CHARACTERISATION OF JASMINE 85 RICE (*Oryza sativa*) VARIETY FROM DIFFERENT SOURCES OF SEED PRODUCTION IN GHANA

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BY

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DECLARATION

I hereby declare that this thesis is my own work towards the Master of Philosophy degree (Seed Science and Technology) and to the best of my knowledge. It contains no material previously published by another person or material which has been accepted for the award of any other degree of the University except where due acknowledgment has been made in the text.

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ABSTRACT

Following the observation that rice variety Jasmine 85 from two sources in Ghana were different in morphology, samples of Jasmine 85 were collected from seven sources in Ghana. A reference source was obtained from Africa Rice Centre in Senegal for comparison. An experiment was set up in a Randomized Complete Block Design and under standard conditions in Nobewam (Ashanti region) to ascertain if there were any differences among the sources. Morphological data was taken in the field to identify off types and characterise the sources. All the sources were not significantly different in terms of aroma, anthocyanin coloration, leaf pubescence, and ligule shape, but the sources showed significant differences with regards to pericarp colour, days to 50% heading, plant height, number of tillers, seed length, seed width, panicle length, and number of secondary branches. Physico-chemical analyses were done for further characterisation. Grain size and shape, grain chalkiness, cooking time, head rice yield, gelatinisation temperature, amylose content, and viscosity properties were significantly different among the sources. Grain hardness was not significantly different among the sources. Molecular characterisation using 15 SSR markers was done to establish the genetic resemblance among the sources. The sources differed significantly, although the result also showed that they are closely related. A cluster analysis run on the morphological and physico-chemical data gave four clusters: (GBEWAA and TONO), (SARI), (PV), (KIP, CRI, ARI, DARTEY). The molecular data on the other hand gave three clusters: (TONO, DARTEY, PV), (SARI, ARI, CRI), (KIP, GBEWAA). The results imply that seeds from different sources should not be mixed for production, and these varieties should be treated separately in future evaluations.

DEDICATION

I dedicate this work to my parents Dr. Akintayo Inussa and Mrs. Brym Modesta.

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CHAPTER ONE

INTRODUCTION

Rice is the most important basic food in the world. It serves as the staple food of more than 2.5 million people in developing countries with an annual consumption going beyond 100kg/habitant (Courtois, 2007). It is one of the most important cereals worldwide, classified second after maize in terms of cultivated area and quantity produced (IRRI, 2005a). According to FAOSTAT (2013), the world production of paddy rice in 2011 was 722,760,295 tons for a cultivated area of 106,412,497 hectares while the African production was 24,511,877 tons for a cultivated area of 9,383,330 hectares.

Asia dominates the rice economy with 90% of cultivated area of rice, with the United States of America, Latin America and Africa sharing the other 10% (Courtois, 2007). Many scientific discoveries show that rice is a good source of food for human consumption. This is because the grain's proteins, lipids and glucose content are at an equilibrium quantity. According to Food and Agriculture Organization (FAO, 2008), rice represents 27% of energy and 20% of alimentary protein. The germ and the husk which are eliminated during threshing are rich in vitamins – especially vitamin B1 – minerals, fibre and enzymes.

By using the slogan "RICE IS LIFE", 2004 was proclaimed by FAO as the international rice year, underlining the importance of this commodity in human nutrition. In developing countries it represents a good ally of the fight against hunger (FAO, 2004).

Since the middle 70s, rice consumption in Africa far exceeds local production necessitating huge imports (WARDA, 2007). Recently, importation into Africa has reached 10 million tons (FAO, 2011), causing a huge loss of currency in African countries. Factors causing this growing importation are overpopulation in consumer areas, urbanization and the change in

the diet of people (WARDA, 1993). Other phenomena leading to this huge importation are genetic, biotic and abiotic problems that reduce rice integrity and production. It is therefore important that rice production be raised to face this growing demand.

Seed is the first factor for increasing production and contributes up to 40% of production increase (Ministry of Agriculture water, and Fisheries, Burkina-Faso, 2011). Without seed there is no agricultural production (Sidibé, 2007). The use of quality seed provides guaranteed regularity of production and at the same time increases it, which amounts to an increase in farmers' income. To tackle the above problems, it is important that each African country comes out with a strategy to raise their seed production.

In the case of Ghana, with a paddy rice production of 463,975 tons for a cultivated area of 197,480 hectares giving average yield of 2.3 tonne/hectare in 2011 (FAOSTAT, 2013), rice seed production is a subject of varietal purity and integrity problems, such that Ghanaian consumers find satisfaction in foreign rice. Ghana is the largest export market for the United States of America in West Africa. In 2009, local rice farmers produced only 30% of the country's rice requirement; the remaining 70% was imported. The importation of rice into Ghana has increased from $100 million in 1999 to $500 million in 2009 (Ghana business news, 2011),

In Ghana, aromatic rice is the most desired rice type. Thus, Jasmine 85 is grown in almost all parts of the country. Jasmine 85 is an aromatic rice variety developed by the International Rice Research Institute (IRRI) from a cross between IR262-43-8-11 and KHAO DAWK MALI 4-2-105 (Marco *et al*, 1997). It was released in Ghana as Gbewaa rice in 2009 by CSIR-Savannah Agricultural Research Institute (CSIR-SARI) (Diako *et al*, 2011).

The current Jasmine 85 grown in Ghana is not a single uniform variety. It has been noticed that Jasmine 85 grown by Prairie Volta Co. Ltd was morphologically different from that

grown by Crops Research Institute, and both were different from that obtained from CSIR-SARI (Dartey, personal communication). A seed company that purchases Jasmine 85 from different sources in Ghana might inadvertently mixed different varieties.

This study therefore seeks to use morphology, physico-chemical and molecular tools to characterise Jasmine 85 from different sources and compare with a check from Africa Rice Centre (AfricaRice).The true signature of Jasmine 85 will be identified from the study, but in addition the other variants will be characterized.

The main objective of this study was to establish the most reliable source of Jasmine 85 in terms of seed quality in Ghana.

The specific objectives included:

- to characterise the Jasmine 85 collected from the different seed production sources,
- to determine which of the Jasmine 85 seeds collected is the true one, and
- to determine the level of purity of each of the Jasmine85 seeds collected.

In response to these, some hypotheses have been formulated:

- Jasmine 85 seeds in Ghana are pure
- Jasmine 85 in Ghana from different seed sources are similar

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy and geographical location of rice

Rice is an annual grass which belongs to the genus *Oryza* and the family poaceae (Agropedia, 2009). This cereal is a monocot crop that can self-pollinate (Smith, 1998). In the taxonomy data base, there are 24 species under the genus *Oryza* (thus genomes A to K) and a chromosome number of 2n = 24 (IRRI, 2005b). Among its species only two are cultivated; these are *Oryza sativa* which originates from Asia and *Oryza glaberrima* which originates from Africa (Vaughan *et al*., 2003).

Oryza sativa is the most commonly grown species throughout the world. It is native to Southeast Asia, but has spread throughout tropical and sub-tropical environments (Vaughan *et al*, 2003). *Oryza sativa* is differentiated into two sub-species based on geographical conditions: the *indica* type and the *japonica* type (Linares, 2002). *Oryza glaberrima* is limited to Western Africa. This species is less yielding compared to *Oryza sativa* but resistant to several stresses (WARDA, 1996; Jones *et al*., 1997; Sarla *et al*, 2005; Futakuchi *et al*, 2009). Although they are distinctively different from each other, *Oryza glaberrima and Oryza sativa* are used as the background parents in varieties amelioration programmes.

2.2 Morphology of the rice plant

Rice is an annual grass, with round and hollow jointed culms. It is a semi-aquatic plant and consists of parenchymatic tissues (Agropedia, 2009). This crop is the only cultivated cereal plant adapted to growing in both flooded and non-flooded soils. The plant is grown under a

wide range of climatic and geographical conditions on all five continents. The major parts of the mature rice plant are: panicles, leaves, leaves sheath, tillers, stem and roots.

The presence of parenchymatic cells on leaf, culm and roots can diffuse oxygen from aerial parts downward to roots (Agropedia, 2009).

2.2.1 Panicle

The panicle is the top part of the rice plant, carried on the last inter-node. Panicles are composed of the primary ramifications that carry secondary branches themselves carrying the pedicels that carry the spikelets. The number of primary and secondary ramifications depends on species and Treatment. One single panicle can bear between 50 and 500 spikelets (Wopereis *et al.*, 2009).

2.2.2 Leaves

The leaves grow alternately on the stem, with one leaf per node. The last leaf wrapping the panicle is the panicle leaf or flag leaf. The leaves are the growth engine of the plant, as they capture solar radiation and produce carbohydrates. The plant breathes and transpires through its leaves. Leaf architecture may be erect, oblique or drooping depending on the variety. The sheath is the leaf part that wraps the tiller. At the junction between the leaf and the collar, two elements can be found (thus, the auricle and the ligule) (Wopereis *et al*, 2009).

2.2.3 Tillers

Just after the node of the main stem come the tillers also called secondary stem, which can in turn produce tertiary tillers. The group of tillers produced by a single plant constitute a rice hill. Tillering ability is a function of the variety, but is also influenced by growing conditions and crop management practices (Wopereis *et al*, 2009).

2.2.4 Stems

The stem is composed of a series of nodes and internodes. The internodes are hallow, with a smooth surface. The lower internodes are shorter than the upper ones. The main function of the stem is to transport water and nutrients and to bring air to the roots (Wopereis *et al,* 2009).

2.2.5 Roots

The roots constitute the underground portion of the plant. They serve as support, draw food and water from the soil, and store food. They are fibrous and consist of rootlets and root hairs. The embryonic roots produce few branches; they live for only a short time after germination. Secondary adventitious roots emerge from the underground nodes of the young culm and replace the embryonic roots (Agriquest, 2012).

