EVALUATION OF AGRONOMIC PERFORMANCE AND GENOTYPIC MAPPING USING SNP MARKERS OF GRAIN IRON AND ZINC CONTENT AMONG RICE ACCESSIONS IN EASTERN DEMOCRATIC REPUBLIC OF CONGO

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Evaluation of Agronomic Performance and Genotypic Mapping Using SNP Markers of Grain Iron and Zinc Content Among Rice Accessions in Eastern Democratic Republic of Congo

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This work is dedicated to my wife, Mrs. Zawadi Wa Bahati Charlotte, and my daughter, Ms. Marie-Laure Binja, for providing me with moments of ease during my study, as well as for their encouragements. I also dedicate this work to my parents, Mr. Norbert Bukomarhe and Mrs. Antoinette Bafunyembaka, and the entire Bukomarhe family for their prayers and moral support.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CGIAR	Consultative Group on International Agricultural Research
CIAT	International Centre for Tropical Agriculture
DArTseq	Diversity Array Technology sequencing
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of the Congo
FAO	Food and Agriculture Organization
GWAS	Genome-Wide Association Studies
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
INERA	Institut National pour l'Etude et la Recherche Agronomiques
IRRI	International Rice Research Institute
MAS	Marker Assisted Selection
QTL	Quantitative Traits Loci
SNP	Single Nucleotide Polymorphisms

ABSTRACT

Rice (*Oryza sativa* L.) is among the most important food crop in the Democratic Republic of Congo (DRC). The crop has historically been neglected by local researchers. However, recent urbanization and changes in dietary habits have elevated its significance. The identification of rice varieties with high yield and reasonable micronutrient contents, especially iron (Fe) and zinc (Zn), could be important in rice varieties for the DRC. This study aimed to (a) determine agronomic performance of a subset of 36 rice accessions to identify high yielding and stable genotypes for cultivation in eastern DRC and (b) associate SNP markers with Fe and Zn content in rice grains for application in marker assisted selection. For the molecular markers study, 85 diverse rice accessions from the DRC maintained at the Kalemie were analysed for variation in Fe, Zn and SNP markers. The data were analysed using rMVP package of R software for Genome Wide Association Study (GWAS) to identify genomic regions linked to grain Fe and Zn content. Candidate genes associated with Fe and Zn content were identified and annotated from the *Oryza sativa* reference genome version 7.0 of the MSU-Rice Genome Annotation Project (RGAP) database. In the evaluated rice grains, Fe content ranged from 0.95 to 8.68 mg/100g (dry weight basis), while Zn content ranged from 0.87 to 3.8 mg/100g. Two significant SNPs were identified, with one on chromosome 11 associated with Fe and another on chromosome 4 associated with Zn. Candidate genes involved in transcription regulation and transporter activities related to Fe and Zn homeostasis, including the bZIP family genes and MYB family genes were pinpointed. For the agronomic trial, 36 rice accessions were evaluated in a 6x6 triple lattice design with three replications in Bwegera and Sange sites, in Eastern DRC. Data on various agronomic traits such as Day to Flowering (DTF), Plant Height (PH), Effective number of Tiller (ET), Panicle Length (PL), Grain per Panicle (GP), Thousand Grain weigh (TGW), Grain Length (GL), Grain Width (GW), Ratio Grain Length and Grain Weight (RGLGW), Grain Yield (GY) were recorded during growth and analysed using R software. Across locations, no significant differences were recorded in various agronomic variables, except for 1000-grain weight (TGW). Within locations, significant differences were observed for the evaluated variables except for panicle length, grain length, grain width, and the ratio of grain length to grain weight. Based on grain yield, accessions IR990-48-B-B-12, IR841, IR88638 and MR254 were the best across and within individual sites making them favorable recommendations for farmers. The identified markers and candidate genes can be utilized in Marker Assisted Selection (MSA) in rice breeding programs, aiming to enhance Fe and Zn content. The study is offering opportunities for developing biofortified rice varieties to combat malnutrition among consumers.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Rice (*Oryza sativa* L.) is the most important food crop, feeding more than half of the world's population (Mohidem et al., 2022). Other than satisfying the caloric requirements of millions of people daily (Sweeney & McCouch, 2007), rice serves as the primary source of protein, thiamine, riboflavin, niacin, and essential micronutrients, such as Fe and Zn in the diet (Ali & Wani, 2021; and Mohidem et al., 2022). Globally, rice cultivation expanded to cover approximately 115 million hectares of land. Over the period from 1961 to 2019, the global rice cultivation area witnessed a modest increase of slightly over 40%. Notably, Africa experienced the most substantial surge in rice cultivation areas. Throughout this period, global rice (paddy) production surged by more than threefold, soaring from 215 million tonnes to 755 million tonnes, with Asia contributing significantly to this production (Bin Rahman & Zhang, 2023). The rice productivity rate in Africa varies significantly, with irrigated areas achieving yields of 2.5–5.6 t/ha compared to 0.6–2.3 t/ha in rainfed areas (Arouna et al., 2021). These yields are generally below the global average productivity of 2.76 t/ha (Parameswari et al., 2017). The low productivity in Africa could be attributed to several factors, including the use of poor-quality seeds, lack of improved varieties, pests and diseases, and poor soil, water, and weed management practices (Zenna et al., 2017). Majority of rice growers in Asia and Sub-Saharan Africa are smallholder farmers with farm holdings of between 0.5 and 3 hectares (Dzudzor, 2013). In the Democratic Republic of Congo (DRC), rice is among the main crops produced after cassava, maize, and beans (FAO, 2023b) and its production is around 1t/ha (FAO, 2023a). Rice production in the DRC faces a range of challenges, including the utilization of outdated farming methods, limited access to improved seed varieties, high input costs, as well as biotic constraints such as pests and abiotic constraints related to environmental factors (Andriatsiorimanana et al., 2023).

The global population is projected to reach approximately 9.7 billion by 2050 (United Nations Department of Economic and Social Affairs & Population Division, 2022),

and a significant proportion of it faces a major problem of malnutrition. Malnutrition manifests itself in various forms and it impacts not only on an individual's health and well-being but also imposes significant burdens on families, communities, and states (Food and Agriculture Organization of the United Nations, 2017). It continues to be a significant public health concern in many developing countries (Elmighrabi et al., 2023) in the world, with the African continent being the most affected. In the DRC, deficiencies in micronutrients, such as iron and zinc, contribute significantly to mortality rates among women and children (Gupta et al., 2020). Iron (Fe) is vital for various biological functions in the human body, including the synthesis of oxygen transport proteins (Abbaspour et al., 2014; Jones, 2020). Its deficiency leads to anemia (WHO, 2014) while zinc (Zn) deficiency affects multiple systems in the human body, including epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems (Roohani et al., 2013).

1.2 Statement of the Research Problem

In the Democratic Republic of Congo, rice ranks seventh among food crops in terms of quantities consumed after cassava, plantain, fruit, maize, groundnut and vegetables (Ulimwengu et al., 2012). It has been observed that as time goes by, and with increased urbanization (Eric et al., 2010), the eating habits of communities have changed and rice consumption has increased. According to recent FAO statistics, the annual rice consumption in the DRC as of 2019 amounted to 19 kg per person per year. However, local production has stagnated at 350,000 metric tons since 2018, while estimated consumption is close to 700,000 metric tons (Gregoire et al., 2023). This implies that the national demand for rice has been increasing at a higher rate than that of other food crops. Unfortunately, rice growing in the country has remained the prerogative of small scale farmers cultivating on average 0.50 ha under rainfed conditions or 0.20 ha under flooded cultivation and achieving yields of 1 ton of paddy per hectare (FAO, 2023a). Moreover, majority of the rice growers use low yielding traditional varieties, poor quality seeds, poor agronomic practices and with low inputs of fertilizers and pesticides (Ministry of Agriculture and Rural Development, 2013). In addition, information on the micronutrient contents of the available germplasm remains

unknown. So far, no measures have been undertaken in the country purposely directed towards improving the crop through plant breeding.

1.3 Justification of the Study

Plants play a critical role as pathways for nutrient movement from soil to humans (Philipo et al., 2020). Major efforts have been deployed by the Consultative Group on International Agricultural Research (CGIAR) institutes worldwide, such as the International Food Policy Research Institute (IFPRI) and the International Centre for Tropical Agriculture (CIAT) under the HarvestPlus program in developing and introducing biofortified varieties of crops (Bouis et al., 2000). In this context, cultivars of beans, maize, sweet potato, and cassava have been introduced into the DRC to alleviate hidden hunger and reduce its consequences (Moumin et al., 2020).

Being the second most consumed cereal after maize (Food and Agriculture Organization of the United Nations et al., 2019), rice could constitute an alternative source of micronutrients in the DRC. Iron and Zinc content in grain rice are variable depending upon its variety and processing. The target levels for Fe and Zn content in polished rice grains, according to HarvestPlus, should be 1.3 and 2.4 mg/100 g, respectively (Bouis & Welch, 2010).

However, it is important to note that the performance of any character is a combined result of the genotype (G), the environment (E), and the interaction between genotype and environment (GE) (Sharifi et al., 2017). Consequently, the agronomic performance of any genotype varies from one environment to another.

This study has been proposed as a pre-breeding undertaking to generate genetic and agronomic information that will be useful for rice plant breeders in the country.

1.4 Objectives

1.4.1 General Objective

This study aimed to determine agronomic performance of a panel of available rice accessions and identify genomic regions associated with Fe and Zn content in rice grains.

1.4.2 Specific Objectives

i. To determine agronomic performance of a subset of 36 rice accessions to identify high yielding and stable genotypes for cultivation in Bwegera and Sange, the Eastern Democratic Republic of Congo.
ii. To associate SNP markers with Fe and Zn content in rice grains for application in marker assisted selection.

1.5 Null Hypotheses

i. There are no differences among rice accessions for agronomic performance across locations in Bwegera and Sange, Eastern Democratic Republic of Congo.
ii. There are no associations between SNP markers with Fe and Zn content in rice grains.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy and Origin of Rice

The cultivated rice belongs to the grass family Poaceae, and genus Oryza (Acquaah, 2012). There are two cultivated species of rice in the world namely, *Oryza sativa*, the Asian rice that is grown worldwide and forms the bulk of what is traded in the world; and *Oryza glaberrima*, the African rice, that is grown on a limited scale in West Africa (Khush, 1997). The Asian rice was first domesticated in southern China and northeastern India about 8,000 years ago (Khush, 1987).

The domesticated rice (*Oryza spp*) has wild relatives of the genus Oryza which is divided into four species namely, *O. sativa*, *O. ridleyi*, *O. granulata, and O. officinalis*. Six wild species are included in *O. sativa,* i.e. *O. rufipogon* (established throughout Oceania and Asia), *O. barthii* (is African species and widespread in West Africa), *O. glumaepatula* (is widespread in Central and South America), *O. nivara* (considered to be an ecotype of *O. rufipogon*), *O. meridionalis* (is inhabitant to Australia), and *O. longistaminata* (is also African species and found throughout Africa); Crossings between Asian rice (*O. sativa* L.) and African rice (*Oryza glaberrima* Steud.), have produced "New Rice for Africa" (NERICA) (Somado & Keya, 2008).

2.2 Rice Production

Rice is the most important food crop, feeding more than half of the world's population (Mohidem et al., 2022), across six continents in the world, and is grown in more than 100 countries. It is produced on an estimated area of about 158 million hectares and produces over 700 million tonnes per year (470.6 million tonnes of milled rice) in 2015. Nearly 640 million tonnes (90%) of the world's rice is grown in Asia. China and India are the main rice producers. Latin America and Africa produce around 25 million tonnes each. In sub-Saharan Africa and Asia, irrigated and upland rice are grown on smallholdings of 0.5 to 3 ha per household and in many different environments (Chauhan et al., 2017).

2.3 Rice in the Democratic Republic of Congo (DRC)

In the DRC, rice is one of the principal food crops (Sharp, 2016). It is the seventh most important crop, in terms of consumption, after cassava, plantain, fruits, maize, groundnut and vegetables (Ministry of Agriculture and Rural Development, 2013) and is grown on an estimated area of about 450,000 ha (Sharp, 2016). According to the Ministry of Agriculture and Rural Development (2013), both rainfed and irrigated rice genotypes are grown in the country. In terms of area planted, the rainfed types occupy 98% of the land while the irrigated types occupy 2% of the land. The highest quantities of rice come from four provinces (Orientale Province, Maniema, Equator, and Oriental Kasai). Irrigated rice is found in Kinshasa, in Bas-Congo (Mbanza-Ngungu, Mawunzi), in Orientale Province (Kisangani), in Equator Province (Mbandaka, Bumba) (Table 2.1.), and the Ruzizi plain.

Table 2.1: Paddy Rice Production (in tons) by Province from 2016 to 2019 in the DRC

	Years			
Provincies	**2016**	**2017**	**2018**	**2019**
Kinshasa	7983	8,550	9158	9,809
Kongo – Central	65,125	69,756	74,717	80,031
Kwango	7926	8,489	9,092	9,739
Kwilu	55,833	59,807	68,314	73,172
Maï – Ndombe	16,008	17,146	18,365	19,671
Equateur	52	55	59	63
Sud – Ubangi	6,884	7,374	7,894	8,455
Nord – Ubangi	6,455	6,914	7,406	7,933
Mongala	49,122	52,615	56,357	60,365
Tshuapa	15,935	17,068	18,282	19,582
Tshopo	104,247	111,661	119,602	128,108
Bas – Uele	83,397	89,328	95,681	102,486
Haut – Uele	158,975	170,281	182,391	195,363
Ituri	52,127	55,834	59,805	64,058
Nord – Kivu	42,871	91,945	49,184	52,682
Sud – Kivu	81,920	87,746	93,986	100,670
Maniema	163,108	174,708	187,133	200,442
Haut – Katanga	3,707	3,771	4,039	4,326
Lualaba	5,552	5,946	6,369	7,275
Haut – Lomami	3,288	3,521	3,771	4,039
Tanganika	30,170	32,315	34,613	37,075
Kasaï – Oriental	-	-	-	-
Sankuru	117,806	126,184	144,132	154,383
Lomami	2,229	2,546	2,727	2,921
Kasaï – Central	24,815	26,579	2,847	3,049
Kasaï	26,975	28,893	30,948	33,149
DRC	**1132,510**	**1,213,006**	**1,286,872**	**1,378,846**

Source: (National Institute of Statistics, 2020)

2.3.1 Constraints of Rice Cultivation

The main constraints of rice growing in the DRC include low soil fertility; diseases (blast and brown spot) predators (birds, rodents and insects); climatic disturbances (drought, reduction in sunshine following the strong cloud cover); and poor cultivation

systems (in particular shifting cultivation on slash-and-burn, the use of inefficient space to exploit) (Ministry of Agriculture and Rural Development, 2013).

