MORPHOLOGICAL, MOLECULAR AND QUALITY CHARACTERIZATION OF RICE VARIETIES AND LANDRACES FROM EASTERN AND SOUTHERN AFRICA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

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ABSTRACT

This study was carried out to evaluate the genetic diversity of rice germplasms collected from Eastern and Southern Africa countries (Burundi, Kenya, Malawi, Tanzania including Zanzibar and Rwanda) based on morphological, molecular and quality traits for utilization in breeding programmes. A total of 191 rice germplasms were characterized in this study. Twenty four qualitative and quantitative morphological traits, eight grain quality traits and 18 Simple Sequence Repeat (SSR) markers were used for analysis. The plant morphological traits viz., basal leaf sheath color, leaf blade color, panicle exsertion, panicle type, apiculus color, lemma/palea color, awning, number of days to 50% flowering, days to maturity, leaf length and width, panicle length, plant height, spikelet fertility showed most variation among the genotypes. Among the grain quality traits, milling recovery, degree of milling, 1000-grain weight, brown rice length and shape exhibited most variation among the evaluated genotypes. Genetic variation analysis of morphological and grain quality traits resulted in grouping of the germplasms into seven clusters. Principal component analysis showed that 75.37% of the variability was contributed by the first six principal components. A total of 18 SSR's markers were used and 16 found to be polymorphic. A total of 121 alleles were obtained on polymorphic SSR with an average of 7.56 allele per marker and the number of alleles ranged from 2 to 20. The Polymorphism Information Content (PIC) values ranged from 0.01 to 0.89 with an average value of 0.49. The genetic diversity of each SSR locus appeared to be associated with number of allele detected per locus. The cluster analysis based on similarity index of simple matching grouped the studied rice genotypes into six

clusters. The information obtained will be very useful in identification and selection of suitable parents for use in breeding programmes to develop unique germplasms that complement existing varieties.

DECLARATION

I, Judith Joachim, do hereby declare to the Senate of Sokoine University of Agriculture that this Dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institutions.

_____ _____

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DEDICATION

To my Almighty God who allowed me to accomplish this work without Him this work could be impossible. Most to my parents Mr. and Mrs. Joachim Mmassy and my brother Juvenal and sisters Jacqueline and Immaculate. Also I dedicate to my lovely husband Johnson John because he was there for me in so many ways and my future children will be our great delight.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
APS	Ammonium Persulfate
BecA	Biosciences for Eastern and Central Africa
CTAB	Cetyl Trimethyl Ammonium Bromide
DAP	Diammonium Phosphate
DNA	Deoxyribonucleic Acid
DUS	Distinctiveness, Uniformity and Stability
EDTA	Ethylenediamine Tetraacetic Acid
ESA	Eastern and Southern Africa
EtOH	Ethanol
FAO	Food and Agriculture Organization
GCP	Generation Challenge Program
H_2O	Water
ILRI	International Livestock Research Institute
IRRI	International Rice Research Institute
Kg ha^{-1}	Kilogram per hectare
$MgCl_2$	Magnesium Chloride
Min	Minutes
mM	milliMolar
ng	Nanogram
NJ	Neighbor Joining
PAGE	Polyacrylamide Gel Electrophoresis

PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
QTL	Quantitative Traits Loci
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolution per minute
SES	Standard Evaluation System
SNP	Single Nucleotide Polymorphism
SSR's	Simple Sequence Repeats
$t\ ha^{-1}$	Tone per hectare
TBE	Tris Borate EDTA
TE	Tris EDTA
V	Volts
µl	Microliter
µM	microMolar

CHAPTER ONE

1.0 INTRODUCTION

The cultivated rice plant (*Oryza sativa L.*) belongs to the tribe Oryzeae under the sub-family Pooideae in the grass family Gramineae (Poaceae). It is distributed throughout the tropics and subtropics however it is grown more easily in the tropics (Acquaah, 2007). Two species of rice are considered important as food species for humans: *Oryza* L., *sativa*, which is grown worldwide and indigenous to Asia; and *Oryza glaberrima* Stoud, the cultivated species of Africa.

The two species can be distinguished in the field especially by differences in ligule shape and panicle branching. *Oryza sativa* have long (40-45 mm), pointed and thin ligules and many panicle branches, while *oryza glaberrima* has short (6 mm), oblong and thick ligules and lack secondary branching on the primary branches of the panicle (Sarla and Swamy, 2005). Both of these belong to a bigger group of plant the genus *Oryza* that includes about 20 other (wild and weedy) species (Maclean, *et al.,* 2002).

Rice is the world's most important staple crop. It is the staple food for more than half of the world's population, most of them in the developing countries (Beverley *et al.,* 1997). It is ranked second to maize among the most cultivated food crops in the world (Moukoumbi *et al*., 2011). Human consumption accounts for 85% of total production for rice, compared with 72% for wheat and 19% for maize (Maclean *et al.,* 2002). According to Oteng and Sant'Anna, (1999), during the past three

decades, the rice crop has seen consistent increase in demand and its growing importance is evident in the strategic food security planning policies of many countries. However, self-sufficiency in rice production is declining as demand increases. This has seen Africa become a big rice importer in international markets, accounting for 20% of global imports, with a record level of 10 million tonnes per year (Hannon and Cassell, 2012). This is due to population growth at 4% per annum, rising incomes and a shift in consumer preferences from traditional food eating habits in favor of rice, especially in urban areas; hence the relative growth in demand for rice is faster in Africa than anywhere in the world (Balasubramanian *et al.,* 2007). In 2012, Africa rice consumption was 24 million tonnes of milled rice (Seck *et al.,* 2013) of which 10 million tonnes was imported, hence there is need to increase rice production in order to meet the rice demand in the continent.

The need for improving rice production does not only depend on rice crop and management technologies, but also on the suitability of rice varieties, which must be drawn from existing germplasm that has been collected and conserved by national, regional or international genetic resource centers. Detailed evaluation and characterization of available rice genotypes is one of the main prerequisites in conservation and sustainable utilization of rice genetic resources. This ensures that maximum variation is captured in designing breeding strategies aimed at increasing productivity. Information on diversity and population structure is expected to assist plant breeders in selection of parents to be used in hybridization programmes, provide a more rational basis for expanding the gene pool and for identifying plant materials that harbor more valuable alleles for genetic improvement (Semon *et al.,*

2005). Therefore, rice diversity is the foundation for variety improvement programmes, and better use of this diversity can both help solve current biotic and abiotic production problems and create rice cultivars resilient to these constraints. Almost in all major crop species, morphological and physiological descriptors are available to establish the uniqueness of a variety (Moukoumbi, *et al.,* 2011). Hence, characterization and identification of rice cultivars are crucial for the genetic varietal improvement, release and seed production programmes.

However, the utilization of the genetic resources of the rice crop are mostly being used for higher yields and early maturity (Ogunbayo *et al.,* 2005). But, in most East and Southern Africa countries, a variety will not be fully accepted only for its high yielding properties until it's combined with good acceptable grain qualities that meet farmers' needs and culinary preferences. Therefore, there is need to understand available genetic resources for better quality traits since inferior grain quality of the currently high yielding varieties in the domestic market is a dominant phenomenon (Samado *et al*., 2008).

For acceptance and economic reasons, grain quality aspects of rice grains such as size, shape, chalkiness, translucency, color, milling quality, eating and cooking quality call for more consideration in breeding programmes. According to Bhattacharya (2004), there is a wide variety divergence in rice quality and this has attracted the attention of researchers in developing high quality rice. Thus, rice grain quality should not be neglected in rice breeding programmes because of its crucial importance of culinary preference to consumers and hence market-driven demand

and premium price. Ndour (1998) revealed that, techniques such as plant characterization have been successfully used in identifying elite individual genotypes. It is an indispensable tool for selecting varieties or lines based on agronomical, morphological, genetic or physiological characters. Therefore, in this study, the same technique (characterization) was used to identify the diversity that exists among the improved varieties and landraces collected from Eastern and Southern Africa (ESA) region. Thus characterization of these varieties will further contribute towards creating genetic database for breeding programmes strategies in the region.

1.1 Overall Objective

Morphological, molecular and quality characterization of the cultivated rice varieties and land-races from ESA region for their better utilization in breeding programmes.

1.2 Specific Objectives

The specific objectives of the study were to;

i. Phenotype the diversity of the collected rice varieties and landraces from ESA countries using morphological traits.
ii. Evaluate the genetic diversity of the cultivated rice varieties and landraces from ESA region using molecular markers.
iii. Determine grain quality characteristics among rice varieties and landraces collected from ESA.

CHAPTER TWO

2.0 LITERATURE REVIEW

About half of the world's population depends upon rice as food and it accounts for 20% of the global human per capita energy and 15% per capita protein. Besides its importance as food, rice is also the most important crop to millions of small farmers who grow it on millions of hectares and to the many landless workers who obtain income from working on these farms (Maclean, *et al.,* 2002).

Rice is rapidly becoming an important crop in Africa as it accounted for 4.3% of world's rice production area, 2.5% of rice productivity and 20% of the world's rice imports (FAO, 2000). According to Aniekwe (2010), rice consumption in Africa, is growing at even faster rates, and gradually replacing more traditional food crops such as cassava, sweet potatoes, millet and sorghum.

Although rice is the main agricultural crop in most tropical countries, majority of the ESA countries are well below 2 t ha^{-1} paddy productivity (Tanzania, 1.9 t ha^{-1}; Uganda, 1.4 t ha^{-1}; and Mozambique, 1.2 t ha^{-1}). Kenya, Burundi and Rwanda have a little bit higher than 2 t ha^{-1} average productivity however, they have comparatively limited rice area (< 50,000 ha) (Singh *et al.,* 2013). Rice production in ESA region cannot keep up with rising demand and much of this demand has come from urban consumers who prefer imported rice from Asia because it is cheaper than locally grown rice (Rickman, 2008). The data generated from this work will assist breeders in strategizing breeding programmes so as to increase the rice

production in the region. Increased local production with good grain qualities would reduce reliance on imported rice and meet rice demand in region.

2.1 Morphological Characterization

Evaluation of germplasm accessions in any genetic material collections is essential to ensure the principles of conservation and utilization of germplasm hence characterization of morphological traits of rice is important (Riley *et al*., 1995). According to Thimmanna *et al.* (2000) the characters such as leaf length and width, pubescence of leaf, leaf angle, ligule shape and colour, panicle type, secondary branching, exertion, awning, seed length and width and 1000 grain weight can be used in differentiating the parental lines of rice cultivar.

In addition, based on the study done by Mehla and Kumar (2008) on various morphological characters responsible for identification of rice cultivars, they concluded that there exists wide variation among the rice cultivars in respect to morphological characters viz. awn length, panicle length, leaf blade colour and leaf sheath colour, node base colour, awning, distribution of awns, stigma colour, anthocyanin colouration of stem nodes and internodes, hence, these characters can be used for identification of rice cultivars.

Moreover, when Ashfaq *et al.* (2012) associated various morphological traits with yield, there was a strong association revealed between the plant yield and the other yield component traits namely panicle length, number of seeds per panicle, productive tillers per plant and seed weight per panicle. The yield component traits

were associated with other traits that also had a great contribution to the improvement of yield. For instance, panicle length was associated with flag leaf area, number of primary branches per panicle, number of spikelets per panicle, number of seeds per panicle and grain weight per panicle were directly or indirectly associated with the plant yield, leading to increased rice yield.

2.2 Genetic Diversity

Genetic diversity is generally defined as the amount of genotypic (on the DNA level) variability present in a group of individuals. This genetic diversity gives species the ability to adapt to changing environments, including new emerging biotic (pests and diseases) and abiotic stresses such as global warming (Parmesan and Yohe, 2003). Molecular/DNA marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular trait (Kumar, 2009). This approach is based on DNA polymorphism among tested genotypes, and thus reveals sites of variation in genomic DNA.

Genetic divergence is an efficient tool for an effective choice of parents for hybridization programme and molecular markers have been used to verify the diversity. It assists in selection of the genetically divergent parents to obtain desirable combinations in the segregating generations (Banumathy *et al.,* 2010). Knowledge regarding the amount of genetic variation in germplasm accessions and genetic relationships between genotypes are important considerations in designing effective breeding programmes.

DNA/molecular marker offers many advantages over other categories of markers such as morphological, cytological or biochemical markers. Molecular markers can cover large number of loci detectable with a single procedure. There is more polymorphism in DNA markers, which are able to reveal the variation and allelism. Many DNA markers are co-dominant and can differentiate between the homozygous and heterozygous genotypes. Furthermore, DNA markers are neutral, and they have no effect on phenotype, no epistatic effect, are not influenced by environmental conditions and are normally expressed in all tissues and can be scored at all developmental stages (Kesawat and Das, 2009).

There are several DNA markers available that are being used for detection of genetic variations among populations and individuals as mentioned hereunder. Restriction Fragment Length Polymorphism (RFLP) is defined as the variation(s) in the length of DNA fragments produced by a specific restriction endonuclease from genomic DNAs of two or more individuals of a species. It requires the presence of high quantity DNA, is not amenable for automation, level of polymorphism is low and few loci are detected per assay. Random Amplified Polymorphic DNA (RAPD) is generated using synthetic short oligomers (usually 10-mers size) of arbitrary primers in the polymerase chain reaction (PCR) that will amplify anonymous genomic sequences or regions from a DNA template (Park *et al.,* 2009). Amplified Fragment Length Polymorphism (AFLP) combines the power of RFLP with the flexibility of PCR based technology, in which a subset of restriction fragments are selectively amplified using oligonucleotide primers complementary to sequences that have been ligated to each other (Maheswaran, 2004). Another PCR-based DNA marker is

Simple Sequence Repeats (SSR). SSR or microsatellites are short tandem repeats (1-10bp), interspersed throughout the genome and can be amplified using primers that flank these regions (Park *et al.,* 2009). Single Nucleotide Polymorphism (SNP's) is a DNA sequence variation occurring when a single nucleotide in the genome differs between members of a species. It is widely used as a genetic marker in mapping experiments and Quantitative Trait Loci (QTL) analyses (Kim *et al.,* 2009).

DNA markers that differentiate genotype are more reliable and convenient than physiological characters in the identification and characterization of genetic variation (Zeng *et al.,* 2004). Seetharam, *et al.* (2009) concluded that, the best measure to analyze genetic diversity among genotypes would be with the use of all information; both from morphological characters and DNA based markers.

Simple sequence repeats (SSRs) have been used for characterizing genetic diversity in several crop species including sorghum (Dean *et al.,* 1999; Smith *et al.,* 2000), wheat (Prasad *et al.,* 2000; Sehgal *et al.,* 2012), maize (Senior *et al.,* 1998; Legesse *et al*., 2007) and cotton (Liu *et al.,* 2000; Islam *et al*., 2012). Susan McCouch developed the first molecular map of rice genome in 1988, since then many maps have been published utilizing different kinds of populations and many types of molecular markers. In rice, SSRs have been used to assess the genetic diversity of both wild and cultivated species (Siwach *et al.,* 2004; Neeraja *et al.,* 2005; Joshi and Behera, 2006; Lapitan *et al.,* 2007; Sajib *et al*., 2012). SSRs are more popular because they are highly abundant, co-dominant, cost effective, highly reproducible

and exhibit a high degree of allelic variation at each locus (Panaud *et al.,* 1996; Temnykh *et al*., 2000). Moreover, SSRs are the best molecular marker system for many types of genetic analyses, including germplasm surveys, linkage mapping, and phylogenetic studies (Ghneim *et al.,* 2008).

In the study done by Giarrocco *et al*. (2007) on genetic diversity, the SSR markers grouped the *Oryza sativa* accessions of Argentine rice cultivars into two major groups, *indica* and *japonica*. On the other hand, Jayamani *et al.* (2007) analysed 178 rice accessions of Portuguese origin using 24 SSR covering two loci per chromosome, and all the loci were polymorphic among the accessions and clearly distinguished the *indica* and *japonica* subspecies.

Also, Ghneim *et al.* (2008) evaluated genetic diversity in 11 Venezuelan rice cultivars using simple sequence repeats markers, the results showed that all 48 SSRs were polymorphic across the 11 genotypes and a total of 203 alleles were detected.

According to Seetharam *et al*. (2009), thirty rice genotypes comprising land races, pure lines, somaclones, breeding lines and varieties specifically adapted to coastal saline environments were characterized by SSR markers and morphological characters, when out of 35 primers of SSR markers, 28 were polymorphic.

Thus, from the studies done by various researchers it shows that SSR markers are efficient in detecting genetic polymorphisms on rice genotypes.

2.3 Quality Characterization

Rice grain quality is an important criterion in most rice breeding programs because it exerts large effects on market value and consumer acceptance. According to Traore (2005), rice grain quality is considered second most important problem following yield, although it is rarely mentioned in Africa as a constraint. However, in several cases, even varieties with high yield are rejected by consumers because of their poor appearance, cooking and eating qualities. As such development of cultivars with good grain qualities is an important objective to emphasize in rice improvement programmes (Lapitan *et al.,* 2007). Grain appearance and culinary grain quality (milling, cooking and eating qualities) are the major criteria considered in evaluation of grain quality in a breeding programme.

Grain appearance consists of size and shape of the kernel, translucency and chalkiness of endosperm. Size and shape is a stable varietal property that can be used to identify a variety and are among the first criteria of rice quality that breeders consider in developing new varieties (Traore *et al.,* 2011). Rice varieties with little or no chalkiness in their endosperm are more preferred by consumers, because percentage grain chalkiness is closely related to milling quality. Chalky grains have a lower density of starch granules and are therefore more prone to breakage during milling, hence end up with poor quality rice and low milling recovery (Hai-mei *et al.,* 2011). When the rice grains are more broken, consumers do not prefer them and they fetch low market prices. Grain appearance is therefore essential as it attracts the attention of the consumer, and although it has no effect on cooking and eating quality, it is the first basis on which a consumer accepts or rejects a variety.

The aim of milling rice is to remove the husk, the bran layers and the germ with minimum breakage to the grain hence to produce an edible, white rice kernel that is sufficiently milled and free of impurities (IRRI, 2009). It is also one of the most important criteria of rice quality and a crucial step in post-production of rice. The degree of milling is another quality characteristic of rice and it is defined as a measure of the percentage bran removed from the brown rice kernel. Apart from the amount of white rice recovered, it influences the color and the cooking behavior of rice (IRRI, 2009). The accurate measurement of the amounts and classes of broken grains is very important to consumers and breeders (Mutters, 2003).

Bhonsle and Sellappan (2010) evaluated the grain quality of traditionally cultivated rice varieties of Goa and concluded that some of the traditional rice varieties were with high grain quality characteristics, which could be used in rice breeding programmes and biotechnological research for further improvement of rice.

Subudhi *et al*. (2012) evaluated forty one rice varieties of different ecologies to find out those with better grain quality characters and yield, for use in varietal development programme and were further popularized among farmers.

Moreover, a study was conducted by Kanchana *et al*. (2012) to know the physical qualities of 41 rice varieties and seven varieties were found to be the best according to the length, breadth, bulk density and 1000 grains weight.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

One hundred and ninety two rice genotypes from five ESA countries (Burundi, Kenya, Malawi, Tanzania (including Zanzibar) and Rwanda and advanced breeding lines from regional multi environment trials (MET)) comprising of landraces and improved varieties provided by collaborators in the above captioned countries, were used in this study (Appendix 1).

3.2 Methods

3.2.1 Assessment of phenotypic diversity using morphological traits

The germplasms were grown and evaluated for morphological traits at Bagamoyo Irrigation Scheme, Pwani-Tanzania, during wet season starting March to July 2013. The seeds were sown in the nursery bed before they were transplanted at 21-day old. The experiment was laid out in an Alpha Lattice Design with three replications with ensured irrigation. One seedling was transplanted per hill, spaced at 20 cm x 20 cm and the inter-plot spacing was 40 cm. A plot size of 0.8 m x 3 m was used for each germplasm in the field. Recommended cultural practices like fertilizer application were adopted. Diammonium Phosphate (DAP) (18% N, 46% P_2O_5, 0% K_2O) and urea as basal application at a rate of 15 kg ha^{-1} and 8 kg ha^{-1} were top dressed 5 days after sowing, and urea at the rate of 20 kg ha^{-1} was applied as top dressing at tillering and at panicle initiation stages. Weeding was done by hand whenever weeds appeared.

Morphological data were collected for both quantitative and qualitative characters at appropriate growth stage of rice. The procedures and descriptors for characterization and evaluation of the morphological traits of rice was based on the IRRI descriptors (1996) and Bioversity International-IRRI-AfricaRice (2007). Twenty four (24) major traits for DUS testing were recorded for the morphological characterization of 191 genotypes as one failed to grow (Appendix 2).

3.2.2 Evaluation of the molecular diversity using SSR markers

Laboratory work on genetic variation was done using SSR markers at ILRI-BecA hub in Nairobi. Seeds of all genotypes were grown in a screen house at ILRI in small plastic containers and sampled after 20 days for DNA extraction.

