GENETIC DIVERSITY IN LIBERIAN AND GHANAIAN RICE (*Oryza sativa L., Oryza glaberrima Steudi*) GERMPLASM USING MORPHOLOGICAL AND SIMPLE SEQUENCE REPEAT (SSR) MARKERS

BY

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GENETIC DIVERSITY IN LIBERIAN AND GHANAIAN RICE (O*ryza sativa L.*, *Oryza glaberrima Steudei*) GERMPLASM USING MORPHOLOGICAL AND SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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ZOGBO LUTHER

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DECLARATION

I, **Zogbo Luther**, hereby declare that the work presented in this thesis is the result of my own effort and findings, and no such previous application for a degree in this University or elsewhere has the same work been presented.

All sources of information have been acknowledged by reference to authors.

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ABSTRACT

Rice is an important staple food crop that feed over half of the global population and it has become the cereal that provides a major source of calories for the urban and rural poor in Africa. However, little attention has been paid to the improvement of Liberian and Ghanaian rice germplasm evaluation and the genetics of some quality traits. The need for increasing rice cultivation depends not only on cultural/traditional practices but also on their inbuilt genetic potential to withstand stresses. Therefore, these varieties have to be collected and evaluated for their exploitable genetic variability and conserved. The first step in achieving this is to evaluate and characterize available rice germplasm or genotypes at both morphological and molecular levels to reveal important traits or accessions of interest to plant breeders for crop improvement. In experiment, arranged in Completely Randomized Design was conducted to study the genetic variability among and within 48 genotypes or accessions obtained from Central Agricultural Research Institute (CARI), Suakoko, Liberia and Plant Genetic Resources Research Institute (PGRRI), Bunso, Ghana. DNA was extracted from 48 plants per accession without bulking to check the purity of the accession using the 16 SSR markers. Field data taken included 28 qualitative and 14 quantitative traits scored using the IRRI descriptor list. Analysis of variance revealed highly significant difference ($P \leq 0.01$) among the accessions for all quantitative traits studied except for grain width. Qualitative data revealed some variations among the traits. Four significant principal components analysis were identified and accounted for 55.3%. PC1 had Eigen-value of 0.44 explaining 18.5% of the total variation. Next was PC2 which had 0.31 as its Eigen-value, explained 14.5% to the total variation. Correlation analysis indicated that length of ligule was highly significant and positive with leaf width of blade. Similar observation was made with grain length and length of ligule. The SSR markers were highly informative as generated by the powerMarker V3.25 software. At the

similarity coefficients 90%, the highly distance genetic diversity was found between two accessions, ACSS37 and ACSS1. Cluster X was the largest of all the clusters while Clusters VII and VIII were the second largest clusters with 7 accessions each. The outcome of this study should be useful to manage the gremplasm conservation and future rice genetic improvement. However, all the accessions may be cultivated over time at differe nt locations on the field to ascertain their stability and purity.

DEDICATION

To God be the glory, I dedicate this thesis to my beloved mother, Korto Mama Luther, for her

prayers and encouragement during my study.

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LISTS OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
CARI	Central Agricultural Research Institute
CRD	Completely Randomized Design
CRIG	Cocoa Research Institute of Ghana
CTAB	Hexadecyl Trimethyl-Ammonium Bromide
DNA	Deoxyribonucleic Acid
ESA	East and Southern Africa
FAO	Food and Agriculture Organization
GY	Grain Yield
IRRI	International Rice Research Institute
MOFA	Ministry of Food and Agriculture
NPGS	National Plant Germplasm System
NTSYS	Numerical Taxonomy and Multivariate Analysis
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PGR	Plant Genetic Resources
PGRRI	Plant Genetic Resources Research Institute
SSR	Simple Sequence Repeat Markers
UPGMA	Unweighted Paired Group Method Using Arithmetic Averages
UV	Ultra Violet Light
WARDA	West Africa Rice Development Association

WSA

West and Southern Africa

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CHAPTER ONE

1.0 INTRODUCTION

Rice (*Oryza sativa* L., 2n = 24), a member of Poaceae (Gramineae) is the world's most important staple food crop that feeds over half of the global population (Khush, 2005). It is cultivated in tropical and subtropical regions. Rice is grown in more than 114 countries, over an area of 161.4 m ha in a wide range of ecosystems under varying temperature and water regimes with the production of 466.7 mt (on milled basis) (FAO, 2011). According to Nwanze *et al*. (2006) approximately 20 million farmers are engaged in rice production in sub-Sahara Africa (SSA) and about 100 million people depend on it directly for their livelihoods on the continent.

Rice is rapidly becoming a staple food in the African diet; and its production in SSA continues to be outpaced by consumption as a result of low and stagnated production. Imported rice accounts for 50 percent of sub-Saharan Africa's rice requirement (FAO, 2006a). Rice is no longer a luxury food but has become the cereal that constitutes a major source of calories for the urban and rural poor. Rice production in SSA has been bedeviled with conditions such as environmental degradation due to pesticide usage, excessive water usage, and nutrient contamination, methane emission and ammonia volatilization and these conditions require urgent attention (Newmah, 2010).

A wide range of technologies are, however, available and can be used as tools for reducing these adverse consequences of rice production but they are, however, not extended to majority of rice growers or farmers (FAO, 2006b). Self-sufficiency in rice production is, however, declining as demand increases. Little attention has been paid to the improvement of Liberian and Ghanaian rice

germplasm evaluation and the genetics of some quality traits. Thus, there is very little information available on the genetic diversity of Liberian and Ghanaian rice germplasm for crop improvement and conservation purposes. There is an urgent need to increase and improve the production of rice in Africa in order to meet up with the high demand. The need for increasing rice cultivation depends not only on cultural/traditional practices, but also, on their inbuilt genetic potential to withstand stresses. A successful breeding programme will depend on the genetic variability of a crop for achieving the goals of improving the crop and producing high yielding varieties (Padulosi, 1993). The first step in achieving this is to evaluate and characterize available rice germplasm or genotypes at both morphological and molecular levels; as phenotypic and genotypic diversity will reveal important traits or accessions of interest to plant breeders (Singh, 1989).

1.1 Main objective

The overall objective of this study is to evaluate the genetic and morphological variability of rice germplasm from Liberia and Ghana using morphological and simple sequence repeat (SSR) markers.

1.2 Specific objectives

1. To assess the extent of genetic diversity and relatedness among rice germplasm collections in Liberia and Ghana at both the morphological and molecular levels.
2. To estimate correlations among measured quantitative traits.
3. To identify some morphological traits that are discriminating and contribute most to the total variability among rice germplasm accessions using principal components analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany, Classification and Taxonomy of Rice

Rice (*Oryza sativa, O. glaberrima*), morphologically, is an annual grass and one of the most essential crops cultivated worldwide. Rice is the seed of a monocot plant which is in the family called Gramineae (Anon, 2004). Fageria *et al.* (2003) reported on rice as a main source of 35-60% dietary calories consumed by more than 3 billion people and probably the most versatile crop. The two species of domestic rice *Oryza sativa*, grown throughout the world and *Oryza glaberrima*, cultivated mostly in West Africa. According to Calpe (2002), rice is a major staple food for the rural population, and mainly cultivated by small farmers in holdings of less than 1 ha. Rice provided a "wage" commodity for workers in the cash crop and non-agricultural sectors. Its duality gave rise to conflicting policy objectives, with policy-makers to save farmers when prices drop, and or defended consumer purchasing power with sudden price increases.

Rice can grow in diverse environments; but it is noted to grow faster and more vigorously in wet and warm conditions. This plant develops a main stem and many tillers and may range from 0.6 to 6 meters (floating rice) in height (Anon, 2004). The tiller bears a ramified panicle that measure between 20 and 30 centimeters wide. Each panicle has 50 to 300 flowers (floret or spikelet), which form the grains. The fruit obtained is a caryopsis and has great food security potentials.

The essentiality of rice crop can be seen in its nutrition to much of the population in Asia, Latin America, the Caribbean and Africa. Calpe (2002) reported that rice is central to the food security of over half the world population, not to mention the culture of many communities. Rice is therefore considered a "strategic" commodity in many countries and is, consequently, subject to a wide range of government controls and interventions.

Rice which is a genetic resource can be morphologically and physiologically characterized and evaluated in order to enable breeders understand and appreciate its wide range of genotypic diversities for relevant crop improvement practices. It is therefore important to consider some morpho-agronomic characters that are associated with the yielding ability of the crop (Yoshida, 1981). Based on the "plant type" concept with regard to low and high yielding varieties of rice in Japan, Tsunoda (1964) compared and summarized their morphological characteristics as follows:

1. Low nitrogen responders possess long, broad, thin, drooping, pale-green leaves, and tall, weak stems.
2. High nitrogen responders have erect, short, narrow, thick, dark-green leaves, and sturdy stems. Hence, morphological characterization can be useful in selecting rice genotypes that have the potential for high yields.

Rice crop in the African continent is considered as the main staple food in at least 8 of the 17 countries of West Africa and rapidly gaining importance as a major food among other crops (Jones *et al.*, 1998a). West African rice production is however declining in its self-sufficiency because demand is increasing faster (5.9% annual growth since 1970) than its production (4% annual growth over the period (Zafar *et al.,* 2004). They also indicated that rice growth environments in West Africa are extremely diverse, varying as a function of agro-ecological, geo-morphological, hydrological, and socio-economic factors. Upland rice occupies 80% of the rice area and accounts for 75% of regional production (Jones *et al*., 1998b). It is estimated that over 80% of the resource poor rice farmers in West Africa grow rice as a subsistence crop. Most rice farmers in West Africa grow landraces which are generally tolerant to environmental stresses but whose yield potential is

lower than that of improved varieties. On the other hand, improved varieties bred for improved management conditions generally are not well adapted to traditional farming systems (Morris and Bellon, 2004). New improved rice varieties better adapted to the resource-poor farmers conditions are required and must draw on existing germplasm or be developed by combining desired characters from various germplasm sources. In 2015, production in West Africa have increased little since December. Overall, the campaign is assessed to have yielded 14.6 million tones (9.2 million tones, milled basis), representing a 4 recent year-on year expansion and a new record. The excellent turnout was facilitated by a generally favorable climate, which provided a further boost to state efforts to raise output (FAO, 2016). According to officials, Senegal reaped a record 906 000 tones (634 000 tones, milled basis), up 62 percent, or nearly 350 000 tones, from a year earlier. According to (FAO, 2016), prospects also point to an increase for Ghana to 660 000 tones (396 00 tones, milled basis).

Vaughan *et al.* (2003) considered rice crop as a major source of nutrition for about two-third of mankind. This phenomenon involuntarily provides an avenue for an increased production in order to keep pace with the growing population in spite of its productivity seriously being affected by biotic and abiotic stresses (Zafar *et al.,* 2004). Ogunbayo *et al*. (2005) reported that, Africa's inability to produce rice to self-sufficiency levels is indicative of the presence of major challenges in the rice industry requiring urgent attention. It is necessary to stem the trend of over-reliance on imports to meet the increasing demand for rice. Local potential resources for production should be exploited with sustainable strategies at all levels of the rice industry (Ogunbayo *et al*., 2005). This creates an urgent need to increase and improve the production of rice in Africa in order to meet the high demand. According to Ng *et al.*(1988), expansion of rice production does not only depend on

cultural practices and management, it also depends on the suitability of rice varieties, which must be drawn from existing germplasm that has been collected and conserved by genetic resources centers. During the past three decades, there has seen a steady increase in demand for rice and its growing importance is evident, given its important place in the strategic food security planning policies of many countries and governments to maintain minimum food reserves to ensure food security. Its adoption as a principal staple food is increasing in Africa every day whereas self-sufficiency in rice production declines as demand increases. According to Jamal *et al.* (2009) rice is grown extensively in tropical and sub-tropical regions of the world. More than half of the people on the globe depend on rice as their basic diet and generally extensively consumed in the producing countries. It is estimated that the world population will increase by about 2 billion in the next two decades, and half of this increase will be in Asia where rice is the staple food (Gregory *et al*., 2000), and other continents of the world particularly Asia, Africa and Latin America, where the demand for rice is a top priority as reported by Sasaki (1999, 2002).

According to the Millennium Development Authority on Economic Growth and Poverty Reduction (MoFA, 2005) rice accounts for nearly 13% of total cereal consumption in Ghana. As a growing diet and major staple, it is increasingly replacing other traditional staples of rural and urban dwellers. For instance, the per capita consumption of rice increased from 7 kg/year in 1988/89 to 18.7 kg/year during 1998-2000 as reported by the Ghana living standard survey. MoFA (2005) estimates put per capita consumption of rice at 25 kg/year. Besides, an IRRI/FAO report estimated domestic output of milled rice in 2004 at 14,000 MT against demand of 440,000 MT (MoFA 2005) leaving a domestic deficit in supply. The huge demand for rice productivity amidst associated environmental factors in developing countries coupled with its high population is

evident of these countries incapability to meet world-wide demand. This left the sub-region of West Africa to begin experiencing rice shortages and fluctuation in prices.

Evaluating and characterizing landraces of rice germplasm will help plant breeders in the subregion to better embark on specific selection to improve rice accessions based on agromorphological traits and variations as a result of differences in their DNA sequences. It is likely to foster increased rice production and productivity if the trend is supported. With the foregoing estimates, MoFA (2005) reported small scale farmers are primary producers, and they continue to contribute towards hunger alleviation by cultivating rice crop over a wide range of environments; but yet still limited in terms of yields due to a number of factors ranging from poor agronomic practices to mainly lack of high yielding varieties. Ogunbayo *et al*. (2005) studied phylogenetic evaluation of forty rice accessions using morphological and molecular techniques within cluster similarities and between clusters morphological differences were observed. Landraces of rice accession differ from improved cultivars in adaptation to soil type, sowing and ripening periods and yield stability particularly, in regions where seasons are unpredictable. This re-enforced the importance of investigating local germplasm for breeding purposes. Therefore, accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including;

1. Analysis of genetic variability in landrace genotype and cultivars (Cox *et al*., 1986),
2. Identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barret and Kidwell, 1998)
3. Introgression of desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998).

Frankel *et al.* (1995) and Qualset *et al.* (1997) reported that landraces are the most diverse populations of cultivated rice plants. Besides being adapted to their natural and man-made environments, landrace genotypes tend to be co-adapted. Genetic variation within a landrace may be considerable, but is far from random (Qualset *et al*., 1997). Guei and Traore (2001) described the rice to constitute a good source of unique genes for stress tolerance and genetically dynamic. The genetic diversity among and within landraces makes them a valuable resource as potential donors of genes for the development and maintenance of modern crop varieties, and for direct use by farmers (Soleri and Smith, 1995). The utilization of these rice genetic resources had been limited to only adaptable genotypes as reported by Caldo *et al*. (1996). Despite these positive attributes, little efforts have been made to characterize and evaluate landrace rice accessions of West Africa origin.

2.2 Socio-economic importance of rice

According to Leviton and McMahon (1996), rice is the most common cereal grain consumed, and offers considerable economic and agricultural importance. Rice is the only cereal crop, almost entirely consumed by humans, unlike wheat and corn. The global, annual rice production is 562, 260 thousand metric tons (tmt), a yield that is a close third to wheat (584, 874 tmt) and maize (576, 821tmt) (Mullins, 1999).

Africa has become a big player in international rice markets, accounting for 32% of global imports in 2006 Pardey *et al*. (2006), at a record level of 9 million tones that year. Africa's emergence as a big rice importer is explained by the fact that during the last decade rice has become the most rapidly growing food source in sub-Saharan Africa (Sohl, 2005). Indeed, due to population growth

(4% per annum), rising incomes and a shift in consumer preferences in favor of rice, especially in urban areas (Balasubramanian *et al.*, 2007), the relative growth in demand for rice is faster in this region than anywhere in the world (WARDA, 2005). This is occurring throughout the sub-regions of sub-Saharan Africa (SSA).

Rice production has been expanding at the rate of 6% per annum (2001–2005), with 70% of the production increase due mainly to land expansion and only 30% being attributed to an increase in productivity (Fagade, 2000; Falusi, 1997; Africa Rice Centre, 2007). Much of the expansion has been in the rain fed systems, particularly the two major ecosystems that make up 78% of rice land in West and Central Africa (WCA): the upland and rain fed lowland systems (Dingkuhn, 1997). Demand for rice in WCA has far outstripped the local production (Africa Rice Centre, 2007). According to Dingkuhn (1997), land expansion of the West and Central Africa (WCA) mainly seen in the rain fed systems (constituting 78% upland and rain fed lowland).

According to OSIRIZ (CIRAD's Observatory of International Rice Statistics), Africa cultivated about 9 million hectares of rice in 2006, a production figure that surpassed 20 million tonnes for the first time but with a future increment of 7% per annum (WARDA, 2005; FAOStat, 2002a). The production-consumption gap in this region is being filled by imports, valued at over US$ 1.4 billion per year. The share of imports in consumption rose from an average of 43% from 1991 to 2000, to an average 57% by 2002-2004 (WARDA, 2005; FAOStat, 2002b).

2.3 Rice production and productivity in West Africa

While rice is very much a cash crop for small-to medium-scale farmers in the East and Southern Africa (ESA) region, it is more of a subsistence crop in West Africa where most of the continent's

rice is produced. In West Africa, 75% of the total production of rice in 1999/2003 was from upland, hydromorphic and lowland ecosystems, with about 25% from irrigated fields. Rice is also produced in mangrove production systems and in flooded environments. Research on the mangrove ecology is coordinated by the Rokupr Rice Research Station in Sierra Leone. Low yield constitutes one of the main challenges of rice production in SSA. From 2001–2005, average rice yields in SSA exhibited a highly variable trend, positive or negative across subregions and countries (Africa Rice Trends, Center, 2007). The overall rice production increase during the same period was mainly due to the expansion of rice production into marginal areas in West Africa where most production occurs.

2.4 Rice production in Ghana

In Ghana, rice is a major food crop grown in most communities. It is cultivated both as food crop and cash crop. Rice production presently in Ghana is estimated from "200,000 to 300,000 MT of paddy or roughly 120,000 to 180,000 MT of milled rice, the bulk of which comes from the Upper East, Northern and Volta Regions" (Global Food Security Response, 2009)(Table 1). The crop accounts for nearly 13% of total cereal consumption and it is increasingly replacing indigenous staples of both rural and urban dwellers. Small scale farmers are the major producers of rice in Ghana. They grow rice over a wide range of different yields under different agro-ecological and environmental conditions (MoFA, Inland Valleys Rice Development Project 2005). The differences in production have been attributed to rainfall which remains the foundation of rice production. Between 1994 and 2004, production increased from expansion of land area under cultivation. By the end of 2008, rice production in Ghana was estimated at 301,921 MT of paddy, yielding roughly 181,000 MT of milled rice, produced on 132,921 hectares, resulting in an average

yield of 2.27 MT/Ha of paddy for upland and lowland rice aggregated (GFSR, 2009). It is generally agreed that current domestic production accounts for between 30 to 40 percent of domestic consumption (approximately 600,000 MT of milled rice), leaving almost 70% supply deficit to be filled by domestic import supply (Table 2.1).

Table 2.1: Domestic Rice production in the Republic of Ghana

	Lowland rain-fed	Upland Rain-fed	Irrigated	Total
Planted Area (Ha)	93,750 ha	18,750 ha	10,200 ha	122,700ha
Paddy (MT/Ha)	2.4 ton/ha	1.0 ton/ha	4.5 ton/ha	2.4ton/ha
Paddy Production(MT)	224,700 ton	18,750 ton	45,900 ton	289,650ton
% in Area	77 %	15 %	8 %	100 %
% in Production	78 %	6 %	16 %	100 %

Source: Asante *et al*., (2013)

2.5 Morphological diversity of rice

Morphological characterization and physiological traits evaluation are particularly useful in revealing economic importance of crop. It is needed for the sub region of West Africa since evaluation of germplasm accessions in any collections is essential to ensure the principles of conservation and utilization of germplasm (Riley *et al*., 1995). Qualset and Shands (2005) indecated that characterization was needed to handle the diversified genetic resources of plants to improve the nutritional value of foods to meet changing consumers demand, combat pest and diseases and adapt to the environmental changes. Nevertheless, the amount of data related to agronomic traits on crop germplasm is limited. This is due to relatively high costs and difficulties of large scale experimental trials, the use of agronomic evaluation to characterize germplasm collections is far from the actual necessity of uncovering the phenotypes of agronomic interest in accessions of a collection. More should definitely be done in this area in order to stimulate a higher use of stored germplasm in breeding programs. A

complete agronomic trait evaluation of crop germplasm remain to be achieved though, seems to be practically impossible (IRRI, 1996).

Morphological traits are used as a preliminary evaluation tool due to their easiness and can be employed as a common approach for assessing genetic variability among phenotypically distinguishable rice accessions. The evaluation of morphological traits usually reveals important traits which are essential to characterize the genetic resources. Rice (*Oryza sativa* L.) is considered as one of the most important cereal crops and the staple food for more than half of the world's population (Jiang *et al.,* 2013). It is considered as the ideal model plant to study grass genetics and genome organization due to its diploid nature, comparatively small genome size (Causse *et al*., 1994; Kurata *et al.*, 1994), considerable level of genetic polymorphism (McCouch *et al.,* 1998), a large amount of well conserved genetically diverse materials and fully sequenced genome (Pervaiz *et al.*, 2010; Rabbani *et al*., 2010).

Due to the importance of rice as one of the major world food crops, its genetic diversity has attracted great interest of researchers. The genetic diversity of rice has been used effectively to increase productivity. Majority of the programs involved in improvement of rice productivity has been mainly focused on the yield aspects; meanwhile other important agro morphological characters have been neglected. It is essential to not only conserve the existing genotypes but also to explore the gene-pool characteristics to capture such important agro-morphological characters.

In favor of this reason, it is crucial to discover the genetic diversity and the relationships among cultivars. Morphological evaluation is a preliminary step to estimate the variability and relationship among cultivars although several other tools are also used extensively (Smith *et al*., 1991).

Therefore, to establish a country specific successful crop improvement programme, it is very much essential to discover the morphological variability of existing rice cultivars within the country.

Suriyagoda *et al.* (2011) have reported variability of rice varieties. They had similar observations in terms of dendrogram analysis, clustering groups and principle component analysis with some exceptions. These exceptions were identified in terms of variations in cluster formation and it grouping behaviors of their tested rice varieties. According to Steel (1972), the exceptions were possible due to the variations in external conditions such as soil types, and soil fertility levels and soil moisture regimes associated with two cropping systems. Furthermore, the genetic make-up of seed, environment and field management practices has been reported to influence the morphology of a crop (Singh and Rachie, 1985).

2.6 Grain yield and characters associated with grain yield in rice

Yan *et al*. (2002) reported grain yield (GY) in cereals as one of the most important and complex traits in plant breeding experiments. Continued improvement of GY remains the top priority in most breeding programs. Grain yield in rice depends on various growth and component traits, and is the final outcome of a combination of different yield components, such as the panicle number per plant, the filled grain number per panicle, and the weight per grain (Yoshida, 1983). Therefore, it is of significance to reveal the genetic contribution of yield component traits to GY. Many breeders have paid much attention to the concept of plant ideotypes and proposed several models for high-yielding rice, such as the ‘heavy-panicle’ and the ‘multi-panicle’ types (Donald, 1968). It was suggested that an increase in GY could be effectively achieved through yield component improvement since yield components have higher heritability than GY (Xiong *et al*, 1992).

Correlation and path analyses have revealed the relationships between GY and its yield components at both the phenotypic and genetic levels (Xiong *et al*, 1992). However, the implications in those studies to breeding practice were limited due to complicated correlations between GY and its yield components, which were effected to varying degrees by numerous factors such as environmental effects and experimental error (Risch, 2000; Darvasi and Pisant´eShalom, 2002). Selection of yield components was not highly effective in increasing GY, because of their negative correlations to each other (Ribaut *et al.* 1997). Until now, understanding of the genetic basis of correlation among quantitative traits has remained unresolved. Molecular marker techniques and statistical methods facilitated the analysis of quantitative trait loci (QTLs) (Lander and Botstein 1989; Wang *et al.*, 1999). Many QTL mapping experiments for GY and its yield components have been conducted based on the separate phenotypic values (Chen *et al.*, 2000; Lehmensiek *et al*., 2006).