A seed is a small embryonic plant enclosed in a covering called the seed coat, usually with some stored food. It is the product of the ripened ovule of gymnosperm and angiosperm plants which occurs after fertilization and some growth within the mother plant (Agriquest, 2012). The physical, physiological, phytosanitary and genetic qualities of the seed require attention so that farmers are provided with quality seed of the appropriate crops and the appropriate Treatment. Also, farmers depend on quality seed of appropriate varieties to attain food security (FAO, 2010).

Basically four quality aspects of seed are usually considered. These are:

- Genetic quality: Gene composition of the seed
- Analytical quality: percentage of undamaged seed of the desired Treatment of a specific crop or inert matter
- Physiological quality: ability of seed to germinate at the desired time and

- Phyto-sanitary or pathological quality: presence or absence of plant diseases in or on the seed (Adetumbi *et al,* 2014).

For the genetic quality the DUS (distinct- uniform- stable) characteristics is the most important.

- The distinctness of a variety is the ability to clearly distinguish it from any other plant variety.
- A plant variety must be sufficiently uniform in its characteristics. For uniformity assessment, a population standard deviation of 1% and an acceptance probability of at least 95% should be applied. For a sample of 20 plants, one off-type plant is accepted by Ghanaian authorities.
- A Treatment is stable if its relevant characteristics remain unchanged after repeated propagation. If required or in case of doubt, stability can be tested either by cultivating an additional generation, or by testing a new seed or a new plant material so as to check whether it presents the same characteristics as the material previously provided (Adetumbi *et al*, 2014., Crochemore *et al*,2004).

Genetic purity testing is conducted to ascertain the purity of the genetic make-up of the seed samples. Usually, this is conducted through grow-out test. Seed samples are grown alongside a known reference sample, with attention focused on the descriptive morphological characteristics. This method is being criticized as time consuming and subjective to environmental conditions. Recently, genetic purity testing is being conducted by molecular means. This involves the use of DNA extracted from either leaf or seed samples of the crop. It is faster and precise but involves a high level of biotechnology expertise (Adetumbi *et al,* 2014).

In Ghana, some scale production of Jasmine85 stated that they increase their output and get maximum satisfaction from their production by paying particular attention to their grain

quality including managing their own seed production sometime based on imported foundation seed (IFPRI, 2013).

2.3 Rice life cycle

Rice undergoes three phases in its life cycle. The three phases are grouped into ten stages. These are:

2.3.1 Vegetative phase

2.3.1.1 Stage0: Germination stage.

The embryo will germinate as soon as it finds sufficient moisture. Germination marks the start of metabolic activity and covers the period from the emergence of the coleoptile or the radicle to the emergence of the first leaf (Wopereis *et al*, 2009).

2.3.1.2 Stage 1: Seedling

This is the period that follows germination and takes about 14 days. During this stage the young seedling essentially feeds on the food reserve in the endosperm. Leaf production follows a rhythm of one leaf every three or four days. The seedling stage covers the period from the first leaf to the fifth leaf. During this stage the seedling also produces roots (Wopereis *et al*, 2009).

2.3.1.3 Stage 2: tillering

This is the period during which the seedling produces tillers. This stage starts with the emergence of the fifth leaf. The number of tillers increases until maximum tillering is reached (Wopereis *et al*, 2009; A.O.G.T.R, 2005).

2.3.1.4 Stage 3: internode elongation

When the tillering stage comes to its end, the plant's internodes start to grow, leading to an increase in plant height (Wopereis *et al*, 2009).

2.3.1.5 Stage 4: panicle initiation

This stage is marked by the emergence of the panicle. The young panicle that emerges inside the bottom of the last node become visible only 10 days after it is formed. At this stage the number of grains in the panicle is already determined. Timing of panicle initiation in rice is influenced by many factors, such as variety, temperature and photoperiod (Wopereis *et al,* 2009).

2.3.2 Reproductive phase

2.3.2.1 Stage 5: panicle development

This stage is characterized by the swelling of the bottom of the panicle leaf, which is due to the panicle growing upwards inside the stem. After initiation the panicle grow towards the top of the stem, causing a swelling in the stem called elongation. The organs of the flower develop and the panicle grows on until it reaches its final size before appearing from the flag leaf (Wopereis *et al*, 2009).

2.3.2.2 Stage 6: Heading and flowering

Heading is characterized by the emergence of the panicle from the bottom of the flag leaf. The panicle takes two to three weeks to emerge from the stem completely. Three days after heading, flowering occurs and the process goes on progressively until the panicle has completely appeared (Wopereis *et al,* 2009).

2.3.3 Maturity phase

2.3.3.1 Stage 7: milky stage

After fertilization, the ovary swells and the caryopsis (grain) develops until it reaches its maximum size after seven days. The grain is first aqueous and then reaches a milky consistency, which is perceptible when the grain is squeezed. At this stage the panicle are still green and erect (Wopereis *et al,* 2009).

2.3.3.2 Stage 8: Dough stage

The milky part of the grain becomes soft and then reaches a hard paste consistence about two weeks after flowering. The panicle begins to droop while the colour of the grains progressively changes into the colour that is characteristic of the variety (Wopereis *et al,* 2009).

2.3.3.3 Stage 9: Maturity

The grain is ripe when it has reached its final size and maximum weight, giving the panicle its droopy appearance. Grains become hard and develop characteristic colour dependant on the variety. This stage is reached when 85 to 90% of the panicle grains are ripe (Wopereis *et al,* 2009).

2.4 Aromatic rice

Aromatic rice has become popular because of its aroma. Growing demand for aromatic rice has spurred interest in the development of domestic cultivars that offer similar combinations of grain attributes such as texture, cooking characteristics, aroma, and taste. Aroma in rice is associated mainly with the presence of 2-acetyle-1-pyrroline. This compound is most closely associated with the aroma of Basmati and Jasmine types of rice (Buttery *et al*. 1983; Hien *et al*. 2006a; Lorieux *et al*. 1996; Widjaja *et al*. 1996; Yoshihashi *et al*. 2004).

Many other compounds are also found that cause aroma in aromatic rice cultivars (Widjaja *et al*. 1996). Methods for smelling leaf tissue, grains after heating in water, and reacting with solutions of 1.7% KOH are available (Sood and Siddiq 1978). The identification of 2-acetyle-1-pyrroline, using gas chromatography mass spectrometry selected ion monitoring (GC-MS-SIM) is also available (Hien *et al*. 2006b; Lorieux *et al*. 1996; Widjaja *et al*. 1996; Yoshihashi *et al*. 2004).

Molecular markers, such as single nucleotide polymorphism (SNP) and simple sequence repeats (SSR) that are genetically linked to aroma have been developed for the selection of aromatic rice (Cordeiro *et al*. 2002; Jin *et al*. 2003). The availability of rice genome sequences provided an opportunity to discover the gene responsible for aroma in rice by comparing the sequences of aromatic and non-aromatic genotypes (Goff *et al.* 2002; IRGSP 2005).

2.5 Rice seed production in Ghana

Rice has become a staple food in Ghana and much of West Africa where it serves as an important convenience food for urban consumers (Tomlins *et al.*, 2007). Rice is used in a wide range of food products in Ghana; the most common include jollof rice, rice balls, rice porridge, fried rice and plain-cooked rice. The various rice products are fundamentally different in their appearance and characteristics. Although rice forms a major part of the Ghanaian diet, locally grown rice is not patronized because of its variable quality. Several factors account for the variability in rice quality; the most dominating factors are the poor sensory and physical qualities (Tomlins *et al.*, 2005). These quality defects is not as a result of only inappropriate post-harvest handling, but also from poor planting materials and poor agronomic practices (Gayin *et al.*, 2009). Perhaps if the quality of local rice is improved and made comparable to that of the imported rice, then it will be possible to increase the market for local rice.

According to the 2010 budget statement of Ghana, the country imports about 70% of the domestic rice requirement because domestic rice is not competitive, accounting for about USD 600 million per annum (Duffuor, 2009). Buah *et al* (2011) reported that Ghana depends largely on imported rice to make up the deficit in rice supply. To overcome this, governments over the years have been promoting domestic rice production and consumption. However,

due to the poor grain quality of the locally produced rice, consumers usually prefer imported rice. Availability of improved rice varieties with good grain quality is therefore necessary in order to boost domestic rice production and consumption (Asante *et al.*, 2013).

Although many varieties of rice have been developed, few have been adopted possibly because researchers have not considered farmers' preferences and perceptions of varieties during the development process (Efisue *et al.*, 2008). Adoption of improved rice varieties may differ depending upon the preferences of the farmers. Farmers assess a new technology such as a new rice cultivar, in terms of a range of attributes, including grain quality, straw yield, and input requirements in addition to grain yield (Joshi and Bauer, 2006).

Previous studies on rice grain quality preference in Ghana and other parts of the world have focused on consumers (Bam *et al.*, 1998; Diako et *al.*, 2010; Abansi *et al.*, 1992; Choudhury *et al.*, 1992; Juliano and Duff, 1991). However, little has been done to assess grain quality in relation to the farmer in order to infer how grain quality could influence farmers' preference for improved rice varieties.

2.6 Jasmine85 background

Jasmine85 is an aromatic rice variety developed in Thailand in 1966 by Doctor Ben Jackson a rice breeder at the International Rice Research Institute. In 1989, the USDA in collaboration with IRRI, University of Arkansas, Louisiana State University, and Texas A&M University, released Jasmine 85 to American farmers. This variety grows rapidly, gives high yield, and carries good resistance to pests in southern United States. It also suppresses the growth of weeds in the surrounding area. All these desirable features allow US farmers to grow Jasmine85 as Organic Rice, for which health-conscious US consumers are willing to pay a premium price (Tanasugarn, 1998). One main problem with Jasmine 85 is the many broken grains found after milling, which tend to drive the price down (Hagrove, 1997).

