.500 L of water (2–3 times the quantity required by wheat or maize) are needed to produce 1 kg of paddy rice (Bouman, 2009). Rice is therefore highly vulnerable to limited water availability.

During drought, alteration of mitosis and meiosis limits cell elongation and expansion and hinders the development of reproductive cells resulting in reduced growth and altering grain yield-related traits (Pandey & Shukla, 2015, Li et *al.*, 2015). Drought can negatively affect crop yield at any developmental stage. For example, drought during the vegetative stage hinders plant development by limiting photosynthetic activities and accumulation of assimilates (Okami *et al.*, 2015). In fact, reduced relative water content causes a decrease in leaf water potential, affects stomatal conductance and transpiration rate and triggers an increase in leaf temperature. Thus, gas exchange is disturbed and photosynthesis - related genes are down-regulated (Fahad *et al.*, 2017). Moreover, leaf rolling and leaf senescence caused by drought reduce leaf size, limit carbon dioxide (CO_2) availability and induce loss of photosynthetic pigments (Serraj *et al.*, 2009). Adenosine triphosphate (ATP) synthesis and phosphorylation processes decline under drought stress (Fahad *et al.*, 2017). Furthermore, under drought stress conditions the uptake and movement of minerals decrease, limiting plant nutrition and development (Pandey & Shukla, 2015). Drought stress results in a reduction in dry weight accumulation in all organs of the plant (Kamoshita *et al.*, 2008). Under drought or heat, reduction and oxidation (redox) homeostasis is disturbed by the accumulation of reactive oxygen species (ROS) to a higher level than the antioxidants, thus causing oxidative stress (Gill &Tuteja, 2010).

Similarly to drought stress, high temperatures impede growth and development of rice plants by inhibiting photosynthetic activities, with reduced chlorophyll pigments as a consequence, and blocking circulation of assimilates (Lamaoui *et al.*, 2018). Protein degradation, ion leakage from their binding proteins and limitation of electron transport are consistently reported in plants stressed by drought or heat (Zandalinas *et al*., 2018). The synthesis of heat shock proteins is enhanced, at the expense of normal protein production (Bray *et al*., 2000). In addition, the production of protective molecules such as phytohormones and antioxidants is accelerated in the presence of heat stress (Maestri *et al*., 2002).

Drought and/or heat at the reproductive stage are detrimental to rice reproductive organs and limit grain yield and yield components (Zu *et al*., 2017). Drought and heat inhibit anther dehiscence, pollen shedding, pollen germination on stigma and fertilisation, as well as pollen tube growth and panicle exertion (Zhang *et al*., 2018), causing high spikelet sterility (Serraj *et al*., 2009). Moreover, high temperature reduces the grain filling period, causing a 10% reduction in rice yield for every 1°C increase above the optimal temperature (Tenorio *et al*., 2013, Peng *et al.*, 2004). Besides reducing rice yield, drought or temperature stress also affects the physical, nutritional and cooking qualities of the grain, such as protein content, amylose content and starch structure (Wassmann *et al*., 2009).

1.3 Mechanisms of plant adaptation to drought and extreme temperatures

Plants have developed different mechanisms to maintain their growth and grain yield under abiotic stress. Drought resistance mechanisms can be divided into (Fang & Xiong, 2015; Shavrukov *et al.*, 2017):

(i) *Drought avoidance*, which is the capacity of plant tissues to maintain a high water content either by root morphological traits making them able to absorb water from soil, or by physiological traits that reduce water losses.

(ii) *Drought tolerance*, which is the capacity of the plant to maintain physiological processes in spite of low leaf water status.

(iii) *Drought recovery*, which is the capacity to resume growth after loss of turgor and leaf desiccation due to severe drought.

(iv) *Drought escape*, which is an adaptive mechanism involving early flowering and a shorter vegetative phase thus allowing cultivars to produce grains before being exposed to terminal drought.

In the presence of heat stress, plants respond by transpiration cooling by stomata opening and water evaporation (Crawford *et al.*, 2012), a process that may accelerate leaf water loss. However, transpiration cooling sustains leaf gas exchange and maintains the photosynthetic process under high temperature (Scafaro *et al.*, 2016). Other tolerance mechanisms such as accumulation of osmo-protectants and antioxidants, are common under both drought (Farooq *et al.*, 2008) and heat stress (Chakraborty & Pradhan, 2011).

1.4 Improving rice for adaptation to drought and extreme temperature

In addition to challenges like water scarcity, an annual yield increase of more than 1.2% is required to feed a growing population (Normile, 2008). So far, rice has been improved for drought resistance either by selecting for grain yield (Raman *et al.*, 2012) or indirectly by selecting for secondary traits (Wu *et al.*, 2011). However, strong genotype by environment (G×E) interactions limit the performance of selected cultivars in adapting to large scale environmental changes. In reality, there are major variations in the intensity and frequency of climate stresses between different environments and seasons. Successful cultivar improvement for such varying weather scenarios would require a complex G×E adaptation (Hammer & Jordan, 2007).

Understanding the basic processes involved in drought resistance and how they are affected by various weather scenarios might be an alternative to overcome the large G×E interactions. Efforts to determine drought resistance; by identification and characterisation of traits that could be transferred through plant breeding into cultivars with high-yielding genetic backgrounds, have had very limited success because of possible links between grain yield and undesirable traits (Kadam *et al.*, 2018). Moreover, in field conditions, drought is often accompanied by other abiotic stresses such as heat and salinity. Multiple stresses may have interactive effects that cannot be deduced from the effects of individual stresses (Zandalinas *et al.*, 2018). Co-occurrence of drought and heat stress is predicted to increase both in intensity and frequency (Wassaman *et al.*, 2009), particularly in the tropics, where the main rice production regions are found.

Although drought and heat stress often occur in combination and each separate stress is known to be very damaging for rice production and quality

(Seneviratne *et al.*, 2010), studies on the interactive effects of both stresses are still rare. Understanding the responses of rice to concurrent drought and heat is crucial for developing cultivation strategies and resilient cultivars that maintain grain yield production and good quality characteristics.

2 Objectives

The overall aim of this thesis was to contribute substantial scientific knowledge to allow a progress towards the development of high yielding and nutritionally rich rice cultivars adapted to rising temperatures and frequent dry spells.

To reach this aim, the specific objectives of the thesis were to:

- Determine and understand the interplay of quality and yield attributes of Rwandan rice cultivars grown in conditions of satisfactory water supply and temperature.
- Asses how high temperature stress in combination with repeated drought treatments during different developmental stages of rice affect plant growth, grain yield, eating and nutritional quality.
- Identify quantitative trait loci (QTL) linked to grain yield traits under drought at different plant developmental stages.
- Define actions and tools for breeding and production of rice in Rwanda despite the predicted climate change scenarios.

3 Methodology

3.1 Plant material

Seven cultivars released by Rwanda Agricultural Board (RAB) – formerly known as ISAR (Institut des Sciences Agronomiques du Rwanda) – were used to determine the grain quality and yield attributes of rice under recurrent drought treatments and high temperature. The seven cultivars are 'Ingwizabukungu', 'Intsindagirabigega', 'Jyambere', 'Mpembuke', 'Ndamirabahinzi', 'Nemeyubutaka' and 'Kigoli'. All the cultivars are *indica* type except 'Kigoli', which is a *japonica* type. The main characteristics of the seven cultivars as described by ISAR (2010) are presented in Table 1 of Paper II.

For quantitative trait loci (QTL) identification, a cross between IR64, known as drought sensitive and 'Moroberrekan', a drought resistant cultivar (Liu et *al*., 2006) was performed at AfricaRice, Tanzania. Twelve F_2 lines obtained from AfricaRice were grown for multiplication in a greenhouse at the Swedish University of Agricultural Sciences (SLU), Alnarp and thereafter the $F_{3:2}$ seeds were grown in open fields in Rwanda. The parental lines of the cross were included in both of the cultivations.

3.2 Experimentation

In order to understand opportunities to contribute to high yielding and nutritional rich rice cultivars with good adaptation to high temperatures and drought conditions, a total of four experiments were conducted.

- The first experiment characterized the Rwandan rice cultivars for their quality characteristics and grain yield (and its components) and established the interplay between grain yield components and quality characteristics (Paper I)

- The second experiment evaluated the effect of concurrent drought and temperature at different growing stages on yield and quality traits of rice cultivars. The rice plants were grown in two biotron chambers differing in temperature range: a low-temperature chamber (22/26 °C day/night temperature) and a high-temperature chamber (27/30 °C day/night temperature; Paper II)
- The third experiment evaluated the Rwandan rice cultivars in field conditions for their responses to intermittent drought at different plant development stages in two sites with contrasting temperatures (Paper III).
- The fourth experiment sought to identify QTL and genes associated with grain yield and its components under repeated drought in a $F_{2:3}$ mapping population obtained from a cross between drought sensitive and drought resistant parents (Paper IV).

Thus, in Papers II, III and IV, the cultivars (Paper II and III) and the mapping population (Paper IV), were evaluated for drought sensitivity and growth parameters during the plant development. Grain yield and its components were evaluated after maturity in all the four papers (I-IV). Cooking and eating qualities of the grain and health-promoting compounds were measured after harvesting, following analytical methods described in Papers I, II and III.

3.2.1 Drought treatments

Drought treatments were applied as single or repeated events at different plant growth stages (Papers II, III and IV). The treatments were: well-watered (no drought, D0) during the whole growing period, a treatment that served as a control; drought at seedling stage (DS); drought at seedling and tillering stages (DST); drought at seedling and reproductive stages (DSR); drought at tillering stage (DT); drought at tillering and reproductive stages (DTR); drought at reproductive stage (DR); and drought at seedling, tillering and reproductive stages (DSTR).

3.2.2 Experimental design

The first experiment was conducted in a factorial design, while the other experiments were laid out in a split-plot design, with drought treatment as main plots and cultivars (Papers II and III) or $F_{2:3}$ population (Paper IV) as subplots.

3.2.3 Data recording in the biotron and in the field

Data were recorded on all plants grown in the biotron (Papers I and II). In experiments in the open field (Paper III and IV), data were recorded for a sample of five plants taken along the diagonals in each subplot. Drought sensitivity (leaf rolling, leaf drying) and plant recovery rate after drought at seedling and tillering stages were scored using the 0-9 scale proposed in the standard evaluation system for rice (IRRI, 2002), where the score 0 means no symptoms of drought sensitivity and 9 means that the cultivar is highly sensitive to drought and has a recovery rate below 20% as presented in Table S3 of Paper III. The number of tillers was counted after drought treatment at seedling and tillering stages. The time to flowering was counted as days from sowing up to 50% of plants flowered. At harvesting time, each plant was individually harvested. The number of panicles per plant, number of spikelets per panicle, panicle length, spikelet sterility and total number of grains per plant were counted at harvesting time. After manual threshing, the grain yield per plant and the 100-grain weight were measured using a precision balance (Paper I-IV).

3.2.4 Analysis of quality characteristics

Preparation of samples

In order to have sufficient samples for milling and to carry out quality characteristics analyses, rice grain samples with the same treatment were pooled, i.e. for the first experiment - from each of the cultivar × fertilizer dose (Paper I), and from the second experiment - cultivar × drought × temperature treatment (Paper II, III). Each of these samples was thereafter individually stored at -20^{o}C. A working sample was thereafter taken from each of these stored samples and freeze-dried for 48 h. After freeze-drying, the whole grains were grinded using an IKA-WERKE grinder type A10, (SKAFTE medlab, Mindelheim, Germany). The flour was stored at -20^{o}C until further analyses. All analyses of quality characteristics were then made in triplicate from the flour.

Mineral and heavy metal content

The mineral and heavy metal concentrations in plant materials (Paper I) were analysed using an ICP-OES, Optima 8300, PerkinElmer at Lund University, following the methods described by Hussain *et al*. (2010) and Hussain *et al*. (2012), respectively.

Amount and size distribution of polymeric and monomeric protein

The quantity and size distribution of polymeric and monomeric proteins (Paper I) were determined by size exclusion high performance liquid chromatography (SE-HPLC) following Johansson *et al*. (2002).

Total protein content

Total protein content (Papers II and III) was determined from total nitrogen content obtained following the combustion method described by McGeehan and Naylor (1988). Total protein content was then calculated from total nitrogen content using a conversion factor of 5.7 (ISO16634 - 2: 2016).

Total antioxidant capacity

Total antioxidant capacity (Paper I-III) was determined following the method of Pérez-Jiménez & Saura-Calixto (2005) with small modifications as described in Paper I.

