FINGERPRINTING AND RELATING JUVENILE CHARACTERS OF RICE TO YIELD AND YIELD COMPONENTS IN KIAMBU AND KIRINYAGA COUNTIES

By

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DECLARATION

I declare that this thesis is my original work and has not been presented for the award of a degree in any other University or for any award.

_____ _____

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Supervisors' Approval

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as University supervisors.

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DEDICATION

To my dear husband Lawrence, and my children Reagan, Dave, Tiffany and Tehilla for their unconditional love and support during the course of this work

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To God be all the glory.

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ACRONYMS AND ABBREVIATIONS

AMOVA	-	Analysis of molecular variance
ANOVA	-	Analysis of Variance
ASARECA	-	Association of Strengthening Agricultural Research in Eastern and Central Africa
DNA	-	Deoxyribonucleic acid
JKUAT	-	Jomo Kenyatta Univesity of Agriculture and technology
KALRO	-	Kenya Agricultural Research Institute
PIC	-	Polymorphism information content
PCR	-	Polymerase chain reaction
SSR	-	Simple sequence repeat
UPGMA	-	Unweighted pair group method with arithmetic

ABSTRACT

Rice is increasing as an important food and cash crop in Kenya and is third to maize and wheat in terms of consumption. Consumption has continually outstripped production resulting in massive importation. The main problem affecting productivity involves lack of proper information on best storage time, use of poor quality seed, varietal admixtures which has contributed to low yields. There is need to characterize genotypes. Microsatellit are useful in various plant genetic studies and plant characrerization. Accelerated aging is considered an excellent option as a vigor test due to shortest time of acquisition and efficient results. In the proposed study, seed quality experiments including germination, dormancy, purity, were carried out in the laboratory using eight rice varieties in a Completely Randomized Design. Accelerated ageing tests at 0,24,48 and 72hrs(45^{o}C and 98%RH) were carried out JKUAT post-harvest laboratory using eight rice varieties' in four replications of100 seeds each. Data was collected on dates to plumule and radical emergence and height. Five varieties were used to produce seeds which were stored for 1,2,3,4,5 and 6 months respectively. The seedlings were transplanted into fields in a Randomized Complete Block Design using 5*6 factorial arrangement. The study also determined the relationship between juvenile characters and adult characters of rice in Kiambu and Kirinyaga counties. All the data were analyzed using GENSTAT statistical package. ANOVA and LSD at 5% significant level. Seed quality results showed that There was significant variation in both coleorrhiza and coleoptile formation among rice varieties ($p<0.001$), treatments ($p<0.001$) and interaction between rice varieties and treatment (($p<0.001$). The difference between all treatments was significant with 72 hours treatment having the highest number of days to coleorhiza and coleoptile formation. Results showed that the effect of genotype on coleorhiza and colleoptille formation was significant ($p<0.001$). Genotype B317 had the earliest colleoptile formation 8.75days, coleorhiza formation (9.81), B317 also had the highest germination percentages at 7(33.69), 14 (52.44) and at 21 days after sowing (56.62). Mzungu had the latest number of days to coleorhizae (12.44), coleoptiles (11.18) and lowest germination percentages at 14(27.52) and 21days (31.31). The genotype had significant effect on germination index, seed length and seedling vigor index. In Mwea, there was a significant effect of storage time on germination, with 4 months giving the highest seedling vigor index (673.6), height at 1 month after transplanting (73.3cm), height at maturity (72.3cm) and 3 months giving the highest days to flowering(58.87) .The interaction between storage time and genotype on was significant, with genotype B217 stored for 3 months leading in height at one month after transplanting (87.7), height at maturity(91.8cm) .in Kiambu.The interaction between storage time and genotype was significant with 5months of storage having the highest seedling vigor index (759.5), 1000grain weight (69) and yield (15.5kg/ha). Analysis of molecular variance results indicated that the five polymorphic markers used in this study showed a total of 11 alleles across the loci of the 18 rice genotypes studied. Juvenile characters such as seedling vigor and height at transplanting had a positive and significant correlation to 1000grain weight.Genotypes TXD and B217 were identified by the 5 markers. TXD (Saro 5) was the best fit for fingerprinting as it was identified by only 1 marker. These results could be used in preparing rice seed certification procedures, in gene bank management and other seed storage ventures.

CHAPTER ONE: INTRODUCTION

1.1. Background information

Rice is an annual grass being used as an important food crop throughout the world and is believed to have originated from China (Molina *et al.*, 2011). In 2009, world rice production was estimated at about 680 million tons with a projected record harvest of 710 million tons (FAO, 2010), alongside an increase in consumption of about 8 million tons.

Rice is a vital cereal in supplying higher than 20% of calories used globally in the human diet and serves as a staple cereal to more than fifty percent of human beings in the globe. With reference to energy consumed in the world, rice gives 20%, wheat 19% and maize gives 5% (FAO, 2009).

Worldwide intake of rice continues to surpass world production from 2002 and got as high as 4% yearly rise in market requirement in some African countries (Africa Rice, 2008). Rice was introduced in Kenya from Asia around 1500-1800BC and was very useful in conquering famine (FAO, 2003). In Kenya, 95% of rice is grown under irrigation. About 800 million people are ailingfrom malnourishment and hunger worldwide, thus creating the need for a sustainable increase in rice production to improve global food security and contribute to poverty alleviation (Badawi 2004). Rice is becoming an important food and cash crop in East Africa and is second to maize in terms of consumption. The variation in consumption behaviour emanate from rises in population, and urban development. For instance, in Kenya rice consumption increased by 12 percent in 2003 while for wheat and maize it was 4% and 1%, respectively.