Some QTLs associated with GY, coinciding with those for yield components, were usually regarded as pleiotropic QTLs or closely linked loci (Chen *et al.* 2000). It was revealed that correlated traits often have QTL(s) at the same chromosomal locations (Huang *et al*. 2006; Paterson *et al*., 1991; Julier *et al.*, 2007). Force *et al*. (1999) reported that pairs of traits with higher genetic correlations would share more common QTL regions than those with smaller genetic correlations. Zhuang *et al.* (1997) suggested that pleiotropism rather than the close linkage of different QTLs might be the major reason for the correlation among related traits. A common problem of these analyses is that QTL mapping for related traits was conducted by considering the phenotypic values rather than conditioning one trait on the other related traits. However, to condition one trait on the

other(s) can provide statistical estimates of the effects and positions of QTLs, and detect their genetic relationships at the QTL level (Melchinger *et al.*, 1998).

The genetic basis and relationship of GY with its yield components at the QTL level in rice are still poorly understood and needs to be determined. Melchinger *et al.* (1998) proposed conditional analysis methods, which can be used to exclude the contribution of a causal trait to the variation of the resultant trait. The remaining variation of the resultant trait is defined as conditional variation, or net variation, which indicates the extra effects of genes that are independent of the causal trait (Atchley and Zhu, 1997). This method has been used to study the dynamic behavior of developmental traits in cotton (Melchinger *et al.*, 1998) and mice (Atchley and Zhu, 1997). Liu *et al.* (2008) applied this method to identify the QTLs for yield in rice with different component influences, but the QE effects were ignored due to the data being derived only from one environment in their study. Understanding expression patterns of QTLs is one of the major goals in quantitative genetics.

According to the theory of developmental genetics, genes are expressed differently at different times and growth stages (Atchley and Zhu, 1997). Many studies indicated that the expression of QTLs is affected by many factors, such as environments (Zhuang *et al*., 1997), genetic background (Ribaut *et al.*, 1997; Xing *et al.*, 2010), developmental stages (Cao *et al*. 2001), and related traits (Liu *et al*., 2008). According to expression patterns, QTLs can be classified into at least 5 types, namely non-specific, environment-specific, genotype-specific, stage-specific and trait-specific QTLs. Non-specific QTLs are the most stable since their expression is not influenced by inner and external factors, and they can be used under various conditions. The other 4 types of QTLs are

unstable, and are suitable only under specific conditions. Comparing both the unconditional and conditional QTL mapping methods, some new QTLs controlling the target trait could be detected by conditional QTL mapping (Cao *et al.*, 2001; Liu *et al.*, 2008).

Wu *et al*. (2004) and McCarty *et al.* (2008) extended and applied this method for the analysis of conditional variation of the resultant trait on multiple related traits in cotton. A statistical procedure for analyzing of conditional genetic effects combined with the QTL mapping method, namely the conditional QTL mapping method was proposed to investigate the genetic relationship between 2 traits at the QTL level (Melchinger *et al.*, 1998). It could distinguish whether the QTL of the target trait is associated with its component trait or not. The conditional QTL mapping method has been used to study the net QTL effects at different developmental stages of plant height and tiller numbers in rice (Cao *et al.*, 2001), and to explore the QTLs contributed to the conditional variation of a resultant trait on its related traits (Liu *et al*., 2008). According to Yoshida (1983) grain yield in rice is a complex trait, which is the combination of different yield components. The yield component traits, however, are less environmentally sensitive and have higher heritability than grain yield (Yano and Sasaki, 1997). In the classical Mendelian approach, it was very difficult to identify the individual genes controlling a quantitative trait (Comstock, 1978).

Recent advances in QTL mapping facilitated the analysis of the genetic basis of quantitative traits at the single locus level (Lander and Botstein, 1989; Zeng, 1994; Li *et al.*, 2003; Yang *et al.*, 2007). Quantitative trait loci for rice yield and its components have been reported (Champoux *et al*., 1995; Yadav *et al.*, 1997; Xing and Zhang 2010), and pleiotropy or close linkage of QTLs has been assumed as the basis of relationships among them (Paterson *et al.* 1991; Zhuang *et al.* 1997; Julier

et al., 2007). However, a common problem associated with the QTL mapping analyses regarding yield and its components reported so far was based on the separate analysis of each trait. There is no clear evidence that pleiotropy or close linkage of QTLs would result in genetic correlation among the traits of interest. It is nonetheless impossible to reveal the complex genetic basis of trait correlation by these methods (Melchinger *et al.*, 1998; Liu *et al*., 2008).

2.7 Germplasm collection and characterization

According to Riley *et al*. (1995), characterization of morphological and physiological traits and evaluation is particularly essential in revealing economic importance of a crop. It is important for the sub region of West Africa since appraisal of germplasm accessions in any collections is essential to ensure the principles of preservation and utilization of germplasm. Characterization is needed to handle the diversified genetic resources of plants to improve the nutritional value of foods, meet changing consumers demand, combat pest and diseases and adapt to the environmental changes (Qualset and Shands, 2005). The amount of data related to agronomic traits on crop germplasm is limited. Due to reasons that include relatively high costs and difficulties of large scale experimental trials, the use of agronomic evaluation to characterize germplasm collections is far from the actual necessity of uncovering the phenotypes of agronomic interest in accessions of a collection. More should definitely be done in this area in order to stimulate a higher use of stored germplasm in breeding programs. A complete agronomic trait evaluation of crop germplasm remains to be achieved.

2.8 Core collection and their use in germplasm management

There are a variety of settings where it is necessary to select a subset of lines, populations, or individuals to represent some larger set of germplasm. For instance, conservation geneticists may

want to identify a “core set” of populations that maximize the total amount of genetic variation, subject to an upper limit on the number of lines they can maintain (Frankel 1984; Frankel and Brown 1984; Schoen and Brown 1983). Plant breeders may wish to select a subset of available breeding lines or populations for breeding stock maintenance purposes (Gouesnard *et al.* 2000). The concept of a core collection was introduced by (Brown *et al*. 1989) with the intent of using the core collection to minimize the cost of germplsam conservation whilst ensuring maximum genetic diversity. Accordingly, Van Hintum (1996) further modified the concept of core collection to accommodate a germplasm collection optimally representing specific genetic diversity. This latest modification allows substantial flexibility for the composition of core collections, and justifies the formation of multiple core subsets of a target species in space and time. Recent time, Johnson and Hodgkin (1999) reviewed the current status of core collections and examples of core development and utilization. Overall, they found that experience with core collections has increased as cores for many crops have been developed. In addition, (Brown *et al*.1999) stressed principles, Procedures, progress, problems and promise of the core collections, while Van Huntum 1999) described a general methodology for creating a core collection. Many approaches to constructing core collections have been developed through the years. Several sampling methods to select entries for the core collections have been suggested ranging from random sampling (Brown 1989; to stratified sampling (Peeters and Martinelli, 1989).

2.9 Conservation of genetic materials and importance of genetic diversity studies in rice

Genetic diversity is a study undertaken to classify an individual or population compared to other individuals or populations. Determining genetic diversity can be based on morphological, biochemical, and molecular types of information (Mohammadi and Prasanna, 2003; Sudre *et al*., 2007. Genetic fingerprinting is the unambiguous identification of an individual (based on the

presence or absence of alleles at different markers) or a population (based on frequencies of alleles of the markers). This is an absolute measure and does not differ depending on other individuals or populations under study. Both genetic diversity and fingerprinting studies are done using molecular markers. Molecular markers have advantages over other kinds, where they show genetic differences on a more detailed level without interferences from environmental factors, and where they involve techniques that provide fast results detailing genetic diversity (Binneck *et al*., 2002; Gracia *et al*., 2004; Saker *et al*., 2005).

Preservation of genetic resources entails many activities, several of which may greatly benefit from knowledge generated through applying molecular marker technologies. This is the case for activities related to the acquisition of germplasm (locating and describing the diversity), its conservation (using effective procedures) and evaluation for useful traits. The availability of sound genetic information ensures that decisions made on conservation will be better informed and will result in improved germplasm management. For all the activities related to genetic resources, those involving germplasm evaluations and the addition of value to genetic resources are particularly important as they help identify genes and traits, and thus provide the foundation on which to enhance use of collections (de Vicente, 2005).

Merriam-Webster (1991) reported that 'Characterization' is the description of a character or quality of an individual and the word 'characterize' is also a synonym of 'distinguish', that is, to mark as separate or different, or to separate into kinds, classes or categories. Characterization of genetic resources thus refers to the process by which accessions are identified or differentiated. This identification may, in broad terms, refer to any difference in the appearance or make-up of an accession. In the agreed terminology of gene banks and germplasm management, the term

'morphological characterization' stands for the description of characters that are usually highly heritable, easily seen by the eye and equally expressed in all environments (IPGRI/CIP, 2003). Characterization in genetic terms refers to the detection of variation as a result of differences in either DNA sequences or specific genes or modifying factors.

According to de Vicente *et al.* (2005), genetic characterization clearly offers an enhanced power for detecting diversity (including genotypes and genes) that exceeds that of traditional methods. Likewise, genetic characterization with molecular technologies offers greater power of detection than do phenotypic methods. This is because molecular methods reveal differences in genotypes, that is, in the ultimate level of variation embodied by the DNA sequences of an individual and not influenced by environment. Standard characterization and evaluation of accessions may be routinely carried out by using different methods, including traditional practices such as the use of descriptor lists of morphological characters. They may also involve evaluation of agronomic performance under various environmental conditions. In contrast, genetic characterization refers to the description of attributes that follow a Mendelian inheritance or that involve specific DNA sequences. In this context, the application of biochemical assays such as those that detect differences between isozymes or protein profiles, the application of molecular markers and the identification of particular sequences through diverse genomic approaches all qualify as genetic characterization methods.

2.10 Genetic diversity or characterization studies

Molecular characterization, by itself or in conjunction with other data (phenotypic traits or georeferenced data), provides reliable information for assessing, among other factors, the amount of genetic diversity (Perera, *et al.,* 2000), the structure of diversity in samples and populations

(Shim and Jørgensen (2000), rates of genetic divergence among populations (Maestri *et al.*, 2002).

Negash *et al.* (2002) reported on Abyssinian banana or ensete (*Ensete ventricosum* (Welw.) Cheesman) from Ethiopia, which was analyzed with AFLP markers. Out of the 146 clones from five different regions, only 4.8% of the total genetic variation was found between regions, where as 95.2% was found within regions. Negash *et al.* (2002) reported a reduced number of clones for conservation and indicated the existence of a common practice of exchange of local types between regions, which, in its turn, emphasized the need to collect further in different farming systems.

A study on taro (*Colocasia esculent* (L.) Schott) genetic diversity in the Pacific, using SSR markers, showed that many of the accessions from countries of the Pacific region were identical to those of Papua New Guinea. According to Mace *et al.* (2005), the cultivars may originally have been introduced throughout the region from Papua New Guinea and that collection of taro genetic diversity could focus on Papua New Guinea alone. According to work done by Papa and Gepts (2003), molecular characterization also aids determine the breeding behavior of species, individual reproductive success and the existence of gene flow, i.e., the movement of alleles within and between populations of the same or related species, and its consequences. Molecular data is noted to improve and allow the elucidation of phylogeny, and thus assist in the basic knowledge for understanding taxonomy, domestication and evolution. Information from molecular markers or DNA sequences offers a good basis for better conservation approaches (Nwakanma *et al.*, 2003). Börner *et al.* (2000) analyzed bulk seed of wheat accessions to test their genetic integrity after 24 cycles of regeneration and after more than 50 years of storage at room temperature in a gene bank. They found neither contamination nor incorrect manipulation effects such as mechanical mixtures,

but did identify one case of genetic drift in one accession. The fact that IPK-Gatersleben gene bank (Germany) splits its germplasm samples into either almost or completely pure lines, i.e. accessions, is expected to have contributed to this very positive finding (de Vicente, 2005). However, in the same gene bank, a study examined the genetic constitution of rye accessions that underwent frequent regeneration. Results showed that

1. A significant number of alleles present in the original sample was lacking in the newly regenerated material,
2. New alleles in the new material were not present in the first regeneration sample (Chebotar *et al.,* 2003). Thus, the use of molecular markers can quickly help check whether changes in alleles or allele frequencies are taking place.

Management of germplasm established in a collection (usually a field, seed or *in vitro* gene bank) comprises several activities. Usually, such activities seek to ensure the identity of the individually stored and maintained samples, to ensure the safeguarding of genetic integrity and genetic diversity and to have the material available for distribution to users. These tasks are primarily a responsibility of gene bank managers and curators, and involve the control of accessions on arrival at the facilities, as well as their continuous safeguarding for the future through regeneration and multiplication. For all these routine activities, information about the genetic constitution of samples or accessions is critical and provides the most important means of measuring the quality of the work being performed.

Molecular information has been used to assess the need for decreasing the size of germplasm collections, which otherwise would add costs to the long-term conservation of germplasm. Dean *et*

al. (1999) used microsatellite markers to analyze the genetic diversity and structure of 19 sorghum accessions known as 'Orange' in the USDA's national sorghum collection. Two redundant groups (involving five entries) were found among the 19 accessions evaluated. It was also realized that much of the total genetic variation was partitioned among accessions. Dean *et al.* (1999) concluded that the number of accessions held by the US National Plant Germplasm System (NPGS) could be significantly reduced without risking the overall quantity of genetic variation contained in these lots.

According to Barcaccia (2010), selected molecular technologies render cost-effective and comprehensive genotypic profiles of accessions ('fingerprints') that may be used to establish the identity of the material under study. Simultaneously, these technologies can detect contaminants (and, in the case of material mixtures, contamination with introgressed genes from other accessions or commercial varieties), as well as the presence of redundant materials (or 'duplicates'). Molecular data has been reported by Chwedorzewska *et al.* (2002) to provide the baseline for monitoring natural changes in the genetic structure of the accession, or those occurring as a result of human intervention (e.g. seed regeneration or sampling for replanting in the field). Whatever the case, analysis of molecular information allows the design of strategies for either purging the consequences of inappropriate procedures or amending them to prevent future inconveniences (van Treuren and de Vicente, 2005). Markers were also helpful in examining genetic identities and relationships of *Malus* accessions (Hokanson *et al.*, 1998). Eight primer pairs unambiguously differentiated 52 of 66 genotypes in a study that calculated the probability of any two genotypes being similar at all loci analyzed as being about 1 in 1,000 million. The results not only

discriminated among the genotypes, but were also shown to be useful for designing strategies for the collection and *in situ* conservation of wild *Malus* species.

Characterization has benefited from several procedures resulting from advances in molecular genetics such as genetic and QTL mapping, and gene tagging (Yamada *et al.,* 2004; Xing and Zhang, 2010). Research in this field has led to the acknowledgement of the value of wild relatives, in which modern techniques have discovered useful variation that could contribute to varietal improvement (van de Wouw *et al.,* 2010; de Vicente *et al.* 2006). A small number of potential duplicates were identified in a core collection of cassava (*Manihot esculenta* Crantz) when isozyme and AFLP profiles were compared (Chavarriaga-Aguirre *et al.,* 1999). The core collection had been assembled with information from traditional markers, which proved to be highly effective for selecting unique genotypes. Molecular data were used for efficiently verifying the previous work on the collection and ensure minimum repetition. The taro core collection for the Pacific region was treated in a similar manner (Mace *et al.,* 2005). Thus, gene bank managers can easily realize the potential value of using molecular methods to support and possibly modify or improve a gene bank's operations.

A special and increasingly important role of genetic characterization is that of identifying useful genes in germplasm, that is, of maximizing conservation efforts. Because the major justification for the existence of germplasm collections is use of the conserved accessions, it is important to identify those valuable genes that can help develop varieties that will be able to meet the challenges of current and future agriculture (de Vicente *et al.*, 2005).

2.11 Genetic erosion

The germplasm or genetic resources among the natural resources of crop plants have suffered a sharp depletion in both the number of crop species and the genetic diversity expressed by the amount of genetic variation within a species since the beginning of scientific breeding (Frankel, 1973; Harlan, 1975). The genetic resources of crop plants have been dwindling at an alarming pace along with the rapid pace of development during the last three decades. The genetic base of the major food crops suffered a sharp reduction when the farmers, consumers, crop breeders, and government demanded genetic uniformity among the new varieties. Paradoxically, genetic erosion is a by-product of successful plant breeding (Paddock, 1970; Hawkes, 1983). The rapid spread of improved varieties has intensified the displacement of the traditional unimproved cultivars (land races) and accelerated their extinction. The trend toward greater uniformity has increased the genetic potential vulnerability of the major crops to epidemics of diseases and insects (National Academy of Sciences, 1972).

2.12 Molecular diversity of rice

Having a better knowledge of the molecular basis of the important biological phenomena in crops is essential for the effective preservation, management, and efficient utilization of plant genetic resources (PGR). According to Barcaccia *et al.* (2010), an adequate knowledge of existing genetic diversity, where in plant population is found and how to best utilize it, is of fundamental interest for basic science and applied aspects like the efficient management of PGR. The improvement of PGR is dependent on continuous infusions of wild relatives, traditional varieties and the use of modern breeding techniques.

These processes all require an assessment of diversity at some level, to select resistant, highly productive varieties (Barcaccia *et al.,* 2010). The assessment of genetic diversity within and between populations, according to Barcaccia *et al.* (2010), is routinely performed at the molecular level using various laboratory-based techniques such as allozyme or DNA analysis, which measure levels of variation directly. Genetic diversity may be also gauged using morphological, and biochemical characterization and evaluation: Each of the techniques has its particular bottlenecks / problems / limitations:

1. Morphological characterization does not require expensive technology but large tracts of land are often required for these experiments, making it possibly more expensive than molecular assessment. These traits are often susceptible to phenotypic plasticity; conversely, this allows assessment of diversity in the presence of environmental variation.
2. Biochemical analysis is based on the separation of proteins into specific banding patterns. It is a fast method which requires only small amounts of biological material. However, only a limited number of enzymes are available and thus, the resolution of diversity is limited.
3. Molecular analyses comprise a large variety of DNA molecular markers, which can be employed for analysis of variation. Different markers have different genetic qualities (they can be dominant or co-dominant, can amplify anonymous or characterized loci, can contain expressed or non-expressed sequences, etc.). However, the methods require expertise and high initial investment.

Barcaccia *et al.* (2010) reported that the concept of genetic markers is not a new one and further stated that in the nineteenth century, Gregor Mendel employed phenotype-based genetic markers in his experiments. Later, phenotype-based genetic markers for *Drosophila melanogaster* led to the founding of the theory of genetic linkage, occurring when particular genetic loci or alleles for

genes are inherited jointly. The limitations of phenotype-based genetic markers led to the development of DNA-based markers, i.e., molecular markers. A molecular marker can be defined as a genomic locus, detected through probe or specific starters (primer) which, by virtue of its presence, distinguishes unequivocally the chromosomic trait which it represents as well as the flanking regions at the 3' and 5' extremity (Barcaccia *et al.*, 2010).

Molecular markers may or may not correlate with phenotypic expression of a genomic trait. They offer numerous advantages over conventional, phenotype-based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell. Additionally, they are not confounded by environmental, pleiotropic and epistatic effects. According to Barcaccia *et al.* (2010), an ideal molecular marker should possess the following features:

- be polymorphic and evenly distributed throughout the genome;
- provide adequate resolution of genetic differences;
- generate multiple, independent and reliable markers;
- be simple, quick and inexpensive;
- need small amounts of tissue and DNA samples;
- link to distinct phenotypes;
- Require no prior information about the genome of an organism. Nevertheless, no molecular marker presents all the listed advantages.

Mondini *et al*. (2009) stated that molecular techniques have been applied in the analysis of specific genes, as well as to increase understanding of gene action, generate genetic maps and assist in the development of gene transfer technologies. Molecular techniques have also had critical roles in studies of phylogeny and species evolution, and have been applied to increase our understanding

of the distribution and extent of genetic variation within and between species. These techniques are well established and their advantages as well as limitations have been realized (Mondini *et al.*, 2009).

Agarwal *et al.* (2008) and Primmer (2009) stated that different methods of molecular assessment differ from one another with respect to important features such as technical requirements, genomic abundance, and level of polymorphism detected, locus specificity, reproducibility and cost. Depending on the need, modifications in the techniques have been made, leading to a second generation of advanced molecular markers. Genetic or DNA based marker techniques such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) are now in common use for ecological, evolutionary, taxonomical, phylogenic and genetic studies of plant sciences. These techniques are well established and their advantages and limitations have been documented (Mondini *et al.*, 2009; Agarwal *et al*., 2008; Primmer, 2009).

2.6.1 DNA Based Molecular Techniques in genetic diversity studies.

Simple Sequence Repeats (SSR) are sets of repeated sequences found within eukaryotic genomes (Katti *et al.*, 2001; Bell and Ecker, 1994; Morgante and Olivieri, 1993). These consist of sequences of repetitions, comprising basic short motifs generally between 2 and 6 base-pairs long.

Polymorphisms associated with a specific locus are due to the variation in length of the microsatellite, which in turn depends on the number of repetitions of the basic motif. Variations in the number of randomly repeated units are mainly due to strand slippage during DNA replication where the repeats allow matching via excision or addition of repeats (Schlotterer and Tautz, 1992). As slippage in replication is more likely than point mutations, microsatellite loci tend to be hyper

variable. Microsatellite assays show extensive inter-individual length polymorphisms during PCR analysis of unique loci using discriminatory primers sets. Microsatellites are highly popular genetic markers as they possess: co-dominant inheritance, high abundance, enormous extent of allelic diversity, ease of assessing SSR size variation through PCR with pairs of flanking primers and high reproducibility (Gupta and Varshney, 2000). However, the development of microsatellites requires extensive knowledge of DNA sequences, and sometimes they underestimate genetic structure measurements, hence they have been developed primarily for agricultural species, rather than wild species (Gupta and Varshney, 2000). Initial approaches were principally based on hybridization techniques, whilst more recent techniques are based on PCR (Gupta and Varshney, 2000).

Molecular markers are segments of chromosomes which don't necessarily encode any traits and are not affected by the environment but which are inherited in a Mendelian fashion. Some segments of the chromosome change faster than others (i.e. coding vs. non coding DNA). As a result it is recommended to use fast changing markers for closely related individuals and slow changing markers for less related individuals (different species). Different marker types therefore have different usefulness in fingerprinting individuals and populations. Moreover; a good marker for fingerprinting studies will be cheap to run, or gives a lot of information per run; very repeatable between assays; experience very low error rate and easy, unambiguous to score; and contain many alleles (high information content). The following techniques are those most used in genetic diversity studies and listed in chronological order: RFLP (restriction fragment length polymorphism) (Botstein *et al.*, 1980), SSR (simple sequence repeats or just microsatellites) (Tautz, 1989), RAPD (randomly amplified polymorphic DNA) (Williams *et al*., 1990) or AP-PCR (arbitrarily primed PCR) (Welsh and McClelland 1990), ISSR (inter-simple sequence repeats)

(Zietkiewicz *et al.*, 1994), AFLP (amplified fragment length polymorphism) (Vos *et al.*, 1995), SNPs (single nucleotide polymorphisms) (Chen and Sullivan, 2003) and, more recently, DarT (diversity array technology) (Mace *et al.*, 2009) and other high throughput platforms. These different types of molecular markers are also different as to their potential to detect differences between individuals, their cost, facilities required, and consistency and repetition of results (Schlotterer 2004; Schulman, 2007; Bernardo, 2008).

Arif *et al.* (2011) presented a review summary of various tools of DNA marker technology for application in molecular diversity analysis with special emphasis on wildlife conservation. As a laboratory methodology, fingerprinting and diversity studies require the following steps:

- isolation of DNA
- digestion, hybridization, and/or amplification of DNA into specific fragments
- sizing /or separation of DNA fragment combinations or patterns into a set of individual DNA fingerprints
- comparison of DNA fingerprints from different individuals
- calculation of similarity (or dissimilarity) coefficients for all pairs of entries in the genetic study
- Creation of a dendrogram or graph to visualize the differences.

This investigation combined morphological and molecular techniques (using SSRs) to assess the variation in a collection of rice germplasm from Liberia and Ghana.