2.4 Variation in Agronomic Traits

The crop variety, the phenology, and the crop species's growth stage determine the different responses of the crops observed in varying environments. Those responses of genotypes across environments are some of the main challenges facing plant breeders (Zewdu et al., 2020). Thus, when studying the stability of rice accessions, traits such as days to flowering, plant height, 1000-grain weight, number of panicles per plant, panicle length, number of grains per panicle, and grain yield are usually considered (Al-kordy et al., 2019; Aryawati et al., 2021).

Limited knowledge exists regarding the environmental influences on the timing of flowering in rice. In a recent study, Julia & Dingkuhn (2012) sought to elucidate the climatic effects on the timing and duration of anthesis. Their study revealed that the timing of flowering in rice is controlled at the individual spikelet level. Anthesis events within a panicle are dispersed across an extended period, likely attributable to variations in physiological age and topological position among spikelets. The size and shape of grains constitute crucial factors influencing both the yield and quality of grains, and they have been subjects of selection since the early domestication of cereals. During the rice grain filling stage, the *OsSPL16* gene is responsible for encoding a protein that acts as a positive regulator of cell proliferation. Elevated expression of this gene promotes cell division and enhances grain filling, leading to positive effects on both grain width and overall yield in rice (Wang et al., 2012). The performance of rice genotypes in terms of grain yield is significantly influenced by environmental factors such as climate and soil conditions. The variability in climatic factors plays a substantial role in the observed variations in grain yield (Shrestha et al., 2020).

Yao et al. (2012) in their study on agronomic performance of high-yielding rice variety grown under alternate wetting and drying irrigation showed that the “Super” hybrid rice varieties do not necessarily require more water input to produce a high grain yield.

The increasing of the number of spikelets per panicle should be a primary target for breeding high-yielding rice varieties for alternate wetting and drying conditions.

A study by Rono (2018) showed that at $P<0.05$, rice genotypes were significantly different for the evaluated agronomic variables and yield traits except the filled grains and 1000 grain weight. And the significant variation was observed between seasons for plant height and filled grains.

2.5 Rice Grain Nutritional Quality

Other than satisfying the caloric requirements of millions of people daily (Sweeney & McCouch, 2007), rice serves as the primary source of protein, thiamine, riboflavin, niacin, and essential micronutrients, such as Fe and Zn in the diet (Ali & Wani, 2021; Mohidem et al., 2022). However, rice has been reported to have a relatively low content of Fe and Zn, particularly when the paddy undergoes processing (Yadav et al., 2021).

2.5.1 Micronutrients

Micronutrients include essential minerals and vitamins required in small quantities and that have great importance for growth and healthy development. In the maintenance of tissue function, healthy immune functions and in metabolism, micronutrients play a central role and they are subdivided into two groups (macro and micro minerals). The required amount of macro minerals in the body is 100mg/day while micro minerals (iron, cobalt, chromium, copper, fluoride, iodine, manganese, selenium, zinc and molybdenum) are required in the body in amounts less than 50mg/day (Celep et al., 2017) .

The intake insufficiency of micronutrients such as Fe and Zn through staple foods results in malnutrition. The basis for the productive, good health, and longevity of life is nutrient sufficiency (Narwal et al., 2017). Soil fertilizer and water management are responsible for the micronutrients concentration in grain, and Fe and Zn are affected by the combination of those both factors (Damian, 2016).

2.5.1.1 Fe and Zn Micronutrients

Iron is the fourth most abundant element on earth (Frey & Reed, 2012). Despite of this, it is one of the most widespread nutritional disorder in the world among humans. Iron is vital for various biological functions in the human body, including the synthesis of oxygen transport proteins (Abbaspour et al., 2014 and Jones, 2020) while Zn deficiency affects multiple systems in the human body, including epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems (Roohani et al., 2013)..

Humans obtain their Fe and Zn requirements through the food eaten. Breeding for higher concentrations of Fe and Zn in the grains is possible as there is sufficient genotypic variation in the rice germplasm. Previously, researchers mainly aimed at production of high yielding varieties. But nowadays the focus has been shifted to enrichment of micronutrients (Fe and Zn) in staple food crops that helps in ameliorating the micronutrient deficiency problem (hidden hunger) in the human population (Kiranmayi et al., 2014).

2.6 Micronutrients Analysis

2.6.1 Methods of Analysis for Iron

. There are different techniques for measuring iron in food:

- Iron spot test: Performed to determine the presence of iron from fortification, regardless of its type, in flour. It is a qualitative method (Nichols et al., 2012).
- Quantitative method for determining soluble iron from ferrous sulfate (Lee & Ydesdale, 1979).
- Quantitative method for determining total iron. This method is highly selective; visible spectrophotometry or atomic absorption spectrophotometry (AAS) are utilized for quantifying (Shongwe, 2007).

The procedure used in those analysis include the digestion of a sample to destroy organic matter and reduce complex molecules to their elements, using either wet or

dry digestion. A solution from the digested product is then prepared with diluted acid and then the iron content is measured using Atomic Absorption (AA) (USAID, 2010).

2.6.2 Method of Analysis for Zinc

Under pressure in a closed vessel, a sample to be analyzed for Zn content is digested using HNO_3 and H_2O and heated in a microwave. The solution is diluted by adding H_2O in the digested sample and then Zn is determined by Atomic Absorption Spectrophotometer (Jorhem & Engman, 2000).

2.6.3 Variation in Iron and Zinc Content in Rice Grain

One hundred and fifty nine rice genotypes were studied by Maganti et al. (2019) in a single season and their iron and zinc content analyzed for both brown and polished rice. The results indicated that the concentration varied from 6.9 to 22.3 mg/kg for Fe, and from 14.5 to 35.3 mg/kg for Zn, in unpolished, brown rice.

Pippal et al. (2018) investigated the substantial variability in grain yield-related traits and Fe/Zn contents in rice. This study focused on seeds harvested from the F_4 and BC_1 F_3 generations, where the F_5 (278) and BC_1 F_4 (212) plants were developed through the crossbreeding of PAU201 (high yielding) and Palman 579 (iron-rich) varieties, specifically selected for their Fe-Zn richness. The parents exhibited notable differences in iron and zinc content. Palman 579 demonstrated higher levels with 332.8 µg/g iron and 51.4 µg/g zinc, whereas PAU201 displayed lower values at 51.5 µg/g for iron and 25.0 µg/g for zinc. Substantial variations in iron and zinc content were observed within the F_5 and BC_1 F_4 populations. The mean performance of the F_5 population indicated elevated values for both iron and zinc content.

A panel of 192germplasm lines was evaluated by Parikh et al. (2019) for iron and zinc content and the variation recorded among the diverse rice germplasm accessions of brown rice were 6.3 µg/g -24.5 µg/g for Iron and 15.4 µg/g -39.40 µg/g for Zinc, while polished rice grains had 0.1 µg/g -6.7 µg/g for Iron and 13.1 µg/g -32.6 µg/g for Zinc. Similar results were reported by Anuradha et al. (2012) with the concentration ranging from 6.2 ppm to 71.6 ppm for iron and from 26.2 ppm to 67.3 ppm for Zinc.

A significant correlation (-0.25) was recorded between Zn concentration and grain elongation. Further, the wild accessions had the highest Fe and Zn contents.

2.7 Genetic Mechanisms of Iron and Zinc Uptake in Rice

Plants have specialized mechanisms for the uptake and accumulation of Fe and Zn from the soil. They secrete phytosiderophores (PSs) to chelate Fe(III) and form Fe(III)-PS complexes, which are then transported into the plant through specific plasma membrane transporters (Tsednee et al., 2012). Rice also has mechanisms to directly uptake Fe(II) and chelated Zn, using the mugineic acid (MA) family. Zinc uptake occurs through specific plasma membrane transporters, and it can also enter the root directly as ionized Zn(II) (Gao & Xiong, 2018).

The genetic control for metal transport has been studied in rice and *Arabidopsis*, which identified the founding members of metal transporters, specifically focusing on Fe and Zn. These include the zinc-regulated transporter/iron-regulated transporter (ZRT/IRT)-related protein (ZIP) family (Guerinot, 2000), the natural resistance-associated macrophage protein (NRAMP) family, the cation diffusion facilitator (CDF) family, the major facilitator superfamily (MFS), the P_{1B}-type heavy metal ATPase (HMA) family, the vacuolar Fe transporter (VIT) family, and the cation exchange (CAX) family (Bashir et al., 2016).

The identification of the molecular factors influencing mineral uptake and transport as well as understanding the genes that regulate mineral homeostasis and localization in rice, would greatly contribute to the development of focused breeding approaches for biofortification purposes.

2.8 Molecular Markers

Molecular marker-assisted breeding is the application of molecular biotechnologies to alter and improve living organism (plants or animals) traits on the basis of genotypic assays (Jiang, 2013). The molecular markers assist indirectly the selection of a target traits; that are closely linked to underlying genes or that have been developed from the actual gene sequences (Xu & Crouch, 2008).

Molecular markers have a major role in molecular breeding. Depending on the type of DNA markers, molecular markers are divided into three groups namely a) hybridization-based markers; b) polymerase chain reaction (PCR) based markers; and c) DNA sequence-based markers among which the Single Nucleotide Polymorphisms (SNPs) markers are found (Shabir et al., 2017).

Single nucleotide polymorphism is based on differences between DNA sequences or individuals, also is among the techniques that have been used for application to plant breeding. SNPs can be placed into nucleotide substitutions either as transitions (G/A or C/T) or transversions (C/G, A/T, T/G or C/A). Practicaly, single base variants in cDNA (mRNA) are SNPs. They can occur through insertions and deletions in the genome. SNPs may be present within coding or non-coding regions of genes or in the intergenic regions (Jiang, 2013). The identification of SNPs markers for bio fortification in rice has been performed by Giraldo et al. (2008).

SNPs are linked to genes with a simplest form for polymorphism. They are a potential genetic markers in plant breeding and genetic study. They are also co-dominant markers (Jiang, 2013).

2.9 Association Mapping in Plants

Genome-wide association studies (GWAS) are a valuable method for identifying the genetic basis of phenotypic variation (Burghardt et al., 2017). This approach has the potential to pinpoint single polymorphisms within genes responsible for phenotypic differences. It involves searching for genotype-phenotype correlations among unrelated individuals, such as natural populations and collections of landraces, breeding materials, and varieties (Cerda & Cloutier, 2012).

GWAS is a highly effective tool for elucidating the genetic basis of traits in plants (Brachi et al., 2011). It has been widely utilized for identifying quantitative trait loci (QTL) for grain quality traits, including Fe and Zn content in rice (Gao & Xiong, 2018; Islam et al., 2022; Nawaz et al., 2015), wheat (Rathan et al., 2022), Aegilops tauschii (the wild progenitor of bread wheat) (Arora et al., 2019), and beans (Delfini et al., 2021). Additionally, QTLs play a pivotal role in controlling a wide array of traits with

significance in both medical and agricultural domains, encompassing factors like disorders, crop yield, and plant resilience against stress (Isobe et al., 2007).

To detect relationships between marker loci and numerous phenotypes, several statistical models, including the Mixed Linear Model (MLM) (Zhang et al., 2010) and Fixed and random model Circulating Probability Unification (FarmCPU) (Liu et al., 2016), are available and commonly employed. The inclusion of covariates for structure and kinship in the statistical model can effectively control for confounding factors (Kaler et al., 2020).

2.10 Candidate Gene Selection

Gene identification entails the in silico process of selecting genes with prior associations to the trait of interest. The candidate gene selection approach initiates by selecting a potential gene candidate, guided by its relevance within the mechanism of the investigated trait (Patnala et al., 2013).

Linkage Disequilibrium (LD), which represents the nonrandom association of alleles at different loci (Flint-Garcia et al., 2003), describes how the correlation between alleles at different loci weakens with increasing physical distance between those loci along a chromosome (Vos et al., 2017). It plays a pivotal role in pinpointing candidate genes in Genome-wide association studies (GWAS). Li et al. (2018) pioneered a cluster-based GWAS method leveraging LD network analysis and principal component analysis for streamlined association tests. Teo et al. (2009) introduced a technique for genome-wide examination of LD disparities among populations, aiding in the identification of regions harboring candidate genes exhibiting distinct LD patterns.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Agronomic Performance Experiment of Rice Accessions of the Eastern DRC

3.1.1 Plant Materials and Field Experiment

The experiment was conducted using a subset of 36 rice accessions from different sources (Table 3.1) and maintained by the rice breeding program of the Institut National pour l'Etude et la Recherche Agronomiques (INERA), in Eastern DRC. The experiment was conducted in two rice growing sites of the Ruzizi plain in the Eastern DRC, namely Bwegera site (at S 02º54.510' and E 029º01.639' altitude : 932m) and Sange site (at S03°04.360′ and E029°08.243′, altitude : 910m) under irrigated condition. The PBTools (Version 1.4) software developed by IRRI were used to randomize treatments in each replication and each site and produce the layouts per site. The experiment was laid out in a 6 x 6 partially balanced lattice design with three replications of 10m x 8.5m separated from each other by 1m path. And in each replication, 6 blocks were separated from each other by 0.5m. Each block consisted of 6 plots each measuring 1m x 1.25m separated by 0.5m from each other. Each plot of 1m x 1.25m represents a treatment. During transplanting, in each plot, 30 seedlings (21 days old) were transplanted at 25cm x 25cm spacing. To identify plots within replications, labels were produced and installed in each sites. The soils in Bwegera and Sange are clay loam soils. Two fractions of urea (46% N) at a rate of 60 kg ha^{-1} were utilized for fertilization purposes, at 4 weeks and 8 weeks after transplantation. These quantities were kept constant in each site. Weed control was done by hand picking each time before the application of fertilizer while harvesting was done manually.