3.2.2.1 Genomic DNA extraction from leaves

DNA was extracted from all the genotypes following the procedure of CTAB method by Mace *et al.* (2003). Freshly harvested leaves were cut into small pieces (approximately 10-20 mg) and put inside 2ml sterile extraction tubes with 2 autoclaved steel balls. The tubes were closed, arranged in the racks and placed on dry ice to prevent degradation of DNA. The collected samples were stored at -80°C freezer until DNA extraction was done. A couple of racks were removed from freezer and placed onto cryogenic container where liquid nitrogen was poured over the tubes followed by grinding using the GenoGrinder (SPEX SamplePrep 2010 Geno/Grinder) for 5 minutes under 1500 rpm. Six hundred microliters of 2X CTAB (Cetyl Trimethyl Ammonium Bromide) buffer, pre-warmed to 65°C was added to each tube using micropipette and mixed thoroughly. The mixture was incubated in

water bath at 65°C for 1 hour with intermittent mixing after every 15 minutes. From the water bath, the mixture was briefly cooled and thereafter 600 µl of chloroform-isoamyl alcohol (24:1) was added and mixed at room temperature. Racks containing tubes with mixtures were placed into centrifuge and spun at 3500 rpm for 10 minutes. The aqueous phase was aspirated into new tube. Six hundred microliters of ice-cold isopropanol was added to each tube and incubated at -20°C for 1 hour. From the freezer, the tubes were spun at 3500 rpm for 10 minutes. The solution was decanted and the pellet washed with 600 µl of 70% ethanol (EtOH), followed by spinning at 3500 rpm for 5 minutes and air-dried. The dried pellets were dissolved into 200 µl of TE (Tris-EDTA) buffer and 2 µl of RNAase (10 mg/ml) was added and incubated at 37°C for 1 hour. Twenty microliters of 3M sodium acetate (1/10 volume solution) and 400 µl of absolute EtOH were added and the mixture was incubated at -20°C for 1 hour before spinning at 3500 rpm for 5 minutes. The liquid was drained and pellets were washed with 600 µl of 70% EtOH. The EtOH was discarded, pellets air dried and then dissolved in 100 µl TE. DNA was stored at 4°C until use. The DNA quality and concentration were checked on 0.8% agarose gel and the spectrophotometer (NanoDrop 2000c UV-Vis spectrophotometer, Thermo Fisher Scientific Inc., USA). The gel was run at 100 V for 30 minutes and bands were visualized and documented using a gel documentation system (InGenius, Syngene, USA).

3.2.2.2 SSR analysis

The Generation Challenge Program (GCP) standard panel of SSR markers was used for genetic diversity analysis. A panel of 22 SSR markers was used for the PCR

amplification. The sequence and details of the primer pairs/markers used are given in Table 1.

Table 1: Sequence and allele size of primer pairs/markers used

Primer Name	Linkage group	Expected Allele size	Forward Primer	Reverse Primer	Tm	Reference
RM11	7	140	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	55	Panaud *et al*.,1996
RM19	12	226	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	55	Panaud *et al.,*1996
RM44	8	99	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	55	Chen *et al*., 1997
RM105	9	134	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	55	Temnykh *et al*., 2000
RM118	7	156	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG	67	Temnykh *et al*., 2000
RM124	4	271	ATCGTCTGCGTTGCGGCTGCTG	CATGGATCACCGAGCTCCCCCC	67	Temnykh *et al*., 2000
RM125	7	127	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	55	Temnykh *et al*., 2000
RM133	6	230	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC	61	Temnykh *et al*., 2000
RM154	2	183	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	61	Temnykh *et al*., 2000
RM161	5	187	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG	61	Temnykh *et al*., 2000
RM162	6	229	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG	61	Temnykh *et al*., 2000
RM178	5	117	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC	67	Temnykh *et al*., 2000
RM215	9	148	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	55	Chen *et al*., 1997
RM237	1	130	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	55	Chen *et al*., 1997
RM252	4	216	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	55	Chen *et al*., 1997
RM271	10	101	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	55	Temnykh *et al*., 2000
RM277	12	124	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	55	Temnykh *et al*., 2000
RM273	4	207	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC	55	Temnykh *et al*., 2000
RM283	1	151	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG	55	Temnykh *et al*., 2000
RM307	4	174	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	55	Temnykh *et al*., 2000
RM334	5	182	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	55	Temnykh *et al*., 2000
RM413	5	79	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	55	Temnykh *et al*., 2000

T_m – Annealing temperature (°C)

Then, cocktail for PCR amplification was prepared as seen in Table 2 below.

Table 2: Cocktail for PCR amplification

Components of cocktail	Stock concentration	Final concentration	1 Reaction (10 µl)
Sterile nanopure H_2O			4.35 µl
10X PCR Buffer (+25 mM $MgCl_2$)	10X	1X	1.8 µl
dNTP mix	2.5mM	0.1 mM	0.8 µl
Primer Forward	5µM	0.25µM	1.0 µl
Primer Reverse	5µM	0.25µM	1.0 µl
Taq polymerase	5U/µl	1 U/10µl	0.05 µl
DNA (5-25 ng)			1.0 µl
Total			10 µl

The reaction mixture was spun for thorough mixing of the cocktail components before being aspirated in a 96-well PCR plate, which was then placed in the thermal cycler (Applied Biosystems GeneAmp PCR system 9700, Thermo Fisher Scientific Inc., USA). The PCR profile used is as indicated below in Table 3.

Table 3: PCR profile

1.	Initial denaturation	95 °C	3 min
2.	35 cycles of the following steps;		
	• Denaturation	95 °C	30 sec
	• Primer annelling	56 °C	1 min
	• Extension	72 °C	2 min
3.	Final extension	72 °C	30 min
4.	Storage	4 °C	∞

3.2.2.3 Polyacrylamide gel (PAGE)

After the PCR amplification, the PCR products were separated using 8% polyacrylamide gel. Glass plates were thoroughly washed and air-dried. Ninety five percent ethanol was sprayed on surfaces of plates, which were then wiped with lint-free tissue. The gasket was attached on the round bottomed plate and spacers were put along the inside edges of the gasket. Then the other plate was placed on top of the bottom assembly and clamps were set on both sides of the plates. The gel solution was prepared in a beaker following the order of reagents as shown in Table 4.

Table 4: Composition of PAGE reagents used to prepare gel solution

Reagents	Final concentration required	Volume used to make 8%
Sterile reagent H_2O		34.5 ml
5x TBE buffer	0.5x	5.0 ml
40% Acrylamide	8%	10.0 ml
10% APS	0.1%	0.5 ml
TEMED	0.0833µl/ml	41.5 µl
Total		50.0 ml

The prepared gel was smoothly and continuously poured in between glass plates starting from one corner until it reached the top portion of the plates. A comb was gently inserted between the plates and the gel was allowed to polymerize for 30 minutes. Tris Borate EDTA (1xTBE) buffer was added in the base of the electrophoresis tank and the plates (with polymerized gel) were attached with clamps on sides of the tank after the gasket was removed. TBE buffer was added on top of the tank and the comb was removed from the gels. Three microliters of PCR product was mixed well with 3 µl of 1X loading dye and loaded into the gel well.

DNA ladder (O'GeneRuler Low Range ladder) for size determination was loaded into first gel well. Gel was run at 100 V for 45 minutes. Acrylamide gel was gently removed and stained with GelRed for 30 minutes. The stained gel was put in the exposure box of the gel documentation system (InGenius, Syngene, USA) for visualization of bands and photographed.

3.2.3 Determination of grain quality characteristics among rice varieties and landraces

The physical grain quality analysis was carried out at IRRI research station in Dakawa-Morogoro, Tanzania.

3.2.3.1 Grain physical dimensions

The brown rice (rice that hasn't been milled to remove the outer layer of bran and germ) size i.e. length and width was measured by randomly picking five fully matured grains from each genotype and the size was determined by graphical method (Kanchana *et al.,* 2012). Each grain was placed horizontally along the X-axis of the graph sheet. Then length and width were recorded and their means were calculated. One square grid in graph sheet was equal to 10 mm. Brown rice shape was determined by the length-width ratio. Based on the length of the grains, the brown rice grains were classified into four classes; extra long (>7.5mm), long (6.6 to 7.49mm), medium (5.51 to 6.6) and short (<5.5mm). The grains were also classified into four classes depending on their length to width ratio as slender (>3), medium (2.1 to 3), bold (1.1 to 2) and round (<1.1) (IRRI, 1996).

3.2.3.2 1000-Grain weight

Thousand grains of each genotype were counted and weighed using a weighing machine. Based on 1000-grain weight obtained for each genotype, they were classified into very low (<15g), low (15-20g), medium (21-25g), high (26-30g) and very high (>30g). Grain weight is a key determinant of grain yield in rice.

3.2.3.3 Milling quality

Hundred grams of rough rice/paddy (rice that the husk/hull have not removed) were weighed. The moisture content was determined using a digital grain moisture meter. The measured paddy was dehulled with testing husker machine and the resulting brown rice and husks were weighed separately. The brown rice was milled in a testing miller for 30 seconds. The bran was weighed and the weight of the total milled rice was determined. The following equations were used to determine the milling characteristics of rice samples (Graham, 2002).

$$\% \text{ Milling recovery} = \frac{\text{Weight of milled rice}}{\text{Weight of paddy}} X\ 100 \quad \text{.....(1)}$$

$$\% \text{ Degree of milling} = \frac{\text{Weight of milled rice}}{\text{Weight of brown rice}} X\ 100 \quad \text{.....(2)}$$

3.2.3.4 Grain chalkiness and translucency

A visual rating of the chalky proportion of the grain was used to determine the degree of chalkiness. The rating was based on the Standard Evaluation System [SES] scale as described by IRRI (1996), also presented in Table 5 below.

Table 5: Rating scale of chalkiness in milled rice grains

Scale	% area of chalkiness
0	None
1	Small (less than 10%)
5	Medium (11% to 20%)
9	Large (more than 20%)

The translucency was also determined visually at daylight and the grains where categorized as either translucent, opaque or cloudy.

3.2.4 Data analysis

3.2.4.1 Morphological and quality traits

The morphological and grain quality traits were given scores as per the IRRI Standard Evaluation System (SES) and Bioversity International-IRRI-AfricaRice descriptors for wild and cultivated rice. Data analysis was done using Plant Breeding Tools (PB Tools 1.2) software. The mixed model used was Yijk = $\mu + \tau i + \rho j + \beta jk + \varepsilon ijk$ where Yijk is value of the observed traits, τi is effect of the treatments (varieties), ρ j is effect of the 3 replicates, βjk is effect of incomplete block within the 3 replicates and εijk is the experimental error. Frequency distribution was computed to group the genotypes into different classes.

3.2.4.2 Cluster analysis

A dendrogram for the 191 rice genotypes was drawn using Statistical Tool for Agriculture Research (STAR 2.0.1) for cluster analysis based on morphological and grain quality traits.

3.2.4.3 Principal component analysis (PCA)

Principal components were computed using correlation matrix to examine the percentage contribution of each trait to total genetic variation by XLSTAT 2014.5.04.

3.2.4.4 Molecular data

3.2.4.4.1 Marker polymorphism

The clear and unambiguous alleles of SSR markers were scored manually and were coded as their integer size in base pair. Polymorphism Information Content (PIC) for each SSR was calculated by the formula PIC= $1-\Sigma X^2_k / n$ developed by Nei *et al*. (2002) where, X^2_k represents the frequency of the k^{th} allele, n represents the number of genotypes. Genetic diversity parameters that are heterozygosity, number of alleles for each marker, major allele frequency and genetic diversity within and among the accessions were done using PowerMarker version 3.0.

3.2.4.4.2 Cluster analysis

The SSR allele segregation data were used to construct similarity index between genotypes using simple matching coefficient. The similarity index was used for clustering the genotypes based on Neighbor-Joining (NJ) method by DARwin 5.0.

CHAPTER FOUR

4.0 RESULTS

4.1 Morphological Characterization Based on Qualitative Traits

4.1.1 Seedling vigor

Seedling vigor among the rice genotypes was recorded. In extra vigorous category, most were the improved varieties while the normal category were the landraces genotypes. Fifty nine (30.89%) genotypes were extra vigorous, 121 (63.35%) were vigorous and 11 (5.76%) were normal.

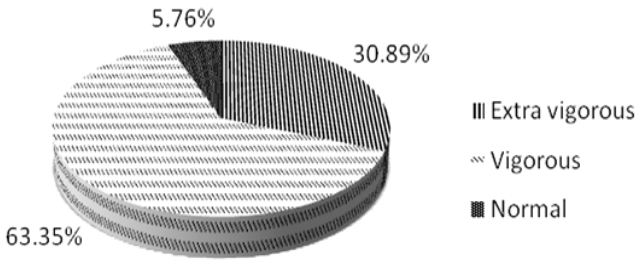

Figure 1: Seedling vigor of 191 rice genotypes as observed in seedling stage

4.1.2 Ligule shape and color

In this study, there was no variation on ligule shape, as all genotypes were observed to be 2-cleft. Moreover, two different colors were observed on ligules of the genotypes being characterized. Most of the genotypes were observed to have white ligule, whereby out of 191 genotypes, 177 (92.67%) were white and only 14 (7.33%) had ligule with purple lines.

4.1.3 Basal leaf sheath color

Most of the genotypes 174 (91.10%) were observed to have green basal leaf sheath color. The remaining genotypes had purple or mixture of purple color, whereby 3 (1.57%) genotypes had purple lines, 3 (1.57%) had light purple color and 11 (5.76%) were observed to have purple coloration.

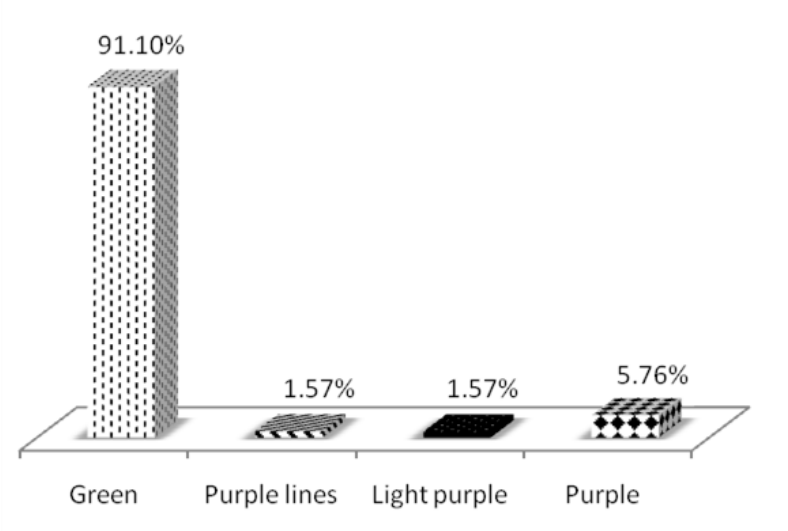

Figure *2:* Basal leaf sheath color of studied rice genotypes

4.1.4 Leaf blade color

Leaf blade color was another qualitative trait that was used to characterize the rice genotypes. In this study the germplasms were differentiated into light green 15 (7.85%), green 158 (82.72%) and dark green were 4 (2.09%). Leaf blade color of 2 (1.05%) accessions was observed to have purple tips and 12 (6.28) had purple margins.

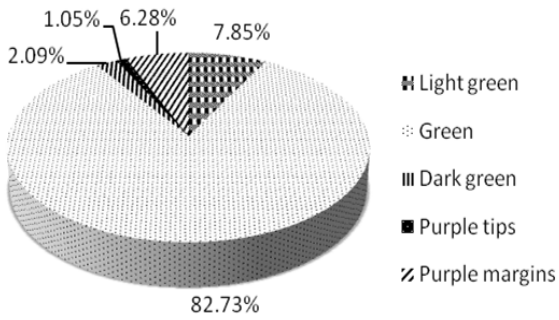

Figure 3: Leaf blade color as observed from the 191 rice genotypes

4.1.5 Leaf blade pubescence

The leaves of most rice varieties are pubescent but those of a few are glabrous. Fourteen (7.33%) genotypes had glabrous leaf blade pubescence and the rest of the studied genotypes 177 (92.67) were pubescent. Only one genotype (Mwangaza) from Tanzania was among the genotypes that were observed to have the glabrous leaf blade pubescence, the rest were from Kenya (8 genotypes) and Rwanda (5 genotypes).

4.1.6 Auricle presence/absence and color

Auricles were present in all genotypes studied although variation in color was observed. Most of the genotypes had light green auricles (184) while the rest (11) had purple auricles.

4.1.7 Stigma color

One sixty seven (87.43%) of the genotypes evaluated had white stigma, whereas 22 (11.52%) had purple and only 2 (1.05%) had light purple.

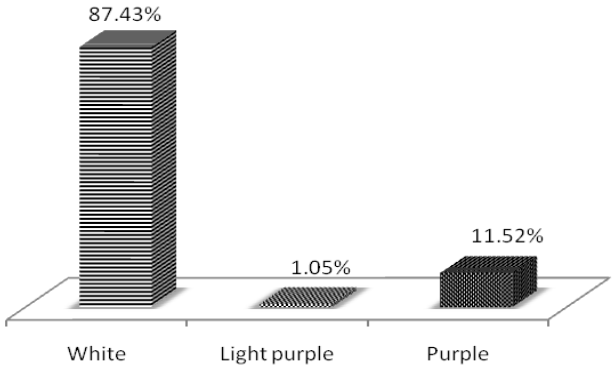

Figure 4: Stigma color of studied rice genotypes as observed at heading stage

4.1.8 Panicle exsertion

Based on panicle exsertion, the studied germplasms were distinguished into well exserted 7 (3.66%), moderately well exserted 51 (26.70%), just exserted 56 (29.32%) and partly exserted 77 (40.31%). It is a conspicuous character for identification of the rice cultivars.

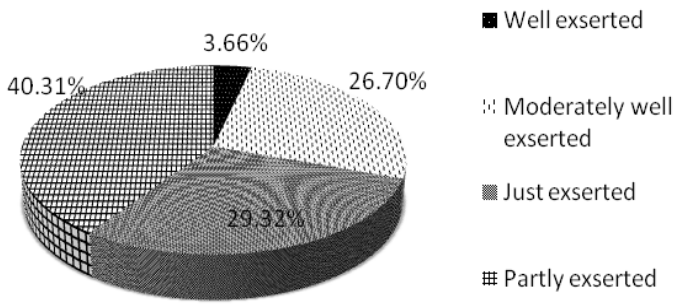

Figure *5:* Panicle exsertion of the studied rice germplasms

4.1.9 Panicle type

One thirty three (69.63%) genotypes had compact panicles, 54 (28.27%) had intermediate type of panicles whereas 4 (2.09%) had open panicles. Most of the genotypes with open panicles were the improved varieties.

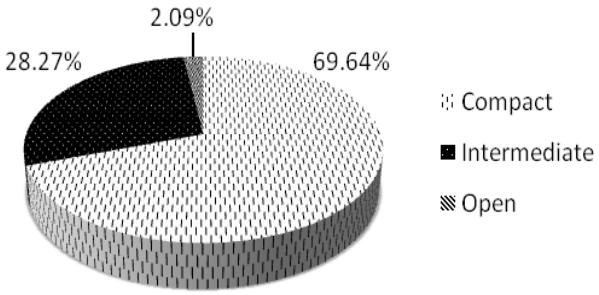

Figure 6: Panicle type of 191 rice germplasms as observed in dough stage

4.1.10 Apiculus color

Apiculus is the extending tip of the lemma or palea. The apiculus color of 145 (75.92%) genotypes was straw, 21 (10.99%) had brown apiculus, 7 (3.66%) had red, 15 (7.85%) had purple and the apiculus color of the remaining 3 (1.57%) was purple on the apex (purple apex). The genotypes with red apiculus were the landraces from Tanzania while the purple and purple on the apex were both improved and the landraces.

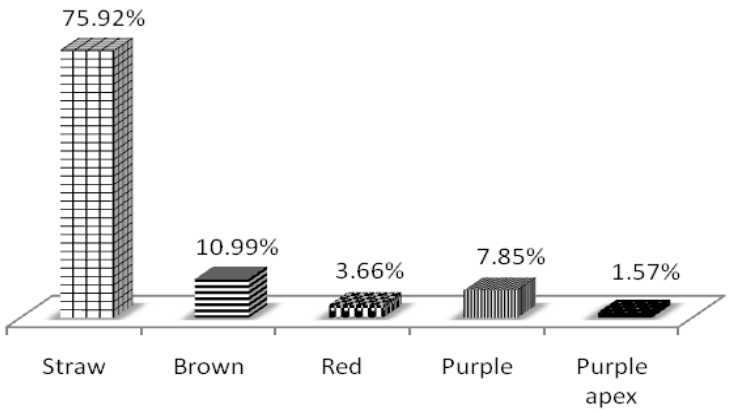

Figure *7:* Apiculus color of the studied rice genotypes

4.1.11 Awning

Majority of the genotypes 172 (90.05%) had no awns. Twelve (6.28%) had short and partly awns, 4 (2.09%) had long and fully and 3 (1.57%) had long and partly awns. Most breeders select awnless grains because the awns are tough, persistent and objectionable in milling and threshing.