Demand in Kenya was 240,000 tons and the country managed to produce only 70,000 tons, importing 170,000 tons at a cost of KES 7 billion (Kega and Maingu 200. A lot of smallholders are engaged in rice growing in Kenya. Rice is grown mainly for the local market consumption in Kenya and there is no clear information regarding export. Low productivity remains one of the major challenges to be overcome if rice is to remain a viable cropping enterprise.

There are two cultivated rice species, *Oryza sativa and Oryza glaberrima*. The most commonly cultivated species in Kenya is *Oryza sativa*. The production of rice in Kenya has faced several challenges, which results in low yields that include diseases, pests, prolonged seed storage (African Rice 2013).

Seed storage period can be classified as long term, short term or medium. Good storage maintains the quality of seed for example; storage in air tight conditions has been found to be useful in maintaining initial moisture content and prevents thriving of insects and pests The viability of seeds stored in open conditions in the tropics rarely exceeds 2years. Other information from the IRRI gene bank indicates that the viability of rice seed can decline very quickly, over months when rice is stored under typical conditions (Ortiz, 2002). The moisture content sometimes is constantly maximal in some places irrespective of the air dryng practices for (Berjak and Pammenter, 2008; Mohamed-Yasseen *et al*., 1994).

Evaluating variability, keeping passport data and characterization of heritable morphological and molecular traits of germplasm is a key factor in germplasm collection and storage (Duvick, 1990, Bretting and Widrlechner, 1995). Markers are extensively

used in biotechnology and for taking care of germplasm (Duvick, 1990). They are useful in gene mapping and finger printing commercial germplasm (Smith and Smith, 1992; Bretting and Widrlechner, 1995).

Polymerase chain reaction (PCR) is a process by which specific portions of the sample DNA can be amplified almost indefinitely (Saiki *et al*. 1985, 1988). The process, the polymerase chain reaction (PCR), imitates the biological process of DNA replication, but confines it to specific DNA sequences of interest. This helps to enhance the discriminating power and the ability to recover information from very tiny initial samples (McKie, 2009).

Africa produced an average of 14.6 million tons of rough rice per year on 7.3 million hectares between 1989 and 1996 (Traore, 2005). Out of the vast available areas, West Africa has the largest planted rice area of about 4.1 million hectares. Yet, production remains at low levels. This is probably due to poor crop management techniques, lack of research and extension system, and limited utilization of productive varieties (Badawi, 2004; Anon, 2007). One of the major concerns in rice production has to do with seed and grain quality (Traore, 2005). While many components contribute to rice quality, the most important are cooking and eating qualities. These parameters primarily involve the physical and chemical characteristics of starch. The constituents that play important roles in cooking and eating quality are amylose content, gelatinization temperature, and gel consistency (Traore, 2005). According to Horna *et al* (2007) grain quality is one of the key selection criteria highly prioritized by farmers and consumers of rice and therefore farmer select rice with traits that are desirable for consumption as well as for production

and sale. In the future, grain quality will be more important as very poor consumers, who depend largely on rice for their daily food, demand higher quality rice (Traore, 2005). However, defining quality is very difficult since it is defined by the end user and their preferences are highly variable. For instance, in the Middle East, consumers prefers long grain, well milled rice with aroma while the European community generally prefers long grain rice with no scent because the presence of any scent signals spoilage and contamination (Troare, 2005).

There are several varieties of rice under cultivation worldwide. These are selected based primarily on the quality of their seed and grain by consumers as well as producers (Horna *et al*., 2007). To increase local production, good quality seed must be sown (Rickman *et al*., 2006). Rickman *et al*. (2006) estimated that good quality seed is expected to increase yield by 5-20 % and that the extent of this increase is directly proportional to the quality of seed that is being sown. Seed quality can be considered as the summation of all factors that contribute to seed performance (Rickman *et al*., 2006). These factors can be grouped as genetic, physical, and physiological quality. The current study examined some of the physical qualities and one aspect of the physico-chemical quality. The aim of this research was to study seed and grain characteristics of 46 rice accessions.

The seeds take to the agriculturist all the genetic potential of a cultivar with major characteristics. New improved cultivars become agricultural raw material whenever their high quality seeds are available to agriculturists and are sowed by them (Peske *et al*., 2006). For rice seeds in Brazil, the minimum germination percentage required for production and commercialization is 80%, regardless of the category (Brasil, 2005).

Types of Seed vigor tests include Accelerated aging (Marcos-Filho,2015), Tetrazolium tests (Franca-Neto and Krzyza, 2009)and Electrical conductivity tests (Marcos-Filho, 2015),In comparison to all these, the accelerated aging test is indicated to define the seeds' vigor (Marcos- Filho, 2005) and as a consequence, their storage potential, because it delays the germination process and the embryo's growth (Maia *et al*., 2007). This test is used in order to evaluate the physiological potential of seeds after certain storage period (Panobianco *et al*., 2007). Accelerated ageing test has been used to evaluate the seeds' vigor in several species for being simple to execute, of low cost, fast, replicable and with easy interpreting results (Vieira and Krzyzanowski, 1999; Abreu *et al*., 2011; Panobianco *et al*., 2007).

Therefore, this project aimed at evaluating the dormancy and the behavior of the seeds physiological quality in rice cultivars of lowlands and highlands during the storage under different environmental conditions

1.2 Statement of the problem

As a traditional food plant in Africa, rice cultivation declined in colonial times, but its production has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care. It helped Africa conquer its famine of 1203 (Rangel *et.al,* 2006). Cuevas-Pérez *et al*., 1992 and Rangel *et al*., 1996 showed that the genetic bases of new rice varieties were narrowing in Latin America. Breseghello *et al.,* (1999) and Rangel *et al.,* (2000) demonstrated that the genetic progress made in various rice breeding programs was decreasing over time.