Table 3.1: List of Rice Accessions Used for the Agronomic Performance Trial

Sr. No	Accession name	Source/program	N°	Genotype name	Source/program
1	ARS134-B-1-1-5-B	AfricaRice	19	ARS79-5-11-11	AfricaRice
2	Orylux7-1	AfricaRice	20	V18/RRS126-48-1-13-2	AfricaRice
3	WAHX14N-926	AfricaRice	21	Orylux11	AfricaRice
4	MR254	AfricaRice	22	ARS134-B-B-B	AfricaRice
5	Golmy	AfricaRice	23	Magoti	Local landrace
6	ARS848-15-3-2-4	AfricaRice	24	Runingu	Local landrace
7	IR93348:32-B-15-3-B-B-B-1	IRRI-Burundi	25	ARS169-2-B-3-B	AfricaRice
8	ARS168-3-B-1-B	AfricaRice	26	ARS134-B-1-1-4	AfricaRice
9	IR88638	IRRI-Burundi	27	IR82574/643-1-2	IRRI-Burundi
10	ARICA12	AfricaRice	28	Orylux5	AfricaRice
11	ARICA3	AfricaRice	29	SAHEL210	AfricaRice
12	IR64-sub-1	IRRI-Burundi	30	IR841	IRRI-Burundi
13	HHZSAL6	AfricaRice	31	ARS39-145/EP-3	AfricaRice
14	ARS755-3-3-1-B	AfricaRice	32	ARS101-4-B-1-1-B	AfricaRice
15	ARS134-B-1-1-5	AfricaRice	33	ARS101-4-B-1-3	AfricaRice
16	IR990-48-B-B-12	IRRI-Burundi	34	NERICA-L-19-Sab-1	AfricaRice
17	IR64-biofortified	IRRI-Burundi	35	ARS756-1-1-3-B-2-2	AfricaRice
18	IR107015-37	IRRI-Burundi	36	ARS563-425-1-B-2-3	AfricaRice

3.1.2 Field Data Collection

Data on different agronomic variables such as Day to Flowering (DTF), Plant Height (PH), Effective number of Tiller (ET), Panicle Length (PL), Grain per Panicle (GP), Thousand Grain weigh (TGW), Grain Length (GL), Grain Width (GW), Ratio Grain Length and Grain Weight (RGLGW), Grain Yield (GY) were taken on five randomly selected rice hills at various stages of plant growth for the subset of the 36 rice accessions.

Days to flowering (days) was recorded after counting the number of days from the transplanting date to the time of 50% of plants in a plot bloomed.

Plant height (cm) was recorded (from five plants) at the time of 50% flowering by measuring the length from the base of the plant to the peak of the main panicle,

Effective number of tillers per plant was recorded (from five plants) after anthesis by counting manually.

Panicle length (cm) was recorded (from five plants) at the mature stage by measuring the length of the panicles.

Grains per panicle was determined by counting manually the number of grain at the entire panicle the harvest time.

Grain yield (g/plot) was recorded (from a maximum of 30 plants and minimum of 10 plants) as the mass of harvested grains from each plot at physiological maturity.

1000-grains weight was recorded after counting manually and weighting using a digital weighing balance.

Grain size (length and width) was recorded (from five grains) (mm) using a digital caliper.

3.1.3 Data Analysis

Analysis of agronomic data for individual site and across two locations (Sange and Bwegera) was performed in the R software. Data for each trait was subjected to analysis of variance (ANOVA) for each site separately and for the combined sites. The traits means were separated by Fischer's unprotected least significant difference LSD at the 5% probability level.

3.2 Genome-wide Association Analysis

3.2.1 Plant Materials and Experiment

The experiment was conducted using a panel of 85 rice accessions from diferent origins (Table 3.2) and maintained by the rice breeding program of the Institut National pour l'Etude et la Recherche Agronomiques (INERA), in Eastern DRC. The accessions were grown in Kalemie (S05°49.770′ and E029°17.442′, altitude: 778 m) and in the Ruzizi plain (S03°04.360′ and E029°08.243′, altitude 910 m) under irrigated conditions. The experiment was laid out in a 17 × 5 lattice design with three replications. The soils at Kalemie are clay loam soils as well as in the Ruzizi plain. Two fractions of urea (46% N) at a rate of 60 kg ha^{-1} were utilized for fertilization

purposes. The Fe and Zn concentration in the soils were 178.26 mg/kg and 17.62 mg/kg; and 118.2 mg/kg and 8 mg/kg, at the Kalemie and Ruzizi plain, respectively.

Table 3.2: List of Accessions Used in the GWAS Experiment

Sr. No	Accession name	Source/ program	Sr. No	Accession name	Source/program
1	Komboka	IRRI-Burundi	44	ARICA2	AfricaRice
2	IR64	IRRI-Burundi	45	NL19	AfricaRice
3	IBEI6	AfricaRice	46	NL14	AfricaRice
4	GIZA128	IRRI-Burundi	47	NL17	AfricaRice
5	Nipponbare	IRRI-Burundi	48	D20-ARS-3-2	AfricaRice
6	Jasmine	IRRI-Burundi	49	IR96279-33-3-1-2	IRRI-Burundi
7	NL59	AfricaRice	50	ARS134-B-1-1-5-B	AfricaRice
8	FKR	AfricaRice	51	Orylux7-1	AfricaRice
9	08FAN10	IRRI-Burundi	52	WAHX14N-926	AfricaRice
10	WAB2066-TGR2	AfricaRice	53	MR254	AfricaRice
11	WAB2066-TGR3	AfricaRice	54	Golmy	AfricaRice
12	IR99084-B-B-13	INERA-DRC	55	ARS848-15-3-2-4	AfricaRice
13	IR127229	IRRI-Burundi	56	IR93348:32-B-15-3-B-B-B-1	IRRI-Burundi
14	IR106172-78 :1-B-B	INERA-DRC	57	ARS168-3-B-1-B	AfricaRice
15	ARS848-15-3-2-3	AfricaRice	58	IR88638	IRRI-Burundi
16	IR106364-B-B-CNUS	INERA-DRC	59	ARICA12	AfricaRice
17	ARS844-24-10-2-B	AfricaRice	60	ARICA3	AfricaRice
18	ARS168-1-B-3-B	AfricaRice	61	IR64-sub-1	IRRI-Burundi
19	ARS851-1-3	AfricaRice	62	HHZSAL6	AfricaRice
20	IR87638-10-2-2-4	INERA-DRC	63	ARS755-3-3-1-B	AfricaRice
21	IR98419-B-B-11	INERA-DRC	64	ARS134-B-1-1-5	AfricaRice
22	IR97071-24-1-1-1	INERA-DRC	65	IR990-48-B-B-12	IRRI-Burundi
23	ARS803-4-5-4-3	AfricaRice	66	IR64-biofortified	IRRI-Burundi
24	IR93856-23-1-1-1	INERA-DRC	67	IR107015-37	IRRI-Burundi
25	ARS790-5-11-1-1	AfricaRice	68	ARS79-5-11-11	AfricaRice
26	IR17015-6-5-3-B1	INERA-DRC	69	V18/RRS126-48-1-13-2	AfricaRice
27	IR106359-B-18-5	INERA-DRC	70	Orylux11	AfricaRice
28	IR95624-B-138-3	INERA-DRC	71	ARS134-B-B-B	AfricaRice
29	IR13A461	IRRI-Burundi	72	Magoti	Local landrace
30	Mugwiza	IRRI-Burundi	73	Runingu	Local landrace
31	Vuninzara	IRRI-Burundi	74	ARS169-2-B-3-B	AfricaRice
32	IR97045-24-1-1-1	IRRI-Burundi	75	ARS134-B-1-1-4	AfricaRice
33	Kigoma	Local landrace	76	IR82574/643-1-2	IRRI-Burundi
34	Makasane	IRRI-Burundi	77	Orylux5	AfricaRice
35	Rukaramu	Local landrace	78	SAHEL210	AfricaRice
36	Musesekara	IRRI-Burundi	79	IR841	IRRI-Burundi

Sr. No	Accession name	Source/program	Sr. No	Accession name	Source/program
37	Yasho-Yasho	Local landrace	80	ARS39-145/EP-3	AfricaRice
38	Kasozi	IRRI-Burundi	81	ARS101-4-B-1-1-B	AfricaRice
39	IR7525	IRRI-Burundi	82	ARS101-4-B-1-3	AfricaRice
40	Orylux7	AfricaRice	83	NERICA-L-19-Sab-1	AfricaRice
41	ART29	INERA-DRC	84	ARS756-1-1-3-B-2-2	AfricaRice
42	Sipi	INERA-DRC	85	ARS563-425-1-B-2-3	AfricaRice
43	CRS36	IRRI-Kenya			

For each genotype, seed samples from the two sites were pooled for micronutrient analysis.

3.2.2 Fe and Zn Content Profiling

Fifteen grams (g) of paddy rice were sampled in duplicate from each accession, dehusked, and milled for Fe and Zn concentration analysis at the Mycotoxin and nutrition platform at the International Livestock Research Institute (ILRI), Nairobi, Kenya. The AOAC official method 985.01 for metals and other elements in plants and pet foods, as described by (Hou et al., 2016), was used. In brief, 500 mg of the milled rice grain samples were weighed in duplicates into 50 mL microwave digestion tubes, then 8.0 mL of concentrated nitric acid and 2.0 mL of 30% hydrogen peroxide were added. The digestion was performed using an Anton Paar Multiwave GO plus microwave digestor (Graz, Austria). The samples were heated at 100 °C for 10 min, the temperature was then increased to 180 °C at a rate of 10 °C/min followed by a 10 min hold. The samples were quantitatively transferred to 25.0 mL flasks and topped to the mark with 2% HNO_3. The extracts were analyzed using the Perkin Elmer Avio 550 Max ICP-OES instrument. For the quantification of Fe and Zn in the extract, ICP-OES mix standard CatNo.43843 (Sigma-Aldrich, Buchs, Switzerland) was used. The serial dilution of the standard was performed using 2% HNO_3 to obtain the calibration standards of 80, 320, 800, and 1600 µg/L, and the external standard calibration method was then applied. The calibration was performed using Perkin Elmer syngistix™ software version 5.1. The obtained data were used to calculate the content for each element in mg/100 g using Microsoft® Excel® by applying the formulae below:

$$X\ (mg/100\ g) = \frac{(C - B) * V * 100}{W * 1000}$$

where X is the individual elemental composition, mg/100 g; C is the concentration of the individual elements, µg/L after external calibration; B is the concentration of the reagent blanks, µg/l used in the extraction; V is the volume digest topped up to 25 mL; 100 is the conversion factor to mg/100 g dilution factor after extraction with 1% HNO_3; W is the weight of the sample used; 1000 is the conversion factor from µg/l to mg/l. The results were corrected for moisture content and reported on the dry weight basis (dwb).

For Quality Control (QC) purposes after every batch of 30 samples (Appendix I), a QC sample T18106QC (infant formula) obtained from Fera Science Ltd. Sand Hutton, York. YO41 1LZ. UK was subjected to the whole pipeline of analysis in duplicate.

3.2.3 Genotypic Data Acquisition and Analysis

Rice grains from 85 genotypes were sown and raised in trays in the nursery at JKUAT. Seedlings of 15 days old were transferred to ILRI and kept before DNA extraction and sequencing at SEQART Platform in ILRI where the harvested and conserved leaves were submitted to the Geno grinder machine to grind samples, and genomic DNA was extracted from the ground rice sample using the NucleoMag®Plant Kit for DNA extraction. Genotypic data were then acquired using DArTseq technology, as previously mentioneded by (Kimwemwe et al., 2023). Briefly, The DArTseq procedure utilized complexity reduction approach for library construction, which involved digesting genomic DNA from rice accessions with two restriction enzymes (PstI and MseI), ligating barcoded adapters, and amplifying the adapter-ligated fragments using polymerase chain reaction (PCR) to generate a library of DNA fragments for sequencing (Kilian et al., 2012).

The libraries were then sequenced with HiSeq2500 (Illumina, San Diego, CA, USA) and the resulting sequences were scored for DArTseq markers using an in-house marker scoring pipeline, DArTsoft14. The sequenced reads were then aligned to the rice reference genome version 7.0, from the Rice Genome Annotation Project (RGAP)

database (Kawahara et al., 2013) to detect Single Nucleotide Polymorphism (SNP) markers and their corresponding chromosome and physical positions.

3.2.4 Evaluation of Linkage Disequilibrium Decay

The genome-wide linkage disequilibrium pattern for our diverse panel, based on the retained SNP markers after filtering, were estimated using Trait Analysis by Association Evolution, and Linkage (TASSEL) software version 5.2.88 (Bradbury et al., 2007). Pairwise association among all the SNP markers were calculated to obtain the correlation coefficient (r^2). The averages of all the r^2 across each of the 12 rice chromosomes were plotted against the physical distance of the SNPs using R software (R Core Team, 2022) to estimate the Linkage Disequilibrium (LD) decay simulation curve.

3.2.5 Fe and Zn Content Data Analysis

The distribution of the evaluated rice accessions based on Fe and Zn content was plotted using rcompanion package of R software (Mangiafico, 2016). The descriptive statistics including the median, mean, range, and the standard deviation were computed using the moments package of R software (Komsta & Novomestky, 2015). To understand the central tendency in the dataset used in this study, the mode was calculated using the dplyr package in R software (Wickham et al., 2019). The average values from the two replicates for each micronutrient were used for the Genome-Wide Association Analysis.

3.2.6 Genome-Wide Association Analysis

Genome-Wide Association Studies (GWAS) for each micronutrient was performed with rMVP package of R software (Yin et al., 2021) using two linear models; Mixed Linear Model (MLM) and fixed and random model circulating probability unification (FarmCPU). According to VanRaden (VanRaden, 2008), the kinship matrix and the principal components (top five) internally generated by the rMVP package were used as covariates within the rMVP package. The association between Fe and Zn content and genotype data were visualized using Manhattan plots. To obtain the estimated

number of independent tests, the total length of the chromosomes (43,225,920 bp) was divided by the average LD decay distance (401,947 bp) observed in the association panel utilized for this study, resulting in 107.54 independent tests.

To obtain a type I error probability of 5%, a significance threshold was calculated by dividing 0.05 by the estimated number of independent tests (107.54) to obtain a threshold of 4.649×10^{-4}.

3.2.7 Candidate Genes Identification

After the GWAS, candidate genes for Fe and Zn content in rice grains were identified and annotated from the Oryza sativa reference genome version 7.0, available in the MSU-Rice Genome Annotation Project (RGAP) database (Kawahara et al., 2013). All the genes within the LD decay window, upstream and downstream, of the significant SNPs were treated as potential candidate genes associated with Fe and Zn content. Based on functional annotations of the identified genes, the genes belonging to transporter activity and transcription regulator activity involved in Fe and Zn homeostasis were identified and selected. Publicly available RNA-seq data for rice found in Rice Expression Database (RED) (Xia et al., 2017) were mined for potential expression changes across all the selected candidate genes.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Analysis of Variance (ANOVA) for Agronomic Traits

The results of the combined ANOVA for all the measured agronomic traits variables are summarized in Table 4.1. The accession x site interaction effects were found to be highly significant ($p < 0.001$) for only the trait Thousand Grain Weight (TGW). Highly significant differences were observed among the accessions and sites for all the measured agronomic variabless, except for Grain Yield (GY), Panical Length (PL), Grain Length (GL), Grain Width (GW), and Ratio Grain Length and Grain Width (RGLGW).