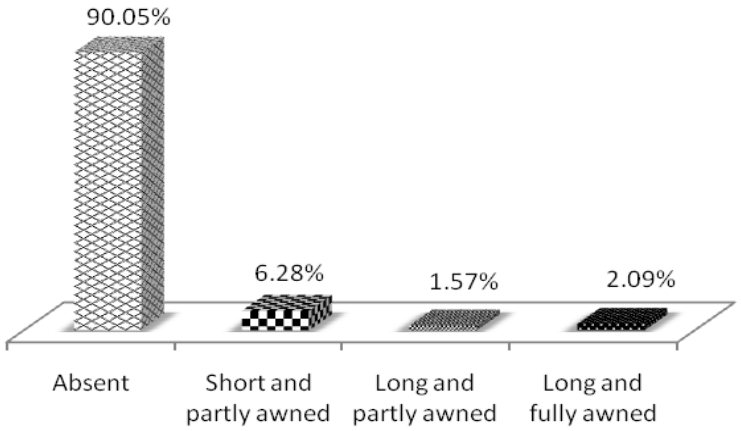

Figure 8: Awning of rice germplasms as observed at maturity stage

4.1.12 Lemma and palea color

Lemma and palea of most of the genotypes evaluated had straw color 155 (81.15%). Nineteen (9.95%) genotypes had brown furrows and 13 (6.81%) had brown color on lemma and palea. In addition, 2 (1.05%) of the genotypes had black color, 1 (0.52%) had purple and 1 (0.52%) had purple spots.

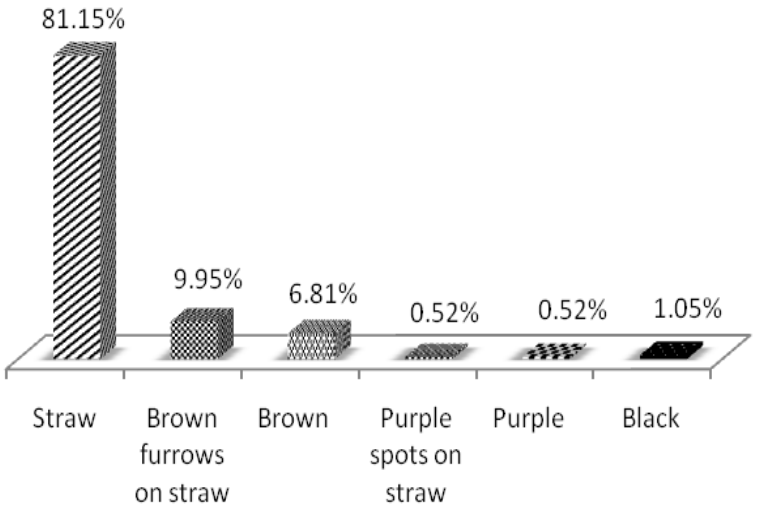

Figure *9:* Lemma and palea color as observed at maturity stage of rice

4.1.13 Sterile lemma color

Sterile lemma is the flowerless bract at the base of spikelet. Straw color on sterile lemma was observed in majority of the genotypes 187 (97.91%), while 3 (1.57%) and 1 (0.52%) had purple and gold color respectively.

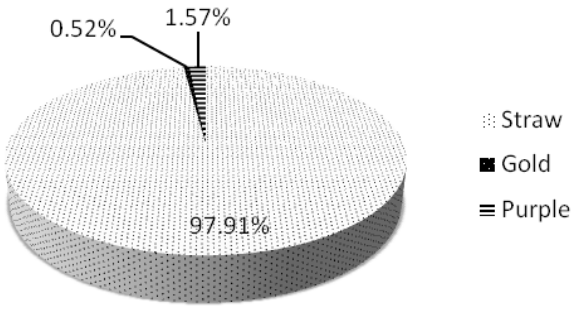

Figure 10: Sterile lemma color of rice genotypes as observed at maturity stage

4.2 Morphological Characterization Based on Quantitative Traits

Appendix 3 represents means of nine traits as obtained for each of the 191 rice

genotypes. Also it shows the grand means, F-probability and LSD (5%) for each trait. All the traits show a significant difference among the genotypes that were characterized. Seedling height varied from 35.10 cm to 57.66 cm and these were recorded for CH_164 (TXD 307) and CH_37 (Kaniki) genotypes respectively.

The leaf length (the leaf just below the flag leaf) varied from 29.89 cm (CH_167; IR 64) to 59.90 cm (CH_83; Tondogoso). The leaf length was classified as short, medium and long. Moreover, the genotypes were classified as narrow, medium and broad based on the leaf width, and it varied from 0.63 cm (CH_34; Singano) to 2.09 cm (CH_89; Lifumba). CH_172 (Mwangaza) was recorded the earliest flowering date (65 days) based on days taken for 50% flowering as well as earliest maturity date (96 days). However, CH_ 58 (JYAMBERE; Bug 2013A) was recorded the latest at 50% flowering and maturity dates of 111 and 142 days respectively. The shortest panicle length of 18.43 cm was recorded for CH_27 (Kachambo) whereas the longest panicle length of 30.63 cm was recorded for CH_123 (Jambo Twende). The genotypes were grouped as short, medium, long and very long based on panicle length. Moreover, the panicle number per plant ranged from few (4) to medium (17).

Based on plant height, the genotypes heights varied from 66.47 cm to 129.02 cm and these were recorded for CH_8 (IR 2793-80-1) and CH_113 (Afaa Melela). The evaluated genotypes were classified as partly sterile, fertile and highly fertile on percentage spikelet fertility basis. However, it varied from 69.70% for CH_173 (KUNGAHARA; Bug 2011A) to 96.17% for CH_180 (Yunyin).

4.3 Quality Characterization

4.3.1 Chalkiness

Majority of the genotypes 113 (59.16%) were observed to have less than 10% of chalky texture and 48 (25.13%) genotypes had no chalky texture in grains. The remaining 17 (8.90%) and 13 (6.81%) genotypes had medium (11%-20%) to large (>20%) chalky texture in grains.

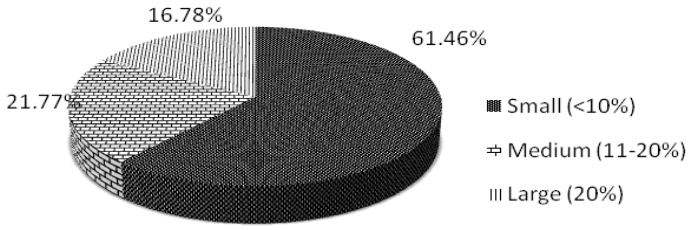

Figure 11: Chalkiness of 191 rice genotypes

4.3.2 Translucency

Based on translucency of the rice grain, 162 (84.82%) genotypes had opaque grains, 16 (8.37%) were translucency and 13 (6.81%) genotypes had cloudy grains.

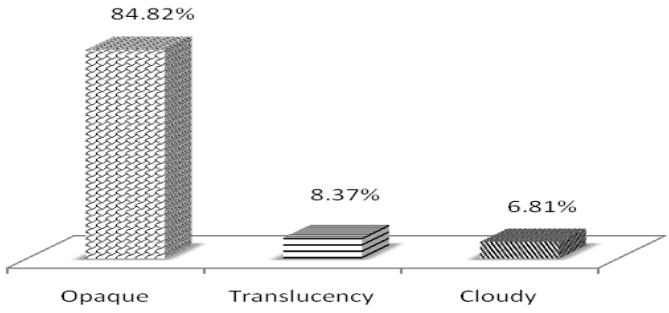

Figure 12: Translucency of the studied rice germplasms

4.3.3 Brown rice size

The shortest brown rice (de-hulled grain) length (5.15 mm) was found in CH_184 (Zhongeng) and the longest length (11.77 mm) was found in CH_78 (Kia la ngawa) (Appendix 4). Based on Standard Evaluation System for rice (IRRI, 1996), the varieties were grouped into four classes: short, medium, long and extra long. The dominant class was long (77 genotypes) and extra long (100 genotypes). Brown rice width varied from 2.2 mm to 5.0 mm.

4.3.4 Brown rice shape

Based on brown rice shape, the varieties were grouped in three classes: bold (7 genotypes), medium (166 genotypes) and slender (18 genotypes). The length-width ratio varied from 1.6 mm, which was recorded in CH_180 (Yunyin) and CH_184 (Zhongeng) to 4.2 mm in CH_78 (Kia la ngawa).

4.3.5 Milling recovery and degree of milling

The lowest milling recovery (61.28%) was obtained in CH_53 (Ringa nyekundu 2) and highest (71.93%) in CH_17 (Nerica 10). Moreover, based on degree of milling, the genotypes varied from 84.73% to 91.57% and these were recorded for CH_64 (IB 26, Bug 2013A) and CH_142 (Supa BC Improved).

4.3.6 1000-Grain weight

Thousand grain weight of evaluated genotypes varied from 16.52 g to 38.44 g. The lowest grain weight was in CH_53 (Ringa nyekundu 2) while the highest in CH_161 (Rumbuka).

According to their grain weight, genotypes were grouped into four classes: low, medium, high, and very high.

4.4 Cluster Analysis

Agglomerative clustering performed on the Euclidean distance matrix utilizing the Ward's linkage method and the resulting dendrogram is presented in Fig. 13. Quantitative morphological and grain quality traits were used to construct the dendrogram and 191 rice genotypes formed seven clusters. Distribution pattern indicated minimum number (14) of genotypes were included in cluster 1 and the maximum (37) in cluster 4. Cluster 2, 3, 5, 6 and 7 consisted of 29, 33, 32, 26 and 20 genotypes respectively. The list of entire seven clusters along with the genotypes included is presented in Appendix 5. The clustering pattern of some genotypes under this study did not follow their geographical distributions.

Moreover, in this study similar name genotypes from either same or different collection regions were grouped into same cluster. For example, Zambia from Malawi and Tanzania were grouped together while Rumbuka from Rwanda were grouped into same cluster. Also varieties named Supa Surungai, Supa Kijivu, Supa, Supa India and Supa Katrin all from Tanzania were grouped in the same cluster. However, the genotypes with similar names were grouped into quite different clusters. For example, varieties named Kia la ngawa were grouped into different clusters.

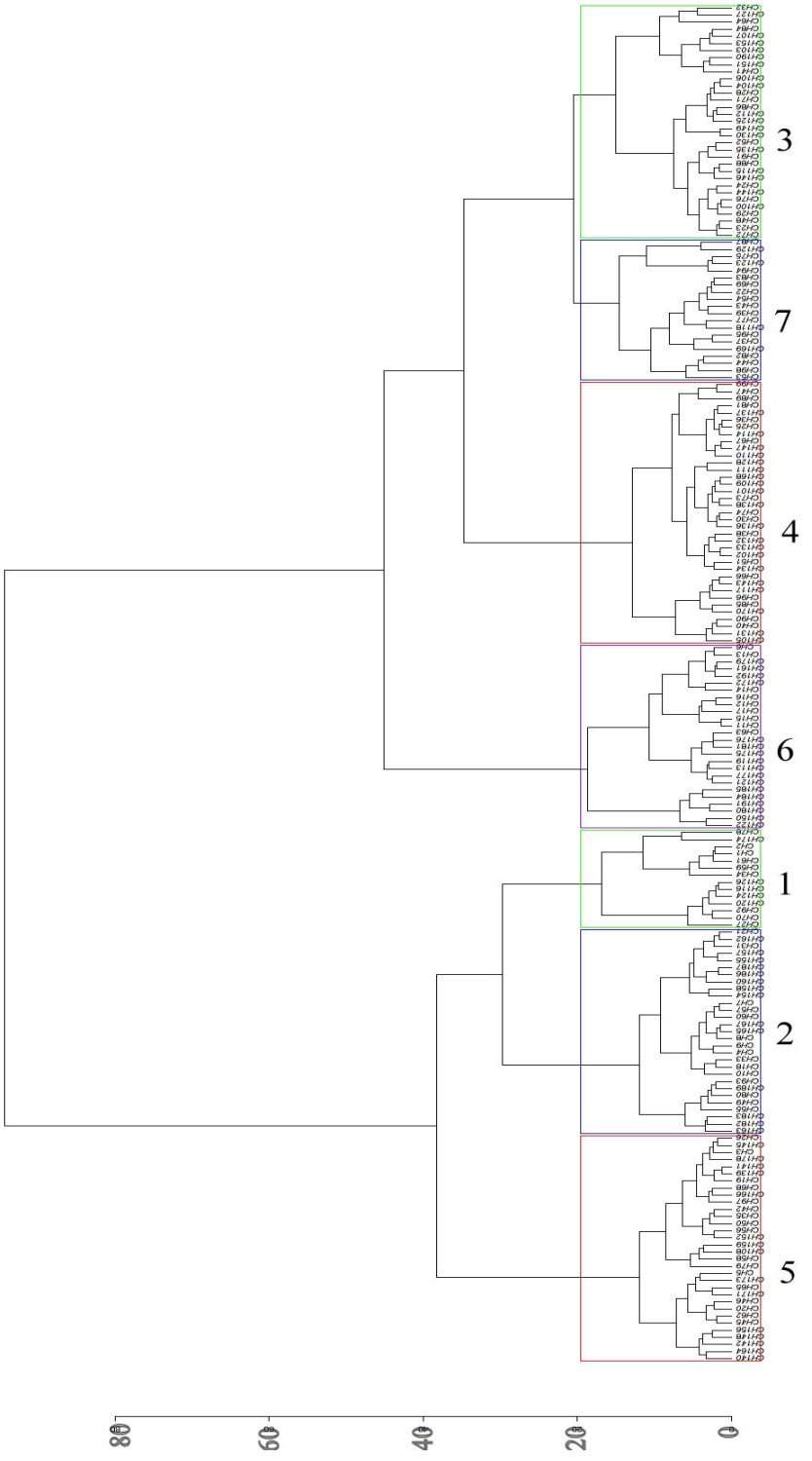

Figure 13: Dendrogram using agglomerative clustering method representing distribution of 191 rice genotypes based on morphological and grain quality traits

The highest cluster mean value was observed in cluster 1 for seedling height, brown rice length and shape; in cluster 2 for number of panicle per plant; in cluster 4 for leaf width and degree of milling; in cluster 5 for 50% flowering and days to maturity; cluster 6 for 1000-grain weight, milling recovery and brown rice width and in cluster 7 for leaf length, panicle length, plant height and percentage spikelet fertility (Table 6). Cluster 3 comprised of the genotypes with third highest mean for leaf length, panicle length, 1000-grain weight and brown rice shape. However, the lowest performed genotypes for more number of characters were included under cluster 2.

Table 6: Cluster mean of 15 morphological and grain quality traits of 191 rice genotypes

Characters	1	2	3	4	5	6	7
Seedling height (cm)	49.65	41.87	49.08	49.17	42.49	46.68	49.47
Leaf length (cm)	42.33	38.46	49.17	50.69	39.33	47.13	54.05
Leaf width (cm)	0.98	1.01	1.17	1.42	1.18	1.34	1.32
50% Flowering (days)	80.35	87.99	90.47	91.38	99.64	80.44	97.06
Panicle length (cm)	23.25	22.29	24.04	23.86	22.06	24.37	25.90
Plant height (cm)	97.69	78.75	98.74	101.58	80.31	104.78	110.40
Number of panicle/plant	10.51	12.15	8.72	7.54	10.06	5.46	7.77
Days to maturity	111.02	118.56	121.07	122.00	130.21	111.07	127.65
Spikelet fertility (%)	89.28	90.09	89.35	85.79	83.87	89.45	90.14
1000-grain weight (g)	26.42	24.16	26.97	30.83	26.61	32.27	23.67
Milling recovery (%)	66.29	67.72	66.23	67.79	67.44	69.23	65.63
Degree of milling (%)	87.95	88.39	87.74	88.92	88.58	87.68	88.70
Brown rice length (mm)	8.49	7.09	7.48	8.07	7.50	7.25	7.12
Brown rice width (mm)	2.73	2.71	2.82	2.95	2.94	3.08	3.04
Brown rice shape (mm)	3.15	2.65	2.71	2.76	2.61	2.39	2.47

4.5 Principal Component Analysis (PCA)

Table 7 shows the correlation matrix of 15 traits that were used to characterize 191 rice genotypes. The correlation matrix showed that there was positively correlation between leaf length and seedling height (0.66), leaf width and leaf length (0.48) and panicle length and leaf length (0.59). Also plant height was positively correlated with panicle length (0.64), leaf length (0.74) and seedling height (0.58). Also brown rice shape was positive correlated to brown rice length (0.75) but negatively associated with brown rice width (-0.57). Negatively correlation was observed in number of panicle per plant and leaf length, leaf width, 1000-grain weight and plant height.

Table 7: Correlation matrix of 15 traits used in characterizing the rice germplasms

	Seedling height	Leaf length	Leaf width	50% Flowering	Panicle length	Plant height	No. of panicle/ plant	Days to Maturity	Spikelet fertility	1000 Grain weight	Milling recovery	Degree of milling	Brown rice length	Brown rice width	Brown rice shape
Seedling height	1.00														
Leaf length	0.66	1.00													
Leaf width	0.20	0.48	1.00												
50% Flowering	-0.16	0.14	0.06	1.00											
Panicle length	0.38	0.59	0.27	-0.02	1.00										
Plant height	0.58	0.74	0.38	-0.08	0.64	1.00									
No. of panicle/plant	-0.28	-0.51	-0.66	0.17	-0.37	-0.52	1.00								
Days to maturity	-0.16	0.14	0.06	1.00	-0.02	-0.08	0.17	1.00							
Spikelet fertility	0.09	0.15	0.01	-0.23	0.14	0.14	0.00	-0.23	1.00						
1000-Grain weight	0.26	0.23	0.45	-0.37	0.13	0.25	-0.56	-0.36	-0.03	1.00					
Milling recovery	-0.17	-0.16	0.27	-0.28	-0.01	-0.03	-0.27	-0.28	0.05	0.41	1.00				
Degree of milling	-0.06	0.03	0.16	0.20	-0.18	-0.09	0.00	0.20	0.00	-0.05	0.17	1.00			
Brown rice length	0.16	0.12	0.10	-0.01	0.18	0.12	-0.12	-0.01	-0.10	0.29	0.02	0.01	1.00		
Brown rice width	0.06	0.18	0.32	-0.04	0.02	0.10	-0.28	-0.04	-0.01	0.25	0.06	-0.03	0.01	1.00	
Brown rice shape	0.08	-0.06	-0.19	0.01	0.09	0.00	0.15	0.01	-0.09	-0.02	-0.10	0.00	0.75	-0.57	1.00

The principal component analysis (PCA) showing the factor scores of each character among 191 rice genotypes, eigen value and percentage total variance accounted by six principal components is presented in Table 8. The six principal components accounted by about 75.37% of total variance with the first principal component taking 24.26%. The relative discriminating power of the principal axes as indicated by eigen values was high (3.64) for axis 1 and low (1.04) for axis 6. The first principal component that accounted for the highest proportion (24.26%) of total variation was mostly correlated with plant height, leaf length, panicle length, seedling height and grain weight. In second principal component the leaf length, days to 50% flowering and maturity were the most related traits. The third principal component was dominated by traits such as brown rice length and shape. Character that was mostly correlated with the fourth principal component was milling recovery. The fifth principal component was more related to degree of milling. Only spikelet fertility made substantial contribution to the sixth principal component.

The biplot of first and second principal component revealed that leaf length, panicle length, plant height and seedling height loaded more on the first component and accounted for more variation compared to other traits. Days to 50% flowering and days to maturity loaded more on second component (Fig. 14a). Moreover, the biplot of second and third component showed that days to 50% flowering and maturity loaded more heavily on second principal component than others. While brown rice shape and length loaded more to the third component (Fig. 14b).