Total phenolic content

Total phenolic content (Paper I, II) was measured following the Singleton *et al*., (1999) method with small modifications (Paper I).

Amylose content

The amylose content (Papers II and III) was determined using the spectrophotometric iodine method following Hofvander *et al*. (2004), with the exception that the starch was defatted with 95% ethanol according to Perez and Juliano (1978).

Gel consistency

The gel consistency test for eating quality of rice (Cagampang *et al*., 1973) was followed to determine the gel consistency of the rice samples (Paper II).

Gelatinisation temperature

Gelatinisation temperature was determined by differential scanning calorimetry (Paper II; DSC, Seiko 6200), following the method of Gunaratne *et al*. (2011).

4 Results and discussion

Overall, the results obtained in this thesis showed that a combination of drought and extreme temperatures impacted yield components negatively and resulted in a considerable reduction in yield. Thus, the predicted climate change constitutes a clear threat to rice growth and grain yield production. However, the nutritional quality characteristics of the grain were not affected by climate extremes of the present study. This indicates opportunities to produce high quality rice independent of the climate change provided that cultivars with a high nutritional content are selected. The results also showed that genetic variability existed within the rice cultivars evaluated, contributing opportunities for breeding climate tolerant lines. The QTLs identified in this thesis as impacting grain yield after drought treatments may be helpful and used to improve rice adaptation to stressful environment.

4.1 Interplay of quality and yield attributes of rice cultivars grown in conditions of satisfactory water supply and temperature

Under satisfactory water and temperature conditions, significant differences existed between the cultivars for grain yield and yield traits (Paper I) and for quality characteristics (Paper I-III).

The cultivar ‘Jyambere’ had a high number of panicles per plant, long spikes, high number of spikelets per spike and high yield (Paper I). Thereby, ‘Jyambere’ showed the most suitable performance for high yield among the rice cultivars while weather conditions were satisfactory. The cultivar ‘Jyambere’ also showed a high proportion of soluble protein, together with ‘Mpembuke’, whereas ‘Ingwizabukungu’, ‘Ndamirabahinzi’, and ‘Nemeyubutaka’ had a high percentage of total unextractable polymeric proteins (%UPP), *i.e*. high dough mixing strength (Paper I).

Relatively high concentrations of minerals, notably iron (Fe), magnesium (Mg), phosphorus (P) and zinc (Zn), were found in 'Ingwizabukungu', 'Jyambere' and 'Nemeyubutaka' (Paper I). However, despite variations in minerals among the cultivars, none of the cultivars showed high enough iron and zinc contents to meet human requirements for these two elements (Paper I). Thus, there is clearly room for improvements in mineral contents in all rice cultivars evaluated in the present thesis. The cultivar 'Intsindagirabigega' showed high amylose content (Paper II and III) whereas 'Mpembuke' and 'Ndamirabahinzi' showed high total phenolic content (Paper I and II) and total antioxidant capacity (Paper I-III) at satisfactory environmental conditions.

Basically no significant correlations were found between grain yield and quality traits. The only significant correlations obtained were negatively between total antioxidant capacity (TAC) and iron (Fe) content, and positively between content of P and K (Table 1).

Table 1. *Correlation coefficient between grain yield and its components and quality traits in rice grown at satisfactory environmental conditions in the biotron*

	Panicles	Spike length	Spike -lets	Grain yield	Poly prot	% UPP	TAC	Fe	K	Mg	P	Zn
Poly prot	-0.45	-0.50	-0.26	-0.35								
%UPP	-0.21	0.05	0.07	-0.09	0.52							
TAC	-0.01	0.45	-0.24	-0.49	-0.35	0.01						
Fe	0.03	-0.52	0.02	0.43	0.05	-0.21	-0.89*					
K	0.15	0.17	0.24	0.42	-0.30	0.25	-0.21	0.30				
Mg	-0.69	-0.49	0.38	-0.60	0.17	0.26	-0.36	0.26	0.30			
P	0.15	-0.09	-0.31	-0.05	0.03	0.25	-0.29	0.42	0.78*	-0.06		
Zn	-0.03	-0.36	-0.28	-0.07	0.64	0.48	-0.63	0.52	0.35	0.03	0.73	
Amc	0.53	0.17	0.07	0.24	0.32	-0.06	-0.32	0.15	-0.56	-0.55	-0.27	0.21
Tot prot	0.69	0.22	-0.35	0.02	0.02	-0.08	-0.22	0.30	0.13	-0.76	0.57	0.54

*Significant correlation at $P \leq 0.05$.

Previous results have reported positive correlation between amylose content and grain yield traits (Abacar *et al.* 2016), and mineral elements (Jiang *et al.*, 2007) in rice grains. However, the different traits evaluated are mainly the result of different genes. Thus, eventual correlations between different analysed traits will need also explanations as to how the traits are interplaying. Yield is mainly a result of starch accumulation in the grain harvested. Thereby a relationship between yield and amylose content in the grain might be reasonable if amylose is produced in higher amounts than amylopectin when grain starch accumulation is increased. Mechanisms for a simultaneous increase of amylose and minerals are more difficult to explain.

The results of this thesis, indicating mainly no correlation among the evaluated traits, suggest the potential to improve grain quality traits such as amylose content, protein content and mineral content without jeopardising grain yield. Thus, breeding strategies should be undertaken to combine high yield, high amylose content, and good mineral content and high antioxidant capacity.

4.2 Effects of heat and drought on yield and quality

The different experiments of the present thesis clearly showed that the environment, here constituted by temperature (Paper II) and drought (Papers II and III) treatments and site (Paper III) differences, impacted highly on yield while cultivars, here a total of 7 Rwandan cultivars (Papers II and III), mostly determined the quality of rice.

4.2.1 Rice growth and grain yield

Effect of cultivation temperature and drought on a number of different growth and yield components was evaluated in the present thesis (Papers II-IV). Drought treatment, alone or in combination with temperature or cultivar, significantly contributed to variations in the evaluated traits, i.e. in number of panicles per plant, spike length, spikelet fertility, 100-grain weight and grain yield per plant (Papers II-IV).

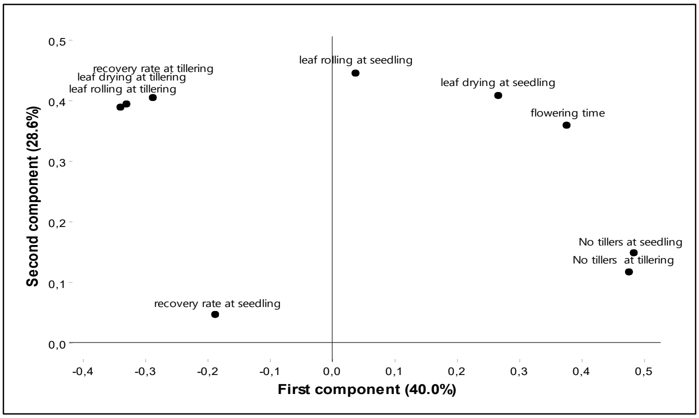
4.2.1.1 Drought sensitivity and recovery rate

Drought treatment, especially repeated drought at seedling and tillering stages, explained most of the variation in leaf rolling and leaf drying (Papers II and III). This is indicated also in Figure 1 with the most positive values on the second principal component for these characters and treatments in the loading and score plot, respectively. A combination of drought and temperature or cultivar also significantly affected drought sensitivity (Papers II and III). Generally, high levels of leaf rolling and leaf drying were obtained when low temperatures were applied during the seedling stage and when high temperatures were applied during the tillering stage. Low temperatures affect photolysis activity and reduce chlorophyll content (Kim *et al.*, 2009). This results in reduced plant growth and weaker competitiveness to stress (Ali *et al.*, 2006), leading to leaf drying and plant death (Biswas *et al.*, 2017) during earlier plant growth. During tillering stage, increased photosynthetic activity (Salam *et al*., 1997) combined with high transpiration (Crawford *et al.*, 2012) at high temperature will result in reduced leaf water content, thus increasing the leaf rolling and drying.

4.2.1.2 Growth parameters

Flowering was delayed by low temperatures and repeated drought treatment (Paper II) as also indicated in Figure 1 with positive values on both first and second principal component in loading and score plot, respectively. Low temperatures affect photolysis activity and reduce chlorophyll content (Kim *et al*., 2009). This results in reduced plant growth and weaker competitiveness to stress (Ali *et al*., 2006), leading to leaf drying and plant death (Biswas *et al*., 2017). However, the results from this thesis showed a higher number of tillers per plant with low temperatures during both seedling and tillering stages (Paper III). Decreasing number of tillers due to high temperatures has been reported previously in rice (Manalo *et al*., 1994). High temperatures speed up plant growth at the expense of assimilates accumulation, and consequently the number of tillers is reduced (Glaubitz *et al.*, 2014). In this thesis, drought sensitivity and plant death increased to a higher extent at high temperatures during tillering stage, as a result of a high requirement for photosynthetic activity.

(1a)



(1b)

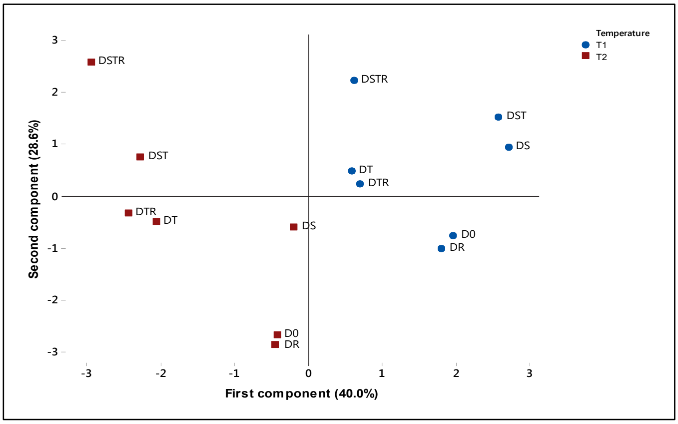


Figure 1. Principal component analysis: (1a) loading plot and (b) score plot of leaf rolling, leaf drying, recovery rate, number (no.) of tillers and time to flowering of drought-stressed rice subjected to low/high temperatures during seedling and/or tillering stages. Key to treatments: D0: well-watered (no drought), DS: drought at seedling stage, DST: drought at seedling and tillering stages, DT: drought at tillering stage, DTR: drought at tillering and reproductive stages, DR: drought at reproductive stage, DSTR: drought at seedling, tillering and reproductive stages. T1: low temperatures, T2: high temperatures.

4.2.1.3 Yield components

The number of panicles per plant was significantly reduced by drought treatment at seedling stage (Paper IV) or at combined drought treatments at seedling and tillering stages (treatments DST and DSTR) and high temperatures (Paper II). This was a result of low tillering capacity and failure of panicle initiation during drought at vegetative stages, which led to a 30% grain yield reduction in the field and to 100% yield loss in the biotron (Papers II and III). These results confirm previous findings of panicle and spikelet development failure under high temperatures (Fu *et al*., 2008, Shi *et al*., 2016).

Although the number of panicles per plant is an important yield attribute (Rajeswari & Nadarajan, 2004), an excessive number of panicles per plant at low growing temperatures at the Tonga site did not translate into increased grain yield, but rather contributed to spikelet sterility (Paper III), as noted elsewhere (Kobata *et al*., 2013). Thus, grain yield was significantly lower at Tonga (60%) than at the Bugarama site (Paper III; Figure 3). Spikelet fertility was significantly affected by a combination of drought treatment at reproductive stage (DR, DTR, DSTR) and low temperatures (Papers II and III) leading to significant grain yield losses in the biotron (Paper II) and, in particular, in the field (Paper III; Figure 2).

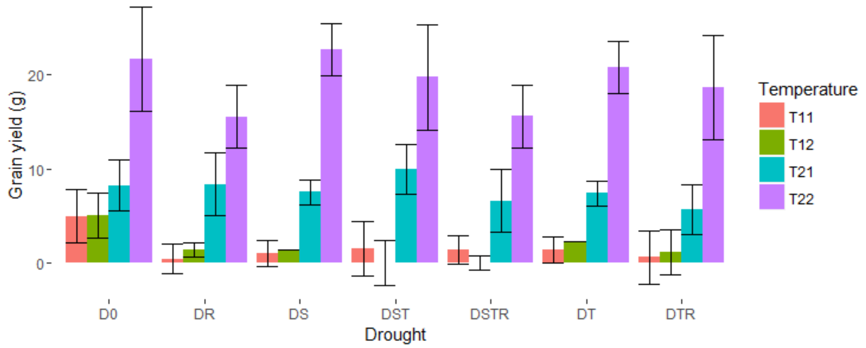


Figure 2. Grain yield per plant under different drought treatments in combination with low/high temperature. Key to treatments: D0: well-watered (no drought), DS: drought at seedling stage, DST: drought at seedling and tillering stages, DT: drought at tillering stage, DTR: drought at tillering and reproductive stages, DR: drought at reproductive stage, DSTR: drought at seedling, tillering and reproductive stages. T11: low temperatures in biotron, T12: high temperature in biotron, T21: Tonga site (low temperatures in the field in Rwanda), T22: Bugarama site (high temperatures in the field in Rwanda). Error bars represent standard deviation of mean values for grain yield.