2.13 Genetic distance

Genetic distance refers to the genetic divergence between species or between populations within a species. It is measured by a variety of parameters. Smaller genetic distances indicate a close genetic

relationship whereas large genetic distances indicate a more distant genetic relationship. In its simplest form, the genetic distance between two populations is the difference in frequencies of the traits. Genetic distance is a quantitative measure of genetic difference between individuals, population or species at the allelic level (Coulson *et* al., 1998). Many distance measures are available but the choice depends on the kind of data, to be precise, interval data obtained from morphological evaluations, allele frequency data from isozyme or DNA amplification products and presence or absence data. The most common distance measure for morphological data is Euclidean distance or straight line measure, estimated as similarity or dissimilarity. The Euclidean distance between two individuals is given by the sequence root of the sum of all squares of pairwise differences between two individuals, A and B, having morphological measures (i) where i=1…,P represented by X1, X2,…,Xp and Y1,Y2…and Yp is shown in equation 2.1.

…. (2.1)

Unlike Euclidean distance which is based on a metric character, Gower's genetic distance (Gower, 1971) coefficient estimates distance on both qualitative, where a match between two individuals is scored 0 and a mismatch is assigned 1, and quantitative traits which are calculated as the difference in trait value divided by the overall range of trait. Then follow a sum of the individual trait distance for each pair of individuals divided by the number of trails scored in both individuals. The distance measure based on correlation coefficient is a powerful estimation when considering multivariate variables. Distance measure based on correlation coefficient is able to increase accuracy scores when data is not normalized. Moreover, it has the power to detect associations between two or more variables than the classical methods such as Pearson measure as liner relationships.

For SSR molecular data analysis, in which repeat amplification products represent alleles, varation in calculated allele frequencies may be estimated or bands may be scored as presence or absence to generate a binary data. A common distance measure which employs allele frequencies is Roger's distance, Cavalli-Sforza and Edward's (1967) distance *inter alia.* Roger's distance, RDij is given by Equation 4.2 (Mohammadi and Prasanna, 2003)

RDij= ½ [£ (Xai –Xaj)2]1/2…..4.2

Where Xai and Xaj = frequency of the allele a for individual I and j, respectively.

When a bibary data matrix is constructed from s molecular data ,four measures of genetic distance are often used, namely, the Modified Roger's distance GD_{MR} Nei and Li's (1979) coefficient, GD_{NL}, Real and Vargas (1996) coefficient GD_J, and simple matching coefficient GD_{SM} of (Hubalek,1982), where X_{11} is the number of bands or alleles present in individuals: X_{00} is the number of bands or alleles absent in both X_{10} is the number of bands or alleles present in individual *i* only, and X_{01} is the number of bands or alleles present in individual *j* only. Simple matching and

Modified Roger's are example of Eucledean distance measures. The formula for estimation for the genetic distance of a binary matrix data are presented in equations 2.4, 2.5 and 2.6.

$$GD_{NL} = 1 - \left(\frac{2X_{11}}{2X_{11}+X_{10}+X_{01}}\right) \quad \ldots 2.4$$

$$GD_J = 1 - \left(\frac{X_{11}}{X_{11}+X_{10}+X_{01}}\right) \quad \ldots 2.5$$

$$GD_{SM} = 1 - \left(\frac{X_{11}+X_{00}}{X_{11}+X_{10}+X_{01}+X_{00}}\right) \quad \ldots 2.6$$

Roger's genetic distance is also less sensitive to the overestimation of distance produced by heterozygous loci and finite sample size than the Manhattan metric, Cavalli-Sforza and Edwards's distance and the modified Nei's distance. The choice of a genetic distance measure for microsatellite marker is subject to many factors, especially models that make assumptions on the

evolutionary forces, particularly mutation and drift that drive genetic change in the population under study. The stepwise mutations model assumes that mutations are cumulative, like a growing chain typical of microsatellites and tend to increase or decrease value of the allele one step at a time rather than viewing very mutation event as resulting in a totally new allele of the infinite allele model. In effect, alleles resulting from stepwise mutations are related to the one before it, making it possible to trance evolutionary events in a population. In contrast, the infinite alleles model alludes that a mutation event changes an allele from a given state into a totally new allele unrelated to the previous allele which can then be subject to random selection or drift.

Microsatellite development is based on the stepwise mutations model, its distance measures incorporate small changes arising from mutation, while not ignoring drift also. For example, the Nei and Li (1979) genetic distance measure for co-dominant markers is a linear function of the coancestry (Jung, 1993), the Modified Rogers distance is widely preferred because of its statistical and genetic properties. Though the simple matching distance measure has Euclidean properties which facilitate use in analysis of molecular variance, its major demerit is undifferentiated scores for 0-0 and 1-1 matches (Mohammadi and Prasanna, 2003).

2.14 Multivariate techniques for interpretation of genetic distance

Regardless of population size, genetic distance among accessions is better visualized by application of various multivariate statistical techniques that analysis relationships among accessions and traits and group them into clusters on the basis of their genetic distance from multiple measurements on individual operative taxonomic units. The most common multivariate techniques include cluster analysis, principal component analysis or principal coordinate analysis and multidimensional scaling (Thompson *et al.,* 1998).

2.14.1 Cluster analysis

Cluster analysis Darrock (2003) groups individuals on the basis of similarity in their characteristics such that accessions within clusters are homogeneous and among clusteras are heterogeneous. Two cluster methods based on *i*) distance measurement by Johnson and Wichern (1992) and the more robust maximum likelihood estimation and Bayesian methods of Pritchard *et al*. (2000) developed to overcome the constraints of distance –based methods are commonly applied . Mohammadi and Prasanna (2003) compared the most used hierarchical tree-producing cluster method to the less commonly used nontree- generating non-hierarchical methods. Hierarchical cluster is founded upon assessment of relatedness and distance among individuals such that objects that are nearby are more related than those that are far apart. Each cluster connotes the maximum distance which connects members so that different cluster have different maximum distances. Essentially, the hierarchical algorithm is agglomerative as it successively groups individuals and then merges them on the basis of their similarities in three distance categories, Viz., minimum (single linkage), maximum (complete linkage), and the average distance , Unweighted Paired Group with Arithmetic Means, UPGMA (Blashfield *et al*., 1978).

The second most common clustering method is the Ward's minimum variance method (Ward 1963). Mohammadi and Prasanna (2003) gave an extensive review of seven methods of clustering namely, (1) single linkage, (2) compete linkage , (3) UPGMA, (4) Unweighted pair Group method based on centroids (UPGMC) , (5) Median clustering , (6) Ward's method, and (7) Principal Component Analysis (PCA) . Comparison of the clustering methods when applied to classification of barley germplasm collections based on both qualitative and quantitative data on disease resistance (Peeters and Martinelli, 1989) and for the assessment of genetic diversity in dent and

popcorn maize based on inter-simple sequence repeats (Kantety et *al*., 1995) demonstrated that UPGMA provided results that were consistent with known heterotic groups and pedigree information while PCA clearly separated the dent corn lines from the popcorn varieties. Moreover, Rohlf and Wooten (1978) also confirmed that UPGMA gives most accurate clustering method for classification in contrast to Lebeda and Jendrulek (1986) who report of performance of the UPGMA clustering method is its sensitivity to unequal evolutionary rates leading to faulty tree plots.

Melman *et al*. (2009) used the Euclidean distance and the UPGMA in estimating genetic variation in inbred lines from Kenya, CIMMYL and U.S.A. by means of morphological traits and SSRs. Analtsis of genetic diversity with SSRs among 42 CIMMYT maize inbred lines and 48 individuals from each of 7 populations were performed by simple matching coefficient distance measure on the binary matrix of the inbred lines and Nei's ,Rogers' and Modified Rogers distance measures on the 53 SSR allele frequency matrix , respectively. On both sets of data, clustering was done by the UPGMA method (Warburton *et al.,* 2002).

Investigation of genetic diversity and assignment into heterotic groups of one hundred fifty-five lowland tropical CIMMYT inbred lines were determined on 79SSR allele frequencies by the Modified Rogers distance measure follow by UPGMA clustering (Xia *et al*., 2004). Enokai *et al* (2000) studied genetic diversity among 55 inbred lines comprising 41 Japanese highland maize and 14 lines introduced from U.S.A., Canada, and Europe by estimation of the Dice genetic distance measured on 60 SSR loci binary data followed by UPGMA clustering , Similarly, genetic diversity and relationship among 35 Ethiopien and 21 CIMMYT- Zimbbabwe inbred lines was determined on 27 SSR binary , matrix data through a Euclidean dissimilarity coefficient distance matrix and

UPGMA clustering. Beyene *et al.* (2006) used the Ward's minimum variance (Ward, 1963) method and Nei and Li's (1979) coefficient too assess genetic similarities on 20 SSR marker data among 62 traditional Ethiopian highland maize accessions. Magorokosho (2006) estimated the genetic distance between maize population originating from Zambia, Zimbabwe, and Malawi by means of Euclidean coefficient and Ward's modified location module. Dziedzic *et al.* (2014) evaluated over 500 Ghana maize populations with 20 SSRs, modified Rogers distance and clustering by UPGMA. There is scarcity of information on the use of the Dice coefficient for coefficient for estimating genetic distance in the African maize germplsm.

2.14.2 Principal Component Analysis (PCA)

Multivariate data analysis was developed by Pearson (1901) for the social sciences. It was later developed again by Hotelling (1933) for the field of Educational Psychology. Other important recent authors of PCA analysis are Jonson and Wichern (2007) and Joliffe (2002). The application of PCA has been relevant in areas of agriculture, genetics (Menozzi *et al*., 1978) biology, chemistry, ecology food research, just to mention a few. The main idea of PCA is to reduce the dimensions of a data set with large numbers of variables while conserving the variance of the original data. PCA being linear, transforms the original data to new data sets of linear variables (PC) (Johnson and Wichern, 2007; Wilks, 2006). Generally the first PC has the maximum variance, followed by the second, third, etc. The PCA generates three important products, the eigenvalues, eigenvectors and scores, the dominant modes representing the most important characteristics from the original data. Generation of a scatter plot from two or more PCs in space reveals sets of similar individuals (Warburton and Crossa, 2000) and relationships between two or more variables (Mohammadi and Prasanna, 2003).

PCA has been one of the major tools used in genetic diversity studies. PCA was applied to maize genetic diversity assessment by Qi-Lun *et al*. (2008). Similarly maize researchers in Africa such as Beyene *et al*., (2006), also used the PCA technique in their respective maize genetic diversity studies. The statistical power of PCA in genetic diversity studies is evident with the use of descriptor list in which it is common practice to evaluate large number of both morphological traits and molecular parameters. The method reduces the large variables into only few ones that carry majority of the variance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Sites

The experiment was conducted to study the genetic diversity of rice accessions in Liberia and Ghana using morphological characteristics and SSR markers. The experiment comprised two components; namely, morphological and molecular characterizations. The morphological characterization was carried out in pots behind the Insectary Laboratory while the molecular work was also done at the Biotechnology and Tissue Culture Laboratory both at the Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana.

3.2 Seed Sources

Forty-eight (48) upland rice accessions were evaluated (Appendix I). Thirty-five Ghanaian genotypes were obtained from Plant Genetic Resources Research Institute (PGRRI) at Bunso Ghana, while thirteen Liberian accessions were obtained from the Central Agricultural Research Institute (CARI), Suakoko in Bong County, Liberia.

3.3 Evaluation of morphological characteristics of the rice genotypes.

The field experiment was conducted in 12 litre pots at the Department of the Crop and Soil Sciences experimental field at the Faulty of Agriculture, KNUST, Kumasi. It lies between latitude 6.68° N, and longitude 1.57° W.

3.4 Soil used for the pot experiment

Soil collected from Deduako community, Kumasi, was analysed for its physical and chemical properties, and the results are shown in (Appendix III). Soil samples were thoroughly mixed and air-dried and composite sample taken for physico-chemical analysis and biological assaying using standard protocols. Soil pH was determined according to the electrometric method described by Page *et al*. (1982) in a suspension of 1:2.5 soil to distilled water (soil: water) ratio. The modified Walkley and Black procedure as described by Nelson and Sommers (1982) was used to determine organic carbon content in soil samples. The Kjeldahl method involving digestion and distillation was used to determine total nitrogen. The readily acid-soluble forms of phosphorus were extracted with Bray No. 1 solution (HCl; NH4F mixture) (Bray and Kurtz, 1945). Practicle size distribution was determined by the hydrometer method (Day, 1953). Potassium was determined in 1 M ammonium acetate (NH_4OAc) extract (Black *et al*., 1986).

3.5 Experimental setup for morphological characterization

The experiment was laid out in Completely Randomized Design (CRD) with three replications (Appendix II). Each replication was 52 rice accessions as treatment. Out of the 52 accessions, 48 genotypes were successful.

3.6 Agronomic Practices

Standard agronomic practices such as soil sterilization, site preparation, filling of pots, seed preparations, seed sowing and thinning-out, fertilizer application, pest and weed control, watering and harvesting were adhered to.

Soil sterilization was done using the Steaming method. The mental barrel was filled with a Sandy loam soil with 12 litre buckets filled with water added to soil for maximum steaming. Fire wood was added under the mental barrel to help produced excessive heat to kill or reduced the soil borne diseases. The soil was boiled to about 90oC for 30 minutes and later cooled to a room temperature level. The sterilized soil was then distributed into each of the pots.

The site for the pot experiment was cleared using cutlass and a hoe. Plastic sheets were used to cover the area to avoid soil micro infestation. A total of one hundred and forty-four 12-litre buckets (pots) were filled with sterilized soil. The pots (buckets) were arranged/ grouped into three replications for the different rice accessions. Each replicate had 48 pots. Germination test was carried out to ascertain their viability.

The 48 samples of rice accessions under each group were soaked for 24 hours thereafter, incubated for three (3) days for faster germination and emergence. Each of the 48 pots was planted with 4 seeds and later thinned to one. The plants were watered at least once a day manually to prevent waterlog. The compound fertilizer of NPK (15-15-15) was applied by ring method 7 days after planting at the rate of 1.5 g in each pot as a first dose. Second rate of the compound fertilizer (NPK; 15-15-15) was applied one month after the first dose was applied at same rate of 1.5 g per pot. The third dose was the urea fertilizer. The urea was applied in the maximum tillering stage one month

after the second dose of NPK was applied at the rate of 1.5 g per pot. Pests (worms, leaf miners and flies) infestation were avoided using a systemic pesticide, Lambda Master 2.5 EC (25g lambda-cyhalothrin/litre) at a dosage of 100ml / 15 Litre of water (600mls/ha). Spraying of insecticide was done twenty five (25) days after planting. Weed control was done manually by uprooting when necessary. Birds were scared from reproductive stage with bird net to harvesting time.

3.7 Harvesting

Harvesting was done per pot when 90% of plants on a given replication in full were physiologically matured (ripe seeds and droopy panicle). Harvesting was done by carefully cutting panicles with a pair of scissors or harvesting knifes. Panicles from each plant were placed in a separate envelope and labelled to reflect the accession name and replication.

3.8 Morphological characters evaluated

3.8.1 Standard evaluation system for rice data collection

The sources of materials used as a guide for rice data collection is IRRI and WARDA Rice Descriptor (2007). Morphological characters collected were both qualitative (27) and quantitative (14).

3.8.2 Qualitative characters

The qualitative characters studied were basal leaf sheath color, leaf sheath (anthocyanin coloration), leaf blade attitude (angle of the penultimate leaf prior to heading), leaf (auricle color; shape of ligule; color of ligule), flag leaf (attitude of blade), culm (kneeing ability; habit angle), awns (color of awns; distribution of awns), lemma (color of apiculus), lemma and palea color, lemma (anthocyanin coloration of area below apiculus), spikelet (color of stigma), culm (anthocyanin coloration of nodes; internode anthocyanin), culm logging resistance (culm strength), panicle (attitude of main axis; attitude of branches and secondary branching), sterile lemma length, scoring of these traits was based on IPGRI rice descriptor manual (Darwin, 2003) as shown in (Appendix IV).

3.8.3 Quantitative characters measured

3.8.3.1 Culm diameter at basal internode (DABI)

The culm diameter at basal internode (mm) was measured using vernier calipers and the mean was computed.

3.8.3.2 Flag leaf length and width

Leaf length was measured from the base to the tip of the flag leaf, rounded off to the nearest millimetre, while the width was measured at the widest part of the flag leaf and recorded to the nearest mm.

3.8.3.3 Leaf width and length of blade

The leaf width was measured from the widest side of the leaf end of each of the randomly selected plants using the measuring tape. Length of bla de was also measured transversely from the collar to the tip of the leaf blade.

3.8.3.4 Length of ligule (LOL)

Leaf length of ligule of the replicated accessions was obtained when measured by measuring from the base of the collar to the tip.

3.8.3.5 Panicle number per plant (PNPP)

The total number of panicles per plant was counted and recorded at the maturity stage before harvest.

3.8.3.6 Plant height

Plant height (cm) was measured from soil surface to tip of the plant at reproductive stage using the measuring tape.

3.8.3.7 Productive tillers per plant

3.8.3.8 Awn length (mm)

The awn is a long slender extension of the lemma in rice. It was measured from the tip of the spike to the tip of the longest awn.

3.8.3.9 Panicle length of main axis

The panicle length of main axis was measured from the base of the panicle to the tip of the lemma or palea using the measuring tape.

3.8.3.10 One hundred grain weight

One hundred well developed seeds were randomly selected per replication for each accession. The seeds were obtained from the harvested samples of accessions after harvest; dried to 13% moisture content and weighed on a balanced precision scale (METTER PM 400) to determine the 100 grain weight.

3.8.3.11 Grain length

The grain length was measured as the distance from the base of the lowermost glume to the tip (apiculus) of the fertile lemma or palea.

Productive tillers/ Plant was obtained by counting the number of tillers per plant and averaged across replications for each accessions during the maturity stage.

3.8.3.12 Grain width

To obtain the grain width, it was measured as the distance across the fertile lemma and palea at the widest point using the callipers at post-harvest stage.

3.9 Morphological data analysis

Microsoft Excel was used to record and organize the data. The qualitative and quantitative data were subjected to Analysis of Variance (ANOVA) using the GenStat Statistical package version (12th edition, VSN international, Hemel Hempstead) to calculate the relationship between the traits of the genotypes. Least Significant Difference (LSD) at 5% was used to separate the treatments means. MINITAB®17 Statistical Software was used to perform analyze the principal component analysis (PCA).

3.10 Laboratory Experiment: Evaluation of 48 Rice Genotypes from Liberia and Ghana using SSR Markers

3.10.1 Molecular analysis

After the morphological characterization of the accessions, the 48 rice accessions were examined at the molecular level for the estimation of the genetic diversity among and within the genotypes. Sixteen SSR primers designed for rice DNA fingerprinting were used. The primers were obtained from Metabion International Laboratory, Germany. These are shown in Table 3.1.

3.10.2 DNA extraction and purification

About two grams of the young, fresh and healthy leaves of three week old plants from each of the 48 rice accessions were sampled, cleaned with 70% Ethanol and placed in 2ml eppendorf tubes. They were immediately frozen in liquid Nitrogen and ground to a fine powder. The total DNA was isolated from the leaf tissue using CTAB method (Dellapora *et al.* 1983) with slight modification by Takrama (2000) adopted by Cocoa Research Institute of Ghana (CRIG) / Kirkhouse Trust

Mobile Laboratory. Each tube had 800 µl of CTAB buffer containing 2% CTAB, 2%, PVP, 1.4M NaC1, 20mM EDTA pH 8.0 and 0.2% 2 -mercaptoethanol and incubated in a water -bath at 65ºC for 30min with intermit tent vortexing . An equal volume of 24: 1 ratio of Chloroform; Iso -amy1 alcohol was added and spun in 5804 centrifuge (Eppendorf, Germany) for 30 minutes at 14000 rpm.

The supernatant was pipetted into 1.5 ml eppendorf microfuge tube and the Chloroform Iso-amyl alcohol wash was repeated .The supernatant was again pipetted into a new 1.5 ml eppendorf microfuge tube and placed on ice. Iso - propanol was added and kept in the freezer overnight to enhance DNA precipitation. The precipitate was centrifuged at 14000 rpm for 5 minutes and the ice _cold 2-propanol was decanted. DNA Pellets were washed with 10 mM Ammonium acetate _ Ethanol buffer, and 80% ethanol was added to pellets and centrifuged at 6000rpm for 4 minutes. The ethanol was decanted and pellets were dried at 37 ºC in DNA mini centrifuge. The precipitated DNA was re-suspended in 50 µl of 1.0 mM Tris- HCI pH 8.0 and 0.1 mM EDTA pH 8.0 1X TE buffer.

Table 3.1a: List of rice SSR markers used in the DNA fingerprinting

S/No	Marker name	Primers Sequence (5'- 3')	No. of bases	Motif	Number of repeats	Chromosomes	Amplified product size
1	RM 105-F	GTCGTCGAC CCA TCG GAG CCAC	22	CCT	6	9	134
	RM105-R	TGGTCG AGGTGG GGA TCG GGT C	22				
2	RM 125-F	ATCAGCAGCCATGGCAGCGACC	22	GCT	8	7	127
	RM 125-R	AGGGGATCATGTGCCGAAGGCC	22				
3	RM 11-F	TCTCCTCTTCCCCCGATC	18	GA	17	7	140
	RM 11-R	ATAGCGGGCGAGGCTTAG	18				
4	RM 25-F	GGAAAGAATGATCTTTTCATGG	22	GA	18	8	146
	RM 25-R	CTACCATCAAAACCAATGTTC	21				
5	RM 408-F	CAACGAGCTAACTTCCGTCC	20	CT	13	8	128
	RM 408 -R	ACTGCTACTTGGGTAGTCGACC	22				
6	RM 536-F	TCTCTCCTCTTGTTTGGCTC	20	CT	16	11	243
	RM 534-R	ACACACCAACACGACCACAC	20				
7	RM 552-F	CGCAGTTGTGGATTTCAGTG	20	TAT	13	11	195
	RM 552-R	TGCTCAACGTTTGACTGTCC	20				
8	RM 484-F	TCTCCCTCCTCACCATTGTC	20	AT	9	10	299
	RM 484-R	TGCTGCCCTCTCTCTTCTTC	20				
9	RM 171-F	AACGCGAGGACACGTACTTAC	21	GATG	5	10	328
	RM 171-R	ACGAGATACGCTCGCCTTTG	20				
10	RM 271-F	TCAGATCTACAATTCCATCC	21	GA	15	10	101
	RM 271-R	TCGGTG AGACCTAGAGAGCC	20				
11	RM 474-F	AAGATGTACGGGTGGCATTC	20	AT	13	10	252
	RM 474-R	TATGAGCTGGTGAGCAATGG	20				
12	RM 215-F	CAAAATGGAGCAGCAAGAGC	20	CT	16	9	148
	RM 215-R	TGAGCACCTCCTTCTCTGTAG	21				
13	RM316-F	CTAGTTGGGCATACGATGGC	20	GT	8	9	192
	RM 316-R	ACGCTTATATGTTACGTCAAC	21				
14	RM 447-F	CCCTTGTGCTGTCTCCTCTC	20	CTT	8	8	111

	RM 44-R	ACGGGCTTCTTCTCCTTCTC	20				

Table 3.1 continued

15	RM 433-F	TGCGCTGAACTAAACACAGC	20	AG	13	8	224
	RM 433-R	AGACAAACCTGGCCATTCAC	20				
16	RM118-F	CCAATCGGAGCCACCGGAGAGC	22	GA	8	7	156
	RM 118-R	CACATCCTCCAGCGACGCCGAG	22				

F=forward primer, R=Reverse primer. Source: Metabion International Laboratory, Germany

The DNA integrity was assessed by loading 10 at DNA samples (plus 2 µl loading dye) on a 1% agarose gel submerged in 1X TBE buffer, and run at 120 volts for 45 minus. The agarose gel was stained with gel red for DNA band visualisation under a UV transilluminator.

3.10.3 DNA quality control

After extraction, the purity and concentration of the extracted DNA were determined using a spectrophotometer (Biochrom Libra S12) and the absorbance reading taken at 260 mn and 280 mn levels. The DNA quality was assessed using the absorbance ratio at 260 to that at 280 nm wavelengths (A260/A280). A ratio of 1.8-2.0, was considered of good quality, whereas a ratio of less than 1.8 indicated proteins contamination and /or other UV absorbers in the sample, and a ratio higher than 2.0 indicated the sample may be contaminated with RNA or phenols (CIMMYT, 2005). DNA quality was calculated according to Weising *et al.* (1995) as DNA (µg/µ1) =A260x50 where, A260 is the absorbance at 260nm. Therefore, the concentration of DNA in µg/µ1 was calculated as DNA (µg/ml) = [A260x50] x DF, where DF is the dilution factor.