There are several problems affecting productivity of local rice varieties which include diseases, poor seed delivery systems, limited national breeding capacities, weeds, floods, poor water management, soil fertility challenges, soil salinity and iron toxicity lodging, erratic weather patterns and high cost of production and poor seed which results in low yields.

Seed quality and storage can affect seed quality and productivity in the long run (IRRI 2010). The use of poor quality seed results in poor performance of a crop, including poor initial crop stand, poor nursery productivity and poor ability to withstand hash environmental conditions. Another study also indicated that the viability of rice seed can decline very quickly, over months when rice is stored under typical conditions (Bewley and Black 2012). The dormancy period for most Kenyan varieties has not been documented, and many farmers and produces simply store seed until the convenient time of usage. A relatively small amount of information is available in the literature describing the effects of environment and genotype on rice yield (Aggarwal *et al*., 1996; Gravois and Bernhardt, 2000; Yang *et al*. 2001; Fageria and Barbosa Filho, 2001) and little is known about their effects on seed quality. A World Bank – FAO study claims 8% to 26% of rice is lost in developing nations, on average, every year, because of post-harvest problems and poor infrastructure. The post-harvest losses may exceed 40%, not only do these losses reduce food security in the world, the study claims that farmers in developing countries such as China, India and others lose approximately US$89 billion of income in preventable post-harvest farm losses, poor transport, the lack of proper storage and retail. The proposed study seeks to address the storage problem of the seed with regard to length of time taken, from harvesting to planting (IRRI, 2010).

Another problem is lack of proper information on quality rice seed in Kenya. The available records concerning storage are not recent and the bestperiod of rice storage and its effect on viability and yield remains unclear. Also limited is information regarding how juvenile characteristics of rice may correlate to yields.

There is wide genetic variability available in rice among and between landraces leaving a wide scope for future crop improvement. Most other cases leaf sheath color, inflorescence color, awn presence and other conventional morphological traits, together with stress resistance, have been, and continue to being used to distinguish the uniqueness of a new cultivar. However, since more cultivar receives protection, and thereby increases the size of the database, it becomes more difficult to distinguish new cultivars from those in the database. There therefore need to fingerprint varieties in Kenya in order to avail clear information regarding relatedness among varieties since this has an effect on genetic vulnerability. Genetic vulnerability is the replacement of local landraces with improved varieties of narrow genetic base which has resulted in genetic erosion and rapid reduction in genetic diversity (Ford-Lioyd and Jackson, 1986; Rubenstein *et al*., 2005; Smolders, 2006).

1.3 Justification of the study

Productivity and expansion in agriculture entail the vigor and nature of the germination of the seed as its components. Maintaining the seed quality during the storage period is a factor that must be considered in the production process of any crop, for the farming success depends, mainly, on the use of high quality seeds (Freitas *et al*., 2004). Best

quality seeds result in best quality crops subsequently yielding highest value yields (Mbora *et al.*, 2009). The quality of the seed is of great value to the farmers as it enables them to determine the seed potential performance when subjected to conditions that are optimal. High-quality seeds are expected to produce seedlings which are healthy and do not have any initial disease traits because the high-qualityseeds tend to be better in health and are free from many diseases

As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. According to FAO of the UN, it is the grain with the third-highest worldwide production, after (FAOSTAT, 2012). Low productivity remains one of the major challenges to be overcome if rice is to remain a viable cropping enterprise. The major problem is the lack of a proper seed certification scheme for rice, which has resulted in admixtures and most farmers are unable to distinctly separate one genotype from another.

Less is known about the pre-harvest factors, seed production environment and degree of seed maturity which influence begining quality, final storage longevity of seeds.

The proposed study seeks to address quality aspects of the seed and to use fingerprinting to distinguish the uniqueness of each cultivar. Since more cultivars continue to receive protection and thereby increases the size of the database, it becomes more difficult to distinguish new cultivars from those in the database. Studying juvenile characteristics variability is one great way of assessing yield factors.

Although seed storage period is also believed to have great impact on yields, very limited information is available to guide farmers on the best seed age to use for planting. The present study seeks to study the performance of seeds after storage which will generate

information that will assist the farmers in selecting seeds. Molecular marker techniques using powerful tools have been developed for assessing and characterizing genetic resources. Genetic marker screening is based on the survey of genetic diversity as revealed by variation at specific gene loci and provides information about the amount and distribution of genetic diversity within and among populations. One of the solutions that can be undertaken is the use of biotechnological and breeding methods in rice improvement. The present study seeks to use DNA based molecular techniques applications in assessing the genetic diversity of rice in Kenya.

The degree of genetic differentiation between populations is important because the status of the locally grown rice is unclear and this study hopes to unveil this as it finger prints the available varieties. Further scope of crop improvement depends on the conserved use of genetic variability and diversity in plant breeding programme and use of new biotechnological tools. Plant scientists have used morphological, pigmentation, quality or other characteristics to classify and distinguish plant genotypes within a species. But this is becoming a challenge with continuous introduction of new varieties .The use of molecular genetic markers would therefore provide one solution to the problem of providing unique DNA profiles for the protection of new rice cultivars (Chen *et al*., 1997). The information obtained from this study will be useful to breeders and other rice stakeholders, especially farmers on the most productive storage period for seeds, and the characters to expect from each genotype in Kenya when grown. The adaptation to the environmental conditions of every seed is not yet established for different regions (Donohue *et al.*, 2005; Donohue, 2009).