4.2.2 Quality characteristics

Cooking and eating qualities, and nutritional content, together with the grain appearance, are the main determinants of consumer acceptance and the quality of cooked rice, and therefore the commercial value of rice. The present experiments showed that variation in the evaluated quality characteristics was generally more dependent on cultivar than on temperature or drought treatment.

4.2.2.1 Eating and cooking quality

Starch properties such as amylose content, gelatinisation temperature and gel consistency are major determinants of eating and cooking qualities (ECQs) of rice (He *et al*., 2006). Starch properties evaluated in this thesis were found to be impacted by cultivar and environmental conditions, i.e. drought, temperature and site, as well as their interactions, as revealed by ANOVA (Papers II and III). However, simple regression analyses clearly indicated cultivars contributing a higher proportion of explanation for variation in starch properties of the evaluated rice cultivars than the used environmental influences (Papers II and III). Despite an inconsistent variation in starch properties for each of the cultivars dependent on environmental cultivation conditions (Papers II and III), the cultivar 'Intsindagirabigega' showed high amylose content in both biotron and field conditions and in most temperature, drought and site conditions (Papers II and III). Based on their amylose content, the rice cultivars included in this thesis can generally be classified as having low to intermediate amylose content (10-25%) independent of temperature, drought and site conditions (Papers II and III). This implies that these cultivars are soft and may be sticky (low amylose; Oko *et al*. 2012). However, several of the cultivars showed amylose content of above 30% at specific drought and temperature conditions (Paper III). Previous studies are available indicating an increase in amylose content in rice during drought stress (Emam *et al.* 2014). Although, the results from this thesis did not indicate such a corresponding consistent relationship in rice between any drought or temperature conditions and amylose content (Papers II and III); instead, similar significant cultivar differences in amylose content was obtained in both biotron and field cultivations (Papers II and III). Similarly, consistent differences in gel consistency and gelatinisation temperature were not found among temperature, drought or site conditions (Paper II). Instead significant cultivar differences were obtained also for these starch related properties (Paper II). Gel consistency was high in the cultivars ‘Intsindagirabigega’, ‘Ingwizabukungu’ and ‘Zong geng’ (Paper II), whereas ‘Jyambere’ showed a high gelatinisation temperature (Paper II). Cultivars with a high gelatinisation temperature are known to require more energy for cooking (Oko *et al*., 2012).

4.2.2.2 Total protein

Protein content was mostly cultivar-dependent (Papers I and II), but was also influenced by the environment (Paper III). The cultivar ‘Jyambere’ showed a high content of total protein (Papers II and III).

A combination of drought and high temperature seemed to slightly increase the total protein content in rice grains (Paper III). In previous studies, the genetic composition and environmental factors such as the level and timing of nitrogen application (Kaur *et al*., 2016), drought (Liang *et al*., 2018) and temperature regime (Mitsui *et al*., 2013) have been consistently reported to be factors influencing the total protein content and properties of rice grain. In fact, under different environment stresses, certain proteins are upregulated whereas others are downregulated, thus affecting the content and functionality of proteins (Feki & Brini, 2016, Ke *et al*., 2009).

4.2.2.3 Total phenolics and total antioxidant capacity

The total phenolic content and total antioxidant capacity were influenced by cultivar, environment (temperature, drought and site) and their interaction as shown by ANOVA analyses (Paper III). However, simple regression analyses showed clearly cultivar determining the largest proportion of variation as to both total phenolic content and total antioxidant capacity (Papers II and III). Also, independent of biotron or field cultivation, as well as while grown at satisfactory or drought/temperature stressing conditions, the same cultivars were ranked significantly better or worse than the others (Papers I-III). Thus, the cultivars ‘Mpembuke’ and ‘Ndamirabahinzi’ consistently showed high total phenolic content and antioxidant capacity, while significantly lower values were found for ‘Intsindagirabigega’ and ‘Jyambere’ (Papers II-III). The importance of genetic factors in determining phenolic content and antioxidant capacity has been reported elsewhere for rice (Pang *et al*., 2018) and for other plants (Scalzo *et al*., 2005). Phenolic compounds are reported to play a role in plant defence (Parr & Bolwell, 2000). Also, a beneficial stress memory was observed in ‘Mpembuke’ and ‘Ndamirabahinzi’ (Paper II), the high-phenolic containing cultivars (Papers I-III). Thus, an association between phenolic compounds and stress memory might prevail, which could be exploited to produce cultivars that can withstand repeated stress events.

4.3 Quantitative trait loci for grain yield and its components under repeated drought

The detrimental effect of climate change for rice yield is obvious from drought treatments at high cultivation temperature (Paper II and III). Grain yield was significantly associated with number of panicles per plant and spikelet fertility (paper I-IV). Drought during vegetative stage affected panicle development whereas drought during reproductive stage affected the spikelet fertility (Paper II-IV). Efforts to improve rice for high yield under drought should then target optimal number of panicles per plant and high spikelet fertility.

The present thesis was able to identify QTLs associated with the number of panicles per plant, spikelet fertility and grain yield under drought treatment at different stages of plant development (Paper IV). Thus, QTL for the number of panicles per plant under drought treatments were found on chromosomes 1, 4 and 8. Furthermore, QTL for spikelet fertility under drought treatment were located on chromosomes 1, 5 and 9 whereas QTL for grain yield were found on chromosome 1, 4, 6, 7, 8, 9, 11, and 12. These QTL could be pyramided in elite cultivars (Swamy *et al.*, 2013) in order to improve grain yield under recurring drought. In fact, the QTL defined in this thesis as a major contributor for yield during temperature and drought stress, has by previous studies (Lusser *et al.*, 2012) been shown to carry several genes associated with stress tolerance (Paper IV). This QTL may thereby be exploited for durable rice production in the changing environment using genomic breeding tools.

4.4 Actions and tools for breeding and production of rice in Rwanda despite the predicted climate change scenarios

The results in this thesis consistently showed that drought at seedling stage is detrimental to rice growth at low temperatures and when repeated at tillering stage (Papers II and III) at high temperature (paper II-IV). Dramatic yield reductions were observed due to repeated drought at every plant development stage (Papers II-IV). The results suggest possible increase in rice production with increasing temperatures in inland valleys of Rwanda. Nevertheless, there is urgent need to breed high yielding cultivars adapted to recurring drought and high temperatures in order to sustain rice production under the climate change scenarios. Moreover, proper production planning should be done to avoid periods of low temperatures during seedling stages. Also, water saving techniques such as alternate watering and drying (De Laulanié, 2011) should be implemented to save water simultaneously as drying during critical stages such as tillering, panicle initiation and flowering should be avoided.

The present thesis clearly showed the need for various types of cultivars for sites at different altitudes and with different temperature conditions in inland Africa, as e.g. in Rwanda. The cultivars ‘Intsindagirabigega’ and ‘Jyambere’ showed adaptation to high temperature cultivation and to drought at early stage (Papers II and III). However, these two cultivars were poorly adapted to low temperatures cultivation (Table 2). In contrast ‘Ndamirabahinzi’ was sensitive to drought during seedling stage but tolerant at later stage (Papers II and III). Moreover, this cultivar showed relatively high grain yield at the low temperatures (although inferior to yield at high temperatures; Table 2). Thus, ‘Ndamirabahinzi’ could be proposed for cultivation at low temperatures. In general, breeding for highland valleys require at present cultivars adapted to cold nights. Although the climate change may contribute to increased temperature, still cultivars adapted to relatively cold nights or spells of cold nights might be a requirement. As to the lowland valley requirements, climate change will for sure contribute to higher temperature, more drought and spells of extremes. To be able to grow rice in these areas, novel cultivars are a desire, adapted to such environment. For such breeding requirements, novel breeding methods using QTL and marker assisted selection or genomic selection will be a benefit. The present thesis presents suitable QTL to be used in breeding for the lowland valleys rice. Unfortunately, the highland valley material was flooded (Paper IV) and additional field trials will be needed to define suitable QTL for breeding of rice for this region.

Table 2. *Grain yield and its components for rice cultivars gown in Bugarama and Huye, Rwanda*

Site	Cultivar	Panicles per plant	Spike length (cm)	Spikelet fertility (%)	100-grains weight (g)	Grain yield (g)
Bugarama	'Ingwizabukungu'	4.3 c	21.0 bcd	79.1 abc	3.6 ab	16. 2 c
Bugarama	'Intsindagirabigega'	9.1 b	21.7 abc	85.5 a	3.7 a	24.1 a
Bugarama	'Jyambere'	11.3 b	21.8 ab	81.8 ab	3.5 abc	23.3 ab
Bugarama	'Mpembuke'	4.9 c	22.7 a	73.7 bc	3.6 ab	18.4 bc
Bugarama	'Ndamirabahinzi'	5.0 c	21.8 ab	76.2 abc	3.6 ab	16.6 c
Bugarama	'Nemeyubutaka'	3.4 c	22.7 a	69.9 c	3.7 a	16.9 c
Bugarama	'Zong geng'	5.6 c	22.0 ab	76.4 abc	3.3 abcd	19.0 abc
Huye	'Ingwizabukungu'	9.6 b	18.6 ef	26.8 d	3.5 abc	9.4 de
Huye	'Intsindagirabigega'	16.6 a	18.3 f	21.0 de	2.9 de	7.8 de
Huye	'Jyambere'	14.9 a	18.6 ef	13.2 e	2.5 e	4.0 e
Huye	'Mpembuke'	11.3 b	19.7 def	32.1 d	3.1 bcd	9.4 de
Huye	'Ndamirabahinzi'	11.4 b	20.1 cde	29.2 d	3.2 abcd	9.8 d
Huye	'Nemeyubutaka'	11.5 b	19.6 def	23.1 de	3.1 bcd	7.3 de
Huye	'Zong geng'	11.5 b	18.5 f	26.4 d	3.0 cd	8.5 de

Means followed by the same letter within a column are not significantly different according to Turkey's test at $P \leq 0.05$

Correlation coefficient between grain yield and quality characteristics under drought and temperature conditions (Paper II and III, Table 4), reveal the potential to increase grain yield without impairing amylose content. However, significant negative correlations existed between total protein content and grain yield, and amylose content. Drought conditions limiting nitrogen uptake after anthesis have been identified as the factor influencing this relationship (Parry et *al.*, 2007). Thus, breeding for high nitrogen uptake at anthesis and post-anthesis could be an alternative to overcome the grain yield-protein content unbalance (Bogard et *al.*, 2010).

Table 3. *Correlation coefficient between rice grain yield and amylose content (Amc), total protein content and total antioxidant capacity (TAC)*

	Amc	Total protein	TAC
Total protein	-0.612***		
TAC	0.050 ns	-0.110 ns	
Grain yield	0.336***	-0.447***	0.018 ns

***Significant at $P \leq 0.001$, ns = non-significant

5 Conclusions

The predicted rising temperatures and frequent dry spells as a result of climate change constitute a major threat to rice production in Rwanda and elsewhere in the tropics. Even a small increase in temperature resulted in dramatic grain yield losses when combined with repeated droughts during plant development. The main conclusions and key findings are the following:

- Drought sensitivity, growth and grain yield parameters were mainly influenced by drought treatment as single or in combination with cultivation temperature.
- An increase in night temperatures due to climate change might be beneficial for rice cultivation in highland inland valleys.
- Repeated drought during vegetative stages and drought at the reproductive stage were the most detrimental to rice growth and production.
- Grain yield reductions were highly linked to high spikelet sterility and/ or to poor panicle development. Any effort to improve rice for high grain yield under stressful environment should then target a combination of number of panicles per plant and high spikelet fertility.
- Quantitative trait loci harbouring genes of interest for stress tolerance exist on chromosome 1, 4, 8, 9 particularly for improving the number of panicles and spikelet fertility under recurring dry spells
- Grain quality characteristics were mainly cultivar dependent and less influenced by drought and/ or cultivation temperature.
- The Rwandan rice cultivars included in this research displayed a genetic diversity that may be exploited toward improving rice adaptation to stressful climate and better quality characteristics:
    - ‘Intsindagirabigega’ and ‘Jyambere’ are adapted to high temperature cultivation conditions,
    - ‘Intsindagirabigega’ has high amylose content,

- ’Ndamirabahinzi’ and ‘Mpembuke’ are rich in total phenolics and have high total antioxidant capacity, and display a beneficial stress memory,
- ‘Jyambere’ has high total protein content and high content in mineral elements together with ‘Nemeyubutaka’ and ‘Ingwizabukungu’.