From the quantities of DNA calculated, the appropriate volumes were pipetted into tubes and topped up with sterile distilled water (SDW) to make final concentration of 20 ng/µl for polymerase chain reaction amplifications. For samples with very weak concentration, no further dilutions were done. DNA samples with no visible shearing were then selected for subsequent PCR amplification.

3.10.4 Polymerase chain reaction with molecular markers

PCR Amplifications were carried out in Techne prime thermal cycler (Labnet International Inc., California, USA) and GeneAmp PCR System 9700 (Applied Biosystems, USA) of 96 well plates with heated lid to reduce evaporation. The DNA from the 48 tagged rice samples were fingerprinted using SSR markers. 9µl of premix of 3.3 µl double distilled water, 5.0 µl buffer +dNTPs

(Deoxynucleotide Triphosphates), 0.02 µl $MgCL_2$, 0.6 µl forward and reverse primers and 0.08 µl Taq polymerase was pipetted into 200 µl tube. After that 1 µl of 4ng/ µl DNA was added to make a total volume of 10 µl. The reaction mixtures were short spun and placed in the thermal cycler and ran with the following

held at 4 ºC.

programme: 3.0 minutes at 94 ºC followed by 30 sec at 94 ºC for denaturing, 30 sec at 55ºC for annealing, 1min at 72 ºC for extension, repeated for 35 cycles, an d final extension of 7.0mins at 72 ºC amplified samples were

3.10.5 Agarose gel electrophoresis

The PCR products were separated using horizontal Agarose gel electrophoresis. The amplified DNA fragments were separated on 2.0 % agarose gel stained with 0.005% gel-red solution. After that 2 µl loading dye (6X Bromophnol blue) was added to the PCR products. When the gel was set, the PCR products were added in the wells submerged in 1X TBE buffer. The samples were then run at 120 volts for 2 hours, observed on the UV transilluminatar and photographed.

3.10.6 Gel scoring of DNA fragment

After staining with GelRed, size matching/ calling was done using a 100 bp DNA marker ladder (KAPA Universal DNA Ladder Kit) The bands on the gel were scored for presence (1) or (0) of bands together with their respective sizes. For each markers, alleles for the data set were scored according to size of base pairs of the 100bp ladder DNA marker. This procedure was conducted for each marker until all alleles were scored with the smallest and largest-sized alleles representing the start of the first scoring and end of the last scoring respectively.

3.11 Molecular Data Analysis

The DNA applicant size was considered as a unique characteristic and scored as presence (1) or absence (0) for cluster analysis. A sequential agglomerative and hierarchical clustering dendrogram, which illustrates genetic relationship among the rice genotypes was constructed using unweighted pair group method with arithmetic mean (UPGMA) algorithm for clustering and simple Matching (SM) similarity coefficient in NTSYS software (2.2). Sequential and hierarchical Nested (SAHN) option was employed (Rohlf, 2000). Gene diversity, allele frequency, number of alle les, heterozygosity and polymorphic information content (PIC) values of the markers were calculated using PowerMarker V3.25 computer software.

CHAPTER FOUR

RESULTS

4.1 Morphological Characters

The morphological characters taken included both qualitative and quantitative characters.

4.1.1 Qualitative characters

The results of some qualitative traits assessed among the forty-eight rice accessions for basal leafsheath color; leaf blade attitude (angle of the penultimate leaf prior to heading); leaf (auricle color); leaf (color of ligule); leaf (shape of ligule) and leaf sheath (anthocyanin coloration) are as follows:

Figure 4.1: Basal leaf-sheath colour type for the 48 rice varieties

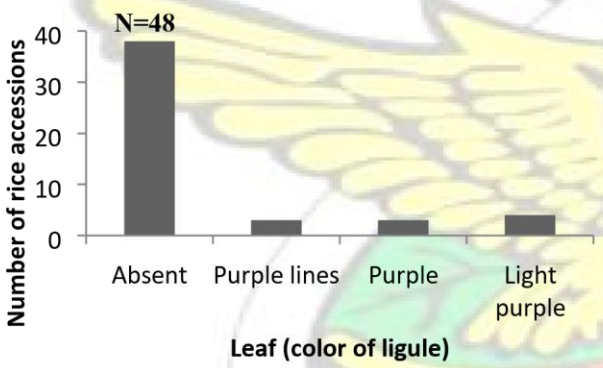
Figure 4.2: Leaf Blade Attitude (Angle of the Penultimate Leaf Prior to Heading)

Figure 4.1 shows the distribution of basal leaf sheath colour in the germplasm. There were five types for the forty-eight rice accessions, namely; green, purple, light purple, purple line and

mixture. Thirty-five rice accessions were of the green colour type while two, seven, three and one of the accessions were of the purple, light purple, purple line and mixture types respectively.

The leaf blade attitude (angle of the penultimate leaf prior to heading) was of three types; erect, horizontal or drooping. Seventeen of the rice accessions studied were of the erect type while ten and twenty-one of the accessions were of the horizontal and drooping Leaf Blade Attitude (Angle of the Penultimate Leaf Prior to Heading) type (Figure 4.2).

According to Figure 4.3, the ligule (colour of ligule) was of four types for the forty-eight rice accessions, namely; purple, light purple, purple line and those that were not having (absent). Three of the rice accessions were of the purple and purple line type, while four and thirty-eight of the accessions were of the light purple and absent types respectively.



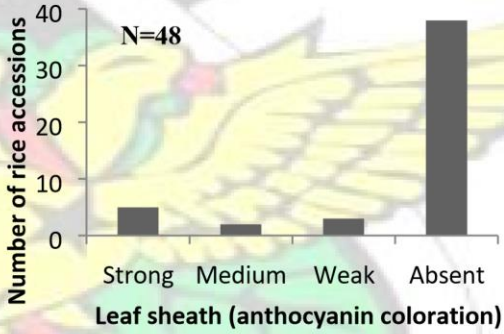


Figure 4.3: Ligule (colour of ligule) type for the 48 rice varieties

Figure 4.4: Leaf sheath (anthocyanin coloration) type for the 48 rice varieties

The leaf sheath (anthocyanin coloration) was of four types for the forty-eight rice accessions, namely; strong, medium, weak and those that were of the absent type. Five of the rice accessions were of the strong type, two, three and thirty-eight of the accessions were of the medium, weak and absent types respectively (Figure 4.4).

According to Figure 4.5, the leaf (auricle colour) was of four types for the forty-eight rice

accessions, namely; purple lines, purple, light purple and those that were of the type absent. Three of the rice accessions were of the purple lines type, while two, six and thirty-seven of the accessions were of the purple, light purple and absent types respectively.

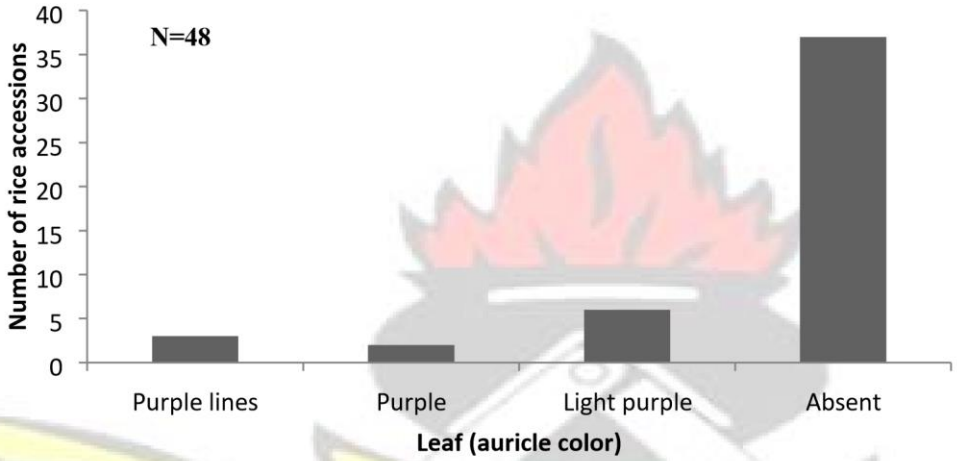


Figure 4.5: Auricle (auricle colour) type for the 48 rice varieties

4.1.2 Morpho-agronomic analysis of quantitative characters

The mean, standard error, range, coefficient of variation, standard error of deviation, F probability of the least significant difference at 5% were computerized for 12 quantitative characters are shown in the Table 4.1 below.

Table 4.1: Summary statistics of 14 quantitative traits measured on 48 rice accessions from Liberia and Ghana

Traits	Mean ±S.E	Range	CV (%)
Culm (DABI)	7.19 ±1.03	1.79 – 13.00	14.3
Flag leaf (FLL)	34.04 ±2.25	13.17 – 67.50	6.6
Leaf (LOB)	51.47 ±1.44	34.67 – 71.83	2.8
Leaf (LOL)	1.83 ±0.17	1.13 – 2.50	9.5
Leaf (WOB)	1.50 ±0.16	1.17 – 2.00	10.5
Panicle (PNPP)	11.47 ±1.10	4.67 – 25.00	9.6
Plant height, cm (PH)	129 ±11.14	66.00 – 187.5 3-29	8.6
Produ. Tillers /plt	11.06±4.6		42.0
Awn Length (AL)	8.04 ±0.12	1.5 – 62.00	14.3
Grain Length(GL)	1.46 ±0.35	0.96 – 2.08	24.2
Grain width (GW)	1.55 ±0.65	1.16 – 3.10	41.6
Grain (WOFDG)	2.17 ±0.40	1.10 – 2.88	18.4
Panicle (LOMA)	24.58 ± 3.27	19.47 – 30.63	13.3
Sterile (LL)	1.30±1.23	1.00-9.00	28.1

WOB=Leaf width of blade, LOL=Leaf length of ligule, FLL= Flag leaf length, DABI=Culm diameter at basal internode, AL= Awn length, LOMA= Panicle length of the main axis, WOFGD=100 grain weight of fully developed grain, GL= Grain length, GW=grain weight, PH= plant height, Sterile (LL) =.sterile lemma length.

4.2 Phenological Studies of Some Early Reproductive Traits among Rice Accessions The following qualitative traits were also studied in the early reproductive stage to characterize their diversity among the 48 rice accessions. These early reproductive traits were lemna colour, lemma length, colour of awns, distribution of awns, culm habit (angle), culm (kneeing ability), flag leaf (attitude of blade), lemma (anthocyanin coloration of area below apiculus), lemma (colour of apiculus), lemma and palea colour and spikelet (colour of stigma)] are as follows:

From Figure 4.6, the sterile lemma colour was of three types for the forty-eight rice accessions, namely; straw, brown and black. Thirty-nine rice accessions were of the straw type while eight and one of the accessions were of the brown and black types respectively.

The sterile lemma length was of two types; either short or medium. Forty-four of the rice accessions

studied was of the short (≤1.5mm) sterile lemma length type while four of the accessions were of the medium (1.6-2.5mm) sterile lemma length type (Figure 4.7).

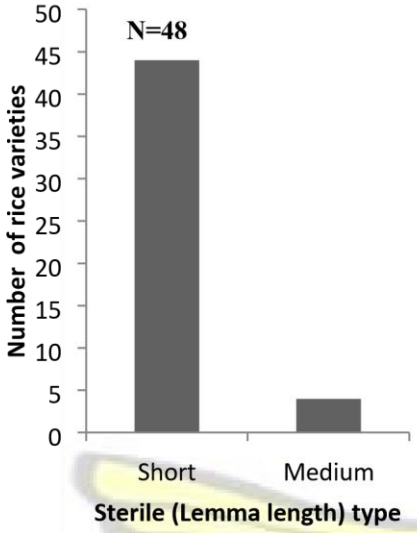


Figure 4.6: lemma length types for the 48 rice varieties studied

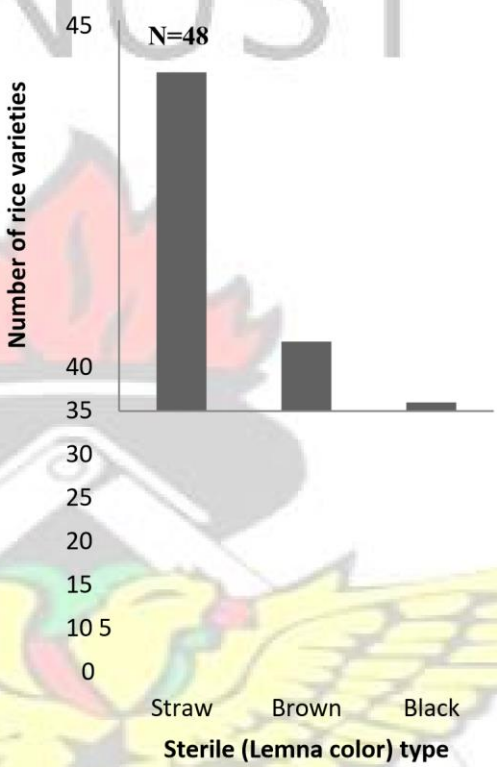


Figure 4.7: lemma color types for the 48 rice varieties studied

The colour of awns was of six groups; namely, those that were absent (20 rice accessions), gold (19 rice accessions), red (4 rice accessions), purple (1 rice accession), whitish (3 rice accessions) and straw (1 rice accession) (Figure 4.8).

The distribution of awns was of six groups; namely, those that were absent (24 rice accessions), tip only (10 rice accessions), upper quarter (9 rice accessions), upper half (1rice accession), upper

three quarters (2 rice accessions) or whole length (2 rice accessions) (Figure 4.9).

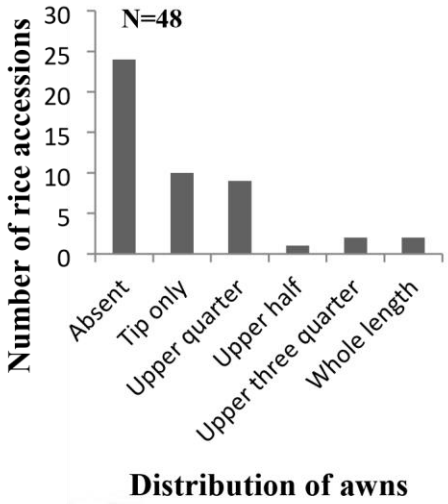


Figure 4.8: Distribution of awns for the 48 rice accessions

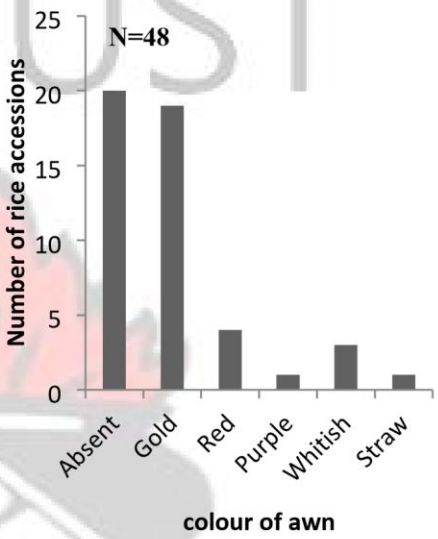


Figure 4.9: Colour of awns for the 48 rice accessions

According to Figure 4.10, the culm habit (Angle) for the 48 rice accessions was of four types, namely, open (29 rice accessions), spreading (1 rice accession), semi-erect (14 rice accessions) and erect (4 rice accessions).

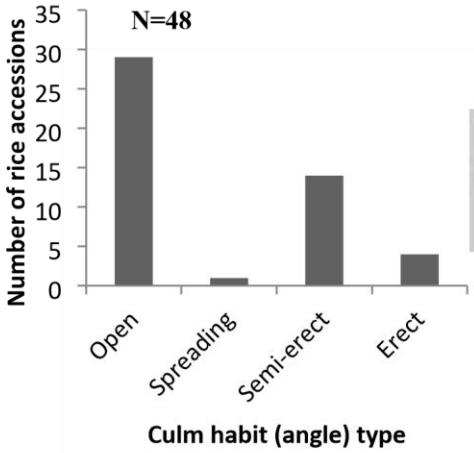


Figure 4.10: Culm habit (Angle) for the 48 rice accessions

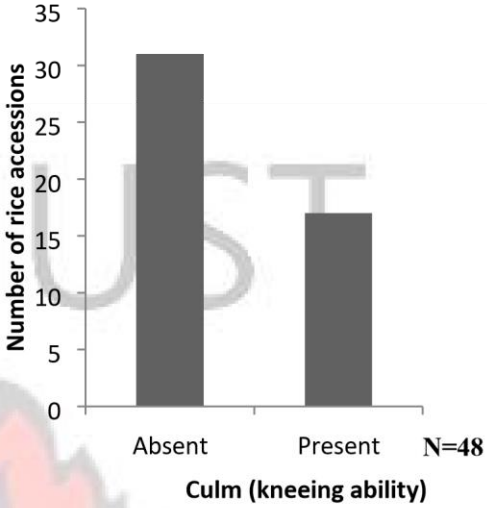
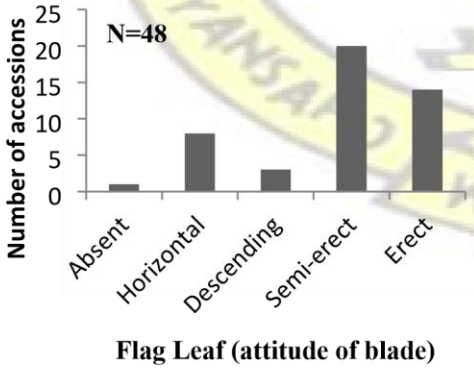
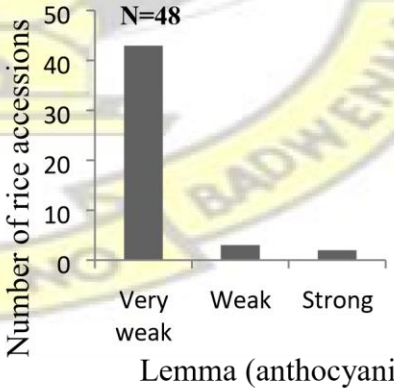


Figure 4.11: Culm kneeing ability of the 48 rice accessions

The Culm (kneeing ability) was of two groups; either absent or present. Thirty-one of the rice accessions studied had the Culm (kneeing ability) absent while seventeen of the accessions had the Culm (kneeing ability) present (Figure 4.11).

The Flag leaf (attitude of blade) was of five groups; namely, those that were absent (1 rice accession), horizontal (8 rice accessions), descending (3 rice accessions), semi-erect (20 rice accessions) or erect (14 rice accessions) (Figure 4.12).





Lemma (anthocyanin coloration of area below

Figure 4.12: Flag Leaf (attitude of blade) of the 48 rice accessions

Figure 4.13: Lemma (anthocyanin of the coloration of area below apiculus) type of the 48 rice accessions

According to Figure 4.13, the Lemma (anthocyanin coloration of area below apiculus) for the 48 rice accessions was of three types. The three types included very weak (43 rice accessions), weak (3 rice accessions) or strong (2 rice accessions).

The Lemma (colour of apiculus) was of five colours; namely, those that were white (30 rice accessions), green (2 rice accessions), brown (8 rice accessions), purple (6 rice accessions), straw (1 rice accession) or red (1 rice accession) (Figure 4.14).

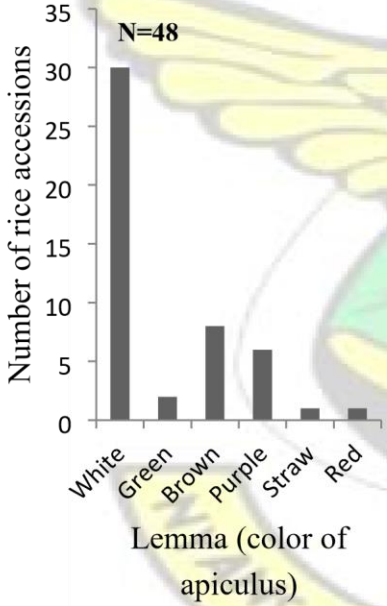


Figure 4.14: Lemma (colour of apiculus) of the 48 rice accessions

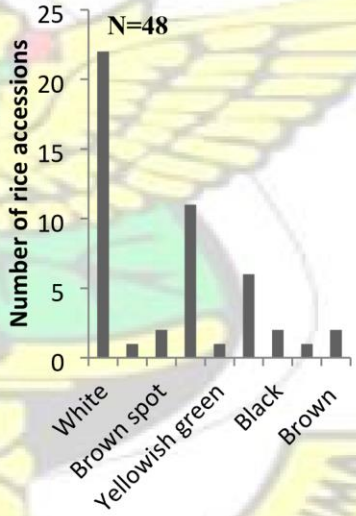


Figure 4.15: Lemma and palea colour of type of the 48 rice accessions

According to Figure 4.15, the lemma and palea colour for the 48 rice accessions was of nine types. There were some that were white (22 rice accessions), blackish brown (1rice accession), brown spot (2 rice accessions), green (11 rice accessions), yellowish green (1rice accession), gold (6 rice accessions), black (2 rice accessions), purple (1rice accession) and brown (2 rice accessions).

Colours such as white (35 rice accessions), yellow (10 rice accessions), light purple (2 rice accessions) and purple (1rice accession) were recorded for the Spikelet (Colour of Stigma) for the 48 rice accessions (Figure 4.16).

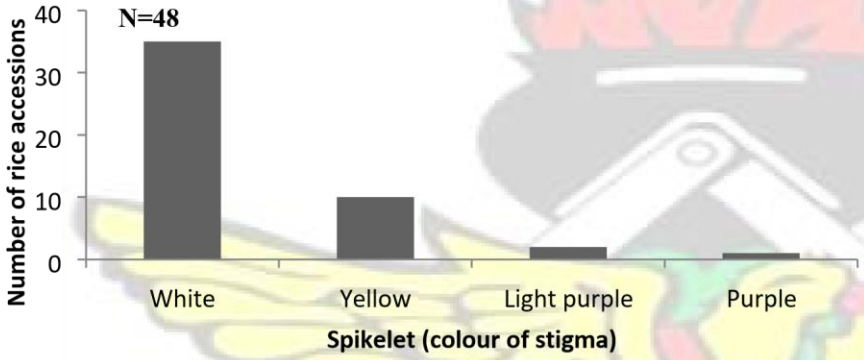


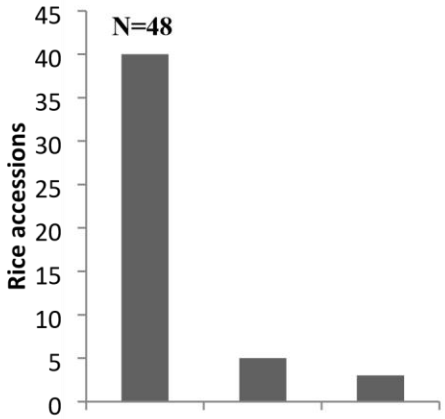
Figure 4.16: Spikelet (colour of stigma)

4.3 Phenological Studies of Some Late Reproductive Traits among Rice Accessions

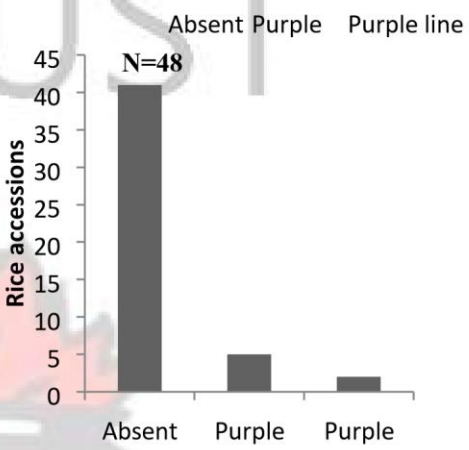
Qualitative traits that were measured at the late reproductive stage were culm (anthocyanin coloration of code), culm (internode anthocyanin), culm lodging resistance (culm strength), culm (underlying node colour), flag leaf (attitude of blade), panicle (attitude of branches), panicle (attitude of main axis) and panicle (secondary ranching).

According to Figure 4.17, the Culm (Anthocyanin Coloration of Node) of the 48 rice accessions was of three types; those that were absent (40 rice accessions), purple (5 rice accessions) or purple

line (3 rice accessions).