The registration number of this variety is CV-1O7, PI 595927 and is characterised as:

- Irrigated and rain-fed Lowland rice
- Midseason aromatic
- Long-grain cultivar
- Medium grain cooking quality
- Soft in texture and cohesive
- Highly resistant to some pest and disease.
- intermediate and standard height cultivars
- Straw-coloured, awn-less, colourless
- Plant with erect tillers and upright flag leaves (IRRI, 1998).

In the 1998, Ghana's Savana Agricultural Research institute (CSRI-SARI) introduced this rice variety in their system with the purpose of reducing milled rice importation in Ghana. This variety has been chosen because of the characteristics it has, especially its aroma, grain size and grain length (Diako *et al.,* 2011).

2.7 Varietal Characterisation

The aim here is to find by using different strategies and tools, the fingerprint of the variety, bearing in mind that the fingerprint should not be similar from one variety to another. There are several approaches in assessing genetic similarity or diversity between different materials, which include analysis of pedigree, physical and chemical properties, morphological or molecular (Li *et al.,* 2002).

Plant morphological characters have been the universally undisputed as descriptors applied for testing distinctness, uniformity and stability (DUS) of crop varieties (Adetumbi *et al.*, 2013). Many studies on genetic diversity using agro-morphological characterisation have been conducted and led to the identification of the phenotypic variability in rice. However,

these descriptors, other than being limited in number, make the process time-consuming and also less reliable due to its interaction with the environment in which the cultivar is grown and subjectivity in decision-making (Ogunbayo *et al.*,2005; Bajracharya *et al.*,2006; and Barry *et al.*,2007)

Molecular marker technology is the powerful tool for determining genetic variation in rice

(Xu *et al*., 1974). In contrast to morphological traits, molecular markers can reveal abundant difference among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterisation. Also, this technology is untouched by environmental influence. Among various Polymerase Chain Reaction (PCR) based markers, the Simple Sequence Repeat (SSR) markers are more popular in rice because they are highly informative, and relatively less expensive (Gracia *et al.,* 2004). SSR markers are class of repetitive DNA sequences usually 2.6 base pairs that are distributed throughout whole genome and are flanked by highly conserved regions. (Chambers and Avoy 2000).

Molecular and phylogenetic techniques have become more important in recent works in plant taxonomy compared to morphological techniques which were traditionally used. Nevertheless, the use of morphology should not simply be dismissed but used in tandem with the recent molecular techniques as this provides a strong basis for phylogenetic hypotheses and classification (Sennblad *et al.,* 1998). Molecular analysis can rather be used as complement to the field morphological analysis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Field location

The fieldwork took place at Nobewam (North 06° 37’ 06.2”, West 001° 17’ 00.4”, 200 m above sea level) in the Ashanti region of Ghana. This area is characterized by double maximum rainfall lasting from March to July and from September to the latter part of November with mean annual rainfall of 1200mm (Anon, 2002).

3.2 Laboratory location

The physico-chemical and molecular analyses were conducted at the Grain quality laboratory and Biotechnology laboratory of AfricaRice in Cotonou. AfricaRice is one of 15 international agricultural research institutions under the Consultative Group for International Agricultural Research (CGIAR) with its headquarters in Cotonou, Benin. (http://www.africarice.org).

3.3 Seed sources

Rice variety Jasmine 85 from eight sources were used as treatments. Seven of these materials were obtained from seven different seed sources in Ghana whilst the check was from AfricaRice, Senegal. Although Jasmine 85 was originally bred by IRRI, seed sample could not be obtained from them.

After harvesting, true to type seeds obtained from the field work were used to carry out the laboratory analyses.

The sources of Jasmine 85 seeds are presented in the Table 3.1.

Table 3.1: Seed source, location and designation

Source of seed	Location	Designation
Savana Agricultural Research Institute	Tamale	GBEWAA
Savana Agricultural Research Institute	Tamale	SARI
Kpong Irrigation Project	Asutsuare	KIP
Crops Research Institute	Nobewam	DARTEY
Crops Research Institute	Fumesua	CRI
Prairie Volta Co Ltd	Aveyime	PV
Tono Irrigation Project	Tono	TONO
AfricaRice	Senegal	ARI

3.4 Experimental designs

The field experiment was arranged in a Randomized Complete Block Design (RCBD) with four replications. The layout of the field is presented in appendix 1.

Complete randomized design was used for statistical analyses of the physico-chemical, varietal purity and molecular properties of the treatments.

3.5 Agronomic practices

Standard agronomic practices: nursery, land preparation, transplanting, fertilizer application, pest and weed control and harvesting; were adhered to.

3.5.1 Nursery

Seed beds were prepared 14 days before sowing. Weeds and other plants (previous crop plants) were uprooted before the beds were seeded. Seeds (300 grams from each source) were pre-germinated and sown by broadcasting on a wet nursery bed. Seeds from each source were sown in a nursery plot measuring 10 m x 2 m.

3.5.2 Land preparation and transplanting

The land was sprayed with total weed killer Sunphosate (410g/litre glyphosate) at the rate of 5 litres/hectare. The field was rotovated 10 days after herbicide application. The field for the experiment measured 25 m x 16 m; the field was demarcated to create four blocks, each measuring 2m x 25m and separated by 2 m wide space. Within each block, eight plots measuring 2m x 2m were demarcated and randomly allocated to a seed source (treatment). Randomization was generated from the software Cropstat. Plots within each block were separated by 1 m wide space (Appendix 1). Transplanting was done 14 days after rotovation. Seedlings from a given seed source were planted in plots allocated to that source at a planting distance of 20cm x 20cm. A total of 100 plants (10 rows x 10 columns) was planted in each plot.

3.5.3 Fertilizer application

The following fertilizer regimes were adhered to:

- 60-60-60 kg/ha (160g/plot) of NPK (15-15-15) applied by broadcasting five days after transplanting
- 30 kg (N)/ha (26g (Urea)/plot) equally split applied at tillering (25 days after transplanting) and at booting (70 days after transplanting).

3.5.4 Pest and weed control

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 Liter of water (600mls/ha). Spraying of insecticide was done;

- at vegetative stage just after tillering

- before reproductive stage at the panicle initiation

- and after reproductive stage at the grains ripening

Weed control was done manually by uprooting when necessary.

Bird pests were continually scared especially after the reproduction stage to harvesting time.

3.6 Harvesting

Harvesting was done per plot when 90% of plants on a given plot are physiologically matured (ripe seeds and droopy panicle). Harvesting was done by carefully cutting panicles with a pair of scissors. Panicles from each plant were placed in a separate envelope and labelled to reflect the plot (seed source), block and row.

3.7 Data analysis

Microsoft Excel was used to record and organize the data. GenStat statistical package (version 12) was used for the statistical analysis of variation among means at 5% degree of freedom. *Statistica* software was used for the data clustering. DARwin 5.0 software was used to analyse the molecular data.

3.8 Field Data

A total of 32 plants were randomly selected from each plot and tagged. Selection was done by randomly selecting 4 plants from each row. Border plants were ignored during the selection process. Off types when recognised were tagged as such and ignored during subsequent data collection.

The percentage purity was calculated by the formula:

$$\% \text{ Varietal Purity} = \frac{\text{Number of off type}}{\text{Total number of plants}} \times 100$$

- The following data were taken using the Standard Evaluation System for Rice (Bioversity International, 2007).

3.8.1 Aroma test

This test was done in the early vegetative stage (30 days after transplanting). To detect the presence of aroma and its level in the plant, leaf sample was taken and put in an eppendorf tube containing alcohol (90% ethanol), closed for 24 hours and well labelled. This allowed the aroma to be released. After this period the tube was opened and smelt by a panel of 4 persons in order to detect the presence as well as the level of aroma. The level of aroma were measured using the following scale: 0 (no aroma), 1 (lightly scented), 2 (strongly scented).

3.8.2 Basal leaf sheath colour

It was observed at the late vegetative stage. The Colour of the outer surface of the leaf sheath was observed and recorded.

3.8.3 Leaf blade pubescence

It was observed at the late vegetative stage and was assessed either visually or by touch, by rubbing fingers over the leaf surface from the tip downwards.

3.8.4 Ligule shape

The ligule of the plant is located between the leaf blade and the leaf sheath. Its shape was observed and recorded.

3.8.5 Plant height

This parameter was taken at maturity stage. It was taken from the base of the plant to the last seed of the highest panicle.

3.8.6 Heading days

This parameter was taken during the reproductive stage. It is the time between the transplantation day and the day when 50% of the plants have headed.

3.8.7 Tiller number, spikelet number

They were taken respectively at the vegetative stage and at the maturity stage by counting.

3.8.8 Panicle length

This was measured on the field at the maturity stage of the plant. It was done by measuring the distance between the panicle neck (collar) and the tip of the last seed on the panicle.

3.8.9 Seed coat (pericarp) colour

This was recorded by opening manually the seed husk and identifying the coat or pericarp colour.

3.8.10 Seed width, seed length

The seed width and length of 50 seeds per plot were measured using calliper at the maturity stage (just after harvesting).

3.9 Physico-chemical data

Seeds from the selected plants of a given plot (source) were combined, labelled and submitted to Quality Grain Laboratory for the physico-chemical analysis. For each sample, all laboratory analysis were repeated four times.

The moisture content of the seeds was taken using single kernel tester. Seeds were then milled because their moisture contents were appropriate (11-13%) for milling.

The dried samples were dehusked in a THU-34A Satake testing Rice Husker. The brown rice weight was taken and then polished in a BS08A Satake single pass friction Rice pearler. Broken and whole grains were separated. The weights of the whole grains and the broken grains were also taken after separation in a rotating cylinder.

