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GENETIC DIVERSITY ANALYSIS OF RICE GERMPLASM (*Oryza sativa, Oryza glaberrima*) USING MORPHOLOGICAL AND MOLECULAR MARKERS

IBRAHIM SOE

[B.Sc. (HONS) CROP SCIENCE]

AUGUST, 2015

GENETIC DIVERSITY ANALYSIS OF RICE GERMPLASM (*Oryza sativa, Oryza glaberrima*) USING MORPHOLOGICAL AND MOLECULAR MARKERS

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MPHIL. AGRONOMY (PLANT BREEDING)

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IBRAHIM SOE

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AUGUST, 2015

DECLARATION

I hereby declare that this submission is my own research work towards the Mphil. Agronomy (Plant breeding) and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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(Head of Department)

DEDICATION

This work is dedicated to my late Mother Fatmata Soe and Father Safayou Soe.

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ABSTRACT

This experiment was carried out to determine the genotypic variation among rice accessions using phenotypic traits and SSR markers and the relationship between phenotypic traits and yield of rice accessions. A total of 87 accessions from six countries were laid out in a completely randomised design with 3 replications. Seventeen quantitative and 11 qualitative traits were recorded based on the internationally accepted standard evaluation system for rice. Genstat, NTSYS-pc and PowerMarker were the software's used for data analysis. Highly significant ($P < 0.001$) differences were observed among the accessions for all the quantitative traits. Differences were also observed among the accessions regarding the 11 qualitative traits. First five principal components accounted for 75.01 % of the total genetic variance among the accessions. Significant positive correlation with grain yield was noticed for some of the morphological traits. At 21 % similarity coefficient, the 87 accessions from six countries were grouped into seven clusters based on the morphological traits. Accessions from these seven clusters have tiller number 10-20, erect culm angle, no awn, 90-120 days to 50 % flowering, semi erect flag leaf, well exserted panicle and grain width 2-3.5 mm respectively. Following successful field experiment, the genetic diversity were again examined using 12 SSR markers. Six primers out of these 12 primers showed DNA amplification and polymorphism among the 87 rice accessions. The number of alleles detected by these six primers ranged from 2-9 with an average of 6.83 while PIC ranged from 0.34-0.79 with an average of 0.55. The UPGMA cluster dendrogram generated based on the 6 SSR markers grouped the accessions into 4 clusters at 41 % similarity coefficient. Accessions from these four clusters have late maturity, green basal leaf sheath colour, no awn and fewer tillers respectively. This experiment has proven that both

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morphological and SSR markers are effective tools in assessing genetic diversity in rice accessions. The genetic diversity revealed by the morphological and SSR markers in this study would be very important to select potentially good genotypes for future rice improvement programmes.

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LIST OF ACRONYMS

AD	-	After the Death of Christ
AFLP	-	Amplified Fragment Length Polymorphism
ANON	-	Anonymous
BC	-	Before Christ
bp	-	Base Pair
BP	-	Before Present
CRI	-	Crop Research Institute
CSIR	-	Council for Scientific and Industrial Research
CTAB	-	Cetyl Trimethyl Ammonium Bromide
DNA	-	Deoxyribonucleic Acid
dNTPs	-	deoxynucleotide triphosphates
FAO	-	Food and Agricultural Organisation
FAOSTAT	-	Food and Agricultural Organisation Statistics
IBPGR	-	International Board for Plant Genetic Resources
IRRI	-	International Rice Research Institute
MoFA	-	Ministry of Food and Agriculture
NERICA	-	New Rice for Africa
NTSYS	-	Numerical Taxonomy Multivariate Analysis System
PC	-	Principal Component
PCA	-	Principal Component Analysis
PCR	-	Polymerase Chain Reaction
PIC	-	Polymorphic Information Content
RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism

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RNAase	-	Ribonuclease
SARI	-	Savanna Agricultural Research Institute
SNP	-	Single Nucleotide Polymorphism
SSA	-	Sub Saharan Africa
SSR	-	Simple Sequence Repeats
TE	-	Tris EDTA (ethylenediaminetetra-acetic acid)
UNCTAD	-	United Nations Conference on Trade and Development
UPGMA	-	Unweighted Pair Group Method with Arithmetic Averages
USDA	-	United States Department of Agriculture
UV	-	Ultra Violet
WARDA	-	West Africa Rice Development Association

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CHAPTER ONE

INTRODUCTION

Rice (*Oryza sativa* or *Oryza glaberrima*) is consumed by more than fifty percent of the world's population especially in developing countries. The third highest cereal produced is rice after wheat and maize (FAOSTAT, 2012). As maize is cultivated for purposes other than human use, rice is grown solely for human consumption and therefore the most important grain with regard to man's sustenance. Rice provides about half of the world calories for human beings (Khush, 2003). Jianguo *et al.* (2003) reported that rice is a protein source and a staple food in many countries, though rice does not contain complete protein. By-products of rice have many uses: Tatamin mat for example is made from rice straw, beer or sake brewed from rice, rice bran is used to feed farm animals and rice hull for energy generation; making synthetic fibres and also fertilizers (FAO, 2000).

Many countries in Africa consume rice as their staple food and constitute part in other foods. One third of cereal calories consumed in West Africa comes from rice and up to 85 % in countries like Sierra Leone, Guinea-Bissau, Gambia, Liberia, Guinea, and Côte d'Ivoire. During the last three decades, this crop has seen increase in demand throughout the African continent, FAO (1996) projected that West African rice consumption will increase at 4.5 % through the year 2000 and beyond, and therefore many African governments have made it a priority in their food security planning policies (WARDA, 2005). Africa consumes 11.6 million tonnes of rice per year more than half of which is imported at huge cost (FAO, 1996). According to FAO (2008) African countries import more rice because of their population growth and urbanization. The cultivation of rice is mainly associated with countries and regions with high rainfall and low labour cost. Many countries in

Africa experience high rainfall and also pay less for labour but still import more than half of the rice they consume. Rice is mainly cultivated on land areas less than one hectare usually with low yielding varieties in Africa. Ninety-five percent of the total world production of rice is by developing countries, China and India alone producing more than half (FAO, 2003).

The inability of Africa to reach food self-sufficiency especially in rice production is the result of several constraints which require urgent redress to stem the trend of over dependence on imports and to satisfy the increasing demand for this commodity in areas where the potential of local production resources is exploited at very low level. In this 21st century, the world is facing many security problems of which food is a part. Gregory *et al*. (2000) reported that world population will increase by about two billion in the next two decades and nearly half of this population will be in developing countries where rice is the staple food. By the year 2025 global demand for rice will be 880 million tonnes which is 70 % more of the present world production (IRRI, 2010). In years to come expanding the areas of rice cultivation will be limited because of land and water resource scarcity due to climate changes, urbanization and population growth especially in Asia where more than fifty percent of the world rice is produced. The average growth rate of rice yield was 3.68 % annually in the 1980s but it decreased to 0.75 % per year in the late 1990s (Nguyen and Ferrero, 2006). Pressure from biotic and abiotic factors, declining productivity in intensive rice production systems, increasing cost of production in industrialized countries, low yielding varieties and increasing public concern for the protection of natural resources are some of the factors responsible for the low yield of rice obtained by farmers.

One of the major ways of addressing the issues affecting rice production and also increasing the average yield of this rice crop is through scientific research and subsequent dissemination of the resulting data obtained from the research (Nguyen and Ferrero, 2006). Breeding for rice varieties that produces higher yields of grain per unit land area is the only way to achieving increased rice production because of the reduction in land for rice production (Paterson *et al*., 2005), meeting the world's rice requirements will depend upon the development of high yielding genotypes that have resistance against biotic and abiotic stresses using conventional and biotechnological approaches and increasing rice grain yield also depends on reducing plant height, increasing effective tillers per plant, increasing kernel number per panicle and increasing 1000 grain weight (Paterson *et al*., 2005).

The effectiveness of any rice improvement programme depends on the utilization of different germplasm stock available in research organizations/institutes around the world. This will enable breeders to evaluate and select high yielding varieties for breeding programmes. The strength of association/relationship of rice grain yield with yield components should be considered in determining the selection criteria of rice germplasm on the basis of available phenotypic and physiological traits, yield being a major objective in any breeding programme.

There have been extensive efforts by rice breeders around the world to improve the quantity and quality of rice by crossing varieties. These efforts have produced many rice varieties. It is foreseen that more will follow as environmental conditions and consumers' desires are changing. However, for breeding efforts to be successful, the genetic resources available to plant breeders need to be assessed accurately using molecular markers. In contrast to morpho-agronomic traits, molecular markers can reveal differences existing among genotypes at the DNA level (Prabakaran *et al*.,

2010). Molecular markers have been proven to be very objective and independent of environmental conditions (Se-Jong *et al*., 2012).

1.1 General objective

The general objective was to evaluate rice germplasm using morpho-agronomic traits and SSR markers for further crop improvement.

1.2 Specific objectives

i. To determine the genotypic variation among rice accessions from different countries using phenotypic traits.

ii. To evaluate the relationship between phenotypic traits and yield of the accessions.

iii. To determine the magnitude of genetic variation among the accessions by means of SSRs profiling.

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CHAPTER TWO

REVIEW LITERATURE

2.1 Origin and distribution of rice

There have been plenty of deliberations around the world/Globe on the origins of the domesticated rice. Most people believe that, rice originated from India in 3000 BC where natives discovered the crop growing in the wild and began to study it (IRRI, 2013). Cultivation and cooking means are thought to have reached the Western countries rapidly and by medieval times, Europeans saw the introduction of this crop as a hearty grain. All forms of Asian rice both indicas and japonicas were obtained from a single domestication that occurred between 8200-13500 years ago in China, of the wild rice *Oryza rufipogon* (Molina *et al*., 2011). Vanghan *et al*. (2008) reported that the commonly acknowledged view, based on archaeological evidence, is that rice was first domesticated in the region of Yangtze River in China.

Morphological studies of the phytoliths of this crop (rice) from the Diaotonhuan archaeological site shows the changes from the collection of wild rice plant to the growing of the domesticated rice. Large number of wild rice phytoliths at the Diaotonhuan level dating from 12000-11000 BP indicates that wild rice gathering and cultivation was part of the indigenous means of human survival. According to MacNeish and Libby (1995) changes in the morphology of Diaotonhuan phytoliths dating from 10000-8000 BP shows that rice had been domesticated by this time. Soon after, the two major rice varieties of japonica and indica were being cultivated in central China (Harris, 1996). In the late 3rd millennium BC, there was an increase of rice cultivation into mainland Southeast Asia and Westwards across India and Nepal (Harris, 1996).

Some other people believed that rice cultivation began simultaneously in several countries over 6500 years ago (Molina *et al*., 2011). The first rice crop was seen in China (Emu Du region) around 5000 BC and in Thailand around 4500 BC. They were later seen in Cambodia, Southern India and Vietnam. Later derived species indica and japonica expanded to other Asian countries such as Indonesia, Myanmar, Pakistan, Philippines, Sri Lanka, Korea and Japan (Molina *et al*., 2011). The Asian rice (*Oryza sativa*) became adapted to farming in the Middle East and Mediterranean Europe around 800 BC. The Moros brought rice to the Spanish European country when they conquered the country around 700 AD (Huang *et al*., 2012). After the mid-15th century, rice cultivation spread throughout Italy and then France, later to other parts of the continents during the great age of the European exploration. The Spanish people took this crop to South America at the beginning of the 18th century (UNCTAD, 2010).

The first cultivators of rice in the United States of America did so by coincidence after storm damaged a ship docked in the Charleston South Carolina port (USDA, 2008). The captain of the ship gave over a small bag containing rice to a local planter as a gift and by 1726 Charleston was exporting more than 4500 metric tonnes of rice annually (USDA, 2008). In America, farmers have been successfully growing and harvesting rice for more than 300 years now. There are thousands of rice genotypes grown in America today (USDA, 2008).

The African rice species (*Oryza glaberrima*) was grown long before Europeans came to the continent. At present, the African species (*Oryza glaberrimais*) is being replaced by the familiarized Asian species (*Oryza sativa*). Some West African countries like Senegal still grow the African rice species for use in ritual contexts (Linares, 2002). Around 1500-800 BC, the African species (*Oryza glaberrima*)

spread from its centre of origin, Niger River Delta and extended to Senegal (Moorman and Veldkamp, 1978). However, this species never developed too far from its original region. Its cultivation even dropped in favour of the Asian species, possibly brought to Africa from the East Coast between the 7th and the 11th centuries by the Arabians (Paterson *et al*., 2005).

2.2 Diversity of rice

The genus *Oryza* contains 25 known species, 23 are wild species and the remaining two are *Oryza sativa* (genome AA, 2n = 2× = 24), the Asian rice and *Oryza glaberrima* (genome A^gA^g, 2n = 2× = 24) the African rice, are the cultivated species (Brar and Khush, 2003). The Asian rice species, *Oryza sativa* is more consumed, present in several countries, especially Asia and Africa and more diverse than *Oryza glaberrima* (Sarla and Swamy, 2005). *Oryza sativa* is broadly divided into subgroups: indica, japonica and javanica based on morphological and physiological characteristics. The indica and the japonica subgroups are by far the most important. Both *Oryza sativa* and *Oryza glaberrima* are grown as annuals, although *Oryza sativa* can be maintained for many years if protected from frost and drought (Sohl, 2005).

There is diverse opinion regarding the ancestry of the *Oryza sativa* and *Oryza glaberrima*. Sarla and Swamy (2005) reported that *Oryza sativa* and *Oryza glaberrima* have a common progenitor which is unknown and may not exist following a sequence from wild perennial to wild annual to cultivated annual ancestors. It is established fact that *Oryza rufipogon* and *Oryza nivara* gave rise to *Oryza sativa*, while *Oryza longistaminata* and *Oryza barthii* are the progenitors of *Oryza glaberrima* (Ishi *et al*., 2001). The two wild species are diploid weedy species containing the AA genomes and are found throughout south eastern Asia where they

hybridize freely with the cultivated rice (Sohl, 2005). *Oryza glaberrima* differs from *Oryza sativa* in many quantitative and qualitative traits on the field (Sarla and Swamy, 2005). On the field, *Oryza glaberrima* differs from *Oryza sativa* by its roundish, short, tough ligules and the small number of secondary branches on its panicles (Hore, 2005). Because of the great genetic variability between *Oryza sativa* and *Oryza glaberrima* problems of sterility can be experienced after crossing them (Rubenstein and Heisey, 2003). However, this problem can be eliminated after many crosses and selection.

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2.3 Rice production, importation and consumption in Sub Saharan Africa

Rice is a commodity of strategic significance and the fastest growing food source on the African continent, such that its availability and cost are a major determinant of the welfare of the poorest people, who are the least food-secure consumers in Africa (Nwanze *et al*., 2006). The growing demand for this crop poses an economic challenge for the continent. It is now produced and consumed in more than 40 African countries. This crop is ranked as the fourth most important in terms of cultivation after maize, sorghum and millet (FAO, 2006). Annual rice production on the African continent (SSA) is estimated at 14.5 million metric tonnes, comprising fifteen percent of the region's cereal production (FAO, 2006). Much of this quantity is being produced by smallholder farmers on land areas less than one hectare. Nwanze *et al*. (2006) reported that approximately 20 million farmers are engaged in its production in SSA and about 100 million people depend on rice in SSA directly for their livelihoods. Between 1961 and 2003, consumption of rice increased by 4.4 % annually and among the major cereal crops grown on the continent, rice is the fastest growing food source (Kormawa *et al*., 2004). Also between 1985 and 2003,

annual rice production increased by 4 %, while production growth for maize and sorghum were 2.4 % and 2.5 %, respectively.

African consumption of rice exceeds its production. Only 49 % of the rice consumed in Africa is supplied locally (Ininda, 2008). Africa consumes about 21 million metric tonnes of rice, about 10.3 million metric tonnes of which are imported annually valued at US$ 2.7 billion (WARDA, 2008). Importation of rice accounts for more than 40 % of Africa's local rice consumption. The world's largest proportionate increase in rice consumption over the next decade will occur in Africa (FAO, 2008). The insufficient rice production in Africa affects the well-being of over 20 million smallholder farmers mostly resident in rural areas, who depend upon rice for their livelihood. The more than half production deficit of rice along with the subsequent large outflow of foreign exchange presents a great developmental challenge to governments and development agencies in Africa.

Chipili *et al*. (2003) reported that rice is the number one staple food in Ghana. The country experienced a dietary shift to rice particularly in the urban areas starting from early post-independence period. This shift was partly due to increased income, favourable government pricing policies of rice and ease of cooking (Nyanteng, 1987). According to MoFA (2009) individual rice consumption in Ghana increased from 17.5 Kg to 38 Kg between the years 1999-2008 and is estimated to rise to 63 Kg by the end of 2018. This country depends largely on imported rice to balance the deficit in supply. Ghana rice imports increased from 240,000 metric tonnes in 1998 to 425,150 metric tonnes in 2003 and by 2003, Ghana was consuming 561,400 metric tonnes of rice annually (MoFA, 2009). The quantity of rice produced in Ghana currently stands at 107,900 metric tonnes leaving a huge gap of 453,500 metric tonnes which is met by importation mostly from Asia (Kunateh, 2009).

The increased consumption of rice in Sub Saharan Africa can be attributed to factors such as population growth, urbanization, consumer preference, cheapest food poor households can afford, diet change, increased consumption of food away from home, the convenience of cooking and also the ease of storage. Nwanze *et al*. (2006) reported that rice is the most important rapidly growing food source in Sub Saharan Africa with consumption growing at an alarming rate of 5 % annually since 1961.

2.4 Principal constraints to rice production in West Africa

According to Nwanze (1996), demand for rice in West Africa has been growing at an alarming rate since 1973. The increased consumption is mainly due to population expansion and the increased proportion of rice in the West African diet. In an effort to keep pace with increasing demands, production of rice in West Africa has been expanding rapidly, growing at 4.9 % annually, faster than any of the other staple food in the region (WARDA, 2008). In spite of the increase in production, importation of rice is growing, averaging 9 % annually for the last twenty years mainly due to low production (Nwanze *et al*., 2006).

Upland ecosystem which is the most extensive in Africa tends not to use fertilizer and rely solely on rain. Under this system rice grain yield decreases considerably when there is inconsistent and poor rains. Rice growth needs 600 mm of rain to complete its growth (Moormann and Veldkamp, 1978). Water is a major yield limiting factor in upland rice cultivation, although soil characteristics also play an important role in these areas. Varietal traits that can avoid drought condition are very important on upland areas. According to DeVries and Toenniessen (2001) the release of early maturing progenies from inter-specific crosses involving *Oryza glaberrima* (Africa species) and *Oryza sativa (*Asia species) named New Rice for Africa (NERICA) has given farmers the opportunity to cultivate rice in areas having limited

rainfall. Weed competition, pests and disease causing organism are another important yield reducing factors followed by blast, soil acidity and general soil infertility in the upland ecosystem. Farmers traditionally continue to control these stresses through bush-fallow methods. Increasing population growth on the continent has dramatically reduced fallow periods.

Lowland systems obtain water from three different sources: Direct rainfall, high water table and surface water. It is estimated that a total of 50 million hectares of inland valleys are available for rice cultivation in West Africa (WARDA, 2008). By using organic and inorganic fertilizers and controlling pests, lowland areas have the opportunity of producing higher grain yield than the upland ecosystem (WARDA, 1993). Notwithstanding, high levels of Fe^{2+}, poor drainage and Mn^{2+} may occur and often produce iron toxicity symptoms (Cherif *et al*., 2009). However, iron toxicity in rice production is influenced by several environmental factors, thus complicating studies on this cropping constraint (Asch *et al*., 2005). Virmani (1979) reported that iron toxicity exists in many West African countries, including Sierra Leone, Benin, Burkina Faso, Liberia, Nigeria, Senegal and Côte d'Ivoire where rice grain yield losses resulting from iron toxicity range from 12 % to 88 %. Where resistant lines are not cultivated in such conditions total crop failure may result. One of the major physical constraints in the lowland ecosystem is uncontrolled flood water which can engulf the crop or produce flash floods capable of carrying away huge quantity of harvestable yield. The ability to remove weeds before seeding largely determines the area that can be grown by family labour and the effectiveness of weeding, controlling pests and diseases after sowing largely affects grain yield to be harvested in this ecosystem.

2.5 Morphology of rice

Assessing diversity existing in a collection of crops is very important for identifying genes of interest for breeding programmes (Jayamani *et al*., 2007). Morphological, biochemical and molecular markers are the method used to study these diversities. The study of morpho-agronomic traits is the conventional way of evaluating genetic variability in crops (Bui and Nguyen, 1999). For many breeders it is still the only method used for crop improvement.

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Morphologically, rice is an annual plant and may be characterized as a grass with round and hollow culm, flat leaf blades and a terminal panicle. Rice plant varies in height from dwarf to very tall varieties of more than 3 m (Maji and Shaibu, 2012). A greater majority of commercial varieties around the world ranges from 1 to 2 m in height. The vegetative parts of rice plant consist of roots, culms and leaves (Tandekar and Koshta, 2014). The tiller bears leaves, roots and often a panicle (productive tiller). The leaves appear on the culm in two ranks, one at each node. The leaf consists of the blade and sheath. The leaf sheath is continuous on the culm with the blade. The leaf sheath envelops on the culm above the node in varying length, form and tightness. Sheath pulvinus is the swelling around the base of the leaf sheath just above the point of its insertion with the culm (Anjan and Mishra, 2013). The sheath pulvinus usually appears above the nodal septum and is frequently mis-termed the node. The leaf blades are usually flat and sessile. Accessions differ in their blade length, width, shape, colour, angle and pubescence. The top most leaf seen on the rice plant is the flag leaf. Accessions also differ in flag leaf area, colour, pubescence and angle. Variability in tiller and leaf numbers is a common attribute among rice accessions (FAO, 2000).

2.6 Genetic diversity and molecular markers

Genetic diversity arises primarily because of variation in the linear sequence of nucleotides in DNA. Mutations generally happen in the coding region of genes, or the spacer regions within and among genes, in the number of gene copies, the linkage relationship between several genes or indeed in the whole chromosomes. According to Brown (2008), a small portion of these changes translates into protein variation, characters, into marker polymorphisms, morphological and physiological variation in agronomic characters and ultimately genotypes given different names by scientists and farmers. The magnitude of genetic diversity in crops depends on: recombination, random genetic drift, selection and mutation. Mutation and recombination carries new variations to a population, while selection and genetic drift remove some alleles from agronomically important lines (Pervaiz *et al*., 2010).

Genetic diversity studies are carried out for several purposes including: phylogeny, breeding, variety identification and germplasm conservation. Mwirigi *et al*. (2009), reported that identification of genetic diversity among accessions is vital to the maintenance and utilization of germplasm resources. According to Sharma and Jana (2002), assessing the amount of genetic diversity existing in a crop species is a precondition for initiating an effective breeding programme, as it offers the basis for tailoring desirable genotypes. Genetic diversity studies afford the understanding of genetic variation existing among populations and allows grouping of lines to precise heterogenous groups from which identification and selection of parents can be done for hybridization (Mostafa *et al*., 2011). Knowledge of genetic diversity structure within a large collection of accessions may be of abundant help to make decisions on management procedures, as well as on breeding policies to use in present and future breeding programmes (Kumar *et al*., 2007). Genetically diverse parents are likely to

segregate and or to produce higher heterotic genoypes. The more diverse parental lines are the greater the chances of attaining higher heterotic F_1s and broad spectrum of variability within segregating generations. Genetic diversity studies of rice also allows selecting the genetically divergent parents to achieve the desirable recombinant in the segregating generations of rice. The study of diversity in plant breeding exposes genetic variability in diverse populations and offers justification for introgression and ideotype breeding programmes to enhance crop improvement (Aremu *et al*., 2007).

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As said earlier, studying of morpho-agronomic diversity among genotypes is the traditional way of assessing genetic variability for plant breeders. For many species, especially crops like rice, it is still the only method used by majority of breeders. Majority of morpho-biochemical traits are quantitative in nature and it frequently misguides the plant breeder to recognize a particular genotype and it is often challenging to use their criteria (Latif *et al*., 2011). However, with the introduction molecular techniques, powerful tools have been developed by scientists so that genetic resources can be accurately evaluated and characterized, purity testing of hybridized seeds for quality control and DNA fingerprinting (Chambers and Avoy, 2000).