Culm (anthocyanin coloration of line node)



Culm (internode anthocyanin)

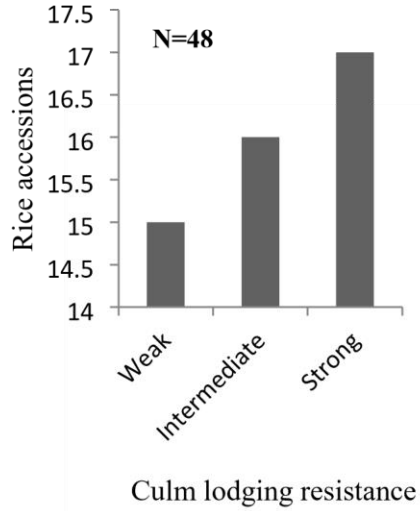
Figure 4.17: Culm (anthocyanin coloration of node) of the 48 rice accessions the 48 rice accessions

Figure 4.18: Culm (internode anthocyanin) of

The Culm (internode anthocyanin) of the 48 rice accessions was of three types; those that were absent (41 rice accessions), purple (5 rice accessions) or purple line (2 rice accessions) (Figure

The Culm lodging resistance culm strength) of the 48 rice accessions was of three forms; namely, those that were weak (15 rice accessions), intermediate (16 rice accessions) and strong (17 rice accessions) (Figure 4.19).

4.18).



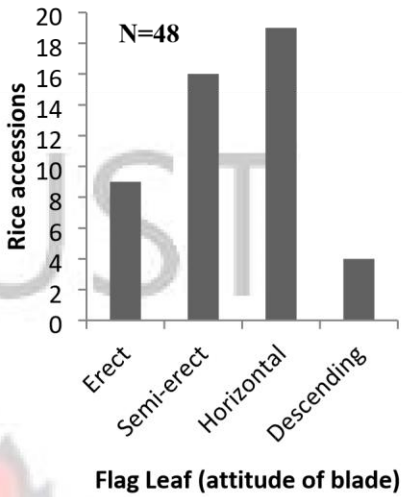


Figure 4.19: Culm lodging resistance Figure 4.20: Flag leaf (attitude of blade) (culm strength) of the 48 rice accessions of the 48 rice accessions

According to Figure 4.20, the Flag leaf (attitude of blade) of the 48 rice accessions was of four types; those that were erect (9 rice accessions), semi-erect (16 rice accessions), horizontal (19 rice accessions) or descending (4 rice accessions).

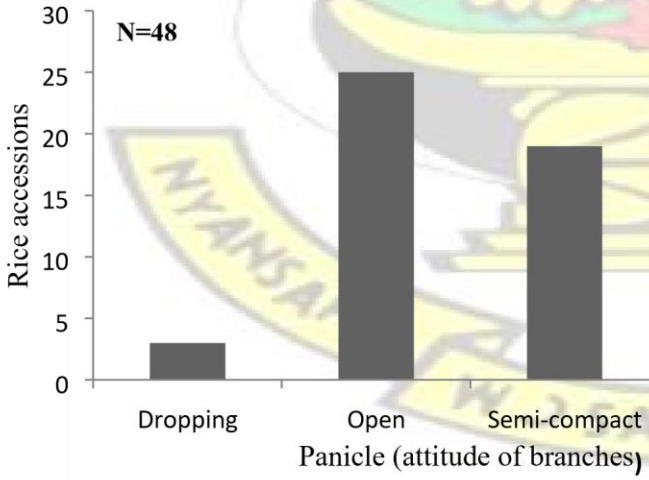


Figure 4.21: Panicle (attitude of branches) of the 48 rice accessions

The Panicle (attitude of branches) of the 48 rice accessions was of four types; those that were dropping (3 rice accessions), open (25 rice accessions), semi-compact (19 rice accessions) or compact (1 rice accessions) (Figure 4.21).

According to Figure 4.22, the Panicle (attitude of main axis) of the 48 rice accessions was of three types; those that were slightly dropping (1 rice accessions), semi-upright (16 rice accessions) or upright (31 rice accessions).

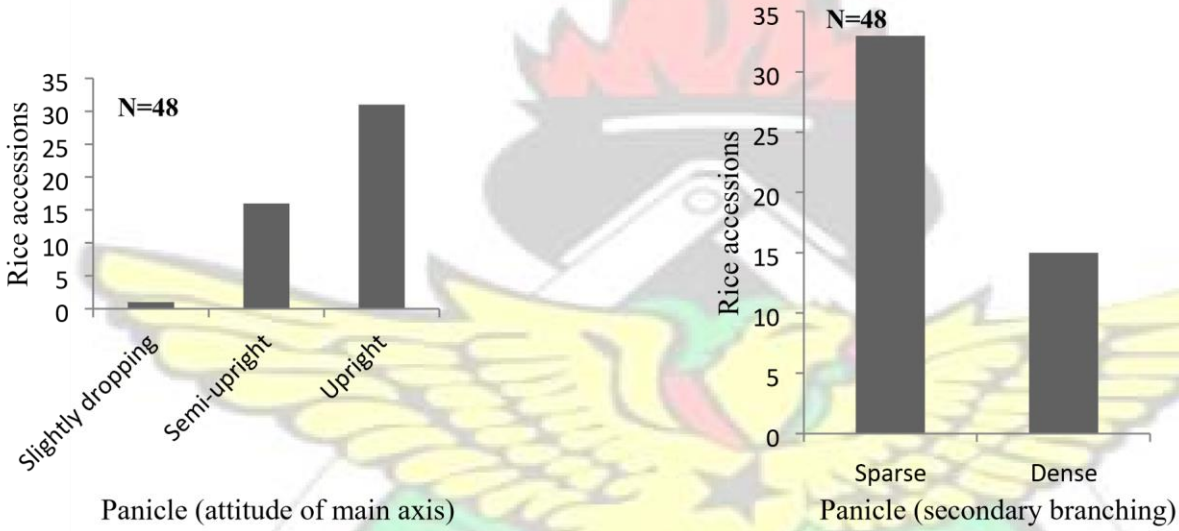


Figure 4.22: Panicle (attitude of main axis) of the 48 rice accessions

Figure 4.23: Panicle (secondary branching) of the 48 rice accessions

The Panicle (secondary branching) of the 48 rice accessions was either sparse (33 rice accessions) or dense (15 rice accessions) (Figure 4.23).

4.4 Principal Component Analysis (PCA)

The principal components analysis based on the 14 quantitative traits was performed individually to determine the relative contribution of the different traits to the total variation in rice. The PCA is an ordination multivariate technique that allows the use of biplots to visualize the relationship between the accessions and measured traits.

Four significant principal components were identified and accounted for 55.3% of the total variation. PC1 had Eigen-value of 0.44, explaining 18.5% of the total variation (Table 4.2).

Quantitative traits such as panicle per plant (0.43), panicle length of the main axis (0.43), flag leaf (0.34), sterile lemma length (0.39), leaf length of ligule (0.27) and plant height (0.25) contributed greatly to PC1, which accounted the highest for the total variation. PC2 depicted proportion of variance as 14.5%, PC3 contributed 12.2% to the total variation and PC4 had 10.1% to the total variation (Table 4.2). PC2 was associated with leaf length of blade (0.48), sterile lemma length (0.43), grain width (0.34), plant height (0.33) and flag leaf (Table 4.2). PC3 was associated with Culm diameter at the basal internode (0.47), awn length (0.46), leaf width of blade (0.41), and flag leaf (w) (0.35). The fourth PC had 0.16 as its Eigen-value and it explained 10.1% of the total variation. PC4 was associated with leaf width of blade (0.62), grain weight of 100 fully developed grain (0.45), leaf length of ligule (0.32), and grain length (0.29).

Table 4.2: Principal components analysis based on the 14 quantitative traits

Variable	PC1	PC2	PC3	PC4
LEAF (LOB)	0.25	-0.48	-0.04	-0.08 LEAF (WOB)
0.07 0.03	0.41	-0.62		
LEAF (LOL)	0.27	0.01	-0.18	0.32
FLAG LEAF (L)	0.34	0.28	0.04	0.11
FLAG LEAF (W)	0.11	0.33	0.35	0.01
CULM (DABI)	-0.15	0.11	-0.47	-0.28
PANICLE/PLT	-0.43	-0.29	0.01	0.18
STERILE (LL)	-0.39	-0.43	0.09	-0.04
AWN LENGTH	0.21	0.11	-0.46	-0.26
PANICLE LOMA	0.41	-0.14	-0.28	-0.05
GRAIN (WOFDG)	0.27	-0.22	0.05	-0.45
GRAIN L	-0.29	0.29	-0.26	- 0.29
GRAIN WIDTH	0.19	0.34	0.23	0.09
Plt Height	0.25	-0.33	0.18	0.27
Eigenvalue	0.44	0.31	0.24	0.16
Proportion	0.03	0.02	0.02	0.01
Cumulative	0.95	0.97	0.99	1.00

WOB=Leaf width of blade, LOL=Leaf length of ligule, FLL= Flag leaf length, FLW=Flag leaf width, DABI=Culm diameter at basal internode, PAN=panicle number per plant, SLL=Sterile lemma length, AL= Awn length, LOMA= Panicle length of the main axis, WOFGD=100 grain weight of fully developed grain, GL= Grain length, GW=grain weight, PH= plant height

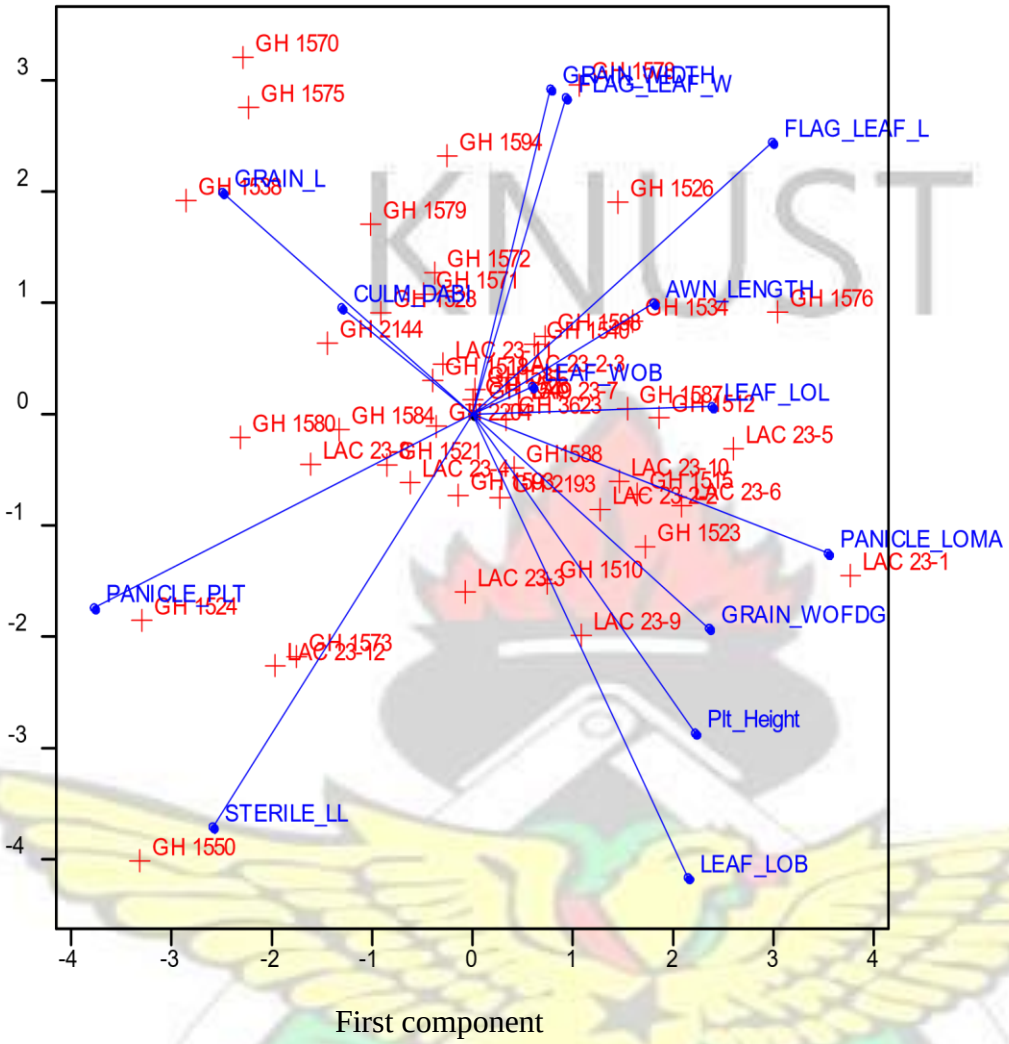


Figure 4.24: Grouping genotypes based on PCA biplot among the 14 quantitative traits
Trait abbreviations as given in Table 4.2

The principal components analysis based on PCA biplot of the rice accessions revealed diverse grouping pattern among the 14 quantitative traits (Figure 4.24). While some accessions showed longer vector distances , shorter vector distances were observed for others. For PC1, Gh1578 recorded the longest vector distances. Gh1526 and LAC 23 -1, recorded the moderate distances from the vector origin while GH3623, GH1588 and LAC23 -2-3 recorded the shortest. GH1570

and GH1550 had the longest distances for PC2. GH5173 and LAC 23-12 had similar vector in PC2 and were separated from the rest of the accessions (Figure 4.24).

4.5 Cluster Analysis of the Morphological Data

A dendrogram was constructed for the forty eight (48) rice genotypes based on their morphological characteristics. Figure 4.25 shows at a similarity index of 68% the accessions clustered into 7 main clusters. The most distant genotype were Gh1540 and LAC23-1, which were found in the first and last of position of the dendrogram. Cluster I was the largest of all the clusters and contained 20 accessions with five sub -clusters. Cluster III was the second largest cluster with 16 accessions including two sub -clusters and cluster IV had 8 accessions with two sub -clusters respectively. Cluster VI had three accessions with two sub -clusters and next was cluster II which had two accessions only. Cluster V and VII had one accession each, which were LAC23 -9 and GH1540. Cluster VI had three accessions and comprised of two sub-clusters. Accessions in the same cluster have the same morphological characteristics and sub -clusters indicate that the accessions have some distinct traits from other members of the clusters. The 48 rice accessions (from Liberia and Ghana) showed no distinctive morphological characteristics based on geographical origin, as the analysis showed no group of accessions from either of the geographical locations divergently clustered.

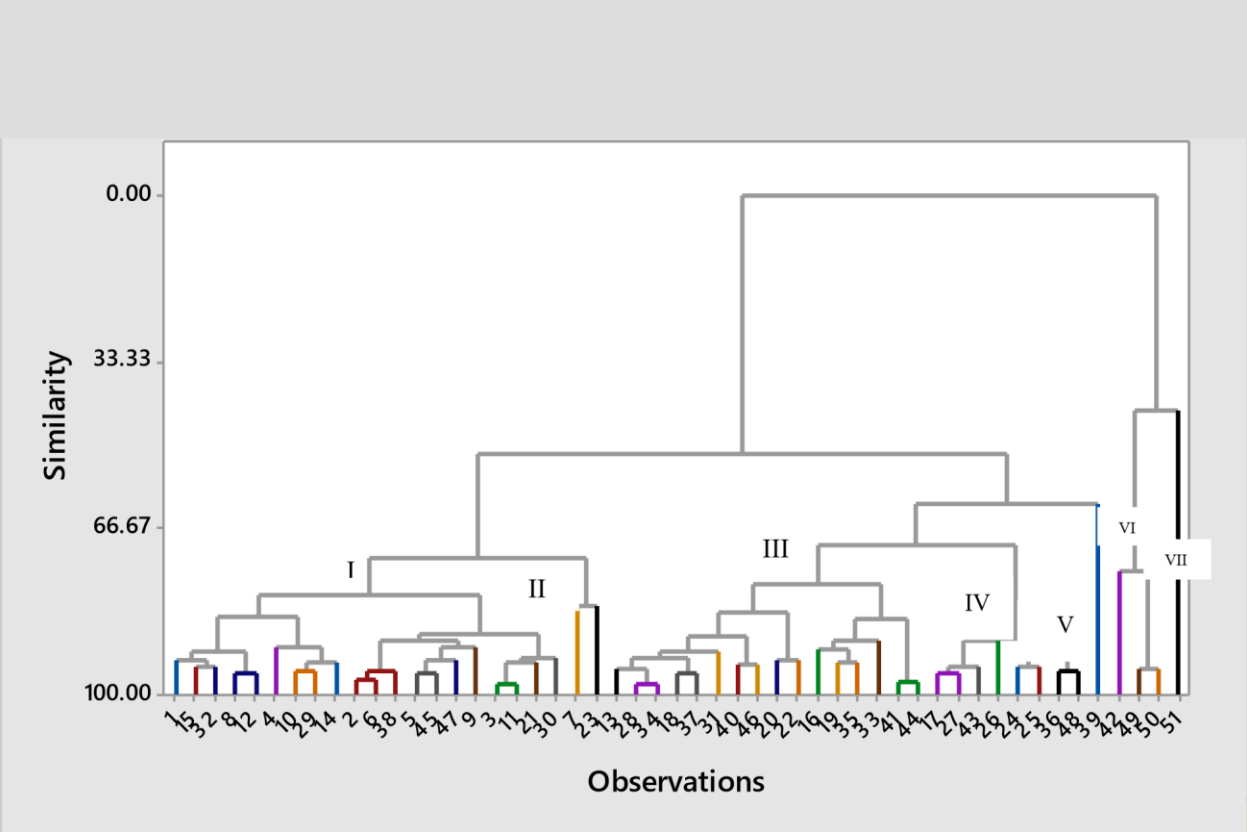


Figure 4.25: Dendrogram generated based on the 14 traits of the forty eight (48) rice genotypes using UPGMA.

Table 4.3: Peason correlation coefficients among the quantitative traits studied

	LOB	WOBL	W	DABI	PAN	LL	AWN	LOMA		WOFGD	GL	GW
	LOL											
WOB	-0.316											
LOL	-0.237	0.93**										
FLL	0.44**	-0.21	-0.12									
FLW	-0.10	0.17	0.18	0.22								
DABI	0.02	0.37*	0.38*	-0.05	-0.13							
PAN	0.22	0.04	0.04	-0.04	-0.16	0.14						
SLL	0.46**	0.97**	0.92**	-0.31*	0.11	0.33*	-0.02					
AL	-0.05	0.05	0.08	0.03	-0.14	0.05	-0.32*	0.07				
LOMA	0.78**	-0.14	-0.02	0.54**	0.09	0.21	0.19	-0.30*	0.08			
WOFGD	-0.33*	0.98**	0.94**	-0.29*	0.114	0.37*	-0.03	0.97**	0.06	-0.16		
GL	-0.43*	0.98**	0.93**	-0.26	0.13	0.37**	-0.01	0.99**	0.09	-0.25	0.98**	
GW	-0.45**	0.97**	0.93**	-0.27*	0.13	0.34**	-0.04	0.99**	0.08	-0.28*	0.98**	0.99**
PH	0.71	-0.28**	-0.23*	0.39	-0.05**	-0.09	0.14	-0.40	-0.22*	0.69**	-0.31*	0.38**	-0.38**

P≤0.05*, P≤0.01** WOB=Leaf width of blade, LOL=Leaf length of ligule, FLL= Flag leaf length, FLW=Flag leaf width, DABI=Culm diameter at basal internode, PAN=panicle number per plant, SLL=Sterile lemma length, AL= Awn length, LOMA= Panicle length of the main axis, WOFGD=100 grain weight of fully developed grain, GL= Grain length, GW=grain weight, PH= plant height.

4.6 Pearson Correlation among the Quantitative Traits

Highly significant positively correlation was observed between length of ligule and leaf width of blade (r =0.93). Relationship between sterile lemma length and length of ligule was highly positively correlated (r = 0.92) (Table 4.3). Plant height was significantly positively correlated (r = 0.69). Grain length and length of ligule is highly significantly positively correlated (r =0.99) (Table 4.3). However, correlation between culm diameter at basal internode and length of ligule, sterile lemma length and culm diameter at basal internode , 100 grain weight of fully developed grain and culm diameter at basal internode , plant height and grain length were positively correlated (Table 4.3). Correlation between awn length and culm diameter at basal internode, panicle length of the main axis and length of ligule, plant height and 100 grain weight of fully developed grain were negatively correlated (Table 4.3).

Correlation studies provide information on nature and extent of association between two pairs of metric characters. However, it could be possible to bring genetic improvement in one character by selection of the other of a pair. Traits that show significant positive correlation in this study could be improved simultaneously. However traits that exhibit negative relationships could be improved independently in the future

4.7 Summary Statistics about the SSR Markers Used

DNA bands were scored for DNA fingerprinting analysis with the molecular data generated using 16 SSR markers. Summary statistics of each SSR marker was calculated using Powermaker

Software version 3.25. The results obtained are presented in Table 4.4. Out of the 20 primers pairs, 16 gave polymorphic bands and therefore were used for the analysis. Allelic frequency revealed by the markers across the 48 rice accessions ranged from 0.50 to 0.97 with a mean of 0.76. SSR Marker RM474, RM484, RM11, RM105, and RM125, recorded the highest number of allele number detected (10.00, 7.00, 7.00, 7.00, 6.00) Table 4.4. This was followed by RM536 and RM 552 (5.00, 5.00) in that order, the least were RM 215, RM 316, RM 447s, RM43 and RM 118. The genetic diversity revealed by expected heterozygosity (HE) ranged from 0.11 for RM447 to 1.00 for RM474 with a mean of 0.47 (Table 4.4).

Table 4.4 Summary Statistics of the sixteen (16) SSR Markers

Marker	Allele No	Heterozygosity	PIC	Gene Diversity	Allele Frequency
RM105(SSR1)	7.00	0.28	0.32	0.84	0.27
RM125(SSR1)	6.00	0.49	0.52	0.70	0.47
RM11(SSR3)	7.00	0.54	0.69	0.66	0.52
RM25 (SSR4)	4.00	0.16	0.17	0.92	0.15
RM408(SSR5)	6.00	0.63	0.87	0.57	0.59
RM536(SSR6)	5.00	0.23	0.25	0.88	0.22
RM552(SSR7)	5.00	0.58	0.87	0.56	0.51
RM484(SSR8)	7.00	0.68	0.98	0.50	0.65
RM171(SSR9)	6.00	0.55	0.74	0.63	0.50
RM271(SSR10)	6.00	0.16	0.12	0.92	0.16
RM474(SSR11)	10.00	0.69	1.00	0.50	0.66
RM215(SSR12)	3.00	0.38	0.50	0.75	0.33
RM316(SSR13)	3.00	0.20	0.22	0.89	0.19
RM447(SSR14)	3.00	0.10	0.11	0.95	0.10
RM43 (SSR15)	3.00	0.06	0.06	0.97	0.06
RM118(SSR16)	3.00	0.16	0.17	0.92	0.15
Mean	**5.25**	**0.37**	**0.47**	**0.76**	**0.35**

PIC: Polymorphic information content

The mean PIC value for all markers used was 0.35 and ranged between 0.06 and 0.66 in loci RM43 and RM474, respectively. Gene diversity ranged from 0.06 in RM43 to 0.69 in RM11 with a mean

of 0.37. Generally, the allele frequency of all the markers was below 0.95 indicating that they were all polymorphic in character (Table 4.4).

4.7.1 Diversity based on SSR markers in rice varieties using the dendrogram

A dendrogram was constructed to ascertain the genetic diversity and relatedness among forty-eight (48) rice genotypes based on SSR analysis using the unweighted pair group method with arithmetic means (UPGMA). The dendrogram indicated similarity values ranging from 1.00 to 0.72 with eleven main clusters: I, II, III, IV, V, VI, VII, VIII, IX, X and XI at similarity coefficients of 90%. The highest genetic distance was found between the accessions, ACSS37 and ACSS1. These accessions had the first and last positions of the dendrogram respectively. Cluster X was the largest of all the clusters and it contained 19 accessions with four sub-clusters. Clusters VII and VIII were the second largest clusters with 7 accessions each. Cluster VII had three sub-clusters while cluster VIII comprised of four sub-clusters. Clusters I to V contained 1 accession each, and cluster VI had 5 accessions with three sub -clusters. Cluster IX comprised of 2 accessions and was sub divided into two sub-clusters. The accessions 17, 9, 47, 8, 6, 44, 40, 26, 22, 21, 19, 18, 39, 15, 14, 42, 13, 10, 7, 4, 3 and 2 were very similar with 100% similarity coefficient.