1.4 Objectives

1.4.1 General objective

To fingerprint and assess the relationship between storage and juvenile characteristics on the yield of Kenyan Rice

1.4.2 Specific objectives

i. To evaluate the quality of selected rice genotypes in Kenya
ii. To determine the effect of storage time and genotype on growth and yield of rice.
iii. To determine the relationship between rice juvenile characters and yield.
iv. To establish relationship among rice genotypes using SSR markers

1.5 Hypotheses

i. The genotype and ageing time have no significant effect on quality of rice.
ii. Farmer storage time does not affect the growth and yield of rice seeds.
iii. The juvenile characteristics of rice have no significant relationship with yield of rice.
iv. There is no significant difference between Kenyan rice varieties

1.6 Conceptual framework

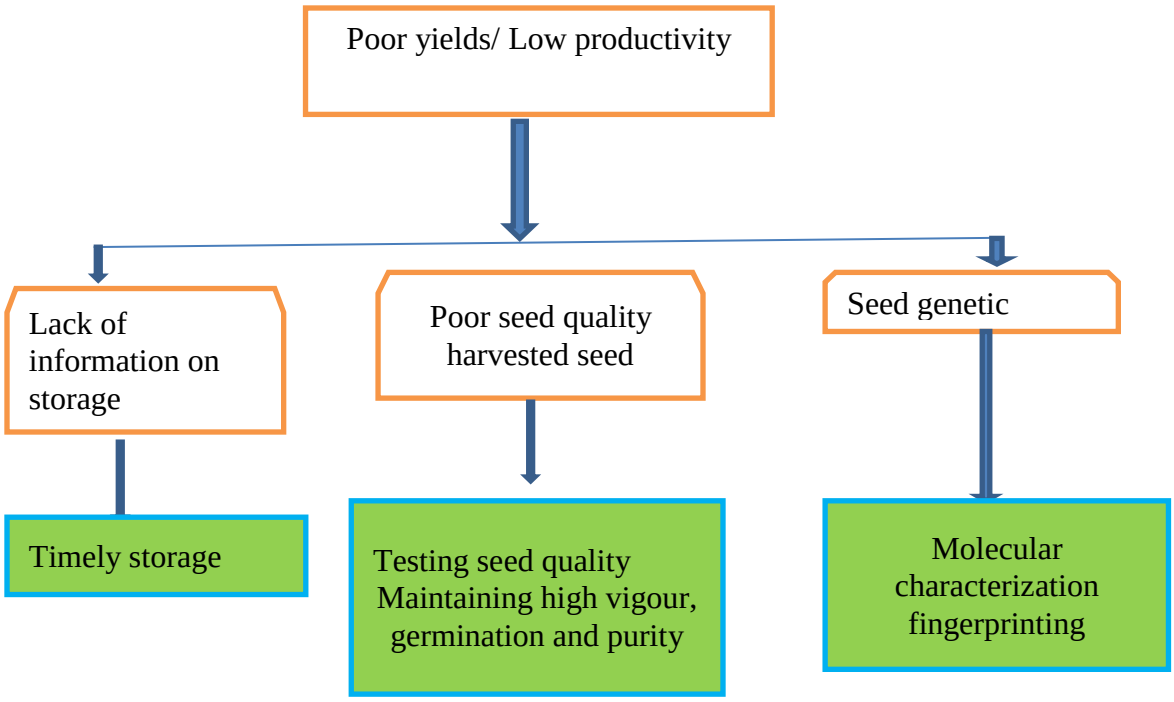

Figure 1.1: Conceptual framework

CHAPTER TWO: LITERATURE REVIEW

2.1 Rice origin and botany

Rice belongs to the genus *Oryza* and has two species cultivated throughout the globe: *Oryza glaberrima*, whose other name is African rice, and *Oryza sativa*, also commonly referred to as Asian rice (Lanares, 2002). The origin of *O. glaberrima*is is believed to have been the attempts of the indigenous inhabitants (who lived along the banks of Niger River) to domesticate *Oryza barthii*. The species is thought to have been a wild crop before domestication which might have taken place about three and a half millenniums ago.

On the other hand, the two strains of *O. sativa*, *Oryza japonica* and *Oryza indica* are believed to have been domesticated in Asia. *Oryza glaberrima* has undergone great enhancement over the past years making it hardier and drought resistant though less yielding and prone to lodging and losing yield through grain shattering before harvest. *Oryza sativa,* however, has higher returns though does not thrive in many areas since it originated from Africa. This is mostly due to the stresses of disease, pests, drought or soil problems (Jones *et al.*, 2008).

Of the grains with the highest productions rates in the world, rice can only be rivaled by maize. (Boumas, 1985). Poaceae, also known as "true grass," family is composed of the above-mentioned of domesticated rice species; *Oryza sativa* and *Oryza glaberrima*. These two species trace their origin from parts of Africa and Asian Research shows that the specific regions are Subtropical and Tropical Southern Asia and Southeastern Africa (Linares, 2002).

Rice is grown as monocarpic annual plant though it can survive in the tropical as a perennial with a lifespan of about 20 years in addition to being able to produce a ratoon crop (Boumas, 1985). The crop thrives well in the subtropical and temperate zones where temperatures are slightly higher as compared to regions within the tropics (Boumas, 1985).

Rice can grow up to heights of between 1m and 1.8m or even more in soil that is fertile enough, or its genotype is one that thrives. Just like other crops within the grass family, rice has long, slender leaves, of between 50cm and 100cm long with a breadth of 2 to 2.5 centimeters (Boumas, 1985). Branched arching enables the production of the small wind-pollinated flowers to the pendulous inflorescence 30-50cm long. The part of rice that is suitable for human consumption is the grain. The grain is averagely 8mm long and 3mm thick. It contains the embryo, glumes, and endosperm (Boumas, 1985). Certain varieties even contain awns at the tips of the grain. The awn is at times very long in some varieties thus requiring special machines to break them off and remove them before de-husking is done (Tokpah, 2010).