Molecular markers are small portions of DNA that are part of a gene of interest or closely linked. The use of molecular markers is centred on the naturally occurring DNA polymorphism, which forms the foundation for manipulative methodologies to explore for practical purposes (Juneja *et al*., 2006). Weising *et al*. (1995) reported that an ideal molecular marker must have some desirable characteristics such as high reproducibility, selective neutral behaviours, easy access: It should be easy and fast to detect, highly polymorphic, Co-dominant inheritance and numerous occurrences in

genome. The DNA based markers are promising and effective for genetic diversity studies in plant germplasm and reveal their evolutionary relationships. They are more reliable, less labour input and remain unaffected across different seasons, growth stages, agronomic practices and locations (Ren *et al*., 2003). Information regarding genetic diversity at molecular level could be used to aid, identify and develop genetically unique genotype that compliments existing cultivars (Ni *et al*., 2002). Melchinger (1999) reported since 1990's, several types of DNA molecular markers are being increasingly utilized by plant breeders for investigating germplasm diversity and genetic relationships. These DNA markers are Restriction Fragment Length Polymorphism (RFLPs), Random Amplified polymorphic DNA (RAPDs), Simple sequence repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs) and Single nucleotide polymorphisms (SNPs).

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2.6.1 Restriction fragment length polymorphism (RFLPs)

Restriction Fragment Length Polymorphism (RFLPs) were the first DNA markers to be developed and used for plant genome analysis (Vos *et al*., 1995). Its technique exploits variations in homologous DNA sequences. In RFLPs development, genomic DNA is exposed to restriction enzymes and the subsequent fragments separated by gel electrophoresis, followed by transfer to a filter by Southern blotting and probed. RFLPs markers are co-dominant, reproducible, difficult to automate and labour intensive. Variability of RFLPs markers in plants is caused by procedures that result in the elimination or addition of restriction sites in the genome (Semagn *et al*., 2006). According to IBPGR and Cornell University (2003), RFLPs analysis results in a series of bands on a gel, which can then be recorded either for absence or presence of particular bands or as co-dominant markers. RFLPs are co-dominantly inherited,

estimating heterozygosity and as such requires large amounts of DNA (IBPGR and Cornell University, 2003).

2.6.2 Random amplified polymorphic DNA (RAPDs)

Random Amplified polymorphic DNA (RAPDs) profiling is one of the polymerase chain reactions (PCR) based DNA markers. Vinita *et al*. (2013) reported that PCR based RAPDs technique corresponds with straight DNA fingerprinting and its analysis is theoretically simple. The two methods of identifying RAPDs markers are DNA Amplification Fingerprinting (DAF) and Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) (Caetano-Anolles *et al*., 1991), nearly all RAPDs markers are dominant and so not possible to differentiate whether a DNA segment is amplified from a locus that is homozygous or heterozygous. Co-dominant RAPDs markers detected as different sized DNA segments amplified from identical locus, are detected only rarely (Weising *et al*., 2005). Differences in varieties are assigned due to the absence or presence of PCR products pictured on a gel. RAPDs development is easy to automate and does not require previous information of the target sequences used to design primers (Vanniarajan *et al*., 2012). It is a dominant marker but its main problem is that, reproducibility is very low.

2.6.3 Single nucleotide polymorphisms (SNPs)

A single-nucleotide polymorphism is a DNA sequence disparity occurring when a single nucleotide A, T, C or G in the genome varies between members of a species or paired chromosomes in a human being (Barreiro *et al*., 2008). SNPs represent the most recurrent form of polymorphism in the human being genome (Wang *et al*., 1998). Though there are several methods of SNPs discovery and genotyping, they essentially make a distinction among a probe of known sequence and the targeted DNA, which contains the SNP site. Mammadov *et al*. (2012) reported that although

SNPs are important tools in genetic mapping and diversity studies, high costs are involved since a large number is required to compensate for their bi-allelic nature and increase genome coverage. While these huge costs are reduced through genotyping by sequencing, extensive investment in manpower and equipment is needed to process, compute and store the large amount of sequencing data produced with this methodology (Semagn *et al*., 2006).

2.6.4 Simple sequence repeats (SSRs)

Simple sequence repeat molecular markers are also called microsatellites or short sequence repeats. SSRs markers are developed using primers designed to flank the repeat sequences and are also highly polymorphic, easy to use and automate, co-dominant, low cost and multi allelic (Varshney *et al*., 2009). They are short cycle repeats with 1-10 bp, most typically, 2-3 bp. SSRs are thus one of the utmost used molecular markers in genetic diversity studies of many plants such as rice, maize and wheat. According to Hajeer *et al*. (2000), these markers are particularly variable and consistently distributed throughout the genome.

There are more than 20,000 available microsatellite markers that have been mapped to specific positions in rice genome (Pervaiz *et al*., 2009). Microsatellites markers (SSR) have been effectively used for several purposes including: genome mapping (Mccouch *et al*., 2002), assessment of the genetic variability and relatedness between various rice cultivars, including both aromatic and non-aromatic (Ghneim *et al*., 2008), identification and purity testing of varieties and determination of the genetic relationship among several sub-species (Ni *et al*., 2002). Nayak *et al*. (2004) reported that the working group on Biochemical and Molecular Techniques (BMT) of the International Union for the Protection of New Varieties of Plants (UPOV) has identified SSRs as the most widely utilized molecular marker for plant variety

characterisation. Therefore because of this vital information, ease of use and high polymorphism, microsatellites were selected for use in this study.

2.7 Morpho-agronomic traits relationship with rice grain yield

2.7.1 Tillers

A rice plants generally consists of main shoot and tillers which initiate at different times and differ in growth and development patterns depending on the time of their initiation and genotype (Krishna and Nidhi, 2014). Tiller number plays a significant role in influencing rice grain yield since it is closely related to number of panicles per unit ground area. Rahman *et al*. (2011) reported that tillers contribute a great portion of grain yield and the extent of contribution varies among genotype and planting density. Tillers can generally be divided into different categories according to the type of culm from which the tiller is initiated, that is a primary tiller initiated from a main culm, a secondary tiller from a primary tiller, a tertiary tiller from a secondary tiller, a quaternary tiller from a tertiary tiller (Counce *et al*., 1996). Keisuke *et al*. (1995) reported that a synchronous association exists in the time of appearance and growth between a given tiller and leaf. A marked variation could be found in the time of leaf appearance and panicle emergence and grain development among tillers within a plant (Florent *et al*., 2001). Therefore grain yield and its contributing traits vary greatly with tiller type and early initiated tillers produce more grains than late initiated tillers (Peltonen and Jarvinen, 1995).

According to Senapati *et al*. (2009) high tillering capacity is regarded as an important trait in rice production since the number of tillers per plant is closely correlated to number of panicles per plant. Wu *et al.* (1998) yield potential of a rice genotype may be considered by its tillering capacity. Miller *et al*. (1991) reported that the plants with more tillers shows a greater inconsistency in mobilizing assimilates and

nutrients. Therefore variation in grain development and yield between tillers is unpredictable over the genotypes differing in tillering ability. Variation in grain yield among tillers has been regarded the most important factor affecting yield potential for a given rice variety. Lafarge *et al*. (2002) reported that grain quality could be also affected by tillering ability due to different grain development characteristics. But however, the effect of tillering ability on grain quality still remains unclear.

2.7.2 Flag leaf

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Among the many leaves on rice plant the top three leaves particularly flag leaf contributes mostly to grain yield (Yoshida, 1981). The morphological traits of flag leaf such as size and shape and physiological traits of flag leaf such as chlorophyll content and photosynthesis ability have been considered to be the most important determinants of grain yield in cereals (Chen *et al*., 1995; Hirota *et al*., 1990). Flag leaf is therefore one of the utmost important yield components in determining grain yield potential of cereal crops (Xue *et al*., 2008). Flag leaf plays an important role in rice yield by increasing grain weight in amount of 41 to 43 % (Yoshida, 1972). According to Davood *et al*. (2009) flag leaf area could be chosen as a factor for increasing rice grain yield. For this reason flag leaf is an activist leaf at grain filling period. Flag leaf strongly contribute to grain filling after flowering, while flag leaf shape is one of the main factors determining its photosynthetic capacity (Fan *et al*., 2007). The leaf (source) being the organ of photosynthesis is regarded to be the most important determinant which is characterized for higher photosynthetic capacities (Prakash *et al*., 2011). Flag leaf help in maintaining photosynthesis during the grain filling period as this could increase grain yield capacity because photosynthesis during ripening contributes to grain carbohydrate by 60–100 % (Yoshida, 1981). It has been established that the flag leaf, stem and head are the closest source to the

grain (Prakash *et al*., 2011). Grain yield increase would be effectively rested on the basis of the capabilities of yield contributing traits and other closely related traits (Xue *et al*., 2008).

Among the flag leaf characters (Erect, horizontal and droopy) associated with yielding ability, erect leaf habit seems the most important (Yoshida, 1972). Erect leaves allow better penetration and more even distribution of light into the crop and thus greater photosynthesis activity (Jennings, 1979). Erect leaves mostly have higher leaf area index (single-side leaf area per unit of land area) which increases the capturing of light for photosynthesis and nitrogen use in crowded plantings (Yang and Hwa, 2008). This later improves dry matter accumulation in panicles and eventually increases grain yield (Sinclair and Sheehy, 1999). The higher, the photosynthetic capacity of an erect leaved character, the higher grain yield it produces (Yoshida, 1972). A canopy that has more erect leaves characters and a smaller extinction coefficient with better light intercepting structure has a larger critical or optimum leaf area index and a higher maximum canopy photosynthetic rate (Monneveux *et al*., 2004). Studies have shown that erect flag leaves intercept more solar radiation than horizontal and droopy leaves characters (Monneveux *et al*., 2004). Yoshida (1981) reported that there is only one rice experiment indicating that the droopy leave character has lower crop photosynthesis than the erect leaves at higher light intensities. In a canopy structure with flat leaves the total area of the top leaf is more exposed to sunlight than lower leaves (Yoshida, 1972).

2.7.3 Tall, weak culms

The culms are the jointed rice stem that develops from the plumule (primary bud of the seed embryo) and is composed of solid centres and hallow internodes. Culm height varies according to environment, management practices and variety. The

length of the growing season determines how many nodes the culm develop (usually 13-16). Generally the top internodes are the longest and bear the head (panicle).

Generally tall and weak culms of rice plants are associated with lodging (Faruq *et al*., 2011). In lowland rice production, lodging is characterized by bending of stem, stem breakage and root lodging (Kono *et al*., 1995). Stem bending type is the major type of lodging in lowland rice. It is mostly caused by the increase in panicle weight during maturation and by environmental effects (rain and wind). The culms of rice plant become weakened under heavy nitrogenous fertilizers application, deep submergence and sunlight deficiency (Lang *et al*., 2012). Lodging tends to occur in vigorously growing plants after flowering, when ripening progresses and panicles drop.

Lodging is a major problem in the production of rice because it causes decrease in yield by reducing photosynthesis in the canopy structure. In a lodged rice plant, the normal canopy structure is damaged resulting in reduced photosynthesis capacity and dry matter production (Mahbub *et al*., 2006). Lodging prevents the transport of water, nutrients and assimilates through the xylem and phloem resulting in a reduction of assimilates for grain filling (Kashiwagi *et al*., 2005). High moisture levels in a lodged plant community may be a favourable condition for fungal growth and also for the development of diseases which have detrimental effects on grain quality and quantity (Kono *et al*., 1995). Mojith *et al*. (2011) reported that lodging limits the opportunity of increasing rice yields through heavy fertilizer application and dense planting. Lodging also causes complications in harvesting operations, increases demand for grain drying and subsequently results in increased production cost (Kashiwagi *et al*., 2005). According to Setter *et al.* (1997) lodging of rice plants during the ripening period results not only in yield reduction, but also in a decrease

of grain quality due to the increased colouring of brown rice and or decreased flavour.

The importance of lodging resistance genotypes has long been recognized; the development of semi-dwarf rice genotypes by introducing the sd-1 gene in the early 1960s represented the greatest achievement in improving lodging resistance and yield potential of rice (Chandler, 1969). Reducing rice plant height by the introduction of sd-1 gene has decreased the effects of the upper part of the plant on the lower part thereby improving resistance to lodging. Ookawa and Ishihara (1992) reported that plant height is not necessarily the most important factor in determining lodging resistance in rice. They found that resistance to lodging differs among cultivars with similar heights. Studies have also shown that the culm characteristics supporting lodging resistance in rice include basal internodes length and thickness, culm wall thickness and leaf sheath wrapping and thickness (Matsuda *et al*., 1983). Duan *et al*. (2004) reported that aside the culm thickness, the culm vascular bundle number in rice also plays contributes to lodging resistance. Zhu *et al*. (2008) have found that a large number of quantitative trait locus alleles affecting culm strength, length and thickness in indica and japonica crosses of rice are related to lodging resistance. Kashiwagi *et al*. (2008) obtained similar results and recommend that increasing culm diameter in rice breeding programmes can improve lodging resistance. Aside improving lodging resistance in rice, a thick culm may also act as a carbohydrate store for high yield (Hirose *et al*., 2006).

2.7.4 Panicle number and length

A panicle is borne on the uppermost internode of the culm which is often mis-termed a peduncle. The architectural design of the panicle covers several aspects such as spikelet density, panicle length and panicle curvature (Sharief *et al*., 2006). The

degree to which the panicle and a portion of the uppermost internode extend beyond the flag leaf sheath decides the exsertion of the panicle. Accessions differ in degree of panicle exsertion. The nearly solid node between the uppermost internode of the culm and the axis of the panicle is the panicle base. This node does not usually bear a leaf or a dormant bud but may give rise to the first 1-4 panicle branches. The panicle base frequently appears as a ciliate ring and is used as a dividing point in measuring culm length and panicle length. The region about the panicle base is often called the neck. Panicles are perhaps most frequently found on grasses such as rice, oats and rye. Accessions differ greatly in the length, shape and angle of the primary branches and in the weight and density (number of spikelets per unit of length) of panicles.

The number of panicle with filled grains generally determines the ultimate yield of the crop. Therefore, effective panicles should have high ripening percentage and high grain to straw ratio (harvest index). Panicles affect yield capacity, as grain yield in rice is determined by the number of grains per area and potential size of grains (Feil, 1992). The number of panicles in cereals determines the ultimate yield of the crop, so also the length of a panicle determines the number of grain to be accommodated (Surek and Beser, 2003). This gives an indication that accessions with long panicles will accommodate more grain than short panicles accessions. Efisue *et al*. (2014) reported that accessions with long panicles possess high yielding ability. Kumar *et al*. (2007) by studying traits in rice varieties reported that there are positive and significant correlations between panicle length and grain yield.

Studying the relationship between morpho-agronomic traits of crops and their yield will reveal accessions to breeders for hybridization particularly for yield increases.

2.8 Genetic erosion of rice germplasm

Rice germplasm has evolved through several millennia of cultivation and selection by our farming ancestors. An important consequence of the domestication of rice plants is a reduction of genetic variability (Hore, 2005). Replacement of older varieties by modern improved varieties has accelerated in the past 50 years in what is now commonly known as the Green Revolution (Pervaiz *et al*., 2010). Extensive adoption of modern high yielding rice genotypes by farmers worldwide has led to a concern about genetic resource erosion in local rice and loss of diversity. High yielding rice varieties have almost completely replaced land races in most rice growing countries in Asia and Africa (Hamilton and Raymond, 2005). Erosion of local germplasm has also resulted from the various land preparation methods, overgrazing, cutting and burning of forests for human settlement, the indiscriminate use of fertilizers and pesticides, civil strife, improper means of storage, extensive plantations of cash and export-oriented trees and industrial crops and large-scale vegetable production, have all resulted in losses of local rice varieties from farmers' fields (Hamilton and Raymond, 2005). To maintain crop genetic diversity, collection, evaluation, characterization and conservation of traditional land races are vital. Maintaining biodiversity, especially crops in the ecosystem is of great importance to human's survival (Ishwaran and Erdelen, 2005).

2.9 Importance of characterization

Characterization is the description of a quality of an individual (Merriam-Webster, 1991). The word characterize is also a synonym of distinguish, meaning to mark as separate or to separate into type, kinds, classes or categories. Characterization of genetic resources therefore refers to the process by which accessions are identified or distinguished. It may also involve the evaluation of morph-agronomic performance

of accessions under a wide range of environmental conditions. In genetic terms characterization of accessions refers to the detection of variation as a consequence of differences in either DNA sequences or modifying factors or specific genes. Standard evaluation and characterization of accessions may be regularly carried out by using different approaches including traditional practice, involving the use of standard descriptor lists of morphological characters or characterization based on DNA using molecular markers. Rubenstein and Heisey (2003) reported that descriptor lists are very important tools used for ensuring that those who are documenting the characteristics of conserved species are using the same language and standards.

Grain yield per hectare is the most important consideration in any rice breeding programme (Ashura, 1998), but yield is a complex character to be inherited and may involve several related components. The need for increasing rice cultivation depends not on cultural practices alone, but also the magnitude of genetic variability among accessions. Therefore a successful breeding programme of crops will depend on characterization of genetic diversity for achieving the goals of improving the crop and producing high yielding varieties (Padulosi, 1993). To achieve this goal, the first step is to collect and evaluate germplasm at both morphological and molecular levels as phenotypic diversity will reveal very important traits of interest to the plant breeders (Singh, 1989). Evaluation of germplasm collections is vital for maintenance of the diversity and identification of valuable genes. Molecular and phenotypic characterization can disclose the maximum genetic diversity or genetic relatedness found in a population and hence duplicated breeding lines can be removed (Dias *et al*., 2013; Xu *et al*., 2000). Removal of duplicates from conserved genotypes ensures that only genetically distinct genotypes are maintained, save space and maintenance cost (Yada *et al*., 2010). Once variability has been ascertained, crop improvement

can be made through the use of appropriate selection method and increasing total yield would be made easier by selecting for yield attributing traits because they are more often easily inherited than total yield itself.

2.10 Constraints and solutions of germplasm characterization

Characterization of conserved germplasm is the greatest problem in many national, regional and global collections. Comprehensive evaluation and characterization for a number of plant descriptors have been affected by the sheer number of accessions particularly those involving grain quality and resistance to abiotic and biotic stresses which involves sophisticated instruments and significant resources. It is also very difficult to characterize very large collection of accessions using morphological and molecular markers (Vinita *et al*., 2013). The core collection concept was introduced in the 1980s for practical evaluation and effective management of large collections in crops (Brown, 1989). A core collection is a subset of a large number of germplasm collections that contains chosen accessions capturing most of the genetic variability within the entire gene bank. Crops like rice with large gene pool, the development of core collections will assist plant breeders in having access to and effective use of genetic diversity preserved. Lacking knowledge of a large collection of genetic materials, it would be difficult to evaluate and categorize such genetic resources. Hence greater effort is essentially needed to evaluate and characterize genetic resources available in national, regional and international by capacitating the human resource (FAO, 2002).

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental sites

The field experiment was conducted behind the insect laboratory (N 6.68º, W 1.57º) and laboratory experiment at the Biotechnology laboratory, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana.

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3.2 Planting materials

A total of 87 accessions of rice germplasm from Ghana (CRI and SARI), Thailand, Mali, Benin (Africa rice), Cameroon and Philippines (IRRI) were collected and used in this study (Table 3.1).

Plate 3.1: Rice accessions growing in buckets

Table 3.1: Plant materials and their source

No.	Accession	Source	No.	Accession	Source
1	WAB 2081-WAC B-TGR4-B	Africa Rice	24	TOX 3107	CSIR-SARI
2	WAB 2125-WAC B-1-TGR3-WAT B1	Africa Rice	25	ANYOFULA	CSIR-SARI
3	IR 841 (CHECK)	Africa Rice	26	NABOGU	CSIR-SARI
4	DKA-M2	Africa Rice	27	GR 21	CSIR-SARI
5	JASMINE 85	CSIR-SARI	28	PHKA RUMDON	Cameroon
6	FAROX 508-3-10-F43-1-1	Africa Rice	29	MLI 20-4-1-1-1	Mali
7	FAROX 508-3-10-F44-2-1-1	Africa Rice	30	DKA-M2	Mali
8	WAB 2098-WAC2-1-TGR2-WAT B2	Africa Rice	31	SIK 353-A-10	Mali
9	WAB 2056-2-FKR2-5-TGR1-B	Africa Rice	32	DK 3	Mali
10	WAB 2060-3-FKR1-WAC2-TGR4-B	Africa Rice	33	MLI 6-1-2-3-2	Mali
11	TXD 88	Africa Rice	34	MLI 25-1-2	Mali
12	WAB 2098-WAC3-1-TGR1-4	Africa Rice	35	DKA 4	Mali
13	WAB 2076-WAC1-TGR1-B	Africa Rice	36	DKA- M8	Mali
14	WAB 2081-WAC2-2-TGR2-WAT B3	Africa Rice	37	SIK 350-A-150	Mali
15	GBEWAA	CSIR-SARI	38	DKA-M11	Mali
16	PERFUME IRRIGATED	Thailand	39	DKA 22	Mali
17	WAS-122-13-WAS-10-WAR	Africa Rice	40	DKA-M9	Mali
18	LONG GRAIN ORDINARY 2	Thailand	41	DKA 1	Mali
19	EXBAIKA	CSIR-SARI	42	DKA 21	Mali
20	WAS-163-B-5-3	Africa Rice	43	MLI 20-4-3-1	Mali
21	FAROX 15	CSIR-SARI	44	SBT 70	Cameroon
22	PERFUME SHORT	Thailand	45	BASMATI 113	Thailand
23	KATANGA	CSIR-SARI	46	AGRA RICE	CSIR-CRI

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Cont't Table 3.1: Plant materials and their source

No.	Accession	Source	No.	Accession	Source
47	BASMATI 123	Thailand	68	AFRK- 4	Africa Rice
48	CRI-30	CSIR-CRI	69	IR 74963-2-6-2-5-1-3-3	IRRI
49	CRI-2	CSIR-CRI	70	IR 81412-B-B-82-1	IRRI
50	CRI-45	CSIR-CRI	71	IR 55419-04	IRRI
51	CRI-73	CSIR-CRI	72	IR 79913-B-179-B-4	IRRI
52	CRI-48	CSIR-CRI	73	APO	IRRI
53	NERICA 1	Africa Rice	74	N22	IRRI
54	AFRK-7	Africa Rice	75	IR 77298-14-1-2-10	IRRI
55	AFRK-8	Africa Rice	76	KALIAUS	IRRI
56	AFRK-5	Africa Rice	77	UPL RI 7	IRRI
57	AFRK-13	Africa Rice	78	KALIA	IRRI
58	NERICA 4	Africa Rice	79	IR 74371-46-1-1	IRRI
59	AFRK-6	Africa Rice	80	IR 74371-54-1-1	IRRI
60	AFRK-2	Africa Rice	81	IR 80411-49-1	IRRI
61	AFRK-11	Africa Rice	82	IR81023-B-116-1-2	IRRI
62	NERICA 14	Africa Rice	83	WAY RAREM	IRRI
63	AFRK-9	Africa Rice	84	VANDANA	IRRI
64	AFRK-3	Africa Rice	85	IR 77298-5-6-18	IRRI
65	AFRK-1	Africa Rice	86	IR 74371-70-1-1	IRRI
66	AFRK-10	Africa Rice	87	UPL RI 5	IRRI
67	AFRK-5	Africa Rice			

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3.3 Field experiment

3.3.1 Experimental design and agronomic practices

Completely randomised design (CRD) with three replications was used. Rubber buckets with top and button diameters of 30 cm and 18.5 cm respectively and also with height of 28 cm were filled with sterilized top soil to avoid contamination. Two (2) seedlings of twenty one (21) days old were transplanted on the 7th July 2014 and later thinned to one (1) after ten (10) days. Each accession of the 87 was transplanted into a separate bucket (Plate 3.1). Two gram (2 g) of NPK (15:15:15) was top dressed three (3) days after transplanting in each bucket. Urea was also top dressed at each stage of tillering and panicle initiation, 1.5 g in each bucket. Other routine operations like irrigation, application of insecticides and hand weeding in and around the buckets were employed whenever necessary.