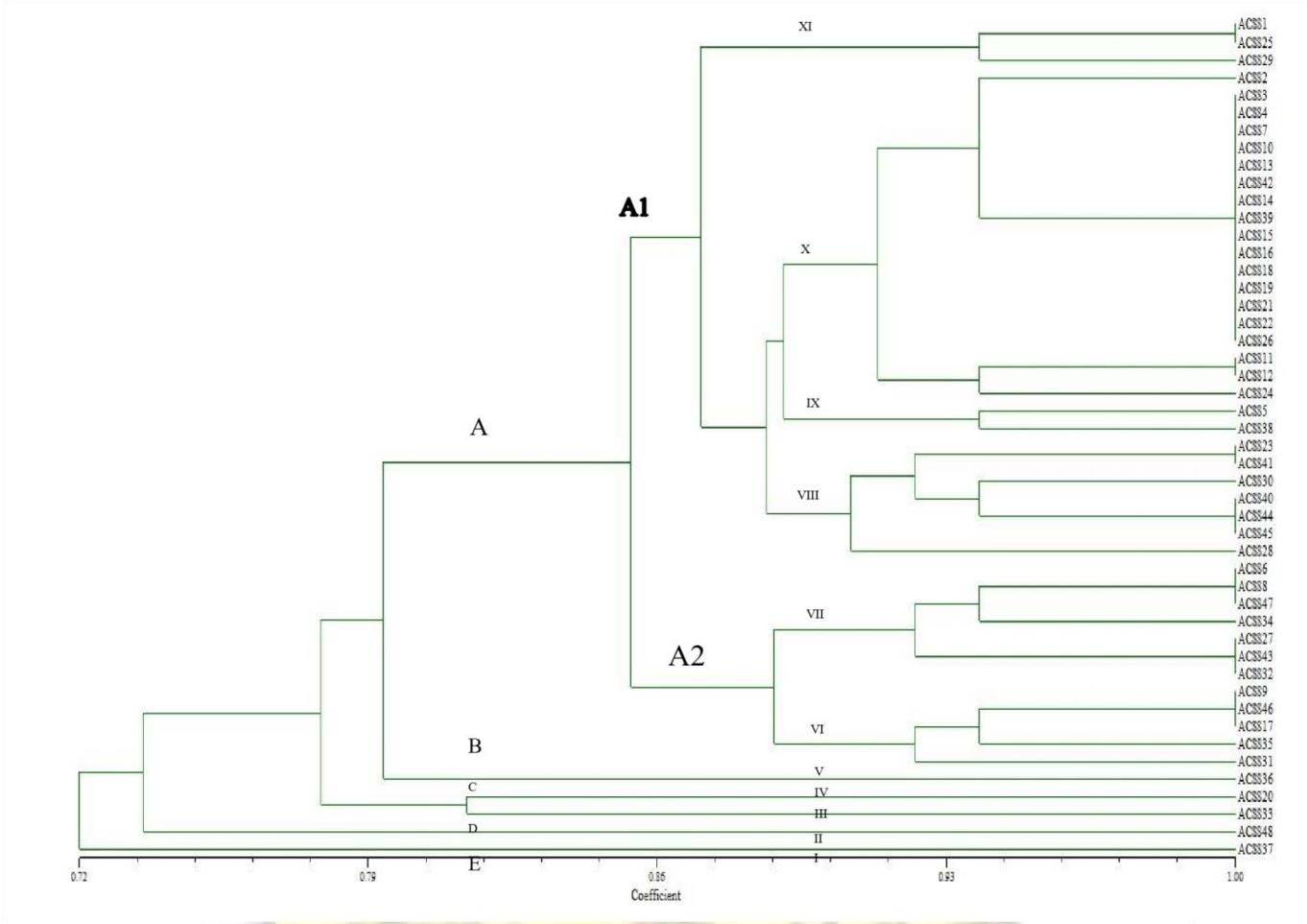


Figure 4.26: Dendrogram based on 16 SSR markers 48 rice accessions using SM similarity coefficient and UPGMA clustering.

CHAPTER FIVE

5.0 DISCUSSION

Information on genetic diversity and relatedness in crop germplasm is useful for plant breeders because it assists them in planning crosses (Venter *et al*., 2001). Such information could be used to design strategies to improve traits, maintain and manage germplasm in Genetic Resource Centres, or enhance the genetic base of future varieties. Hence, to effectively maintain, evaluate and utilize germplasm, it is important to investigate the extent of available diversity. In the present study, a set of rice genotypes from Liberia and Ghana were subjected to diversity analysis based on variation in morpho-phenological traits and SSR molecular profiles.

5.1 Qualitative Characters

Qualitative traits are important parameters for plant description and evaluation, and are greatly influenced by the consumers' preference. Charts showing distribution for ten qualitative traits are displayed in Figure 4.1 to Figure 4.10. For leaf blade intensity of green color, it was observed that a significant portion of 47 accessions were medium and 1 accession was dark. For leaf blade attitude angle of the penultimate leaf prior to heading, erect type was in 17 accessions, horizontal type was in 10 accessions and the drooping type in 21 accessions. Breeding for erect leaf angle has been suggested as a method of increasing grain yield in cereal crops (Mwangi *et al*., 2013). There are great possibilities of increasing light penetration into crop canopy, which is one of the ways of obtaining higher grain yield. Duncan (1971) demonstrated that increased penetration of light into canopy would increase photosynthetic rate and perhaps enhance grain yield. Also, Chang and Tagumpay (1970) found erect leaf angle was associated with high yield in rice (*Oryza sativa* L).

Some variations existed among the accessions with respect to some qualitative data; notably, leaf blade; distribution of anthocyanin coloration (DAC), pubescence of blade surface (PBS) and leaf

(shape of ligule (SL). Fischer (1998) reported that Jasmine 85 has pubescent leaves, with no anthocyanin colouration and light brown pericarp; also the variety is aromatic. With respect to pericarp colouration, all the seeds match a true Jasmine 85 variety. Studies carried by Bisne and Sarawgi (2008) to characterize 32 aromatic rice accessions of Badshah Bhog group from IGKV, Raipur, Chhattisgarh germplasm, found the highest variation among accessions for the traits leaf blade colour, lemma and palea colour, apiculus colour, and lemma and palea pubescence.

With regards to the culm (DABI), the flag leaf, leaf (LOB; LOL; WOB), panicle (PNPP; LOMA), awn length, and grain (WOFDG), significant differences existed among seeds for the various accessions. There is a variation of the characteristics among the accessions. Ideally, a single variety from different sources should not vary (Daniel *et al*., 2014). These variations affect grower or seed dealer satisfaction.

For plant height, the study recorded a range from 66.0-187.5 cm and this was contrary to what IRRI (1998) reported for an intermediate height (110 cm) for Jasmine 85. According to (Butler *et al*., 2005), intermediate plants have heights ranging from 75 cm to 160 cm.

Grain size and shape are among the first rice quality criteria that breeders consider when developing new varieties, because preferences for grain size and shape vary from one group to another (Senqupta *et al.*, 2006). Rice grain quality depends on physicochemical properties which are greatly influenced by genotype of the plant (Kishine *et al*., 2008). In this study, the grain length and width were different for all the rice accessions studied.

5.2 Flag Leaf (Attitude of Blade)

Leaf angle is measured near the collar as the angle of attachment between the flag leaf blade and the main panicle axis. Flag leaf angle is believed to influence the degree of light saturation of the upper leaves of rice crop (Yoshida, 1981). Its distribution had 14 accessions with erect, 20 accessions semi-erect, 3 accessions descending, 8 accessions horizontal and the flag leaf attitude of blade was absent in 1 accession. Mwangi *et al*. (1988) did observe differences in the flag leaf angle, varying from erect to semi-erect. It is widely established that erect leaves allow the deeper penetration and more even distribution of light which results in crop photosynthesis (Yoshida, 1981). The crop photosynthesis of an erect-leaved canopy is about 20% higher than that of the droopy-leaved canopy when the leaf area index is extremely high (Keulen *et al*., 1989). This model assumes that all the leaves are uniformly oriented at angles of 0 or 90^{0} with respect to the horizontal plane. Hence, selection for the ideal ideotype (erect and semi-erect flag leaf) that can intercept light and has the potential for high grain yield will be effective in the collections.

Breeders can select such ideotypes for further improvement.

5.3 Awning Characteristic

Awning characteristic is another trait recorded among accessions evaluated. Majority of the accessions had absent of awns color, 20 accessions out of 48 had; gold, red, purple, whitish and straw revealed 19, 4, 1, 3 and 1 awn color type respectively. Awning is considered a nuisance during milling by many farmers but it has been reported to play a role in preventing birds from sucking the milk-stage rice during grain filling. Breeders may therefore select the short-awned types as a compromise during cultivar development.

5.4 Plant Height

Analysis of data revealed plant height mean value of 129±1.14cm and a wide range of 66.0-187.5 cm, with coefficient of variation (8.6%). Plant height in rice is complex character and the end product of several genetically controlled factors called internodes (Cheema *et al*., 1987). Tall plant type is very typical of landrace genotypes which exceed in their capacity to support panicle growth by large stem reserve mobilization. Ali *et al*. (2008) observed relatively greater range in plant height than the other characters. The smallest plant height was recorded for accession GH1571cm and accession GH1550cm recorded the highest value.

A break-through was realized in plant breeding with speedy development of semi dwarf cultivars with displayed characteristics of lodging resistance and nitrogen responsiveness in erect leaves pattern. This was why Newmah (2010) confirmed the success of the "Green Revolution" to be directly related to intensive use of semi dwarf varieties. This was true because the semi dwarf plant type was extensively utilized in the rice (*Oryza sativa*) cultivars throughout the world. However, depending on the part of the world with improvement in farmers' lives, there is a growing desire to combine desirable characteristics of tall varieties' with yielding ability and a new type of architecture: intermediate plant height as stated by Zafar *et al*. (2004).

5.5 The Culm (DABI)

The culm (DABI) had mean of 7.19 cm and a range of 1.79-13.00 cm was recorded. As a component of plant height, there were variations in the culm lodging resistance measured, with a significant portion of the accessions of (17) being strong, 16 intermediate and 15 being weak (Figure 4.14). It was observed that a significant amount of the accessions possessed green underlying node colour for the culm while 15 were of light gold type (Figure 4.15). Similarly,

Oppong-Konadu *et al.* (2005) reported such short sturdy culms could be exploited for breeding purposes because they minimize lodging, thus creating little yield or no yield loss with welldeveloped panicles. These genetic attributes are essential when high yielding varieties for warm tropical environments where rice yields generally tend to be low due to high temperature that increases respiratory losses in carbohydrate reserves**.**

5.6 Grain Length and Grain Width

In breeding applications, according to Yu *et al.* (2002), grain size is usually evaluated by the grain weight, which is positively correlated with several characters including grain length, grain width and grain thickness. It is a major determinant of grain weight, one of the three components (number of panicles per plant, number of grain per panicle and grain weight of grain yield). The grain length ranged from 0.96-2.08 cm and width 1.16-3.10 cm (Table 4.2), thus inferring rice accessions were largely long-grain. Although the preference for rice grain characteristics varies with consumer groups, long and slender grains are generally preferred and are good valuable attributes that could be exploited to improve the grain characteristics of local rice accessions (Table 4.2) (Tamu 2015).

Similar variability were reported by Tamu (2015) who studied twelve genotypes of coarse rice to check their yield performance in Kallar tract and reported highly significant variation for different traits. This variation in the grain yield might be due to the environment and genetic constitution of accessions (Jamal *et al.*2009) or the correlation of grain yield per plant with various yield contributing characteristics such as; fertility of soil, flag leaf area, number of grains per panicle and grain weight which showed positive correlations. Similarly, Mirza *et al*. (1994) reported positive correlation among number of panicles per plant, panicle length, number of grains per panicle, 100grain weight and grain yield per plant-type. The grain shape character also showed the highest

variation in studies conducted in Pakistan by Siddiqui *et al.* (2007). Although this character is mentioned as qualitative, its evaluation is carried out as a quantitative trait, according to the grain dimensions.

5.7 Panicle (Attitude of Blade, Main Axis and Secondary Branching)

Panicles are also important parameters for plant description and evaluation, and are greatly influenced by the consumers' preference. Thirty-three (68.75%) of accessions showed sparse secondary branching and 15 (31.25%) secondary branching. Mwangi *et al*. (2013) found panicle exsertion a conspicuous character for identification of the rice cultivars. Breeding for erect leaf angle has been suggested as a method of increasing grain yield in cereal crops.

5.8 Productive Tillers per Plant

Tiller is one of the main attributing plant traits as indicated by Abbasi *et al.* (1995). Based on statistical data analyzed, there was high significant difference of $P< 0.011$. The coefficient of variation and standard deviation recorded next 42.0% and 3.53 respectively. The accessions had a great variability with a high range (3-29) for number of productive tillers (Table 4.2). Better tillering capacity is a desirable feature to upgrade the yield potential of upland varieties. Trials in Nigeria (Ogata *et al.*1972) and the Philippines (Jana and De Datta 1971; De Datta and Beachell 1972 generally indicated that when rainfall is plentiful and the soil has good water-retention capacity, the high-tillering and short-statured varieties definitely respond better to nitrogen and yield higher than do the taller types (Jana and De Datta 1971; De Datta and Beachell 1972. The accessions that produced more productive tillers will contribute to increased yield in a breeding program and could be selected as base genotypes for further improvement.

5.9 Core Collection

Core collection is important in germplasm characterisation. Accessions selected for this study were 48 in total from Liberia and Ghana. Among the accessions studied, 18 out of the 48 accessions were distant from the rest, and were selected to constitute a core collection for further improvement. The concept of a core collection was introduced by Frankel and Brown (1984) and Brown *et al* (1989) with the intent of using the core collection to minimize the cost of germplasm conservation while ensuring maximum genetic diversity.

5.10 Cluster and Principal Component Analysis (PCA)

Hierarchical cluster analysis based on the morphological data grouped the accessions into four distinct clusters suggesting diversity among the accessions of rice in Ghana and Liberia. The clustering of accessions from different origin into different clusters suggests diversity of populations within a geographical origin and similarity of populations beyond geographical limits. This finding agrees with results of Alemayehu and Becker (2002) and Zada *et al.* (2013). The variability among the accessions from diverse origin could be related primarily to their morphological differences and their uses or selection.

The scattering of genotypes in all quarters in the biplots of the principal components analysis suggest the high level of genetic diversity in the evaluated genotypes. The biplots also disclosed a comprehensive understanding of the relationship between accessions and traits evaluated. Divergence studies of morphological and reproductive traits using principal components analyses have been reported by different researchers (Balkaya *et al.*, 2005; Warwick *et al.*, 2006; Dawood *et al*., 2009; Jatoi *et al*., 2011; Zada *et al*., 2013). The levels and patterns of genetic diversity

observed among accessions of rice in this study provides the basis to identify desirable parents to create segregating progenies with maximum genetic variability for further selection, conserve genetic resources of the plant and to introgress desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998).

5.11 Evaluation of the SSR Markers in Rice Genotypes using Dendrogram

A greater proportion (96%) of the markers used in this experiment had moderate to high informativeness for linkage analysis in rice. The informativeness of a locus is based on the expected values. Locus with expected heterozygosity of 0.5 or less are not very useful for largescale parentage analysis (Otoo *et al.,* 2009). However, the results observed for heterozygosity in the experiment were highly informative which denotes that they will be useful in good parentage analysis derived from the molecular analysis.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusions

There were significant diversity among the accessions of rice from Ghana and Liberia evaluated. This should help strengthen the background necessary for the promotion and breeding of improved varieties of rice. Differences were observed at the morphological and molecular level for the 48 rice accession from both Ghana and Liberia. Though the differences between them are not much, they are significant. This difference in seeds of the same variety can be explained by several factors. In breeding it is called genetic evolution. Differences could also be attributed to a mixture of seeds in the seed industry and also the informal way of sharing seeds. In the seed industry, it is

the responsibility of seed producers to take measures to ensure the genetic purity of the seed crop. This often requires cooperation among different companies and growers producing seed of the same varieties in the same area and coordination of planting location and dates. Among the accessions studied, 18 out of the 48 accessions were distant from the rest, and were selected to constitute a core collection for further improvement.

The studies of the forty-eight rice accessions also revealed a significant amount of information for breeding programmes interventions. Differences among accessions were observed for characters such as flag leaf angle, awning, leaf blade pubescence and a low variability for leaf patterns, leaf blade color, panicle type and basal leaf sheath color. These phenotypic traits could be explored for rice improvement. Majority of the accessions classified were associated with the dark green and erect leaf pattern with sturdy stems and the rest were pale green with droopy leaf pattern and weak stems. Based on the "plant type" concept, the latter group will be low nitrogen responders and will require improvements. Cluster analysis performed established accessions with regard to N response based on their morpho-agronomic characteristics. Regardless of the accessions response to N uptake, there were variations in their grain yield and grain characteristics. Additionally, variation in the grain length and width did exist as shown in their values obtained. The long grain and translucent types could be used in quality grain development.

6.2 Recommendations

To obtain enough information on the 48 accessions studied from Liberia and Ghana, I recommend for further evaluation of the morphological characterization at field level.

Based on the groupings from the dendrogram, accessions should be selected from each of the groups for molecular studies to gather additional information on their distinctiveness as expressed in the analysis. The accessions should be analyzed for their phylogenetic relationship and variation based on molecular markers, to complement the morpho-agronomic findings.

To obtain complete information on the genetic diversity of Liberian and Ghanaian rice accessions, future studies should employ high number of SSR loci covering all the 12 chromosomes in rice. Moreover, biochemical characterization such as mineral and protein analysis should also be included.

In comparison with previous studies on landraces, the diversity revealed in this study is narrow. It is, therefore, recommended that rice breeding programs in Liberia and Ghana should include new genetically unrelated genotypes in order to broaden the genetic base of Liberian and Ghanaian rice germplasm.

REFERENCES

Abbasi, F. M., M.A. Sagar, M. Akram and Ashraf M. (1995). Agronomic and quality traits of some elite rice genotypes. *Pakistan J. Sci. Indust. Res*., 38: 348- 50.

Africa Rice Center (2007). Nerica: The New Rice for Africa-a Compendium. *Available at issuu.com/Africaricecenter/docs/nericacompedium.*

Agarwal, M., Shrivastava, N. and Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant cell reports*, *27*: 4, 617-631. aided by assessment of genetic markers. *Proc Natl Acad Sci* USA, **22**: 10623-10627.

53: 297-312.

Alemayehu, N. and Becker. H. (2002). Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun). *Genet. Resour. Crop Evol,* 49: 573-582.

Ali, S. S., Jafri S. J. H., T. Z. Khan, A. Mahmood and Butt M.A. (2008). Heritability of yield and yield components of rice. *Pakistan J. Agric.Res*. 16: 89–91.

Anonymous. (2004). Bioversity International, IRRI and WARDA. Descriptors for wild and cultivated rice (*Oryza* spp.). Bioversity International, Rome, Italy; International Rice Research.

Arif, M., Choudhary, A., Bechtold, M. L., Szary, N. M., Puli, S. R., Othman, M. O. and Roy, P. K. (2011). Pancreatic stents for prophylaxis against post-ERCP pancreatitis: a meta-analysis and systematic review. *Gastrointestinal endoscopy*, *73*:2, 275-282.

Asante, M. D., Asante, B. O., Acheampong, G. K., Offei, S. K., Gracen, V., Adu-Dapaah, H. and Danquah, E. Y. (2013). Farmer and consumer preferences for rice in the Ashanti region of Ghana: Implications for rice breeding in West Africa. *Journal of Plant Breeding and Crop Science*, *5*:12, 229-238.

Atchley, W. R. and Zhu, J. (1997). Developmental quantitative genetics, conditional epigenetic variability and growth in mice. *Genetics*, *147*:2, 765-776.

Balasubramanian, V., Sie M., Hillmans R. J., and Otsuka K. (2007). Increasing Rice Production in the Sub Saharan Africa: Challenges and Opportunities in Agronomy, 94: 55-133.

Balkaya, A., Yanmaz, R., Apaydýn, A. and Kar. H. (2005). Morphological characterization of the whitehead cabbage (Brassica oleracea var. capitata subvar. alba) populations in Turkey. NZ *J. Crop Hort. Sci.*, 33: 333-341.

Barcaccia, G. (2010). Molecular markers for characterizing and conserving crop plant germplasm.

In: *Molecular Techniques in Crop Improvement*, Springer Netherlands. pp. 231-254

Barcaccia, G. (2010). Molecular markers for characterizing and conserving crop plant germplasm. In *Molecular Techniques in Crop Improvement* (pp. 231-254). Springer Netherlands.

Barrett, B.A. and Kidwell K.K. (1998). AFLP–based genetic diversity assessment among wheat cultivars forms the Pacific Northwest. *Crop Sci*. 38: 1261–1271.

Bell, C. J. and Ecker, J. R. (1994). Assignment of 30 microsatellite loci to the linkage map of Arabidopsis. *Genomics*, *19*:1, 137-144.

Bernardo, R. (2008). Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Science*, *48*(5), 1649-1664.

Beyene, A., Gibbon, D., and Haile, M. (2006). Heterogeneity in land resources and diversity in farming practices in Tigray, Ethiopia. *Agricultural systems*, *88*:1, 61-74.

Binneck, E., Nedel, J. L. and Dellagostin, O. A. (2002). Análise de RAPD na identificação de cultivares: uma metodologia útil. *Revista Brasileira de Sementes*, *24*:1, 183-196.

Bisne, R. and Sarawgi, A. K. (2008). Agro-morphological and quality characterization of Badshah Bhog group from aromatic rice germplasm of Chhattisgarh. *Bangladesh Journal of Agricultural Research* 33: 479-492.

Black, J. H., Meyer, D. M., York, D. G., Chaffee Jr, F. H., and Foltz, C. B. (1986). An upper limit on the microwave background temperature at z= 1.776. *The Astrophysical Journal*, *308*, L37L41.

Blashfield, R. K., and Aldenderfer, M. S. (1978). The literature on cluster analysis. *Multivariate Behavioural Research*, *13*:3, 271-295.

Borner, R., Kampmann, G., Chandler, J., Gleißner, R., Wisman, E., Apel, K. and Melzer, S. (2000). A MADS domain gene involved in the transition to flowering in Arabidopsis. *The Plant Journal*, *24*:5, 591-599.

Botstein, D., White, R. L., Skolnick, M., and Davis, R. W. (1980). Construction of a genetic linkage map in man using Restriction Fragment Length Polymorphisms. *American Journal of Human Genetics*, 32:314-331.

Bray, R. H. and Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil science*, *59*:1, 39-46.

Brown A.H.D, Frankel O.H, Marshall D.R, and Williams J.T. (1989). A case for core collections in the use of plant genetic resources Cambridge: Cambridge University press; 136156.

Brown, A. H. D., Spillane, C., Johnson, R. C. and Hodgkin, T. (1999). Implementing core collections-principles, procedures, progress, problems and promise. *Core collections for today and tomorrow*. 1-9.

Butler, J. D., Byrne, P. F., Mohammadi, V., Chapman, P. L., and Haley, S. D. (2005). Agronomic performance of alleles in a spring wheat population across a range of moisture levels. *Crop Science*, *45*:3, 939-947.

Caldo, R.A, L.S. Sebastian and Hernandez J.E. (1996). Morphology-Based Genetic Diversity Analysis of Ancestral Lines of Rice in Philippine Rice Cultivars. Philipp. J. *Crop Sci*.,21:3: 86-92.

Calpe, C. (2002). Part II. RICE IN WORLD TRADE, Status of the world rice market, In Proceedings of the 20th Session of the International Rice Commission, Bangkok, Thailand, 23-26 July 2002.

Cao, T., Duprez E., Borden K.L.B, Freemont P.S, and Etkin L.D (2001). Ret finger protein is a normal component of PML nuclear bodies and interacts directly with PML. J. Cell Sci. 111: 1319-1329.

Causse M.A, Fulton T.M, Cho Y.G, Ahn S.N, Chunwongse J., Wu K, Xiao J., Yu Z., Ronald P.C, Harrington S.E, Second G., McCouch S.R, and Tanksley S.D (1994). Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251- 1274.

Cavalli-Sforza, L. L., and Edwards, A. W. (1967). Phylogenetic analysis. Models and estimation procedures. *American journal of human genetics*, *19*:3, 233.

Center, A. R. (2007). Africa rice trends. *Overview of recent developments in the sub-Saharan Africa rice sector, Africa Rice Center Brief, Cotonou, Benin: WARDA*, *8*.

Champoux, M. C., Wang, G., Sarkarung, S., Mackill, D. J., O'Toole, J. C., Huang, N. and McCouch, S. R. (1995). Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. *Theoretical and Applied Genetics*, *90*:7-8, 969-981.