The description of Li (2003) illustrates that the rice grains are coarse or paddy, comprising brown rice (or caryopsis) and the hull. The brown rice consists of three major parts. These include the embryo, the endosperm, and layers (usually thin) of differentiated tissues. These layers are the pericarp, the five nucleoli and the seed coat. According to Li (2003), the seed coat has six layers of cells. The aleurone layer occupies the mid-center of all the layers. The embryo, on the other hand, has the plumule (referred

to as embryonic leaves) covered by coleoptiles and the radical or embryonic root unsheathed by the coleorhizae. The plumule and the radical are joined by the mesocotyl (Li, 2003). Rice endosperm chiefly consists of starch granules in a proteinaceous matrix, fused with sugar, fats, crude fiber and organic matter.

Hull weight contributed to about 20% of the total grain weight. Some rice grains have hulls with three parts namely: rachilla, lemmas, and palea. Others possess rudimentary glumes whereas others have a unique portion referred to as the pedicel within their structures. A perfect understanding of grain quality demands a careful study of the physical structure of each grain. It begins with observing the anatomy of a single grain irrespective of the purpose that the grain may serve ultimately (Hammermeister, 2008). The study is required especially in grading of grains meant for human consumption.

From seed germination, several plants go through a level of growth and development which enables them to shift to final yielding stage. One way to account for the low productivity in rice is to study the growth stages and correlate the juvenile characters to adult characters and yield. There are three distinct phases of growth that can be discerned including juvenile vegetative phase, an adult vegetative phase, and an adult reproductive phase (Lawson and Poethig, 1995; Sylvester *et al.,* 1996; Kerstetter and Poethig, 1998). The phases occur in a distinct manner where the change from across the phases may be sudden or gradual (Hackett, 1985; Sylvester *et al.,* 1990; Greenwood, 1995; Bongard-Pierce *et al.,* 1996).

2.2 Distribution of rice.

The origin of rice cultivation remains parts of Africa and Asia. In Asia, the more evidence shows that Southwest regions such as Eastern India, Southern and Indo China are the most likely areas that the crop might have originated. The main rice growing regions are found in Africa, Latin America, and, Asia though the major exporting countries are Thailand, theUnited States, Pakistan, Vietnam and India (Boumas, 1985). It is claimed that not all the rice produced in these regions are fit for human consumption. However, a larger percentage (85%) is used for human consumption as the rest as channeled to other uses. Li, (2003) observed that Antarctica is the only continent in the world which does not produce rice. Another recent study estimated by De Datta (1981) listed some 112 rice-growing cross the globe. Three international research centers are currently working on studies regarding the extensive distribution of rice (De Datta, 1981).

2.3 Rice production

A large population of the world consumes rice. It is the second most crucial cereal after maize in the world today. Of the cereals, rice is the second most commonly consumed. Its popularity can be because it is very easy to digest. More than 90% of the rice grown and consumed worldwide happens in the Asian communities. Li (2003) states that approximately 155 million hectares are used globally to produce rice amounting to 596.5 million metric tons annually. Most people prefer rice over other food crops. The preference rate, however, vary from one region to another (Juliano, 1993).

Table 2.1: Current rice production and projections based on area, yield and consumption in 2008 by Agro-ecological conditions.

2008				
	Rainfed Upland	**Rainfed Lowland**	**Irrigated Total**	**Total**
Area (Ha)	2,150	3,180	12,500	17,830
Yield (tons/ha)	2.72	2.76	4.7	4.1
Production (tons)	5,851	8,777	58,513	73,141
2013				
Area (Ha)	3,000	4,000	18,216	25,216
Yield (tons/ha)	3.11	3.2	5.1	4.6
Production (tons)	9,330	12,800	92,902	115,032
2018				
Area (Ha)	4,100	5,050	26,000	35,150
Yield (tons/ha)	3.7	3.76	5.6	5.1
Production (tons)	14,800	18,180	145,600	178,580

(Agricultural Economic Review 2008; National Irrigation Board Strategic Plan 2008 – 2013 and Vision 2030).

The figures represented in the above tables are based on the report of actual production in Kenya in 2008 in the various former provinces namely: Nyanza, Coast, Central, and Western. The predictions are founded on rehabilitating and expansion of the National Irrigation Schemes with existing infrastructures to increase rice production under irrigation. Additionally, this will also include the use of non-aromatic high yielding varieties. The rain-fed rice production will be increased by expanding the acreage and use of NERICA varieties besides other high-quality seed varieties. These interventions are

adaptable and rise in overall production can be realized during the planned period (Agricultural Economic Review 2008).

Millions of people in West Africa now depend upon rice as their staple food (Basorun, 2009). It is estimated that rice demand in the Sub-region stands at about 8 million metric tonnes. It shows evidence of an upward trend in rice consumption even as the population grows. The ease of preparation of rice compared to other types of food has also accelerated the consumption rate. It also easy to preserve rice.

The expansion rate of consumption was estimated to be 5.1 %. Most of the increase is attributed to population growth and increased cultivation of rice in different areas (Anon, 2008). Currently, the cultivated land area is approximately 4.4 million hectares solely dedicated to rice production in West Africa (Somado *et al*., 2008). All the areas cultivated produce nearly 6.2 million metric tonnes of rice (Anon., 2008). Nonetheless, the demand for rice in West Africa is not yet met by these production levels thus necessitating importation from other rice growing regions. The revenue used for such imports amounts to over $1.5 billion annually. Imports of this extent signify a significant obstruction for the broader development and poverty alleviation efforts (Somado *et al*., 2008). A study of the specific countries from West Africa reveals how the countries are dependent on rice production for economic development and provision of employment.