3.3.2 Morpho-agronomic data collection

Morphological and agronomic traits were collected to generate morpho-agronomic data for the eighty seven (87) rice accessions studied. The following were collected during the different growth stages and after harvest: leaf blade pubescence, leaf blade; intensity of green colour, basal leaf sheath colour, ligule shape, auricle colour and ligule colour at vegetative stage. At the early reproductive stage culm angle and days to 50 % flowering were the data collected, while flag leaf angle, awns presence, days to 80 % grain maturity, panicle exsertion, attitude of panicle branches, leaf length, leaf width, ligule length, flag leaf length, flag leaf width, culm length, culm diameter, tiller number, panicle number, plant height and panicle length were collected at late reproductive stage. After harvest, grain yield, grain length, grain width and 100 grain weight were also collected.

IRRI and WARDA rice descriptors were accessed and used as guide for the data collection. Vernier calliper, meter rule and precision balance scale were used to measure quantitative traits as appropriate with each parameter involved.

3.3.2.1 Days to 50 % flowering

This was determined by counting the number of days taken from nursery date to when 50 % of the culms of a plant in the pot flowered (heading).

3.3.2.2 Days to 80 % grain maturity

To obtain this data, days from nursery date were counted to when 80 % of the grains on the panicles of the plant were fully matured and ripened.

3.3.2.3 Culm diameter

Culm diameter at the basal internode was measured and recorded in millimetres. It was measured at the outer diameter of the basal portion of the main culm. Accessions were coded as follows: Thin <5 mm and Thick ≥5 mm.

3.3.2.4 Panicle number

This parameter was obtained by physically counting the number of culms that produced panicles of fertile grains per accession in a bucket. It was coded as: 3-Low (<10 culms), 5-Intermediate (~15 culms) and 7-High (>20 culms).

3.3.2.5 Tiller number

The number of tillers was obtained by physically counting the number of productive and unproductive tillers per accession in a bucket. This parameter was also coded as: 3-Low (<10 culms), 5-Intermediate (~15 culms) and 7-High (>20 culms).

3.3.2.6 100 Grain weight

To obtain 100 grain weight of each accession, well developed 100 seeds were randomly selected from harvested samples dried at 13% moisture content and weighed on a balanced precision scale at the soil laboratory, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana.

3.3.2.7 Grain width

This parameter was measured in millimetre. It was measured as the distance across the fertile lemma and palea at the widest point. 10 representative seeds randomly selected from harvested samples after sun drying at 13% moisture content were measured and averaged.

3.3.2.8 Grain length

Ten representative seeds randomly selected from harvested samples after sun dried at 13% moisture content were measured and averaged. It was done by measuring the distance from the lowermost glume to the tip (apiculus) of the fertile lemma or palea, whichever is longer. On awned cultivars, measurements were taken to a point comparable to the tip of the apiculus (excluding the awn). This parameter was also measured in millimetre.

3.2.2.9 Plant height

To obtain plant height of the evaluated accessions in centimetres (cm), measurements were taken from the soil surface to the tip of the tallest plant part. It was coded as: Short (<110 cm), Intermediate (~120 cm) and Tall (>130 cm).

3.3.2.10 Leaf length

Measurement of the penultimate leaf length (highest leaf below the flag leaf) on the main culm was taken from the ligule to the tip of the leaf blade. This parameter was

taken in centimetres (cm) and coded as: Very short (<21cm), Short (~30 cm), Intermediate (~50 cm), Long (~70 cm) and Very long (>80 cm).

3.3.2.11 Leaf width

Measurement of leaf width on the penultimate leaf (highest leaf below the flag leaf) was done in centimetres at the widest portion and coded as: Narrow (<1cm) and Broad (>2 cm).

3.3.2.12 Culm length

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To obtain the culm length of evaluated accessions, measurements were taken from ground level to the base of the panicle. The culm length was measured in centimetres and coded as: 1-Very short (<50 cm), 2-Very short to short (51–70 cm), 3-Short (71–90 cm), 4-Short to intermediate (91–105 cm), 5-Intermediate (106–120 cm), 6-Intermediate to long (121–140 cm),7-Long (141–155 cm), 8-Long to very long (156–180 cm) and 9-Very long (>180 cm).

3.3.2.13 Flag leaf length

Measurement of the flag leaf length on the culm was taken from the ligule to the tip of the blade. Flag leaf length was measured and recorded in centimetres and coded as: Very short (<21cm), Short (~30 cm), Intermediate (~50 cm), Long (~70 cm) and Very long (>80 cm).

3.3.2.14 Flag leaf width

Measurement of flag leaf width was done in centimetres at the widest portion and coded as: Narrow (<1cm) and Broad (>2 cm).

3.3.2.15 Ligule length

Ligule length was measured and recorded in centimetres from base of collar to the tip of the ligule of the penultimate leaf (highest leaf below the flag leaf).

3.3.2.16 Panicle length

Length of main axis of panicle was measured and recorded in centimetres from the panicle base to the tip. This parameter was also coded as: Very short (<11 cm), Short (~15 cm), Medium (~25 cm), Long (~35 cm) and Very long (>40 cm).

3.3.2.17 Grain yield

Grain yield of the evaluated accessions were weighed on the precision balance scale at the soil laboratory, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana. This was done after harvest, threshing, winnowing and sun drying at 13% moisture content. The grain yield per accession was converted into tonnes/hectare using the top surface area of the bucket.

3.3.2.18 Leaf blade pubescence

To determine this qualitative trait, leaves of accessions were rubbed with fingers from the tip downwards on the surface. The presence and absence of hairs on the blade surface was classified as: 1-glabrous (smooth), 2-intermediate and 3-pubescent.'

3.3.2.19 Panicle exsertion

Panicle exsertion was determined by observing the extent to which the panicle is exserted above the flag leaf sheath. This trait was scored as: 1-Enclosed (panicle is partly or completely enclosed within the leaf sheath of the flag leaf blade), 3-Partly exserted (panicle base is seen slightly beneath the collar of the flag leaf blade), 5-Just exserted (panicle base coincides with the collar of the flag leaf blade), 7-Moderately well exserted (panicle base is seen above the collar of the flag leaf blade) and 9-Well exserted (panicle base is seen well above the collar of the flag leaf blade).

3.3.2.20 Culm angle

This trait was determined by observing, the estimated average angle of inclination of the base of the main culm from vertical. It was scored as: 1-Erect (<15º), 3-Semi erect (intermediate) (~20º), 5-Open (~40º), 7-Spreading (>60-80º, culms not resting on the ground) and 9-Procumbent (culm or its lower part rests on ground surface).

3.3.2.21 Ligule shape

This qualitative trait was also determined by observation and scored as: 0-Absent (no ligule), 1-Truncate, 2-Acute to acuminate and 3-2 cleft.

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3.3.2.22 Flag leaf angle

To determine this data, flag leaf of evaluated accessions were observed and scored as: 1-Erect, 3-Semi erect (intermediate), 5-Horizontal and 7-Descending.

3.3.2.23 Basal leaf sheath colour

This qualitative trait was determined by observing the colour of the outer surface leaf sheath and it was scored as: 060-Green, 084-Green with purple lines, 081-Light purple and 080-Purple.

3.3.2.24 Leaf blad; Intensity of green colour

This trait was carefully observed and scored as: 0-N0 green colours visible due to anthocyanin, 061-Light, 060-Medium (green) and 063-Dark.

3.3.2.25 Attitude of panicle branches

This trait was obtained by observing the compactness of the panicle and classified according to its mode of branching, angle of primary branches and spikelet density and scored as: 1-Erect (compact panicle), 3-Semi erect (semi compact panicle), 5-Spreading (open panicle), 7-horizontal and 9-Drooping.

3.3.2.26 Ligule colour

Ligule colour was also observed and scored as: 0-Absent (liguleless), 011-Whitish, 062-Yellow green, 080-Purple, 081-Light purple and 084-Green with Purple lines.

3.3.2.27 Auricle colour

To obtain the auricle colour of evaluated accessions, it was determined by observation and scored as: 000-Absent (no auricle), 011-Whitish, 062-Yellow green, 080-Purple, 081-Light purple and 084-Green with Purple lines.

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3.3.2.28 Awn presence

To obtain awn presence, the apiculus of the grain was observed to determine its presence or absence. It was score as: 0-Absent, 1-Partly awned and 2-Fully awned.

3.4 Laboratory experiment

Following successful field experiment of the rice accessions, the genetic diversity were examined at the DNA level using SSRs markers. This was carried out to confirm the diversity revealed by the morpho-agronomic traits.

3.4.1 Genomic DNA extraction

Genomic DNA was extracted from young fresh leaves of 87 rice accessions using the CTAB method described by Doyle and Doyle (1990) with slight modifications. Twenty (20) milligrams of leave samples with liquid nitrogen in 2.0 ml Eppendorf tubes were ground into fine powder using Teflon pestle. To each sample, was added 800 µl of 2 % CTAB and 0.5 µl of 0.1 % mercaptoethanol and then incubated at 65 ^{0}C in a water bath for 30 minutes, with intermittent vortexing at 10 minutes interval. The samples were then cooled at room temperature and an equal volume (800 µl) of chloroform: isoamyl-alcohol (24:1) added. To obtain thorough mixture of samples, tubes were inverted several times and centrifuged for 15 minutes at 14000 rpm. The aqueous phase

containing DNA was transferred into clean 1.5 ml Eppendorf tubes and an equal volume of chloroform: Isoamyl-alcohol solution added and then centrifuged for another 15 minutes at 14000 rpm. To the precipitated nucleic acid was added two thirds volume of ice cold isopropanol (400 µl) and shaken gently. Precipitation of samples was enhanced by storing at -20 °C for 8 hours or overnight in a freezer. Another centrifuging at 14000 rpm for 5 minutes was done to pellet nucleic acid. The isopropanol was decanted and the pellet was washed with 500 µl of washing buffer, centrifuged for 4 minutes at 6000 rpm. The washing buffer was also decanted and the pellet was washed in 400 µl of ethanol (80 %) and then centrifuged for 4 minutes at 6000 rpm, 80 % ethanol was decanted and the pellet air dried until the smell of ethanol was no longer detected. The DNA samples were then suspended in 100 µl of TE buffer with 10 mg/ml RNAase and centrifuged for 30 seconds at high speed and then stored at 4°C. The DNA samples of each rice accession was confirmed by electrophoresis on 1% agarose gel.

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3.4.2 Molecular markers and polymerase chain reactions

Twelve simple sequence repeats markers (SSR) presented in Table 3.2 were used to detect polymorphism among the rice accessions. The SSRs markers were procured from Metabion International AG (Germany). The PCR reactions were carried out in eppendorf mastercycler (Eppendorf, Hamburg, Germany) of 96-well plates. PCR kits (KAPA 3G Fast Ready Mix) procured from KAPA Biosystems (Pty) Ltd (South Africa) was used for the reaction. Total volume of 15 µl with final concentrations of 1 X KAPA Plant PCR Buffer + dNTPs and 1.5 mM $Mgcl_2$, 0.3 µM forward and reversed primers, 0.1 X KAPA Plant PCR Enhancer, 1 U/µl KAPA 3G Plant DNA Polymerase and 10 ηg/µl of crude DNA.

PCR amplification was subjected to initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95°C for 30 sec, 57°C for 30 sec and 72°C for 1 minutes and a final

extension at 72°C for 7 minutes. The reactions (PCR products) were then held at 4°C until electrophoresis.

Table 3.2: SSR Primers and their sequences used in DNA fingerprinting

SSR locus and chromosome location	Primer sequence (5' To 3')	Type of SSRs
Xtxp 149 (1)	F=AGCCTTGCATGATGTTCC R=GCTATGCTTGGTGTGGG	$(CT)_{10}$
Xtxp 284 (1)	F=CCAGATTGGCTGATGCATACACACT R=AAGGGTAATTTATGCACTCCAAGGTAGGAC	$(AAG)_{19}$
Xtxp 201 (2)	F=GCGTTTATGGAAGCAAAAT R=CTCATAAGGCAGGACCAAC	$(GA)_{36}$
Xtxp 197 (2)	F=GCGTCAATTAATCCAAACAGCCTC R=GAGTTCCTATTCCCGTTCATGGTGAT	$(AC)_{10}$
Cba (3)	F=AAAGCTCGGCGTTAGAAATA R=CGTTTAACAACTCGTACCATC	$(TA)_{18}$
Xtxp 51 (4)	F=TCTCGGACTCAAGAGCAGAGG R=GGACAGCAGCGGCTTCAG	$(TG)_{11}$
Xtxp 274 (6)	F=GAAATTACAATGCTACCCCTAAAAGT R=ACTCTACTCCTTCCGTCCACAT	$(TTC)_{19}$
Xtxp 278 (7)	F=GGGTTTCAACTCTAGCCTACCGAACTTCCT R=ATGCCTCATCATGGTTCGTTTTGCTT	$(TTG)_{12}$
Xtxp 295 (7)	F=AAATCATGCATCCATGTTCGTCTTC R=CTCCCGCTACAAGAGTACATTCATAGCTTA	$(CT)_{19}$
Xtxp 258 (9)	F=CACCAAGTGTCGCGAACTGAA R=GCTTAGTGTGAGCGCTGACCAG	$(AAC)_{19}$
Xtxp 10 (9)	F=ATACTATCAAGAGGGGAGC R=AGTACTAGCCACACGTCAC	$(CT)_{14}$
Xtxp 217 (10)	F=GGCCTCGACTACGGAGTT R=TCGGCATATTGATTTGGTTT	$(GA)_{23}$

F: Forward primer and R: Reverse primer. (Missihoun *et al*., 2015)

3.4.3 Gel electrophoresis

PCR products obtained were electrophoresed using 2 % agarose gel stained with ethidium bromide solution. The tracking dye in the PCR premix (KAPA 3G) made visual tracking of the PCR products through the gel easier. Approximately 10 µl of the amplified products and 5 µl 100 bp molecular ladder (Universal ladder) obtained from KAPA Biosystems (Pty) Ltd (South Africa) were electrophoresed at 120 V for 120 minutes using Galileo Bioscience (81-2325) tank. A control was loaded in the first well

and the molecular ladder was loaded into well two and the DNA of the rice accessions were loaded in the adjacent wells. The gel was visualized by illumination on Benchtop UV transilluminator. The gels were photographed under UV light.

3.4.4 Scoring of DNA bands

Scoring of DNA bands for each primer that yielded scorable amplification products was done using KAPA universal ladder (100 bp-10000 bp).

3.5 Data analysis

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Quantitative traits data collected during this experiment were subjected to analysis using GenStat 12th edition package. The package helped to calculate mean, standard error, standard deviation, coefficient of variation (CV), least significant difference (LSD) test at 5 % probability and correlation analysis with grain yield. Frequency distribution was used to classify the genotypes into groups based on the qualitative traits and to show grain yield produced by the different morphological traits categories of the rice accessions. The number of alleles per locus, major allele frequency, gene diversity, heterozygosity and polymorphism information content (PIC), values were all calculated using PowerMarker version 3.25 (Liu and Muse, 2005). NTSYS-pc version 2.21q was used to construct UPGMA (unweighted pair group method with arithmetic averages) dendrograms to show the distance-based relationship among the rice accessions based on both morphological traits and SSR markers. It was also used to do principal component analysis (PCA) to examine the percentage contribution of each quantitative traits to total genetic variation.

CHAPTER FOUR

RESULTS

4.1 Morphological and physiological analysis of quantitative traits

Highly significant ($P < 0.001$) differences were observed among the various quantitative traits analysed in this experiment (Table 4.1), revealing that based on morpho-agronomic traits, variation exists among the 87 accessions of rice. The coefficient of variation and standard deviation for days to 50 % flowering (heading) were 3.80 % and 12.33 respectively. Days to 50 % flowering in this study ranged from 74 days to 126 days. The minimum days was observed in genotype AFRK-13, while the maximum value was recorded in SIK 353-A10 (Appendix 1). Coefficient of variation (CV) and standard deviation for days to 80 % grain maturity were 4.60 % and 9.97 respectively. The minimum days to 80 % maturity was observed in genotype AFRK-3 (113 days), while SBT 70 had the highest (149 days). Minimum tiller number was produced by genotypes CRI-45 and CRI-2 from the same research institute, while N22 produced the maximum tiller per plant and their coefficient of variation and standard deviation were 21.00 % and 5.50 respectively. The standard deviation and coefficient of variation for panicle number were 5.13 and 20.80 % respectively in this study. Panicle number per plant among these accessions ranged from 7 to 26. The minimum panicle number per plant (7) was observed with rice genotype CRI-30, FAROX 508-3-10-F43-1-1 produced the maximum panicle per plant (26) followed by EXBAIKA and DKA 21 with 24 each. Standard deviation and coefficient of variation (CV) for panicle length were 3.73 and 11.10 % respectively. The maximum panicle length was in DKA-M2 (34.13 cm) followed by CRI-73, while KALIA (16.67 cm) recorded the minimum. The analysis revealed plant height of 125.70 cm as ground mean with a standard error of 9.57, coefficient of variation of 13.20 % and standard deviation of 21.28. The minimum plant

height was associated with accession AFRK-10 (86.40 cm) and AGRA RICE (171.70 cm) for the maximum. Ground mean of 2.86 g was obtained for the genotypes with means which ranged from 1.81 g-4.50 g for 100 grain weight and their standard deviation and coefficient of variation (CV) were 0.45 and 8.60 % respectively. CRI-2 had the maximum 100 grain weight and N22 the minimum. The coefficient of variation (CV) for grain length and width were, grain length (6.80 %) and width (12.30 %). The coefficient of variation and standard deviation for flag leaf length were recorded as 21.90 % and 9.42 respectively with a ground mean of 35.79 cm.

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Table 4.1: Mean + S.E., maximum and minimum means, coefficient of variation (CV) %, standard deviation (SD) and F probability of 87 rice accessions

Trait	Mean ± S.E	Max	Min	CV%	StdDev	F-prob
Days to 50 % flowering	95.69 ± 2.10	126	74	3.80	12.33	0.001
Days to 80 % maturity	127.98 ± 3.42	149	113	4.60	9.97	0.001
Panicle number	14.50 ± 1.74	26	7	20.80	5.13	0.001
Tiller number	15.31 ± 1.86	26	8	21.00	5.50	0.001
100 grain weight (g)	2.86 ± 0.14	4.05	1.81	8.60	0.42	0.001
Grain width (mm)	2.56 ± 0.18	3.10	2.13	12.30	0.40	0.001
Grain length (mm)	9.27 ± 0.36	10.80	6.77	6.80	0.86	0.001
Culm diameter (mm)	7.00 ± 0.82	9.43	4.43	20.30	1.62	0.001
Plant height (cm)	125.70± 9.57	171.70	86.40	13.20	21.28	0.001
Leaf length (cm)	50.68 ± 5.91	76.20	30.27	20.20	12.41	0.001
Leaf width (cm)	1.46 ± 0.14	2.47	0.77	16.70	0.30	0.001
Culm length (cm)	94.19 ± 5.76	138.17	63.40	10.60	17.44	0.001
Flag leaf length (cm)	35.79 ± 4.52	55.27	20.80	21.90	9.42	0.001
Flag leaf width (cm)	1.79 ± 0.13	2.40	1.40	12.70	0.27	0.001
Ligule length (cm)	1.91 ± 0.34	3.57	0.60	30.90	0.79	0.001
Panicle length (cm)	26.10 ± 1.68	34.13	16.67	11.10	3.73	0.001
Grain yield (t/ha)	8.42 ± 1.11	12.88	3.55	22.80	2.50	0.001

SE= Standard Deviation, Max = Maximum, Min = Minimum F Prob = Probability and CV = coefficient of Variation.

4.2 Principal component analysis of quantitative traits

Principal component analysis grouped the 17 quantitative traits into 10 components. The first 5 components, with eigen values higher than 1.0, accounted for 75.01 % of the total variance in which PC1 explained 28.47 % (Table 4.2) of the total variation. Based on eigen vectors with values greater than or equal to 0.50, traits such as days to 50 % flowering, 100 grain weight, culm length, panicle number, tiller number, flag leaf width and leaf width were the major discriminatory characters associated with the first PC while days to 50 % flowering, days to 80 % maturity, culm length, plant height, ligule length, leaf length, culm diameter and grain yield were associated with the second PC, which accounted for 22.12 % of the total variation. The third PC which explained 9.47 % of the total variation, was dominated by 100 grain weight, flag leaf length and grain length while flag leaf width dominated the forth PC. Grain width dominated the fifth PC which accounted for 6.71 % of the total variation.

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Table 4.2: Principal component analysis of quantitative traits in 87 rice accessions

Traits	PC1	PC2	PC3	PC4	PC5
Days to 50 % flowering	**0.51**	**0.68**	0.12	0.07	0.28
Days to 80 % maturity	0.32	**0.85**	0.10	0.08	0.14
100 grain weight (g)	**-0.66**	0.17	**0.59**	0.08	-0.10
Culm length (cm)	**-0.54**	**0.57**	-0.16	0.34	-0.07
Panicle length (cm)	-0.25	0.48	-0.03	0.42	-0.49
Plant height (cm)	-0.48	**0.62**	-0.35	0.15	0.00
Panicle number	**0.93**	0.14	0.19	-0.19	-0.02
Tiller number	**0.93**	0.11	0.10	0.16	0.00
Flag leaf width (cm)	**-0.65**	-0.15	0.02	**0.54**	0.10
Flag leaf length (cm)	-0.43	0.24	**-0.53**	0.49	0.03
Ligule length (cm)	0.30	**0.68**	0.18	0.08	0.17
Leaf width (cm)	**-0.51**	-0.13	0.47	0.36	0.23
Leaf length (cm)	-0.42	**0.62**	-0.23	0.24	0.27
Culm diameter (mm)	-0.40	**0.53**	0.09	0.17	-0.12
Grain yield (t/ha)	0.48	**0.54**	0.09	0.41	-0.11
Grain length (mm)	-0.35	0.24	**0.66**	0.19	-0.33
Grain width (mm)	-0.30	-0.09	0.18	0.17	**0.70**
Eigen value	4.84	3.76	1.61	1.40	1.14
Proportion (%)	28.47	22.12	9.47	8.24	6.71
Cumulative (%)	28.47	50.59	60.06	68.30	75.01

4.3 Variability in qualitative phenotypic traits of rice accessions

The genotypes from different countries in this study showed variability for basal leaf sheath colour, leaf blade; Intensity of green colour, leaf blade pubescence, auricle colour, ligule shape, ligule colour, culm angle, flag leaf angle, attitude of panicle branches, panicle exsertion and awn depending on their colour or type observed. Frequency distributions for these eleven qualitative traits are presented in Table 4.3. Significant amount of the 87 accessions had no awn 72 (82.76 %), 6 (6.90 %) partly awned and 9 (10.34 %) fully awned. Sixty six (75.86 %) accessions had whitish ligule colour, 12 (13.79 %) yellowish green colour, 2 (2.30 %) purple colour, 3 (3.45 %) light purple colour and 4 (4.60 %) no ligule at the stage of observation. Fifty seven (65.52 %) accessions showed intermediate leaf pubescence, 20 (22.99 %) glabrous leaf and 10 (11.49 %) pubescent leaf. Intermediate leaf pubescence accessions were the second highest rice grain yield producer after the pubescent ones (Figure 4.4). Fifty one (58.62 %) accessions had erect flag leaf, 30 (34.48 %) semi erect flag leaf, 5 (5.75 %) horizontal flag leaf and 1 (1.15 %) descending flag leaf. Accessions with erect and semi erect flag leaf had the maximum yield of rice grain in this study than the other flag leaf categories (Horizontal and Descending) (Figure 4.2). On the basis of ligule shape more than half of the accessions had Acute to acuminate ligule shape 48 (55.17 %). In terms of culm angle, 41 (47.13 %) of the accessions were having semi erect angle. Regarding panicle exsertion, 36 (41.38 %) of the accessions had their panicles moderately well exserted (panicles base was above the collar of the flag leaf blade). Forty one (47.13 %) of these accessions studied also had medium green colour on their leaf blade, while 37 accessions (42.53 %) showed yellowish green auricle colour. Four (4.60 %) of the evaluated accessions had no ligule at the vegetative stage of this study.