The expression used for the yield determination was:

- Head rice yield (%) $= 100 \times \frac{\text{Weight of whole grains}}{\text{Weight of paddy}}$

3.9.1 Grain hardness

Grain hardness was measured using a grain hardness tester. Ten grains were used in the determination of hardness for each sample. The handle of the equipment was initially turned anticlockwise to make room to place a grain on the sample table. After this, the handle was turned clockwise until a cracking sound was heard. At this time, the black pointer returns to the zero point and the red pointer remained. The reading of the red pointer in (Kg) indicated the hardness of the grain.

3.9.2 Grain dimension and chalkiness

The grain dimension and chalkiness were determined using a Rice Statiscal Analyzer of type TechnologiaS21 LKL. This device is equipped with a camera and is connected to a computer. The process was to introduce into the equipment 50g of whole grain; the grains pass under the camera that captures their image. These were transmitted to the memory of the computer that calculates and displays the average of the dimensions (length and width) and the percentage chalky area.

3.9.3 Alkali spreading value and gelatinisation temperature

For this test, 10 ml of potassium hydroxide solution (KOH 1.7%) was poured on 6 rice grains placed in a transparent petri dish. The containers were closed and left at room temperature for 23 hours. The samples were then observed to assign scores (1-2-3-4-5-6 or 7) according to the digestibility of the grain, in comparison with standards. From these scores the gelatinisation temperatures were derived.

3.9.4 Pasting properties

The pasting properties of rice flour samples were measured using a Rapid Visco Analyzer model RVA- super4. Three grams of rice flour was weighed directly into the aluminium RVA canister and 25g of distilled water was added and mixed with the rice flour. The sample was held at 50°C for 1 minute, heated to 95°C at a rate of 12°C/minute, held at 95°C for 2.5 minute, cooled to 50°C at a rate of 12°C/minute and held at 50°C for 2.5 minutes. The rotating speed of the paddle was kept at 160 rpm throughout the run except that the paddle speed was 960 – 1038 rpm at the first 10 seconds. The viscosity graph appears on the computer screen and helps to determine some parameters like the viscosity chart, the peak viscosity and the final viscosity.

3.9.5 Cooking time

Five grams of milled rice was taken and poured into 135ml of boiling distilled water in a 400 ml beaker and covered with a watch glass. After 10 minutes of further boiling, 10 grains were taken out every two minutes with a ladle. The ten grains were pressed between two petri dishes and the grains were considered cooked when at least 9 out of the ten grains no longer had opaque centres. The time was then recorded. The cooking time was then calculated with the formula:

- Cooking time = Initial time – Final time

3.9.6 Amylose content

For the amylose content the iodine absorbance method was used. 100mg of rice floor was weighed and put into a 100ml volumetric flask. 1ml ethanol was added, in addition to 9ml of NaOH (1N). The flask was put into 500°C boiling water for 10 minutes for the flour to digest. After this, the flask was removed from the water and cooled for 2hrs. Distilled water was then added to the digested solution up to flask mark. During that time, 30ml of stock iodine and 10ml of acetic acid were poured in a 1000 ml volumetric flask, and distilled water was added up to the flask mark. The melange was then put on an agitator for 10 minutes in order to mix the iodine solution. The digested flour was then poured into small cups and disposed in the auto sampler according to the computer program. The iodine solution was also connected to the pumping machine.

When the process was activated, the solution was moved into the pumping machine using the auto sampler, where complex amylose iodine was formed and then sent to the spectrophotometer. The spectrophotometer read the complex's information and communicated it to the computer

3.10 Molecular analysis

The molecular test was done using fresh leaves from 3 weeks old rice seedlings. Each treatment was repeated 4 times. 15 SSR rice primers (Appendix 5) were used. The following methodology was used for each treatment:

CTAB METHOD FOR DNA EXTRACTION

20 mg of rice fresh leave was grounded in 2.0 ml micro tubes until it became fine paste, then liquid nitrogen was added. After this, 800 µl of 2% CTAB with 0.1 % of mercaptoethanol was added. The mixture was incubated in a sand bath at 65°C for 30 min with intermittent vortexing. It was cooled at room temperature and an equal volume (800 µl) of chloroform isoamyl alcohol was added. The solution was mixed gently by inversing the tube several times. It was then centrifuged at 14000 rpm for 15min. The aqueous phase of the sample was then transferred into a clean 1.5ml tube.

The nucleic acid was precipitated by adding two thirds volume of ice cold isopropanol (400 µl) and was shaken gently. The ice was kept on for 30 min. To enhance the precipitation, the sample in the tube was stored overnight at -20°C. After this, the pelleted nucleic acid was centrifuge at 14000 rpm for 5 min. When the pellets were fully formed, the isopropanol was decanted and the pellet washed with 500 µl of washing buffer on a rocking surface for 15 min. It was then centrifuge at 6000rpm for 4min.

The washing buffer was decanted and the pellet washed in 400 µl (80%) ethanol and then centrifuged at 6000rpm for 4 min. The ethanol was then decanted and the pellet dried in vacuum at 37°C for 10 min. After this, the DNA was suspended in 50µl 1X TE buffer and centrifuged at high speed for 30sec to remove all insolubles. During that time 1.0% of agarose gel was prepared with (3µl) 0.003% Ethidium bromide. 5µl of the sample was taken using pipette, and added to 1µl loading buffer.

The sample was loaded in the wells on gel submerged in 1X TAE buffer and the sample was run at (90) volts for 45 minutes. The computer connected to the machine recorded the data and pictured the gel.

CHAPTER FOUR

RESULTS

4.1 Seed genetic purity

The analysis of variance (ANOVA) shows significant difference among the sources (appendix 2). ARI had the highest percentage purity of 99.09% follow by PV and DARTEY with percentage purity of 98.76% and 98.16% respectively. GBEWAA and CRI had the least percentage purity of 82.23% and 85.1% respectively. Seeds from the remaining sources had percentage purity ranging from 94.11% to 88.51% (Table 4.1).

Table 4.1: Percentage purity of the sourced seeds

Source	Genetic purity (%)
SARI	89.36[c]
KIP	88.51[c]
GBEWAA	85.1[b]
DARTEY	98.16[e]
PV	98.76[e]
CRI	82.23[a]
TONO	94.11[d]
ARI	99.09[e]
LSD	1.27
CV(%)	1

Means with the same alphabet within columns are not significantly different at 5%.

4.2 Qualitative data: Aroma, Anthocyanin coloration, Leaf pubescence, Pericarp colour and Ligule shape

The eight treatments presented similar characteristics for aroma, anthocyanin coloration, leaf pubescent and ligule shape. The pericarp colour was of two types: the red pericarp from SARI and the light brown pericarp from the seven others.

Table 4.2: Aroma, anthocyanin coloration, leaf pubescence, pericarp colour, ligule shape of the various treatments

Treatment	**Aroma**	**Anthocyanin coloration**	**Leaf pubescence**	**Pericarp colour**	**Ligule Shape**
ARI	Lightly scented	Absent	Pubescent	Light brown	2-cleft
CRI	Lightly scented	Absent	Pubescent	Light brown	2-cleft
DARTEY	Lightly scented	Absent	Pubescent	Light brown	2-cleft
GBEWAA	Lightly scented	Absent	Pubescent	Light brown	2-cleft
KIP	Lightly scented	Absent	Pubescent	Light brown	2-cleft
PV	Lightly scented	Absent	Pubescent	Light brown	2-cleft
SARI	Lightly scented	Absent	Pubescent	Red	2-cleft
TONO	Lightly scented	Absent	Pubescent	Light brown	2-cleft

4.3 Quantitative data: 50% heading days, Plant height, Number of tillers, Seed length, Seed width, Panicle length, Number of secondary branches

The sample from ARI took the least number of days to reach the heading stage however; it was not significantly different from KIP and TONO. DARTEY reached the heading stage with the highest number of days. The difference between the number of days taken by ARI and DARTEY to reach the heading days was significantly different (15.75 days).

For the plant height, PV was tallest with height of 115.7 cm follows by DARTEY (113.7 cm). The source with shortest plants was ARI with 95.1 cm. The difference in height between PV and DARTEY was not significant (2 cm); however, the difference in height between PV and ARI was significant (20.6 cm).

The number of tiller varies between an average of 16.34 tillers for PV and 6.28 tillers for TONO; the difference between their numbers of tillers was significant.

The treatment that had the longest seed was ARI with a seed length of 10.57 mm while GBEWAA with a seed length of 9.56 had the shortest seed. The difference between the two seed length was 1.01.

For the seed width, it varied between 2.28 mm for KIP and 2.05 mm for PV. The difference between these two widths was significant (0.23 mm).

DARTEY had the longest panicle with 25.42 mm and the treatment with the shortest panicle size (21.14 mm) was TONO. The difference between the two sizes (4.28 mm) was significant.

Lastly, DARTEY recorded the highest number of secondary branches (11.75 branches) while GBEWAA (8.50 branches) recorded the lowest. The differences between the two means was also significant.