Table 4.1: Mean Squares and F-tests for Agronomic Traits among 36 Rice Genotypes Evaluated at two Locations in Ruzizi Plain of Eastern DRC

Source of variation	Df	DTF	PH (cm)	ET	PL (cm)	GP	TGW (g)	GL (cm)	GW (cm)	RGLGW	GY (Tone/ha)
Site	1	450.7 ***	3707 ***	153.4 **	6.469	8325 ***	21.282 **	0.08	0.0157	0.0343	13.108 ***
Replication	2	88.4 **	228	182.6 ***	10.36 *	2065 *	0.254	0.045	0.04281 **	0.3373 *	1.36 **
Accession	35	86.0 ***	611 ***	44.69 ***	20.749 ***	3961 ***	27.795 ***	2.8069 ***	0.02257 ***	0.8919 ***	0.181
Block	15	9.3	64	17.34	1.925	432	5.819**	0.227	0.007	0.0787	0.166
Accession x site	35	20.4	58	23.04	2.635	954	4.043*	0.289	0.0103	0.1265	0.136
Residual	132	17.5	114	20.33	2.538	636	2.536	0.263	0.0085	0.0993	0.192
Minimum		84	55	11	18.5	67	20.89	7.69	1.68	3.55	0.12
Maximum		119	137.6	40	31.5	270	33.76	12.41	2.28	6.44	2.52
Mean		103.7	86.26	21.14	24.2	155.35	26.31	9.61	2.02	4.78	1.22
CV (%)		5.39	16.39	24.43	9.75	22.89	10.19	8.55	5.28	10.09	40.87

Df : Degree of freedom, DTF: Day to flowering, PH: Plant height, ET: Effective number of tiller, PL: Panicle length, GP: Grain per panicle, TGW: Thousand Grain weigh, GL: Grain Length, GW: Grain width, RGLGW: Ratio Grain Length and Grain Weight, GY: Grain Yield, CV: Coefficient of Variation. * significant at .05 , ** significant at .01, ***Significant at .001

4.1.2 Performance of Accessions for Agronomic Traits

The accessions displayed varying agronomic performance across and within the two sites (Table 4.2, Table 4.3 and Table 4.4). The DTF ranged between 99 to 114 days at Bwegera, and between 87 to 111 days at Sange. Accession IR88638 was the earliest to reach 50% flowering, after 99 and 87 days, at the Bwegera and Sange site, respectively. The same accession (IR88638) was the earliest across both sites. Accession ARS169-2-B-3-B and HHZSAL6 were the slowest to flower, taking 114 and 111 days at the Bwegera and Sange sites, respectively.

In the Bwegera site the PH ranged between 61.9 and 118.3 cm, with a mean of 82.1 cm, and in the Sange site it ranged between 71.7 and 122.1 cm, with a mean of 90.9 cm. Accessions ARS168-3-B-1-B, ARS563-425-1-B-2-3, ARS134-B-1-1-5, ARS39-145/EP-3 and NERICA-L-19-Sab-1 were among the top 10 shortest accessions with a PHs of 65.7 cm, 68.7. cm, 71.5 cm, 72.5 cm and 72.9 cm; and accessions RUNINGU and MAGOTI were the tallest in both sites while accessions ARS168-3-B-1-B, ARS134-B-1-1-5, ARS101-4-B-1-3, ARS848-15-3-2-4 and ARS134-B-1-1-5 were the shortest accessions with a PHs of 71.7 cm, 76 cm, 76.1 cm, 77.1 cm and 76.6 cm, respectively.

In terms of tillering, the accessions ARS134-B-1-1-5, WAHX14N-926, IR88638, ARS101-4-B-1-3 and NERICA-L-19-Sab-1 produced the most tillers per hill at the Bwegera site, and accessions ARS101-4-B-1-3, WAHX14N-926, SAHEL210, and ARS848-15-3-2-4 produced less tillers per hill while the commercial varieties ARS134-B-B-B and Runingu produced 15 and 15 tillers, respectively, in the Bwegera and Sange site respectively.

The trait PL ranged from 19.1 to 27.7 cm and from 21.3 to 28.4 cm, with the mean of 24 cm and 24.4 cm in the Bwegera and Sange site, respectively. Accessions ARS134-B-1-1-5 and IR841 had a shortest and highest PL value of 27.7 cm and 28.4 cm, respectively, in both sites.

Table 4.2: Mean Values for Agronomic Traits of 36 Rice Accessions Evaluated at Bwegera Ranked Based on GY

Sr. No	Accession	DTF	PH (cm)	ET	PL (cm)	GP	TGW (g)	GL (mm)	GW (mm)	RGLGW	GY (T/Ha)
16	IR990-48-B-B-12	106	88.6^{abc}	23^{ab}	23.8^{abcdefghi}	185^{abcd}	26.84^{bcdefghi}	9.35^{efghij}	2.140	4.373^{fgh}	1.81^{a}
23	Magoti	113	98.7^{ab}	19^{ab}	24.6^{abcdefgh}	174^{abcde}	28.74^{bcde}	9.274^{fg}hij	2.093	4.433^{fgh}	1.35^{ab}
9	IR88638	99	83.3^{bc}	25^{ab}	19.1^{j}	129^{cde}	29.4^{abc}	9.387^{efghij}	2.150	4.373^{fgh}	1.32^{ab}
30	IR841	103	82.9^{bc}	23^{ab}	27.8^{a}	214^{ab}	22.96^{lmno}	9.447^{efghij}	1.940	4.877^{cdefg}	1.28^{ab}
3	WAHX14N-926	103	75.2^{bc}	27^{ab}	23.3^{defghi}	141^{bcde}	22.29^{no}	8.624^{ijk}	1.997	4.327^{gh}	1.21^{ab}
34	NERICA-L-19-Sab-1	104	72.6^{bc}	24^{ab}	23.1^{defghij}	124^{cde}	25.82^{fghijk}	9.18^{ghij}	1.990	4.62^{defgh}	1.19^{ab}
31	ARS39-145/EP-3	100	71.5^{bc}	20^{ab}	22^{efghij}	110de	28^{bcdef}	9.504^{efghi}	2.017	4.72^{cdefg}	1.18^{ab}
27	IR82574/643-1-2	108	88.9^{abc}	20^{ab}	24.4^{abcdefgh}	226^{a}	24.16^{ijklmno}	9.63^{cdefghi}	1.960	4.92^{bcdefg}	1.16^{ab}
35	ARS756-1-1-3-B-2-2	103	81.4^{bc}	21^{ab}	25.8^{abcde}	153^{abcde}	29.35^{abc}	10.724^{bc}	2.010	5.347^{bcde}	1.15^{ab}
15	ARS134-B-1-1-5	103	68.7^{bc}	31^{a}	20.8^{hij}	125^{cde}	24.08^{ijklmno}	9.044^{ghij}	1.947	4.653^{defgh}	1.12^{ab}
11	ARICA3	103	84.6^{bc}	21^{ab}	26.6^{abcd}	167^{abcde}	24.27^{hijklmno}	9.134^{ghij}	2.083	4.397^{fgh}	1.11^{ab}
12	IR64-sub-1	101	77.3^{bc}	22^{ab}	22.7^{defghij}	108de	27.12^{bcdefg}	10.124^{bcdefg}	2.093	4.853^{cdefg}	1.1^{ab}
4	MR254	109	81^{bc}	17^{ab}	25.5^{abcdef}	153^{abcde}	27.07^{bcdefgh}	10.317^{bcdef}	2.007	5.147^{bcdefg}	1.04^{ab}
26	ARS134-B-1-1-4	108	89.2^{abc}	16^{b}	25.3^{abcdefg}	168^{abcde}	26.61^{cdefghij}	9.234^{fghij}	2.010	4.597^{efgh}	1.01^{ab}
17	IR64-biofortified	109	93.9^{abc}	16^{b}	26.2^{abcd}	189^{abcd}	26.19^{efghijk}	8.82^{hijk}	1.997	4.427^{fgh}	0.99^{ab}
22	ARS134-B-B-B	112	82.2^{bc}	15^{b}	23.9^{abcdefghi}	180^{abcde}	29.47^{ab}	9.51^{efghi}	2.143	4.45^{fgh}	0.98^{ab}
7	IR93348:32-B-15-3-B-B-B-1	102	84.5^{bc}	23^{ab}	21.6^{fghij}	132^{bcde}	21.59^{o}	9.797^{cdefgh}	1.943	5.05^{bcdefg}	0.97^{ab}
18	IR107015-37	103	82.2^{bc}	16^{b}	22.6^{defghij}	150^{abcde}	26.5^{defghij}	9.55^{efghi}	2.040	4.683^{cdefgh}	0.96^{ab}
2	Orylux7-1	101	78.2^{bc}	21^{ab}	22.5^{defghij}	101^{e}	24.83^{ghijklmn}	10.03^{bcdefg}	1.993	5.037^{bcdefg}	0.92^{ab}
5	Golmy	101	78.7^{bc}	22^{ab}	25.7^{abcdef}	113^{cde}	29.3^{abcd}	9.96^{bcdefg}	2.127	4.697^{cdefgh}	0.92^{ab}
13	HHZSAL6	110	93.8^{abc}	21^{ab}	26.6^{abcd}	160^{abcde}	24.87^{ghijklmn}	8.54^{ijk}	1.950	4.38^{fgh}	0.91^{ab}
24	Runingu	106	118.3^{a}	18^{ab}	24.2^{abcdefgh}	138^{bcde}	31.85^{a}	10.427^{bcde}	2.027	5.203^{bcdef}	0.9^{ab}

Sr. No	Accession	DTF	PH (cm)	ET	PL (cm)	GP	TGW (g)	GL (mm)	GW (mm)	RGLGW	GY (T/Ha)
10	ARICA12	109	86.4^{abc}	21^{ab}	23.5^{bcdefghi}	162^{abcde}	28.25^{bcdef}	10.007^{bcdefg}	2.030	4.947^{bcdefg}	0.88^{ab}
20	V18/RRS126-48-1-13-2	107	80.1^{bc}	18^{ab}	23.3^{cdefghi}	129^{cde}	26.34^{efghijk}	9.61^{defghi}	1.897	5.103^{bcdefg}	0.88^{ab}
19	ARS79-5-11-11	104	81.8^{bc}	18^{ab}	25.8^{abcde}	134^{bcde}	27.55^{bcdefg}	10.664^{bcd}	1.930	5.53^{abc}	0.86^{ab}
32	ARS101-4-B-1-1-B	106	88^{abc}	23^{ab}	27.4^{abc}	159^{abcde}	22.59^{mno}	10.957^{b}	1.903	5.76^{ab}	0.84^{ab}
6	ARS848-15-3-2-4	111	72.9^{bc}	18^{ab}	21.3^{ghij}	114^{cde}	22.4^{mno}	7.904^{k}	2.063	3.843^{h}	0.8^{ab}
1	ARS134-B-1-1-5-B	108	81^{bc}	20^{ab}	27.6^{ab}	195^{abc}	23.9^{jklmno}	10.297^{bcdef}	1.883	5.477^{abcd}	0.77^{ab}
28	Orylux5	102	77.2^{bc}	19^{ab}	26.6^{abcd}	108de	27.42^{bcdefg}	12.207^{a}	1.943	6.29^{a}	0.76^{ab}
29	SAHEL210	104	88^{abc}	24^{ab}	23.5^{bcdefghi}	148^{abcde}	26.58^{cdefghij}	9.597^{defghi}	2.053	4.677^{cdefgh}	0.76^{ab}
21	Orylux11	101	83.6^{bc}	18^{ab}	24.1^{abcdefgh}	159^{abcde}	25.14^{ghijklm}	9.787^{cdefgh}	2.057	4.763^{cdefg}	0.75^{ab}
25	ARS169-2-B-3-B	114	74.2^{bc}	19^{ab}	23.6^{bcdefghi}	173^{abcde}	25.8^{fghijkl}	9.124^{ghij}	2.043	4.477^{fgh}	0.74^{ab}
14	ARS755-3-3-1-B	109	86.3^{abc}	23^{ab}	26.2^{abcd}	175^{abcde}	25.02^{ghijklmn}	9.144^{ghij}	1.957	4.69^{cdefgh}	0.7^{ab}
33	ARS101-4-B-1-3	111	74.6^{bc}	23^{ab}	24.4^{abcdefgh}	137^{bcde}	27.5^{bcdefg}	9.194^{ghij}	2.043	4.5^{efgh}	0.69^{ab}
36	ARS563-425-1-B-2-3	104	65.8^{bc}	17^{ab}	21.6^{fghij}	126^{cde}	23.55^{klmno}	8.397^{jk}	1.883	4.51^{efgh}	0.67^{ab}
8	ARS168-3-B-1-B	101	62^{c}	18^{ab}	19.9^{ij}	121^{cde}	22.76^{mno}	8.83^{hijk}	1.947	4.543^{efgh}	0.49^{b}
	Minimum	99	62	15	19.1	101	21.59	7.904	1.883	3.843	0.49
	Maximum	114	118.3	31	27.8	226	31.85`	12.207	2.150	6.29	1.81
	Mean	106	82.2	21	24.1	150	26	9.593	2.008	4.796	0.98
	LSD at 5%	-	33.1	15	4.2	83	2.85	1.095	-	0.868	1.18
	P-value	1	0.1	1	0.1	1	0.01	0.001	5E-02	0	0.06

Table 4.3: Mean Values for Agronomic Traits of 36 Rice Accessions Evaluated at Sange Ranked Based on GY