For instance, Berisavljevic *et al*. (2003) stated that rice is key to Ghana's economy and agriculture, contributing to closely 15% of the Gross Domestic Product. This sector of agriculture creates employment for many rural dwellers. The Ghanians have changed

their dietary programs and demand more rice that cannot be produce by the local farmers, it forces the government to import rice to feed the population of the country (Shabbir *et al*., 2008). The Ghanians import their rice from the Americas and parts of Far East such as Pakistan (Berisavljevic *et al*., 2003). The only problem is that the imported rice has to be sold at higher prices compared to the local rice since they are of better qualities and the logistical implications of making them available has to be borne by the consumers. At the moment, the annual demand for rice in Ghana is hardly met by the domestic production (Tokoradi, 2008).

In West Africa, quality is associated with the type of food people prepare for eating. In Ghana, long grain and aromatic rice are used with sauces or to prepare jollof or fried rice (Takoradi, 2008). Long grain aromatic rice has the greatest demand and is the most expensive rice on the Ghanaian market. Short grain rice is used to prepare omo tuo; this is tenderly cooked rice that is molded into balls and taken with palm butter soup or ground nut paste soup. Short grain and medium grain rice are used in porridge mixed with sugar, salt and milk, while broken rice is used for fried rice in the three African countries of Senegal, Mali, and The Gambia (Anon, 1994).

2.4 Effect of environment **on rice production**

A relatively small amount of information describing the effects of environment and genotype on rice yield is available in the literature (Aggarwal *et al*., 1996; Yang *et al*., 2001; Fageria and Barbosa, 2001; Gravois and Bernhardt, 2000) though the effect remains unknown especially on the seed quality and viability. Seed quality can only be known from the yield, grain measured weight, how proportioned the seed setting is, and

the possible grain longevity. Other characteristics observed are the seed weight against density, and seed membrane permeability. Some also take note of the seed discoloration when determining the quality of the seed. Of these, seed weight is mostly used since it is the most straightforward parameter to determine (Naylor 1993).

2.5 Rice cultivation

Flooding is the ancient method that has been used over the ages to cultivate rice in fields after young seedlings have attained maturity. While flooding is not mandatory for the cultivation of rice, all other methods of irrigation require higher effort in weed and pest control during growth periods and a different approach for fertilizing the soil (Stoop, 2002).

Rice is mostly grown as an annual plant though it can survive in the tropical as a perennial with a lifespan of about 30 years in addition to being able to produce a ratoon crop. Depending on its genotype and the soil fertility, rice can grow up to heights of between 1m and 1.8m. The crop has same characteristics as other crops that fall under the grass family. The leaves are slender and measure up to 100 cm long. The breadth varies between two and two and half centimeters. Tradition method is still widely used in growing rice. The fields are flooded where the seedlings are taken care of in the setting of nurseries. Today flooding may not be mandatory since other methods have been developed which also ensure optimum yields are realized. Nonetheless, the methods require frequent irrigation which also necessitates improved programs to curb prevalence of weeds and pests. The soil fertility must also be taken care of through other alternative means as opposed to traditional methods (Stoop, 2002).

2.5.1 Rice seedling phase

Germination of rice occurs when the first leaves and roots start to appear from the seed and the rice plant starts to grow. The germination requirements are specified amount of water and suitable temperatures. The temperature must be maintained above 10 degrees and not more than 40 degrees celcius. When these conditions are provided, the seed naturally halts the domancy stage and the germination process begin. Once the seed is planted in the soil after the domancy is broken, the shoot emerges and grows to reach heights where it can access light and air before the root begins to grow. The shoot develops through the flooding without support root. The main support in this case is the portion of the grain that has not fully disintergrated. Whenever the seed is transplanted in a field with non-flooded soil, it is likely that the root will first emerge followed by the shoot (Araki, 2001).

2.5.2 Vegetative growth phase

The development of tillers and more leaves and a gradual rise in plant height normally characterize the vegetative phase. The rice genotype normally determines the number of days the vegetative stage takes, though the standard timeframe ranges from 55 to 85 days. The early vegetative phase is marked by initial stages of seed germination into seedlings, and it continues until the time tilling ends (Lawson and Poethig, 1995). The indications of the seedling stage are when the first shoot and root emerge.The seminal roots grow simultaneous with the leaves. The stage proceeds until the young plant has grown at least five leaves. The early stages of growth exhibits a leave growth rate of averagely one leaf within a maximum of four days. Other crops may have a growth rate of one leaf at three days when the growth conditions are improved. Tilling marks the end of the early phase

and the beginning of the late phase. Transition between the phase depends on the growth rate of the crop over a span of days. The tillers grow to maximum number and the process may take nearly six weeks. Afterward, the stem begins to lengthen as we approach the last stages of the development. Growth in length stops once it reaches the desired height. This may take an additional of 2 weeks after the maximum numbers of tillers are attained. The vegetative phase ends at after a span of 52 days from the time sowing is done (Sylvester *et al.*, 1996).

2.5.3 Reproductive phase

The leaf stem begin to bulge the developing panicle as an indication for the commencement of reproductive phase.The tip of the panicle in developing phase sprouts from the stem and grows outward. The‘heading’ stage depends on the visibility of the panicle. Rice is at this stage when the panicle becomes visible. The farmer determines the shift in change from heading stage to flowering a day after he or she is able to see the panicle. As the flowers open, they shed their pollen on each other thus making pollination possible. Flowering can continue for about a week (Borchert 1983).