Table 8: Eigen values, % variance and cumulative % variance of 15 morphological and grain quality traits for the first six principal components

Traits	Principal 1	Principal 2	Principal 3	Principal 4	Principal 5	Principal 6
Seedling height	0.67	0.19	0.05	-0.38	0.20	-0.30
Leaf length	0.74	0.52	-0.10	-0.09	0.16	-0.05
Leaf width	0.32	0.17	-0.16	0.33	-0.48	0.47
50% Flowering	-0.38	0.85	0.02	0.25	0.05	-0.02
Panicle length	0.69	0.32	0.00	-0.11	-0.14	0.22
Plant height	0.81	0.32	-0.02	-0.09	0.04	0.09
No. of panicle/plant	-0.71	-0.01	0.18	-0.41	0.12	0.05
Days to maturity	-0.38	0.85	0.03	0.26	0.05	-0.02
Spikelet fertility	0.21	-0.18	-0.14	-0.42	0.38	0.59
1000-Grain weight	0.60	-0.37	0.11	0.39	-0.01	-0.29
Milling recovery	0.23	-0.47	-0.02	0.59	0.17	0.21
Degree of milling	-0.12	0.10	0.00	0.42	0.74	0.15
Brown rice length	0.27	0.01	0.75	0.09	-0.04	-0.15
Brown rice width	0.22	-0.09	-0.50	0.06	0.24	-0.35
Brown rice shape	0.16	-0.01	0.86	-0.01	0.13	0.15
Eigen value	**3.64**	**2.40**	**1.66**	**1.44**	**1.12**	**1.04**
% Variance	**24.26**	**16.00**	**11.04**	**9.63**	**7.49**	**6.94**
Cumulative % variance	**24.26**	**40.27**	**51.31**	**60.94**	**68.43**	**75.37**

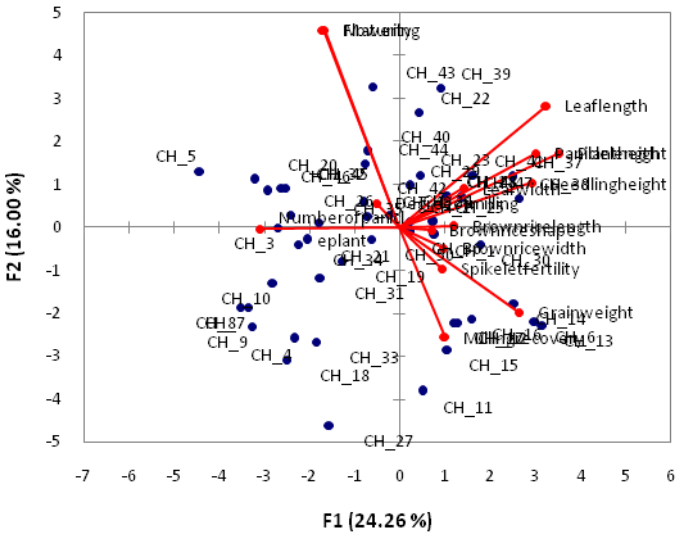

Figure 14a: Biplot of first and second principal components of morphological and grain quality traits

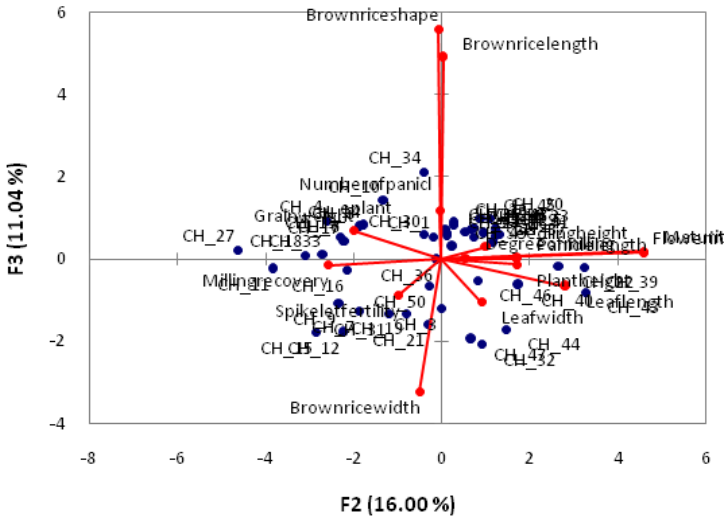

Figure 14b: Biplot of second and third components of morphological and grain quality traits

4.6 Molecular Characterization

4.6.1 SSR polymorphism

A total of 22 SSR primers were used for 187 rice genotypes and out of those only 18

SSR had specific amplifications using PAGE. Example of SSR allele for RM 307 and RM 413 are shown in Fig. 15. Sixteen of these primers were polymorphic among the studied materials while the remaining 2 primers (RM 277 and RM 283) were monomorphic. A total of 121 alleles were obtained on polymorphic SSR with an average of 7.56 alleles per primer (Table 9). The number of alleles ranged from 2 (RM 215) to 20 (RM 252). The frequency of major allele at each locus varied from 0.16 to 0.99 with average of 0.63.

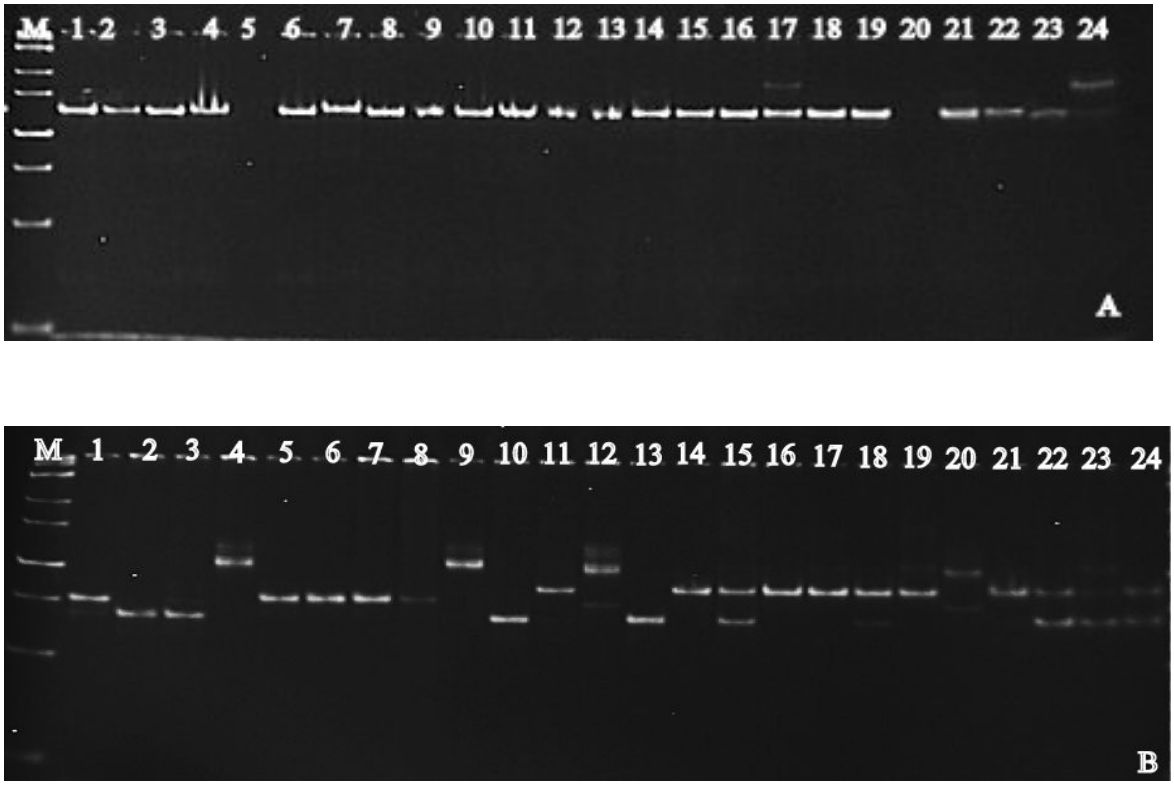

Figure 15: Allelic variations of some rice genotypes generated by SSR markers RM 307 (block A) and RM 413 (block B) observed on 8% PAGE. M: 25-700bp DNA ladder

The Polymorphism Information Content (PIC) was calculated for each marker as a relative measure of in formativeness. It ranged from 0.01 (RM 215) to 0.89 (RM 252) with an average value of 0.49. The genetic diversity of each SSR locus appeared to be associated with number of allele detected per locus. An average genetic diversity of 0.52 was obtained indicating a high level of genetic variation among the genotypes.

Table 9: Data on major allele frequency, allele number, gene diversity, heterozygosity and PIC obtained among 187 rice genotypes for 16 SSR markers

SSR Marker	Major allele frequency	Number of alleles	Gene Diversity	Heterozygosity	PIC
RM11	0.16	16	0.89	0.27	0.88
RM19	0.34	13	0.79	0.18	0.77
RM44	0.95	3	0.09	0.00	0.09
RM105	0.66	3	0.49	0.01	0.42
RM118	0.57	3	0.55	0.00	0.47
RM125	0.48	8	0.64	0.70	0.58
RM133	0.70	3	0.45	0.00	0.38
RM161	0.55	3	0.59	0.00	0.53
RM162	0.60	10	0.61	0.10	0.58
RM178	0.97	3	0.06	0.00	0.06
RM215	0.99	2	0.01	0.00	0.01
RM252	0.17	20	0.90	0.35	0.89
RM273	0.58	10	0.61	0.12	0.58
RM307	0.97	3	0.05	0.00	0.05
RM334	0.21	14	0.89	0.27	0.88
RM413	0.39	7	0.74	0.15	0.70
Mean	**0.63**	**7.56**	**0.52**	**0.13**	**0.49**

4.6.2 Clustering

The genetic similarity index among the rice germplasms used led to construction of Neighbor-Joining (NJ) tree depicted in Fig. 16a and 16b. The NJ-tree revealed six

clusters in the 187 rice genotypes studied. Cluster 4 was the largest containing 50 genotypes while cluster 3a had the least number of genotypes (10). Cluster 1, 2, 3b, 5 and 6 contained 34, 13, 12, 34 and 34 genotypes respectively.

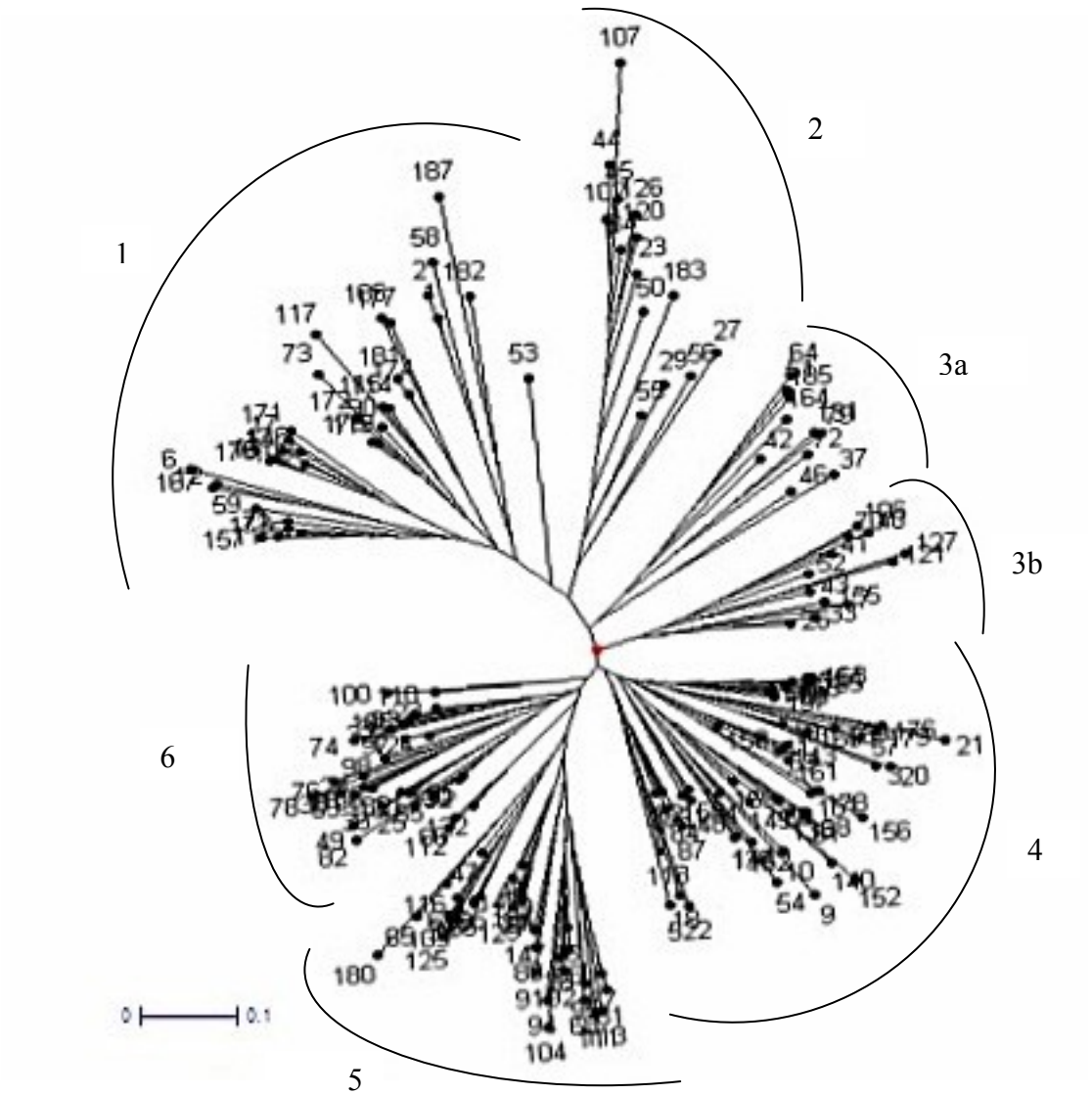

Figure 16a: Neighbor-Joining (NJ) tree of simple matching similarity coefficient using SSR data for rice genotypes. The number as identifier represents the S/N number of genotypes as shown in Appendix 6

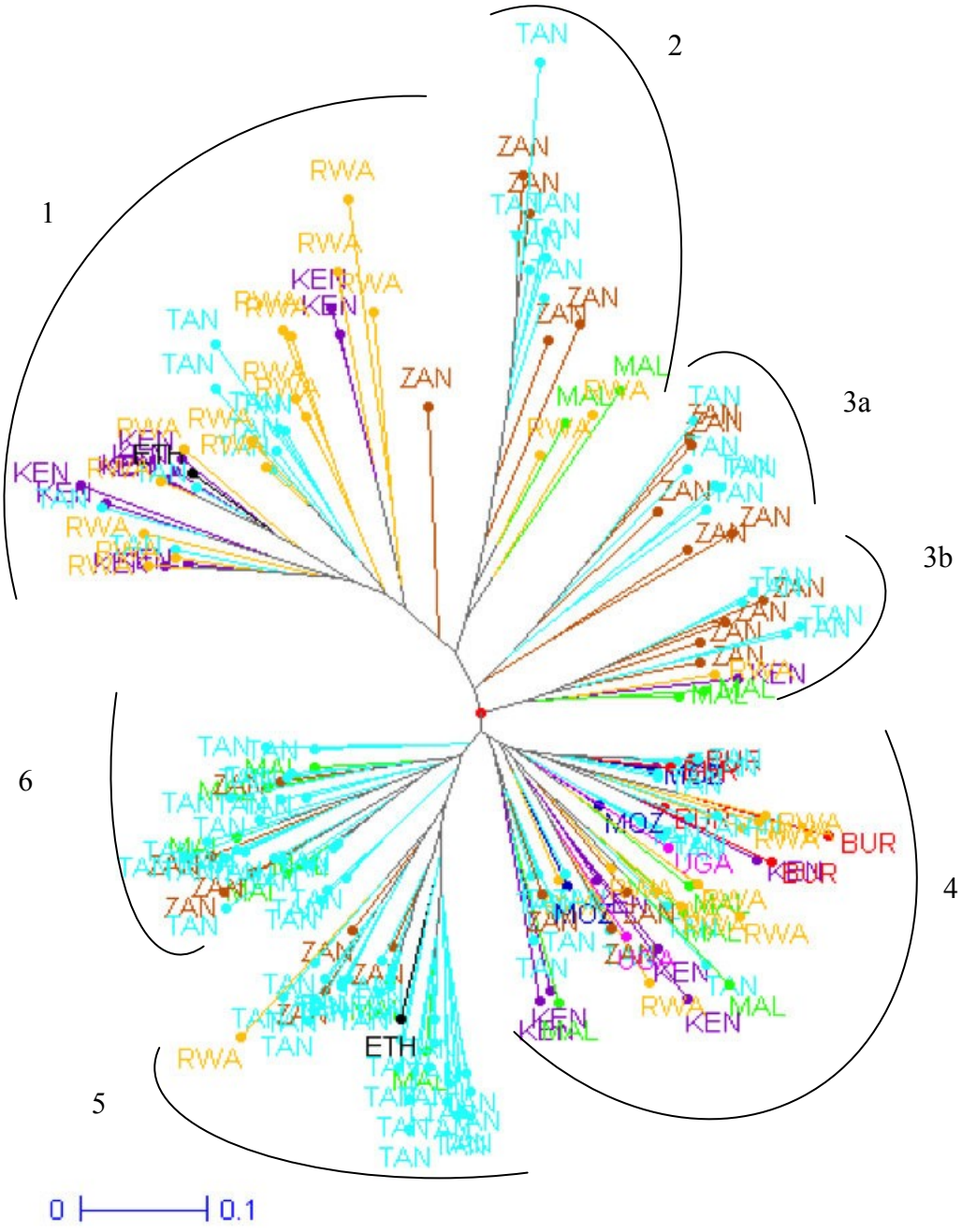

Figure 16b: Neighbor-Joining (NJ) tree of simple matching similarity coefficient using SSR data for rice genotypes classified by geographical origin, Burundi (red), Ethiopia-MET (black), Kenya and Uganda-MET (purple), Malawi (green), Mozambique-MET (blue), Tanzania (light blue), Rwanda (yellow), Zanzibar (brown)

Based on geographic origin, genotypes from the same country clustered in all the groups although there were more of the genotypes from the same country clustered in the same group as indicated by the color coding for different countries. This indicated that the distribution pattern did not follow the geographical origin of the genotypes. Basmati 370 variety from Kenya and Rwanda, and Rumbuka from Rwanda grouped together by both phenotypic as well as genotypic clustering. Also, the genotypes Kia la ngawa were clustered in different groups. The Supa varieties from Tanzania that were in the same cluster using the phenotypic analysis, were clustered in different groups using molecular analysis.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Morphological Characterization

Characterization of morphological traits will enable rice breeders to exploit a wide range of genotypic diversities to further crop improvement practices hence increase the rice productivity. In this study, 191 rice genotypes were characterized based on twenty four quantitative and qualitative traits which were observed in different stages of rice growth.

Ligule is a thin, upright, papery membrane that lies at the junction between the sheath and the blade. It can have either a smooth or hairy-like surface (IRRI, 2009). In this study, there was no variation on ligule shape, as all genotypes were observed to be 2-cleft. Moreover, two different colors were observed on ligules of the genotypes being characterized. Most of the genotypes were observed to have white ligule, whereby out of 191 genotypes, 177 were white and only 14 had ligule with purple lines. Ligule shape can serve as a unique character in identifying genotypes and hence could be of importance in every rice breeding programme.

No character has received as much attention, with so little justification as the pigmentation patterns of different plant parts. Pigmentation in any of its possible combinations does not appear to be related to crop development, pest resistance or grain yield (Jennings *et al.,* 1979) but it has been found useful in recognizing, removing off-types and maintaining the genetic purity of seed. High variation was

observed among the genotypes based on basal leaf sheath color. Most of the genotypes 174 were observed to have green basal leaf sheath color, and the remaining genotypes had purple or mixture of purple color. Based on leaf blade color the studied genotypes were differentiated into light green, green, dark green, purple tips and purple margins. However, it will not be reliable for identification of cultivars, because the intensity of green color of many cultivars gets bleached when the plant are left in the field to dry in sun or as a result of influence of fertilizers and environmental conditions (Kooistra, 1964).

Panicle exsertion is an essential physiological process for obtaining high grain yield in rice and is mainly driven by peduncle (uppermost internode) elongation. When some of the spikelets at lower down the panicle are trapped inside the flag leaf sheath, it increases the sterility in the lower unexserted spikelets hence reduce the grain yield (Muthurajan *et al.,* 2010). In present study, panicle exsertion of more than half of the genotypes was recorded as well exserted hence they are good for grain yield improvement. However, the extent of panicle exsertion is largely influenced by the agro-climatic condition and cropping seasons (Hoan *et al.,* 1998).

Panicle type of rice refers to the mode of branching, the angle of the primary branches and the spikelet density (IRRI, 2009). Based on panicle type, three groups were obtained, compact panicles, intermediate type of panicles and open panicles. More than half of the genotypes (69.63%) were observed to have compact panicles. Crop breeders usually selectively breed for a compact panicle type; open panicle type is actively selected against, for reasons of maximizing crop grain production

and harvest. Hence the genotypes with compact panicle types can be used in breeding programmes for the purpose of increasing rice production.

Another trait that was recorded among the genotypes is awning. Majority of the genotypes 90.05% were awnless, short and partly awned were 6.28% and the rest, long and fully, long and partly awned were 2.09% and 1.57% respectively. Acharya *et al.* (1991) stated that awns appear to be equipped with physiological and biological buffers that enable them to adjust to changes in the environment although many farmers consider it a nuisance during milling. Therefore, most breeders tend to select awnless grains, however, lines with partly awned panicles, short-awned types present no problem and should not be discarded because of that character alone during cultivar development.

Moreover, observations were taken for quantitative traits and the variation within the varieties were observed as discussed underneath. Leaf length varied from 29.89 cm (IR 64) to 59.90 cm (Tondogoso), which led to the genotypes being classified as having short, medium and long leaves. According to Mehla and Kumar (2008), the length of leaf had been known to vary between rice genotypes. Moreover, short leaves are more erect than long ones and are evenly distributed throughout the canopy so mutual shading is reduced and light is more efficiently used hence contributing in yield (Jennings *et al.,* 1979). Based on the width of leaf observed, the genotypes were grouped as narrow, medium and broad. Leaf width is less variable than length but obvious differences are found in rice genotypes. Although little attention has been paid to width in relation to yielding ability, field observations

suggested that leaves that are narrower are desirable, as they are assumed to contribute to higher yields since they are more uniformly distributed than wide leaves and cause less shading within the canopy (Fujino *et al.,* 2008).

Flowering duration is an important character that is frequently considered before release of a variety for commercial cultivation (Shahidullah *et al.,* 2009). Number of days to 50% flowering ranged form 65 to 111 that suggested significant variation in days to flowering that could be exploited for cultivar development. Mwangaza was recorded the earliest in flowering (65 days) and Jyambere; Bug 2013A was the latest (111 days). This type of variation might be due to genetic makeup of genotypes and genotypic environmental interactions. Almost fifty percent of genotypes evaluated showed very early to early days to 50% flowering i.e. <71 days represent very early flowering and 71-90 days represent early flowering. Moreover, days to maturity also exhibited high range of variation (96-142 days). Minimum value for days to maturity represents that the variety has a benefit of early ripening. Early maturity genotypes could be selected for areas with short rain seasons and in areas where farmers grow a second crop to take advantage of residual water after harvesting the early rice crop.