6 Future perspectives

Climate change is expected to increase the frequency of extreme weather events. Concurrent climate change and the growing world population constitute major challenges for agricultural production. Increasing the quantity and quality of food and animal feed, using environmentally friendly practices is essential in order to sustain the life of the human population. Breeding cultivars for resiliency and sustainability is therefore an ultimate goal.

Understanding the genetics behind the stability and robustness of rice cultivars is a crucial tool in maintaining rice production despite unpredictable climate conditions. Moreover, a full understanding of the interplays between different mechanisms contributing to varietal resilience is needed. Thus, some aspects that were not considered in this thesis, such as root traits, plant tissue status and osmolytes production under combined stresses, should be investigated in future work.

The data presented in this thesis on genetic variations in yield and quality traits in existing rice cultivars, and on changes in these traits under stress, constitute a good starting point for developing sustainable and resilient lines. Further crossing and selection are to be carried out using the two best rice cultivars identified in this thesis. The F_4 population that is already phenotyped and genotyped can act as a training population from which genomic models are developed to select promising lines based on their estimated breeding value (Heffner *et al*., 2009). Moreover, genome editing techniques could be used to silence or knock out undesirable traits linked to grain yield (Belhaj *et al*., 2013). Furthermore, speed breeding (Ohnishi *et al.*, 2011, Watson *et al.*, 2018) is recommended to reduce the generation time of rice in the biotron.

The complexity of combined stress resistance calls for accurate identification and pyramiding of the genes involved, in order to assemble a broad spectrum of resistance capabilities in new cultivars and to achieve concurrent expression of these for durable resistance (Malav *et al*., 2016). Thus future research should focus on understanding the molecular aspect of stress memory noted in this thesis and use it, in combination with data on grain yield and quality genes, to develop robust, high-yielding and nutritionally rich cultivars.

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Popular science summary

Rice is a food that is regularly consumed by almost half the world's population. Rice production requires a huge quantity of water and a narrow temperature range (between 25 and 30° C). Limited water availability and temperature beyond the ideal range are serious concerns that limit rice grain production and affect grain quality and content of nutritional elements. To feed the increasing human population on Earth, there is a need to increase rice production by more than 1.2% every year, while at the same time water resources and possibilities to cultivate new land are progressively diminishing. Moreover, future climate change is predicted to bring more frequent soil drying and rising temperatures.

To overcome these challenges, researchers have been trying to develop rice varieties that grow and produce grains with less water, or when the temperature range is not optimal. However, prior to the work described in this thesis, the effects of a combination of frequent soil drying and very high temperature on rice growth and production had not been clarified. Understanding how the rice plant reacts to concurrent soil drying and very hot environments is necessary in order to devise strategies for developing suitable rice varieties and production practices that can overcome these constraints. This thesis evaluated how seven varieties of rice currently grown in Rwanda respond to intermittent soil drying combined with high temperature.

Experiments were conducted in controlled spaces (growth chambers), where environmental factors can be manipulated by the researcher, and in the open field. The growth chambers differed only in temperature, 26/23 °C and 30 /27 °C day/night temperature, respectively, for low temperature and high temperature chambers. Experiments in the open field were performed at two sites in Rwanda, Tonga (temperature: 14.5 - 27 °C) and Bugarama (temperature: 16.9 - 32.9 °C), representing low temperature and high temperature sites, respectively. During experimentation, water supply was stopped during different plant growth stages, as a single treatment or repeated during consecutive stages. The treatments tested were: D0, where water was adequately supplied along the growing season, DS: drought during seedling stage, DST: drought during seedling and tillering stages, DT: drought during tillering stage, DTR: drought during tillering and reproductive stages, DR: drought during reproductive stage, and DSTR: drought during seedling, tillering and reproductive stages. The plants were evaluated for leaf rolling, leaf drying, plant recovery percentage, number of tillers per plant, time to flowering, and grain production and its related traits. After harvesting, grains were analysed for quality characteristics, *i.e*. amylose content, gelatinisation temperature, gel consistency, total protein content and content of health-promoting compounds.

Besides the visual and measurable characters listed above, regions of rice chromosomes that contain transmissible features responsible for water scarcity tolerance were examined.

The results showed that a temperature increase of even a few degrees, when combined with drought at the seedling and tillering stages, resulted in dramatic grain yield decreases for all varieties tested, as a result of plant death or failure to develop reproductive organs. The percentage of empty and aborted grains relative to the total number of grains was the factor that contributed most to low grain production when water was withheld during the reproductive stage. Grain quality characteristics were found to be mostly influenced by the rice cultivar and very little of the variation was caused by limited water supply, temperature or their combination. High amylose content was found in 'Intsindagirabigega'. 'Mpembuke' and 'Ndamirabahinzi' had high phenolic content and high total antioxidant capacity. 'Jyambere' displayed high total protein and high mineral content together with 'Intsindagirabigega' and 'Nemeyubutaka'. A possible link between amylose content and grain production was observed, indicating the possibilities to combine high grain yield with high eating and cooking properties. Regions of rice chromosomes which are associated with grain yield traits at recurring water scarcity were found. Chromosome regions controlling the number of panicles per plant were found on chromosomes 1, 4 and 8, whereas regions for spikelet fertility were located on chromosomes 1, 5 and 9. Chromosome regions associated with grain yield were found on chromosome 1, 4, 6, 7, 8, 9, 11, and 12. These chromosome regions may be integrated into elite varieties for improving grain yield under limited water availability at different growth stages.

The results in this thesis provide clues to key parameters determining grain production and quality characteristics under combined recurring water scarcity and high temperatures. Thorough screening of rice breeding lines and populations could help to select those with these features, thereby enabling development of high-yielding and nutritionally rich cultivars under combined limited water resources and increasing temperatures.

Populärvetenskaplig sammanfattning

Ris konsumeras regelbundet av nästan hälften av världens befolkning. Risproduktionen är beroende av stora mängder vatten och bedrivs inom ett smalt temperaturspann på mellan 25 och 30 ^{o}C. Begränsad vattentillgång och temperaturer utanför det optimala spannet leder till lägre avkastning samt påverkar risets kvalitet och innehåll av näringsämnen. För att kunna försörja jordens ökande befolkning, måste risproduktionen årligen ökas med mer än 1,2%, samtidigt som våra vattenresurser och möjligheter att uppodla nya landområden gradvis minskar. Framtida miljöförändringarna förväntas resultera i återkommande perioder då jordarna torkar ut samt ökande temperaturer.

För att övervinna dessa utmaningar har forskare försökt utveckla rissorter som trots mindre vattenmängder och högre temperaturer kan växa och ge avkastning. Hittills vet vi mycket lite om hur upprepad uttorkning av jorden i kombination med mycket höga temperaturer påverkar tillväxt och avkastning hos ris. Att förstå hur risplantan reagerar på en kombination av torra jordar och hög temperatur är nödvändigt för att kunna lägga upp strategier för utveckling av nya rissorter och produktionsmetoder som kan möta dessa hinder. I denna avhandling utvärderades hur sju sorter av ris som idag odlas i Rwanda svarar på återkommande marktorka i kombination med höga temperaturer.

Försök utfördes både under kontrollerade förhållanden (i tillväxtkammare) där miljöfaktorer kan manipuleras av forskaren, och under fältförhållanden. Två tillväxtkamrar ställdes in på olika temperaturer motsvarande låg temperatur 26 °/23 °C (dag/natt) och hög temperatur 30 °/27 ^{o}C (dag/natt). Fältförsöken genomfördes på två platser, Tonga (14,5 °– 27 ^{o}C) och Bugarama (16,9 °– 32,9 ^{o}C), vilka representerade odling vid låg respektive hög temperatur. Under försöken stoppades vattenförsörjningen under risplantans olika tillväxtstadier, i vissa fall endast under ett stadium, i andra fall under flera av utvecklingsstadierna. De olika försöken omfattade D0: jämn vattentillförsel under hela växtsäsongen, DS: torka under fröplantsstadiet, DST: torka under fröplantsstadiet och under det stadium då plantorna satte rotskott, DT: torka då plantorna satte rotskott, DTR: torka då plantorna satte rotskott samt under reproduktionsstadiet, DR: torka under reproduktionsstadiet, och DSTR: torka under samtliga stadier, dvs under fröplantsstadiet, då plantorna satte rotskott samt under reproduktionsstadiet. Därefter utvärderades plantorna för i vilken utsträckning bladen krullade sig och torkade ihop, procent plantor som återhämtade sig efter torkperioden, antal rotskott per planta, antal dagar till blomning, samt avkastning och avkastningsrelaterade egenskaper. Efter skörd analyserades kvalitetsegenskaper hos riskornen, dvs amylosinnehåll, gelatineringstemperatur, gelkonsistens, innehåll av protein och hälsofrämjande ämnen. Förutom utvärdering av de ovannämnda karaktärerna, utvärderades regioner på riskromosomerna som innehåller överförbara egenskaper kopplade till torktolerans.

Resultaten visade att en temperaturökning på endast ett par grader, i kombination med torka på fröplantsstadiet eller då plantan sätter rotskott, resulterade i en dramatiskt minskad avkastning för alla sorter som en följd av plantdöd eller att plantan inte lyckades utveckla fortplantningsorgan. Om vattentillgången stoppades under reproduktionsfasen var andelen tomma och aborterade riskorn i relation till det totala antalet korn den faktor som bidrog mest till låg avkastning. Det var huvudsakligen sorttillhörigheten som var avgörande för kvalitetsegenskaperna hos riskornen och mycket litet av variationen orsakades av begränsad vattenförsörjning, hög temperatur eller en kombination av dessa. ’Intsindagirabigega’ uppvisade ett högt amylosinnehåll. ’Mpembuke’ och ’Ndamirabahinzi’ hade ett högt fenolinnehåll och en hög antioxidativ kapacitet. ’Jyambere’, ’Intsindagirabigega’ och ’Nemeyubutaka’ hade ett högt innehåll av proteiner och mineraler. En möjlig koppling mellan amylosinnehåll och avkastning observerades, vilket pekar på en möjlighet att kombinera hög avkastning med egenskaper som relaterar till ätupplevelsen och tillagningsegenskaperna hos sorten. Regioner på de olika riskromosomerna som kan kopplas till avkastningsrelaterade egenskaper under perioder av torka identifierades. Regioner kopplade till antal vippor per planta identifierades på kromosom 1, 4 och 8, medan regioner kopplade till småaxens förmåga att sätta frö var lokaliserade på kromosom 1, 5 och 9. Kromosomregioner associerade med avkastning identifierades på kromosom 1, 4, 6, 7, 8, 9, 11, och 12. Dessa regioner skulle kunna föras in i elitsorter för att på så sätt öka avkastningen vid begränsad vattentillgång under olika utvecklingsstadier. För att övervinna dessa utmaningar har forskare försökt utveckla rissorter som trots mindre vattenmängder och högre temperaturer kan växa och ge avkastning. Hittills vet vi mycket lite om hur upprepad uttorkning av jorden i kombination med mycket höga temperaturer påverkar tillväxt och avkastning hos ris.

Resultaten från avhandlingen ger ledtrådar angående viktiga parametrar som bestämmer avkastning och kvalitetsegenskaper vid återkommande perioder av vattenbrist i kombination med höga temperaturer. En genomgripande screening av förädlingslinjer och populationer skulle kunna leda till en identifiering av genotyper med lovande egenskaper och på så sätt möjliggöra en utveckling av högavkastande och näringsrika sorter för odling i ett allt varmare och torrare klimat.