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Table 4.3: Frequency distribution for eleven qualitative traits in rice accessions

Trait	Colour/Type	Frequency	Percent (%)
Basal leaf sheath colour	Green	57	65.52
	Green with purples lines	4	4.60
	Light purple	21	24.14
	Purple	5	5.75
Lead blade: Intensity of green colour	Light	11	12.64
	Medium (green)	41	47.13
	Dark	35	40.23
Leaf blade pubescence	Glabrous (smooth)	20	22.99
	Intermediate	57	65.52
	Pubescent	10	11.49
Auricle colour	Whitish	13	14.94
	Yellowish green	37	42.53
	Purple	19	21.84
	Light purple	17	19.54
	Purple lines	1	1.15
Ligule shape	Absent	4	4.60
	Truncate	26	29.89
	Acute to acuminate	48	55.17
	2-cleft	9	10.34
Ligule colour	Absent	4	4.60
	Whitish	66	75.86
	Yellowish green	12	13.79
	Purple	2	2.30
	Light purple	3	3.45
Culm angle	Erect	29	33.33
	Semi erect	41	47.13
	Open	15	17.24
	Spreading	2	2.30
Flag leaf angle	Erect	51	58.62
	Semi erect	30	34.48
	Horizontal	5	5.75
	Descending	1	1.15
Attitude of panicle branches	Semi erect	3	3.45
	Spreading (open panicle)	13	14.94
	Horizontal	40	45.98
	Drooping	31	35.63
Panicle exsertion	Partly exserted	2	2.30
	Just exserted	24	27.59
	Moderately well exserted	36	41.38
	Well exserted	25	28.74
Awn	Absent	72	82.76
	Partly awned	6	6.90
	Fully awned	9	10.34

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4.4 Correlation analysis of quantitative traits with rice grain yield

Correlation analyses of fifteen (15) quantitative traits collected during this study are presented in Table 4.4. Seven (Panicle number, Days to 50% flowering, Days to grain maturity, Tiller number, panicle length, ligule length and 100 grain weight) of these quantitative traits showed significant positive relationship with rice grain yield, one (flag leaf width) showed significant negative correlation with grain yield, while four (Leaf length of blade, Grain width, flag leaf length and culm length) had non-significant positive association with grain yield and two (grain length and plant height) showed non-significant negative correlation with rice grain.

Panicle number (0.58***), Days to 50% flowering (0.43***), Days to grain maturity (0.26***), Tiller number (0.52***), panicle length (0.24***), ligule length (0.29***) and 100 grain weight (0.30***) showed significant positive correlation with grain yield, while significant but negative correlation existed between grain yield and flag leaf width (-0.19***) (Table 4.4). Leaf length of blade (0.03), Grain width (0.01), flag leaf length (0.06) and culm length (0.04) showed non-significant positive correlation with grain yield, While grain length (-0.05) and plant height (-0.09) had non-significant negative correlation with grain yield (Table 4.4).

For quantitative traits with significant and positive correlation with grain yield, it means that any increase in these quantitative traits caused increase in the grain yield. Rice grain yield produced by the different culm number categories are presented in Figure 4.1. It shows that a unit increase in culm number resulted into increase in the grain yield as significant and positive correlation existed between the two traits (Table 4.4). The non-significant negative relationship observed between yield and plant height in this study, revealed that unit increase in one of the trait resulted into reduction in the other.

Table 4.4: Correlation coefficient between rice yield and its quantitative traits

Trait	100GW	CL	TN	D50%F	D80%GM	FLL	FLW	GY	GW	GL	LIL	LLB	PL	PH	PN
100GW	_														
CL	0.31***	_													
TN	-0.42***	-0.37***	_												
D50 %F	-0.02ns	0.21***	0.34***	_											
D80%GM	-0.14*	0.02ns	0.42***	0.73***	_										
FLL	0.02ns	0.13*	-0.26***	0.02ns	-0.11ns	_									
FLW	0.23***	0.12*	-0.38***	-0.15*	-0.26***	0.40**	_								
GY	0.30***	0.04ns	0.52***	0.43***	0.26***	0.06ns	-0.19***	_							
GW	0.16**	0.01ns	-0.16**	-0.08ns	-0.05ns	0.06ns	0.14*	0.01ns	_						
GL	0.43***	0.20***	-0.20***	0.08ns	-0.01ns	-0.09ns	0.04ns	-0.05ns	0.01ns	_					
LIL	-0.02ns	0.10ns	0.18**	0.54***	0.42***	0.04ns	-0.08ns	0.29***	-0.04ns	0.11ns	_				
LLB	0.16**	0.43***	-0.25***	0.27***	0.13*	0.29***	0.14*	0.03ns	0.06ns	0.01ns	0.28***	_			
PL	0.14*	0.25***	-0.11ns	0.18**	0.01ns	0.28***	0.16**	0.24***	-0.04ns	0.12*	0.12ns	0.12ns	_		
PH	0.17**	0.61***	-0.28***	0.22***	0.10ns	0.35***	0.14*	-0.09ns	-0.02ns	0.11ns	0.10ns	0.39***	0.29***	_	
PN	-0.43***	-0.36***	0.97***	0.37***	0.41***	-0.25***	-0.38***	0.58***	-0.16**	-0.17**	0.21***	-0.23***	-0.08ns	-026***	_

*, ** and*** significant at 0.05, 0.01 and0.001 probability levels respectively, ns = non-significant. 100 GW= 100 Grain weight, CL = Culm length, TN = Tiller number, D50%F = Days to 50% flowering (heading), D80%GM = Days to 80% grain maturity, FLL = Flag leaf length, FLW = Flag leaf width, GY = Grain yield, GW = Grain width, GL = Grain length, LIL =Ligule length, LLB = Leaf length of blade, PL = Panicle length, PH = Plant height and PN = Panicle number.

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4.5 Yield of rice accessions based on morphological parameters

Frequency distribution showing grain yield of accessions studied based on the different categorises of plant height, leaf blade pubescence, Culm number and flag leaf angle. From the study, accessions with the following quantitative and qualitative characters produced the highest grain yield, high tillering followed by intermediate, erect flag leaf, short and pubescent leaf followed by intermediate (Figure 4.1, 4.2, 4.3 and 4.4 respectively).

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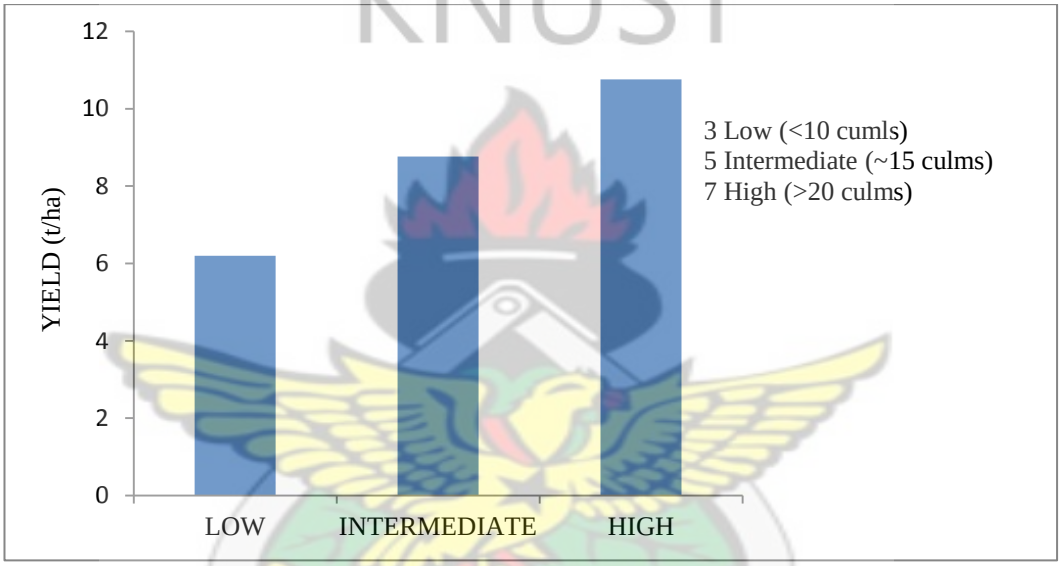

Figure 4.1: Yield in relation to different culm categories

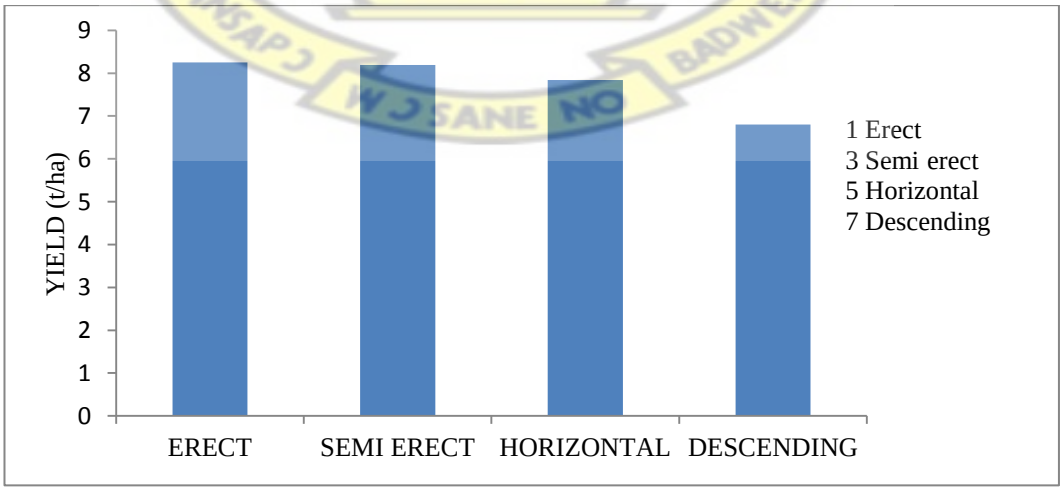

Figure 4.2: Yield in relation to different flag leaf angles

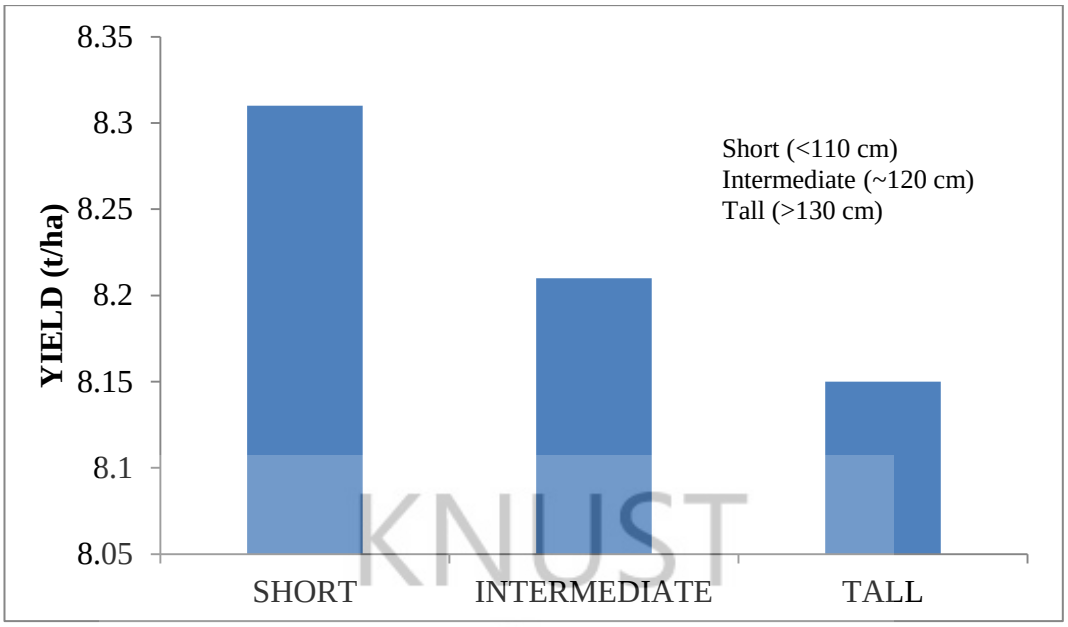

Figure 4.3: Yield in relation to different plant height categories

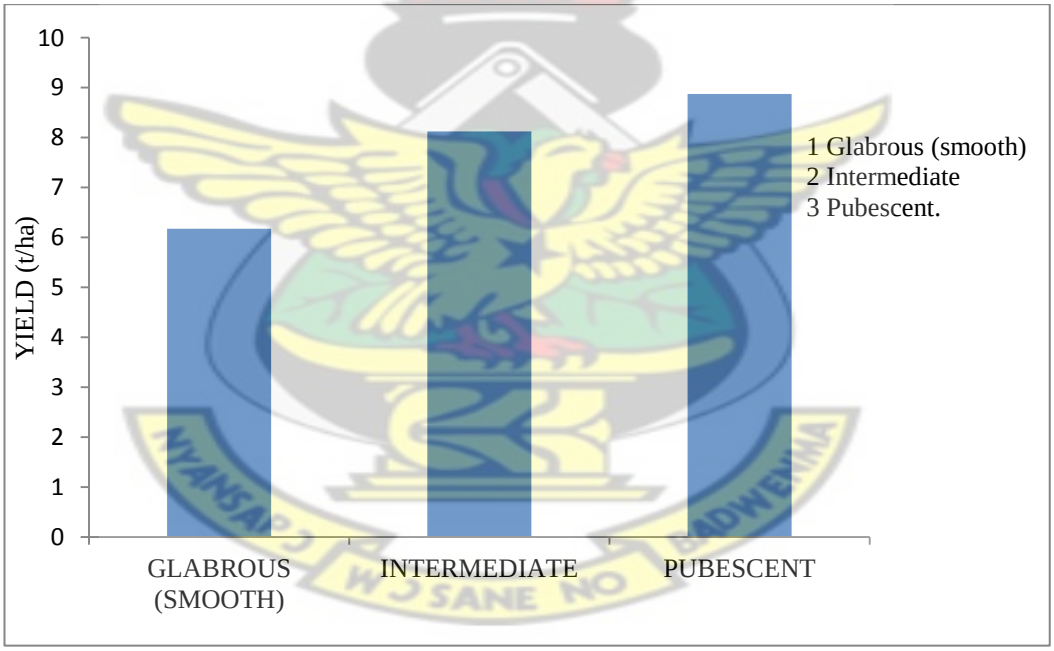

Figure 4.4: Yield in relation to different leaf pubescence categories

4.6 Clustering of rice accessions based on morphological traits

Genetic relationship revealed by morphological traits using similarity coefficients based on unweighted pair group method with arithmetic mean (UPGMA) is shown in Figure 4.5. From the figure, the 87 rice accessions were clustered into seven major groups at 21 % similarity coefficient. Cluster I contained 28 accessions from Ghana, Philippines, Benin and Mali. These accessions have tiller number ranged from 10-20 and their days to 80 % grain maturity also ranged from 100-130 days in this study. Cluster II 2 accessions 2 (WAB 2125-WAC B-1-TGR3-WAT B1) and 86 (IR 74371-70-1-1) both from Benin and Philippines respectively. WAB 2125-WAC B-1-TGR3-WAT B1 and IR 74371-70-1-1 are accessions that have green basal leaf sheath colour, erect culm angle, medium green leaf blade; intensity of green colour, just exserted panicle exsertion and absent of awn. Cluster III contained sixteen accessions from all the six countries in which the 87 accessions where sourced. These sixteen accessions have the following characteristics such as no awn on their grains, tiller number 10-23, height 130-160 cm and panicle number 10-20 (Appendix 1 and 2). Cluster IV contained four accessions from Mali, Thailand and Philippines. These four accessions includes DKA-M11, MLI 20-4-3-1, PERFUME IRRIGATED and IR 81412-B-B-82-1, they are accessions with also no awn, green basal leaf sheath colour, 90-120 days to 50 % flowering. Cluster V contained 22 accessions which have erect and semi erect flag leaf. The sixth cluster contains nine accessions from Mali, Ghana and Benin. These nine accession have the following characteristics such erect flag leaf, moderately well exserted panicle exsertion and whitish ligule colour. Finally cluster seven contains six accessions which have no awn and their grain width ranged 2.00-3.50 mm in this study.

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Further at 39 % similarity coefficient, the seven major clusters, except cluster II were divided into sub-clusters. Cluster I (IA, IB and IC), cluster III (IIIA, IIIB, IIIC and IIID), cluster IV (IVA and IVB), cluster V (VA and VB), cluster VI (VIA and VIB) and cluster VII (VIIA, VIIB and VIIC). At 90 % similarity coefficient, only accession 56 (AFRK-5) and 67 (AFRK-5) both from Benin (Africa Rice) showed similarity.

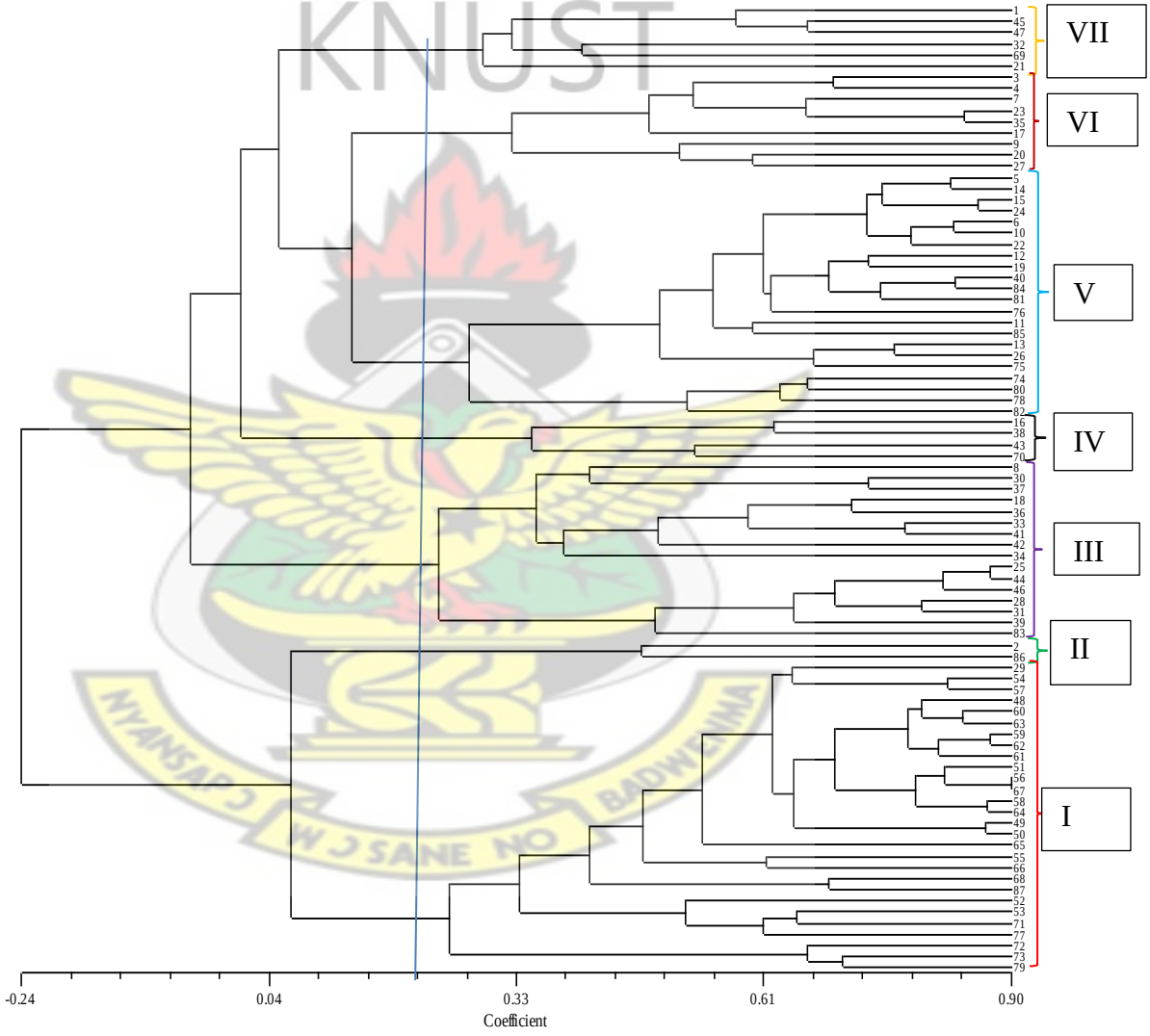

Figure 4.5: An UPGMA cluster dendrogram showing genetic relationships among 87 rice accessions based on morphological traits

Number follows details in Table 3.1

4.7 Genetic diversity assessment of rice accessions using SSR markers

A total of 12 SSR loci were evaluated for their efficiency of polymorphism across 87 accessions of rice received from different countries (Table 3.1). Among the 12 SSR primers used in this study, 6 yielded scorable amplification products (Table 4.5). Forty one alleles with a mean of 6.83 alleles per locus were obtained from these 6 SSR primers. These number of alleles per locus ranged from 2 [Xtxp 284 (1)] to 9 [Xtxp 10 (9)]. Allele frequency ranged from 0.31 to 0.73 with an average of 0.54. The locus Xtxp 149 (1) was the most informative since it had the highest level of polymorphism with PIC value of 0.79 and gene diversity value of 0.81. Xtxp 149 (1) also had the highest heterozygosity of 0.91 followed by Xtxp 201 (2) 0.87 and Xtxp 284 (1) scoring 0.00 heterozygosity.

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Table 4.5: Allele number, major allele frequency, gene diversity, heterozygosity and polymorphism information content (PIC) values generated from six SSR molecular markers

Marker	Allele No.	Major allele frequency	Gene diversity	Heterozygosity	PIC
Xtxp 149 (1)	8.00	0.31	0.81	0.91	0.79
Xtxp 284 (1)	2.00	0.69	0.43	0.00	0.34
Xtxp 201 (2)	8.00	0.52	0.61	0.87	0.55
Xtxp 197 (2)	8.00	0.73	0.44	0.27	0.43
Xtxp 278 (7)	6.00	0.54	0.57	0.31	0.50
Xtxp 10 (9)	9.00	0.47	0.71	0.42	0.68
Mean	6.83	0.54	0.60	0.46	0.55

4.8 Clustering of rice accessions based on SSR primers

Genetic relationship revealed by 6 SSR primers using similarity coefficients based on unweighted pair group method with arithmetic mean (UPGMA) is shown in Figure 4.6. From the figure, the 87 rice accessions were clustered into four major groups at 41 % similarity coefficient. Cluster I contained 10 accessions from Benin (Africa rice), Thailand, Mali and Philippines (IRRI) which are late maturing, cluster II 6 accessions from Ghana (CSIR-SARI), Thailand and Mali, they have green basal leaf sheath colour and cluster IV 14 accessions from Benin (Africa rice), Ghana (CSIR-SARI and CRI), Mali, Thailand and Philippines (IRRI), they have short culm length and fewer tillers. Majority of the accessions (57) studied were grouped into cluster III, they are accessions with no awn and broad leaf width. This cluster contained accessions from all the six countries in which collections were done. Further at 58 % similarity coefficient, the four major clusters were divided into sub-clusters. Cluster I (IA and IB), cluster II (IIA and IIB), cluster III (IIIA, IIIB, IIIC, IIID, IIIE, IIIF and IIIG) and cluster IV (IVA, IVB and IVC). More than half of the accessions studied (49) from all six countries showed closest resemblance at a similarity coefficient of 88 %. Accession [73(APO) and 76(KALIAUS) from Philippines (IRRI)], [66 (AFRK-10) from Benin (Africa Rice) and 70(IR 81412-B-B-82-1) from Philippines (IRRI)], [35(DKA 4) and 36(DKA- M8) from Mali], [32(DK 3) from Mali and 70 (IR 55419-04) from Philippines (IRRI)], [1(WAB 2081-WAC B-TGR4-B) and 3 (WAB 2125-WAC B-1-TGR3-WAT B1) from Benin (Africa Rice)] and finally [80 (IR 74371-54-1-1), 83(WAY RAREM) and 84(VANDANA) all three from Philippines (IRRI)] showed 100 % similarity. The lowest genetic similarity of 0.00 % existed between [2(WAB 2125-WAC B-1-TGR3-WAT B1) and 54(AFRK-7)], [4(DKA-M2) and 50(CRI-45)], [4(DKA-M2) and 65(AFRK-1)], [6(JASMINE85)

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and 68(AFRK-4)], [6(FAROX 508-3-10-F43-1-1) and 68(AFRK-4)], [6(FAROX 508-3-10-F43-1-1) and 47(BASMATI 123)], [16(PERFUME IRRIGATED) and 47(BASMATI 123)], [18(LONG GRAIN ORDINARY 2) and 68(AFRK-4)], [19(EXBAIKA) and 68(AFRK-4)], [61(AFRK-11) and 79(IR 74371-46-1-1)], [53(NERICA 1) and 79(IR 74371-46-1-1)], [40(DKA-M9) and 68(AFRK-4)] and [27(GR 21) and 79(IR 74371-46-1-1)] (Appendix 4).