The panicle length and number of panicle per plant directly control the yield of a particular variety (Ashfaq, *et al.,* 2012). Thirty nine genotypes were observed having long panicle and very long for only one genotype; as length of greater than 25 cm and less than 30 cm represent long panicle and greater than 30 cm is very long. The remaining genotypes were observed to be short and medium. Similar results were obtained by Sarma *et al.* (2004) where they characterized 142 ahu rice genotypes of

Assam and found that eight genotypes showed more than 25 cm panicle length and the remaining genotypes were recorded lesser panicle length. Moreover, the panicle number per plant ranged from few (4) to medium (17).

Analysis of data revealed that plant height mean value was 95.13, and a wider range of 66.47 cm to 129.02 cm. Similar range was observed with Zafar *et al.* (2004) and this is typical of landrace genotypes which excel in their capacity to support panicle growth by large stem reserve mobilization. Ali *et al.* (2000) has also observed relatively greater range in plant height than the other characters. In this study, the genotypes were classified as semi dwarf and intermediate as per IRRI standard evaluation system for rice (1996) with majority of landraces having high heights. Hirano *et al*. (1992) confirmed the success of Green Revolution to be directly related to intensive use of semi dwarf varieties. Since the semi dwarf plant type was greatly utilized in the improvement of rice cultivars throughout the world, then this was true. However, depending on the part of the world where farmers live and their need for tall rice, there is a rising need to combine desirable characteristics of tall varieties with yielding ability and a new type of architecture: intermediate plant height as explained by Zafar *et al.* (2004).

Agbo and Obi (2005) observed that percentage of fertile spikelets had higher correlation values with yield. With good crop management and growth, high yields are obtained with normal spikelet sterility as much as 10% to 15%. The evaluated genotypes were classified as partly sterile, fertile and highly fertile on percentage spikelet fertility basis. However, the percentage of spikelet fertility varied from

69.70% for Kungahara; Bug 2011A to 96.17% for Yunyin suggesting that the genotypes have higher yielding ability.

5.2 Quality Characterization

Improvement and introduction of varieties with good grain qualities, is one of the major important objective of rice breeding programmes. Grain appearance or marketable quality includes grain length (size), grain shape, grain transparency, grain chalkiness and number of chalky grains (Masoumiasl *et al.,* 2013). Therefore, it is imperative to determine the relevant physical properties of rice grains.

Chalkiness is a grain-appearance trait that affects consumer acceptance of rice. Chalk is the opaque area in the rice grain and is undesirable in almost every market. Chalky areas occur because of malformed starch granules with air spaces between them (IRRI, 2006). In this study, majority of the genotypes (113) were observed to have minor i.e. less than 10% of chalkiness and 48 had no chalk in their grains. It is important to quantify chalkiness in existing cultivars across production environments, and because of its apparent tie to milling quality, this needs to be correlated to milling quality. This information could help in the development of cultivars that are resistant to kernel chalk formation and thus, improve milling and end-use quality (Bautista *et al.,* 2009). Moreover, the genotypes grains were grouped as opaque, translucency and cloudy based on translucency of the rice grain.

Milling recovery is the total milled rice obtained out of paddy, expressed as weight percent of milled rice (including brokens) obtained from a sample of paddy (IRRI,

2009). Milling of rice increases its shelf life and whiteness, a physical property that the consumers have come to desire. According to IRRI (2009), the maximum milling recovery is 69-70% depending on rice variety, but because of grain imperfections and the presence of unfilled grains, commercial millers are happy when they achieve 65% milling recovery. In this study, milling recovery varied from 61.28% to 71.93% and majority of genotypes fell in milling recovery percentage greater than 65, which is very acceptable for commercial millers. Twelve genotypes fell between 61-64%, which is also acceptable to some village mills as they even have 55% or lower (IRRI, 2009).

A high milling degree means that the milled rice is very white with relatively light milling. Degree of milling is influenced by grain hardness, size and shape, depth of surface ridges, bran thickness and mill efficiency (Payakapol *et al.,* 2011). From the results obtained, milling degree of the evaluated genotypes varied from 84.73% to 91.57%. The results show that the percentage milling degree is relatively high hence the whiteness was also high.

The 1000-grain weight varied from 16.52 g to 38.44 g. Grain weight provides information about the size and density of the grain. IRRI (2009) reported that longer grains are lighter in weight than medium or bold grains but the contrary was observed in this study. For example, CH_50 (Nawa Tule na Bwana), CH_163 (IR 03A262) and CH_166 (Shingo ya Mwali) were recorded as longer grains but had higher weight, while CH_43 (Kihogo) had low weight though it was recorded as medium grain, probably this is due to the reason that most of the varieties used had long grains.

Determining the physical dimension of rice varieties is very important, since it is produced and marketed according to grain size and shape. The length and width of rice grain are important attributes that determine the shape of the rice (IRRI, 2009). Based on the brown rice length, the varieties were classified as short, medium, long and extra long using a scale set by IRRI (1996). Takoradi (2008) reported that long grain rice is highly demanded by the rice consuming populace. Hence the long grains obtained in this study can be used in breeding programmes so as to meet the consumers' need. The ratio of the length and the width is used internationally to describe the shape of the variety. From this study, the varieties were categorized into three groups as bold, medium and slender. Although the preference for rice grain characteristics varies with consumer groups, long and slender grains are generally preferred and are good valuable attributes that could be exploited to improve the grain characteristics.

5.3 Cluster Analysis

The cluster analysis of both quantitative morphological and grain quality traits revealed the grouping of genotypes into seven clusters. Minimum number (14) of genotypes were included in cluster 1 and the maximum (37) in cluster 4. Cluster 2, 3, 5, 6 and 7 consisted of 29, 33, 32, 26 and 20 genotypes respectively.

In this study similar name for some genotypes from either same or different collection regions were grouped into same clusters e.g. Zambia and Rumbuka varieties, hence indicating that they seem to be originated from the same genetic materials. Also varieties named Supa Surungai, Supa Kijivu, Supa, Supa India and

Supa Katrin all from Tanzania were grouped in the same cluster (cluster 4). Although they have different names in some cases but it shows that they are all Supa variety. This can be due to free exchange of materials and the farmers tend to change/add names of the varieties. However, the similar name genotypes (Kia la ngawa) were grouped into quite different clusters, therefore indicating that they are not duplicates and suggesting that similar names are not always same as a rule of a thumb.

The distribution pattern of genotypes in this study into different clusters revealed in few cases no parallelism in morphology, grain quality traits and geographic location as genotypes collected form same geographic region was found in different clusters as well as in same cluster. This indicates that although genetic diversity is generally associated with geographical diversity, but factors other than geographical separation are also responsible for divergence, which might be due to selection, genetic drift and continuous exchange of genetic materials among the countries. Similar results were also reported by Chandra *et al.,* (2007), Hosan *et al.,* (2010), Sharma and Koutu (2011) and Chakma *et al.,* (2012). Considering this, parents should not be selected on basis of geographic diversity but on genetic diversity.

Cluster 1 exhibited highest mean value for seedling height, brown rice length and shape and lowest in leaf width, 50% flowering and days to maturity. This indicated that genotypes in this cluster could be used as parents for grain quality improvement and for developing short duration varieties. The highest mean value in cluster 2 was observed for number of panicle per plant and second highest for spikelet fertility.

Cluster 3 comprised of the genotypes with third highest mean for leaf length, panicle length, 1000-grain weight and brown rice shape. Thus the genotypes in these clusters are good for improving yield contributing traits. In cluster 4 the highest mean was observed for leaf width and degree of milling and second highest for leaf length, 1000-grain weight, milling recovery, brown rice length and shape, which is encouraging for improving grain quality traits. The mean value for cluster 5 ranked the first for 50% flowering and days to maturity and lowest in spikelet fertility and panicle length. From the results, it shows the genotypes in this cluster may not be good as parents for improving yield and yield components. Cluster 6 had the highest mean value for 1000-grain weight, milling recovery and brown rice width. Cluster 7 recorded highest mean for leaf length, panicle length, plant height and percentage spikelet fertility and second highest for seedling height, 50% flowering, days to maturity, degree of milling and brown rice width. Genotypes belonging to this group can be used in breeding programmes for improving yield and its components.

5.4 Principal Component Analysis (PCA)

The results of the PCA explained the genetic diversity of the studied rice germplasms. It measures the importance and contribution of each component to total variance whereas each coefficient of proper vectors indicates the degree of contribution of every original variable with each principal component is associated. The higher the coefficients, the more effective they will be in discriminating between germplasms, regardless of the positive or negative sign (Nachimuthu *et al.,* 2014).

Variation did exist among the 191 rice germplasms with respect to the 15 traits that were evaluated. Panicle length, leaf length and seedling height were observed to greatly influence in plant height. The study done by Khan *et al*. (2009) concluded that plant height has direct effect on grain yield. Therefore, this study suggested that when the mentioned traits will be improved, then plant height is improved hence grain yield is increased. On the other hand, leaf length, leaf width, 1000-grain weight, and plant height did not influence number of panicle per plant. Hence these characters have no effect on number of panicle per plant.

The principal component analysis (PCA) revealed the total contribution of characters to the variation. The six components accounted for 75.37% of the total variation. According to Gana, (2006) and Aliyu *et al.,* (2000) characters with high variability are expected to provide high level of gene transfer during breeding programs. The factor scores of seedling height, leaf length, panicle length, plant height and 1000-grain weight were correlated with the first principal components of principal component axes. From the study, it showed that the mentioned traits contributed mostly to genetic diversity of the studied rice genotypes. High level of variability existing within the varieties and traits will make room for further improvement of the cultivars in breeding programs. However, mostly of the yield contributing traits were good in Principal 1 and Principal 2 compared to the remained Principals. Therefore, good hybridization breeding program can be initiated by selection of the genotypes from Principal 1 and Principal 2.

5.5 Molecular Characterization of Rice Genotypes

Molecular markers have been proven to be powerful tools in the assessment of genetic variation within and among the species. A total of 121 alleles were obtained on polymorphic SSR with an average of 7.56 alleles per primer, ranged from 2 to 20. The results obtained were comparable to the range of 2 to 17 alleles per locus reported by Lapitan *et al.* (2007) and the range of 3 - 22 allele per locus with a mean of 7.8 as reported by Jain *et al.* (2004).

The Polymorphism Information Content (PIC) value is an evidence of allele diversity and frequency among the varieties (Pervaiz *et al.,* 2009). In present study, PIC ranged from 0.01 to 0.89 with an average value of 0.49. The highest PIC value was obtained for RM 252 and was considered as the best marker. However, 10 SSR markers showed PIC values of 0.5 or more that indicated they were highly informative. According to DeWoody *et al.* (1995), the markers with PIC value of 0.5 or higher indicate that they are highly informative and extremely useful in distinguish the polymorphism rate of a marker at a specific locus. Similarly, earlier studies on genetic diversity in rice also observed higher PIC values as reported by Hossain *et al.,* 2007; Lapitan *et al.,* 2007; Joshi *et al.,* 2010 and Etemad *et al.,* 2012. In this study, the genetic diversity of each SSR locus appeared to be associated with number of allele detected per locus, i.e. the higher the PIC value of a locus, the higher the number of allele detected. This observation was compared with the report of Yu *et al.* (2003) based on much larger SSR markers.

Based on the differences among the studied rice genotypes, the NJ-tree revealed six major clusters. Cluster 4 was the largest containing 50 genotypes and the least

number of genotypes (10) was in cluster 3a. Cluster 1, 2, 3b, 5 and 6 contained 34, 13, 12, 34 and 34 genotypes respectively. Based on geographic origin, genotypes from the same country clustered in all the groups although there more of the genotypes from the same country clustered in the same group as indicated by the color coding for different countries. Hence, no geographical isolation was observed except in cluster 5 where out of 34 genotypes 28 were from Tanzania and this could be a significant observation regarding geographical isolation. Frequent exchange of genetic materials among breeders and farmers of different countries for cultivation and development of improved rice varieties might be the reason for the observed lack of geographical isolation.

Moreover, the DNA analysis of genotypes has proven that the varieties (Basmati 370) that were collected from different locations are the same while the varieties that bare the same name (Kia la ngawa) are different genetic materials. However, the Supa varieties from Tanzania that were observed to be phenotypically similar, the genetic analysis confirmed that they are not similar and this might be due to genetic drift of the materials.

Both morphological, quality and SSR markers were able to group rice genotypes into distinct groups. Comparison of the morphological, grain quality and SSR dendrograms showed that some genotypes clustered together for both analyses while some were clustered in different groups. For example, CH_1, CH_2, CH_8, CH_155, CH_154, CH_165 clustered together in both morphological, grain quality and molecular dendrograms. The genotypes that were clustered together based on

morphological and grain quality traits, but clustered separately in SSRs dendrogram are CH_74, CH_109, CH_110, CH_137 and CH_128. However, the data from this study showed that morphological traits alone still cannot be regarded as critical indicators to identify individual rice genotype because most of the traits are influenced by the environment and nutrition available to the plant from the soil where it grows. SSRs were able to separate genotypes which where identified as morphologically the same into distinct groups, different from the associations derived from morphological descriptors. Therefore, when possible, use of morphological descriptors should be backed with DNA markers for efficient and reliable genetic diversity studies and germplasm management. Otherwise, morphological descriptors should be used with caution.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The present study was done with an objective of characterizing the rice varieties in morphological, molecular and quality basis for utilization in improvement programmes. The salient findings of the study are summarized below:

i. Out of 24 morphological traits observed, basal leaf sheath color, leaf blade color, panicle exertion, panicle type, apiculus color, lemma/palea color, awning, number of days to 50% flowering, days to maturity, leaf length and width, panicle length, plant height, spikelet fertility showed most variation among the genotypes.

ii. Among the grain quality traits, milling recovery, degree of milling, 1000-grain weight, brown rice length and shape exhibited most variation among the evaluated genotypes.

iii. The one hundred and ninety one genotypes characterized were grouped into seven clusters based on morphology and grain quality traits. However, the clustering pattern of the genotypes did not follow the geographical distributions.

iv. Principal component analysis has identified leaf length, days to 50% flowering, panicle length, plant height, days to maturity, seedling height and 1000-grain weight as the most important for classifying the variation existing in the germplasms.

v. Out of 18 Simple Sequence Repeat (SSR) markers used, 16 were found to be polymorphic with an average of 7.56 allele per marker.

vi. The cluster analysis based on similarity index of simple matching grouped the studied rice genotypes into six clusters, and no geographical isolation was observed.

vii. The information about the genetic diversity of studied ESA rice varieties will be very useful in identification and selection of suitable parents for use in breeding programmes to develop unique germplasms that compliment existing varieties.

6.2 Recommendations

i. Future work to be carried out on bio-chemical characterization of grain quality, cooking and eating properties of the studied rice genotypes to meet the consumers demand.

ii. Rice genotypes with lowest genetic similarity and traits of interest can be selected and used in breeding programmes and screening for higher yield and superior grain quality rice varieties.

iii. Further work on molecular basis by using SNP markers to carry out fingerprinting study, as SNPs are highly abundant and less susceptible to mutations than SSRs.

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APPENDICES

Appendix 1: Rice genotypes used in this study

Entry No	S/N	Designation	Country	Source
CH_1	1	BASMATI 217	KENYA	
CH_2	2	BASMATI 370	KENYA	
CH_3	3	BG90-2	KENYA	
CH_4	4	BR 153	KENYA	
CH_5	5	BW 196	KENYA	
CH_6	6	DUORADO PRECOSE	KENYA	
CH_7	7	IR 13240-108-2-2-3	KENYA	
CH_8	8	IR 2793-80-1	KENYA	
CH_9	9	ITA 304	KENYA	
CH_10	10	ITA 310	KENYA	
CH_11	11	LINE-8A-2	KENYA	
CH_12	12	LINE 11 WARDA	KENYA	
CH_13	13	LINE 16	KENYA	
CH_14	14	LINE-18-MWUR1	KENYA	
CH_15	15	NERICA 1	KENYA	
CH_16	16	NERICA 4	KENYA	
CH_17	17	NERICA 10	KENYA	
CH_18	18	WAT 317-WAS-B-55-11-3-5-1	KENYA	
CH_19	1	V14	BURUNDI	
CH_20	2	V18	BURUNDI	
CH_21	3	FACAGRO 906	BURUNDI	
CH_22	1	CHIMDIMA	MALAWI	
CH_23	2	CHUPA	MALAWI	
CH_24	3	FAYA 14M69	MALAWI	
CH_25	4	FAYA KARONGA	MALAWI	
CH_26	5	FRX 472	MALAWI	
CH_27	6	KACHAMBO	MALAWI	
CH_28	7	KACHIKOPE	MALAWI	
CH_29	8	KANAMALIA	MALAWI	
CH_30	9	KILOMBERO	MALAWI	
CH_31	10	LIFUWU	MALAWI	
CH_32	11	MTUPATUPA	MALAWI	
CH_33	12	NUNKILE	MALAWI	
CH_34	13	SINGANO	MALAWI	

CH_35	14	WAMBOWE	MALAWI
CH_36	15	ZAMBIA	MALAWI
CH_37	1	KANIKI	ZANZIBAR
CH_38	2	BARAMATA	ZANZIBAR
CH_39	3	DHAHABU	ZANZIBAR
CH_40	4	DOMO LA FISI	ZANZIBAR
CH_41	5	ILIKUWAJE KAMA SI UMBEA	ZANZIBAR
CH_42	6	KIA LA NGAWA	ZANZIBAR
CH_43	7	KIHOGO	ZANZIBAR
CH_44	8	KIJICHO	ZANZIBAR
CH_45	9	MABULA	ZANZIBAR
CH_46	10	MADEVU	ZANZIBAR
CH_47	11	MAUWA MEKUNDU	ZANZIBAR
CH_48	12	MOSHI WA SIGARA	ZANZIBAR
CH_49	13	MWANA MATONGO 2	ZANZIBAR
CH_50	14	NAWA TULE NA BWANA	ZANZIBAR
CH_51	15	NIWAHI	ZANZIBAR
CH_52	16	RINGA KIJICHO	ZANZIBAR
CH_53	17	RINGA NYEKUNDU 2	ZANZIBAR
CH_54	18	TARABINZONA	ZANZIBAR
CH_55	19	WAYA	ZANZIBAR
CH_56	1	JASMINI (Bug 2011B)	RWANDA
CH_57	2	IR 64 (Rujeje) (Bug 2013A)	RWANDA
CH_58	3	JYAMBERE (Bug 2013A)	RWANDA
CH_59	4	NERICA 1 (Bug 2013A)	RWANDA
CH_60	5	FASHINGABO (Bug 2013A)	RWANDA
CH_61	6	BASMATI 370 (Bug 2013A)	RWANDA
CH_62	7	KIGEGA (Bug 2011A)	RWANDA
CH_63	8	RUMBUKA (Bug 2013A)	RWANDA
CH_64	9	IB 26 (Bug 2013A)	RWANDA
CH_65	10	NZAHAHA (Bug 2012B)	RWANDA
CH_66	1	JARIBU 220	TANZANIA
CH_67	2	ZAMBIA	TANZANIA
CH_68	3	AFAA MWANZA 1/159	TANZANIA
CH_69	4	CHAMOTA	TANZANIA
CH_70	5	MAGONGO YA WAYUNGU	TANZANIA
CH_71	6	MZUNGU	TANZANIA
CH_72	7	SOTEA	TANZANIA
CH_73	8	SIFARA	TANZANIA
CH_74	9	SUPA SURUNGAI	TANZANIA
CH_75	10	LINGWELINGWELI	TANZANIA

CH_76	11	SINDANO KUBWA	TANZANIA
CH_77	12	TOSA	TANZANIA
CH_78	13	KIA LA NGAWA	TANZANIA
CH_79	14	KAGIHA	TANZANIA
CH_80	15	KIHOGO RED MOROGORO	TANZANIA
CH_81	16	CHAMBENA	TANZANIA
CH_82	17	MBAWA YA NJIWA	TANZANIA
CH_83	18	TONDOGOSO	TANZANIA
CH_84	19	FAYA MAFUTA	TANZANIA
CH_85	20	AFAA MWANZA	TANZANIA
CH_86	21	MWANZA	TANZANIA
CH_87	22	MPAKA WA BIBI	TANZANIA
CH_88	23	RANGI MBILI	TANZANIA
CH_89	24	LIFUMBA	TANZANIA
CH_90	25	GOMBE	TANZANIA
CH_91	26	SUPA UKEREWE	TANZANIA
CH_92	27	RINGA	TANZANIA
CH_93	28	MSONGA	TANZANIA
CH_94	29	MZINGA	TANZANIA
CH_95	30	SINDANO NYEUPE	TANZANIA
CH_96	31	PISHORI (BROWN)	TANZANIA
CH_97	32	MLEKE ALONGOLE	TANZANIA
CH_98	33	MBAWAMBILI MWEKUNDU	TANZANIA
CH_99	34	NONDO	TANZANIA
CH_100	35	RANGIMBILI NYEKUNDU	TANZANIA
CH_101	36	SUKARI	TANZANIA
CH_102	37	GAMTI	TANZANIA
CH_103	38	USINIGUSE	TANZANIA
CH_104	39	KIVULI	TANZANIA
CH_105	40	KALING'ANAULA	TANZANIA
CH_106	41	SIMZITO	TANZANIA
CH_107	42	THEMANINI	TANZANIA
CH_108	43	KALUNDI	TANZANIA
CH_109	44	SUPA KIJIVU	TANZANIA
CH_110	45	SUPA	TANZANIA
CH_111	46	KALAMATA	TANZANIA
CH_112	47	AFAA KIKANGAGA	TANZANIA
CH_113	48	AFAA MELELA	TANZANIA
CH_114	49	MKIA WA NYUMBU	TANZANIA
CH_115	50	LOYA	TANZANIA
CH_116	51	SHINGO YA MWALI	TANZANIA
CH_117	52	NGADIJA	TANZANIA
CH_118	53	KALIVUMBULA	TANZANIA
CH_119	54	LUNYUKI	TANZANIA