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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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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 ($t·ha^{-1}$)
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 $\mu mol·s^{-1}$ 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 ($g·m^{-3}$) 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

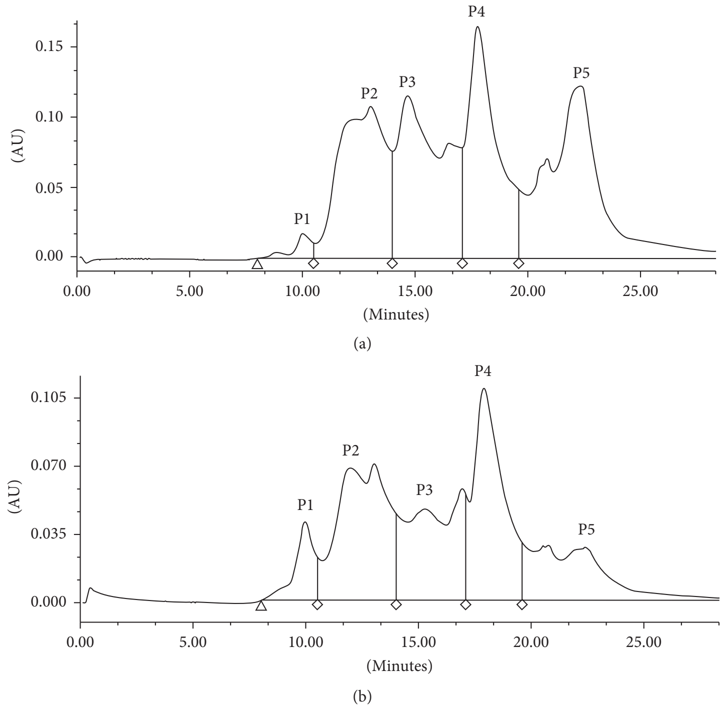

FIGURE 1: The SE-HPLC profile of the protein content in Mpembuke grain: first extraction (a) and second extraction (b). P1–P5 shows the different peaks referred to in Table 6 and in the text.

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,

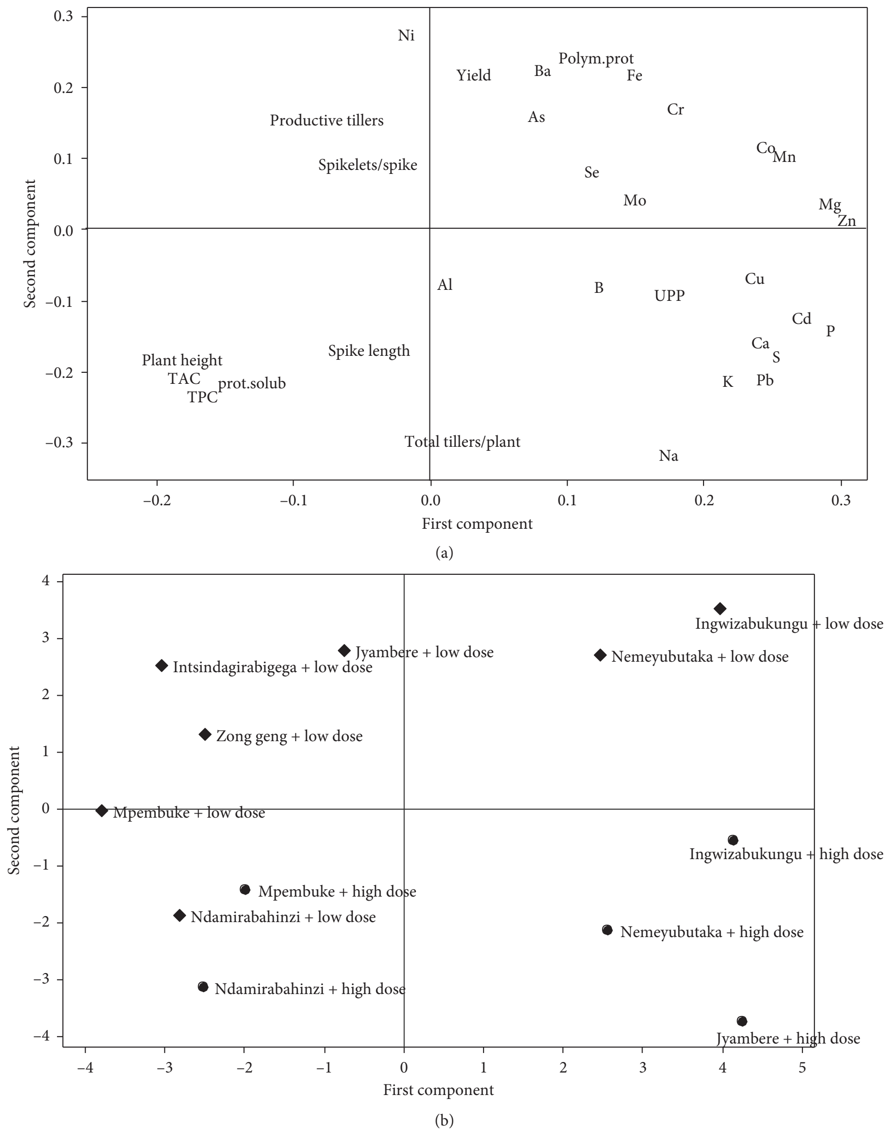

Figure 2: Loading (a) and score (b) plot from principal components analysis of grain yield, its components, mineral elements, total phenolic content, total antioxidant capacity, polymeric protein, protein solubility, and UPP. First principal component explained 32% of the variation while the second principal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

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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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Article

Concurrent Drought and Temperature Stress in Rice—A Possible Result of the Predicted Climate Change: Effects on Yield Attributes, Eating Characteristics, and Health Promoting Compounds

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Abstract: Despite the likely increasing co-occurrence of drought and heat stress, not least in equatorial regions, due to climate change, little is known about the combinational effect of these stresses on rice productivity and quality. This study evaluated the impact of simultaneous drought and temperature stress on growth, grain yield, and quality characteristics of seven rice cultivars from Rwanda, grown in climate chambers. Two temperature ranges—23/26 °C night/day and 27/30 °C night/day—together with single or repeated drought treatments, were applied during various plant developmental stages. Plant development and yield were highly influenced by drought, while genotype impacted the quality characteristics. The combination of a high temperature with drought at the seedling and tillering stages resulted in zero panicles for all evaluated cultivars. The cultivar 'Intsindagirabigega' was most tolerant to drought, while 'Zong geng' was the most sensitive. A "stress memory" was recorded for 'Mpembuke' and 'Ndamirabahinzi', and these cultivars also had a high content of bioactive compounds, while 'Jyambere' showed a high total protein content. Thus, climate change may severely impact rice production. The exploitation of genetic diversity to breed novel rice cultivars that combine drought and heat stress tolerance with high nutritional values is a must to maintain food security.

Keywords: *Oryza sativa*; grain yield; quality; bioactive compounds; drought; temperature

1. Introduction

Rice (*Oryza sativa* L.) is a major staple food crop in the world which contributes to both food security and income generation, particularly within developing countries [1]. Rice has a semi-aquatic phylogenetic origin and thrives in diverse environments if provided with sufficient water [2] and temperatures ranging from 20 to 35 °C. Around 50% of the rice cultivated area is irrigated, whereas rainfed cultivation covers 43% and submerged cultivation covers 7% [2]. In Rwanda, rice growing areas include lowland valleys at around 900 m above sea level with temperature over the growing season ranging from 17 to 33 °C and highland valleys at around 1700 m above sea level with temperatures from 15 to 27 °C [3]. According to Meteo Rwanda measurements, humidity is normally relatively low in Rwanda rice cultivation areas (50–75%). The predicted global warming constitutes a serious threat to rice production as well as to the quality of the produced rice. Temperature stress and drought are predicted to increase to a higher extent in tropical and subtropical regions [4], which are the main rice producing areas. The predicted temperature increase for Rwanda is 0.9–2.2 °C until the mid-21st century, with increasing number of extreme weather events [5].

High temperature stress (above the optimum of 25–30 °C [6]) or drought conditions have negative effects on plant development, including irreversible injury to growth and development of the plant, reduction of photosynthesis [7], reduction in number of panicles per plant and peduncle elongation, limitations of pollen production [4], no swelling of pollen grains, and increased spikelet sterility [8]. Low temperatures (below the optimum of 25–30 °C [6]) lead to inhibited seedling growth, reduced panicle development, delayed heading, poor panicle exertion, low spikelet fertility, and poor grain quality [9]. Besides affecting growth and grain yield, water and temperature stresses change the quality and chemical composition of rice. Levels of starch, phenols, flavonoids, and phytic acid were shown to decrease, whereas antioxidant capacity, content of amylose, oxalic acid, calcium, and iron seemed to increase in drought-stressed grains [10]. In another study, gelatinization temperature decreased in drought stressed rice grains, whereas it increased under heat stress [11]. Therefore, the predicted increase in drought and heat stresses constitute a great threat to rice productivity and quality characteristics, and it may affect lives of millions of the world's population, especially in poor areas of the tropical and subtropical regions [4], where rice constitute a staple food. Consequent to reduction in grain yield, food insecurity, malnutrition, extreme poverty, and other health and social problems will increase if no appropriate measures are taken to mitigate the effects of climate change on food production.

Adaptation measures such as the modification of planting date, alternate wetting and drying, mulching, aerobic rice cultivation [12], selection of cultivars with stress escape/avoidance possibilities [13], and breeding for drought or heat/cold stress are different solutions that have been adopted to mitigate rice productivity at climate change. For example, the International Rice Research Institute (IRRI) has developed drought tolerant cultivars such as 'Sahbhagi Dhan,' 'Sahod Ulan,' and 'Sookha Dhan' grown today in India, the Philippines, and Nepal, respectively [13]. However, multiple stresses may have contrasting effects, and simultaneous occurrence may cause interaction effects that cannot be extrapolated from the effects of individual stress [14]. Treatment strategies need to be, therefore, carefully adopted to fit the circumstances. The co-occurrence of drought and heat stress extremes is likely to increase—both in intensity and frequency—with the actual global warming. Simultaneously, cold nights can still be an issue in rice growing highland valleys of, for instance, Rwanda. Gaps still exist in the knowledge of concomitant environmental effects on rice growth, productivity, and the quality of harvested grains. Hence, understanding rice responses to a combination of temperature and drought stress, as well as their recurrence across plant developmental stages, is needed.

The aim of this study was to investigate how drought and temperature stress combinations during different growth stages affect rice growth, grain yield, eating quality, and health promoting compounds. The acquired knowledge will form the basis for the successful cultivation and breeding of novel rice cultivars that will sustain rice production and nutritional quality within a changing climate, thereby contributing towards human health by reducing poverty in densely populated rice growing areas.

2. Materials and Methods

2.1. Plant Material and Cultivation

Plant Material

Seven rice cultivars (Table 1) obtained from the Rwanda Agricultural Board (RAB) were used in this study. The cultivars were released in 2010 by the Agricultural Research Institute of Rwanda (ISAR), currently known as RAB, except 'Zong geng,' which was introduced from China in the 1960s, and 'Intsindagirabigega,' which was introduced in 2002 from WARDA (currently Africa Rice Centre) and released in 2004. The cultivars were selected based on their medium water requirements, i.e., approximately 909 L of water are needed to produce 1 kg of rough rice [15]. The other main characteristics of the used cultivars [16] are presented in Table 1.

Table 1. Characteristics of rice cultivars included in this study.

ID	Name/code	Popular Name	Characteristics						
			Type	Plant Height	Flag Leaf	Panicle Exertion	Tillering Ability	Lifespan (Days)	Potential Yield (t 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'	*Iindica*	Intermediate	Intermediate	Moderate well exerted	Medium	152	10.9
4	WAB923-B-6-AL1	'Mpembuke'	*Iindica*	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

Source: ISAR, 2010. NA: information not available.

2.2. Growth Chamber

For the present experiment, five replications of a total of seven cultivars were grown using seven different drought treatments and two different temperature treatments. To control the effect of environmental factors, except the selected drought and temperature treatments, the rice cultivars were grown in growth chambers in the biotron of the Swedish University of Agricultural Sciences (SLU) at Alnarp. SLU biotron chambers are known for their high accuracy and low level of between-chamber effects [17]. To avoid the effects of endogenous rhythm in plants [18], all plants in the experiment were sown at the same time. Thus, two growth chambers were used in this experiment: The first chamber was set at a day and night temperature of 26/23 °C (low temperature, T1), whereas the second was set at 30/27 °C (high temperature, T2) for the whole growing period. The selection of temperatures for the experiment were based on Kohl [19] when growing rice in a controlled environment (23–31 °C), the optimum temperature (25–30 °C) in the field according to Wopereis et al. [6], and on the known field conditions of Rwanda [3]. Moreover, we considered the predicted climate change of a 2 °C increase [2] and low night temperatures being present in Rwanda [3], both of which are known to be harmful to rice growth and grain yield [20]. Thus, we used the low optimum temperature of 25 °C as a starting point for the calculation of night temperatures. By decreasing and increasing this optimum temperature by 2 °C, we set the night temperatures to 23 °C and 27 °C, respectively. Thereby, the selected night temperatures can be seen as representatives of an increase in night temperature from climate change in highland and lowland conditions of Rwanda, respectively. Day temperatures were set to 3 °C higher than night temperatures following the procedure by Harrington [21]. Thereby, we ended up with day and night temperatures of 26/23 °C (low temperature, T1) and 30/27 °C (high temperature, T2), representing climate predicted climate changes in the lowland and highland of Rwanda, respectively. For both chambers, a light intensity of 350PAR µmol s^{-1} was used [22] with 11 h of light and 13 h of darkness, whereas the atmospheric relative humidity was 70% [23], the latter also representing the field conditions for rice cultivation in Rwanda.