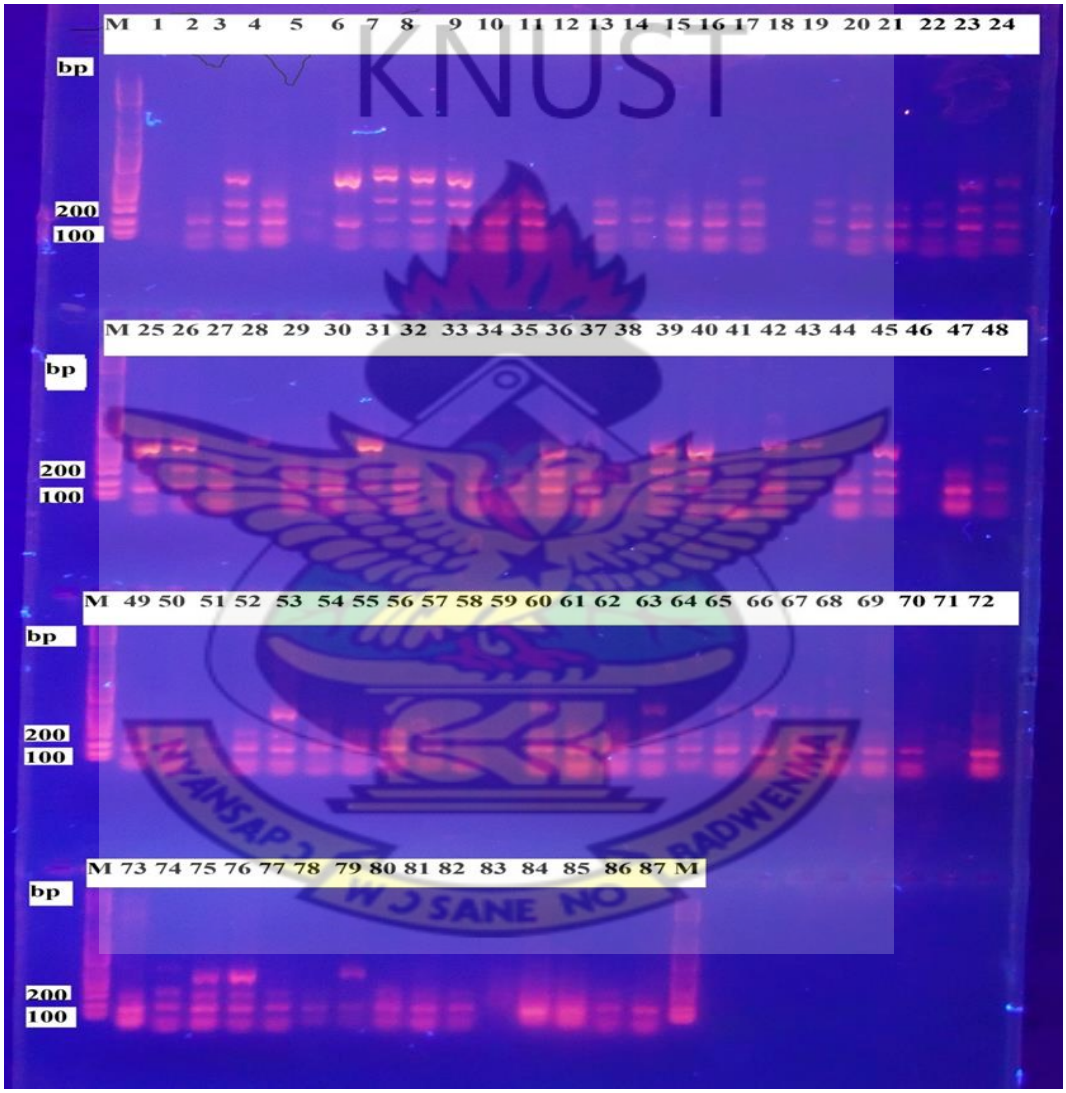

Plate 4.1: Representative SSR profiles of 87 rice accessions using primer Xtxp 149 (1)

M = Marker and number follows details in Table 3.1

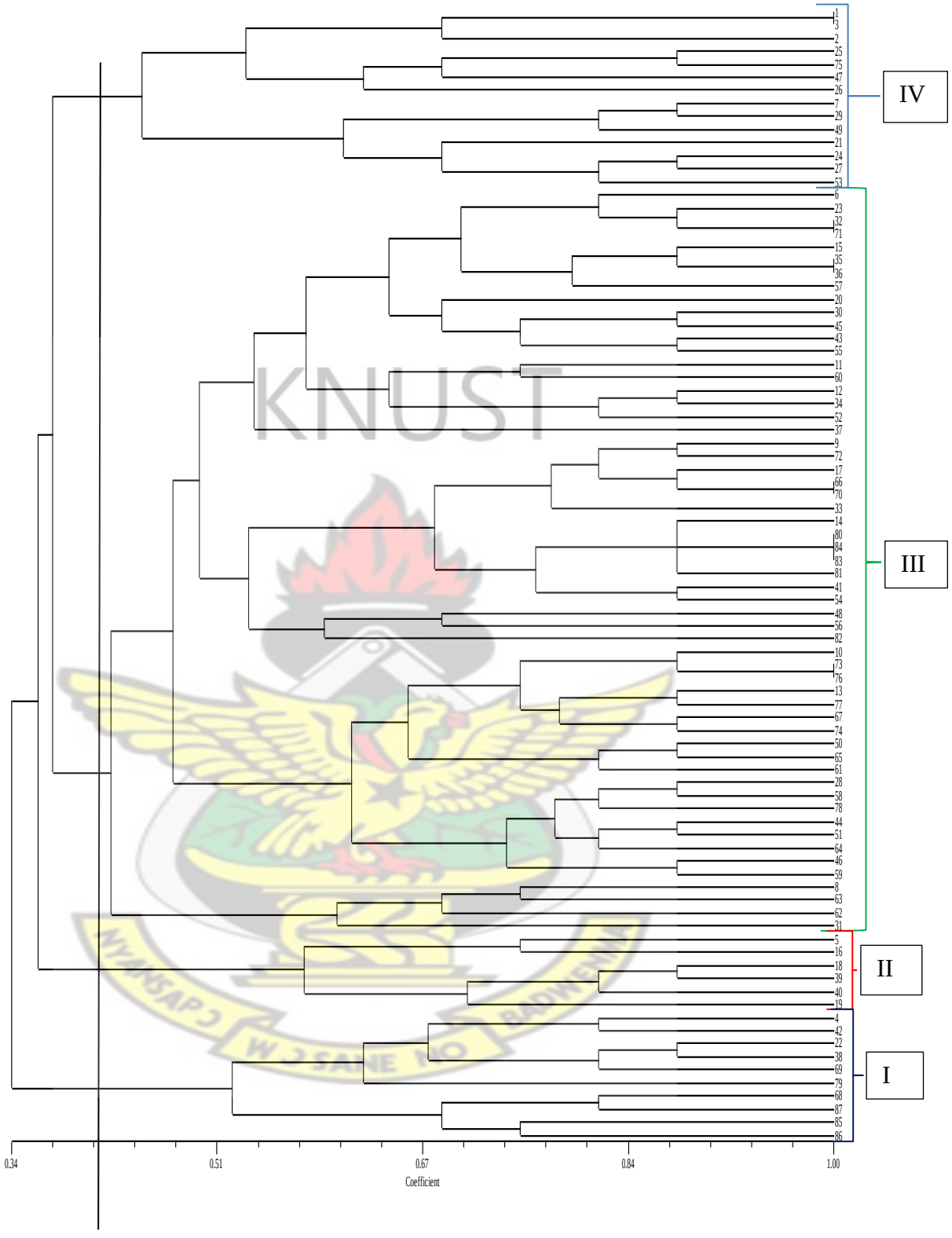

Figure 4.6: An UPGMA cluster dendrogram showing genetic relationships among 87 rice accessions based on six SSR markers

Number follows details in Table 3.1

CHAPTER FIVE

DISCUSSION

The major objective of any breeding programme is to produce quality and high yielding varieties for release to farmers. The requirement to achieving this goal is the presence of sufficient amount of genetic variability within accessions in which desirable lines would be selected for further genetic manipulation that leads to the achievement of targeted objectives. With the desire to have sufficient amount of variability within accessions, the introduction of new planting materials can be made from one country to another easily and may be used for further genetic manipulation to develop breeder lines.

This study was conducted to determine variation among the rice accessions using phenotypic traits, the relationship between morpho-agronomic traits and yield of the accessions and determine the magnitude of genetic variation using SSRs profiling of the accessions from different parts of the world (Ghana, Mali, Thailand, Cameroon, Benin and Philippines). The results from this study are discussed below.

5.1. Quantitative traits

5.1.1 Days to 50 % flowering

The highly significant genetic variation ($P < 0.001$) among the accessions for days to 50 % flowering in this study is similar to those previously reported by Weiya *et al.* (2008), they observed variation in days to flowering of several genotypes and identified a regulatory gene responsible for variation in this physiological trait among rice genotypes. Variation among accessions for this quantitative trait might be due to the genetic makeup of the accession or interaction with the environment. The availability of early flowering and maturing genotypes are important for the avoidance of drought condition.

5.1.2 Days to 80 % grain maturity

The number of days to maturity plays an important role in crop production. Early maturing crops allow multiple cropping of that crop within a year, escape pest and diseases and selection for areas with short rainy season in rain fed environment. Highly significant ($P < 0.001$) variation was also found among the studied genotypes for this physiological trait. Karim *et al*. (2007) studying 41 aromatic genotypes of rice for variability and genetic parameter analysis reported that, the variation within the genotypes for days to maturity was due to genetic makeup rather than environmental conditions. Short duration crops are important to breeders for use as parents in crop improvement.

5.1.3 Tiller number

Differences among the accessions were highly significant ($P < 0.001$) for tiller number. This finding is supported by Rahman *et al*. (2011). Tiller number per plant is an important component of yield in rice and mostly attracts breeders for selection as it correlates positively with grain yield (Table 4.4). Too few tillers result into too few panicles as they are directly related. In rice, the genetic manipulation of tiller number is important to obtain high grain yield, but the physiological basis of the regulation of tiller growth remains unclear.

5.1.4 Panicle number

Variability among the accessions was also highly significant ($P < 0.001$) for this quantitative trait based on the data analysis. This result is in agreement with earlier findings of Zahid *et al*. (2005), they studied twelve (12) genotypes of coarse rice and reported highly significant variation for various morphological traits including number of panicles per plant. Similar findings were also made by Hassan *et al*. (2003); they concluded that genetic variation was responsible for the significant

differences. This trait is an important yield component in rice and correlates positively with grain yield (Khan *et al*., 2009). The panicle number with filled grain determines the yield outcome of the crop. Thus, effective panicles should be once with high ripening percentage and high grain to straw ratio (Harvest index) for selection by breeders (Efisue *et al*., 2014).

5.1.5 Panicle length

Highly significant ($p < 0.001$) variation was also observed among the accessions studied regarding this trait. Significant and positive correlation existed between grain yield and panicle length in this study. Tahir *et al*. (2002) studying genetic variability for different agronomic traits in ten rice genotypes, found out that panicle length is under genetic control and could be selected for breeding purpose. Leaf area index, Photoperiod, sink and source relationship, competition among plant population and plant density contribute to variation among genotypes for this trait (Prakash *et al*., 2011). Exposure to unfavourable environmental condition, time of planting along interrelationship within various traits also contribution to variation among accessions for panicle length (Xue *et al*., 2008).

5.1.6 Plant height

Analysis of variance for plant height was found to be highly significant ($P < 0.001$) among the various accessions studied. This result is in conformity with those of Kole and Hasib (2008). Hussain *et al*. (2005) found out that planting and sowing methods, transplanting date and soil condition affect plant height in rice. Zahid *et al*. (2005) studied 12 genotypes of rice and reported high heritability and genetic advance for this trait and hence desirable trait for crosses. Reduction of plant height in rice may improve its resistance to lodging and eventually reduces yield loses due to this

condition (lodging). A number of short and intermediate height accessions were identified in this study that could be exploited for breeding purposes.

5.1.7 100 Grain weight

Abbasi *et al*. (1995) reported that 100 grain weight is another important yield attributing trait in rice. Highly significant ($P < 0.001$) variation was observed within the accessions studied for this trait. The genotype CRI-2 produced the minimum tiller number but obtained the highest 100 grain weight. N22 also produced the highest tiller number but the lowest 100 grain weight. These two genotypes can therefore be important parents for crosses. Result obtained from this study regarding 100 grain weight are similar to those of Ali *et al*. (2000), they found out that, difference in 100 grain weight among the accessions studied was due to grain size and shape.

5.1.8 Flag leaf length

The analysis of variance for flag leaf length showed highly significant ($P < 0.001$) difference among the accessions studied. This result is in conformity with earlier findings of Ghosh and Sharmam (2012), they studied various agro-morphological traits in rice and they concluded that, the variation was due to both genotype and environmental conditions. Flag leaf length is another important trait, as it is directly involved in photosynthetic activity.

5.1.9 Grain length and width

The analysis of variance showed highly significant ($P < 0.001$) difference for these two traits among the accessions studied in this experiment. This is supported by Bai *et al*. (2010), who studied genetic variability in rice genotypes for their grain quality and reported highly significant differences. Preference for rice grain quality varies among consumers, long and slender grain is generally preferred and is available from

this study that could be exploited to improve grain characteristics of local rice accessions. Genetic analyses of these two quantitative traits have been reported by many scientists and most have shown that grain shape of rice is quantitatively inherited (Zhang *et al*., 2005). Shi *et al*. (2005) showed that rice grain shape is controlled by triploid endosperm gene, cytoplasmic gene and genotype by environment interaction.

5.2 Influence of quantitative traits on total genetic variance as revealed by PCA

In genetic diversity studies using morphological traits, the most important variables describing phenotypic variation are defined by principal component (PC) analysis. Diversity studies using principal component analysis have been carried out in rice by several authors to understand and prioritize the most essential traits which explain much of the variability among the studied accessions (Guei *et al*., (2005). The PC analysis in this study showed that 75.01% of the total genetic variance encountered among the rice accessions was accounted for by the first five principal components taking into account all the 17 quantitative traits studied. The total genetic variance of 75.01 % by the first five components in this study was higher than what previously Bozokalfa *et al*. (2009) reported when accessing genetic variation among 434 landraces of rice for the first five components. In the present study, it can be deduced that days to 50 % flowering, 100 grain weight, culm length, panicle number, tiller number, leaf width, days to 80 % maturity, plant height, ligule length, leaf length, culm diameter, grain yield, grain length, flag leaf width and grain width were the most important traits which accounted for much of the variability among the rice genotypes. These findings agree with Caldo *et al.* (1996).

5.3 Qualitative traits

An important way of describing a plant is by its qualitative traits (Kurlovich, 1998). These characters in plant are mostly genetically controlled and thus less influenced by environmental conditions (Sarawgi *et al*., 2013). According to Hien *et al*. (2007), qualitative traits are mainly influenced by natural selection, socio economic scenarios and consumer preference. Significant portion of the accessions in this study had pubescent leaves. Rutger and Mackill (2001) reported that most rice cultivars around the world have pubescent leaves. This trait has been of little importance in agriculture because of its allergic reaction and itching effect to farmers. However, pubescent leaves provide resistance to biotic and abiotic stresses (Angeles-shim *et al*., 2012; Kim *et al*., 2011). It also produces higher yield (Figure 4.4). A significant portion of the accessions, 48 (55.17 %) showed acute to acuminate ligule shape. Ligules prevent water and insects from entering the leaf sheath (ANON, 2009). For character culm angle, 2 (2.30 %) had spreading culm. Spread culm angles are easily lodged especially during windy conditions resulting into yield loss. Also significant amount of the accessions studied 51 (58.62 %) had erect flag leaf. Erect and semi erect leaf accessions are generally associated with higher yield. Only 2 (2.30 %) of the accessions had partly exserted panicles. Panicles enclosed in leaf sheath consume time in harvesting or processing as the panicles have to be removed from the sheath. Significant amount of the accessions evaluated in this study had no awn 72 (82.76 %). Awn is considered nuisance by many farmers especially during milling, but this trait scare away birds. Sarawgi *et al*. (2013) reported similar qualitative traits variation in rice genotypes.

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5.4 Correlation analysis of some quantitative traits with rice grain yield

Correlation analysis is a measure of the strength of association between two characters (traits). Morphological and physiological traits analysis in plant through correlation can be used as tool for indirect selection. Correlation studies assist plant breeders at the time of selection and provide valuable information about yield and yield related traits. Selection in plant breeding holds major importance in the development of any crop and knowledge about the criteria used for selection of any crop is an asset. Breeders must always have a target in mind before proceeding towards improvement of any crop through selection.

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Correlation analyses revealed that, out of the fourteen (14) morphological and physiological characters only 100 grain weight, tiller number, days to 50 % flowering, panicle number, days to 80% grain maturity, ligule length and panicle length showed significant positive association with grain yield. This suggest that they could be used to predict grain yield. Dourosti (2005) studying 64 lines of rice genotypes reported similar significant positive correlation of panicle length and total tiller number with grain yield. The significant positive correlation of 100 grain weight with grain yield is in agreement with the earlier findings of Rahim (2005). Also, the significant positive relation between grain yield and panicle number are in conformity with the findings of Senapati *et al*. (2009). Results obtained in this study regarding significant positive relationship of grain yield with days to 50 % flowering and 80 % grain maturity are contrary to the findings of Ashfaq *et al*. (2012), they revealed non-significant positive relation for these two traits with rice grain yield, while Maji and Shaibu (2012) also reported non-significant positive correlation between ligule length and grain yield. The contrary result obtained in this study is due to the variation in genotype or genotype by environment interaction. Traits that

had significant positive correlation with grain yield would be ideal for selection to improve rice grain yield. Regarding plant height, non-significant negative association was obtained with grain yield. Senapati *et al*. (2009) reported similar result. Hairmansis *et al*. (2010) studying agronomic characters and grain yield also reported similar result. Mohaddesi *et al*. (2010) obtained significant positive correlation of plant height with grain yield. These differences in result might be due to the genotypes or interaction with environment. According to Singh *et al*. (2000), high yielding types of rice should be of short stature. The non-significant positive association between grain yield and grain width in this study are in conformity with earlier findings of Mirhoseini *et al*. (2013).

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5.5 Genetic relationship based on morphological descriptors

Cluster analysis is very useful in revealing complex relationships among populations of diverse origins in a more simplified manner. It is also effective in indicating accessions with useful traits belonging to different clusters for hybridization. The 87 accessions from six countries in the study were classified into seven main clusters at a similarity coefficient of 0.21 based on morphological traits, which is an indication of genetic variation among the accessions. The variation observed among the accessions suggests that morphological traits can reveal diversity existing among rice accessions. This is in agreement with earlier findings of Efisue *et al.* (2014), they reported that morphological traits can discriminate between rice accessions.

5.6 Genetic diversity studies of rice using SSR markers

Success of rice improvement programmes depends on the amount of genetic variability and the degree to which the desirable traits are heritable (Ravi *et al*., 2003). Hence assessment of genetic variability among genotypes becomes important in establishing relationships among different cultivars. Characterization using

molecular markers is the alternative strategy to overcome the several limitations of morpho-agronomic traits characterization of genetic materials, which are expensive, environmental effects and long evaluation time. In the present investigation, twelve SSR markers were used to characterize and assess the genetic variability among 87 rice accessions collected from six countries. Only six out of these 12 microsatellite markers revealed genetic polymorphism and ensured unambiguous identification of the rice accessions. Small numbers of molecular markers can be used to assess genetic diversity as shown earlier in other studies. Ali *et al*. (2011) reported that a subset of 36 microsatellite markers gave nearly similar results as using 169 SSR markers for genetic diversity studies. These six SSR primers yielded a total of 41 alleles ranging from 2 [Xtxp 284 (1)] to 9 [Xtxp 10 (9)] with an average of 6.83 alleles per locus and were similar to those earlier reported by Ni *et al*. (2002). They used Indian quality rice germplasm and reported an average of 6.80 alleles per locus. The number of alleles detected in the present study are lower than those observed by Chakhonkaen *et al*. (2012), who reported a total of 127 alleles that ranged from 4-12 alleles using 19 InDel (Insertion-Deletion) markers to evaluate genetic diversity in 101 rice accessions. Expected heterozygosity ranged from 0.00 to 0.91 with an average of 0.46. These results are congruent with Chakhonkaen *et al*. (2012), who obtained expected heterozygosity average of 0.42 that ranged from 0.13- 0.76. The average genetic diversity of 0.60 obtained was higher compared to 0.55 previously reported by Sajib *et al*. (2012), they used 9 SSR markers to study genetic diversity among 12 aromatic landraces of rice. Polymorphism information content (PIC), is a measure of polymorphism among varieties for a marker locus used in linkage analysis (Sajib *et al*., 2012). It ranged from 0.34-0.79 with an average of 0.55 in this study. The PIC range and average observed in this study are similar to those reported

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earlier by Meti *et al*. (2014), they reported PIC range of 0-0.74 with an average of 0.58 using 12 SSR markers to estimate genetic diversity in 48 aromatic rice genotypes. Higher values of PIC might be the result of diverse genotypes and lower values may be the result of closely related genotypes (Prabakaran *et al*., 2010).

The dendrogram showed that there was genetic variation among the 87 rice accessions in relation to the SSR primers used. The similarity coefficient of these accessions ranged from 0.34-1.00, which is an indication of the genetic variation among the accessions based on the SSR primers. The variation observed among the accessions is an indication that SSR markers can reveal diversity existing between rice accessions. This is in agreement with earlier findings of Pervaiz *et al*. (2010), they reported that SSR markers are effective tools in discriminating rice genotypes. The accessions were grouped into four main clusters at a genetic similarity coefficient of 0.41. Also at a similarity coefficient of 0.58, each of the four main clusters were divided into sub-clusters. Accession 73 (APO) and 76 (KALIAUS) both from Philippines (IRRI) showed 100 % similarity revealing that no genetic variability exist between these two accessions based on the 6 SSR primers. The similarity could have arisen from informal exchange of seeds (germplasm) among farmers but given different names because of differences in dialect and ethnic groups. It is important to eliminate duplicates to enable effective management and conservation of germplasm.

Broadening the genetic base of rice in breeding programmes is urgently needed to enhance heterozygosity in crosses and create heterotic progenies. The lowest genetic similarity of 0.00 % that existed between [2(WAB 2125-WAC B-1-TGR3-WAT B1) and 54(AFRK-7)], [4(DKA-M2) and 50(CRI-45)], [4(DKA-M2) and 65(AFRK-1)], [6(JASMINE85) and 68(AFRK-4)], [6(FAROX 508-3-10-F43-1-1) and 68(AFRK-

4)], [6(FAROX 508-3-10-F43-1-1) and 47(BASMATI 123)], [16(PERFUME IRRIGATED) and 47(BASMATI 123)], [18(LONG GRAIN ORDINARY 2) and 68(AFRK-4)], [19(EXBAIKA) and 68(AFRK-4)], [61(AFRK-11) and 79(IR 74371-46-1-1)], [53(NERICA 1) and 79(IR 74371-46-1-1)], [40(DKA-M9) and 68(AFRK-4)] and [27(GR 21) and 79(IR 74371-46-1-1)] (Appendix 4) revealed that, they are good parental lines for breeding.