CH_120	55	KATUMAHI	TANZANIA	
CH_121	56	MBEGA	TANZANIA	
CH_122	57	TUNDURU	TANZANIA	
CH_123	58	JAMBO TWENDE	TANZANIA	
CH_124	59	FAYA (CHIKUYU MANYONI)	TANZANIA	
CH_125	60	LIMOTA	TANZANIA	
CH_126	61	WAHIWAHI	TANZANIA	
CH_127	62	MBAWAMBILI RANGIMBILI	TANZANIA	
CH_128	63	KISEGESE	TANZANIA	
CH_129	64	MOSHI	TANZANIA	
CH_130	65	UMANHO	TANZANIA	
CH_131	66	KIHOGO RED	TANZANIA	
CH_132	67	MASANTULA	TANZANIA	
CH_133	68	MWARABU	TANZANIA	
CH_134	69	SUMBAWANGA	TANZANIA	
CH_135	70	MWASUNGO	TANZANIA	
CH_136	71	MBAWAMBILI	TANZANIA	
CH_137	72	SUPA INDIA	TANZANIA	
CH_138	73	UROO 1 IMPROVED	TANZANIA	
CH_139	74	TXD 306 IMPROVED	TANZANIA	
CH_140	75	TXD 85 IMPROVED	TANZANIA	
CH_141	76	TXD 88 IMPROVED	TANZANIA	
CH_142	77	SUPA BC IMPROVED	TANZANIA	
CH_143	78	FAYA DUME 1	TANZANIA	
CH_144	79	FAYA DUME 2	TANZANIA	
CH_145	80	FAYA DUME 3	TANZANIA	
CH_146	81	FAYA DUME 4	TANZANIA	
CH_147	82	FAYA DUME 5	TANZANIA	
CH_148	1	SUPA BC	ZANZIBAR	MET 2012
CH_149	2	BKN/SUPA	ZANZIBAR	MET 2012
CH_150	3	EDIGET (WAB189-B-B-B-HB)	ETHIOPIA	MET 2012
CH_151	4	ROJOMENA 271/10	ETHIOPIA	MET 2012
CH_152	5	IR 80482	MOZAMBIQUE	MET 2012
CH_153	6	IR 77080	MOZAMBIQUE	MET 2012
CH_154	7	HUA 565	MOZAMBIQUE	MET 2012
CH_155	8	FRX 78-12	MALAWI	MET 2012
CH_156	9	FRX 92-14	MALAWI	MET 2012
CH_157	10	IR77713	BURUNDI	MET 2012
CH_158	11	IR79511	BURUNDI	MET 2012
CH_159	12	INTSINDAGIRA-BIGEGA	RWANDA	MET 2012
CH_160	13	INTSINZI	RWANDA	MET 2012
CH_161	14	RUMBUKA	RWANDA	MET 2012
CH_162	15	IR 05N221	TANZANIA	MET 2012
CH_163	16	IR 03A262	TANZANIA	MET 2012

CH_164	17	TXD 307	TANZANIA	MET 2012
CH_165	18	WITA 9	UGANDA	MET 2012
CH_166	19	K5	UGANDA	MET 2012
CH_167	20	IR 64	TANZANIA	MET 2012
CH_168	21	SUPA KATRIN	TANZANIA	SUPA_BGM 2012
CH_169	22	TEMERIN-381	TANZANIA	SUPA_BGM 2012
CH_170	23	KILOMBERO LUPEMBE	TANZANIA	SUPA_BGM 2012
CH_171	24	KDML	TANZANIA	RYT_BGM 2012
CH_172	25	MWANGAZA	TANZANIA	
CH_173	11	KUNGAHARA (Bug 2011A)	RWANDA	
CH_174	12	NDENGARA	RWANDA	
CH_175	13	CYICARO	RWANDA	
CH_176	14	MPEMBUKE	RWANDA	
CH_177	15	INGWIZABUKUNGU UL 26	RWANDA	
CH_178	16	MBAKUNGAHAZE (IRRI6)	RWANDA	
CH_179	17	RUMBUKA	RWANDA	
CH_180	18	YUNYIN	RWANDA	
CH_181	19	NDAMIRABAHINZI	RWANDA	
CH_182	20	TERIMBERE (LL29)	RWANDA	
CH_183	21	FASHINGABO	RWANDA	
CH_184	22	ZHONGENG	RWANDA	
CH_185	23	YUNKENG	RWANDA	
CH_186	24	INTISINZI	RWANDA	
CH_187	25	GAKIRE	RWANDA	
CH_188	26	BASMATI	RWANDA	
CH_189	27	FAC 56	RWANDA	
CH_190	28	BR	RWANDA	
CH_191	29	IRON	RWANDA	
CH_192	30	NEMEYUBUTAKA	RWANDA	

Appendix 2: Descriptors for the morphological traits and their stages of observation adopted from IRRI 1996

S/N	Trait	Scale	Description	Stage of observation
1	Seedling vigor	1	Extra vigorous	Seedling
		3	Vigorous	
		5	Normal	
		7	Weak	
		9	Very weak	
2	Seedling height	3	Short (<30 cm)	Seedling (5-leaf stage)
		5	Intermediate (30 – 60 cm)	
		7	Tall (>60 cm)	
3	Ligule shape	1	Acute to acuminate	Tillering to stem elongation
		2	2-cleft	
		3	Truncate	
4	Ligule color	1	White	Stem elongation to booting
		2	Purple lines	
		3	Purple	
5	Basal leaf sheath color	1	Green	Tillering to booting
		2	Purple lines	
		3	Light purple	
		4	Purple	
6	Leaf blade color	1	Light green	Stem elongation to heading
		2	Green	
		3	Dark green	
		4	Purple tips	
		5	Purple margins	
		6	Purple blotch (purple mixed with green)	
		7	Purple	
7	Leaf blade pubescence	1	Glabrous	Booting to heading
		2	Intermediate	
		3	Pubescent	
8	Auricle (present/absent)			
9	Auricle color	1	Light green	Stem elongation to booting
		2	Purple	
10	Stigma color	1	White	Heading
		2	Light green	
		3	Yellow	
		4	Light purple	
		5	Purple	
11	Leaf length	3	Short (<30 cm)	Heading
		5	Medium (30 – 45 cm)	
		7	Long (>45 cm)	
12	Leaf width	3	Narrow (<1cm)	Heading
		5	Intermediate	
		7	Broad (>2 cm)	
13	50% flowering	1	Very early (<71 days)	
		3	Early (71 – 90 days)	
		5	Medium (91 – 110 days)	
		7	Late (111 – 130 days)	
		9	Very late (>131 days)	
14	Panicle exsertion	1	Well exserted	Milk to maturity
		3	Moderately well exserted	
		5	Just exserted	
		7	Partly exserted	
		9	Enclosed	
15	Panicle type	1	Compact	Dough
		5	Intermediate	
		9	Open	
16	Panicle length	1	Very short (<16 cm)	Milk
		3	Short (16 – 20 cm)	
		5	Medium (21 – 25 cm)	
		7	Long (26 – 30 cm)	
		9	Very long (>30 cm)	
17	Plant height	1	Semidwarf (<110 cm)	Milk to maturity
		5	Intermediate (110 – 130 cm)	
		9	Tall (>130 cm)	

18	Apiculus color	1	White	Milk to maturity
		2	Straw	
		3	Brown	
		4	Red	
		5	Red apex	
		6	Purple	
		7	Purple apex	
19	Awning	0	Absent	Milk to maturity
		1	Short and partly awned	
		5	Short and fully awned	
		7	Long and partly awned	
		9	Long and fully awned	
20	Number of panicle/plant	3	Few (<11)	Maturity
		5	Medium (11 – 20)	
		7	Many (>20)	
21	Maturity	1	Very early (<100 days)	Maturity
		3	Early (101 – 120 days)	
		5	Medium (121 – 140 days)	
		7	Late (141 – 160 days)	
		9	Very late (>160 days)	
22	Lemma and palea color	0	Straw	Maturity
		1	Gold and gold furrows on straw background	
		2	Brown spots on straw	
		3	Brown furrows on straw	
		4	Brown (tawny)	
		5	Reddish to light purple	
		6	Purple spots on straw	
		7	Purple furrows on straw	
		8	Purple	
		9	Black	
		10	White	
23	Sterile lemma color	1	Straw (yellow)	Maturity
		2	Gold	
		3	Red	
		4	Purple	
24	Spikelet fertility	1	Highly fertile (>90%)	Maturity
		3	Fertile (75 – 89%)	
		5	Partly fertile (50 – 74%)	
		7	Highly sterile (<50% to trace)	
		9	0%	

Appendix 3: Means of nine quantitative traits obtained in 191 rice genotypes

Entry No.	SH	LL	LW	FD	PL	PH	NPP	MD	SF
CH1	50.42	47.57	0.88	82	25.42	111.21	10	113	91.67
CH2	45.89	46.48	0.90	83	25.93	112.50	12	114	91.90
CH3	43.39	36.16	1.31	98	20.47	71.10	9	128	83.73
CH4	39.06	30.22	0.99	78	22.24	72.27	12	109	87.63
CH5	44.38	31.39	0.74	109	19.96	69.46	14	139	78.43
CH6	48.43	47.18	1.18	79	24.19	109.86	5	110	90.00
CH7	39.15	31.77	0.95	87	21.08	70.57	12	117	90.60
CH8	35.47	33.85	0.93	89	19.31	66.47 ^{L}	12	119	88.80
CH9	42.37	30.50	1.05	80	20.74	69.46	15	110	91.73
CH10	43.18	36.13	0.83	83	23.31	77.35	14	113	88.03
CH11	41.22	39.15	1.29	76	21.66	91.11	5	106	91.17
CH12	49.17	44.40	1.16	75	24.19	97.16	5	105	87.03
CH13	48.45	46.82	1.15	75	24.25	121.31	5	106	89.20
CH14	49.00	46.63	1.09	73	25.89	122.19	5	104	72.87
CH15	44.14	43.53	1.26	77	23.00	90.07	5	108	92.30
CH16	48.13	45.97	1.11	76	24.92	91.06	6	107	88.03
CH17	41.46	43.40	1.51	79	25.82	97.07	5	109	83.87
CH18	39.04	31.89	1.01	77	21.95	72.35	13	108	92.80
CH19	40.45	43.05	1.40	91	23.03	79.87	9	122	84.93
CH20	40.89	37.17	1.01	105	20.97	80.12	12	135	85.70
CH21	40.81	43.90	1.02	92	22.69	77.06	10	122	90.67
CH22	54.25	54.21	1.43	99	25.42	105.45	9	129	89.03
CH23	50.10	49.61	1.00	96	24.46	96.58	9	127	87.17
CH24	49.59	47.21	1.15	93	23.86	84.79	10	124	89.40
CH25	47.80	47.58	1.37	93	23.83	98.05	8	123	87.57
CH26	43.03	39.56	1.19	100	21.48	74.98	9	130	85.23
CH27	44.69	32.38	0.90	67	18.43 ^{L}	77.44	10	98	84.03
CH28	51.12	52.67	1.11	90	24.17	98.38	9	121	94.50
CH29	48.54	46.60	1.15	93	25.00	101.22	9	124	92.73
CH30	48.52	49.73	1.46	88	23.53	102.74	8	118	91.70
CH31	42.84	38.59	0.99	88	22.88	74.38	9	118	85.23
CH32	44.34	49.32	1.09	99	22.29	78.15	9	128	89.44
CH33	41.91	34.88	1.07	79	22.98	77.55	14	110	95.31
CH34	47.09	40.34	0.63 ^{L}	85	21.53	98.72	13	115	86.91
CH35	42.68	40.25	1.19	101	20.81	76.52	9	131	92.33
CH36	49.54	47.02	1.49	92	23.42	95.04	7	123	85.70
CH37	57.66 ^{H}	58.95	1.12	91	28.91	108.17	8	121	95.76
CH38	47.70	54.06	1.47	88	24.50	105.35	6	118	87.37
CH39	50.64	53.78	1.08	99	29.02	113.92	9	130	87.23
CH40	47.93	51.74	1.19	101	22.50	94.45	8	131	80.47
CH41	48.09	56.12	1.31	92	24.81	118.38	8	123	86.73

CH42	43.34	43.53	1.23	99	23.08	96.46	9	129	94.27
CH43	50.71	49.62	1.12	106	25.03	114.26	11	136	92.27
CH44	44.84	50.51	1.17	92	23.42	102.57	9	122	91.27
CH45	41.12	37.34	1.22	102	22.23	82.69	11	132	85.10
CH46	40.19	40.27	0.96	103	21.98	73.77	12	133	84.33
CH47	50.34	48.48	1.57	87	24.67	109.29	7	118	87.30
CH48	48.66	46.79	1.11	94	23.81	109.87	9	125	87.90
CH49	50.11	51.55	1.22	91	23.60	97.53	16	121	90.87
CH50	41.03	38.56	1.09	95	23.86	86.60	7	125	86.47
CH51	47.01	46.35	1.58	89	21.78	91.20	6	120	83.33
CH52	47.43	47.92	1.31	92	23.25	117.74	8	123	93.43
CH53	42.23	45.81	1.06	107	22.43	111.37	10	138	88.60
CH54	50.13	57.11	1.33	94	25.54	110.67	7	124	93.90
CH55	52.64	41.03	1.00	99	21.91	90.37	15	130	89.43
CH56	46.30	43.22	1.29	97	24.52	72.53	10	128	88.53
CH57	42.34	37.24	1.01	85	22.06	76.18	12	115	89.33
CH58	38.30	39.51	1.17	111 [H]	23.05	81.74	11	142 [H]	90.13
CH59	41.63	42.57	1.02	83	26.31	95.45	11	113	86.97
CH60	40.55	36.54	0.96	90	21.79	85.83	10	121	93.67
CH61	48.57	43.70	0.77	81	28.20	127.25	12	111	89.10
CH62	37.87	39.70	1.17	105	20.65	72.52	10	136	87.77
CH63	43.37	52.29	1.62	85	26.34	110.85	4	116	87.80
CH64	42.31	36.91	1.26	80	22.49	84.02	9	111	89.43
CH65	41.35	38.52	1.19	105	21.50	75.89	12	135	83.03
CH66	50.20	53.31	1.33	91	25.26	99.93	8	122	81.27
CH67	46.77	47.40	1.49	90	23.25	96.03	10	121	91.63
CH68	48.20	41.92	1.17	98	23.67	95.73	11	129	83.70
CH69	51.30	56.96	1.36	96	26.45	122.50	8	127	93.63
CH70	56.75	39.20	0.96	76	23.14	92.10	11	107	93.37
CH71	54.40	53.14	1.33	84	23.46	89.62	7	115	94.33
CH72	47.21	46.64	1.03	99	24.40	109.48	9	130	86.10
CH73	48.37	51.12	1.64	87	24.99	106.76	7	117	92.07
CH74	46.16	53.72	1.41	88	24.44	110.95	7	119	91.97
CH75	44.85	49.02	1.60	99	28.29	107.01	6	130	95.60
CH76	45.42	51.69	1.00	91	25.50	99.33	9	122	93.17
CH77	42.67	48.97	1.61	95	25.76	124.79	6	125	80.87
CH78	46.46	53.72	1.14	110	24.79	114.38	10	140	87.53
CH79	37.25	33.22	1.27	101	20.15	76.92	10	132	85.20
CH80	45.97	50.14	1.07	96	24.84	104.13	12	127	95.47
CH81	48.55	53.10	1.42	95	24.71	94.06	8	126	87.50
CH82	50.71	56.95	1.27	93	21.85	89.04	8	124	89.87
CH83	51.22	59.90 [H]	1.40	104	24.99	121.81	7	134	88.20
CH84	49.54	49.05	1.21	87	25.54	101.36	8	118	81.47
CH85	52.93	58.46	1.21	94	26.39	101.77	8	124	86.47

CH86	47.78	50.62	1.33	90	23.36	92.59	8	121	93.77
CH87	48.33	55.89	1.37	93	25.77	110.94	9	123	91.77
CH88	47.52	49.01	1.38	88	22.09	98.63	9	119	90.63
CH89	56.64	56.40	2.09 [H]	92	24.28	110.72	7	122	85.70
CH90	51.19	53.81	1.23	97	22.87	104.65	8	128	79.40
CH91	52.42	50.67	1.36	93	24.43	95.47	7	124	90.40
CH92	55.70	42.80	1.09	73	21.63	89.22	8	104	86.00
CH93	43.51	43.73	1.14	96	24.04	86.91	12	126	89.83
CH94	46.54	58.81	1.79	105	28.72	110.70	4	135	90.17
CH95	51.79	58.56	1.05	91	28.18	118.77	10	122	93.33
CH96	55.41	53.83	1.21	90	24.62	100.65	9	120	75.73
CH97	44.32	48.63	1.31	91	22.73	94.84	9	122	79.87
CH98	56.87	53.16	1.18	79	23.46	105.11	8	110	86.07
CH99	53.08	53.18	1.50	91	23.35	106.84	7	121	88.50
CH100	48.26	52.48	1.02	88	25.30	101.55	9	118	91.50
CH101	51.09	54.31	1.49	88	22.42	106.19	6	118	88.70
CH102	49.00	47.76	1.35	91	24.58	101.88	7	122	81.10
CH103	48.98	46.19	1.20	91	22.26	82.30	6	121	81.07
CH104	50.79	51.57	1.16	88	25.01	103.33	8	119	90.50
CH105	46.87	49.98	1.42	96	23.86	96.82	7	126	73.40
CH106	50.17	55.80	1.32	89	25.06	98.53	8	120	91.83
CH107	51.04	48.00	1.02	93	23.27	113.00	10	124	83.27
CH108	52.55	52.34	1.23	107	21.47	98.98	10	138	86.57
CH109	49.87	50.34	1.51	86	23.94	104.14	9	116	88.83
CH110	48.68	51.24	1.18	90	23.58	105.15	9	120	88.40
CH111	49.94	51.62	1.51	87	21.38	103.76	6	117	88.83
CH112	47.51	56.32	1.23	94	25.28	99.10	8	125	93.23
CH113	44.04	50.54	1.67	85	24.21	129.02 [H]	6	116	89.77
CH114	49.91	49.11	1.37	90	23.72	99.70	7	121	85.33
CH115	50.40	47.05	1.29	88	23.60	103.13	8	118	91.90
CH116	51.49	38.66	1.07	74	21.71	89.51	11	105	90.73
CH117	49.89	46.19	1.27	98	24.35	103.85	8	129	80.13
CH118	43.14	46.60	1.46	102	25.15	122.46	7	133	90.87
CH119	46.90	45.77	1.47	81	21.10	118.03	7	112	95.07
CH120	49.97	43.61	1.03	75	22.29	87.45	10	105	84.90
CH121	45.48	54.75	1.36	85	25.91	126.20	6	116	95.37
CH122	56.78	56.17	1.20	86	23.30	111.02	6	116	87.77
CH123	43.91	54.15	1.67	100	30.63 [H]	103.45	5	130	91.30
CH124	50.78	44.99	0.98	79	21.23	98.17	10	109	90.70
CH125	47.01	50.63	1.28	91	24.88	91.66	8	122	88.80
CH126	53.43	39.82	1.06	76	21.07	98.92	10	107	95.27
CH127	53.26	57.13	0.99	94	22.69	87.97	13	124	88.57
CH128	46.98	41.18	1.41	85	21.11	81.77	8	116	93.07
CH129	54.39	58.26	1.30	89	22.79	101.57	9	120	82.30