2.3. Sowing and Watering

Pots with the size 6 × 10 × 12 cm were filled with soil and placed in big plastic trays. Two seeds per pot were sown, and, after emergence, the most vigorous seedling in each pot was left to grow. A total of 35 plants (five plants per cultivar) were grown in each tray and seven trays, i.e., a total of 245 plants, were kept in each growth chamber. The soil was kept wet by regular watering from soil surface up to three weeks after sowing. Thereafter, water was added to the plastic trays, and plants got access to the water through holes in the bottom of the pots. Water depth was maintained at 5 cm in the tray by regularly adding water to it.

2.4. Drought Treatments

The drought treatments applied in the present study followed methodology developed at IRRI [23] and also applied at Africa Rice in Tanzania. In this study, the aim was to evaluate the effects of drought at different growth stages, and, therefore, a number of different drought treatments were applied, i.e., well-watered throughout the growth cycle (D0), drought during the seedling stage only (DS), drought during the seedling and active tillering stages (DST), drought at the active tillering stage only (DT), drought at the active tillering and reproductive stages (DTR), drought at reproductive stage only (DR), and drought at every stage, i.e., the seedling, active tillering, and reproductive stages (DSTR). To initiate drought stress, the trays were drained from water using a tiny tube, and water was not added to the trays again until the drought treatment was finalized. For drought during the seedling stage (DS), the plants were drought treated for two weeks. For drought during the active tillering stage (DT), water was withheld for two weeks; plants were then watered from the soil surface for one day and drought stressed again for another two weeks. For drought at the reproductive stage (DR), the drought stress cycle was one week of drought, one day of watering, and one week of drought.

The combined drought treatments (DST, DTR, and DSTR) used the same treatments as for the separate treatments but in combined doses. Soil water content was measured in the pots at the end of each drought treatment period using an AT Delta—T device (Theta probe type HH1). The probe readings were between 36 and 87 in drought stressed pots, depending on drought treatment and cultivar; in non-stressed pots, it was 513, i.e., a soil moisture content of 7–17% in drought treated pots. After each drought treatment described above, watering was resumed by adding water to the tray.

2.5. Data Recording in the Growth Chambers

After each drought treatment, the number of tillers plant^{-1} was counted. Drought sensitivity was evaluated applying the 0–9 scoring scale for leaf rolling and drying, as proposed by the IRRI [24], although scoring was carried out separately at the seedling and tillering stages. The recovery rate was calculated 10 days after resuming watering, as the percentage of recovered plants over the total number of plants that were present before the drought treatment. Days to flowering were counted as days after sowing to 50% of plants flowering. Number of panicles plant^{-1}, panicle length, number of spikelets panicle^{-1}, and spikelet sterility rate (percentage of sterile grains over total number of grains panicle^{-1}) were counted at harvesting time. Each mature plant was harvested individually. The panicles were manually threshed, and the grain yield plant^{-1} was measured using a precision balance.

2.6. Analysis of Nutritional Quality

2.6.1. Sample Preparation

In order to obtain large enough samples for milling and nutritional quality analyses, rice grains from each of the cultivar × drought × temperature treatment were pooled, and each of these samples was thereafter stored individually at −20 °C. A working sample was thereafter collected from each of these stored samples for freeze-drying in 48 h. The freeze-dried whole grains were thereafter grinded using an IKA-WERKE grinder type A10, (SKAFTE medlab, Mindelheim, Germany). The flour was stored at −20 °C until further analyses. All analyses of nutritional quality were then made in triplicate from the flour.

2.6.2. Total Protein Content

The total nitrogen content was determined following the nitrogen combustion method [25] using a nitrogen/carbon analyzer (Flash 2000NC Analyzer, Thermo Scientific, Waltham, MA, USA). The total protein content was calculated by multiplying the total nitrogen content by a conversion factor of 5.7 [26].

2.6.3. Total Phenolic Content and Total Antioxidant Capacity

The total phenolic content (TPC) was determined according to Singleton et al. [27], and the total antioxidant capacity (TAC) was analyzed following Pérez-Jiménez's and Saura-Callixto's [28] methods with small modifications, as described in Mukamuhirwa et al. [29].

2.6.4. Amylose Content

The amylose content (AmC) was determined following the spectrophotometric iodine analysis of starch proposed by Hofvander et al. [30], except that the defatting of rice starch was carried out by the use of 95% ethanol [31].

2.6.5. Gel Consistency

The gel consistency (GC) of each sample was measured following the gel consistency test for eating quality of rice developed by Cagampang et al. [32]. Briefly, 0.2 mL of 95% ethanol containing 0.025% thymol blue was added to 100 ± 0.5 mg of rice flour in a culture tube. After vortexing, 2 mL of 0.2N KOH were added, and the mixture was vortexed again. The tubes were covered by concave marble glass and directly put in a boiling water bath (95 °C) for 8 min. The tubes were then cooled at room temperature for 5 min and placed on ice water for 15 min. After cooling, the tubes were horizontally placed on a millimetric paper, and the length of the gel was measured from the bottom of the tube to the top of the gel run.

2.6.6. Gelatinization Temperature

The gelatinization temperature (GT) was determined using a differential scanning calorimetry (DSC, Seiko 6200) method following Gunaratne et al. [33] with small modifications. About 3 mg of rice flour was added into a coated aluminum DSC pan (TA Instruments) in duplicate, and 10 μL of water were gently added to the flour sample. After water addition, the pans were quickly hermetically sealed to avoid water evaporation. For measurement, the scanning rate was set to 10 000 degrees min between 20–150 °C. An empty pan served as reference.

2.7. Statistical Analysis

Simple regression analyses were carried out to understand the proportion of the contribution of drought treatment, temperature regimes, and cultivars on the variation in number of tillers per plant^{-1}, leaf rolling, and leaf drying, similarly as described in Malik et al. [34]. Drought treatments and cultivars were analyzed according to the mean values for each character evaluated by the regression analyses. An analysis of variance (ANOVA), followed by calculations of means separated with Tukey's test ($p < 0.05$), was carried out using the Statistical Analysis Systems (SAS), Cary, North Carolina [35]. A principal component analysis (SAS program) was applied to understand variations and relationships among various parameters and treatments.

3. Results

3.1. Early Plant Growth—Drought Sensitivity Score, Number of Tillers Plant^{-1} and Time to Flowering

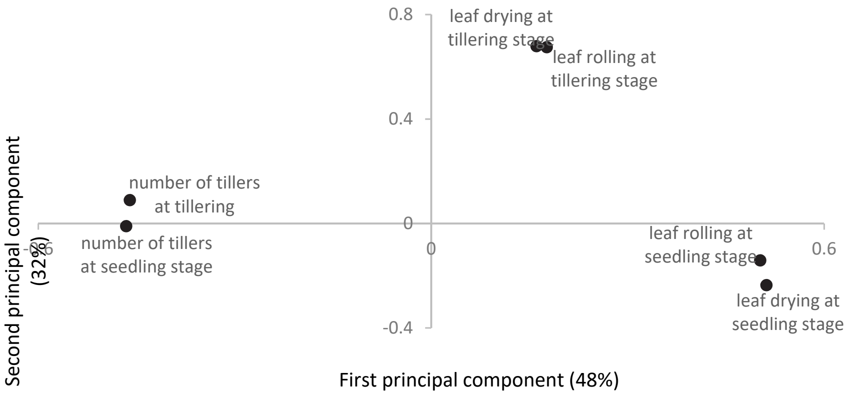
The applied drought treatment explained more than 40% of the variation in leaf rolling and drying at both the seedling and tillering stages, while the cultivar was the major determinant of the number of tillers plant^{-1} (Table 2). The combination of drought and cultivar explained more than 45% of the variation in leaf rolling and drying at the seedling and tillering stages (Table 2). There was no correlation between soil water content in the stressed pots at the end of the drought treatment and leaf rolling or leaf drying.

Table 2. Percentage of explanation (obtained through the coefficient of determination (R^2) from simple linear regression analysis) of temperature (T), drought (D), cultivar (C), and their combinations on numbers of tillers plant^{-1}, leaf rolling, and leaf drying at the seedling (S) and tillering (Tl) stages.

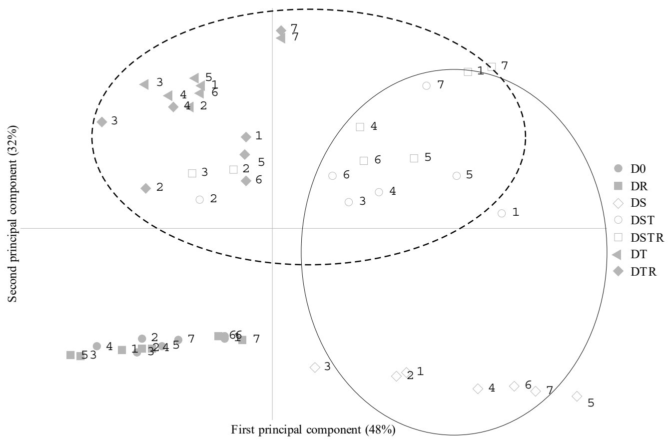
	No of Tillers Plant^{-1}		**Leaf Rolling**		**Leaf Drying**	
	S	**Tl**	**S**	**Tl**	**S**	**Tl**
T	0.49	0.78	0.07	1.2	1.21	2.22
D	7.71	6.88	65.9	43.6	70.40	59.60
C	7.71	13.6	1.0	1.4	1.64	2.16
TD	2.82	0.02	10.6	12.0	5.11	2.77
TC	2.82	0.30	0.38	2.1	0.29	0.72
DC	9.24	8.85	66.9	44.8	71.70	61.00
TDC	3.17	0.08	10.9	12.3	5.31	2.95

T: temperature; D: drought; C: cultivar; TD: temperature and drought; TC: temperature and cultivar; DC: drought and cultivar; TDC: temperature, drought and cultivar.

A principal component analysis (Figure 1) clearly showed the drought at the tillering stage as being the main component determining leaf rolling and drying at the tillering stage, i.e., DST, DT, DTR, and DSTR (encircled by a dotted line) are treatments that have positive values for the second principal component (Figure 1b), as is shown also for leaf rolling and drying at the tillering stage (Figure 1a). Similarly, positive values of the first principal component were found both for samples with drought treatment at the seedling stage (DS, DST, and DSTR; encircled by a full line, Figure 1b), and for leaf rolling and drying at the seedling stage (Figure 1a). The first principal component explained 48% of the variation, while the second principal component explained 32% of the variation. Among the cultivars, 'Zong geng' (No 7) was found to be the most sensitive to drought stress, showing high values of the first principal component (high values of leaf rolling and drying at the seedling stage) during seedling drought and high values of the second principal component (high values of leaf rolling and drying at tillering) during drought at the tillering stage. The number of tillers plant^{-1} decreased, while leaf rolling and leaf drying increased after drought stress at the seedling and tillering stages (Figure 1a).



(**a**)



(**b**)

Figure 1. Loading (**a**) and score (**b**) plots from principal components analysis of leaf rolling, leaf drying, and number of tillers during drought at vegetative stages. Treatments, including drought at the seedling stage, are encircled by a full line, while treatments, including drought at tillering, are encircled

by a dotted line. D0: Well-watered during the whole growing season; DS: Drought treatment at the seedling stage; DST: Drought treatment at the seedling and tillering stage; DT: Drought treatment at the tillering stage; DTR: Drought treatment at the tillering and reproductive stages; DR: Drought treatment at reproductive stage; DSTR: Drought treatment at the seedling, tillering, and reproductive stages. Numbers refer to cultivars as follow; 1: 'Ingwizabukungu'; 2: 'Intsindagirabigega'; 3: 'Jyambere'; 4: 'Mpembuke'; 5: 'Ndamirabahinzi'; 6: 'Nemeyubutaka'; and 7: 'Zong geng'.