Table 4.3: Means of 50% heading (days); plant height (m); number of tiller; seed length (mm); seed width (mm); panicle length (mm); number of secondary branches of the various sources

Treatment	50% Heading (days)	Height (m)	Number of Tillers	Seed length (mm)	Seed width (mm)	Panicle length (mm)	Number of secondary branches
ARI	69.25^{c}	95.1^{e}	7.62^{cd}	10.57^{a}	2.25^{a}	21.85^{de}	10.75^{a}
CRI	84^{a}	111.4^{abc}	10.75^{b}	10.53^{a}	2.22^{a}	25.21^{ab}	11.50^{a}
DARTEY	85^{a}	113.7^{ab}	6.84^{cd}	10.32^{b}	2.213^{ab}	25.42^{a}	11.75^{a}
GBEWAA	74.25^{b}	106.9^{cd}	8.40^{bcd}	9.56^{f}	2.213^{ab}	22.05^{de}	8.50^{b}
KIP	71.50^{bc}	97.1^{e}	7.23^{cd}	10.24^{bc}	2.28^{a}	21.58^{de}	11^{a}
PV	83.50^{a}	115.7^{a}	16.34^{a}	10.12^{cd}	2.05^{c}	24.09^{bc}	11^{a}
SARI	84.25^{a}	109^{bcd}	9.18^{bc}	9.94^{de}	2.123^{bc}	22.88^{cd}	11^{a}
TONO	71.50^{bc}	105.2^{d}	6.28^{d}	9.86^{e}	2.205^{ab}	21.14^{e}	9.50^{b}
LSD	3.02	5.65	2.68	0.18	0.094	1.32	1.08
CV (%)	2.6	3.6	20.1	1.3	2.9	3.9	7

Means with the same alphabet within columns are not significantly different at 5%.

4.4 Physico-chemical properties of the seed accessions

The moisture content of the seeds did not affect the post-harvest processing because they were less than 14%. Table 4.4 shows the moisture content of the treatments.

Table 4.4: Moisture content of the treatments

Treatment	Moisture content (%)
TONO	13.5
PV	13.8
KIP	13.2
SARI	13.4
ARI	13.0
DARTEY	11.7
GBEWAA	11.4
CRI	11.5

4.4.1 Rice grain chalkiness and dimensions

The grain dimension gave a length to width ratio (L/W) between 2.9 and 3.1. The different dimensions obtained were long – medium, medium – medium and medium – slender. The grain chalkiness varied between 7.34% and 20.80%. The table also shows the result of the grain hardness test which varied between 9.02kg for ARI and 7.83kg for DARTEY.

Table 4.5: Means of the Grain length (mm), grain width (mm), grain length to the width ratio (L/W), grain size, grain shape, total chalky area (%), and grain hardness (kg) of the various sources

Treatment	Length (mm)	Width (mm)	L/W	Size	Shape	Total chalky area (%)	Grain hardness (kg)
ARI	6.61[a]	2.208[ab]	3.0	Long	Medium	9.24[e]	9.02
CRI	6.505[ab]	2.198[b]	3.0	Medium	Medium	15.04[b]	8.13
DARTEY	6.38[bc]	2.035[f]	3.1	Medium	Slender	12.41[c]	7.83
GBEWAA	6.308[c]	2.127[d]	3.0	Medium	Medium	20.80[a]	8.89
KIP	6.367[bc]	2.225[a]	2.9	Medium	Medium	10.99[d]	8.09
PV	6.365[bc]	2.035[f]	3.1	Medium	Slender	13.16[c]	8.25
SARI	6.078[d]	2.083[e]	2.9	Medium	Medium	7.34[f]	8.96
TONO	6.44[bc]	2.155[c]	3.0	Medium	Medium	14.30[b]	8.43
LSD	0.156	0.021	-	-	-	1.019	1.22
CV (%)	1.7	0.7	-	-	-	5.4	10

Means with the same alphabet within columns are not significantly different at 5%.

4.4.2 Cooking time and Head rice yield

The cooking times varied from 23 minutes to 15.75 minutes respectively for GBEWAA and for PV. The source which had the shortest cooking time was PV and the source which had the longest cooking time was GBEWAA.

The results of head rice yield showed that PV had the highest head rice yield (30.30%) whilst ARI had the lowest head rice yield 4.30%.

Table 4.6: Means of the cooking time and head rice yield of the various sources

Treatment	**Cooking time (minute)**	**Head rice yield (%)**
GBEWAA	23^{a}	19.64^{d}
SARI	21.75^{ab}	22.36^{c}
ARI	21.25^{b}	4.3^{g}
TONO	19^{c}	20.58^{d}
CRI	18.25^{cd}	27.45^{b}
DARTEY	17.25^{de}	10.83^{e}
KIP	16.75^{de}	6.53^{f}
PV	15.75^{e}	30.3^{a}
LSD	1.53	1.097
CV	5.5	4.2

Means with the same alphabet within columns are not significantly different at 5%.

4.4.3 Amylose content, alkaline spreading value, gelatinisation temperature

The results from the alkaline spreading value gave three classes of gelatinisation temperature: low (< 70°C), intermediate (70-74°C) and high (74.5-80°C).

The amylose content result was significantly different for all the treatments except the results between PV and DARTEY and that between ARI and DARTEY. The highest amylose content was 20.64% for GBEWAA and the lowest amylose content was 10.63% for SARI.

Table 4.7: Alkaline spreading value, gelatinisation temperature (°C) and its classification (°C), and means of the amylose content (%) on the various sources

Treatment	Alkaline spreading value	Gelatinisation temperature (°C)	Temperature classification	Amylose content (%)
PV	7	< 70°C	Low	14.51[e]
GBEWAA	5	70-74°C	Intermediate	20.64[a]
ARI	7	< 70°C	Low	14.85[d]
TONO	6	< 70°C	Low	18.07[b]
DARTEY	7	< 70°C	Low	14.65[de]
SARI	2	74.5-80°C	High	10.63[g]
KIP	7	< 70°C	Low	14.13[f]
CRI	7	< 70°C	Low	15.79[c]
LSD	-	-	-	0.31
CV (%)	-	-	-	1.4

The means with the same alphabet within columns are not significantly different at 5%.

4.4.4 Viscosity

The peak obtained from the viscosity test (figure 1) gave information on the treatments viscosity. The Treatment with the highest viscosity was SARI (3537cP). GBEWAA (2791cP) had the lowest peak viscosity.

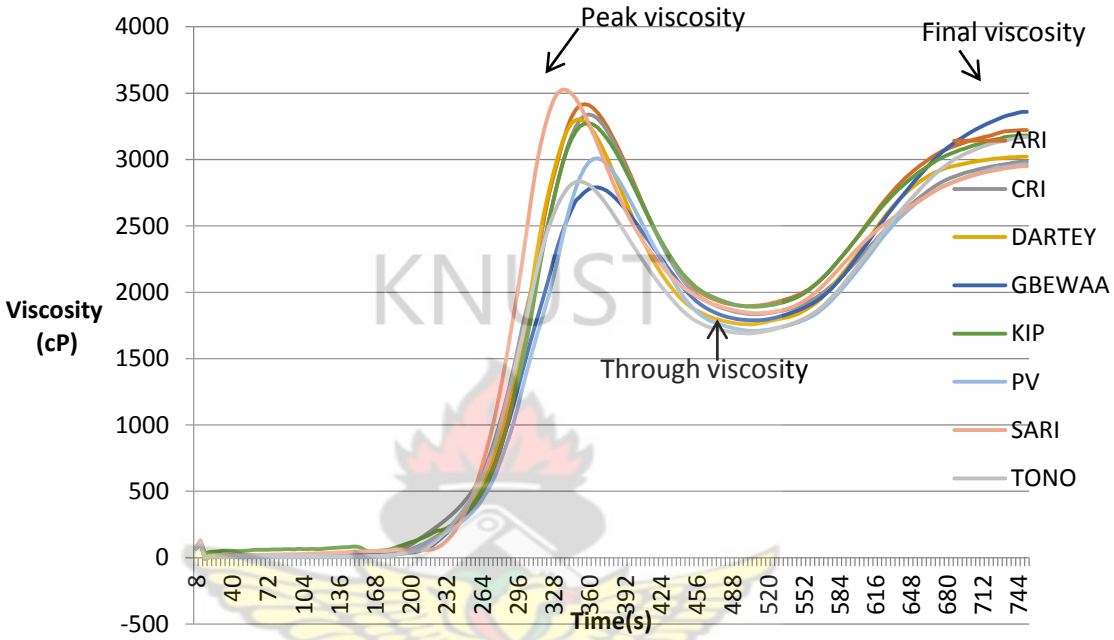

Figure 4.1: Means of the Viscosity properties of the various sources plotted against time (Centipoise)

4.5 Treatment clustering

In order to categorize the eight rice sources, cluster analysis was performed with morphological and grain quality characteristics. The cluster is presented in Figure 2.

This hierarchical tree can be cut in 2 or 3 or 4 groups. The validation measure of Dunn suggests a clustering into 4 classes. The first group comprised CRI, DARTEY, ARI and KIP; GBEWAA and TONO formed the second group, the two remaining classes were SARI and PV in that order.