Sr. No	Accession	DTF	PH (cm)	ET	PL (cm)	GP	TGW (g)	GL (mm)	GW (mm)	RGLGW	GY (T/Ha)
10	ARICA12	109	86.4^{abc}	21^{ab}	23.5^{bcdefghi}	162^{abcde}	28.25^{bcdef}	10.007^{bcdefg}	2.030	4.947^{bcdefg}	0.88^{ab}
20	V18/RRS126-48-1-13-2	107	80.1^{bc}	18^{ab}	23.3^{cdefghi}	129^{cde}	26.34^{efghijk}	9.61^{defghi}	1.897	5.103^{bcdefg}	0.88^{ab}
19	ARS79-5-11-11	104	81.8^{bc}	18^{ab}	25.8^{abcde}	134^{bcde}	27.55^{bcdefg}	10.664^{bcd}	1.930	5.53^{abc}	0.86^{ab}
32	ARS101-4-B-1-1-B	106	88^{abc}	23^{ab}	27.4^{abc}	159^{abcde}	22.59^{mno}	10.957^{b}	1.903	5.76^{ab}	0.84^{ab}
6	ARS848-15-3-2-4	111	72.9^{bc}	18^{ab}	21.3^{ghij}	114^{cde}	22.4^{mno}	7.904^{k}	2.063	3.843^{h}	0.8^{ab}
1	ARS134-B-1-1-5-B	108	81^{bc}	20^{ab}	27.6^{ab}	195^{abc}	23.9^{jklmno}	10.297^{bcdef}	1.883	5.477^{abcd}	0.77^{ab}
28	Orylux5	102	77.2^{bc}	19^{ab}	26.6^{abcd}	108de	27.42^{bcdefg}	12.207^{a}	1.943	6.29^{a}	0.76^{ab}
29	SAHEL210	104	88^{abc}	24^{ab}	23.5^{bcdefghi}	148^{abcde}	26.58^{cdefghij}	9.597^{defghi}	2.053	4.677^{cdefgh}	0.76^{ab}
21	Orylux11	101	83.6^{bc}	18^{ab}	24.1^{abcdefgh}	159^{abcde}	25.14^{ghijklm}	9.787^{cdefgh}	2.057	4.763^{cdefg}	0.75^{ab}
25	ARS169-2-B-3-B	114	74.2^{bc}	19^{ab}	23.6^{bcdefghi}	173^{abcde}	25.8^{fghijkl}	9.124^{ghij}	2.043	4.477^{fgh}	0.74^{ab}
14	ARS755-3-3-1-B	109	86.3^{abc}	23^{ab}	26.2^{abcd}	175^{abcde}	25.02^{ghijklmn}	9.144^{ghij}	1.957	4.69^{cdefgh}	0.7^{ab}
33	ARS101-4-B-1-3	111	74.6^{bc}	23^{ab}	24.4^{abcdefgh}	137^{bcde}	27.5^{bcdefg}	9.194^{ghij}	2.043	4.5^{efgh}	0.69^{ab}
36	ARS563-425-1-B-2-3	104	65.8^{bc}	17^{ab}	21.6^{fghij}	126^{cde}	23.55^{klmno}	8.397^{jk}	1.883	4.51^{efgh}	0.67^{ab}
8	ARS168-3-B-1-B	101	62^{c}	18^{ab}	19.9^{ij}	121^{cde}	22.76^{mno}	8.83^{hijk}	1.947	4.543^{efgh}	0.49^{b}
	Minimum	99	62	15	19.1	101	21.59	7.904	1.883	3.843	0.49
	Maximum	114	118.3	31	27.8	226	31.85`	12.207	2.150	6.29	1.81
	Mean	106	82.2	21	24.1	150	26	9.593	2.008	4.796	0.98
	LSD at 5%	-	33.1	15	4.2	83	2.85	1.095	-	0.868	1.18
	P-value	1	0.1	1	0.1	1	0.01	0.001	5E-02	0	0.06

Sr. No	Accession	DTF	PH (cm)	ET	PL (cm)	GP	TGW (g)	GL (mm)	GW (mm)	RGLGW	GY (T/Ha)
23	Magoti	110a	109^{ab}	21	26.4^{abc}	166^{ab}	28.64^{abcd}	9.617^{abc}	1.95^{ab}	4.933^{abc}	1.37
31	ARS39-145/EP-3	100ab	80.9^{b}	23	22.2^{abc}	123^{b}	27.42^{abcd}	9.743^{abc}	2.18^{a}	4.477^{abc}	1.33
32	ARS101-4-B-1-1-B	104a	90.7^{ab}	19	26.4^{abc}	167^{ab}	24.45^{bcd}	10.59^{abc}	1.893^{b}	5.62^{ab}	1.33
11	ARICA3	103a	87.3^{ab}	22	25.1^{abc}	150^{ab}	24.9^{abcd}	9.337^{abc}	1.96^{ab}	4.767^{abc}	1.32
17	IR64-biofortified	106a	105.4^{ab}	16	26.6^{abc}	215^{ab}	28.07^{abcd}	9.04^{abc}	2.003^{ab}	4.517^{abc}	1.32
8	ARS168-3-B-1-B	100ab	71.7^{b}	25	22^{abc}	144^{ab}	24.57^{abcd}	8.933^{abc}	1.91^{ab}	4.683^{abc}	1.30
13	HHZSAL6	111a	106.4^{ab}	22	25.8^{abc}	159^{ab}	29.14^{abc}	9^{abc}	1.963^{ab}	4.59^{abc}	1.28
26	ARS134-B-1-1-4	106a	96.4^{ab}	19	25.4^{abc}	159^{ab}	31.66^{a}	10.193^{abc}	2.143^{ab}	4.773^{abc}	1.28
35	ARS756-1-1-3-B-2-2	102a	85.9^{ab}	24	23.9^{abc}	154^{ab}	28.05^{abcd}	10.12^{abc}	2.07^{ab}	4.907^{abc}	1.27
33	ARS101-4-B-1-3	101a	76^{b}	30	23.1^{abc}	123^{b}	25.64^{abcd}	8.783^{bc}	1.917^{ab}	4.607^{abc}	1.14
6	ARS848-15-3-2-4	108a	76.1^{b}	26	23.3^{abc}	162^{ab}	26.52^{abcd}	9.313^{abc}	2.03^{ab}	4.597^{abc}	1.10
21	Orylux11	103a	92.6^{ab}	23	23.8^{abc}	191^{ab}	24.46^{bcd}	9.747^{abc}	1.913^{ab}	5.103^{abc}	1.08
15	ARS134-B-1-1-5	98ab	76.6^{b}	25	21.3^{c}	135^{ab}	23.21^{cd}	8.777^{bc}	2.043^{ab}	4.297^{bc}	0.98
	Minimum	87	71.7	15	21.3	123	21.9	8.54	1.893	4.21	0.98
	Maximum	111	122.1	30	28.4	232	31.66	11.173	2.18	5.653	1.95
	Mean	102	90.9	22	24.4	162	26.73	9.615	2.026	4.766	1.47
	LSD at 5%	13	38.4	-	6.7	98	7.11	2.382	0.279	1.346	1.88
	P-value	0	0	0	0	0	0	0.001	0.001	0.001	0.98

Table 4.4: Mean Values for Agronomic Traits of 36 Rice Accessions Evaluated Across Sites (Bwegera and Sange) Ranked Based on GY

Sr. No	Accession	DTF	PH (cm)	ET	PL (cm)	GP	TGW (g)	GL (mm)	GW (mm)	RGLGW	GY (T/Ha)
16	IR990-48-B-B-12	104^{abcd}	91.2^{bcde}	22^{abc}	24.38^{abcdefgh}	194^{abcd}	26.1^{bcdefghij}	9.393^{defghi}	2.098^{ab}	4.485^{efg}	1.72
30	IR841	102^{abcde}	91.1^{bcde}	20^{abc}	28.04^{a}	223^{a}	24.66^{ghijk}	9.443^{defghi}	1.99^{ab}	4.758^{defg}	1.59
9	IR88638	93^{e}	83.83^{bcde}	20^{abc}	20.5^{i}	136^{defg}	29.53^{ab}	9.527^{defghi}	2.135^{a}	4.468^{efg}	1.48
4	MR254	106^{abcd}	88.6^{bcde}	18^{abc}	25.69^{abcdefg}	156^{bcdefg}	28.3^{abcdefg}	10.258^{bcde}	2.032^{ab}	5.062^{bcdef}	1.41
23	Magoti	111^{a}	103.83^{ab}	20^{abc}	25.46^{abcdefg}	170^{abcdefg}	28.69^{abcde}	9.445^{defghi}	2.022^{ab}	4.683^{defg}	1.36
12	IR64-sub-1	101^{bcde}	79.87^{bcde}	22^{abc}	23.25^{bcdefghi}	122^{fg}	27.61^{abcdefgh}	10.08^{bcdef}	2.052^{ab}	4.923^{cdefg}	1.35
3	WAHX14N-926	101^{bcde}	80.27^{bcde}	27^{ab}	22.52^{efghi}	132^{efg}	22.09^{k}	8.807^{ghi}	1.957^{ab}	4.523^{efg}	1.34
14	ARS755-3-3-1-B	106^{abcd}	91.57^{bcde}	24^{abc}	26.15^{abcde}	168^{abcdefg}	25.37^{defghijk}	9.485^{defghi}	2.015^{ab}	4.722^{defg}	1.33
34	NERICA-L-19-Sab-1	102^{abcde}	75.33^{cde}	25^{ab}c	22.36^{fghi}	126^{fg}	25.34^{defghijk}	9.183^{efghi}	2.013^{ab}	4.57^{efg}	1.33
18	IR107015-37	103^{abcde}	95.5^{abcd}	18^{abc}	23.17^{cdefghi}	152^{cdefg}	28.06^{abcdefgh}	9.747^{bcdefgh}	2.06a^{b}	4.733^{defg}	1.32
22	ARS134-B-B-B	109^{abcd}	83.1^{bcde}	17^{bc}	24^{bcdefghi}	176^{abcdef}	28.97^{abcd}	9.295^{defghi}	2.13^{a}	4.37^{fg}	1.31
20	V18/RRS126-48-1-13-2	102^{abcde}	81.6^{bcde}	22^{abc}	23.61^{bcdefghi}	152^{cdefg}	26.07^{bcdefghij}	9.545^{defghi}	1.97^{ab}	4.872^{cdefg}	1.31
7	IR93348:32-B-15-3-B-B-B-1	101^{bcde}	90.87^{bcde}	21^{abc}	22.63^{efghi}	160^{bcdefg}	22.83^{jk}	9.795^{bcdefgh}	1.975^{ab}	4.982^{bcdef}	1.29
27	IR82574/643-1-2	107^{abcd}	88.77^{bcde}	23^{abc}	23.82^{bcdefghi}	213^{ab}	24.85^{fghijk}	9.755^{bcdefgh}	1.992^{ab}	4.907^{cdefg}	1.27
31	ARS39-145/EP-3	100^{cde}	76.2^{cde}	22^{abc}	22.04^{ghi}	117^{g}	27.71^{abcdefgh}	9.623^{cdefghi}	2.098^{ab}	4.598^{efg}	1.26
1	ARS134-B-1-1-5-B	106^{abcd}	86.9^{bcde}	20^{ab}c	26.77^{abc}	189^{abcde}	23.75^{ijk}	10.278^{bcde}	1.922^{ab}	5.368^{abcd}	1.24
10	ARICA12	109^{abcd}	94.23^{bcd}	22^{abc}	23.71^{bcdefghi}	145^{cdefg}	28.67^{abcde}	9.762^{bcdefgh}	2.093^{ab}	4.69^{defg}	1.23
2	Orylux7-1	100^{cde}	81.07^{bcde}	21^{abc}	22.83^{defghi}	123^{fg}	25.54^{cdefghijk}	10.025^{bcdef}	2.043^{ab}	4.913^{cdefg}	1.22
11	ARICA3	103^{abcde}	85.93^{bcde}	21^{abc}	25.84^{abcdef}	158^{bcdefg}	24.58^{ghijk}	9.235^{defghi}	2.022^{ab}	4.582^{efg}	1.22
35	ARS756-1-1-3-B-2-2	103^{abcde}	83.63^{bcde}	22^{abc}	24.81^{abcdefg}	154^{bcdefg}	28.7^{abcde}	10.422^{abcd}	2.04^{ab}	5.127^{bcde}	1.21
19	ARS79-5-11-11	102^{abcde}	87.77^{bcde}	19^{abc}	26.94^{ab}	155^{bcdefg}	26.98^{bcdefghi}	10.918^{ab}	1.973^{ab}	5.54^{abc}	1.20
5	Golmy	99de	81.23^{bcde}	20^{abc}	24.92^{abcdefg}	132^{efg}	28.45^{abcdef}	9.887^{bcdefg}	2.095^{ab}	4.725^{defg}	1.19
24	Runingu	106^{abcd}	120.17^{a}	17^{bc}	25.02^{abcdefg}	151^{cdefg}	31.1^{a}	10.34^{bcde}	2.023^{ab}	5.145^{bcde}	1.17

Sr. No	Accession	DTF	PH (cm)	ET	PL (cm)	GP	TGW (g)	GL (mm)	GW (mm)	RGLGW	GY (T/Ha)
36	ARS563-425-1-B-2-3	102^{abcde}	72.97^{de}	21^{abc}	22.57^{efghi}	138^{defg}	24.65^{ghijk}	8.468^{i}	1.903^{b}	4.473^{efg}	1.16
17	IR64-biofortified	107^{abcd}	99.63^{abc}	16^{c}	26.4^{abcd}	202^{abc}	27.13^{bcdefghi}	8.93^{fghi}	2^{ab}	4.472^{efg}	1.15
28	Orylux5	101^{bcde}	81.97^{bcde}	21^{abc}	26.13^{abcde}	134^{defg}	27.22^{bcdefghi}	11.613^{a}	1.95^{ab}	5.972^{a}	1.15
26	ARS134-B-1-1-4	107^{abcd}	92.8^{bcd}	18^{abc}	25.29^{abcdefg}	164^{bcdefg}	29.13^{abc}	9.713^{bcdefgh}	2.077^{ab}	4.685^{defg}	1.15
29	SAHEL210	104^{abcd}	84.17^{bcde}	25^{abc}	23.75^{bcdefghi}	160^{bcdefg}	26.62^{bcdefghi}	9.455^{defghi}	2.077^{ab}	4.558^{efg}	1.13
25	ARS169-2-B-3-B	107^{abcd}	84.57^{bcde}	21^{abc}	23.77^{bcdefghi}	166^{abcdefg}	24.95^{efghijk}	8.88^{fghi}	2.05^{ab}	4.343^{fg}	1.11
13	HHZSAL6	110^{ab}	100.1^{abc}	21^{abc}	26.17^{abcde}	160^{bcdefg}	27^{bcdefghi}	8.77^{ghi}	1.957^{ab}	4.485^{efg}	1.10
32	ARS101-4-B-1-1-B	105^{abcd}	89.33^{bcde}	21^{abc}	26.88^{abc}	163^{bcdefg}	23.52^{ijk}	10.773^{abc}	1.898^{b}	5.69^{ab}	1.09
15	ARS134-B-1-1-5	101^{bcde}	72.63^{de}	28^{a}	21.05^{hi}	130^{efg}	23.64^{ijk}	8.91^{fghi}	1.995^{ab}	4.475^{efg}	1.05
6	ARS848-15-3-2-4	110^{abc}	74.5^{de}	22^{abc}	22.25^{fghi}	138^{defg}	24.46^{hijk}	8.608^{hi}	2.047^{ab}	4.22^{g}	0.95
21	Orylux11	102^{abcde}	88.1^{bcde}	20^{abc}	23.92^{bcdefghi}	175^{abcdefg}	24.8^{fghijk}	9.767^{bcdefgh}	1.985^{ab}	4.933^{cdefg}	0.91
33	ARS101-4-B-1-3	106^{abcd}	75.3^{cde}	26^{abc}	23.73^{bcdefghi}	130^{efg}	26.57^{bcdefghij}	8.988^{fghi}	1.98^{ab}	4.553^{efg}	0.91
8	ARS168-3-B-1-B	100^{cde}	66.83^{e}	21^{abc}	20.92^{hi}	132^{efg}	23.66^{ijk}	8.882^{fghi}	1.928^{ab}	4.613^{efg}	0.90
	Minimum	93	66.83	16	20.5	117	22.09	8.468	1.898	4.22	0.90
	Maximum	111	120.17	28	28.04	223	31.1	11.613	2.135	5.972	1.72
	Mean	104	86.79	21	24.17	156	26.42	9.605	2.018	4.781	1.23
	LSD at 5%	10	25.12	11	3.75	59	3.75	1.208	0.217	0.742	-
	P-value	0	0	0	0	0	0	0	0	0	0.56

In the Bwegera site, the trait GP ranged from 100 (recorded at accession Orylux7-1) to 226 (recorded at accession IR82574/643-1-2) with a mean 149, and form 123 (recorded at accession ARS101-4-B-1-3) to 232 (recorded at accession IR841) with a mean of 162, in the Sange site.