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CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study revealed important information about the 87accessions as outlined:

- Results obtained indicate highly significant variation among the accessions based on the various morphological and physiological quantitative traits studied.
- The first five principal components accounted for 75.01 % of the total genetic variance among the accessions.
- Differences were also observed among the accessions based on qualitative traits. 82.76 % of the accessions possessed no awn, 41.38 % moderately well panicles exsertion, 45.98 % horizontal panicle branches, 58.62 % erect flag leaf, 47.13 % semi erect culm angle, 75.86 % whitish ligule colour, 55.17 % acute to acuminate ligule shape, 42. 53 % yellowish green auricle colour, 65.52 % intermediate leaf blade pubescent, 47.13 % medium green leaf blade: intensity of green colour and 65.52 % green basal leaf sheath colour.
- Quantitative traits: 100 grain weight, tiller number, days to 50 % flowering, days to 80 % grain maturity, panicle number, panicle length and ligule length showed significant positive association with grain yield, while flag leaf width had significant negative correlation with grain yield. Culm length, flag leaf length, leaf length of blade and grain width showed non-significant positive association with grain yield.
- At 21 % similarity coefficient, the 87 accessions from six countries were grouped into seven clusters based on the morphological traits.

- Only six out of the twelve SSR primers used to assess genetic diversity in the 87 rice accessions yielded scorable amplification products.
- A total of 41 alleles with mean PIC of 0.55 was obtained from the molecular analysis showing the informative nature of the six SSR primers and their superiority in genetic diversity assessment among rice accessions.
- At 41 % similarity coefficient, the 87 accessions from six countries were grouped into four clusters based on the SSR primers.
- The results obtained from this study has proven that both morphological and SSR markers are effective tools in assessing genetic diversity in rice.

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6.2 Recommendations

- Accessions which showed desirable quantitative and qualitative traits in this study should be used for further rice improvement programmes by breeders.
- Breeders should also pay more attention on traits that showed significant positive association with grain yield. From this study, it is observed that accessions with higher tiller numbers could yield more by a corresponding higher number of productive tillers.
- More SSR markers should be used to established genetic diversity among rice accessions than used in the present study.
- Breeding programme should commence with accession 1(WAB 2081-WAC B-TGR4-B) with grain yield of 10.55 t/ha and 74(N22) with grain yield of 3.55 t/ha but short both been genetically diverse as revealed by the morphological and molecular markers

REFERENCES

Abbasi, F.M., Sagar, M.A., Akram, M. and Ashraf, M. (1995). Agronomic and quality traits of some elite rice genotypes. *Pakistan Journal of Scientific and Industrial Research* 38: 348-350.

Ali, M.L., McClung, A.M., Jia, M.H., Kimball, J.A., McCouch, S.R. and Georgia, C.E. (2011). A rice diversity panel evaluated for genetic and agro-morphological diversity between subpopulations and its geographic distribution. *Crop Sci.* 51: 2021-2035.

Ali, S.S., Jafri, S.J.H., Khan, T.Z., Mahmood, A. and Butt, M.A. (2000). Heritability of yield and yield components of rice. *Pakistan Journal of Agricultural Research* 16: 89-91.

Angeles-Shim, R.B., Asano, K., Takashi, T., Shim, J., Kuroha, T., Ayano, M. and Ashikari, M. (2012). A WUSCHEL-related homeo-box 3B gene, depilous (dep), confers glabrousness of rice leaves and glumes. *Journal of Rice* 5: 28-39.

Anjan, K.S. and Mishra, P.K. (2013). Agro-morphological characterization and morphology based genetic diversity analysis of landraces of rice variety (*Oryza sativa* L.) of Bankura district of west Bengal. *International Journal of Current Research* 5: 2764-2769.

Anonymous. (2009). Available at http://en.wikipedia.org//wiki//Ligule. [Accessed 2015 January 17].

Aremu, C.O., Adebayo, M.A., Oyegunle, M. and Ariyo, J.O. (2007). The relative discriminatory abilities measuring genotype by environment interaction in soybean (Glycine max). *Agricultural journal* 2 (2): 210-215.

Asch, F., Becker, M. and Kpongor, D.S. (2005). A quick and efficient screen for resistance to iron toxicity in lowland rice. *J. Plant Nutri. Soil Sci*. 168: 764-773.

Ashfaq, M., Khan, A.S., Khan, S.H.U. and Ahmad, R. (2012). Association of various morphological traits with yield and genetic divergence in rice (*Oryza sativa* L.). *Int. J. Agric. Biol*. 14 (1): 201-231.

Ashura, L.K. (1998). Inter–relationship between yield and some selected agronomic characters of rice. *Africa Crop Science Journal* 6 (3): 323-328.

Bai, X.L., Luo, W. Yan, M.R., Kovi, W., Zhang, H. and Xing, Y. (2010). Genetic dissection of rice grain shape using a recombinant inbred line population derived from two contrasting parents and fine mapping, a pleiotropic quantitative trait locus qGL7. *Journal of Genetics* 11: 16.

Barreiro, L.B., Laval, G., Quach, H., Patin, E. and Quintana-Murci, L. (2008). Natural selection has driven population differentiation in modern humans. *Nature Genetics* 40: 340-345.

Bozokalfa, M.K., Eşiyok, D. and Turhan, K. (2009). Patterns of phenotypic variation in a germplasm collection of rice (*Capsicum annuum*) from Turkey. *Spanish Journal of Agricultural Research*, 7(1): 83-95.

Brar, D.S. and Khush, G.S. (2003). Utilization of wild species of genus *Oryza* in rice improvement. **In**: Nanda, J.S. and Sharma, S.D. (Eds.). Monograph on genus *Oryza*. American Society of Agronomy, Madison, Wisconsin, USA. 283-309.

Brown, A.H.D. (1989). Core collections: A practical approach to genetic resources management. *Journal of Genome* 31: 818-824.

Brown, A.H.D. (2008). Indicators of genetic diversity, genetic erosion and genetic vulnerability for plant genetic resources. Thematic background study, state of worlds plant genetic resources. Food & Agriculture Organization, Rome, Italy. 278-290.

Bui, C.B and Nguyen, T.L. (1999). Using molecular markers in study of rice genetic diversity. *Omonrice* 7: 15-26.

KNUST

Caetano-Anolles, G., Bassam, G.J. and Gressh, P.M. (1991). High resolution DNA amplification fingerprinting using short arbitrary oligonucleotide primers. *Biotechnology* 9: 553-556.

Caldo, R.A., Sebastian, L.S. and Hernandez, J.E. (1996). Morphology-based genetic diversity analysis of ancestral lines of rice in Philippine rice cultivars. *Philippines Journal of Crop Science* 21 (3): 86-92.

Chakhonkaen, S., Pitnjam, K., Saisuk, W., Ukoskit, K. and Muangprom, A. (2012). Genetic structure of Thai rice and rice accessions obtained from the International Rice Research Institute. *Rice* (*A springer open Journal*) 5: 19.

Chambers, M. and Avoy, M. (2000). Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. *Mol. Ecol*. 9: 1037-1048.

Chandler, Jr. R.F. (1969). Plant morphology and stand geometry in relation to nitrogen. **In**: Eastin, J.D., Haskins, F.A., Sullivan, C.Y. and Bavel, C.H.M. (Eds.). Physiological aspects of crop yield. American Society of Agronomy, Madison, Wisconsin, USA. 265-285.

Chen, W., Xu, Z. and Zhang, L. (1995). Physiological bases of super high yield breeding in rice. Liaoning Science and Technology Publishing Company, Shenyang, China. 1-2.

Cherif, M., Audebert, A., Fofana, M. and Zouzou, M. (2009). Evaluation of iron toxicity on lowland irrigated rice in West Africa. *Tropicultra* 88-92.

Chipili, J., Sreenivasprasad, S., Nutsugah, S.K., Twumasi, J.K. and Dartey, K. (2003). Rice blast management in Ghana: Characterization of the pathogen diversity. *Journal of Ghana Science Association* 5: 20-25.

Counce, P.A., Siebenmorgen, T.J, Poag, M.A., Holloway, G.E., Kocher, M.F, and Lu, R.F. (1996). Panicle emergence of tiller types and grain yield of tiller order for direct seeded rice cultivars. *Field Crops Research* 47: 235-242.

Davood, B.T., Ali, G., Hemmal, A.P. and Morteza, N. (2009). Flag leaf morph-physiological response to different agronomical treatment in a promising

line of rice (*Oryza sativa* L.). *American-Eurasian J. Agric and Environ. Sci*. 5 (3): 403-408.

DeVries, J.D. and Toenniessen, G. (2001). Rice's. **In**: Toriyama, K., Heong K.L, and Hardy, B. (Eds.). Securing the harvest biotechnology breeding and seed systems for African Crops. CABI Publishing, Wallingford, USA. 131-138.

Dias, G.B., Gomes, V.M., Moraes, T.M.S., Zottich, U.P., Rabelo, G.R., Carvalho, A.O., Moulin, M., Goncalves, L.S.A., Rodrigues, R. and Cunha, M. (2013). Characterization of capsicum species using anatomical and molecular data. *Genetics and Molecular Research* 4 (2): 1-14.

Dourosti, H. (2005). Assessment of genetic diversity based on agronomic properties of rice promising lines. M.Sc. thesis, Islamic Azad University, Karaj Branch, Iran. 67-69.

Doyle, J.J. and Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 12-15.

Duan, C.R., Wang, B.C, Wang, P.Q., Wang, D.H., and Cai, S.X. (2004). Relationship between the minute structure and the lodging resistance of rice stems. *Colloid Surface B* 35: 155-158.

Efisue, A.A., Umunna, B.C. and Orluchukwu, J.A. (2014). Effect of yield components on yield potential of some lowland rice (*Oryza sativa* L.) in coastal region of Southern Nigeria. *Journal of plant* Breeding *and Crop science* 6 (9): 119-127.

Fan, G., Dong, Y., Wang C., Wan, J., Xie, H., Xu, C., Zhu, J. and Cai, Q. (2007). Analysis of QTLs for Flag leaf shape and its response to elevated CO_2 in Rice (*Oryza sativa* L.). *Elsevier BV* 6: 216-231.

FAO**. (**1996). Food and Agricultural Organisation. Production year book. Food and Agricultural Organisation of the United Nations, Rome, Italy. 12-25.

FAO. (2000). Rice Information. Food and Agricultural Organization of the United Nations, Rome, Italy. 2: 20-27.

FAO. (2002). Food and Agricultural Organisation. Recommendations for sustainable and effective use of rice genetic resources and improvement at national, regional and global level. **Proc**. of the 20th Session of the International rice commission, 23–26 July 2002, Bangkok, Thailand.

FAO. (2003). Food and Agricultural Organisation. Sustainable rice production for food security. Food and Agriculture Organization of the United Nations, Rome, Italy. 23-45.

FAO. (2006). Food and Agricultural Organisation. Rice is life. Available at www fao.org//newsroom//en//news//2006//index. [Accessed 2015 January 15].

FAO. (2008). Food and Agricultural Organisation. Rice marketing monitor, European commission's evaluation of the impact of rice sector reforms. agritrade.cta.int>HomeCommodities>Ricesector. [Accessed 2015 January 15].

FAOSTAT. (2012). Food and Agricultural Organization of the United Nations, Rome, Italy, Statistical Data Base. http://faostat.fao.org. [Accessed 2015 January 12].

Faruq, G.Y., Hui, Y.A., Masitah, N., Afnierna, N.A.M., Norzulaani, K. and Mohamad, O. (2011). Analysis of aroma and yield components of aromatic rice in Malaysian tropical environment. *Australian Journal of Crop Science* 5 (11): 1318-1325.

Feil, B. (1992). Breeding progress in small grain cereals: A comparison of old and modern cultivars. *Plant Breeds* 108: 1-11.

Florent, T., Beatriz, D.S.P., Marcel D.R. and Michael, D. (2001). Leaf blade dimensions of rice (*Oryza sativa* L. and *Oryza glaberrima* Steudl) relationships between tillers and the main stem. *Ann. Bot*. 88: 507-511.

Ghneim, H., Thaura, P.D.D., Pérez, A.I., Torrealba, N.G., Pieters, A.J., Martinez, C.P. and Tohme, J.M. (2008). Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. *Electronic Journal of Biotechnology* 11 (5): 116-121.

Ghosh, S.C. and Sharma, D. (2012) Genetic parameters of agro-morpho-physiological traits in rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding* 3 (1): 711-714.

Gregory, P.J., Ingram, J.S. and Kobayashi, T. (2000). Rice production and global change. *Global Environmental Research* 2: 71-77.

Guei, R.G., Sanni, K.A., Abamu, F.J. and Fawole, I. (2005). Genetic diversity of rice (*Oryza sativa* L.). *Agronomie Africaine* 5: 17-28.

Hairmansis, A., Kustianto, B., Supartopo, F. and Suwarno, S. (2010). Correlation analysis of agronomic characters and grain yield of rice for tidal swamp areas. *Indonessian Journal of Agricultural Science* 11 (1): 11-15.

Hajeer, A., Worthington, J. and John, S. (2000). SNP and microsatellite genotyping: Markers for genetic analysis. Biotechniques: Molecular laboratory methods series. Eaton Publishing, Manchester, UK. 2301-2310.

Hamilton, R.S. and Raymond, R. (2005). Toward global strategy for the conservation of rice genetic resources. **In**: Toriyama, K., Heong, K.L. and Hardy, B. (Eds.). Rice is life: Scientific perspectives for the 21st century. **Proc**. of the World rice research conference held in Tsukuba, 2-3 May, 2005, Japan.

Harris, D.R. (1996). The origins and spread of agriculture and pastoralism in Eurasia. *Psychology Research* 3: 565-574.

Hassan, G., Khan, N.U. and Khan, Q.N. (2003). Effect of transplanting date on the yield and yield components of different rice cultivars under high temperature of D.I. Khan. *Sci. Khy*. 16: 129-137.

Hien, N.L., Sarhadi, W.A., Hirata, Y. and Oikawa, Y. (2007). Genetic diversity of morphological responses and the relation among Asia aromatic rice (*Oryza sativa* L.) cultivars. *Tropics* 16: 343-355.

Hirose, T., Ohdan, T., Nakamura, Y. and Terao, T. (2006). Expression profiling of genes related to starch synthesis in rice leaf sheaths during the heading period. *Physiology of Plant* 128: 425-435.

Hirota, O., Oka, M. and Takeda, T. (1990). Sink activity estimation by sink size and dry matter increase during the ripening stage of barley (*Hordeum vulgare*) and rice (*Oryza sativa* L.). *Annals of Botany* 65 (4): 349–354.

Hore, D.K. (2005). Rice diversity collection, conservation and management in north eastern India. *Genetic Resources and Crop Evolution* 528: 1129–1140.

Huang, X., Kurata, N., Wei, X., Wang, Z., Wang, A., Zhao, Q., Zhao, Y. and Liu, K. (2012). A map of rice genome variation reveals the origin of cultivated rice. *Nature* 490 (7421): 497-501.

Hussain, S.M., Ramzan, M., Aslam, Z.M. and Ehsan, S.M. (2005). Effect of various stand establishment method on yield and yield components of rice. **Proc**. of the International seminar on rice crop, Rice Research Institute, 2-3 October, 2005, Kala Shah Kau, Pakistan.

Ininda, J. (2008). New high yield rice breed to cut rice importation. http://allafrica.com//stories//200806180503.html. [Accessed 2015 January 15].

IBPGR and Cornell University. (2003). Use of molecular markers in diversity studies. www.IPGRIandCornellUniversity.Info.org. [Accessed 2015 May 9].

IRRI. (2010). Annual Report for 2009. International Rice Research Institute, Los Banos, Laguna, Philippines. 179-181.

IRRI. (2013). International Rice Research Institute. Rice knowledge bank. Knowledge bank. www.irri.org. [Accessed 2015 May 8].

Ishi, T., Xu, Y. and Mccounch, S.R. (2001). Nuclear and chloroplast microsatellite variation in A-genome species of rice. *Journal of Genome* 44: 658-666.

Ishwaran, N. and Erdelen, W. (2005). Biodiversity futures. *Frontiers in Ecology and the Environment* 3 (4): 179-180.

Jayamani, P., Negrão, S., Martins, M., Maçãs, B. and Oliveira, M.M. (2007). Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. *Crop Science* 47 (2): 879-886.

KNUST

Jennings, P.R., Coffman, W.R. and Kauffman, H.E. (1979). Rice improvement. International Rice Institute, Manila, Philippines.15-35.

Jianguo, G., Wu, C.S. and Xiaoming, Z. (2003). Estimating the amino acid composition in milled rice by near-infrared reflectance spectroscopy. *Field Crops Research* 75: 1.

Juneja, H., Inagaki, Y.A. and Fujimura, T. (2006). Highly polymorphic microsatellites of rice consist of AT repeats and a classification of closely related cultivars with these microsatellite loci. *Theor. Appl. Genet*. 94: 61-67.

Karim, D.U., Sarkar, M.N.A., Siddique, M.A., Khaleque, M. and Hasnat, M.Z. (2007). Variability and genetic parameter analysis in aromatic rice. *International Journal of Sustainable Crop Production* 2 (5): 15-18.

Kashiwagi, T., Sasaki, H. and Ishimaru, K. (2005). Factors responsible for decreasing sturdiness of the lower part in lodging of rice (*Oryza sativa* L.). *Journal of Plant Production* 2: 166-172.

Kashiwagi, T., Togawa, E., Hirotsu, N. and Ishimaru, K. (2008). Improvement of lodging resistance with QTLs for stem diameter in rice (*Oryza sativa* L.). *Theor. Appl. Genet*. 117: 749-757.

Keisuke, N., Shigenori, M. and Tadashi, B. (1995). Shoot and root development in rice related to the phyllochron. *Journal of Crop Science* 35: 24-29.

Khan, A.S., Imran, M. and Ashfaq, M. (2009). Estimation of genetic variability and correlation for grain yield components in rice (*Oryza sativa* L.). *Amercian-Eurasian Journal of Agriculture and Environmental Science* 6 (5): 585-590.

Khush, G.S. (2003). Productivity improvement in rice. *Nutr. Res*. 61: 114-116.

Kim, H.J., Han, J.H., Kim, S., Lee, H.L., Shin, J.S., Kim, J.H., Cho, J., Kim, Y.H., Lee, H.J., Kim, B.D. and Choi, D. (2011). Tri-chome density of main stem is tightly linked to PepMoV resistance in chili pepper (*Capsicum annuum* L.). *Theor. Appl. Genet*. 122: 1051-1058.

KNUST

Kole, P.C. and Hasib, K.M. (2008). Correlation and regression analysis in scented rice. *Madras Agriculture Journal* 95 (1-6): 178-182.

Kono, M., Matsuo, T., Kumazawa, K., Ishii, R., Ishihara, K. and Hirata, H. (1995). Physiological aspects of lodging, science of the rice plant physiology. *J. Food and Agriculture* 2: 971-982.

Kormawa, P.M, Keya, S. and Touré, A.A. (2004). Rice research and production in Africa. CRC Press, Marseille, France. 29-30.

Krishna, T. and Nidhi, K. (2014). To study the agro morphological variation and genetic variability in rice germplasm. *Middle-East Journal of Scientific Research* 20 (2): 218-224.

Kumar, S.T., Narasimman, R., Eswaran, R., Kumar, C.P.S. and Anandan, A. (2007). Studies on genetic variability, heritability and genetic advance in segregating generations of rice (*Oryza sativa* L.). *International Journal of Plant Science, Muzaffarnagar* 2 (1): 48-51.

Kunateh, M.A. (2009). Aveyime Rice project to produce 2000 mt of rice this year. http://www.ghanadot.com//news.ghanadotKunateh071409E.html. [Accessed 2015 January 16].

Kurlovich, B.S. (1998). Species and intraspecific diversity of white, blue and yellow lupins. *Plant genetic Resource* 115: 23-32.

Lafarge, T.A., Broad, I.J. and Hammer, G.L. (2002). Tillering in grain sorghum over a wide range of population densities: Identification of a common hierarchy for tiller emergence, leaf area development and fertility. *Ann. Bot.* 90: 87-98.

Lang, Y., Yang, X., Wang, M. and Zhu, Q. (2012). Effects of lodging at different filling stages on rice yield and grain quality. *Rice Science* 19 (4): 315-319.

Latif, M., Rahman, M., Kabir, M., Ali, M., Islam, M. and Rafii, M. (2011). Genetic diversity analyzed by quantitative traits among rice (*Oryza sativa* L.) genotypes resistant to blast disease. *Afr. J. Microbiol. Res.* 5: 4383-4391.

Linares, O.F. (2002). The history and future of African rice. www.jstor.org//stable 40175114. [Accessed 2015 January 10].

Liu, K. and Muse, S.V. (2005). PowerMarker: An intregrated analysis environment for genetic marker analysis. *Bioinformatics* 21 (9): 2128-2129.

MacNeish, R.S. and Libby, F. (1995). Origins of rice agriculture. *Journal of education* 5 (13): 57-63.

Mahapatra, K.C. (1993). Relative usefulness of stability parameters in assessing adaptability in rice. *Indian Journal of Genetics and Plant Breeding* 53 (4): 435-441.

Mahbub, M.A.A., Khanam, M., Rahman, M.S., Hossain, M.A., and Gomosta, A.R. (2006). Determination of lodging characters of some BRRI recommended rice varieties at three nitrogen levels during wet season in Bangladesh. *Bangladesh J. Bot*. 35 (2): 117-124.

Maji, A.T. and Shaibu, A.A. (2012). Application of principal component analysis for rice germplasm characterization and evaluation. *Journal, Plant Breed of Crop Science* 4 (6): 87-93.

Mammadov, J., Aggarwal, R., Buyyarapu, R. and Kumpatla, S. (2012). SNP markers and their impact on plant breeding. *International Journal of Plant Genomics* 6 (10): 96-118.

Matsuda, T., Kawahara, H. and Chonan, N. (1983). Histological studies on breaking resistance of lower internodes in rice culm. The roles of each tissue of internode and leaf sheath in breaking resistance. *Crop Science Journal of Japan* 52: 355-361.

Mccouch, S.R., Teytelman, L., XU, Y., Lobos, K.B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., Xing, Y.Z.Q., Kono, I.Y.M., Fjellstrom, R., Declerck, G., Schneider, D., Cartinhour, S., Ware, D. and Stein, L. (2002). Development of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA *Research* 9 (6): 199-207.

KNUST

Melchinger, A.E. (1999). Genetic diversity and heterosis. **In**: Coors, J.G. and Pandey, S. (Eds.). The genetics and exploitation of heterosis in Crops. American Society of Agronomy, Crop Science Society of America, Soils Science Society of America, Madison, Wisconsin, USA. 99-118.

Merriam-Webster. (1991).Webster's ninth new collegiate dictionary. Merriam-Webster Inc., publishers, Springfield, Massachusetts, USA. 81.

Meti, N., Samal, K.C., Bastia, D.N. and Rout, G.R. (2014). Genetic diversity analysis in aromatic rice genotypes using microsatellite based simple sequence repeats (SSR) marker. *African Journal of Biotechnology* 12 (27): 4238-4250.

Miller, B.C., Hill, J.E. and Robert, S.R. (1991). Plant population effects on growth and yield in water seeded rice. *Agron. J*. 83: 291-297.

Mirhoseini, S.M., Daliri, M.S., Moghaddam, M.N., Mohaddesi, A. and Abbasian, A. (2013). Study of agronomic traits in a number of promising rice lines by multivariate statistical methods. *International Journal of Bioscience* 3 (7): 119-125.

Missihoun, A.H., Adoukonou-Sagbadja, H., Sedah, P., Dagba, R.A., Ahanhazo, C. and Agbangla, C. (2015). Genetic diversity of sorghum *bicolor* (L) Moench land race from Northwestern Benin as revealed by microsatellite markers. *African Journal of Biotechnology* 14 (6): 1342-1353.

MoFA. (2009). National rice development strategy, Accra, Ghana. **Proc**. of the Introduction of Strategy development Partners in Ghana, 23rd April, 2009, Accra. jica.go.j//activities//issues//agricul//pdf. [Accessed 2015 January 16].

Mohaddesi, A., Abbasian, A., Bakhshipoor, S. and Mohammad, S.M. (2010). Study of effects of nitrogen fertilizer and planting distance on yield and yield components of promising rice line. *Journal of crop Ecophysiology* 2 (3): 198-203.

Mojith, A., Itsuro, T. and Kiyoaki, K. (2011). Plant architecture and its responses to high planting density and low fertilizer of reduced culm number mutants in

KNUST

rice (*Oryza sativa* L.). *Journal of Plant Breeding and Crop Science* 3 (7): 114-119.