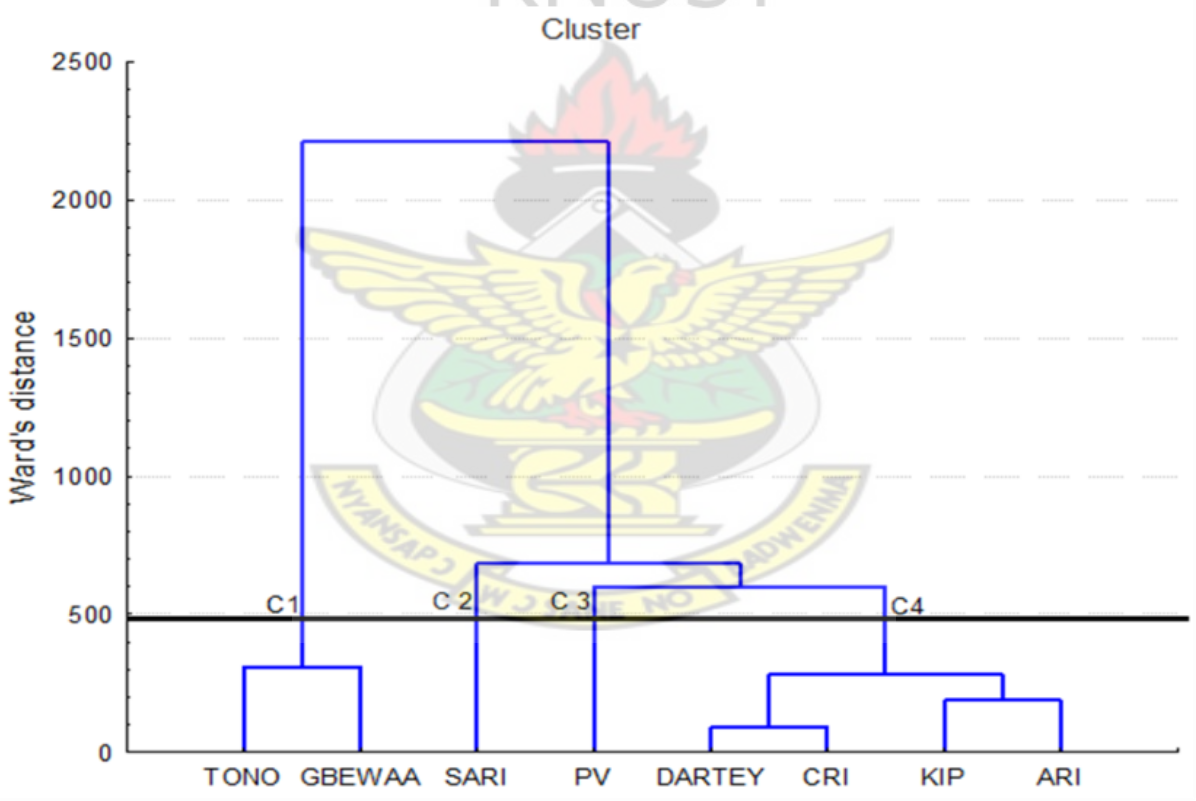

Figure 4.2: Cluster of the various sources using field and physico-chemical data

4.6 Principal component analysis (PCA)

To characterize the different groups, the Principal Component Analysis (PCA) was used. This technique is designed to highlight similarities or differences between the treatments and correlations between variables that describe those treatments. The first two axes cumulate about 61% (Appendix 4) of the total inertia. The resulting correlations circle is presented on the Figure 3.

The x-axis of the figure was explained by two sets of variables positively correlated within each set and negatively linked between the sets. One set contained the amylose, setback viscosity and total chalky area whereas the other set contained breakdown viscosity and peak viscosity. As for the y-axis, it opposed head rice yield, height to width and length. The set of variables that described the x-axis and the one that explained the y-axis were almost uncorrelated.

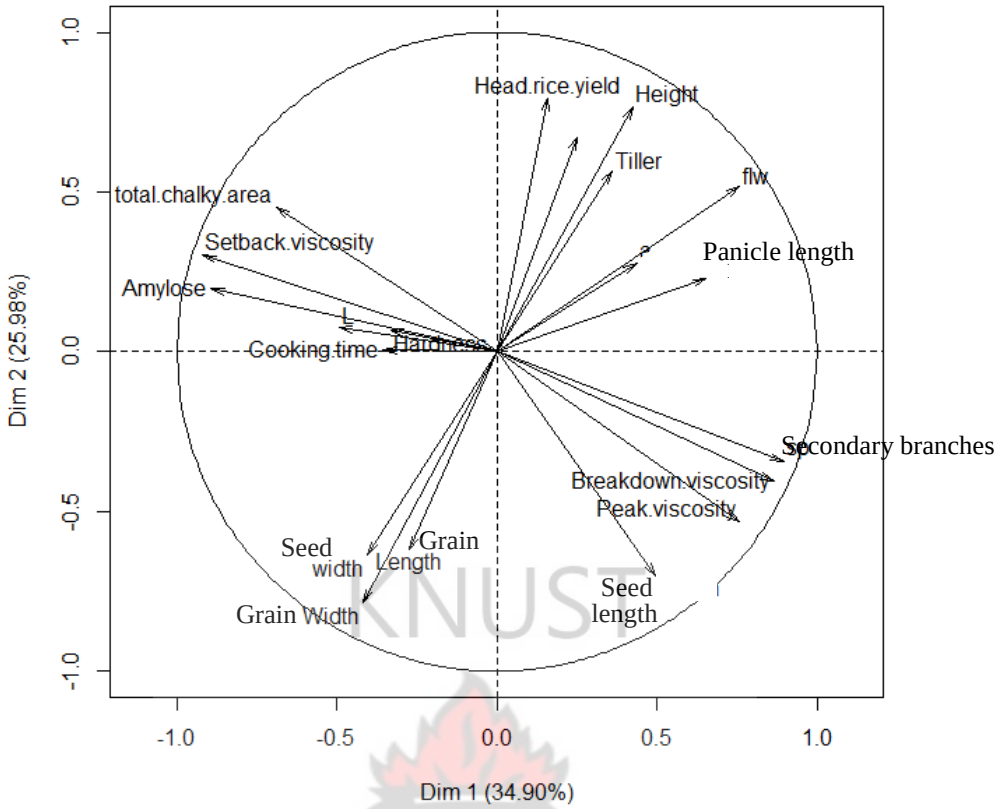

Figure 4.3: Correlation between variate projected in the first factorial plane

Figure 4 represents the scatter plot of the treatments. Its interpretation was done by transiting through the correlations circle. It can be stated that GBEWAA and TONO were characterized by high levels of total chalky area, setback viscosity, amylose and long cooking time whereas CRI and DARTEY recorded great values of secondary branches, breakdown and peak viscosity. While the grain length, the grain width and the seed width of ARI and KIP were very great, SARI displayed large values of heading days (flw). In the case of PV, it recorded the most important levels of head rice yield, plant height and tillers.

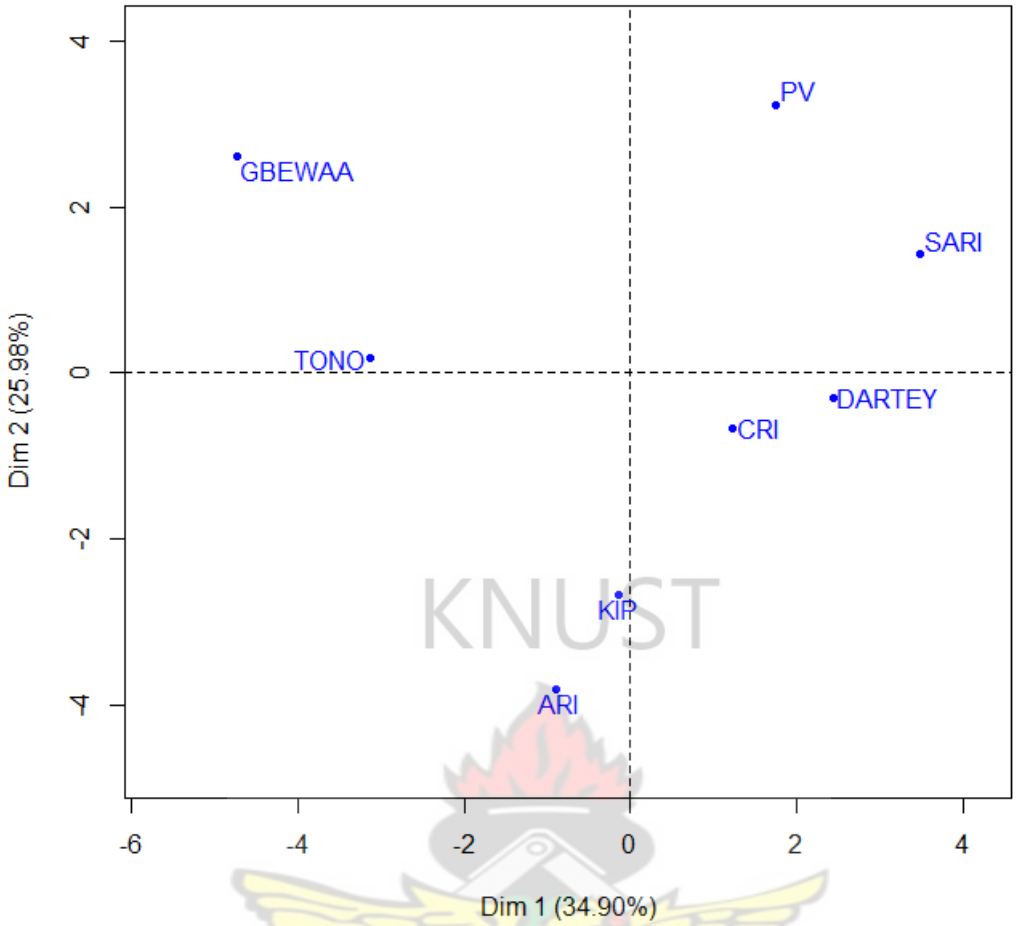

Figure 4.4: Scatter plot of the sources in the first factorial plane

4.7 Molecular results

The molecular analysis showed that GBEWAA was most distant from the other treatments with genetic distance of 0.3 cM between it and CRI. The shortest genetic distance (0.03 cM) was found between ARI and TONO; this meaned that ARI and TONO were closely related genetically. The average distance between the treatments was 0.16 cM. The distances among the sources were not high but they were statistically significant.