The TGW varied from 21.59 to 31.85 g with a mean of 26 g, in the Bwegera site, and from 21.90 g to 31.66 g, with a mean of 26.73 g, in the sange site. The heavier TGW values of 31.85 g and 31.66 g were recorded for the commercial variety RUNINGU and ARS134-B-1-1-4, in Bwegera and in Sange, respectively, and the accessions IR93348:32-B-15-3-B-B-B-1 (21.59 g) and WAHX14N-926 (21.9 g) had the lowest TGW, in Bwegera and Sange sites, respectively.

The GL ranged from 7.903 mm to 12.207 mm with the mean of 9.592 mm in the Bwegera site, and and from 8.54 mm to 11.173 mm with the mean of 9.615 mm in the sange site. In the Bwegera site, the accession ORYLUX5 , ARS101-4-B-1-1-B and ARS756-1-1-3-B-2-2 had the highest GL values of 12.207 mm, 10.957 mm, 10.723 mm and 10.663 mm, respectively, and accessions HHZSAL6, ARS563-425-1-B-2-3 and ARS848-15-3-2-4 had the lowest GL values of 8.54 mm, 8.397 mm and 7.903.623 mm, respectively, while in the sange site, the accessions ARS79-5-11-11, ORYLUX5 and ARS101-4-B-1-1-B had the highest GL values of 11.173 mm, 11.020 mm and 10.59 mm, and the accession ARS563-425-1-B-2-3 had the lowest GL value of 8.54.

The GW, in the Bwegera site ranged from 1.883 mm to 2.15 mm with the mean of 2.008 mm, and the accessions IR88638 , ARS134-B-B-B, and IR990-48-B-B-12 had the highest GW values of 2.15 mm, 2.143 mm and 2.14 mm, accessions ARS563-425-1-B-2-3 and ARS134-B-1-1-5-B had the lowest GW values of 1.883 mm and 1.883 mm. While in the sange site, the GW ranged from 1.893 mm to 2.18 mm with a mean of 2.026 mm, and accessions ARS39-145/EP-3 and ARS39-145/EP-3 had the highest GW values of 2.18 mm and 2.157, and the lowest GW values of 1.893 mm was recorded to accession ARS101-4-B-1-1-B.

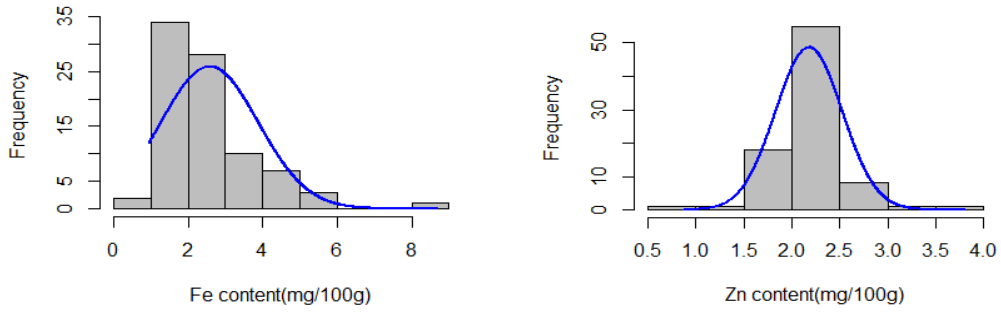
The RGLGW ranged from 3.843 to 6.29 with a mean of 4.796, and accessions ORYLUX5 and ARS101-4-B-1-1-B recorded the highest RGLGW values of 6.29 and 5.76, and accession ARS848-15-3-2-4 had the lowest RGLGW value of 3.843, in the Bwegera site. In the sange site, the RGLGW ranged from 4.21 to 5.653 with a mean of 4.766 and accessions ORYLUX5, , ARS79-5-11-11 and ARS134-B-1-1-5-B had the highest RGLGW values of 5.653, 5.55 and 5.26, and accession ARS169-2-B-3-B had the lowest RGLGW value of 4.21.

The mean value of GY for the test accessions across sites was 1.23 T ha^{-1} and 0.98 T ha^{-1} in Bwegera and 1.46 T ha^{-1} in sange site. In the Bwegera site, the GY ranged from 0.49 T ha^{-1} (accession ARS168-3-B-1-B) to 1.81 T ha^{-1} (accession IR990-48-B-B-12), and from 0.98 t ha-1 (accession ARS134-B-1-1-5) to 1.95 T ha^{-1} (accession ARS755-3-3-1-B) in the Sange site.

Based on grain yield, the top 10 performingaccessios across sites were IR990-48 B-B-12, IR841, IR88638, MR254, Magoti, IR64-sub-1, WAHX14N-926, ARS755-3-3-1-B, NERICA-L-19-Sab-1, IR107015-37 with GY values of 1.72, 1.59, 1.48, 1.41, 1.36, , 1.35, 1.34, 1.33, 1.33 and 1.32 T ha^{-1}, respectively.

4.1.3 Variation in Fe and Zn Content in Rice Grains

The Fe content in rice grains showed a continuous variation that did not follow a normal distribution while the Zn content showed a normal distribution (Figure 4.1). The Fe content ranged between 0.95 and 8.68 mg/100 g (dwb) with a mean of 2.58 ±1.31 mg/100 g (dwb). The Zn concentration ranged between 0.87 and 3.8 mg/100 g (dwb), with an average of 2.18 ±0.35 mg/100 g (dwb) (Table 4.3). The distribution of the variable Fe content exhibited a positive skewness of 1.77 and a kurtosis of 7.46. Similarly, the distribution of the variable Zn content showed a positive skewness of 0.66 and kurtosis of 9.29. For Fe content, the lowest concentration was found in "Musesekara" and the highest in "08FAN10". For Zn, the lowest concentration was found in "ARICA12" and the highest in "Komboka". The individual accessions Fe and Zn values is shown in Table 4.4. Soils chemical characterizations details including Fe and Zn content from three sites used in this study is shown in Table 4.5.



(**A**) (**B**)

Figure 4. 1: Distribution of Accessions Based on Micronutrient Content in Grains of Rice Accessions: (A) Fe Content; (B) Zn Content. The Blue Curves Represent the Fitted Fe And Zn Content Data Distribution, Respectively

Table 4.5: Descriptive Statistics of Fe and Zn Contents in Grains of the Rice Accessions in this Experiment

Trait	Mean ± SD (mg/100g)	Range (mg/100g)	Median (mg/100g)	Mode (mg/100g)
Fe	2.58±1.31	0.95-8.68	2.2	1.61 and 1.66
Zn	2.18±0.35	2.03-2.34	2.16	2.09

Table 4.6: Chemical Characterizations of Soils Collected from Three Sites Used in this Study

Site	pH (H_2O)	EC/µS/cm	% OC	% Ntot.	P-Bray1 mg/kg	K me/100 g	Mg me/100 g	Fe(mg/kg)	Zn(mg/kg)
Bwegera	6.7	19.5	1.41	-	-	0.13	1.05	118.2	0.71
Sange	7.65	165.3	2.26	0.11	7.03	0.64	3.7	73.7	3.81
Kalemie	6.32	167.6	3.12	0.22	16.27	1.00	8.56	178.26	17.62

Table 4.7: Mean Values of Fe and Zn Content in Grains of Rice Accessions in this Experiment

Sr. No	Accession name	Fe (mg/100 g)	Zn (mg/100 g)	Sr. No	Accession name	Fe (mg/100 g)	Zn (mg/100 g)
1	Komboka	2.15	3.8	44	ARICA2	1.12	1.63
2	IR64	4.79	2.34	45	NL19	3.11	2.03
3	IBEI6	2.4	2.44	46	NL14	2.2	2.17
4	GIZA128	1.85	2.52	47	NL17	2.54	2.89
5	Nipponbare	4.36	2.51	48	D20-ARS-3-2	5.6	2.46
6	Jasmine	2.64	2.67	49	IR96279-33-3-1-2	1.09	1.86
7	NL59	2.42	2.31	50	ARS134-B-1-1-5-B	3.66	2.52
8	FKR	3.89	2.21	51	Orylux7-1	2.46	2.14
9	08FAN10	8.68	2.36	52	WAHX14N-926	1.82	1.96
10	WAB2066-TGR2	5.98	2.06	53	MR254	2.82	2.25
11	WAB2066-TGR3	1.9	2.26	54	Golmy	4.32	2.19
12	IR99084-B-B-13	4.21	2.17	55	ARS848-15-3-2-4	2.61	2.11
13	IR127229	2.95	2.09	56	IR93348:32-B-15-3-B-B-B-1	2.15	2.16
14	IR106172-78 :1-B-B	3.44	2.29	57	ARS168-3-B-1-B	2.97	1.79
15	ARS848-15-3-2-3	2.2	1.82	58	IR88638	2.45	2.52
16	IR106364-B-B-CNUS	3.78	2.36	59	ARICA12	2.89	0.87
17	ARS844-24-10-2-B	2.91	2.24	60	ARICA3	2.51	1.95
18	ARS168-1-B-3-B	3.39	1.99	61	IR64-sub-1	1.5	1.81
19	ARS851-1-3	0.96	2.09	62	HHZSAL6	1.66	1.88
20	IR87638-10-2-2-4	2.12	2.2	63	ARS755-3-3-1-B	1.61	1.91
21	IR98419-B-B-11	2.71	2.47	64	ARS134-B-1-1-5	2.71	1.85
22	IR97071-24-1-1-1	5.15	2.33	65	IR990-48-B-B-12	1.54	2.07
23	ARS803-4-5-4-3	2.57	2.58	66	IR64-biofortified	1.96	2.25
24	IR93856-23-1-1-1	1.66	2.32	67	IR107015-37	2.05	2.08
25	ARS790-5-11-1-1	1.72	2.09	68	ARS79-5-11-11	1.87	2.4
26	IR17015-6-5-3-B1	1.61	2.45	69	V18/RRS126-48-1-13-2	1.5	1.85
27	IR106359-B-18-5	1.07	3.01	70	Orylux11	1.28	1.95
28	IR95624-B-138-3	2.57	1.96	71	ARS134-B-B-B	1.79	2.45
29	IR13A461	1.81	2.23	72	Magoti	1.83	2.47
30	Mugwiza	3.71	2.1	73	Runingu	1.95	2.41
31	Vuninzara	4.73	2.09	74	ARS169-2-B-3-B	2.3	1.9
32	IR97045-24-1-1-1	3.52	2.32	75	ARS134-B-1-1-4	1.49	2.22

Sr. No	Accession name	Fe (mg/100g)	Zn (mg/100g)	Sr. No	Accession name	Fe (mg/100g)	Zn (mg/100g)
33	Kigoma	3.99	2.42	76	IR82574/643-1-2	1.64	1.82
34	Makasane	3.23	2.13	77	Orylux5	1.65	1.5
35	Rukaramu	4.89	2.23	78	SAHEL210	1.75	2.07
36	Musesekara	0.95	2.2	79	IR841	1.55	1.67
37	Yasho-Yasho	1.58	2.23	80	ARS39-145/EP-3	2.11	2.16
38	Kasozi	1.47	2.12	81	ARS101-4-B-1-1-B	1.4	2.1
39	IR7525	2.41	2.57	82	ARS101-4-B-1-3	2.12	2.15
40	Orylux7	1.77	2.02	83	NERICA-L-19-Sab-1	1.66	1.67
41	ART29	1.62	2.12	84	ARS756-1-1-3-B-2-2	1.27	2.19
42	Sipi	4.53	2.06	85	ARS563-425-1-B-2-3	1.61	2.12
43	CRS36	2.88	2.09				

4.1.4 Genetic Markers Distribution and Linkage Disequilibrium Analysis

After filtering, 8379 polymorphic SNPs were retained and used for the GWAS and LD decay analysis. The number of markers ranged from 386 SNPs on chromosome 10 to 1018 SNPs on chromosome 1, with an average of 1289 SNPs across chromosomes. The average marker density ranged from 38.61 SNPs/Mb in chromosome 4 to 60 SNPs/Mb in chromo-some 10 (Figure 4.2 and Appendix II).

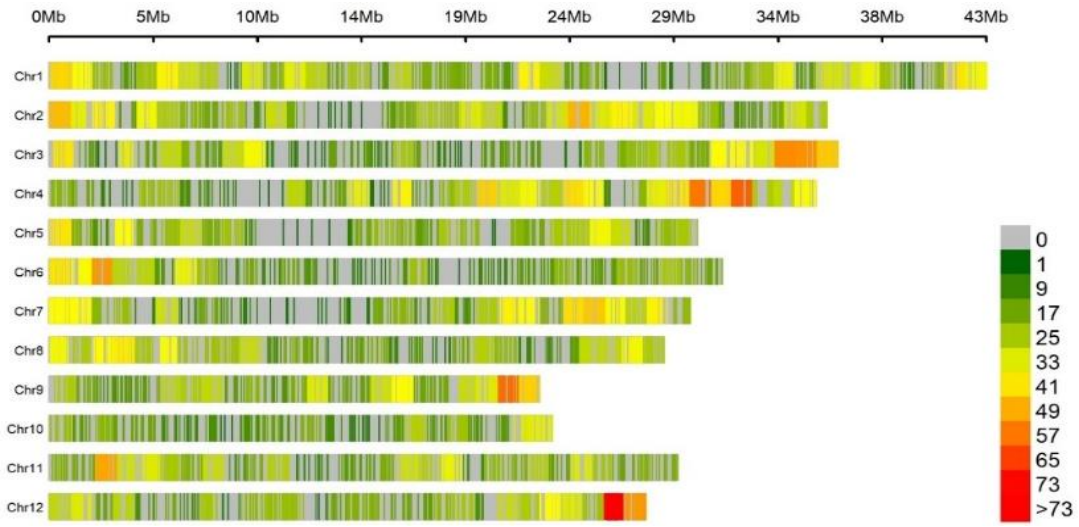


Figure 4.2: Distribution and Density of the 8379 SNP Markers along the 12 Rice Chromosomes. The Horizontal Axis Shows the Chromosome Length and the SNP Density is Represented by Different Colors.