There were no significant interactions between cultivar and drought or between cultivar and temperature regarding recovery rate and flowering time. The recovery rate at the seedling stage was significantly decreased for plants being drought treated at the seedling stage (DS, DST, DSTR; Table 3). Similarly, the recovery rate at the tillering stage was significantly decreased after drought at the seedling stage, followed by drought at the tillering stage (Table 3). Combined drought at the seedling and tillering stages (DST, DSTR) resulted in a significant delay in flowering (39 days) as compared to plants being not drought treated (D0; Table 3). High temperature treated plants flowered significantly earlier (30 days) than low temperature treated plants (Table 3).

Table 3. Recovery rate at the seeding (S) and tillering (Tl) stages and time to flowering.

Cultivar	Recovery Rate (%)		Time to Flowering (DAS)
	S	Tl	
'Ingwizabukungu'	65.0 a	38.0 a	91.6 b
'Intsindagirabigega'	92.5 a	68.0 a	113.1 a
'Jyambere'	87.5 a	52.0 a	112.3 a
'Mpembuke'	62.5 a	50.0 a	113.9 a
'Ndamirabahinzi'	45.0 a	48.0 a	105.8 ab
'Nemeyubutaka'	73.3 a	65.5 a	107.7 ab
'Zong geng'	60.0 a	22.2 a	121.5 a
Temperature			
Low	75.0 a	54.86 a	120.5 a
High	64.3 a	44.57 a	90.3 b
Drought			
D0	95.7 a	87.1 a	94.8 b
DS	38.6 c	32.9 c	111.4 b
DST	64.3 b	35.7 c	133.7 a
DT	100.0 a	41.4 bc	105.8 b
DTR	98.6 a	45.7 bc	111.7 b
DR	95.7 a	77.1 ab	103.7 b
DSTR	80.0 ab	38.6 c	133.2 a

Means followed by the same letter within a column are not significantly different between cultivars or temperatures or droughts according to Turkey's test at $p < 0.05$D0: Plants were well-watered along the growing cycle. DS: Drought stress at the seedling stage; DST: Drought stress at the seedling and tillering stages; DT: Drought at the tillering stage; DTR: Drought at the tillering and reproductive stages; DSTR: Repeated drought at every developmental stage, S: seedling stage, Tl: tillering stage.

3.2. Rice Production—Grain Yield and Yield Components

The means of grain yield and yield components for all cultivars and treatments of this study are presented in Table S1. Generally, the applied drought treatments heavily affected the presence and number of panicles plant^{-1} and, thereby, grain yield (Table 4). The D0 treatment (no drought) at a high temperature resulted in the significantly highest number of panicles plant^{-1}. Combined drought treatment at the seedling and tillering stages (DST, DSTR) at a high temperature had a detrimental effect on the presence of panicles plant^{-1} and, thereby, yield, resulting in zero panicles plant^{-1} for all evaluated cultivars (Table 4). The cultivar 'Intsindagirabigega' showed a high number of plants with panicles, being able to better withstand all the evaluated drought treatments except the combined seedling and tillering treatments (DST and DSTR) at a high temperature (Table 4). The cultivar

'Zong geng' clearly showed the significantly lowest number of panicles plant^{-1}, with a decrease at all drought treatments at a high temperature and at all drought treatments except DS and DST at the lower temperature (Table 4). The cultivars 'Mpembuke' and 'Ndamirabahinzi' both showed sensitivity to drought stress at the seedling stage (DS) at a low temperature, although being able to produce panicles at combined drought stresses at the seedling stage and at later stages (DST, DSTR). The numbers of spikelets panicle$-^{1}$ and spikelets sterility were generally higher at a low temperature than at a high temperature (data not shown). Furthermore, all drought treatments resulted in severe decreases in yield as compared to when the plants were subjected to no drought (Figure 2). In drought stressed pots, a negative correlation (r = −0.28; p = 0.025) was found between soil and water content at the end of the experiment and grain yield.

Table 4. Presence (+) and absence (−) of panicles plant^{-1} (and thereby grain yield) and percentage of plants per rice cultivar and treatment without any panicle. Cultivars 1: Ingwizabukungu; 2: Intsindagirabigega; 3: Jyambere; 4: Mpembuke; 5 Ndamirabahinzi; 6: Nemeyubutaka; and 7: Zong geng were grown at a low or high temperature and with six different drought treatments, D0: No drought stress, DS: Drought at the seedling stage, DST: Drought at the seedling and tillering stages, DT: Drought at the tillering stage, DTR: Drought at the tillering and reproductive stages, DR: Drought at reproductive stage, DSTR: Drought at the seedling, tillering, and reproductive stages.

Treatment	Cultivar							Plants per Temp × Drought with No Panicles (%)
Temp Drought	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Low D0	+	+	-	+	+	+	+	37.1 ab
Low DS	+	+	+	-	-	+	+	80.0 cde
Low DST	+	+	+	+	+	+	+	57.1 bcd
Low DT	+	+	+	+	-	+	-	74.3 cde
Low DTR	+	+	+	-	+	-	-	74.3 cde
Low DR	+	+	-	-	+	-	-	85.7 de
Low DSTR	-	+	+	+	+	+	-	65.7 bcd
High D0	+	+	+	+	+	+	+	11.4 a
High DS	+	+	+	+	-	-	-	74.3 cde
High DST	-	-	-	-	-	-	-	100.0 e
High DT	+	+	+	+	+	+	-	54.3 bcd
High DTR	+	+	+	+	+	+	-	65.7 bcd
High DR	+	+	+	+	+	+	-	51.4 bc
High DSTR	-	-	-	-	-	-	-	100.0 e
Plants per cultivar with no panicles (%)	62.9 ab	50.0 a	61.4 ab	68.6 ab	72.9 bc	58.6 ab	91.4 c	

Means followed by the same letters within a column for treatment or within a row for cultivar are not significantly different at $p < 0.05$ according to Tukey's mean comparison test.

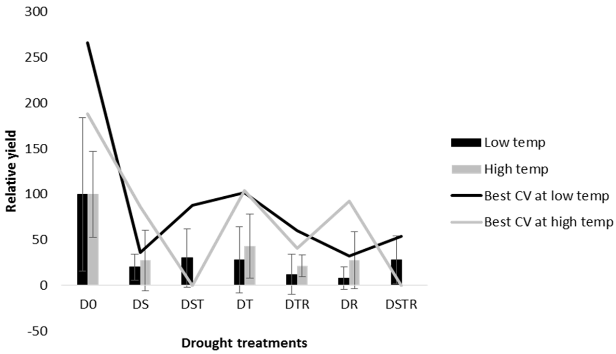
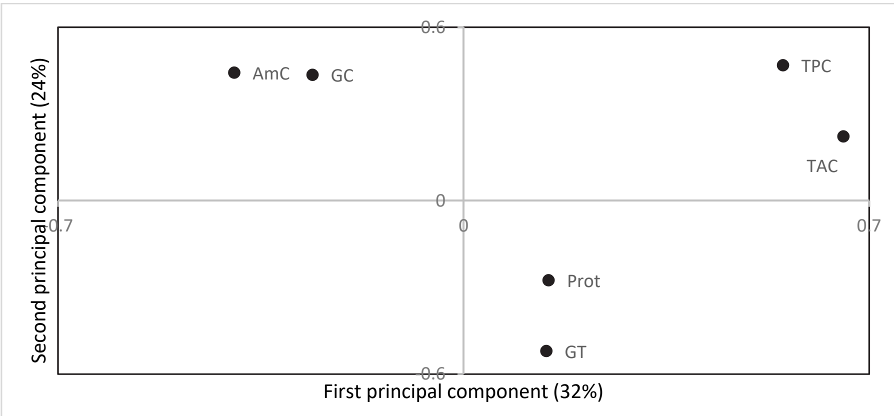


Figure 2. The relative yield (as compared to mean values of the cultivars at no drought treatment at temperatures applied, respectively) of all cultivars at each of the drought treatments at each of the temperatures (bars) and of the cultivar with the highest at each treatment and temperature (line). Error bars represent standard deviation of mean values for yield of all the cultivars.

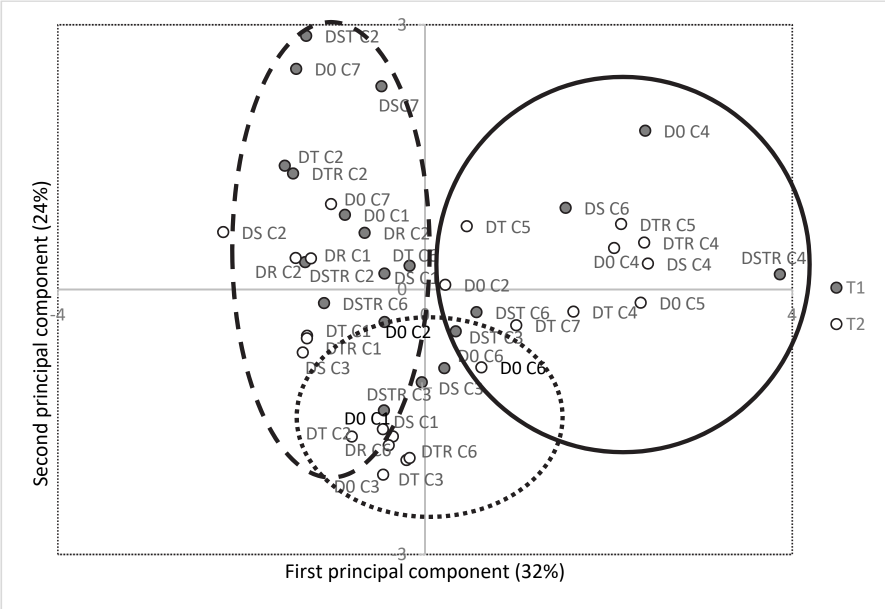
3.3. Eating Quality and Health Promoting Compounds

The means of grain AmC, GC, GT, total protein content, TPC, and TAC for all cultivars and treatments are presented in Table S2. In general, variation in the evaluated quality characteristics seemed to be more dependent on cultivar than on temperature or drought treatment. In a principal component analysis, the cultivars were shown to cluster into three groups (Figure 3b) corresponding to evaluated quality characteristics (Figure 3a). The first and second principal components explained 32% and 24% of the variation, respectively. The cultivars 'Mpembuke' (C4), 'Ndamirabahinzi' (C5), and 'Nemeyubutaka' (C6) showed positive values on the first principal component (encircled by a full line) independent of temperature and drought treatment (Figure 3b), indicating a generally high TAC and TPC content (Figure 3a) in these cultivars. Regression analyses also showed a high degree of cultivar determination for TAC (33.7%) and TPC (16.5%) content, and analysis of variance followed by mean comparisons confirmed that these cultivars have high TAC and TPC contents (results not shown). Furthermore, the cultivars 'Ingwizabukungu' (C1), 'Intsindagirabigega' (C2), and 'Zong geng' (C7) were clustered with negative principal component analyses values (encircled by a dashed line; Figure 3b) independent of temperature and drought treatment, indicating high AmC and GC content (Figure 3a), which was also verified by analyses of variance followed by mean comparisons (results not shown). The cultivar 'Jyambere' (C3), independent of treatments, formed the third cluster (encircled by a dotted line) with negative values on the second principal component (Figure 3b), indicating high protein and GT values (Figure 3a), which was verified by analyses of variance followed by mean comparisons (not shown). The principal component analysis indicates a possible influence of the growing temperature on GT values, with significantly higher GT values at the high temperature treatment (verified by analyses of variance and mean comparisons). In addition, regression analyses showed 10.4% of the variation in GT to be explained by temperature, as compared to 0.1% being determined by cultivar (results not shown).



(**a**)

Figure 3. *Cont.*



(**b**)

Figure 3. Loading (**a**) and score (**b**) plot from principal components analysis of amylose content (AmC), gel consistency (GC), gelatinisation temperature (GT), total antioxidant capacity (TAC), total phenolic content (TPC), and total protein content (Prot) in grains of rice cultivars stressed with drought at different development stages. Treatments corresponding to high TAC, TPC, and protein content are encircled by a full line, whereas a dashed line encircles the treatments corresponding to high or low eating and cooking qualities (AmC, GC, and GT), and a dotted line encircles the treatment corresponding to low content of all analyzed nutritional quality traits. T1: 26/23 °C; T2: 30/27 °C; DS: Drought treatment at seedling stage; DST: Drought treatment at the seedling and tillering stage; DT: Drought treatment at the tillering stage; DTR: Drought treatment at the tillering and reproductive stages; DR: Drought treatment at reproductive stage; DSTR: Drought treatment at the seedling, tillering, and reproductive stages. Numbers refer to cultivars as follow; C1: 'Ingwizabukungu'; C2: 'Intsindagirabigega'; C3: 'Jyambere'; C4: 'Mpembuke'; C5: 'Ndamirabahinzi'; C6: 'Nemeyubutaka'; and C7: 'Zong geng'.