CH130	48.02	41.96	1.10	77	23.78	93.90	9	108	92.13
CH131	42.97	50.65	1.25	104	21.41	106.47	9	134	75.13
CH132	49.44	50.64	1.47	86	25.43	110.35	6	117	81.47
CH133	48.93	47.90	1.56	90	24.41	100.15	6	121	81.10
CH134	44.93	48.87	1.34	91	22.06	90.63	5	121	90.53
CH135	51.47	48.38	1.27	91	21.91	102.36	9	121	93.10
CH136	50.48	48.26	1.44	89	23.33	113.15	7	120	87.73
CH137	48.23	53.66	1.29	99	23.84	107.22	7	130	89.00
CH138	50.69	52.74	1.51	85	25.40	108.14	8	115	90.40
CH139	40.08	43.05	1.23	96	22.94	82.28	8	127	79.90
CH140	39.03	36.35	1.55	97	19.90	66.90	9	127	86.20
CH141	42.12	42.43	1.25	93	22.55	87.48	10	124	80.20
CH142	41.73	33.78	1.14	101	20.60	69.48	12	131	74.40
CH143	46.41	51.74	1.33	97	24.79	96.64	8	128	79.37
CH144	47.13	49.96	1.08	93	23.56	92.17	9	124	93.80
CH145	45.35	41.74	1.11	96	22.52	85.38	7	127	83.93
CH146	47.36	49.11	1.37	90	24.18	96.48	7	121	91.50
CH147	47.33	48.55	1.41	96	23.03	95.06	10	127	90.47
CH148	44.44	35.45	1.16	96	22.11	84.78	11	127	76.00
CH149	47.85	41.35	1.17	82	23.78	101.11	10	113	91.00
CH150	52.25	42.65	1.18	76	23.70	105.44	6	107	92.43
CH151	53.61	50.97	0.98	95	28.53	113.68	10	125	78.47
CH152	45.92	40.30	0.99	90	23.88	72.30	13	121	88.13
CH153	49.93	39.57	1.07	85	22.67	92.32	11	115	82.93
CH154	40.13	39.86	0.93	95	18.53	69.00	10	126	88.70
CH155	44.57	41.56	1.15	85	22.07	78.19	10	116	89.43
CH156	43.35	37.23	1.07	98	22.52	75.38	10	128	82.00
CH157	45.97	39.85	1.07	85	22.16	80.25	10	116	92.77
CH158	41.96	36.44	0.93	97	22.80	74.25	11	128	91.27
CH159	47.14	38.56	1.43	108	20.89	74.85	10	139	80.20
CH160	37.29	35.70	1.08	83	19.68	74.87	9	113	79.83
CH161	45.01	44.02	1.43	80	25.90	103.86	4 [L]	111	90.23
CH162	43.15	43.98	0.99	93	23.45	80.06	10	124	88.33
CH163	40.67	37.82	1.06	88	24.38	86.79	11	119	84.13
CH164	35.10 [L]	32.77	1.07	98	21.76	69.49	9	128	83.33
CH165	38.62	34.99	0.97	87	21.45	73.62	13	117	90.00
CH166	43.65	36.16	1.09	94	23.98	100.37	10	124	83.20
CH167	37.50	29.89 [L]	0.90	86	21.14	72.29	14	117	91.37
CH168	51.92	51.41	1.46	90	23.92	104.35	8	121	89.13
CH169	53.30	53.81	0.99	109	26.20	103.39	7	140	90.77
CH170	47.69	50.06	1.48	90	28.08	94.76	7	121	88.33
CH171	42.69	37.40	1.01	104	22.41	74.22	12	134	83.43
CH172	50.17	46.11	1.24	65 [L]	27.20	108.93	5	96 [L]	83.07
CH173	40.28	36.75	1.11	107	22.39	76.07	10	137	69.70 [L]

CH174	52.17	36.76	1.25	82	23.81	75.31	10	113	90.87
CH175	42.88	49.94	1.48	87	23.49	79.45	5	118	90.30
CH176	48.27	53.31	1.52	84	26.63	113.40	4 [L]	115	94.00
CH177	43.47	45.86	1.24	87	24.53	105.48	6	118	91.80
CH178	42.25	42.35	1.33	96	21.80	90.37	7	127	87.80
CH179	46.99	43.76	1.41	76	28.02	104.22	5	107	86.37
CH180	49.24	45.48	1.19	80	22.46	91.29	10	111	96.17 [H]
CH181	46.49	53.18	1.36	92	25.75	107.18	4 [L]	123	94.07
CH182	39.79	41.63	1.02	94	22.95	70.15	17 [H]	125	88.80
CH183	42.36	43.60	1.02	90	26.26	92.55	13	121	93.27
CH184	45.88	51.12	1.50	88	20.00	109.42	5	119	91.04
CH185	44.32	44.31	1.55	87	23.37	89.54	8	117	87.77
CH186	37.88	39.75	1.01	83	21.45	75.33	11	113	89.73
CH187	40.38	35.08	1.02	86	21.10	72.04	12	116	91.90
CH189	45.00	43.11	1.02	90	23.45	85.92	14	121	93.67
CH190	52.53	52.17	1.01	93	24.69	110.24	8	124	84.47
CH191	46.17	48.15	1.22	78	22.90	93.37	6	108	90.67
CH192	45.91	40.77	1.41	76	24.92	97.65	5	107	87.53
Grand mean	46.66	45.92	1.22	90.39	23.59	95.13	8.82	120.99	87.95
F-probability	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0093
LSD (5%)	5.77	7.68	0.24	5.97	3.15	17.12	2.90	5.98	12.21

SH-Seedling height (cm), LL-Leaf length (cm), LW-Leaf width, FD-50% Flowering days, PL-Panicle length (cm), PH-Plant height (cm), NPP-Number of panicle/plant, MD-Maturity days, SF-Spikelet fertility (%), H (Highest), L (Lowest)

Appendix 4: Means of grain quality traits obtained in 191 rice genotypes

Entry No.	GW	MR	DM	BRL	BRW	BRS
CH1	22.63	66.20	88.23	7.77	2.7	2.9
CH2	20.69	64.86	87.10	7.80	2.5	3.1
CH3	25.52	67.41	87.80	7.10	2.9	2.5
CH4	24.50	66.38	86.17	7.90	2.9	2.7
CH5	22.44	67.82	88.17	7.10	2.7	2.6
CH6	34.45	71.13	88.40	8.23	3.1	2.7
CH7	25.60	65.97	87.50	6.83	2.8	2.4
CH8	24.37	68.22	88.17	7.50	2.9	2.6
CH9	24.61	65.44	87.10	7.27	2.9	2.5
CH10	19.61	66.90	88.03	6.80	2.2 L	3.1
CH11	33.00	71.84	88.87	7.20	2.9	2.5
CH12	24.91	69.90	88.03	7.33	3.1	2.4
CH13	37.31	68.55	87.33	7.77	3.0	2.6
CH14	33.01	69.25	87.10	7.87	3.1	2.7
CH15	30.80	71.15	88.97	7.07	2.8	2.5
CH16	28.90	68.89	89.13	7.23	2.8	2.5
CH17	26.61	71.93 H	89.47	7.60	2.8	2.7
CH18	24.40	67.52	87.87	6.73	2.5	2.7
CH19	31.09	67.65	88.17	6.93	2.9	2.4
CH20	25.14	67.24	88.97	7.70	2.9	2.7
CH21	22.40	67.70	89.77	6.63	2.9	2.3
CH22	21.78	64.09	88.40	6.83	2.7	2.5
CH23	27.17	66.88	87.67	7.50	2.9	2.6
CH24	27.73	67.07	88.37	7.50	2.6	2.9
CH25	29.37	68.63	89.20	7.83	2.9	2.7
CH26	27.91	68.24	88.77	7.57	2.8	2.7
CH27	29.96	68.33	89.13	7.33	2.9	2.5
CH28	28.04	64.63	89.30	7.57	2.8	2.7
CH29	25.05	65.74	87.87	7.73	2.7	2.9
CH30	33.75	67.12	87.87	8.30	2.9	2.9
CH31	26.87	68.23	88.77	6.90	2.9	2.5
CH32	29.69	61.86	85.75	5.75	2.9	2.0
CH33	24.17	67.85	89.22	7.40	2.7	2.8
CH34	20.10	64.39	85.87	7.75	2.2	3.5
CH35	27.87	68.05	88.13	8.07	3.2	2.6
CH36	30.17	68.98	89.30	7.37	2.8	2.7
CH37	25.59	67.79	87.92	8.20	3.1	2.7
CH38	29.30	67.21	88.20	8.97	3.1	2.9
CH39	21.28	64.34	87.40	7.10	2.7	2.6
CH40	28.68	65.76	89.40	8.07	3.3	2.5
CH41	27.54	65.78	85.07	7.90	3.1	2.6
CH42	29.60	66.72	88.90	8.30	3.1	2.7

CH43	19.04	65.16	88.27	6.30	2.5	2.5
CH44	20.72	62.74	88.27	6.67	2.8	2.4
CH45	26.40	65.43	86.73	7.73	3.0	2.6
CH46	23.95	68.36	87.43	7.80	3.2	2.4
CH47	28.89	65.85	88.57	7.30	2.9	2.5
CH48	29.64	66.50	87.83	7.77	2.8	2.8
CH49	27.72	67.85	89.30	7.10	2.8	2.5
CH50	31.43	68.02	88.07	8.17	3.3	2.5
CH51	31.99	67.41	88.43	8.77	2.8	3.1
CH52	24.42	68.20	88.60	7.53	2.8	2.7
CH53	16.52 [L]	61.28 [L]	91.07	6.23	2.9	2.2
CH54	25.88	66.95	89.37	6.40	3.0	2.2
CH55	24.48	65.93	88.33	6.27	2.5	2.6
CH56	27.79	67.05	88.33	7.77	3.1	2.6
CH57	24.20	66.92	87.37	7.07	2.9	2.5
CH58	23.74	66.80	90.57	7.57	3.1	2.4
CH59	23.76	65.10	88.63	9.03	2.7	3.4
CH60	22.87	67.04	87.90	7.03	2.7	2.7
CH61	20.73	66.24	87.97	7.63	2.5	3.1
CH62	24.49	66.40	86.30	7.27	3.0	2.4
CH63	31.96	67.51	86.50	8.23	3.3	2.5
CH64	26.32	64.45	84.73 [L]	7.77	4.2	2.3
CH65	25.68	64.72	88.60	7.33	2.4	3.1
CH66	29.99	67.63	88.17	8.17	2.9	2.9
CH67	29.81	68.65	89.20	7.73	3.0	2.6
CH68	27.93	67.56	88.57	6.97	2.9	2.4
CH69	25.00	65.90	87.60	7.40	2.7	2.7
CH70	31.23	67.73	87.63	7.70	2.7	2.9
CH71	26.45	66.05	89.13	6.97	2.9	2.4
CH72	27.31	64.74	87.57	7.33	2.8	2.6
CH73	35.01	68.79	89.27	8.33	3.1	2.7
CH74	34.00	68.10	88.83	8.57	2.9	3.0
CH75	25.24	68.48	88.17	7.63	3.4	2.3
CH76	25.48	66.31	88.27	7.53	2.5	3.0
CH77	19.97	68.26	86.97	6.67	2.8	2.4
CH78	23.20	65.97	88.67	11.77 [H]	2.8	4.2 [H]
CH79	26.92	67.10	89.40	7.40	4.1	2.2
CH80	24.44	68.08	89.83	7.37	2.7	2.7
CH81	30.79	68.36	90.03	7.50	3.0	2.6
CH82	26.63	63.53	90.87	6.70	2.5	2.7
CH83	25.08	66.56	88.63	6.87	2.7	2.5
CH84	25.45	65.53	87.30	7.87	2.8	2.9
CH85	33.13	67.37	89.07	7.93	2.8	2.8
CH86	25.22	68.57	89.30	7.47	3.0	2.6

CH87	23.60	65.55	89.47	7.23	4.3	2.1
CH88	31.11	66.10	88.53	8.30	2.7	3.1
CH89	29.17	68.91	88.70	7.83	3.1	2.6
CH90	29.05	65.68	87.97	8.57	3.2	2.7
CH91	26.86	68.80	86.43	7.40	2.7	2.8
CH92	31.28	66.55	87.87	7.43	2.5	3.0
CH93	26.17	68.30	89.23	7.13	2.6	2.7
CH94	24.00	66.27	88.33	7.47	3.4	2.2
CH95	25.49	65.65	88.17	7.97	2.8	2.9
CH96	30.28	66.94	87.50	7.93	2.7	3.0
CH97	26.74	67.77	87.37	6.50	3.0	2.2
CH98	23.67	61.91	89.90	7.13	3.1	2.3
CH99	26.80	65.83	87.73	7.43	3.2	2.3
CH100	26.67	66.03	87.77	7.80	2.5	3.2
CH101	31.30	67.78	89.63	7.73	2.9	2.7
CH102	31.40	68.72	89.70	8.57	2.9	3.0
CH103	26.97	63.96	88.20	7.80	2.4	3.3
CH104	27.54	67.37	88.70	7.40	2.9	2.6
CH105	29.81	68.31	88.83	7.60	3.2	2.4
CH106	30.02	66.87	88.77	7.37	2.7	2.8
CH107	24.46	66.29	86.23	7.57	2.6	2.9
CH108	26.25	68.09	90.33	7.20	3.0	2.4
CH109	32.42	70.16	89.33	8.17	3.0	2.8
CH110	30.57	68.23	88.63	7.97	3.1	2.6
CH111	30.35	69.97	91.27	8.40	2.8	3.0
CH112	27.14	67.21	89.47	7.73	3.0	2.6
CH113	32.87	69.10	87.40	7.00	2.8	2.5
CH114	29.35	67.16	89.93	7.70	2.8	2.8
CH115	31.42	66.49	89.00	7.77	2.6	3.0
CH116	31.40	66.12	88.77	8.60	3.1	2.8
CH117	27.82	68.50	88.47	7.33	2.6	2.9
CH118	20.50	68.07	89.17	6.43	2.6	2.5
CH119	32.19	70.57	88.27	7.60	3.0	2.6
CH120	32.26	65.45	88.70	8.83	2.8	3.2
CH121	32.61	69.50	88.67	7.77	3.1	2.5
CH122	35.24	67.23	85.40	6.50	3.3	2.0
CH123	29.51	69.72	88.70	7.03	3.1	2.3
CH124	30.53	66.48	88.43	8.00	2.8	2.8
CH125	23.48	65.29	89.70	7.63	3.0	2.5
CH126	29.78	67.24	88.03	8.50	3.1	2.7
CH127	24.72	62.67	85.47	7.63	3.1	2.5
CH128	32.46	69.04	89.60	7.80	2.9	2.7
CH129	26.35	65.81	88.57	7.47	5.0 [H]	2.1
CH130	25.92	66.73	88.03	7.07	2.9	2.4

CH131	30.37	65.64	88.97	8.33	3.1	2.7
CH132	32.85	66.24	88.03	8.40	2.9	2.9
CH133	30.32	67.40	89.47	8.30	3.0	2.9
CH134	31.93	66.97	88.87	8.57	3.0	2.9
CH135	27.79	67.07	88.43	7.13	2.6	2.7
CH136	34.29	67.19	89.33	8.40	2.8	3.0
CH137	30.74	68.95	89.40	7.87	2.9	2.7
CH138	32.25	68.20	89.03	8.80	3.1	2.9
CH139	30.09	68.26	88.67	7.60	2.9	2.7
CH140	26.79	69.01	89.93	7.57	2.8	2.7
CH141	30.73	68.22	88.33	7.47	2.8	2.6
CH142	25.00	69.10	91.57 [H]	7.40	2.8	2.7
CH143	29.42	66.77	88.10	8.27	2.8	2.9
CH144	26.77	67.98	88.67	8.13	2.9	2.8
CH145	26.79	67.18	88.40	8.07	2.9	2.8
CH146	29.76	66.85	87.37	8.13	2.5	3.3
CH147	29.00	68.76	88.47	8.00	3.0	2.7
CH148	24.86	67.67	89.47	7.40	2.6	2.8
CH149	26.59	66.54	88.60	6.70	2.8	2.4
CH150	35.43	67.96	86.20	6.67	3.6	1.8
CH151	27.36	66.70	86.77	7.13	2.9	2.5
CH152	25.88	66.94	88.63	7.87	3.1	2.6
CH153	23.33	66.73	87.10	7.27	2.6	2.8
CH154	19.18	67.44	89.17	6.80	2.3	3.0
CH155	24.92	67.13	88.27	7.33	2.7	2.8
CH156	29.33	68.32	87.97	7.57	2.5	3.1
CH157	27.49	69.40	88.70	7.50	2.6	2.9
CH158	22.37	68.41	90.07	7.57	2.6	3.0
CH159	27.74	69.08	89.00	7.23	3.2	2.3
CH160	23.65	69.91	89.50	6.67	2.9	2.3
CH161	38.44 [H]	70.07	86.67	7.57	3.0	2.5
CH162	22.22	68.53	88.77	7.07	2.8	2.6
CH163	26.93	67.69	86.53	7.80	2.5	3.2
CH164	28.68	66.36	89.50	8.00	2.9	2.8
CH165	22.28	67.86	88.33	7.20	2.8	2.6
CH166	24.23	69.09	87.60	6.80	2.7	2.6
CH167	25.70	68.37	87.87	7.43	2.8	2.7
CH168	32.04	69.06	89.03	8.17	3.1	2.7
CH169	27.55	64.54	88.73	8.73	2.7	3.2
CH170	32.00	67.85	88.50	7.80	2.8	2.8
CH171	23.46	65.34	86.60	8.07	2.5	3.3
CH172	37.31	69.64	87.63	8.57	2.9	3.0
CH173	22.74	64.73	89.10	7.77	2.9	2.8
CH174	22.40	67.46	86.33	10.67	2.8	4.0

CH175	30.19	69.14	86.30	7.33	2.9	2.5
CH176	29.33	68.30	86.80	7.67	2.9	2.6
CH177	34.60	69.22	88.03	7.37	3.0	2.5
CH178	24.22	68.29	89.10	6.77	2.9	2.4
CH179	37.74	69.45	87.53	7.97	3.2	2.6
CH180	28.12	71.27	88.50	5.73	3.6	1.6 [L]
CH181	33.57	68.09	86.70	8.43	3.2	2.6
CH182	24.64	67.29	87.77	7.53	2.7	2.8
CH183	24.90	67.33	86.60	7.00	2.7	2.6
CH184	27.44	66.48	89.55	5.15 [L]	3.3	1.6 [L]
CH185	29.70	64.63	87.23	5.87	3.2	1.8
CH186	23.61	68.94	89.30	6.70	3.0	2.2
CH187	23.60	69.10	89.30	6.53	2.7	2.5
CH189	22.73	68.10	88.67	6.53	2.6	2.6
CH190	26.65	67.50	85.53	6.63	2.9	2.3
CH191	29.09	69.32	87.13	5.33	3.2	1.7
CH192	34.16	69.92	87.90	7.57	3.0	2.6
Grand mean	27.57	67.31	88.32	7.55	2.90	2.66
F-probability	<0.0001	<0.0001	0.0029	<0.0001	0.3245	<0.0001
LSD (5%)	3.58	2.75	2.73	1.63	0.90	0.72

GW-1000 grain weight (g), MR-Milling recovery (%), DM-Degree of milling (%), BRL-Brown rice length (mm), BRW-Brown rice width (mm), BRS-Brown rice shape (mm), H (Highest), L (Lowest)

Appendix 5: Distribution of rice genotypes in different clusters based on analysis of morphological and grain quality traits

Cluster	Entry No.	Designation	Country	Source
1	CH1	BASMATI 217	KENYA	
1	CH2	BASMATI 370	KENYA	
1	CH27	KACHAMBO	MALAWI	
1	CH34	SINGANO	MALAWI	
1	CH59	NERICA 1 (Bug 2013A)	RWANDA	
1	CH61	BASMATI 370 (Bug 2013A)	RWANDA	
1	CH70	MAGONGO YA WAYUNGU	TANZANIA	
1	CH78	KIA LA NGAWA	TANZANIA	
1	CH92	RINGA	TANZANIA	
1	CH116	SHINGO YA MWALI	TANZANIA	
1	CH120	KATUMAHI	TANZANIA	
1	CH124	FAYA (CHIKUYU MANYONI)	TANZANIA	
1	CH126	WAHIWAHI	TANZANIA	
1	CH174	NDENGARA	RWANDA	
2	CH4	BR 153	KENYA	
2	CH7	IR 13240-108-2-2-3	KENYA	
2	CH8	IR 2793-80-1	KENYA	
2	CH9	ITA 304	KENYA	
2	CH10	ITA 310	KENYA	
2	CH18	WAT 317-WAS-B-55-11-3-5-1	KENYA	
2	CH21	FACAGRO 906	BURUNDI	
2	CH31	LIFUWU	MALAWI	
2	CH33	NUNKILE	MALAWI	
2	CH49	MWANA MATONGO 2	ZANZIBAR	
2	CH55	WAYA	ZANZIBAR	
2	CH57	IR 64 (Rujeje) (Bug 2013A)	RWANDA	
2	CH60	FASHINGABO (Bug 2013A)	RWANDA	
2	CH80	KIHOGO RED MOROGORO	TANZANIA	
2	CH93	MSONGA	TANZANIA	
2	CH154	HUA 565	MOZAMBIQUE	MET 2012
2	CH155	FRX 78-12	MALAWI	MET 2012
2	CH157	IR77713	BURUNDI	MET 2012
2	CH158	IR79511	BURUNDI	MET 2012
2	CH160	INTSINZI	RWANDA	MET 2012
2	CH162	IR 05N221	TANZANIA	MET 2012
2	CH163	IR 03A262	TANZANIA	MET 2012
2	CH165	WITA 9	UGANDA	MET 2012
2	CH167	IR 64	TANZANIA	MET 2012
2	CH182	TERIMBERE (LL29)	RWANDA	
2	CH183	FASHINGABO	RWANDA	
2	CH186	INTISINZI	RWANDA	