Chang, T. T. and Tagumpay, O. (1970). Genotypic association between grain yield and six agronomic traits in a cross between rice varieties of contrasting plant type. *Euphytica*, *19*:3, 356363.

Chavarriaga-Aguirre, P., Maya, M.M., Tohme, J., Duque, M.C., Iglesias, C., Bonierbale, M.W., Kresovich, S. and Kochert, G., (1999). Using microsatellites, isozymes and AFLPs to evaluate genetic diversity and redundancy in the cassava core collection and to assess the usefulness of DNA-based markers to maintain germplasm collections. *Molecular Breeding*, *5* pp.263-273.

Chebotar, S., Röder, M. S., Korzun, V., Saal, B., Weber, W. E. and Börner, A. (2003). Molecular studies on genetic integrity of open-pollinating species rye (Secale cereale L.) after long-term genebank maintenance. *Theoretical and Applied Genetics*, *107*:8, 1469-1476. **Cheema, A. A., Awan, M. A. and Iqbal, J. (1987).** Improvement of plant height architecture in Basmati rice. *Pakistan Journal of Agricultural Research (Pakistan)*.

Chen, Sheng, X. H. Lin, C. G. Xu, and Qifa Zhang (2000). "Improvement of bacterial blight resistance ofMinghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection." pp. 239-244.

Chwedorzewska, K. J., Bednarek, P. T. and Puchalski, J. (2002). Studies on changes in specific rye genome regions due to seed aging and regeneration. *Cellular and Molecular Biology Letters*, *7*:2A, 569-576.

CIMMYT (2005). International Centre for Agricultural Research in the Dry Areas (ICARDA) launched the Global Rust. Initiative (GRI) to tackle this threat .Retrieved from (http://www.cimmyt.org/en/component/docman/doc_download)

Comstock, R. E. (1978). Quantitative genetics in maize breeding. *Maize breeding and genetics, New York*, 191-206.

Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J and Clutton-Brock, T. H. (1998). Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society of London B: Biological Sciences*, *265*:1395, 489-495.

Cox, T.S., J.P. Murphy and D.M. Rodgers. (1986). Changes in genetic, diversity in the red winter wheat region s of the United States. *Proc. Nat'l. Acad. Sci*. 83: 5583–5586 (USA).

Daniel, I. O., Adabale, O. W., Adeboye, K. A., Aladele, E. S., Oduoye, O. T., Adetumbi, J. A., and Boerner, A. (2014). Evaluation of genetic integrity of tomato seeds during ageing by microsatellite markers. *Nigerian Journal of Genetics*, *28*:2, 29-33.

Darroch, J. (2003). Developing a measure of knowledge management behaviours and practices. *Journal of knowledge management*, *7*:5, 41-54.

Darvasi, A. and Pisanté-Shalom, A. (2002). Complexities in the genetic dissection of quantitative trait loci. *TRENDS in Genetics*, *18*:10, pp.489-491.

Darwin, C. (2003). On the origin of species by means of natural selection. 1859. *Ed. Joseph Carroll. Toronto: Broadview*.

Dawood, A., Kikuchi, A., Jatoi, S. A. Kiuchi, F. and Watanabe. K. N. (2009). Genetic variation in the chloroplast DNA (cpDNA) of Zingiberaceae taxa from Myanmar envisaged by the PCR RFLP analysis. *Ann. Appl. Biol.* 155: 91-101.

Day, G. M. (1953). The Indian as an ecological factor in the north-eastern forest. *Ecology*, *34*:2, 329-346.

Day, P.R. (1953). Experimental confirmation of hydrometer theory. Soil Science, 75: 181-186

De Datta, S. K., Malabuyoc, J. A., and Aragon, E. L. (1988). A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. *Field Crops Research*, *19*:2, 123-134. **de Vicente, M. C. and van Treuren, R. (2005).** *Molecular markers for gene bank management* (No. 10). Diversity International.

de Vicente, M. C., Guzman, F. A., Engels, J. and Rao, V. R. (2006). Genetic characterization and its use in decision-making for the conservation of crop germplasm. *The role of biotechnology in exploring and protecting agricultural genetic resources*, 129.

De Vicente, M.C., F.A. Guzmán, J. Engels and Ramanatha, V. R. (2005). Genetic characterization and its use in decision making for the conservation of crop germplasm: The Role of Biotechnology, Villa Gualino, Turin, Italy.p.57

Dean, R. E., Dahlberg, J. A. Hopkins, M. S. Mitchell S. E. and S. Kresovich. (1999). Genetic redundancy and diversity among 'Orange' accessions in the U.S. national sorghum collection as assessed with Simple Sequence Repeat (SSR) markers. Crop Sci., 39:1215-1221.

Dellaporta, S. L., Wood, J., and Hicks, J. B. (1983). A plant DNA minipreparation: version II. *Plant molecular biology reporter*, *1*:4, 19-21.

Dingkuhn, M., Jones, M. P., Aluko, G. K., and Semon, M. (1997). Interspecific *Oryza sativa* L. x O. glaberrima Steud. Progenies in upland rice improvement. *Euphytica*, *94*:2, 237-246.

Donald, C.M.T. (1968). The breeding of crop ideotypes. *Euphytica*, *17*:3, pp.385-403.

Duncan, W. G. (1971). Leaf angles, leaf area, and canopy photosynthesis. *Crop Science*, *11*:4, 482-485.

Dziedzic, K. S., Healey, E. L., Porcheret, M., Ong, B. N., Main, C. J., Jordan, K. P. and McHugh, G. A. (2014). Implementing the NICE osteoarthritis guidelines: a mixed methods study and cluster randomised trial of a model osteoarthritis consultation in primary care-the Management of Osteoarthritis in Consultations (MOSAICS) study protocol. *Implementation Science*, *9*:1, 1.

Fagade, S.O. (2000). Yield gaps and productivity decline in rice production in Nigeria. *In Proceedings of the Expert Consultation on Yield Gap and Productivity Decline in Rice*, FAO, Rome.

Fageria, N. K., N. A. Slaton and Baliges V.C. (2003). Nutrient management for improving lowland rice productivity and sustainability. *Advances in Agron.*80: 63-152.

Falusi, A.O. (1997). Agricultural development and food production in Nigeria: problems and prospects. *In*: B. Shaib, N.O. Adedipe, M. Aliyu and M. Jir, eds. Integrated agricultural production in Nigeria: Strategies and mechanisms. NARP Monograph No. 5. pp. 151-170.

FAO (2006). "Rice is life "International Rice Commission Meets in Peru. *Available at www.fao.org/newsroom/en/news/2006/1000267/index.html. Date accessed: 12:3, 2101*.

FAO (2006b). Food and Agriculture Organization Year Book. Food and Agriculture Organisation, Rome, Italy. pp. 79.

FAO (2016). Rice market monitor/ April 2016. Available at www.fao.org/economic / RMM

FAO STAT - Agriculture (2002a). http://faostat.fao.org/default.aspx

FAOSTAT. (2002b). FAO Statistical Databases. Available online at http://apps.fao.org. Accessed January 2002.

Ferguson, M. E., Burow, M. D., Schulze, S. R., Bramel, P. J., Paterson, A. H., Kresovich, S. and Mitchell, S. (2004). Microsatellite identification and characterization in peanut (A. hypogaea L.). *Theoretical and Applied Genetics*, *108*:6, 1064-1070.

Fischer, K. S. (1998). Toward increasing nutrient-use efficiency in rice cropping systems: the next generation of technology. *Field Crops Research*, *56*:1, 1-6.

Food and Agriculture Organization (FAO) (2011). Rice Market Monitor, Trade and Market, Food and Agriculture Organization of the United Nations Vol.14, Ed.3, pp: 1-31.

Force, A., Lynch, M., Pickett, F. B., Amores, A., Yan, Y. L. and Postlethwait, J. (1999). Preservation of duplicate genes by complementary, degenerative mutations. *Genetics*, *151*:4, 1531-1545.

Frankel O.H, and Brown A.H.D, (1984). Plant genetic resources today: a critical appraisal. In: Crop genetic resources: conservation and evaluation London: George Allen and Unwin; 249-257.

Frankel, O.H. (1973). Survey of crop genetic resources in their centres of diversity. First Report.FAO/IBP, Rome.

Frankel, O.H., J. J. Burdon and W.J. Peacock. (1995). Landraces in transit-the treat perceived. *Diversity*. 11: 14-15.

Global Food Security Response Ghana Rice Study (2009), Micro Report #56

Gouesnard, B., Giauffret, C., Lothrop, J., Dorvillez, D. and Derieux, M. (2000). Genotype× environment interactions in maize hybrids from temperate or highland tropical origin. *Crop science*, *40*:4, 1004-1012.

Gower, J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics*, 857-871.

Gracia, A. A. F., Benchimol, L. L., Antonica, M. M., Geraldi, I. O. and Deuza, A. P. (2004). Comparison of RAPD-RFLP-AFLP and SSR marker for diversity studies in tropical maize inbred lines, *Euphytica,* Vol.108, pp: 53-63.

Gregory, P.J., J. S. Ingram and Kobayashi T. (2000). Rice production and Global Change. *Global Environ. Res*. 2: 71-77.

Guei, R. G. and K. Traore. (2001). New approach to germplasm exchange for sustainable increase of rice biodiversity and production in Africa. *International Rice Commission. Newsletter*. 50: 49-58.

Gupta, P. K. and Varshney, R. K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica, 113*:3, 163-185.

Harlan, J. R. (1975). Crops and man, American Society of Agronomy. Madison, Wisconsin.

Hawkes, J. G. (1983). The diversity of crop plants. Harvard University Press, Cambridge, Mass.

Hokanson, S. C., Szewc-McFadden, A. K., Lamboy, W. F. and McFerson, J. R. (1998). Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a Malus× domestica Borkh. Core subset collection. *Theoretical and Applied Genetics*, *97*(5-6), 671683.

Huang, X. Q., Cloutier, S., Lycar, L., Radovanovic, N., Humphreys, D. G., Noll, J. S. and Brown, P. D. (2006). Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (Triticum aestivum L.). *Theoretical and Applied Genetics*, *113*(4), 753-766.

International Rice Research Institute (IRRI) (1996). Standard Evaluation System for Rice, uly edition, Manila, Philippines.

IPGRI/CIP. (2003). Descriptores del Ulluco (*Ullucus tuberosus*). Instituto internacional de Recursos Fitogenéticos, Roma, Italia; Centro Internacional de la Papa, Lima, Peru.

Jamal, I., Khalil, H., Abdul, B., Khan, S. and Islam, Z. (2009). Genetic variation for yield and yield components in rice. *Arpn. J. Agricultural and Biological Science*, *4*:6, 60-64.

Jana, R. K., and De Datta, S. K. (1971). Effects of solar energy and soil moisture tension on the nitrogen response of upland rice. In *Int Symp Soil Fert Eval New Delhi Proc*.

Jatoi, S. A., Javaid, A., Iqbal, M., Sabal, O. U., Masood, M. S. and Siddiqui, S. U. (2011). Genetic diversity in radish germplasm for morphological traits and seed storage proteins. *Pak. J. Bot.,* 43:2507-2512.

Jiang, W., Zhang, D., Bursac, N. and Zhang, Y. (2013). WNT3 is a biomarker capable of predicting the definitive endoderm differentiation potential of hESCs. *Stem cell reports*, *1*:1, 46-52.

Johnson, R. C., and Hodgkin, T. (1999). *Core collections for today and tomorrow*. International Plant Genetic Resources Institute (IPGRI).

Jolliffe, I. (2002). *Principal component analysis*. John Wiley and Sons, Ltd.

Jones, M.P., Nwanza K.F., Miezan K.M., Singh B.N. and Guei R. G. (1998b). Rice Germplasm Evaluation and Enhancement at WARDA. *In*: *Proceedings of the International Symposium on Rice germplasm Evaluation and Enhancement*, by Dale Bumpers National Rice Research Centre USDA-ARS and Rice Research & Extension Centre Division of Agriculture, University of Arkansas, November,1999.

Julier, Simon J., and Joseph J. LaViola Jr. (2007)."On Kalman filtering with nonlinear equality constraints." *Signal Processing, IEEE Transactions on*, 55:6, 2774-2784.

Jung, C., Pillen, K., and Frese, L. A. melchinger (1993): Phylogenetic relationships between cultivated and wild species of the genus Beta revealed by DNA 'fingerprinting'. *Theor. Appl. Genet*, *86*, 449-457.

Katti, M. V., Ranjekar, P. K. and Gupta, V. S. (2001). Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Molecular biology and evolution*, *18*:7, 11611167.

Keulen, H. V., Goudriaan, J., Seligman, N. G., Russell, G., Marshall, B., and Jarvis, P. G. (1989). Modelling the effects of nitrogen on canopy development and crop growth. *Plant canopies: their growth, form and function. G. Russell, B. Marshall, and P. G. Jarvis (Eds.)*, 83-104.

Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant molecular biology*, *59*:1, 1-6.

Kishine, M., Suzuki, K., Nakamura, S., and Ohtsubo, K. I. (2008). Grain qualities and their genetic derivation of 7 new rice for Africa (NERICA) varieties. *Journal of agricultural and food chemistry*, *56*:12, 4605-4610.

Kurata, N., Nagamura, Y., Yamamot, K., Harushima,Y., Sue, N., Wu, J., Antonio, B. A, Shomura, A., Shimizu, T. and Lin, S. Y. (1994). A 300 kilobase interval genetic map of rice including 883 expressed sequences. *Nature Genet.* 8: 365-372.

Lander, E. S. and Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, *121*:1, 185-199.

Lehmensiek, A., Eckermann, P. J., Verbyla, A. P., Appels, R., Sutherland, M. W. and Daggard, G. E. (2006). Curation of wheat maps to improve map accuracy and QTL detection. *Crop and Pasture Science*, *56*:12, 1347-1354.

Leviton, E. and Mcmahon. K (1996). Plants as a source of food. *Plants and Civilization*.Wm.C Brown Publishers: D ubugue, 1A:176.

Li, Z. K., Yu, S. B., Lafitte, H. R., Huang, N., Courtois, B., Hittalmani, S. and Zhuang, J. Y.

(2003). QTL× environment interactions in rice. I. Heading date and plant height. *Theoretical and Applied Genetics*, *108*:1, 141-153.

Liu, G. F., Yang, J., Xu, H. M., Hayat, Y. and Zhu, J. (2008). Genetic analysis of grain yield conditioned on its component traits in rice (Oryza sativa L.). *Crop and Pasture Science*, *59*:2, 189195.

Mace, E. S., Buhariwalla, K. K., Buhariwalla, H. K. and Crouch, J. H. (2005). A highthroughput DNA extraction protocol for tropical molecular breeding programs. Plant Molecular Biology Reporter 21:4:459-460.

Mace, E. S., Rami, J. F., Bouchet, S., Klein, P. E., Klein, R. R., Kilian, A. and Jordan, D. R. (2009). A consensus genetic map of sorghum that integrates multiple component maps and highthroughput Diversity Array Technology (DArT) markers. *BMC Plant Biology*, *9*:1, 1.

Maestri, E., Malcevschi, A., Massari, A. and Marmiroli, N. (2002). Genomic analysis of cultivated barley (Hordeum vulgare) using sequence-tagged molecular markers. Estimates of divergence based on RFLP and PCR markers derived from stress-responsive genes, and simplesequence repeats (SSRs). *Molecular Genetics and Genomics*, *267*(2), 186-201.

McCarty, J. C., Wu, J. and Jenkins, J. N. (2008). Genetic association of cotton yield with its component traits in derived primitive accessions crossed by elite upland cultivars using the conditional ADAA genetic model. *Euphytica*, *161*:3, 337-352.

McCouch S.R, Temnykh S., Lukashova A., Coburn J., Declerck G., Cartinhour S., Harrington S., Thomson M.., Septiningsi E., Semon M., Moncada P., and Jiming L (1998). Microsatellite markers in rice: Abundance, diversity and applications. In: Rice Genetics IV. IRRI. Manila, Philippines. pp: 117-135.

Melchinger, A. E., Utz, H. F. and Schön, C. C. (1998). Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. *Genetics*, *149*:1, 383-403.

Melman, S. D., Steinauer, M. L., Cunningham, C., Kubatko, L. S., Mwangi, I. N., Wynn, N. B., ... & Secor, W. E. (2009). Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of Schistosoma mansoni. *PLoS Negl Trop Dis*, *3*:8, e504.

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

Mirza, M. S., Akkermans, W. M. and Akkermans, A. D. (1994). PCR-amplified 16S rRNA sequence analysis to confirm nodulation of Datisca cannabina L. by the endophyte of Coriaria nepalensis Wall. *Plant and soil*, *160*:1, 147-152.

MoFA/IVRDP, CSIR/CRI and CSIR/SARI. (2005). Rice Production Guide, Ghana.

Mohammadi, S. A., and Prasanna, B. M. (2003). Analysis of genetic diversity in crop plants—salient statistical tools and considerations. *Crop Science*, *43*:4, 1235-1248.

Mondini, L., Noorani, A. and Pagnotta, M. A. (2009). Assessing plant genetic diversity by molecular tools. *Diversity*, *1*:1, 19-35.

Morgante, M. and Olivieri, A. M. (1993). PCR_amplified microsatellites as markers in plant genetics. *The plant journal*, *3*:1, 175-182.

Morris, M. L., and Bellon, M. R. (2004). Participatory plant breeding research: opportunities and challenges for the international crop improvement system. *Euphytica*, *136*:1, 21-35.

Mullins, I. M. (1999). *Evolutionary relationships in Oryza inferred from the gene that encodes the 10 kDa prolamin (seed storage protein) polypeptide* (Doctoral dissertation, Virginia Polytechnic Institute and State University).

Mwangi, J. K., Murage, H., Ateka, E. M. and Nyende, A. B. (2013). Agronomic diversity among rice (*Oryza sativa* L.) lines in a germplasm collection from Kenya. *In*: *Scientific Conference Proceedings*, 2013.

Mwangi, R. W. and Rembold, H. (1988). Growth_inhibiting and larvicidal effects of Melia volkensii extracts on Aedes aegypti larvae. *Entomologia experimentalis ET applicata*, *46*:2, 103108.

National Academy of Sciences. (1972). Genetic vulnerability of major crops. National Academy of Sciences, Washington, D.C.

Negash, A., Tsegaye, A., van Treuren, R. and Visser, B. (2002). AFLP analysis of onset clonal diversity in south and south-western Ethiopia for conservation. *Crop Science*, *42*:4, 1105-1111.

Nei, M., and Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences*, *76*(10), 5269-5273.

Nelson, D. W. and Sommers, L. (1982). Total carbon, organic carbon, and organic matter. *Methods of soil analysis. Part 2. Chemical and microbiological properties*, (methodsofsoilan2), 539-579.

Newmah, J. T. (2010). *Morpho-Agronomic Characterization of Newly Developed Upland Rice Germplasm (Orzya sativa L., Orzya glaberrima Steudel) From the Africa Rice Center and Ghana* (Doctoral dissertation).

Ng, N.Q., T.T. Chang, D. A. Vaughan and Zuno-Alto V.C. (1988). Africa Rice Diversity:

Conservation and Prospect for Crop Improvement. *Crop Genet. Resource. Afr.* II: 213-227.

Nwakanma, D. C., Pillay, M., Okoli, B. E., and Tenkouano, A. (2003). Sectional relationships in the genus Musa L. inferred from the PCR-RFLP of organelle DNA sequences. *Theoretical and Applied Genetics*, *107*:5, 850-856.

Nwanze, K.F., Mohapatra, S., Kormawa, P.M., Keya, S. and Bruce-Oliver, S. 2006. Rice development in sub-Saharan Africa. Perspective. *J. Sci. of Food and Agric*., 86: 675-677. **Ogata, J. N., Kawano, Y., Bevenue, A., and Casarett, L. J. (1972).** Ketoheptose content of some tropical fruits. *Journal of agricultural and food chemistry*, *20*:1, 113-115.

Ogunbayo, S. A., Ojo, D. K., Guei, R. G., Oyelakin, O. O., Sanni, K. A. (2005). Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques, *African Journal of Biotechnology*, 11:1234-1244.

Oppong-Konadu, E. Y. Akromah R., Dapaah H. K., and Okai A. E. (2005). Genetic diversity within Ghanaian cowpea germplasm based on SDS-page of seed proteins. *African Crop Science Journal*, *13*:2, 117-123.

Otoo, S.K., Tian,S., Stewart, R., Slukvin,I. and Thomson, J. (2009). Human induced pluripotent stem cells free of vector and transgenes seguences. *Science*, 8:797-801. Doi: 10.1126/ science. 1172482.

Paddock, W.C. (1970). How green is the Green Revolution? *Bio Science* 20:897-902.

Padulosi, S. (1993). Genetic diversity, taxonomy and ecogeographical survey of the wild relatives of cowpea (Vigna unguiculata (L.) Walp). Ph.D Thesis. p. 346.

Page, J. M. and Nishimizu, M. (1982). Total factor productivity growth, technological progress and technical efficiency change: dimensions of productivity change in Yugoslavia, 1965-78. *The Economic Journal*, *92*(368), 920-936.

Papa, R. and Gepts, P. (2003). Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (Phaseolus vulgaris L.) from Mesoamerica. *Theoretical and Applied Genetics*, *106*:2, 239-250.

Pardey, P. G., Beintema, N. M., Dehmer, S., and Wood, S. (2006). *Agricultural Research: A growing global divide?* (Vol. 17). Intl Food Policy Res Inst.

Paterson, A. H., Damon, S., Hewitt, J. D., Zamir, D., Rabinowitch, H. D., Lincoln, S. E and Tanksley, S. D. (1991). Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. *Genetics*, *127*:1, 181-197.

Pearson, K. (1901). Principal components analysis. *The London, Edinburgh and Dublin Philosophical Magazine and Journal*, *6*:2, 566.

Peeters, J. P., and Martinelli, J. A. (1989). Hierarchical cluster analysis as a tool to manage variation in germplasm collections. *Theoretical and Applied Genetics*, *78*:1, 42-48.

Perera, L., Russell, J. R., Provan, J. and Powell, W. (2000). Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (Cocos nucifera L.). *Genome*, *43*:1, 15-21.

Pervaiz, Z. H, Rabbani, M.A., Khaliq, I., Pearce, S. R. and Malik, S. A. (2010). Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. Electronic Journal of Biotechnology, 13:3: 1-12.

Primmer, C. R. (2009). From conservation genetics to conservation genomics. *Annals of the New York Academy of Sciences*, *1162*:1, 357-368.

Pritchard, J. K., Stephens, M., Rosenberg, N. A. and Donnelly, P. (2000). Association mapping in structured populations. *The American Journal of Human Genetics*, *67*:1, 170-181.

Qualset, C.O. and Shands H.L. (2005). Safeguarding the future of U.S. agriculture.The need to conserve threatened collections of crop diversity worldwide. Genetic Conservation Program, Univ. California, Davis, CA.

Qualset, C.O., A.B. Damania, A.C.A. Zanatta and S. B. Brush. (1997). locally based crop plant conservation. *In: N*. Maxted *et al.*, (ed.) Plant genetic conservation: The in situ approach. Chapman and Hall, London. p. 160–175.

Rabbani, M. A., Pervaiz, Z. H. and Masood, M. S. (2010). Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electronic Journal of Biotechnology*, 11 (4):123-130.

Real, R. and Vargas, J. M. (1996). The probabilistic basis of Jaccard's index of similarity. *Systematic biology*, *45*:3, 380-385.

Ribaut, J. M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G. O. and Hoisington, D. A. (1997). Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theoretical and Applied Genetics*, *94*:6-7, 887-896.

Riley, K. W., M. Zhou and V. Ramanatha Rao. (1995). Regional and crop networks for effective management and use of plant genetic resources in Asia, the Pacific and Oceania. Proc. XVIII Pacific Science Congress on Population, Resources and Environment: Prospect and Initiative, 5–12 June, Beijing, China.

Risch, N.J. (2000). Searching for genetic determinants in the new millennium. *Nature*, *405*(6788), pp.847-856.

Saker, Mahmoud M., Sawsan S. Youssef, Naglaa A. Abdallah, Hany S. Bashandy, and Ahmed M. El Sharkawy (2005). "Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP." *African Journal of Biotechnology* 4, no.9

Sasaki, T. (1999). Current Status of and Future Prospects for Genome Analysis in Rice. In: Molecular Biology of Rice, Shimamoto, K. (Ed.). Springer-Verlag Publication, Tokyo, pp: 3-4.