4. Discussion

Our results clearly showed a negative concurrent effect of the temperature and single/repeated drought treatments at various growth stages on rice production and yield. Drought impacted to the highest extent the early plant development, while the genetic component—i.e., different cultivars—determined the quality of rice to the largest extent. Thus, our results clearly showed that the predicted increase in temperature and drought due to climate change will impact rice production, which, in turn, might influence food security in vulnerable highly populated areas around the equator.

A combination of a high temperature (27/30 °C) and recurrent drought at the seedling and tillering stages (which might be a predicted climate change scenario for lowland valleys of Rwanda) showed the most severe results for the plant development and yield, with no panicles plant^{-1} resulting in zero yield for all cultivars. However, for the rest of the combinations of drought and temperature treatments, the different cultivars reacted differently. Differences among cultivars towards drought conditions may be the result of a range of various characters in the plant, including both root and leaf characters [36]. Thus, cultivars capable of extracting more water from the soil and maintaining a good water status in the tissues are able to withstand drought and to produce grains. The cultivar 'Zong geng' showed the most severe reaction regarding panicles plant^{-1}, resulting in a total absence after most of the treatments. This genotype is both taller and later flowering than the other cultivars when grown during field conditions (Table 1), which might explain part of its sensitivity to drought conditions, although additional studies are needed for a full understanding of variations in the reactions from the different genotypes. In addition, cultivar interactions with the soil water content and the time course of its' changes may contribute to an understanding of drought treatments on various cultivars. The course of soil water content for each individual plants, cultivars, and treatments was not measured in the present study, and such interactions are an option for further studies. However, previous studies have indicated that leaf characteristics of rice, including leaf water status and leaf elongation rate, are limitedly sensitive to soil water content and deficits; instead, the root character was proposed as the main character related to drought sensitivity [37]. Furthermore, panicle exertion and abortion of secondary rachis branches has been reported as the major causes of yield reduction in rice under drought stress [38], which correspond to the results in the present study. The severe reduction in the number of panicles plant^{-1} and, consequently, reduction in grain yield in the present study indicate that a high temperature and recurrent drought at the seedling and tillering stages were severe for the rice plants. Drought conditions and the cultivation of rice in the present study were based on methodology developed at IRRI [23]. The high temperature combined with recurrent drought at the seedling and tillering stages might have resulted in more severe drought responses in rice than is normally found while rice is grown during field conditions. At present, rice is most likely not recommended to be grown at conditions with severe recurrent drought conditions at a high temperature. However, the severe drought responses from the rice in the present study might also be due to differences in growing conditions in the biotron compared to in field conditions. It is well known that biotron or green-house cultivations are not perfectly comparable to cultivation in field conditions. In the biotron, the rice plants were grown in pots, not allowing the roots to extract water deep in the soil; in addition, microbiota and other biotic factors are known to be different. Despite the fact that biotron conditions are not totally comparable to field conditions, our results predict a high risk of large reduction in rice yield in the predicted climate change scenarios. In previous studies, spikelet sterility has consistently been reported as an indicator of sensitivity to drought around flowering [39] and is directly associated with leaf water potential. However, in the present study, spikelet sterility was mainly found to be correlated to the temperature of rice cultivation and, to a lesser degree, to the drought treatments. The fact that spikelet sterility was not seen as correlated with drought treatments thereby verifies drought treatments as not being severe enough to result in differences in leaf water potential. Extreme temperatures have been found to affect spikelet fertility by causing poor anther dehiscence, pollen grains deficiency, and failure of pollen germination on stigmas [40,41]. Our results primarily showed high spikelet sterility at a low temperature, which has also been shown in other studies [42]. Spikelet sterility under the low temperature regime is a sign of this temperature being in the lower edge of the optimal range. However, we found comparable yields in high and low temperatures treated rice due to an increased spikelet fertility compensating for the low number of panicles plant^{-1} at a high temperature.

Early plant development was affected more by the drought treatments than by differences in temperature. The different drought treatments in the present study resulted in various effects on plant development. Thus, drought at the seedling and tillering stages resulted in leaf rolling and drying at these stages, and repeated drought treatments delayed the flowering—as did the low temperature treatment. A delayed flowering might be associated with reduced early growth. Previous studies have reported a reduction of relative water content from exposure to a low temperature [43], while drought resulted in a reduced synthesis of photosynthetic pigments [44]; both circumstances resulted in a delayed plant development. Early growth rate, leaf emergence, and tillering capacity was found to increase with rising temperatures, from 25 to 31 °C, as a result of increasing photosynthesis [45]. Thus, in our study, the shorter time to flowering in plants grown at a high temperature might be associated with high early growth rate. Drought at the seedling stage significantly reduced the recovery rate of the plants at both the seedling and tillering stages. Thus, this study showed the rice plants to be most sensitive to drought at the seedling stage, especially if followed with drought at the tillering stage and even more so at high temperatures.

The cultivars included in this study reacted differently to the temperature and drought treatments. Effects of drought treatments on different genotypes is known to be the result of a range of various characters in the plant, including both root and leaf characters [36]. However, the only persistent difference recorded was that 'Zong geng' showed significantly higher drought sensitivity as compared to the other cultivars as it showed a higher leaf rolling and drying at both the seedling and tillering stages and the significantly largest decrease in number of panicles plant^{-1}. Grown at a high temperature, 'Zong geng' produced spikelets only while not drought treated; grown at a low temperature, this cultivar produced spikelets only when well-watered and when treated with drought at the seedling stage (also combined with drought at the tillering stage). 'Jyambere', a cultivar reported in a previous study [29] as high yielding, did not show higher performance while drought treated than the other cultivars. Similarly to the present study, leaf rolling and drying were found to increase, while the tillering rate and number of panicles plant^{-1} decreased, with drought stress, although at significantly different extents in different cultivars [46].

The present study showed the limited influence of drought treatment on quality characteristics of rice, where only GT was clearly affected by and positively correlated with temperature. Previous studies have shown that an increased temperature reduces the grain starch and amylose content and also decreases the activity of granule-bound starch synthase [39], which might affect the GT. Independent of drought treatment, the cultivars 'Mpembuke' and 'Ndamirabahinzi' showed (together with 'Nemeyubutaka'), high grain content of TAC and TPC, corresponding to previous results on these cultivars [29]. The results suggest that the content of phytochemicals/bioactive compounds might be under strong genetic control which means that it would be possible to breed for rice high in human health related compounds. High genetic diversity in bioactive compounds has been consistently found in rice cultivars [47,48]. Furthermore, QTLs with significant additive effects have been identified for some bioactive compounds in rice [49], which implies options to use marker assisted selection. In addition, AmC, GC, and protein content were found more related to cultivar than to treatment in the present study. Significant differences between genotypes for AmC and protein content have been observed in rice, whereas differences between drought and irrigation treatments [50] and between temperatures [51] have been negligible. The inheritance of AmC and GC in rice has been revealed to be predominantly governed by additive gene action [52,53]. Therefore, the genetic diversity present in the Rwandan rice cultivars offers opportunities to improve quality characteristics within the material.

Previous results have shown that exposure to stress at an early developmental stage can affect the tolerance and survival of plants when stressed again at a later stage [54]. Thus, plants seem to have a "stress memory" that allows them to cope with recurrent stress [55]. In the present study, the cultivars seemed to have a beneficial stress memory regarding drought stress to different degrees, and the presence of such a memory seemed to be influenced by the temperature treatment. The cultivars 'Mpembuke' and 'Ndamirabahinzi' seemed to have quite a beneficial strong stress memory, especially when grown at a low temperature, since they were not able to produce panicles and yield when drought stressed at the seedling stage but were able to produce panicles and yield after recurrent drought stresses. In addition, 'Ndamirabahinzi' seemed to have a stress memory if subjected to stress at a somewhat later stage, as a similar behavior was recorded for plants stressed at the tillering stage at first and then subjected to recurrent stresses. The sustained accumulation of stress signaling proteins or transcription factors at different levels from the initial state after stress alleviation was proposed as possible mechanism of plant memory [56]. Epigenetic changes involving DNA methylation or chromatin transformation without changing the nucleotide sequence have also been proposed [57] and may confer longer lasting effects than the metabolite accumulation. Further research may be able to elucidate the stress memory mechanisms under combined heat and drought.

The present study was carried out in climate chambers in the biotron at SLU, Alnarp. Despite the fact that climate chamber cultivations are not totally comparable with field cultivations, climate chamber experiments have the advantage of opportunities to control environmental effects on the cultivation. Plants grown in the same chamber have got exactly the same environmental conditions with the exception of treatments given to the plants (e.g., drought treatments in our case). The repeatability of experiments is also increasing with the use of climate chambers, as between-chamber effects are normally low, and, if they exist, they are mostly due to resolvable effects, i.e., the need for the reparation of malfunctioning components [17]. In our study, we used five replications of cultivars and treatments to secure statistically useful results as outcomes. However, a minor risk in our study is that the differences between temperature treatments we see might also partly be a result of between-chamber effects as used temperature treatments were divided in between chambers. Due to the high and well-known accuracy between the biotron chambers with a low level of between-chambers resolvable effects, we see this risk as low. In addition, the temperature results in this study correspond with results from field trials on drought stress for the same cultivars (unpublished results). In the present study, we also wanted to avoid experimental cultivation of the rice plants in various periods over the year; instead, we focused on at the same time planting to secure avoidance of effects of endogenous rhythms in plants [18]. Effects of such rhythms should absolutely have a bigger effect on the results than eventual small results between chamber unresolved effects [17,18].

Though the temperature sets were within the limits for rice development and productivity, this study showed that even small differences in temperature during rice growth may result in severe yield losses under recurrent drought. A small increase in temperature, which might come due to the predicted climate change, might be beneficial for rice production in highland areas of Rwanda, where the temperature currently is at the lower limit for rice production. However, this study shows that the positive effects of a small increase in temperature does not persist at recurrent drought conditions. Therefore, changes in temperatures prevailing in a specific environment should not be left behind when planning for improvement of rice for drought tolerance. There were large differences in panicle development between cultivars and between drought × temperature treatments. Hence, this trait may serve as selection criteria for rice tolerance to concurrent abiotic stresses.

5. Conclusions

The predicted climate change foresees a total increase in temperature combined with frequent dry spells. From this study, we can conclude that this scenario does not in general appear to be favorable for rice production. A moderate increase in temperature in "cool" regions, e.g., in highland valleys of Rwanda, combined with low or moderate drought stress, might result in a yield increase for rice. However, here, a temperature increase of only a few degrees, when combined with drought at the seedling and tillering stages, resulted in dramatic yield decreases for all cultivars. Some of the cultivars could not tolerate drought at all at the higher temperature. Rice is primarily cultivated in tropical and subtropical areas, being the staple food and major income crop for millions of poor people in densely populated areas. A severe decrease in production due to climate change will therefore heavily affect public health in these areas. This calls for a sincere screening to find rice capable of surviving an increased temperature with spells of drought. Here, cultivar differences in survival and production was clearly seen among the evaluated cultivars, although none of them a survived high temperature combined with drought at both the seedling and tillering stages. In this context, understanding the molecular background for some of the cultivars showing better ability to sustain the combined increase in temperature and dry spells is a necessity. Though drought and temperature combination did not affect the quality characteristics to a high extent, the combination of these stresses indirectly affect the nutritional quality and human health promoting compounds, since the quantity of produced grains becomes insufficient to meet the ideal daily intake and to fulfil the required nutritional content resulting in malnutrition. Thus, thorough screenings of cultivars that combine high quality and the capacity to withstand combined stresses at all stages of plants development and understanding involved mechanisms have to capture the attention of rice researchers.

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-4601/16/6/1043/s1, Table S1: Means of grain yield plant^{-1} and grain yield components of rice cultivars grown at low (26/23 °C) or high temperature (27/30 °C) and subjected to different drought treatments. Table S2: Means of quality characteristics: amylose content (AmC), gel consistency (GC), gelatinisation temperature (GT), total protein content (Prot), total phenolic content (TPC), and total antioxidant capacity (TAC) of rice cultivars grown at low (26/23 °C) or high temperature (27/30 °C) and subjected to different drought treatments.

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