The LD decay across the genome was visualized by plotting LD (r^2) values between adjacent markers against the corresponding physical distance in base pairs (bp) (Figure 4.3). A notable observation was the rapid decline in LD as the physical distance increased. At a cut-off value of $r^2 = 0.1$, the average physical distance was 401 kb.

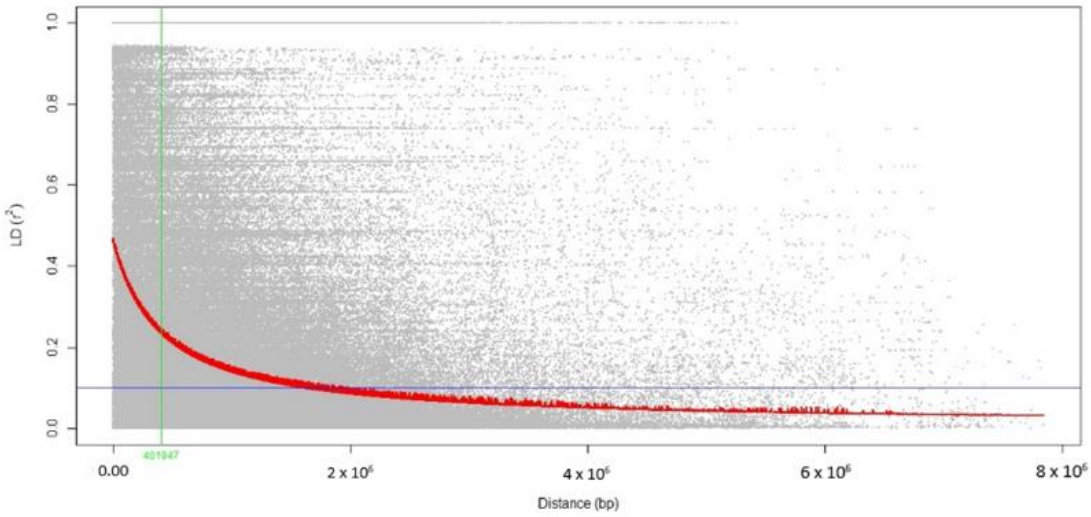


Figure 4.3: The Scatter Plot of Genome-wide Linkage Disequilibrium (LD) Decay Determined Based on the r^2 Values of the Marker Pairs. The Red Curve Line is the Regression Model Fitted to LD Decay. The Horizontal Blue Line is the LD at r^2 = 0.1, Whereas the Vertical Green Line is the Genome-wide LD Decay Rate (~401 kb) at r^2 = 0.1

4.1.5 Marker-Trait Associations for Fe and Zn Content

The GWAS for Fe and Zn content in rice grains was conducted using two multi-locus models, MLM and FarmCPU. Two SNPs significantly associated with Fe content were detected on chromosomes 1 (S1_34232231) and 11 (S11_2567279). Among the two, S11_2567279 was identified by both models and exhibited the strongest association with Fe content (Figure 4.4 and Table 4.8). The SNP S1_34232231 was uniquely identified by FarmCPU (Figure 4.4).

For the Zn content, the MLM model detected one SNP on chromosome 4 (S4_33308504) with a p-value = 2.41×10^{-4}, while the FarmCPU model detected eight SNPs on chromosomes 3, 4, 5, 7, 11, and 12 (Figure 4.5 and Table 4.6). Among the

SNPs significantly associated with the Zn content, S4_33308504 on chromosome 4 was detected by both models.

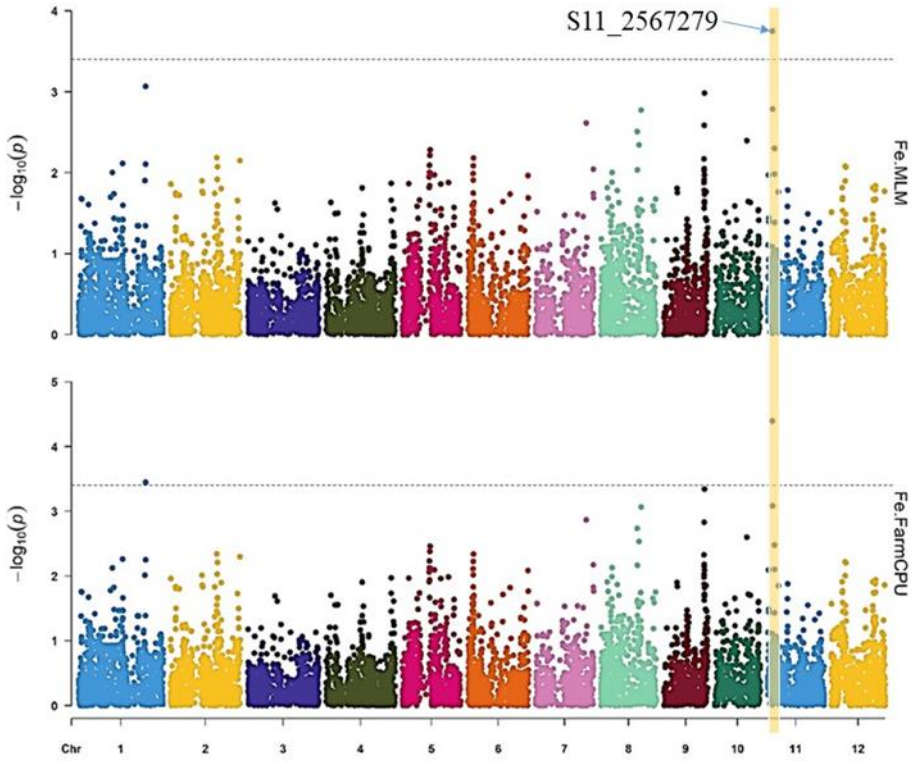


Figure 4.4: Manhattan Plots for the GWAS of Fe Content in Rice Grains Using MLM and FarmCPU. The Significance Threshold ($p \leq 4.649 \times 10^{-4}$) is Represented by the Dashed Horizontal Line. The X-axis Displays the SNP Location along the 12 Rice Chromosomes

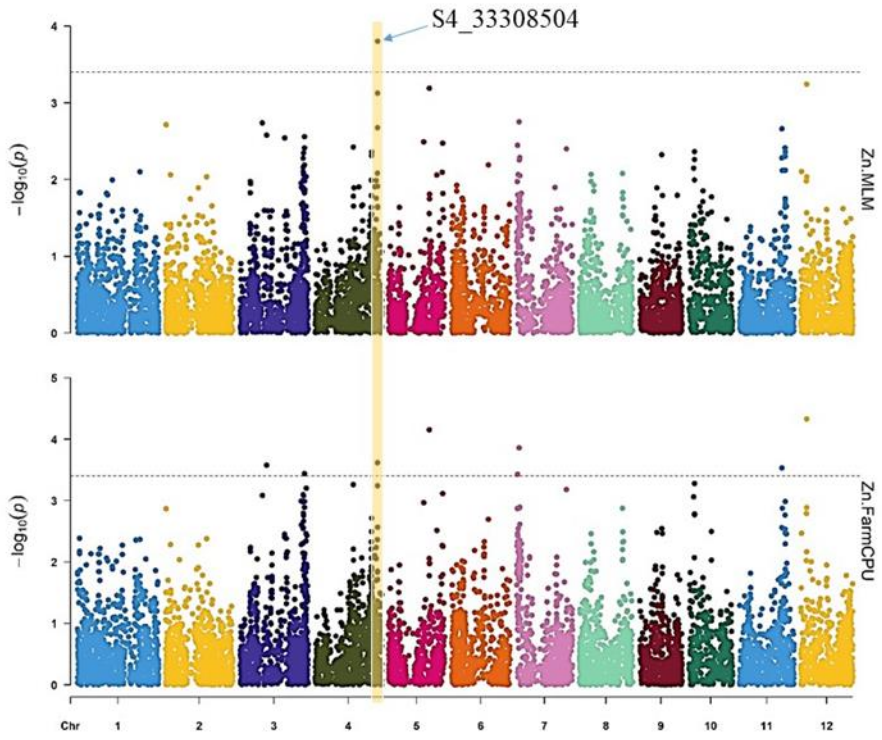


Figure 4.5: Manhattan Plots for the GWAS of Zn Content in Rice Grains Using MLM and FarmCPU. The Significance Threshold ($p \leq 4.649 \times 10^{-4}$) is Represented by the Dashed Horizontal Line. The X-axis Displays the SNP Location along the 12 Rice Chromosomes

Table 4.8: SNP Markers Significantly Associated With Fe and Zn Content of Rice Grains Identified in the GWAS

Trait	Model	SNP	Chr	Position (bp)	*p*-Value
Fe	FarmCPU	S1_34232231	1	34,232,231	3.56×10^{-4}
Fe	FarmCPU	S11_2567279	11	2,567,279	4.01×10^{-5}
Fe	MLM	S11_2567279	11	2,567,279	4.01×10^{-5}
Zn	FarmCPU	S3_14236041	3	14,236,041	2.63×10^{-4}
Zn	FarmCPU	S3_34414350	3	34,414,350	3.62×10^{-4}
Zn	FarmCPU	S4_33308504	4	33,308,504	2.41×10^{-4}
Zn	FarmCPU	S5_21747243	5	21,747,243	7.01×10^{-5}
Zn	FarmCPU	S7_308113	7	308,113	3.72×10^{-4}
Zn	FarmCPU	S7_1159472	7	1,159,472	1.37×10^{-4}
Zn	FarmCPU	S11_22639501	11	22,639,501	2.92×10^{-4}
Zn	FarmCPU	S12_3069954	12	3,069,954	4.66×10^{-5}
Zn	MLM	S4_33308504	4	33,308,504	2.41×10^{-4}

4.1.6 Candidate Genes for Fe and Zn Content

For the Fe content GWAS, two SNPs on chromosomes 1 and 11 harbored 127 and 120 genes, respectively. For the zinc content, 166, 118, 127, 258, 107, and 121 candidate genes were detected on chromosomes 3, 4, 5, 7, 11, and 12, respectively (Table 4.9).

Table 4.9: Fe and Zn Candidate Genes per Significant SNP

Trait	SNP	Chromosome Position	Number of Candidate Gene Identified
Fe	S1_34232231	1	127
	S11_2567279	11	120
Zn	S3_14236041	3	22
	S3_34414350	3	144
	S4_33308504	4	118
	S5_21747243	5	127
	S7_308113	7	117
	S7_1159472	7	141
	S11_22639501	11	107
	S12_3069954	12	121

We further selected all the candidate genes that are involved in Fe and Zn homeostasis based on functional annotations. A total of 36 genes related to metal homeostasis were identified, with a focus on genes associated with transporter activity and transcription regulatory activity.

For the Fe content GWAS, two candidate genes tagged by significant SNP S1_34232231 encode for bZIP transcription factor (*LOC_Os01g58760* and *LOC_Os01g59760*), one in the MYB family transcription factor (LOC_Os01g59660), and one encodes for transcription factor- TGA5 (*LOC_Os01g59350*) (Table 4.10). At the locus of SNP S11_2567279, two candidate genes encode for bZIP transcription factor (*LOC_Os11g05640* and *LOC_Os11g06170*) and two are in the transporter family (*LOC_Os11g05390* and *LOC_Os11g05700*). It is interesting to report that two genes (*LOC_Os11g05480* and *LOC_Os05g37170* for Fe and Zn, respectively) encode for tran-scription factors.

For the zinc content GWAS, 12 candidate genes (*LOC_Os03g25470*, *LOC_Os03g60130*, *LOC_Os03g60850*, *LOC_Os03g61100*, *LOC_Os04g56330*, *LOC_Os04g56470*, *LOC_Os05g37470*, *LOC_Os07g01070*, *LOC_Os07g01560*, *LOC_Os03g60820*, *LOC_Os11g38160*, and *LOC_Os12g05830*) encode for proteins with transporter activity, two encode for proteins with bZIP domains (*LOC_Os07g03220* and *LOC_Os12g06520*) and five encode for MYB (myeloblastosis) transcription factors (*LOC_Os05g37040*, *LOC_Os05g37050*, *LOC_Os05g37060*, *LOC_Os05g37730*, *and LOC_Os07g02800*) (Table 4.10). The other genes functional annotations included a transcription regulator (*LOC_Os03g25430*), transcription elongation factor (*LOC_Os03g60130* and *LOC_Os12g06850*), transcription termination factor nusG (*LOC_Os03g61030*), AP2-like ethylene-responsive transcription factor AINTEGUMENTA (*LOC_Os04g55970*), AP2-like ethylene-responsive transcription factor PLETHORA 2 (*LOC_Os07g03250*), BEL1-like homeodomain transcription factor (*LOC_Os12g06340*), and E2F family transcription factor protein (*LOC_Os12g06200*) as shown in Table 4.10.