Molina, J., Sikora, M., Garud, N., Flowers, J.M., Rubinstein, S., Reynolds, A., Huang, P., Jackson, S., Schaal, B.A., Bustamante, C.D., Boyko, A.R. and Purugganan, M.D. (2011). Molecular evidence for a single evolutionary origin of domesticated rice. *National Academy of Sciences* 108 (20): 51-83.

Monneveux, P., Reynolds, M.P., González-Santoyo, H., Peña, R.J., Mayr, L. and Zapata, F. (2004). Relationships between grain yield, flag leaf morphology, carbon isotope discrimination and ash content in irrigated Wheat. *Journal of Agronomy and Crop Science* 190: 395-401.

Moorman, F.R. and Veldkamp, W.J. (1978). Land and rice in Africa: Constraints and potentials. International Institute of Tropical Agriculture Ibadan, Nigeria. 29-43.

Mostafa, K., Mohammad, H. and Mohammad, M. (2011). Genetic diversity of wheat genotype baspdon cluster and principal component analyses for breeding strategies. *Australian Journal of Crop Science* 5 (1): 17-24.

Mwirigi, P.N., Kahangi, E.M., Nyende, A.B. and Mamati, E.G. (2009). Morphological variability within the Kenyan yam (*Dioscorea spp*.). *Journal of Applied Biosciences* 16: 894-901.

Nayak, A.R., Chaudhary, D. and Reddy, J.N. (2004). Studied on variability and characters association in scented rice over environments. *Indian Jour. Agric. Res.* 38 (4): 250-255.

Nguyen, V.N. and Ferrero, A. (2006). Meeting the challenges of global rice production. *J. Paddy, Water and Environment* 4: 1-9.

Ni, J., Colowit, P.M. and Mackill, D.J. (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science* 42: 601-607.

Nwanze, K.F. (1996). Introductory Remarks: Overview of the rice sector and interspecific hybridization of African and Asian rice species. **Proc**. of

KNUST

Interspecific hybridization: Progress and Prospects, West Africa Rice Development Association, 6-10 May, 1995, Mbe, Cote d'Ivoire.

Nwanze, K.F., Mohapatra, S., Kormawa, P.M., Keya, S. and Bruce-Oliver, S. (2006). Rice development in sub-saharan Africa perspective. *Journal of the Science of Food and Agriculture* 86: 675-677.

Nyanteng, V.K. (1987). Rice in West Africa. Consumption, imports and production of rice with projection to the year 2000. Liberia, Monrovia, WARDA. goliath.ecnext.com//coms2//The-fortunes-and-misfortunes-of.htm. [Accessed 2015 January 15].

Ookawa, T. and Ishihara, K. (1992). Varietal difference of physical characteristics of the culm related to lodging in paddy rice. *Japan Journal of Crop Science* 61: 419-425.

Padulosi, S. (1993). Genetic diversity, taxonomy and ecogeographical survey of the wild relatives of cowpea (*Vigna unguiculata*). Ph.D Thesis, University of Ibadan, Nigeria. 126-128.

Paterson, A.H., Freeling, M. and Sasaki, T. (2005). Grains of knowledge: Genomics of model cereals. *Genome Research* 15: 1643-1650.

Peltonen, S.P. and Jarvinen, P. (1995). Seeding rate effects on tillering, grain yield, and yield components of oat at high latitude. *Field Crops Research* 40: 49-56.

Pervaiz, Z.H., Ashiq, M.R., Ishtiaq, K., Stephen, R.P. and Salman, A.M. (2010). Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. *Electronic Journal of Biotechnology* 13 (3): 0717-3458.

Pervaiz, Z.H., Rabbani, M.A., Pearce, S.R. and Malik, S.A. (2009). Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. *Afr. Jour. Biotech*. 8: 5641-5651.

Prabakaran, A., Paramasivam, K., Rajesh, T. and Rajarajan, D. (2010). Molecular characterization of rice land races using SSR markers. *Electronic Journal of Plant Breeding* 1 (4): 512-516.

Prakash, M., Anandan, A. and Kumar, S.B. (2011). Varietal variations in flag leaf area and yield in mutant lines of PY5 rice. *Karmataka Journal of Agricultural Scien*ce 24 (4): 525-526.

Rahim, S.H. (2005). Study of grain yield stability in rice (*Oryza sativa* L.) promising genotypes. *Iranian Journal of Crop Science* 7 (2): 112-122.

Rahman, M.M., Hussain, A., Sayed, M.A., Ansari, A. and Mahmud, M.A.A. (2011). Comparison among clustering in multivariate analysis of rice using morphological traits, physiological traits and simple sequence repeat markers. *Journal of Agricultural and Environmental Sciences* 11 (6): 876-882.

Ravi, M., Geethanjali, S., Sameeyafarheen, F. and Maheswaran, M. (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica* 133: 243-252.

Ren, F., Lu, B.R., Li, S., Huang, J. and Zhu, Y. (2003). A comparative study of genetic relationships among the AA-genome *Oryza* species using RAPD and SSR markers. *Theo. Appl. Genet*. 108 (1): 113-120.

Rubenstein, K. and Heisey, P. (2003). Plant Genetic Resources: New Rules for international exchange. Bioversity's Regional Office for the Americas, IPGRI, Villa Gualino, Turin, Italy. 63.

Rutger, J.N. and Mackill, D.J. (2001). Application of mendelian genetics in rice breeding. **In**: Khush, G.S., Brar, D.S. and Hardy, B. (Eds.). Rice genetics IV. Science Publishers, Los Baños, Philippines. 27-38.

Sajib, A.M., Musharaf, H., Mosnaz, A.T.M.J., Hossain, H., Islam, M., Ali, S. and Prodhan, S.H. (2012). SSR marker-based molecular characterization and genetic diversity analysis of aromatic land races of rice (*Oryza sativa* L.). *J. BioSci. Biotech.* 1 (2): 107-116.

KNUST

Sarawgi, A.K., Subba-Rao, L.V., Parikh, M., Sharm, B. and Ojha, G.C. (2013). Assessment of the variability of rice (*Oryza sativa* L.) germplasm using agro-morphological characterization. *Journal of Rice Research* 6 (1): 152-178.

Sarla, N. and Swamy, B.P.M. (2005). *Oryza glaberrima*: A source for improving *Oryza sativa*. *Current science* 89 (6): 955-963.

Se-Jong, O.H., Song, J.Y., Lee, J., Lee, G.A., Ko, H.C., Stoilova, T., Krasteva, L., Kim, Y.G., Rhee, J.H., Gwag, J.G., Ro, N.Y., Hur, O.S. and Lee, M.C. (2012). Evaluation of genetic diversity of red pepper landraces (*Capsicum annuum*) from Bulgaria using SSR markers. *Korean Journal of International Agriculture* 24 (5): 547- 556.

Semagn, K., Bjornstad A. and Ndjiondjop, M.N. (2006). An overview of molecular marker methods for plants. *Afr. Jour. of Biotechn*. 5 (25): 2540-2568.

Senapati, B.K., Pal, S., Roy, S.D. and De, K. (2009). Selection criteria for high yield in early segregating generation of rice (*Oryza sativa* L.) crosses. *Journal of Crop and Weed* 5 (2): 12-14.

Setter, T.L., Laureles, E.V. and Mazaredo, A.M. (1997). Lodging reduces yield of rice by self-shading and reductions in canopy photosynthesis. *Journal of Field Crops Research* 49: 95-106.

Sharief, A.E., El-Kalla, S.E., El-Kassaby, A.T., Ghonema, M.H. and Abdo, G.M.Q. (2006). Effect of bio-chemical fertilization and time of nutrient foliar application on growth, yield and yield components of rice. *Journal of Agronomy* 5 (2): 212-219.

Sharma, T.R. and Jana, S. (2002). RAPD variation in *Fagopyrum tataricum* Gaertn accessions from China and the Himalayan region. *Euphytica* 127 (3): 327-333.

Shi, C., Wu, J. and Wu, P. (2005). Genetic analysis of developmental behaviour for amylase content in filling process of rice. *Journal of Agricultural science* 85: 791-796.

Sinclair, T.R. and Sheehy, J.E. (1999). Erect leaves and photosynthesis in rice. *Journal of Sciences* 283: 14-55.

Singh, R.K., Singh, U.S., Khush, G.S. and Rashmi, R. (2000). Genetic and biotechnology of quality traits in aromatic rices. **In**: Reijans, M. and Van, T. (Eds.). Aromatic Rice. New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd, Washington, USA. 44-71.

Singh, S.P. (1989). Pattern of variation in cultivated dry bean. *Bean Improvement Cooperation* 31: 180-182.

Sohl, M. (2005). Rice is life in 2004 and beyond, International year of rice (*Oryza sativa* L.) and its implications. Food and Agricultural Organization of the United Nations, Rome, Italy. 283.

Surek, H. and Beser, N. (2003). Correlation and path coefficient analysis for some yield related traits in rice (*Oryza sativa* L.) under thrace conditions. *Turkey Journal of Agriculture* 27: 77-83.

Tahir, M.D., Wadan, M. and Zada, A. (2002). Genetic variability of different plant yield characters in rice. *Sarhad J. Agric*. 18 (2): 251-255.

Tandekar, K. and Koshta, N. (2014). To Study the Agro morphological variation and genetic variability in rice germplasm. *Middle-East Journal of Scientific Research* 20 (2): 218-224.

UNCTAD. (2010). United Nations Conference on Trade and Development. Rice characteristics. Communicating market information in the commodities area. unctad.org//en//docs//ecn162010d4enpdf. [Accessed 2015 January 10].

USDA. (2008). United State Development of Agriculture. Annual Report for 2007. United States Department of Agriculture, Washington, USA. 25-26.

Vanghan, D.A., Lu, B. and Tomooka, N. (2008). The evolving story of rice evolution. *Journal of Plant Science* 174 (4): 394-408.

Vanniarajan, C., Vinod, K.K. and Pereira, A. (2012). Molecular evaluation of genetic diversity and association studies in rice (*Oryza sativa* L.). *J. Genet*. 91: 9-19.

KNUST

Varshney, R.K., Close, T.J., Singh, N.K., Hoisington, D.A. and Cook, D.R. (2009). Orphan legume crops enter the genomics era. *Current Opinion in Plant Biology* 12: 202-210.

Vinita, P., Nilay, T., Prashant, V., Nagendra, K.S. and Sanjay, S. (2013). Molecular and morphological characterization of Indian farmers rice varieties (*Oryza sativa* L.). *Australian Journal of crop sciences* 7 (7): 923-932.

Virmani, S.S. (1979). Breeding rice for iron toxicity. **Proc**. of the Second varietal improvement seminar, 14-18 September, 1979, Monrovia, Liberia.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van, T., De Lee, M., Hornes, A., Freijters, J., Pot, A., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res*. 23: 4407-4414.

Wang, X.F., Knoblauch, R. and Leist, N. (1998). Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 280: 1077-1082.

WARDA. (1993). Annual Report for 1992. West Africa Rice Development Association. Mbé, Côte d'Ivoire. 277-280.

WARDA. (2005). West Africa Rice Development Association. Rice, a strategic crop for food security and poverty alleviation. www.2.slu.se//cigar//CGIAR WARDAppt. [Accessed 2015 January 8].

WARDA. (2008). West Africa Rice Development Association. Annual Report. Cotonou, Benin. warda.org//warda//impactpub. [Accessed 2015 January 15].

Weising, K., Nybom, H., Wolf, K. and Kahl, G. (2005). DNA fingerprinting in plants. Principles, methods and applications. 2nd Edition. CRC Press, USA. 230-239.

Weising, K., Nybom, H., Wolff, K. and Meyer, W. (1995). DNA Fingerprinting in Plants and Fungi. **In:** Arbor, A. (Ed.). Fingerprinting of plant genotypes. CRC Press, Boca Raton, USA. 1037-1048.

Weiya, X., Yongzhong, X., Xiaoyu, W., Yu, Z., Weijiang, T., Lei, W. and Hongju, Z. (2008). Natural variation in Ghd7 an important regulator of heading date and yield potential in rice. *Nature Genetics* 40: 761-767.

Wu, G.W., Wilson, L.T. and McClung, A.M. (1998). Contribution of rice tillers to dry matter accumulation and yield. *Agron. J.* 90: 317-323.

Xu, R., Norihiko, T., Vaughan, A.D. and Doi, K. (2000). The *Vigna unguiculata* complex: Genetic variation and relationships revealed by RAPD analysis and their implications for In-situ conservation and domestication. *Genetic Resources and Crop Evolution* 47: 123-134.

Xue, D., Chen, M., Zhou, M., Chen, S., Mao, Y. and Zhang, G. (2008). QTL analysis of flag leaf in barley (*Hordeum vulgare*) for morphological traits and chlorophyll content. *Journal of Zhejiang University of Science* 9 (12): 938-943.

Yada, B., Tukamuhabwa, P., Wanjala, B., Kim, D.J., Skilton, R.A., Alajo, A. and Mwanga, R.O.M. (2010). Characterization of Ugandan sweet potato germplasm using fluorescent labelled simple sequence repeat markers. *Hort. Science* 45 (2): 225-230.

Yang, X.C., and Hwa, C.M. (2008). Genetic modification of plant architecture and variety improvement in rice. *Journal of Heredity* 101: 396-404.

Yoshida, S. (1972). Physiological aspects of grain yield. *J. Plant Physiology* 23: 437-64.

Yoshida, S. (1981). Rice plant characters in relation to yielding ability. **In**: Weijiang, T. (Ed.). Fundamentals of rice crop science. International Rice Research Institute Publication, Los Banos, Philippines. 20-55.

Zahid, A.M., Akhtar, M., Anwar, M. and Adil, J. (2005). Genotypic and phenotypic, correlation and path analysis in coarse grain rice. **Proc**. of the International seminar on rice crop. Rice Research Institute, 2-3 October, 2005, Kala Shah Kau, Pakistan.

KNUST

Zhang, M., Guo, B. and Peng, Z. (2005). Genetic effects on grain characteristics of indica black rice and their uses on indirect selections for some mineral element contents in grains. *Gene. Res. Crop Evo*. 52: 1121-1128.

Zhu, X.B., Sun, D.Y., Chen, B.S. and Hong, D.L. (2008). Distribution characterization of leaf and hull pubescences and genetic analysis of their numbers in japonica rice (*Oryza sativa* L.). *Rice Sci*. 15: 267-275.

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APPENDICES

Appendix 1: Quantitative traits data of accessions

ACC. NO.	100GW	CL	TNM	D50%FM	D80%GMM	FLL	FLW	GY	GW	GL	LIL	LLB	LW	PH	PNM	CD	PL
1	3.52	84.87	14.00	100.33	132.00	35.30	1.70	10.55	3.30	9.50	1.97	42.83	1.43	120.53	14.00	8.17	28.00
2	3.25	119.83	13.33	98.33	128.00	43.50	1.73	11.39	3.07	9.60	2.93	62.43	1.70	128.27	13.00	6.40	27.73
3	2.82	87.27	13.67	102.33	133.00	42.77	2.00	10.04	2.40	8.53	2.47	52.73	1.53	123.17	13.33	8.83	28.37
4	2.68	89.67	20.33	100.67	132.00	42.87	1.97	11.07	2.23	8.77	3.27	50.13	1.57	126.17	19.67	8.50	27.50
5	2.66	84.80	19.33	97.33	130.00	26.87	1.67	8.18	2.23	9.77	2.50	41.87	1.70	107.73	18.00	7.07	26.37
6	2.74	72.60	27.67	107.67	138.00	25.40	1.63	8.64	2.33	10.10	1.43	38.47	1.40	103.13	25.67	6.20	24.33
7	2.21	89.97	15.00	96.67	128.00	36.97	1.60	7.84	2.17	8.63	1.97	48.10	1.37	122.63	14.33	7.73	24.50
8	3.02	104.10	17.00	105.33	138.33	40.80	1.87	9.61	2.30	8.90	1.80	46.37	1.47	138.80	16.00	6.63	29.27
9	2.88	91.83	18.33	107.67	138.33	48.67	1.87	10.40	3.03	9.20	1.43	52.77	1.50	137.77	16.00	8.07	31.80
10	2.99	89.30	20.67	99.00	126.67	31.30	1.67	8.99	2.23	9.87	1.87	37.03	1.40	115.50	20.00	6.70	23.13
11	2.38	68.43	20.67	100.67	128.67	37.50	1.87	9.29	2.23	9.07	1.87	48.00	1.33	96.03	20.00	5.30	22.30
12	2.69	89.73	20.67	95.67	130.00	21.17	1.63	12.88	2.83	9.57	2.50	36.07	1.60	117.30	19.33	6.37	22.63
13	2.62	78.40	23.33	102.00	130.67	32.97	1.80	12.38	2.23	8.87	2.10	42.93	1.63	117.97	23.33	5.67	28.63
14	3.03	80.93	20.00	99.00	128.00	29.03	1.63	7.97	2.23	9.73	2.20	45.17	1.47	106.57	18.33	6.40	25.50
15	2.75	82.20	21.00	99.67	131.67	30.40	1.57	10.14	2.23	9.33	2.17	44.33	1.47	110.77	19.00	7.47	24.73
16	2.73	63.40	15.33	105.67	138.00	39.13	1.93	7.59	2.30	10.10	2.67	50.80	1.33	120.93	14.67	7.10	26.50
17	2.72	83.87	19.67	102.67	125.67	39.53	1.53	10.32	2.40	8.67	1.93	47.17	1.70	126.57	19.00	7.80	27.63
18	3.01	109.50	18.67	99.00	128.67	38.17	1.63	10.55	2.33	9.60	1.73	39.63	1.30	132.30	17.33	7.83	27.40
19	2.72	74.83	25.00	99.67	135.00	30.27	1.73	11.56	3.10	9.43	1.53	32.70	1.47	105.00	23.67	5.73	26.37
20	2.54	84.00	19.00	101.67	135.33	39.37	1.80	9.93	3.07	8.97	1.60	47.27	1.50	127.73	19.00	7.03	25.40
21	3.51	85.73	19.67	117.67	133.33	27.00	1.73	9.47	3.03	9.10	2.73	59.07	1.63	93.20	17.00	8.50	23.90
22	2.99	79.73	21.33	98.00	133.00	31.17	1.70	10.36	2.37	9.20	2.10	36.40	1.33	105.63	19.33	6.37	26.60
23	2.52	83.80	21.33	109.00	138.00	45.50	1.83	11.67	2.67	9.03	2.57	60.87	1.47	130.00	20.33	8.17	26.67
24	2.75	79.57	20.67	105.33	139.67	36.67	1.67	11.02	2.13	10.23	2.37	47.53	1.50	115.70	18.33	7.83	26.90
25	3.32	126.20	11.67	121.33	146.00	29.70	1.77	9.25	2.33	10.10	2.70	75.70	1.40	156.13	11.67	8.17	28.23
26	2.52	95.33	22.67	100.33	130.00	36.07	1.97	9.99	2.30	8.87	1.47	48.00	1.43	135.50	22.00	5.30	30.47
27	2.23	75.63	17.00	115.00	136.00	49.27	2.03	8.33	2.80	6.77	1.63	54.37	1.37	119.20	15.33	7.13	22.50
28	2.81	112.57	16.67	118.00	147.67	39.17	1.40	8.23	2.33	10.23	3.03	69.77	1.27	145.53	16.33	7.17	27.17
29	3.34	105.37	9.33	105.33	126.67	40.93	1.93	7.53	3.10	10.17	2.03	56.57	1.60	146.67	9.33	8.17	27.83
30	2.42	129.33	16.00	112.33	136.00	49.97	1.77	10.32	2.33	8.43	2.60	64.50	1.37	166.00	15.67	7.37	34.13
31	2.81	134.33	12.33	126.00	139.33	35.50	1.70	6.11	2.43	9.80	3.57	68.43	1.20	150.00	12.00	7.30	26.10
32	2.97	84.07	14.33	91.33	129.00	30.00	1.77	10.64	2.43	9.80	2.23	50.73	1.43	108.60	14.00	8.83	28.50

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Con't Appendix 1: Quantitative traits data of accessions

ACC. NO.	100GW	CL	TNM	D50%FM	D80%GMM	FLL	FLW	GY	GW	GL	LIL	LLB	LW	PH	PNM	CD	PL
33	2.50	110.33	16.33	102.33	128.00	32.67	1.57	11.38	2.30	9.20	1.20	51.00	1.07	143.93	15.00	8.17	27.83
34	3.03	108.33	16.00	113.33	137.00	39.60	1.77	11.84	2.33	9.07	1.77	63.40	1.33	115.43	15.67	8.50	24.70
35	2.48	87.10	19.67	100.33	133.00	42.50	1.63	11.02	2.70	8.13	2.27	54.00	1.13	131.27	18.67	8.17	25.43
36	2.84	97.77	16.67	104.33	135.00	30.37	1.60	10.75	2.23	8.77	1.57	43.20	1.30	132.87	16.33	8.73	28.43
37	2.58	115.37	17.33	107.00	130.67	50.20	1.67	10.64	2.47	8.40	2.03	65.63	1.30	156.00	15.67	9.17	26.70
38	2.79	85.37	17.00	100.33	127.33	36.17	1.87	7.23	2.30	10.43	2.37	43.63	1.47	112.90	16.00	5.67	25.53
39	2.92	128.57	13.67	105.00	130.00	27.90	1.63	7.43	2.23	9.40	1.90	56.30	1.17	154.70	13.00	7.07	29.57
40	2.27	71.97	24.00	90.00	125.33	20.80	1.53	8.53	2.20	8.40	1.90	34.83	1.40	91.90	23.67	5.93	22.43
41	2.80	103.53	16.33	95.33	125.33	40.77	1.53	10.17	2.20	9.30	1.43	63.83	0.77	142.23	16.00	7.83	26.03
42	3.33	112.77	16.33	96.67	128.67	28.53	1.57	10.57	2.23	10.73	1.70	48.73	1.20	138.13	16.33	6.43	31.37
43	2.70	74.10	11.67	82.00	123.33	35.23	1.50	7.29	2.20	10.23	2.60	54.50	1.17	113.43	11.33	6.37	26.17
44	3.60	129.43	12.33	121.67	148.67	35.87	2.00	7.27	2.67	10.30	3.53	76.20	1.53	158.03	11.67	8.83	27.00
45	2.92	83.40	14.00	100.33	128.67	36.03	1.97	9.14	2.77	9.07	2.13	44.13	1.33	121.07	13.00	8.07	27.90
46	3.29	138.17	12.33	119.00	145.00	32.90	1.87	9.75	2.80	10.30	2.73	68.27	1.73	171.73	12.00	7.73	27.03
47	2.91	94.43	14.33	103.00	134.00	38.03	1.83	8.78	3.03	9.23	2.67	49.83	1.43	132.20	13.67	7.73	28.53
48	2.98	88.40	8.33	83.67	119.00	40.33	2.23	4.78	2.50	9.33	1.17	51.63	1.90	124.93	7.33	7.47	25.10
49	4.05	107.20	7.67	84.67	119.00	29.63	2.17	6.15	2.90	10.27	0.67	58.93	2.47	126.43	7.67	8.17	21.70
50	3.99	103.97	7.67	86.67	126.33	33.53	1.93	5.54	2.57	10.60	1.33	54.53	1.90	132.93	7.67	9.43	24.17
51	3.23	111.63	8.67	77.00	116.00	43.50	2.07	7.21	3.07	10.80	1.27	55.80	1.63	138.60	8.33	7.07	32.20
52	2.85	83.73	13.33	86.67	124.67	37.47	1.83	8.57	3.00	9.77	2.07	57.13	1.70	121.73	13.00	6.63	25.33
53	2.92	86.97	15.33	87.67	120.33	38.13	2.10	9.74	3.07	8.60	1.67	47.33	1.60	123.93	15.33	5.30	25.97
54	2.79	93.13	10.33	76.33	113.00	38.17	1.70	5.63	2.83	9.40	1.57	51.77	1.40	128.47	10.00	7.07	25.57
55	2.94	99.17	10.00	80.00	119.67	43.17	2.13	6.14	2.80	9.40	1.40	48.47	1.40	100.53	9.67	5.73	31.97
56	3.20	93.07	8.33	82.33	116.00	40.03	2.13	5.74	2.93	9.77	1.37	45.47	1.67	124.07	8.00	7.73	30.50
57	2.64	95.33	9.33	74.00	113.00	30.30	1.63	4.78	2.70	8.80	1.17	55.10	1.47	120.57	8.33	6.37	22.27
58	2.91	108.93	9.67	81.67	113.00	36.87	1.93	7.97	2.57	9.43	1.27	47.80	1.50	142.83	9.67	7.83	28.10
59	3.27	91.13	9.33	76.00	113.00	44.17	2.03	5.77	2.33	9.50	1.20	51.60	1.47	130.30	9.00	7.10	21.27
60	3.45	103.80	10.67	85.67	120.33	37.83	2.03	5.18	2.63	9.57	1.47	61.50	1.70	146.87	9.33	6.43	25.73
61	3.23	103.37	8.33	74.67	113.00	45.97	1.83	5.18	2.43	9.20	1.27	55.30	1.73	118.53	5.67	6.80	26.73
62	3.33	99.27	9.00	76.00	113.00	43.30	2.03	5.61	2.57	9.40	1.00	45.47	1.47	123.43	8.00	6.33	25.30
63	3.01	85.30	10.67	84.33	113.00	39.83	2.03	6.76	2.63	9.37	1.60	59.77	1.67	139.97	10.00	6.70	27.00
64	2.87	104.63	9.33	80.67	112.67	32.27	1.87	6.15	2.47	9.30	0.93	44.23	1.63	127.50	9.33	8.17	25.03
65	2.95	101.97	8.67	82.00	116.00	55.27	1.80	6.45	2.67	9.10	1.17	41.97	1.30	131.63	8.67	7.73	26.93
66	3.15	92.03	10.33	79.33	113.00	29.40	2.07	6.91	2.30	9.40	1.07	43.43	1.43	86.43	10.00	7.83	27.47