Sasaki, T. (2002). Rice genomics to understand rice plant as an assembly of genetic codes. *Curr. Sci.*, 83: 834-839.

Schlötterer, C. (2004). The evolution of molecular markers—just a matter of fashion? *Nature Reviews Genetics*, *5*:1, 63-69.

Schlötterer, C. and Tautz, D. (1992). Slippage synthesis of simple sequence DNA. *Nucleic acids research*, *20*:2, 211-215.

Schoen D.J and Brown A.H.D, (1993). Conservation of allelic richness in wild crop relatives is

Schulman, A. H. (2007). Molecular markers to assess genetic diversity. *Euphytica*, *158*(3), 313321.

Sengupta, S., Zhivotovsky, L. A., King, R., Mehdi, S. Q., Edmonds, C. A., Chow, C. E. T., ... and Rani, M. U. (2006). Polarity and temporality of high-resolution y-chromosome distributions in India identify both indigenous and exogenous expansions and reveal minor genetic influence of Central Asian pastoralists. *The American Journal of Human Genetics*, *78*:2, 202-221.

Shim, S. I., and Jørgensen Rikke Bagger (2000). "Genetic structure in cultivated and wild carrots (Daucus carota L.) revealed by AFLP analysis." *Theoretical and Applied Genetics* 101:1-2, 227233.

Siddiqui, S.U., Kumamaru, T. and Satoh, H. (2007). Pakistan rice genetic resources. *In*: Grain morphological diversity and its distribution. *Pakistan Journal of Botany* 39: 841-848.

Singh, S. R. and Rachie, K. O. (1985). Cowpea research, production and utilization. *Annual review of plant biology*, *32*:221-242.

Singh, S.P. (1989). Pattern of variation in cultivated dry bean. Ann. Rep. Bean Improve. Coop, 31: 180-182.

Smith, J S C; Chin, E C L; Shu, H; Smith, O S; Wall, S J; Senior, M L; Mitchell, S E; Kresovich, S and Ziegle, J (1991). Associations among widely used French and U.S. maize hybrids as revealed by restriction fragment length polymorphisms. *Euphytica*, 54:263-273.

Soleri, D. and Smith S. E. (1995). Morphological and phenological comparisons of two hopi maize varieties conserved in situ and ex situ. *Econ. Bot*. 49: 56–77.

Solh, M. (2005). Rice is life in 2004 and beyond. International Rice commission Newsletter, 54:1-10.

Steel, G.G. (1972). The cell cycle in tumours: an examination of data gained by the technique of labelled mitoses. *Cell Proliferation*, *5*:1:87-100.

Sudré, C. P., Leonardecz, E., Rodrigues, R., do Amaral Júnior, A. T., Moura, M. D. C. and Gonçalves, L. S. (2007). Genetic resources of vegetable crops: a survey in the Brazilian germplasm collections pictured through papers published in the journals of the Brazilian Society for Horticultural Science. *Horticultura Brasileira*, *25*:4, 496-503.

Suriyagoda, L. D. B., Thilakarathne, R. M. M. S., Nissanka, S. P. and Samita, S. (2011). Morphological variation in selected rice (*Oryza sativa* L.) germplasm of Sri Lanka. *Journal of the National Science Foundation of Sri Lanka*, 39:2.

Takrama, J. F. (2000). Modified CTAB method for extraction of DNA from leaves. *Cocoa Research Institute, Tafo, Ghana*.

Tamu, A. (2015). *Grain quality characterisation of 87 rice (Oryza sativa) accessions in Ghana* (Masters Dissertation) KNUST.

Tautz, D. (1989). Hypervariabflity of simple sequences as a general source for polymorphic DNA markers. *Nucleic acids research*, 17:16, 6463-6471.

Thompson, J. A., and Nelson, R. L. (1998). Utilization of diverse germplasm for soybean yield improvement. *Crop Science*, *38*:5, 1362-1368.

Thompson, J. A., Nelson, R. L. and Vodkin, L. O. (1998). Identification of diverse soybean germplasm using RAPD markers. *Crop Sci.*, 38:1348-1355.

Tsunoda, S. (1964). *Leaf characters and nitrogen response*. Jons Hopkins Press.

Tsunoda. (1964). A Developmental analysis of yielding ability in field crops (in Japanese, English summary). *Nihono-Gskujitdu-Shinkokai, Maruzen Publ. Co. Tokyo*. pp.135.

Van de Wouw, M., Kik, C., van Hintum, T., van Treuren, R. and Visser, B. (2010). Genetic erosion in crops: concept, research results and challenges. *Plant Genetic Resources*, *8*:01, 1-15.

Van Hintum, T. J. (1996). Core collections in germplasm conservation, evaluation and use.

Van Treuren, R. and de Vicente, M. C. (2005). *Molecular markers for genebank management* (No. 10). Bioversity International.

Vanghan, D.A., H. Morishima and K. Kadowaki. (2003). Diversity in the *Oryza* genus. *Current Opinion in Pl. Biol*. 6: pp.39-46.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Wang, Z. Y., Second, G. and Tanksley, S. D. (1999). Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. *Theor. Appl. Genet*. 83: 565-581.

Warburton, M. L., Xianchun, X., Crossa, J., Franco, J., Melchinger, A. E., Frisch, M. and Hoisington, D. (2002). Genetic characterization of CIMMYT inbred maize lines and open pollinated populations using large scale fingerprinting methods. *Crop Science*, *42*6), 1832-1840.

WARDA. (2005). Rice Trends in Suh-Sahara Africa, Third Editions Cotonous, p. 31.

Warwick, S. I., Gugel, R. K. Mcdonald, T. and Falk. K. C. (2006). Genetic variation of Ethiopian mustard (*Brassica carinata*) germplasm in western Canada. *Genet. Resour. Crop Evol.,*

Weising, K., Atkinson, R. G., and Gardner, R. C. (1995). Genomic fingerprinting by microsatellite-primed PCR: a critical evaluation. *Genome Research, 4*:5, 249-255.

Welsh, J. and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic acids research, 18*:24, 7213-7218.

West Africa Rice Development Association (WARDA) (2007). Tendances rizicoles en Afrique: Vue d'ensemble sur l'évolution du secteur rizicole en ASS, Synthèse du Centre du riz pour l'Afrique, Cotonou, Bénin: West Africa Rice Development Association, p: 10.

World Almanac. (1998).

Wu, N., Fu, K., Fu, Y. J., Zu, Y. G., Chang, F. R., Chen, Y. H., Liu, X. L., Kong, Y., Liu, W. and Gu, C. B. (2004). Antioxidant activities of extracts and main components of pigeonpea

[*Cajanus cajan* (L.) Millsp.] leaves. Molecules 14:1032-1043.

Xia, Q., Zhou, Z., Lu, C., Cheng, D., Dai, F., Li, B. and Pan, G. (2004). A draft sequence for the genome of the domesticated silkworm (Bombyx mori).*Science*, *306*:5703, 1937-1940.

Xing, Y. and Zhang, Q. (2010). Genetic and molecular bases of rice yield. *Annual review of plant biology*, *61*, 421-442.

Xiong, Y., Zhang, H. and Beach, D. (1992). D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell*, *71*:3, 505-514.

Yadav, R., Courtois, B., Huang, N. and McLaren, G. (1997). Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theoretical and Applied Genetics*, *94*:5, 619-632.

Yamada, M., Ishii, M., Hibino, H. and Kurachi, Y. (2004). Mutation in nucleotide-binding domains of sulfonylurea receptor 2 evokes Na-ATP-dependent activation of ATP-sensitive K+ channels: implication for dimerization of nucleotide-binding domains to induce channel opening. *Molecular pharmacology*, *66*:4, 807-816.

Yan, W., F.N. Lee, J.Neil Rutger, K.A.K. Moldenhauer, and Gibbons J.W. (2002). Chinese germplasm evaluation for yield and disease resistance. R.J. Norman and J.-F. Meullenet, (eds.). *B.R. Wells Rice Research Studies 2001*. pp. 349-358, Univ. of AR, Agri. Exp. Sta., Res. Ser. 495.

Yang, S., Wen, P., Ash, G., Harper, J., Carling, J., Wenzl, P., Huttner, E., Zong, X. and Kilian, A. (2007). Low level of genetic diversity in cultivated pigeonpea compared to its wild relatives is revealed by diversity arrays technology. Theoretical and Applied Genetics 113:585595.

Yano, M. and Sasaki, T. (1997). Identification of quantitative trait loci controlling heading date in rice using a high-density linkage. *Theoretical and Applied Genetics*, 95 :7, 1025-1032.

Yoshida, S. (1981). *Fundamentals of rice crop science*. Int. Rice Res. Inst.

Yoshida, S. (1983). Rice Plant Characters in relation to yielding ability. *In:* Fundamentals of Rice Crop Science. International Rice Research Institute Publication. Los Banos, Philippines.

Yu, J., Hu, S., Wang, J., Wong, G. K. S., Li, S., Liu, B and Cao, M. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. indica).*science, 296*:5565, 79-92.

Zada, M., Zakir, N., Rabbani, M. A., Shinwari, Z. K. (2013). Assessement of genetic variation in Ethiopian mustard (*Brassica carinata* A. Braun) germplasm using multivariate techniques.

Zafar, N. A., S. Aziz and S. Masood. (2004). Phenotypic for agro-morphological traits among landrace genotypes of rice (*Oryza sativa* L) from *Pakistan, International Journal of Agriculture and Biology*, 2:335-339.

Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. Genetics, 136:4, 1457-1468.

Zhuang, J. Y., Lin, H. X., Lu, J., Qian, H. R., Hittalmani, S., Huang, N. and Zheng, K. L. (1997). Analysis of QTL× environment interaction for yield components and plant height in rice. *Theoretical and Applied Genetics, 95*:5-6, 799-808.

Zietkiewicz, E., Rafalski, A., and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics, 20*:2, 176-183.

APPENDICES

Appendix I: Accessions of rice, their sources and collection countries

ACC NO	NAME	SOURCE

1	LAC 23-1	AFRICA RICE / LIBERIA
2	LAC 23-2-2	AFRICA RICE / LIBERIA
3	LAC 23-2-3	AFRICA RICE / LIBERIA
4	LAC 23-3	AFRICA RICE / LIBERIA
5	LAC 23-4	AFRICA RICE / LIBERIA
6	LAC 23-5	AFRICA RICE / LIBERIA
7	LAC 23-6	AFRICA RICE / LIBERIA
8	LAC 23-7	AFRICA RICE / LIBERIA

9	LAC 23-8	AFRICA RICE / LIBERIA
10	LAC 23-9	AFRICA RICE / LIBERIA

11	LAC 23-10	AFRICA RICE / LIBERIA
12	LAC 23-11	AFRICA RICE / LIBERIA
13	LAC 23-12	AFRICA RICE / LIBERIA
14	GH 1510	PGRRI/GHANA
15	GH 1515	PGRRI/GHANA
16	GH 1518	PGRRI/GHANA
17	GH 2204	PGRRI/GHANA
18	GH 2193	PGRRI/GHANA
19	GH 1512	PGRRI/GHANA
20	GH 1521	PGRRI/GHANA

21	GH 1534	PGRRI/GHANA
22	GH 1538	PGRRI/GHANA
23	GH 1526	PGRRI/GHANA
24	GH 1528	PGRRI/GHANA
25	GH 1536	PGRRI/GHANA
27	GH 1594	PGRRI/GHANA
28	GH 2144	PGRRI/GHANA
29	GH 1593	PGRRI/GHANA
31	GH1588	PGRRI/GHANA

32	GH 1587	PGRRI/GHANA
33	GH 1580	PGRRI/GHANA
34	GH1581	PGRRI/GHANA

35	GH 1578	PGRRI/GHANA
36	GH 1575	PGRRI/GHANA
37	GH 1572	PGRRI/GHANA
38	GH 1579	PGRRI/GHANA
39	GH 1584	PGRRI/GHANA
40	GH 3623	PGRRI/GHANA
41	GH 1576	PGRRI/GHANA
42	GH 1550	PGRRI/GHANA
43	GH 1598	PGRRI/GHANA
44	GH 1570	PGRRI/GHANA
47	GH 1573	PGRRI/GHANA

48	GH 1523	PGRRI/GHANA
49	GH 1549	PGRRI/GHANA
50	GH 1524	PGRRI/GHANA
51	GH 1540	PGRRI/GHANA
52	GH 1571	PGRRI/GHANA

Appendix II: Field Layout

Rep I

V 28	V 46	V 30	V 19
V 45	V40	V 49	V 23
V 18	V 3	V 33	V 31
V 1	V 35	V37	V 44
V 6	V 15	V 47	V 24
V 36	V 32	V 8	V 12
V 2	V 21	V 16	V 41
V 22	V 27	V 5	V 39
V 48	V 43	V 50	V 17
V 42	V 29	V 38	V 14
V 13	V 10	V 25	V 26
V 20	V 52	V 34	V7
V 11	V 4	V 51	V 9

Rep II

V27	V7	V22	V31
V26	V51	V46	V9
V21	V12	V18	V23
V19	V28	V17	V11
V30	V1	V2	V5
V24	V48	V47	V13
V37	V15	V50	V42
V33	V35	V20	V16
V52	V49	V45	V36
V8	V43	V10	V4
V25	V41	V3	V39
V6	V14	V44	V34
V32	V40	V38	V29

Rep III

V32	V22	V16	V12
V19	V41	V26	V51
V49	V30	V14	V9
V43	V25	V34	V39
V4	V44	V7	V35
V46	V40	V38	V10
V24	V36	V28	V48
V45	V13	V42	V8
V50	V31	V37	V2
V27	V6	V33	V29
V1	V20	V17	V52
V11	V47	V21	V18
V15	V3	V5	V23

Appendix III SOIL ANALYSIS SHEET

SAMPLE ID **SOIL**	**% Org. Carbon**	**% Org. Matter**	**% TOTAL N**	**Exchangeable Cations (cmol/kg)**				**Exch. Acidity (cmol/kg)**		**AVAIL P mg/kg**	**pH**	**ECEC**
				K	**Na**	**Ca**	**Mg**	**Al^+**	**H^+**			
SOIL	0.80	1.38	0.097	0.53	0.31	4.08	0.60	1.00	0.60	6.49	6.88	7.12

Appendix IV: Rice descriptors used for characterization

Descriptor	Descriptor state	Score Code
Vegetative Stage		
Leaf Sheath	Anthocyanin Coloration	
	Absent	0
	Weak	3
	Medium	5
	Strong	7
Leaf Blade Attitude	Angle of the penultimate leaf prior to heading Erect	
		1
	Horzontal	5
	Drooping	7
Leaf	Auricle Color	
	Absent (No auricle)	0
	Whitish	11
	Yellowish green	62
	Purple	80
	Light purple	81
	Purple lines	84
Leaf	Shape of Ligule Absent(no ligule)	0
	Truncate	1
	Acute	2
	Cleft	3
Leaf	Color of ligule	
	Absent(no ligule)	0
	Whitish	11
	Yellowish green	

Early Reproductive

Flag Leaf	Attitude of Blade	
	Erect	1
	Semi-erect	3
	horizontal	5
	Descending	7
Clum	Kneeing ability(Forfloating rice only	

Appendix IV continued

	Absent	0
	Present	1
Clum	Habit(Angle)	
	Erect	1
	Semi-erect	3
	Open	5
	Spreading	7
Awns	Color of awns	
	Absent	0
	Whitish	11
	Straw	20
	Gold	40
	Brown	52
	Light green	61
	Red	70
	Purple	80
	Black	100
Awns	Distributionof awns	
	Absent	0
	Tip only	1
	Upper quarter only	2
	Upper half only	3
	Upper three quarters	4
	Whole length	5

Late Reproductive (Observation)

Flag Leaf	Attitude of Blade	
	Erect	1
	Semi-erect 3 horizontal 5 Descending 7	
Culm	Anthocynin colorationof nodes	
	Absent	0
Purple	80 Light purple 81	
	Purple lines	84

Culm	Internode anthocynin	
	Absent	0
	Purple	80
	Purple lines	84

Appendix IV continued

Culm	Lodging resistance(Culm strength	
	Very weak	1
	Weak	3
	Intermediate	5
	Strong	7
Panicle	Exsertion	
	Enclosed	1
	Partly exserted	3
	Exserted	5
	Moderately- well exserted	7
	Well exserted	9
Panicle	Attitude of main Axis	
	Upright (erect)	1
	Semi-Upright(semi-erect	2
	Slightly drooping	3
	Stronly drooping	4
Panicle	Attitude of branches	
	Compact	1
	Semi-compact	3
	Open 5 horizontal 7	
	Drooping	9
Panicle	Secondary branches	
	Absent	0
	Sparse(light)	1
	Dense(heavy)	2
	Clustering	3
Serile	Lemma color	
	Straw	20
	Gold	40
	Red	70
	Purple	80
Serile	Lemma length	

Short(≤1.5mm) 1 medium(1.5-2.5mm) 3 Long(>2.5mm) 5
Extralong(>=the lemma) 7

Asymmetrical 9 **Appendix V**: ANOVA Tables

Trait: Culm diameter at basal internode_

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	1045.308	21.777	20.62	<.001
Residual	96	101.368	1.056		
Total	144	1146.675			

Trait: Flag leaf length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	17878.857	372.476	73.40	<.001
Residual	96	487.153	5.075		
Total	144	18366.010			

Trait: Leaf length of blade

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	9976.654	207.847	100.61	<.001
Residual	96	198.327	2.066		
Total	144	10174.981			

Trait: Leaf length of ligule

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	16.16648	0.33680	11.07	<.001
Residual	96	2.92000	0.03042		
Total	144	19.08648			

Trait: Leaf width of blade

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	6.52271	0.13589	5.42	<.001
Residual	96	2.40667	0.02507		
Total	144	8.92938			

Trait: panicle per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	3119.444	64.988	53.48	<.001
Residual	96	116.667	1.215		
Total	144	3236.110			

Trait: Plant Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	94913.8	2019.4	16.27	<.001
Residual	96	11915.2	124.1		
Total	144	106829.0			

Trait: Awn length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	20291.2	431.7	3.26	<.001
Residual	96	12727.6	132.6		
Total	144	33018.8			

Trait: Grain Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	7.4175	0.1578	1.27	0.164
Residual	96	11.9549	0.1245		
Total	144	19.3724			

Trait: Grain width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	14.2508	0.3032	0.73	0.888
Residual	96	40.0969	0.4177		
Total	144	54.3477			

Trait: 100 grain weight of fully developed grain

Source of variation	d.f.	s.s.		v.r.	F pr.
Varieties	48	18.5625	0.3949	2.48	<.001

m.s.

Residual	96	15.2765	0.1591		
Total	144	33.8390			

Trait: Panicle length of the main axis

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	1039.14	22.11	2.07	0.001
Residual	96	1023.84	10.66		
Total	144	2062.98			

Trait: Sterile lemma leaf

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	48	119.493	2.542	1.52	0.043
Residual	96	160.667	1.674		
Total	144	280.160			

Trait: Productive Tiller / plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	47	1772.22	37.71	1.75	0.011
Residual	96	2073.33	21.60		
Total	143	3845.56			

LIST OF PLATES

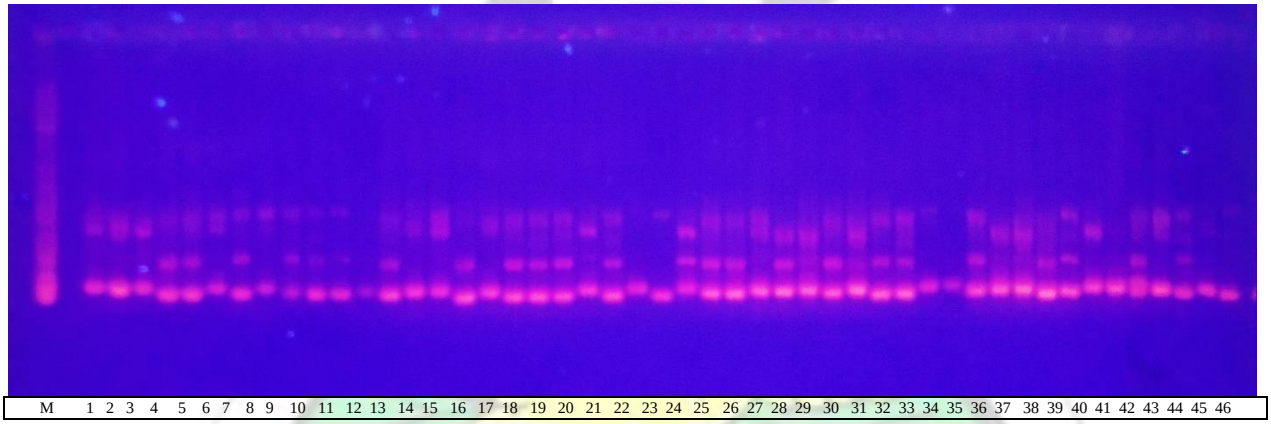


Plate 1: Primer RM 125 showing genetic diversity in 48 DNA samples

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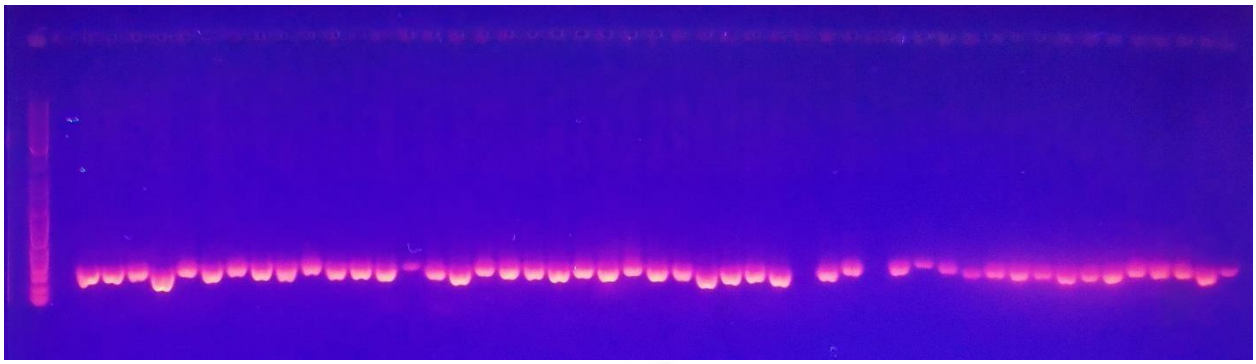

Plate 2: Primer RM 171 Showing genetic diversity in 48 DNA samples

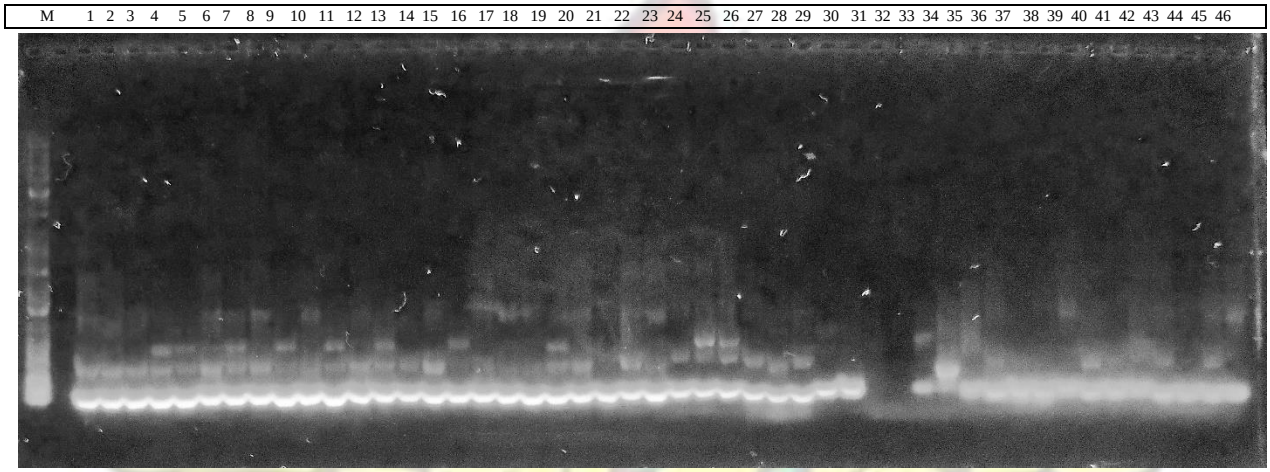

Plate 3: Primer RM 11Showing genetic diversity in 48 DNA samples