2.5.4 Ripening phase

This period usually takes around a month though may be lengthened by low temperatures or rainy days and shortened by sunny and warm days. It begins immediately flowing starts and continues until the grain reaches full maturity where it can now be harvested. This phase is comprised of the three last stages of the growth pattern. The phase can be divided into five stages, milky, dough, yellow, ripe, and maturity, (Araki, 2001). Color and texture of the developing grain are the determinants of the named stages. The length

of the ripening varies between 15 to 40 days for different varieties. This phase is hastened or slowed depending on the changes in temperature (Sylvester, 1990).

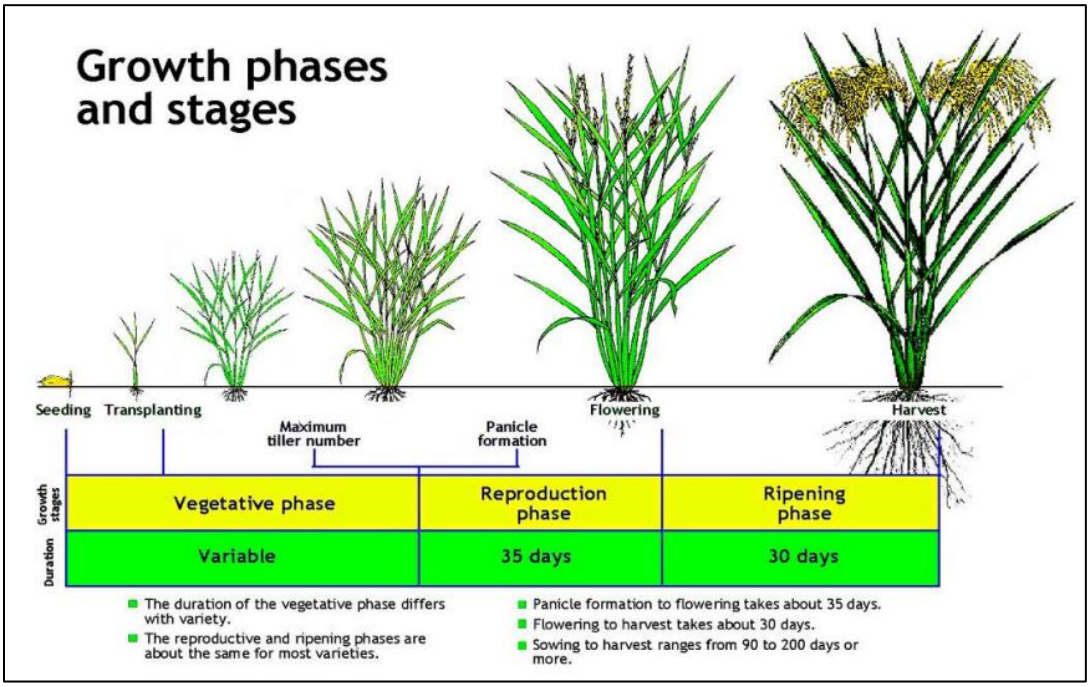

Figure 2.2: Rice phenological phases

2.6 Rice improvement

Infertility resulting from the two varieties of rice being crossed has been overcome through the attempts of use of biotechnology. Upon the two species of rice being cross-fertilized ***embryo-rescue*** in which embryos are withdrawn and grown on some artificial medium is done. This is necessary since embryos that undergo specific cross-fertilizationtend to fall off before they mature resulting in plants most of which are sterile hence wherever possible back-crossing is done with *sativa* parent. Upon the improvement of the fertility of the progeny the male sex cell gene complements are doubled through anther-culture producing plants which are true-breeding. In 1994, the new rice was tried out and just as was anticipated the yield had progeny-combined traits of the sativa parent

with traits of local adaptation from *glaberrima*. From then on there has been the streamlining and refining of the techniques with the aim of generating more new lines yearly (Africa Rice, 2001).

2.6.1 Uses of rice

Majority of the worlds' population approximated to be surpassing 60% feed on rice as their staple food. Cooked rice is the likely way in which it is eaten. The making of custard powder, distilling of portable alcohol and ice-cream making is all dependent on rice starch. Confectionary products such as cookies, snacks, and biscuits make use of the rice bran. The making of food items such as noodles, baby foods, and rice cakes are all made from broken rice. Rice is equally a significant source of energy because it is a carbohydrate. It similarly contains vitamin B in plenty, has some low-fat content with some protein in it. Rice husk is used as an insulator in the building and packing materials. Besides it being used as fuel, itis also used in the manufacturing of paper and board. Chemical derivatives and compost too are made from rice husk. Other uses include the feeding of cattle, making of wax rice, and manufacture of compos (Araki, 2001)

2.6.2 Phenal phases of rice.

Most plants have to attain some level of maturity development before they can reproduce upon seed germination. The widely known growth phases include the juvenile vegetative phase, the adult vegetative phase and the adult reproductive phase (Sylvester *et al.,* 1996; Lawson and Poethig, 1995; Kerstetter and Poethig, 1998). The three stages manifest the varied species of the plants differently. Some of the plants displaying sudden and unique changes in the structure or shape of the leaf with others transiting gradually from the

juvenile phase to the adult phases (Hackett 1985; Greenwood 1995). Despite how a plant displays the change in phase, the plant's regulation is complex encompassing interplay of genetic, environmental and hormonal factors (Poethig 1988; Moose and Sisco, 1994; Araki, 2001).