Table 4.8: Dissimilarity between and within sources (centimogan: cM)

	ARI	DARTEY	CRI	SARI	TONO	PV	GBEWAA
DARTEY	0.17						
CRI	0.13	0.17					
SARI	0.17	6.67E-02	0.17				
TONO	3.33E-02	0.13	0.1	0.13			
PV	0.1	6.67E-02	0.17	0.13	6.67E-02		
GBEWAA	0.23	0.267	0.3	0.2	0.2	0.2	
KIP	0.1	0.13	0.17	6.67E-02	6.67E-02	6.67E-02	0.13

The clustering result obtained from the molecular data, by using the genetic distance between the treatments (Table 9), gave three classes of cluster (Figure 5). Cluster 1 was composed of PV; DARTEY; and SARI, within this cluster PV was distinguished from the other two treatments. Cluster 2 was composed of CRI, ARI, and TONO. Cluster 3 was composed of KIP and GBEWAA, GBEWAA also was distinguished from KIP in this cluster.

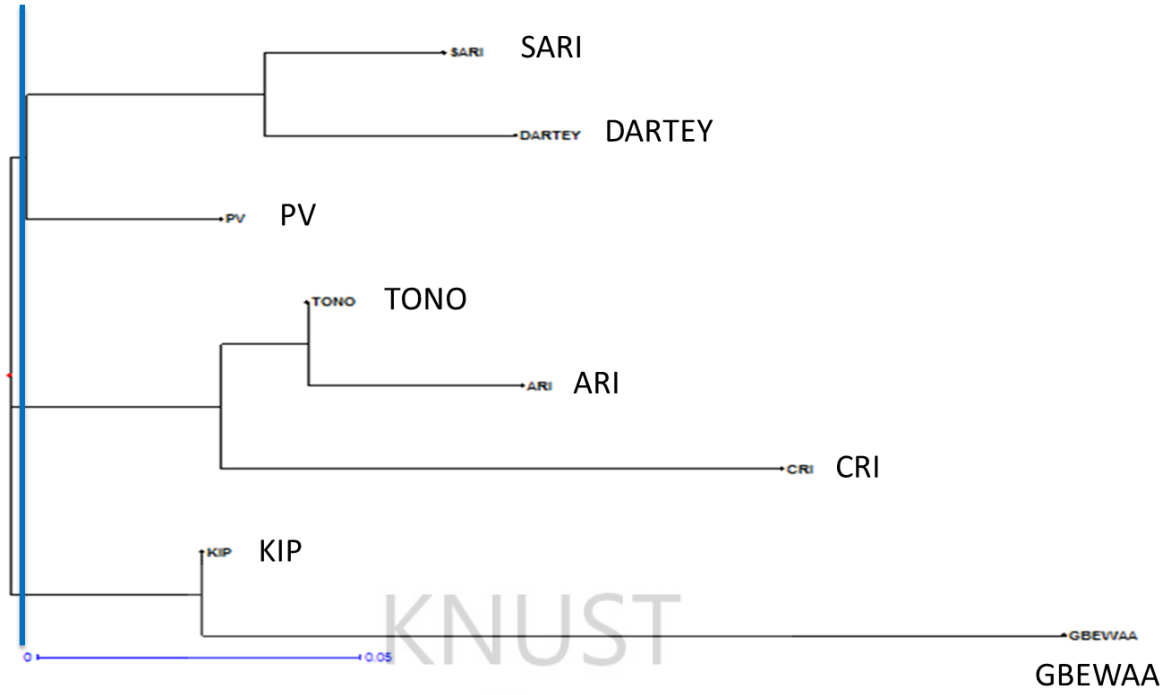

Figure 4.5: Molecular cluster of the sources

CHAPTER FIVE

DISCUSSION

5.1 AGRONOMIC CHARACTERISTICS OF THE TREATMENTS

Based on the argument that good quality seed should be at least 98% pure (Adekumbi *et al*., 2014), the treatment ARI, PV and DARTEY are pure.

No difference exists among the treatments with respect to qualitative data except for pericarp colour, where SARI showed a red pericarp whilst the other treatments indicated light brown pericarp. IRRI (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, seeds from SARI did not match a true Jasmine 85 variety.

The average heading days ranged between 85 days for DARTEY and 69.25 days for ARI; significant differences were found among all the sources, implying that seeds from the various sources may have significantly different maturity days.

With regards to the number of tillers, the seed length, seed weight, panicle length, number of secondary branches, significant difference existed among seeds from the various treatments. There is a variation of the characteristics among the treatments. Ideally, a single variety from different sources should not vary (Adetumbi, 2014). These variations affect grower or seed dealer satisfaction.

For plant height, the study recorded a range from 115.7 cm to 95.1 cm and this was in line with IRRI (1998) which reported an intermediate height (110 cm) for Jasmine 85. According to Morris (1980), intermediate plants have heights ranging from 75 cm to 160 cm.

5.2 PHYSICO-CHEMICAL PROPERTIES OF THE TREATMENTS

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 (Rani *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, three grain dimensions; long-medium, medium-medium and medium-slender were encountered. None of the sources in Ghana had the check dimension. IRRI (1998) reported that Jasmine 85 rice has the typical dimensions of a long and medium grain.

Chalkiness is due to small vesicle structures forming air spaces within endosperm storage substances and it differs from one variety to another depending on the variety chalk5 messenger RNA (Li *et al*., 2014). In terms of chalkiness, no significant difference existed between CRI and TONO; DARTEY and PV, but there was significant differences among the remaining treatments. GBEWAA had the highest value (20.80%) and SARI had the lowest value (7.34%). The grain chalkiness was a highly undesirable quality trait in the marketing and consumption of rice grain (Fitzgerald *et al*., 2009; Siebenmorgen *et al*., 2013; Bowles, 2012; Zhang, 2007; Xing and Zhang, 2010; Fernie *et al*., 2006), however the molecular basis of this trait is poorly understood (Li *et al*., 2014). The research showed that the sources having the lowest chalky area can performed better than those that have the highest chalky area in terms of grain quality.

Rice grain hardness is an important factor in diverse ways in the rice industry in terms of storage (Indudhara *et al*., 1978), drying and handling (Kunze and Hall, 1985), kernel appearance and translucency (Nagato, 1962), resistance to pest and insect attacks (Peng and Hsia, 1984), processing and grain breakage during the milling process (Goodman and Rao, 1983). Results from this study showed no significant difference among the source in terms of hardness; they required almost the same pressure for them to be broken.

Head Rice Yield is the weight of whole white rice grains remaining after milling; as a percentage of the total weight of the paddy. Head rice yield is often the most important quality parameter with regards to millers since the head rice yield is generally linked to the payment they receive. In this study, the head rice yield was significantly different among the sources and the means varied between 30.30% - 4.30%. According to Marshall and Wadsworth (1994) milling yield is determined by both environmental and genetic factors, and is a very complex characteristic. Grains that are cracked, either from moisture cycles in the field or rough handling are also likely to break during milling. Also, Tanasugarn (1998) stated that the weak point of Jasmine 85 is the many broken grains found after milling, which tend to drive the price down.

The cooking time of the treatments varied between 15.75 minutes and 23minutes; the significant differences among treatments implied that mixing grains from two or more of the different sources for boiling would result in inconsistent texture of the meal. The physical appearance of the milled rice is also important in terms of consumer preference because most consumers are selective in what they eat so a mixture of different varieties would not be palatable to most consumers.

Alkaline spreading value were analysed for the various treatments in the laboratory. Gelatinisation temperatures were deduced from the alkaline followed by the classification of the treatment. Values obtained resulted in three temperature classes; below 70°C, low temperature for PV, ARI, TONO, DARTEY, KIP, and CRI; 70-74°C, intermediate temperature for GBEWAA; and 74.5-80°C, high temperature for SARI. IRRI (1998) reported that the rice variety Jasmine 85 has a low gelatinisation temperature (65 to 68%). The gelatinisation temperature is reported to influence the cooking time of rice and samples with high gelatinisation temperature generally require more minutes to cook than samples with lower values ((Kurasawa *et al*., 1963 and Juliano, 1970). This phenomenon was confirmed by

this study as SARI and GBEWAA which had high and intermediate gelatinisation temperatures respectively required more time to cook than TONO, ARI, DARTEY, KIP, CRI, and PV which had lower gelatinisation temperature.

The amylose content ranged from 10.63% to 20.64%. Diako *et al.*, (2011) found in their study that Jasmine 85 has an amylose content of 20.2%. The amylose content influences the cooking and eating qualities of milled rice (Bahmaniar and Ranjbar, 2007). Generally, rice varieties with high amylose content over (25%) cook dry, and are less tender and become hard upon cooling while those with low amylose content (below 20%) cook moist and are sticky (Dipti *et al.*, 2003). GBEWAA, which had an amylose content of 20.64, will therefore cook dry, and become hard upon cooling while the seven others sources will cook moist and sticky. Jasmine 85's amylose content, according to IRRI (1998) is low.

Pasting properties is influenced by amylose content (Tester and Morrison, 1990); the higher the amylose content the less expansion potential and the lower the gel strength for the same starch concentration (Li and Yeh, 2001; Singh *et al.* 2003). The lower the amylose content the starch granules swell much more, and hence a thicker paste is produced (Diako *et al.*, 2011). The viscosity graph gave significant difference among the sources especially with the peak and the final viscosities. SARI had the highest peak viscosity which can be explained by its lowest amylose content; GBEWAA on the other hand expressed the lowest peak viscosity and high amylose content.

5.3 CLUSTERING AND PRINCIPAL COMPONENT ANALYSIS

The clustering and principal component analysis (PCA) was done to regroup the sources in order of resemblance between the field and physico-chemical data of the treatments despite their differences. The clustering gave four different classes represented in the Figure 6. Treatment ARI was obtained from Africa Rice Centre in Senegal with IRRI as its original

source, thus ARI served as the check in this study. Based on the clustering and the PCA; CRI, KIP and DARTEY resembled more to ARI. This suggested that seeds from these sources was closer to Jasmine 85.

With respect to the validation measure of Dunn (Brock *et al*., (2008) and considering the PCA result, the following relationships was established between the sources in each cluster.