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Con't Appendix 1: Quantitative traits data of accessions

ACC. NO.	100GW	CL	TNM	D50%FM	D80%GMM	FLL	FLW	GY	GW	GL	LIL	LLB	LW	PH	PNM	CD	PL
67	2.88	90.80	10.00	86.33	119.33	33.03	1.83	6.84	2.57	9.07	1.40	43.50	1.47	126.13	10.00	7.30	25.90
68	2.52	98.10	11.67	80.00	113.00	36.90	2.40	6.69	2.20	8.53	0.60	44.80	1.80	137.17	11.33	6.63	27.00
69	2.89	75.77	16.67	100.67	129.67	33.87	1.67	8.19	2.47	9.03	2.53	46.00	1.40	119.17	15.33	6.97	31.33
70	2.93	94.23	12.00	92.67	124.67	40.07	1.63	6.67	2.57	9.77	2.13	49.73	1.33	131.93	11.00	6.37	24.33
71	2.73	87.00	11.00	88.67	124.67	33.87	1.90	6.18	2.80	9.27	1.43	47.20	1.47	116.23	11.00	5.30	24.90
72	2.79	92.83	12.00	89.67	124.67	30.53	1.70	7.03	2.53	8.53	1.60	47.23	1.43	122.40	11.67	6.80	22.47
73	3.33	96.90	10.00	92.00	135.00	32.13	1.80	7.70	3.03	8.97	1.90	53.20	1.57	134.83	9.67	6.70	24.57
74	1.81	77.07	25.67	79.33	145.00	29.93	1.53	3.55	2.47	7.53	1.37	50.87	1.27	108.53	22.00	4.43	20.30
75	2.87	74.47	21.00	90.67	126.67	26.90	1.50	8.16	2.27	8.63	2.00	47.47	1.40	125.33	19.67	5.03	27.27
76	2.43	86.20	18.33	98.00	130.67	34.20	1.70	9.60	2.60	10.00	2.90	48.50	1.50	115.77	18.33	6.80	25.63
77	2.79	92.20	12.67	92.33	120.33	41.40	2.10	8.00	2.70	9.17	2.57	46.90	1.53	129.27	12.33	5.93	26.10
78	2.58	83.67	21.00	77.67	135.00	23.47	1.43	6.00	3.03	8.63	1.13	30.27	0.87	110.23	19.00	4.70	16.67
79	2.53	88.47	10.00	87.33	127.67	32.50	1.70	6.14	3.07	8.80	1.57	52.27	1.53	122.90	9.33	6.97	21.90
80	2.46	91.77	18.00	88.67	126.67	31.37	1.67	8.78	2.80	8.40	1.63	55.10	1.40	122.47	17.00	4.93	19.93
81	2.88	82.33	21.67	98.67	130.00	24.97	1.60	7.26	2.80	9.13	2.67	39.47	1.33	110.20	20.00	6.00	25.83
82	2.66	99.77	16.67	87.67	124.67	34.80	1.57	7.47	2.47	8.63	1.97	43.43	1.23	130.93	14.33	5.93	23.00
83	2.98	116.77	13.00	95.33	123.33	26.73	1.60	9.73	2.50	8.47	2.37	56.07	1.37	140.30	12.00	7.83	23.50
84	2.55	80.17	20.67	91.67	126.00	29.00	1.63	8.95	2.43	9.20	2.33	47.83	1.33	103.73	19.67	5.07	23.73
85	2.56	83.00	16.33	95.33	133.67	25.90	1.67	6.94	2.30	8.93	1.37	44.97	1.33	98.77	14.67	5.30	24.67
86	2.52	90.40	12.67	90.33	121.33	34.10	1.73	9.59	2.53	8.30	2.13	70.37	1.30	124.30	12.67	5.30	26.37
87	2.63	97.63	12.67	101.00	127.33	48.67	2.10	8.57	2.43	9.27	1.47	50.10	1.57	141.77	12.67	7.10	25.47
LSD (5%)	0.40	16.06	5.18	5.86	9.54	12.61	0.37	3.09	0.51	1.00	0.95	16.50	0.39	26.73	4.86	2.30	4.68

100 GW = 100 Grain weight (g), CL = Culm length (cm), TNM = Tiller number mean, D50%FM = Days to 50% flowering mean, D80%GMM = Days to 80% grain maturity mean, FLL = Flag leaf length (cm), FLW = Flag leaf width (cm), GY = Grain yield (t/ha), GW = Grain width (mm), GL = Grain length (mm), LIL =Ligule length (cm), LL = Leaf length of blade (cm), LW = leaf width (cm), PH = Plant height (cm), PNM = Panicle number mean, Culm diameter (cm), PL = Panicle length (cm) and ACC. NO. = Accession number which follows details in Table 3.1.

Appendix 2: Qualitative traits scores of accessions

ACC. NO.	AC	APB	BLC	CA	FLA	LBIGC	LBP	CL	PE	LISH	AW
1	081	9	060	3	1	063	2	011	5	1	0
2	062	5	060	1	3	060	2	011	9	2	0
3	062	9	060	5	1	063	3	011	5	2	0
4	062	7	060	3	3	060	1	011	9	3	0
5	011	9	060	3	1	060	2	062	5	3	0
6	011	5	060	1	1	063	1	011	7	1	0
7	062	7	060	1	5	060	2	011	9	2	0
8	062	9	080	1	3	060	2	011	9	1	0
9	062	7	060	1	3	063	2	062	7	2	0
10	062	7	060	3	1	063	2	011	7	3	0
11	081	7	060	3	3	063	2	011	5	3	0
12	080	9	060	1	3	063	2	011	7	1	0
13	080	7	060	1	1	060	2	011	7	3	0
14	062	7	060	3	1	063	2	080	5	2	0
15	011	9	060	3	3	060	2	011	5	3	0
16	062	7	060	5	1	063	2	011	7	3	0
17	062	7	060	1	1	061	2	011	7	1	0
18	080	7	080	1	1	060	2	011	9	2	0
19	062	9	060	3	1	060	2	011	7	1	0
20	062	7	060	1	1	060	3	011	5	1	0
21	080	5	060	3	1	060	2	011	5	3	0
22	011	9	060	1	1	060	2	011	7	1	0
23	062	7	080	1	3	060	3	011	5	1	0
24	062	9	060	1	3	060	1	062	7	2	0
25	081	5	060	1	1	060	2	011	7	2	0
26	062	7	081	1	3	061	2	062	7	2	0
27	081	9	080	3	1	060	2	062	5	2	0
28	011	7	080	5	3	060	2	011	7	2	0
29	080	7	080	3	1	060	2	011	9	1	0
30	011	5	060	1	3	060	2	011	7	1	0
31	011	5	060	3	1	063	2	011	7	2	0
32	081	5	060	5	3	063	2	011	7	2	2

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Con't Appendix 2: Qualitative traits scores of accessions

ACC. NO.	AC	APB	BLC	CA	FLA	LBIGC	LBP	CL	PE	LISH	AW
33	011	9	060	1	3	063	2	011	9	2	2
34	062	5	060	3	3	060	2	011	7	3	0
35	062	7	080	3	1	060	2	011	3	3	1
36	081	7	060	3	1	063	2	011	5	1	0
37	080	5	060	5	1	063	2	062	5	2	0
38	062	7	060	3	5	063	2	081	5	3	0
39	081	7	084	5	3	061	2	011	9	1	0
40	080	7	060	3	3	063	2	011	7	1	0
41	062	9	080	5	3	060	2	080	5	3	2
42	062	9	084	3	1	060	2	011	9	2	0
43	062	9	081	3	3	063	3	011	5	2	0
44	081	7	060	3	3	060	2	011	7	2	0
45	062	9	060	5	1	061	1	062	7	3	2
46	081	7	080	3	5	060	2	011	7	3	0
47	062	9	060	5	5	063	2	011	5	3	0
48	081	3	060	3	3	060	1	011	9	0	1
49	081	9	080	3	1	060	3	011	9	2	0
50	080	9	080	3	1	060	3	011	7	1	0
51	080	9	060	7	1	063	2	062	9	2	0
52	062	9	080	3	1	061	3	011	7	2	0
53	080	5	060	1	1	063	1	011	7	2	0
54	080	7	060	5	3	063	1	011	7	2	1
55	081	9	060	3	1	060	1	0	7	0	0
56	080	5	060	3	1	063	1	062	9	1	2
57	081	9	080	3	1	061	2	011	9	2	0
58	062	7	060	3	1	063	1	011	7	1	0
59	080	9	060	5	3	060	1	011	9	2	0
60	080	9	080	3	1	060	1	011	9	2	0
61	062	9	080	5	3	063	1	011	9	2	0
62	080	7	060	5	3	063	1	011	7	1	2
63	062	9	080	3	1	060	1	011	9	1	0
64	062	9	080	5	1	063	1	011	9	2	0
65	081	7	060	5	1	063	1	011	9	1	1

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Con't Appendix 2: Qualitative traits scores of accessions

ACC. NO.	AC	APB	BLC	CA	FLA	LBIGC	LBP	CL	PE	LISH	AW
66	062	7	081	3	1	063	1	011	7	1	1
67	080	7	080	3	1	063	1	011	9	0	0
68	062	7	060	3	1	063	1	011	9	0	0
69	011	7	084	1	7	060	2	081	5	2	0
70	062	5	060	1	3	060	2	011	9	2	0
71	081	7	060	1	3	060	3	011	7	2	1
72	084	7	080	1	1	063	3	062	7	2	0
73	062	9	080	1	1	060	3	011	5	1	0
74	081	3	081	3	1	060	2	062	5	3	0
75	062	7	060	3	1	061	2	011	5	2	0
76	011	7	060	1	3	063	2	011	5	2	2
77	062	5	060	3	1	061	2	011	7	3	0
78	081	3	084	7	1	063	2	011	3	1	0
79	062	7	060	1	1	063	2	062	7	2	2
80	080	7	060	1	1	060	2	011	5	3	0
81	011	7	060	3	1	060	2	011	9	2	0
82	011	7	060	3	3	061	2	011	9	2	0
83	080	9	080	1	3	061	2	081	7	1	0
84	062	9	060	3	1	060	2	011	7	1	0
85	062	7	060	3	1	063	2	011	7	1	2
86	080	9	060	1	1	060	2	011	5	3	0
87	011	7	081	1	5	061	2	011	5	2	0

AC = Auricle colour, APB = Attitude of panicle branches, BLC = Basal leaf sheath colour, CA = Culm Angle, FLA = Flag leaf Angle, LBC = Leaf blade: intensity of green colour, LBP = Leaf blade pubescence, CL = Colour of ligule, PE = Panicle exsertion, LISH = Ligule shape, AW = Awn and ACC. NO. = Accession number which follows details in Table 3.1.

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Appendix 3: Amplification products of primers

	Xtxp 149 (1)			Xtxp 284 (1)		Xtxp 201 (2)			Xtxp 197 (2)		Xtxp 10 (9)			Xtxp 278 (7)		
ACC. NO.	A	B	C	A	B	A	B	C	A	B	A	B	C	A	B	C
1	0	0	1	0	1	1	0	0	1	0	9	9	9	9	9	9
2	0	0	1	0	1	9	9	9	0	1	9	9	9	9	9	9
3	0	0	1	0	1	1	0	0	1	0	9	9	9	9	9	9
4	9	9	9	0	1	9	9	9	1	0	1	0	0	9	9	9
5	0	0	1	1	0	9	9	9	0	1	0	1	0	0	1	0
6	0	1	0	0	1	9	9	9	0	1	0	1	0	0	1	0
7	0	0	1	1	0	0	1	0	0	1	0	1	0	1	0	0
8	0	1	0	9	9	0	1	0	0	1	0	0	1	1	0	0
9	9	9	9	0	1	1	0	0	0	1	0	0	1	0	0	1
10	0	1	0	0	1	0	1	0	1	0	9	9	9	0	1	0
11	0	1	0	1	0	0	1	0	1	0	0	0	1	0	1	0
12	0	1	0	9	9	0	1	0	1	0	0	1	0	0	1	0
13	1	0	0	0	1	1	0	0	0	1	9	9	9	0	1	0
14	9	9	9	0	1	0	1	0	0	1	0	1	0	1	0	0
15	0	0	1	0	1	1	0	0	1	0	0	1	0	0	1	0
16	0	0	1	0	1	9	9	9	0	1	0	0	1	0	1	0
17	9	9	9	0	1	1	0	0	0	1	1	0	0	0	1	0
18	0	0	1	0	1	9	9	9	1	0	1	0	0	0	1	0
19	0	1	0	0	1	9	9	9	1	0	1	0	0	1	0	0
20	0	0	1	0	1	0	1	0	9	9	0	1	0	0	1	0
21	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0
22	9	9	9	0	1	0	1	0	1	0	0	1	0	9	9	9
23	0	1	0	0	1	0	1	0	0	1	0	1	0	0	1	0
24	0	0	1	0	1	0	1	0	1	0	9	9	9	1	0	0
25	0	1	0	9	9	0	1	0	1	0	9	9	9	9	9	9
26	0	0	1	9	9	0	1	0	1	0	0	1	0	9	9	9
27	0	0	1	9	9	0	1	0	1	0	9	9	9	1	0	0
28	9	9	9	9	9	0	1	0	1	0	9	9	9	0	1	0
29	0	0	1	1	0	0	1	0	1	0	0	1	0	1	0	0
30	1	0	0	0	1	0	0	1	1	0	0	1	0	0	1	0

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Con't Appendix 3: Amplification products of primers

	Xtxp 149 (1)			Xtxp 284 (1)		Xtxp 201 (2)			Xtxp 197 (2)		Xtxp 10 (9)			Xtxp 278 (7)		
ACC. NO.	A	B	C	A	B	A	B	C	A	B	A	B	C	A	B	C
31	9	9	9	9	9	0	1	0	0	1	0	0	1	0	0	1
32	0	1	0	0	1	1	0	0	0	1	0	1	0	0	1	0
33	9	9	9	0	1	1	0	0	1	0	0	0	1	0	1	0
34	0	1	0	9	9	0	0	1	1	0	0	1	0	0	1	0
35	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	0
36	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	0
37	1	0	0	9	9	1	0	0	1	0	1	0	0	0	1	0
38	9	9	9	0	1	0	1	0	1	0	1	0	0	9	9	9
39	0	0	1	9	9	9	9	9	1	0	1	0	0	0	1	0
40	0	0	1	0	1	9	9	9	1	0	1	0	0	0	0	1
41	9	9	9	0	1	0	1	0	1	0	0	1	0	0	1	0
42	9	9	9	0	1	9	9	9	1	0	1	0	0	0	1	0
43	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0
44	9	9	9	0	1	0	1	0	9	9	9	9	9	0	1	0
45	1	0	0	0	1	0	1	0	1	0	0	1	0	0	1	0
46	9	9	9	0	1	1	0	0	1	0	9	9	9	0	1	0
47	9	9	9	1	0	0	1	0	1	0	9	9	9	9	9	9
48	9	9	9	1	0	1	0	0	0	1	1	0	0	1	0	0
49	0	1	0	1	0	0	1	0	1	0	0	1	0	1	0	0
50	0	1	0	9	9	0	1	0	0	1	9	9	9	0	1	0
51	9	9	9	0	1	0	1	0	0	1	9	9	9	0	1	0
52	0	1	0	9	9	0	0	1	1	0	0	1	0	1	0	0
53	0	1	0	9	9	0	1	0	1	0	9	9	9	1	0	0
54	9	9	9	9	9	0	1	0	1	0	0	1	0	0	1	0
55	1	0	0	0	1	0	1	0	0	1	0	1	0	0	1	0
56	0	0	1	0	1	1	0	0	0	1	1	0	0	1	0	0
57	0	0	1	0	1	1	0	0	1	0	1	0	0	0	1	0
58	9	9	9	0	1	0	1	0	1	0	9	9	9	0	1	0
59	9	9	9	0	1	1	0	0	0	1	9	9	9	0	1	0
60	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	0
61	0	1	0	9	9	1	0	0	1	0	9	9	9	0	1	0

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Con't Appendix 3: Amplification products of primers

	Xtxp 149 (1)			Xtxp 284 (1)		Xtxp 201 (2)			Xtxp 197 (2)		Xtxp 10 (9)			Xtxp 278 (7)		
ACC. NO.	A	B	C	A	B	A	B	C	A	B	A	B	C	A	B	C
62	0	1	0	9	9	0	1	0	9	9	1	0	0	0	1	0
63	0	1	0	9	9	0	1	0	0	1	1	0	0	0	0	1
64	9	9	9	9	9	0	1	0	0	1	9	9	9	0	1	0
65	0	1	0	9	9	1	0	0	0	1	9	9	9	0	1	0
66	9	9	9	0	1	1	0	0	0	1	0	1	0	0	1	0
67	0	1	0	0	1	0	0	1	0	1	9	9	9	0	1	0
68	9	9	9	9	9	1	0	0	9	9	9	9	9	9	9	9
69	9	9	9	0	1	1	0	0	1	0	1	0	0	9	9	9
70	9	9	9	0	1	1	0	0	0	1	0	1	0	0	1	0
71	0	1	0	0	1	1	0	0	0	1	0	1	0	0	1	0
72	9	9	9	0	1	1	0	0	0	1	0	0	1	0	1	0
73	1	0	0	0	1	0	1	0	1	0	9	9	9	0	1	0
74	0	1	0	0	1	0	1	0	0	1	9	9	9	0	1	0
75	1	0	0	9	9	0	1	0	1	0	9	9	9	9	9	9
76	1	0	0	0	1	0	1	0	1	0	9	9	9	0	1	0
77	1	0	0	0	1	0	1	0	0	1	9	9	9	0	1	0
78	9	9	9	0	1	0	1	0	1	0	9	9	9	1	0	0
79	9	9	9	0	1	9	9	9	0	1	0	1	0	9	9	9
80	9	9	9	0	1	0	1	0	0	1	0	1	0	0	1	0
81	9	9	9	0	1	0	1	0	0	1	0	1	0	0	0	1
82	9	9	9	0	1	0	1	0	9	9	1	0	0	1	0	0
83	9	9	9	0	1	0	1	0	0	1	0	1	0	0	1	0
84	9	9	9	0	1	0	1	0	0	1	0	1	0	0	1	0
85	9	9	9	0	1	0	0	1	9	9	0	1	0	9	9	9
86	9	9	9	0	1	1	0	0	9	9	0	0	1	9	9	9
87	9	9	9	9	9	1	0	0	9	9	0	1	0	9	9	9

A, B and C are Alleles revealed by the primers, 0 = Absent of band, 1 = Present of band, 9 = No amplification of band and ACC. NO. = Accession number which follows details in Table 3.1.

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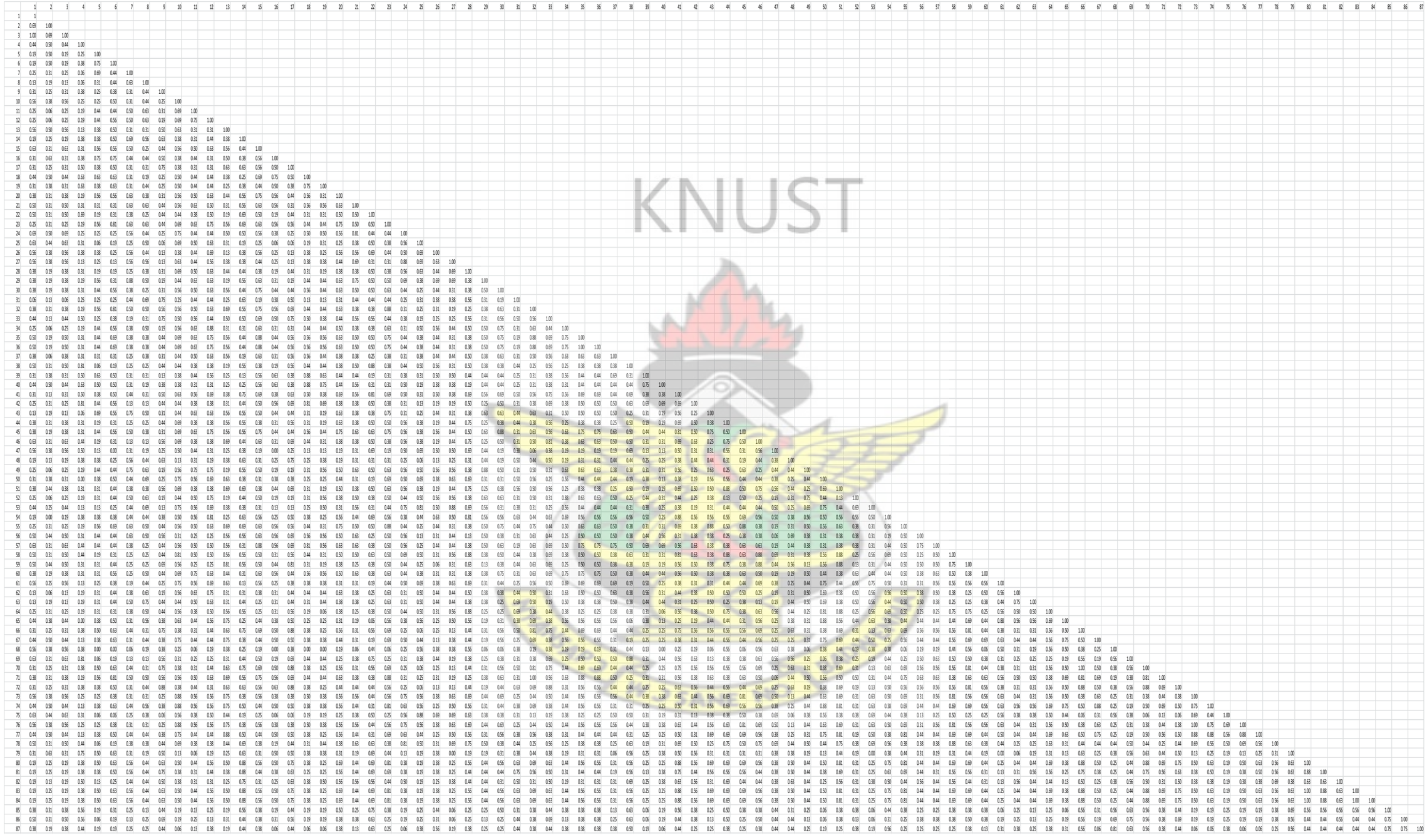
Appendix 4: Similarity matrix of 87 rice accessions based on Dice's similarity index



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ACC. NO. = Accession number which follows details in Table 3.1