Table 4.10: Candidate Genes Associated With Fe and Zn Homeostasis

Trait	SNP	Chr	Candidate Gene			Distance (kb)	Annotation
			Gene ID	Start_Pos	End_Pos		
Fe	S1_34232231	1	*LOC_Os01g58760*	33,962,625	33,963,552	268.679	bZIP transcription factor domain containing protein
		1	*LOC_Os01g59350*	34,306,514	34,313,319	−74.283	transcription factor, TGA5, putative, expressed
		1	*LOC_Os01g59660*	34,508,454	34,512,781	−276.223	MYB family transcription factor
		1	*LOC_Os01g59760*	34,565,292	34,568,290	−333.061	bZIP transcription factor, putative, expressed
	S11_2567279	11	*LOC_Os11g05390*	2,413,596	2,417,533	149.746	transporter, major facilitator family
		11	*LOC_Os11g05480*	2,462,254	2,468,733	98.546	transcription factor
		11	*LOC_Os11g05640*	2,560,816	2,563,634	3.645	bZIP transcription factor domain containing protein
		11	*LOC_Os11g05700*	2,605,745	2,610,544	−38.466	ABC transporter family protein, putative
		11	*LOC_Os11g06170*	2,939,742	2,942,834	−372.463	bZIP transcriptional activator RSG

Trait	SNP	Chr	Candidate Gene			Distance (kb)	Annotation
			Gene ID	Start_Pos	End_Pos		
Zn	S3_14236041	3	*LOC_Os03g25430*	14,538,130	14,539,609	−302.089	transcription regulator, putative, expressed
		3	*LOC_Os03g25470*	14,552,198	14,552,749	−316.157	ctr copper transporter family protein
		3	*LOC_Os03g60130*	34,194,882	34197992	216.358	transcription elongation factor protein
		3	*LOC_Os03g60820*	34,554,642	34,560,824	−140.292	transporter, major facilitator superfamily domain containing protein
	S3_34414350	3	*LOC_Os03g60850*	34,574,307	34,576,973	−159.957	peptide transporter PTR2, putative, expressed
		3	*LOC_Os03g61030*	34,671,286	34,674,494	−256.936	transcription termination factor nusG family protein
		3	*LOC_Os03g61100*	34,708,403	34,711,045	−294.053	GDP-mannose transporter, putative
	S4_33308504	4	*LOC_Os04g55970*	33,341,978	33,346,562	−33.474	AP2-like ethylene-responsive transcription factor AINTEGUMENTA, putative, expressed
		4	*LOC_Os04g56330*	33,580,318	33,582,347	−271.814	ABC transporter, ATP-binding protein
		4	*LOC_Os04g56470*	33,661,879	33,664,246	−353.375	amino acid transporter

Trait	SNP	Chr	Candidate Gene			Distance (kb)	Annotation
			Gene ID	Start_Pos	End_Pos		
Zn	S5_21747243	5	*LOC_Os05g37040*	21,646,920	21,647,702	99.541	MYB family transcription factor
		5	*LOC_Os05g37050*	21,650,252	21,651,057	96.186	MYB family transcription factor
		5	*LOC_Os05g37060*	21,654,182	21,655,380	91.863	MYB family transcription factor
		5	*LOC_Os05g37170*	21,720,654	21,724,490	22.753	transcription factor
		5	*LOC_Os05g37470*	21,926,799	21,931,594	−179.556	transmembrane amino acid transporter protein
		5	*LOC_Os05g37730*	22,081,343	22,083,544	−334.1	MYB family transcription factor
	S7_308113	7	*LOC_Os07g01070*	42,657	44,577	263.536	peptide transporter
		7	*LOC_Os07g01560*	348,475	350,594	−40.362	transporter family protein
	S7_1159472	7	*LOC_Os07g02800*	1,046,017	1,048,052	111.42	MYB family transcription factor
		7	*LOC_Os07g03220*	1,267,975	1,268,550	−108.503	bZIP transcription factor domain containing
		7	*LOC_Os07g03250*	1,299,598	1,304,299	−140.126	AP2-like ethylene-responsive transcription factor PLETHORA 2
	S11_22639501	11	*LOC_Os11g38160*	22,625,530	22,627,579	11.922	transporter family protein
	S12_3069954	12	*LOC_Os12g05830*	2,684,760	2,688,178	381.776	transporter-related
		12	*LOC_Os12g06200*	2,939,684	2,945,048	124.906	E2F family transcription factor protein
		12	*LOC_Os12g06340*	3,029,596	3,034,778	35.176	BEL1-like homeodomain transcription factor
		12	*LOC_Os12g06520*	3,153,015	3,156,795	−83.061	bZIP transcription factor domain containing protein
		12	*LOC_Os12g06850*	3,326,815	3,329,114	−256.861	transcription elongation factor protein

4.2 Discussion

Determining the variation among the subset of 36 rice accessions for agronomic traits across the Bwegera and Sange sites aimed to select high-yielding lines with good agronomic performance for cultivation. The evaluated accessions exhibited significant differences within sites for the traits DTF, PH, ET, GP, TGW and GY , and among accessions, the significant differences was observed for the same traits including PL, GL, GW and RGLGW while GL, GW, RGLGW traits were not significant within sites, and GY trait was not significant as well among accessions, indicating the presence of variation within the population. Similar findings on variation in flowering among plants were reported by Julia & Dingkuhn (2012).

The absence of significant differences in grain yield (GY), grain length (GL), grain width (GW), and the ratio of grain length to grain width (RGLGW) may be attributed to the pivotal role of grain size and shape, crucial factors influencing both yield and quality. These characteristics are known to be under the control of the *OsSPL16* gene. The elevated expression of *OsSPL16*, documented to promote cell division and enhance grain filling, results in positive effects on both grain width and overall grain yield (Wang et al., 2012). The uniform expression of this gene across accessions and sites may contribute to the observed lack of significant differences.

The observed variations among rice accessions in terms of the effective tiller trait per hill are consistent with the findings of Jamal et al (2009). In their study on genetic variation for yield and yield components in six exotic rice genotypes and one check, Jamal et al (2009) specifically investigated traits, including DTF, PH, ET, PL, TGW, and GP. Their results and our study strengthen the evidence for significant genetic variations influencing DTF, PH, ET, and PL traits within the studied rice accessions, but non-significant variation for TGW and GP.

The variations displayed by the accessions may be attributed to their diverse origins, as each accession was developed with distinct breeding goals, resulting in the emergence of substantial variation. In this context, numerous reports have been published regarding phenotypic variation among rice genotypes (Adhikari et al., 2018; Oladosu et al., 2014; Rono, 2018; Suvi et al., 2021). The commercial varieties,

MAGOTI and RUNINGU, demonstrated superior performance compared to most of the tested accessions, which can be attributed to their adaptation to the environmental conditions in the Ruzizi plain.

Genome-wide association mapping is an effective approach that aids the discovery of genes responsible for controlling specific traits of interest. The efficacy of this method depends on the genetic diversity of the association panels used (Uffelmann et al., 2021). In our study, we observed a significant level of phenotypic variation, which was much greater in Fe than in the Zn content in the GWAS panel, as indicated by the descriptive statistics.

In the other hand, for genotypic mapping , we analyzed Fe and Zn content, and genotypic by sequencing for a set of 85 rice germplasm accessions maintained at the rice breeding program of the INERA. For those two important mineral micronutrients, namely, Fe and Zn, the analysis was performed in milled rice and the observed range of variability for Fe in this study (0.95–8.68 mg/100 g dwb) was higher than in the previous studies, 0.118 to 0.787 mg/100 g (Swamy et al., 2018) and 0.65 to 2.31 mg/100 g (Bollinedi et al., 2020). On the other hand, the range for Zn content (0.87–3.8 mg/100 g dwb) in the present study closely aligns with the range previously reported by Rakotondramanana et al. (2022) (1.42 to 4.84 mg/100 g) in whole rice seed. Similar results were also obtained by Descalsota et al. (2018) (0.995 to 2.635 mg/100 g) and Bollinedi et al. (2020) (1.3 to 4.62 mg/100 g), in brown rice. This difference could be attributed to the use of different rice accessions in the study (Bhandari et al., 2017). However, compared to other cereals like maize (8.19–25.65 µg/g for Fe and 17.11–43.69 µg/g for Zn) (Hindu et al., 2018) and Aegilops Tauschii (30.33–69.44 ppm for Fe and 17.54–49.78 ppm for Zn) (Arora et al., 2019), rice exhibited high levels of Fe and Zn content in the grain hence the need for biofortification.

The GWAS panel demonstrated a significant variability for the traits studied. Based on our previous work (Kimwemwe et al., 2023), the observed variation among the evaluated rice accessions and the diversity of the panel used highlight the importance of these genetic resources. This significance extends to their potential contributions in

crop improvement through breeding and the identification of genes that govern these traits (Bhandari et al., 2017).

A total of 8379 high-quality SNPs were obtained from the 85 rice accessions in this diversity panel. This number was relatively higher than what was reported in previous studies by Mogga et al. (2018) and Islam et al. (2022) which reported 525 high-quality SNPs in 59 rice genotypes and 6565 SNPs in 174 rice accessions. This indicates that the 85 rice accessions used in this study exhibited a high coverage hence being suitable for conducting the GWAS (Krishnappa et al., 2022).

Association mapping is a population-based study conducted to identify the relationships between traits and markers using linkage disequilibrium (LD). In this study, the genome-wide LD decays to $r^2 < 1$ within a distance of 401 kilobases (kb). Although the LD decay was relatively slower, these LD decay estimates were lower than the findings of Mather et al. (2007), showing long-range LD in temperate japonica rice (>500 kb). However, the LD of our diversity panel had a longer range than the previously published values of 150 kb (Liu et al., 2019), 109.37 kb, and 214.69 kb in indica rice (Lu et al., 2015). The slow LD decay rates in rice are due to its self-pollinating nature, and a relatively small effective population size (Peringottillam et al., 2022).

GWAS is designed to evaluate the associations between genotypes and phenotypes (Uffelmann et al., 2021), and, thus, detect the location of candidate genes. This study utilized two mixed models, to perform GWAS, namely, MLM (Zhang et al., 2010) in which the population structure and kinship were incorporated, and FarmCPU (Liu et al., 2016) to address false positives by enhancing statistical power and efficiency. In their study, Kaler et al. (2020) mentioned that both models are frequently employed in association mapping. A recent study by Bollinedi et al. (2020) reported that the MLM has gained widespread popularity for GWAS in crop research, specifically in the context of rice. Various investigations have been conducted on rice to map the QTLs related to Fe and Zn concentration. The QTLs responsible for grain Fe and Zn concentration were identified on different rice chromosomes. It was observed that different rice chromosome positions harbored QTLs associated with one of the

micronutrients, revealing their genetic interconnectedness. Swamy et al. (2021) reported QTLs on chromosome 1 and 11 for Fe biofortification in rice. A similar finding was observed in our study whereby the significant SNP markers responsible for grain Fe were identified on chromosome 11 using the MLM and FarmCPU models.

Among the SNPs identified, the ones located on chromosome 11 were found to have the highest impact on Fe content, while those on chromosome 4 had the greatest influence on the Zn content in rice. These SNP markers explained a significant portion of the phe-notypic variation observed in the respective traits.

This study identified genes belonging to the ZIP and MYB transcription factor families. According to Meng et al. (2018), the ZIP family genes play a significant role in Fe and Zn homeostasis in the rice grain. The ZIP family, which includes the zinc-regulated, iron-regulated transporter-like proteins (ZIP) family and iron-regulated transporters (IRTs), plays a crucial role in regulating the absorption and translocation of Zn and Fe in rice. These ZIP transporters are also instrumental in facilitating the cellular uptake and intracellular trafficking of Fe and Zn in plants. Furthermore, they enhance the nutritional content and quality of crops (Ajeesh et al., 2020). Moreover, the MYB family genes identified in the current study were reported by Yan et al. (2021) who clarified that they are important transcriptional regulators playing key roles in the regulation of plant secondary metabolism, as well as contributing to the regulatory network of anthocyanin biosynthesis. Anthocyanins, which are produced through this biosynthetic pathway, serve to improve plant function under mineral imbalance and also act as metal chelating agents (Landi et al., 2015). These gene families are of significant importance in the biofortification of grains since they effectively control Fe and Zn content and the nutritional quality of crops

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The determination of agronomic performance of a subset of 36 rice accessions to identify high yielding and stable genotypes for cultivation in the Ruzizi plain, in the Eastern DRC, demonstrated a variation among the evaluated rice accessions for some agronomic traits in the Bwegera and Sange sites, and no variation for other traits including grain yield traitn (GY). Thus, the null hypothesis saying that there are no differences among rice accessions for agronomic performance across locations was not satisfied for all the evaluated variables at Bwegera and at Sange.

In this study, we conducted a GWAS of Fe and Zn content in rice grains of 85 rice accessions and 8379 DArTseq-derived SNPs. A total of 10 SNPs were significantly associated with Fe and Zn content. Among them, SNP S11_2567279 on chromosome 11 and SNP S4_33308504 on chromosome four were identified for Fe and Zn traits, respectively, using both the MLM and FarmCPU models. Genes belonging to bZIP family genes, MYB family genes, and genes involved in transporter activities were identified within the LD decay window. Thus, there is association between candidate genes and SNP markers with Fe and Zn content in the assessed rice grains.

5.2 Recommendations

1. These results should be taken with caution since the experiments were conducted for only one season. It is possible that the two environments share similar conditions, leading to minimal variation in some specific traits. Alternatively, these traits may be less influenced by the conditions across locations in Bwegera and in Sange, Eastern Democratic Republic of Congo.

2. The accessions ARS755-3-3-1-B, IR841, MR254, V18/RRS126-48-1-13-2 and ARS134-B-1-1-5-B demonstrated high mean values for yield, making them favorable recommendations for farmers in the Sange site. On the other hand, accessions IR990-48-B-B-12, Magoti, IR88638, IR841 and WAHX14N-926 exhibited high mean values for yield, suggesting their suitability for cultivation by farmers in the Bwegera site. Based on grain yield across sange and Bwegera sites, accessionIR88638 emerged as a promising choice for cultivation in both sites.

3. Futher research could explore the broader adaptability of these evaluated accessions to similar agroecological contexts in the Eastern Democratic Republic of Congo or other regions with comparable conditions.

4. The identified markers and candidate genes, after validation and confirmation through experiment, will represent valuable resources that can be utilized in rice breeding programs and implemented in Marker Assisted Selection (MAS).

5. The provided resources (08FAN10 for Fe and Komboka for Zn) can be used to develop improved rice lines with enhanced grain Fe and Zn content, thereby enriching their nutritional value and contributing to enhanced global health outcomes.

6. Additional functional validation of the identified candidate genes will provide deeper insights into their roles in Fe and Zn uptake, transport, and accumulation in rice grains.

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APPENDICES

Appendix I : Summary of Obtained Quality Control (QC Material T18106QC)

Qc data	Fe (mg/100g) *2.01 mg/100g; **1.60-2.42 mg/100g	Zn (mg/100g) *3.14 mg/100g; **2.54-3.73 mg/100g
Batch 1	2.12	3.08
Batch 2	2.11	3.24
Batch3	1.83	2.89
Batch4	2.15	2.94
Batch 5	1.94	2.55

Appendix II: Density of SNPs on Chromosome Based on GBS

Chromosome	Length(bp)	SNP	Density(Mb)
Chr01	43166467	1018	42.4
Chr02	35880116	895	40.09
Chr03	36190712	790	45.81
Chr04	35289647	914	38.61
Chr05	29840269	593	50.32
Chr06	31007247	596	52.03
Chr07	29542077	648	45.59
Chr08	28309384	643	44.03
Chr09	22369061	571	39.18
Chr10	23158805	386	60
Chr11	28877908	651	44.36
Chr12	27521303	674	40.83
Average	31875301.23	1289.08	45.76
Total	**43225920**	**8379**	**51.59**

Appendix III : List of Publications

Peer-reviewed articles:

Bukomarhe C.B.; Kimwemwe P.K.; Githiri, S.M.; Mamati, E.G.; Kimani W.; Mutai C.; Nganga F.; Nguezet PM. D.; Mignouna J.; Civava R.M.; and Fofana M. (2023). Association mapping of candidate genes associated with Iron and Zinc Content in Rice (*Oryza sativa* L.) Grains. *Genes*, 14 (1815). https://doi.org/10.3390/genes14091815

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