2	CH187	GAKIRE	RWANDA	
2	CH189	FAC 56	RWANDA	
3	CH23	CHUPA	MALAWI	
3	CH24	FAYA 14M69	MALAWI	
3	CH28	KACHIKOPE	MALAWI	
3	CH29	KANAMALIA	MALAWI	
3	CH32	MTUPATUPA	MALAWI	
3	CH41	ILIKUWAJE KAMA SI UMBEA	ZANZIBAR	
3	CH48	MOSHI WA SIGARA	ZANZIBAR	
3	CH52	RINGA KIJICHO	ZANZIBAR	
3	CH64	IB 26 (Bug 2013A)	RWANDA	
3	CH71	MZUNGU	TANZANIA	
3	CH72	SOTEA	TANZANIA	
3	CH76	SINDANO KUBWA	TANZANIA	
3	CH84	FAYA MAFUTA	TANZANIA	
3	CH86	MWANZA	TANZANIA	
3	CH88	RANGI MBILI	TANZANIA	
3	CH91	SUPA UKEREWE	TANZANIA	
3	CH100	RANGIMBILI NYEKUNDU	TANZANIA	
3	CH103	USINIGUSE	TANZANIA	
3	CH104	KIVULI	TANZANIA	
3	CH106	SIMZITO	TANZANIA	
3	CH107	THEMANINI	TANZANIA	
3	CH112	AFAA KIKANGAGA	TANZANIA	
3	CH115	LOYA	TANZANIA	
3	CH125	LIMOTA	TANZANIA	
3	CH127	MBAWAMBILI RANGIMBILI	TANZANIA	
3	CH130	UMANHO	TANZANIA	
3	CH135	MWASUNGO	TANZANIA	
3	CH144	FAYA DUME 2	TANZANIA	
3	CH146	FAYA DUME 4	TANZANIA	
3	CH149	BKN/SUPA	ZANZIBAR	MET 2012
3	CH151	ROJOMENA 271/10	ETHIOPIA	MET 2012
3	CH153	IR 77080	MOZAMBIQUE	MET 2012
3	CH190	BR	RWANDA	
4	CH25	FAYA KARONGA	MALAWI	
4	CH30	KILOMBERO	MALAWI	
4	CH36	ZAMBIA	MALAWI	
4	CH38	BARAMATA	ZANZIBAR	
4	CH40	DOMO LA FISI	ZANZIBAR	
4	CH47	MAUWA MEKUNDU	ZANZIBAR	
4	CH51	NIWAHI	ZANZIBAR	
4	CH66	JARIBU 220	TANZANIA	
4	CH67	ZAMBIA	TANZANIA	

4	CH73	SIFARA	TANZANIA	
4	CH74	SUPA SURUNGAI	TANZANIA	
4	CH81	CHAMBENA	TANZANIA	
4	CH85	AFAA MWANZA	TANZANIA	
4	CH89	LIFUMBA	TANZANIA	
4	CH90	GOMBE	TANZANIA	
4	CH96	PISHORI (BROWN)	TANZANIA	
4	CH99	NONDO	TANZANIA	
4	CH101	SUKARI	TANZANIA	
4	CH102	GAMTI	TANZANIA	
4	CH105	KALING'ANAULA	TANZANIA	
4	CH109	SUPA KIJIVU	TANZANIA	
4	CH110	SUPA	TANZANIA	
4	CH111	KALAMATA	TANZANIA	
4	CH114	MKIA WA NYUMBU	TANZANIA	
4	CH117	NGADIJA	TANZANIA	
4	CH128	KISEGESE	TANZANIA	
4	CH131	KIHOGO RED	TANZANIA	
4	CH132	MASANTULA	TANZANIA	
4	CH133	MWARABU	TANZANIA	
4	CH134	SUMBAWANGA	TANZANIA	
4	CH136	MBAWAMBILI	TANZANIA	
4	CH137	SUPA INDIA	TANZANIA	
4	CH138	UROO 1 IMPROVED	TANZANIA	
4	CH143	FAYA DUME 1	TANZANIA	
4	CH147	FAYA DUME 5	TANZANIA	
4	CH168	SUPA KATRIN	TANZANIA	SUPA_BGM 2012
4	CH170	KILOMBERO LUPEMBE	TANZANIA	SUPA_BGM 2012
5	CH3	BG90-2	KENYA	
5	CH5	BW 196	KENYA	
5	CH19	V14	BURUNDI	
5	CH20	V18	BURUNDI	
5	CH26	FRX 472	MALAWI	
5	CH35	WAMBOWE	MALAWI	
5	CH42	KIA LA NGAWA	ZANZIBAR	
5	CH45	MABULA	ZANZIBAR	
5	CH46	MADEVU	ZANZIBAR	
5	CH50	NAWA TULE NA BWANA	ZANZIBAR	
5	CH56	JASMINI (Bug 2011B)	RWANDA	
5	CH58	JYAMBERE (Bug 2013A)	RWANDA	
5	CH62	KIGEGA (Bug 2011A)	RWANDA	
5	CH65	NZAHAHA (Bug 2012B)	RWANDA	
5	CH68	AFAA MWANZA 1/159	TANZANIA	
5	CH79	KAGIHA	TANZANIA	

5	CH97	MLEKE ALONGOLE	TANZANIA	
5	CH108	KALUNDI	TANZANIA	
5	CH139	TXD 306 IMPROVED	TANZANIA	
5	CH140	TXD 85 IMPROVED	TANZANIA	
5	CH141	TXD 88 IMPROVED	TANZANIA	
5	CH142	SUPA BC IMPROVED	TANZANIA	
5	CH145	FAYA DUME 3	TANZANIA	
5	CH148	SUPA BC	ZANZIBAR	MET 2012
5	CH152	IR 80482	MOZAMBIQUE	MET 2012
5	CH156	FRX 92-14	MALAWI	MET 2012
5	CH159	INTSINDAGIRA-BIGEGA	RWANDA	MET 2012
5	CH164	TXD 307	TANZANIA	MET 2012
5	CH166	K5	UGANDA	MET 2012
5	CH171	KDML	TANZANIA	RYT_BGM 2012
5	CH173	KUNGAHARA (Bug 2011A)	RWANDA	
5	CH178	MBAKUNGAHAZE (IRRI6)	RWANDA	
6	CH6	DUORADO PRECOSE	KENYA	
6	CH11	LINE-8A-2	KENYA	
6	CH12	LINE 11 WARDA	KENYA	
6	CH13	LINE 16	KENYA	
6	CH14	LINE-18-MWUR1	KENYA	
6	CH15	NERICA 1	KENYA	
6	CH16	NERICA 4	KENYA	
6	CH17	NERICA 10	KENYA	
6	CH63	RUMBUKA (Bug 2013A)	RWANDA	
6	CH113	AFAA MELELA	TANZANIA	
6	CH119	LUNYUKI	TANZANIA	
6	CH121	MBEGA	TANZANIA	
6	CH122	TUNDURU	TANZANIA	
6	CH150	EDIGET (WAB189-B-B-B-HB)	ETHIOPIA	MET 2012
6	CH161	RUMBUKA	RWANDA	MET 2012
6	CH172	MWANGAZA	TANZANIA	
6	CH175	CYICARO	RWANDA	
6	CH176	MPEMBUKE	RWANDA	
6	CH177	INGWIZABUKUNGU UL 26	RWANDA	
6	CH179	RUMBUKA	RWANDA	
6	CH180	YUNYIN	RWANDA	
6	CH181	NDAMIRABAHINZI	RWANDA	
6	CH184	ZHONGENG	RWANDA	
6	CH185	YUNKENG	RWANDA	
6	CH191	IRON	RWANDA	
6	CH192	NEMEYUBUTAKA	RWANDA	
7	CH22	CHIMDIMA	MALAWI	
7	CH37	KANIKI	ZANZIBAR	

7	CH39	DHAHABU	ZANZIBAR	
7	CH43	KIHOGO	ZANZIBAR	
7	CH44	KIJICHO	ZANZIBAR	
7	CH53	RINGA NYEKUNDU 2	ZANZIBAR	
7	CH54	TARABINZONA	ZANZIBAR	
7	CH69	CHAMOTA	TANZANIA	
7	CH75	LINGWELINGWELI	TANZANIA	
7	CH77	TOSA	TANZANIA	
7	CH82	MBAWA YA NJIWA	TANZANIA	
7	CH83	TONDOGOSO	TANZANIA	
7	CH87	MPAKA WA BIBI	TANZANIA	
7	CH94	MZINGA	TANZANIA	
7	CH95	SINDANO NYEUPE	TANZANIA	
7	CH98	MBAWAMBILI MWEKUNDU	TANZANIA	
7	CH118	KALIVUMBULA	TANZANIA	
7	CH123	JAMBO TWENDE	TANZANIA	
7	CH129	MOSHI	TANZANIA	
7	CH169	TEMERIN-381	TANZANIA	SUPA_BGM 2012

Appendix 6: Distribution of rice genotypes in different clusters based on molecular characterization

Cluster	S/No.	Entry No.	Designation	Country	Source
1	1	CH_1	BASMATI 217	KENYA	
1	2	CH_2	BASMATI 370	KENYA	
1	6	CH_6	DUORADO PRECOSE	KENYA	
1	8	CH_8	IR 2793-80-1	KENYA	
1	11	CH_11	LINE-8A-2	KENYA	
1	12	CH_12	LINE 11 WARDA	KENYA	
1	13	CH_13	LINE 16	KENYA	
1	14	CH_14	LINE-18-MWUR1	KENYA	
1	15	CH_15	NERICA 1	KENYA	
1	16	CH_16	NERICA 4	KENYA	
1	17	CH_17	NERICA 10	KENYA	
1	53	CH_55	WAYA	ZANZIBAR	
1	58	CH_61	BASMATI 370 (Bug 2013A)	RWANDA	
1	59	CH_63	RUMBUKA (Bug 2013A)	RWANDA	
1	63	CH_67	ZAMBIA	TANZANIA	
1	71	CH_75	LINGWELINGWELI	TANZANIA	
1	73	CH_77	TOSA	TANZANIA	
1	90	CH_94	MZINGA	TANZANIA	
1	117	CH_121	MBEGA	TANZANIA	
1	139	CH_143	FAYA DUME 1	TANZANIA	
1	146	CH_150	EDIGET (WAB189-B-B-B-HB)	ETHIOPIA	MET 2012
1	157	CH_161	RUMBUKA	RWANDA	MET 2012
1	166	CH_171	KDML	TANZANIA	RYT_BGM 2012
1	167	CH_172	MWANGAZA	TANZANIA	
1	170	CH_175	CYICARO	RWANDA	
1	171	CH_176	MPEMBUKE	RWANDA	
1	172	CH_177	INGWIZABUKUNGU UL 26	RWANDA	
1	173	CH_179	RUMBUKA	RWANDA	
1	174	CH_180	YUNYIN	RWANDA	
1	177	CH_185	YUNKENG	RWANDA	
1	179	CH_189	FAC 56	RWANDA	
1	181	CH_191	IRON	RWANDA	
1	182	CH_192	NEMEYUBUTAKA	RWANDA	
1	187	CH_197	TOG 5681	RWANDA	
2	27	CH_27	KACHAMBO	MALAWI	
2	29	CH_29	KANAMALIA	MALAWI	
2	44	CH_46	MADEVU	ZANZIBAR	
2	45	CH_47	MAUWA MEKUNDU	ZANZIBAR	
2	50	CH_52	RINGA KIJICHO	ZANZIBAR	
2	55	CH_58	JYAMBERE (Bug 2013A)	RWANDA	
2	56	CH_59	NERICA 1 (Bug 2013A)	RWANDA	

2	102	CH_106	SIMZITO	TANZANIA	
2	107	CH_111	KALAMATA	TANZANIA	
2	120	CH_124	FAYA (CHIKUYU MANYONI)	TANZANIA	
2	123	CH_127	MBAWAMBILI RANGIMBILI	TANZANIA	
2	126	CH_130	UMANHO	TANZANIA	
2	183	CH_193	SHINGO YA MWALI	ZANZIBAR	
3a	37	CH_39	DHAHABU	ZANZIBAR	
3a	42	CH_44	KIJICHO	ZANZIBAR	
3a	46	CH_48	MOSHI WA SIGARA	ZANZIBAR	
3a	51	CH_53	RINGA NYEKUNDU 2	ZANZIBAR	
3a	64	CH_68	AFAA MWANZA 1/159	TANZANIA	
3a	72	CH_76	SINDANO KUBWA	TANZANIA	
3a	75	CH_79	KAGIHA	TANZANIA	
3a	135	CH_139	TXD 306 IMPROVED	TANZANIA	
3a	164	CH_169	TEMERIN-381	TANZANIA	SUPA_BGM 2012
3a	185	CH_196	SUPA KIJICHO	ZANZIBAR	
3b	7	CH_7	IR 13240-108-2-2-3	KENYA	
3b	26	CH_26	FRX 472	MALAWI	
3b	33	CH_35	WAMBOWE	MALAWI	
3b	40	CH_42	KIA LA NGAWA	ZANZIBAR	
3b	41	CH_43	KIHOGO	ZANZIBAR	
3b	43	CH_45	MABULA	ZANZIBAR	
3b	52	CH_54	TARABINZONA	ZANZIBAR	
3b	70	CH_74	SUPA SURUNGAI	TANZANIA	
3b	106	CH_110	SUPA	TANZANIA	
3b	121	CH_125	LIMOTA	TANZANIA	
3b	127	CH_131	KIHOGO RED	TANZANIA	
3b	155	CH_159	INTSINDAGIRA-BIGEGA	RWANDA	MET 2012
4	3	CH_3	BG90-2	KENYA	
4	4	CH_4	BR 153	KENYA	
4	5	CH_5	BW 196	KENYA	
4	9	CH_9	ITA 304	KENYA	
4	10	CH_10	ITA 310	KENYA	
4	18	CH_18	WAT 317-WAS-B-55-11-3-5-1	KENYA	
4	19	CH_19	V14	BURUNDI	
4	20	CH_20	V18	BURUNDI	
4	21	CH_21	FACAGRO 906	BURUNDI	
4	22	CH_22	CHIMDIMA	MALAWI	
4	31	CH_31	LIFUWU	MALAWI	
4	54	CH_57	IR 64 (Rujeje) (Bug 2013A)	RWANDA	
4	57	CH_60	FASHINGABO (Bug 2013A)	RWANDA	
4	60	CH_64	IB 26 (Bug 2013A)	RWANDA	
4	61	CH_65	NZAHAHA (Bug 2012B)	RWANDA	
4	79	CH_83	TONDOGOSO	TANZANIA	

4	87	CH_91	SUPA UKEREWE	TANZANIA	
4	118	CH_122	TUNDURU	TANZANIA	
4	130	CH_134	SUMBAWANGA	TANZANIA	
4	131	CH_135	MWASUNGO	TANZANIA	
4	136	CH_140	TXD 85 IMPROVED	TANZANIA	
4	137	CH_141	TXD 88 IMPROVED	TANZANIA	
4	138	CH_142	SUPA BC IMPROVED	TANZANIA	
4	140	CH_144	FAYA DUME 2	TANZANIA	
4	142	CH_146	FAYA DUME 4	TANZANIA	
4	143	CH_147	FAYA DUME 5	TANZANIA	
4	144	CH_148	SUPA BC	ZANZIBAR	MET 2012
4	145	CH_149	BKN/SUPA	ZANZIBAR	MET 2012
4	148	CH_152	IR 80482	MOZAMBIQUE	MET 2012
4	149	CH_153	IR 77080	MOZAMBIQUE	MET 2012
4	150	CH_154	HUA 565	MOZAMBIQUE	MET 2012
4	151	CH_155	FRX 78-12	MALAWI	MET 2012
4	152	CH_156	FRX 92-14	MALAWI	MET 2012
4	153	CH_157	IR77713	BURUNDI	MET 2012
4	154	CH_158	IR79511	BURUNDI	MET 2012
4	156	CH_160	INTSINZI	RWANDA	MET 2012
4	158	CH_162	IR 05N221	TANZANIA	MET 2012
4	159	CH_163	IR 03A262	TANZANIA	MET 2012
4	160	CH_164	TXD 307	TANZANIA	MET 2012
4	161	CH_165	WITA 9	UGANDA	MET 2012
4	162	CH_166	K5	UGANDA	MET 2012
4	163	CH_167	IR 64	TANZANIA	MET 2012
4	165	CH_170	KILOMBERO LUPEMBE	TANZANIA	SUPA_BGM 2012
4	168	CH_173	KUNGAHARA (Bug 2011A)	RWANDA	
4	169	CH_174	NDENGARA	RWANDA	
4	175	CH_181	NDAMIRABAHINZI	RWANDA	
4	176	CH_183	FASHINGABO	RWANDA	
4	178	CH_187	GAKIRE	RWANDA	
4	184	CH_194	SUPA	ZANZIBAR	
4	186	CH_195	XINAN	RWANDA	
5	28	CH_28	KACHIKOPE	MALAWI	
5	39	CH_41	ILIKUWAJE KAMA SI UMBEA	ZANZIBAR	
5	47	CH_49	MWANA MATONGO 2	ZANZIBAR	
5	48	CH_50	NAWA TULE NA BWANA	ZANZIBAR	
5	62	CH_66	JARIBU 220	TANZANIA	
5	65	CH_69	CHAMOTA	TANZANIA	
5	67	CH_71	MZUNGU	TANZANIA	
5	68	CH_72	SOTEA	TANZANIA	
5	77	CH_81	CHAMBENA	TANZANIA	
5	80	CH_84	FAYA MAFUTA	TANZANIA	

5	81	CH_85	AFAA MWANZA	TANZANIA	
5	83	CH_87	MPAKA WA BIBI	TANZANIA	
5	85	CH_89	LIFUMBA	TANZANIA	
5	91	CH_95	SINDANO NYEUPE	TANZANIA	
5	92	CH_96	PISHORI (BROWN)	TANZANIA	
5	93	CH_97	MLEKE ALONGOLE	TANZANIA	
5	94	CH_98	MBAWAMBILI MWEKUNDU	TANZANIA	
5	96	CH_100	RANGIMBILI NYEKUNDU	TANZANIA	
5	97	CH_101	SUKARI	TANZANIA	
5	101	CH_105	KALING'ANAULA	TANZANIA	
5	103	CH_107	THEMANINI	TANZANIA	
5	104	CH_108	KALUNDI	TANZANIA	
5	105	CH_109	SUPA KIJIVU	TANZANIA	
5	108	CH_112	AFAA KIKANGAGA	TANZANIA	
5	111	CH_115	LOYA	TANZANIA	
5	113	CH_117	NGADIJA	TANZANIA	
5	114	CH_118	KALIVUMBULA	TANZANIA	
5	116	CH_120	KATUMAHI	TANZANIA	
5	119	CH_123	JAMBO TWENDE	TANZANIA	
5	125	CH_129	MOSHI	TANZANIA	
5	129	CH_133	MWARABU	TANZANIA	
5	141	CH_145	FAYA DUME 3	TANZANIA	
5	147	CH_151	ROJOMENA 271/10	ETHIOPIA	MET 2012
5	180	CH_190	BR	RWANDA	
6	23	CH_23	CHUPA	MALAWI	
6	24	CH_24	FAYA 14M69	MALAWI	
6	25	CH_25	FAYA KARONGA	MALAWI	
6	30	CH_30	KILOMBERO	MALAWI	
6	32	CH_34	SINGANO	MALAWI	
6	34	CH_36	ZAMBIA	MALAWI	
6	35	CH_37	KANIKI	ZANZIBAR	
6	36	CH_38	BARAMATA	ZANZIBAR	
6	38	CH_40	DOMO LA FISI	ZANZIBAR	
6	49	CH_51	NIWAHI	ZANZIBAR	
6	66	CH_70	MAGONGO YA WAYUNGU	TANZANIA	
6	69	CH_73	SIFARA	TANZANIA	
6	74	CH_78	KIA LA NGAWA	TANZANIA	
6	76	CH_80	KIHOGO RED MOROGORO	TANZANIA	
6	78	CH_82	MBAWA YA NJIWA	TANZANIA	
6	82	CH_86	MWANZA	TANZANIA	
6	84	CH_88	RANGI MBILI	TANZANIA	
6	86	CH_90	GOMBE	TANZANIA	
6	88	CH_92	RINGA	TANZANIA	
6	89	CH_93	MSONGA	TANZANIA	
6	95	CH_99	NONDO	TANZANIA	
6	98	CH_102	GAMTI	TANZANIA	

6	99	CH_103	USINIGUSE	TANZANIA
6	100	CH_104	KIVULI	TANZANIA
6	109	CH_113	AFAA MELELA	TANZANIA
6	110	CH_114	MKIA WA NYUMBU	TANZANIA
6	112	CH_116	SHINGO YA MWALI	TANZANIA
6	115	CH_119	LUNYUKI	TANZANIA
6	122	CH_126	WAHIWAHI	TANZANIA
6	124	CH_128	KISEGESE	TANZANIA
6	128	CH_132	MASANTULA	TANZANIA
6	132	CH_136	MBAWAMBILI	TANZANIA
6	133	CH_137	SUPA INDIA	TANZANIA
6	134	CH_138	UROO 1 IMPROVED	TANZANIA