- Cluster 1 (GBEWAA and TONO) was characterized by high levels of total chalky area, setback viscosity, amylose and long cooking time

- Cluster 2 which contained two other sub-clusters which were (CRI and DARTEY) with great values of secondary branches, breakdown and peak viscosity and (ARI and KIP) with a great value for the length and the width of their seeds,

- Cluster 3 (SARI) displayed large values of flowering days and pericarp colour

- Cluster 4 (PV) recorded the highest levels of head rice yield, height and tiller.

Variation was found between the various Jasmine 85 sources and it could be due to variation in their genetic and/or environmental influence during its growth and grain handling. Effectively, though those factors are gene dependent they also come as a result of environmental factors. According to some authors such as Viollet *et al.*, (2007), Tousignant and Delorme (2006), Robert *et al.*, (1997), Bos and Caligari (1995) climatic, topography and edaphic parameters play a significant role in plant growth and development.

With regard to these, molecular comparison was used to find relationship between the treatments, in order to confirm the differences found at the physic-chemical and morphology level.

5.4 Molecular characteristics of the treatments

Whenever a seed producer changed a rice variety name, it is not easy to distinguish them, especially when the seeds are similar phenotypically (Sangaré, 2011). The use of molecular markers can however enable seed scientists differentiate the varieties. According to some authors, SSR markers are highly informative and help in the identification of a group of dissimilar genome (Gracia *et al*., 2004). This study found from the 15 molecular markers that there were differences between the treatments. In another words, the Jasmine 85 collected from the different sources were different from each other; there was genetic variation between them.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Differences were observed at the morphological; physico-chemical and molecular level for the collections of Jasmine 85. Though the differences between them are not much, they are significant. There are no sources which look at 100% to ARI (true Jasmine 85), but the most related once to Jasmine 85 are TONO and CRI.

This imply that seeds from different sources should not be mixed for production. This difference in seeds of the same variety can be explained by several factors.

First, natural changes in the genetic material of the variety that can occur. In breeding it is called genetic evolution.

Secondly, it can be attributed to a mixture of seeds in the seed industry and also the informal way of sharing seeds. Effectively, a lot of seed growers think that any variety which is aromatic and has a typical long and slender grain is Jasmine85, leading to mislabelling of seeds.

The genetic purity of any commercial agricultural product propagated by seed begins with the purity of the seed planted. 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.

As recommendation:

- These Jasmine 85 collection need to be re-evaluated to select the best.
- The reason of this varietal problem should be identified

- Train seed growers on the risk that such a problem can bring out, and the need of Ghana to be competitive in their seed production.
- This problem might not be limited to Jasmine 85 or rice. Other rice varieties or other crops should be similarly studied.

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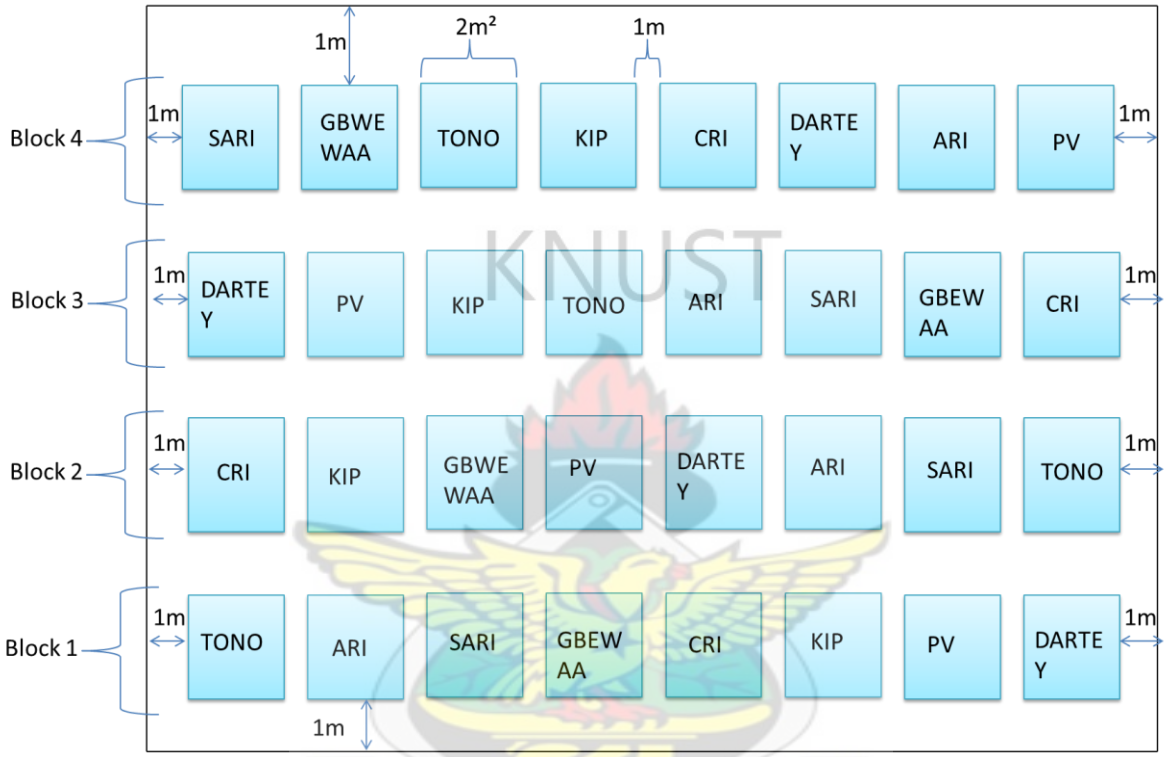
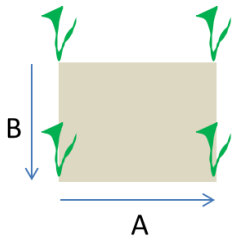
APPENDICES

APPENDIX 1: FIELD DESIGN





A: Distance between between row (30cm)
B: Distance within row (20cm)

100 plants

Plot containing one variety: 10 row/10 column

APPENDIX 2: Analysis of variance table for field data

Variate: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	64.89	21.63	1.46	
Rep.*Units* stratum					
Treatment	7	1539.98	220.00	14.86	<.001
Residual	21	310.87	14.80		
Total	31	1915.74			

Variate: Seed length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.02571	0.00857	0.51	
Rep.*Units* stratum					
Treatment	7	3.30924	0.47275	28.38	<.001
Residual	21	0.34984	0.01666		
Total	31	3.68479			

Variate: Percentage Purity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	1202.0180	171.7169	223.85	<.001
Residual	24	18.4106	0.7671		
Total	31	1220.4286			

Variate: Panicle length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1.8496	0.6165	0.76	
Rep.*Units* stratum					
Treatment	7	78.5825	11.2261	13.85	<.001
Residual	21	17.0160	0.8103		
Total	31	97.4481			

Variate: Number of Tiller

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	16.340	5.447	1.64	
Rep.*Units* stratum					
Treatment	7	297.398	42.485	12.79	<.001
Residual	21	69.745	3.321		
Total	31	383.483			

Variate: Seed width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.016534	0.005511	1.34	
Rep.*Units* stratum					
Treatment	7	0.151447	0.021635	5.27	<.001
Residual	21	0.086191	0.004104		
Total	31	0.254172			

Variate: Secondary branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1.0000	0.3333	0.61	
Rep.*Units* stratum					
Variety	7	33.0000	4.7143	8.61	<.001
Residual	21	11.5000	0.5476		
Total	31	45.5000			

Variate: Days to 50% Heading

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4.594	1.531	0.36	
Rep.*Units* stratum					
Treatment	7	1317.469	188.210	44.58	<.001
Residual	21	88.656	4.222		
Total	31	1410.719			

Appendix 3: Analysis of Variance table for physico-chemical data

Variate: Amylose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	242.86595	34.69514	767.51	<.001
Residual	24	1.08492	0.04520		
Total	31	243.95086			

Variate: Cooking time

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	191.000	27.286	24.71	<.001
Residual	24	26.500	1.104		
Total	31	217.500			

Variate: Grain Hardness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	5.7304	0.8186	1.16	0.364
Residual	24	17.0095	0.7087		
Total	31	22.7399			

Variate: Head rice yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	2556.9153	365.2736	646.20	<.001
Residual	24	13.5664	0.5653		
Total	31	2570.4817			

Variate: Grain length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.67700	0.09671	8.40	<.001
Residual	24	0.27642	0.01152		
Total	31	0.95342			

Variate: Peak viscosity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	2114378.	302054.	19.76	<.001
Residual	24	366850.	15285.		
Total	31	2481228.			

Variate: Setback viscosity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	3895144.	556449.	52.51	<.001
Residual	24	254309.	10596.		
Total	31	4149453.			

Variate: Total chalky area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	468.7200	66.9600	137.23	<.001
Residual	24	11.7103	0.4879		
Total	31	480.4303			

Variate: Grains width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	0.1617875	0.0231125	108.76	<.001
Residual	24	0.0051000	0.0002125		
Total	31	0.1668875			

APPENDIX 4: Principal Component Analysis (PCA) table showing the number of axes to selected

	Inertia	**Cum**	**Ratio (%)**
1	6.980583	6.980583	0.349029
2	5.195758	12.17634	0.608817
3	3.941494	16.11784	0.805892
4	1.831443	17.94928	0.897464
5	1.195956	19.14523	0.957262
6	0.622809	19.76804	0.988402
7	0.231957	20	1

APPENDIX 5: Simple Sequence Repeat (SSR) markers

1. RM252
2. RM511
3. RM316
4. RM8236
5. RM224
6. RM19
7. RM452
8. RM144
9. RM514
10. RM105
11. RM277
12. RM25
13. RM178
14. RM11
15. RM55