2.6.3 Physical characteristics

Those involved in the rice industry have much keen interest on the physical characteristics of the rice kernels (Anon, 2007). The physical components are vital in the grading and marketing, in the development of new varieties of rice and equally in both the grading and cleaning equipment as well as in the processing and drying operations. The physical characteristics of the rice kernels that are of vital importance are the weight and sizes of the rice kernels as well as the size of the grain or seed determined by carefully measuring the grain and seed kernels (Slaton *et al.*, 2000; Richman *et al.*, 2006). The physical appearance of the rice kernels is equally an essential and unique quality. The rice millers, its buyers as well as its consumers, make decisions based on the rice quality whose benchmark is on its shape, size and the physical appearance in relation to its size and shape.

The various rice varieties are grouped based on their sizes; sort, medium or long (Belsnio,1980). Different sizes of rice severely affect the quality of milling and yield hence necessitating proper and thorough separation and segregation of grains based on the size with the aim of improving on the rice milling quality (Mahadevappa and Nandisha,1987; Belsnio,1980).

2.6.4 Seed quality

This involves having seeds with the necessary physiological soundness, essential physical and genetic purity. It involves the selection of rice Genotype that is preferable and cansuite to the conditions of the environment, the practices involved in its management as well as the ultimate rice use (IRRI, 2009). Various varieties of rice tend to have varied chemical and physical characteristics thus necessitating the need of taking into account both the rice genotypes bad and good qualities before deciding on the rice that is most preferable to specific conditions.

The value of the seed is determined by its quality such that if the seed quality is low consequently is value is small,and if the seed quality is the best then its value is highest. (Nguyen, 2001). A seed of superior quality not only helps in the production of uniform crops but also increases per unit area productivity and consequently obtaining high market prices.

When the quality of paddy is high the milling recovery is consequently high thus rice of better quality is released into the market and this increases the profits that are earned (Nguyen; 2001). The decision on the type of rice Genotype to be planted should be based on four characteristics which are the dimension of the seed, period necessary for its maturity and both its uniform filling and its flowering. As a result of these traits that need to be considered a majority of the farmers tend to consider the seed dimension when making their choices on the rice Genotype, they are to plant due to its preference by consumers (IRRI, 2009).

To increase the production of crops, it's necessary to ensure the use of seeds of high quality which are well adapted and of an improved variety. Using seeds of good quality would result in crop yields increasing, the quantity of seeds to be sown reducing in number, reduction in weeds that are carried over as well as diseases and pests. Upon use of seeds of good quality farmers gets end products of high quality, the production cost is minimized resulting from the rate of survival being high and the rate of infection by both pest and diseases being low Rickman *et al.*, (2006).

Farmers can make use of the land to its very best upon the application of seeds whose quality is good,and thus consequently the farmers can attain faster economic returns (Mbora *et al.,* 2009). High-quality seeds have their rates of establishment in the field being very high since the high-qualityseeds havevery little impurities. The viability of the seed, varietal characteristics and the seed lot characteristics are the major criteria through which quality of seed can be described. Conditions such as soil moisture content, light, nutrients availability, the maturity of the seed as well as the temperature under which the parent plant grow influence the quality of the seed.

2.6.5 Seed quality tests.

Farmers tend to determine seed lot quality using the germination test (Richman *et al.,* 2006) with the test being aimed at determining the seeds that develop into healthy seedlings by percentage when subjected to certain conditions for some given period. Germination test results are used in the estimation of planting value of the field and comparing the different seed lot qualities (ISTA, 2007). The number of the seeds in total in proportion to the actual number of germinating seeds in the germination percentage

(ISTA, 2007).Most of the varieties of rice tend to be dormant soon after the harvest for a period that ranges between 0-3months (Mahadevappa and Nandisha 1987; ISTA, 2007; Richman *et al.,* 2006).The rate of germination will tend to deteriorate if it is storedin the traditional open systems (RRI, 2009). In determining the suitability of seeds for planting farmers can only use the germination test from which they can adjust their rates for planting aimed at achieving the population of plants that are desired (ISTA, 2007).

2.6.6 Seed Germination

The process of growing an embryo into a seedling, that is, seed germination entails having the metabolic pathways being reactivated resulting the plumule and radicle growing (Black and Halmer, 2006). The initial phase of growth of the plant is the seedling establishment. The last stage is characterized by the seedling emerging above the surface of the soil. To determine the percentage of germination both the seedlings that are normal and abnormal are counted on the tenth (Richman *et al.,* 2006). A normal seed has all structures that are very necessary of a seedling as it develops whereas abnormal seeds do not have essential structures such as the cotyledon despite germinating during the test period (Schmidt, 2000). For germination to take place it is necessary that seed dormancy is overcome, the embryo is alive, and there exist necessary conditions for germination of the seed in the environment. Change of either of these required conditions will affect the process of germination (Black and Halmer, 2006).

2.6.7 Seed vigor

The very best estimate of the potentiality of the field performance and consequently the seed field planting value is provided by the seed vigor (Saxena 2006). Despite the rate at which seeds germinate varying from the various seed varieties upon the first leaf and root shooting in the very first five days after planting then the seed is said to have sprouted (Rickman *et al.,* 2006). Crop establishment is determined by a germinating seed being able to grow and survive. The fast growth of seedling when subjected to field conditions with the potential of the seedling to grow variedly in relation to background environment and genes is the seedling vigor (Qun and Sun, 2007. The planting value information on the varied range of environment and seed lot potential of storage is provided by the seed vigor (ISTA, 2007). High-vigor seeds are responsible for the uniform and early seedling stand that necessitates competition against the stresses from the environment that are varied. It helps in ensuring that the end product has the best quality attainable within the available means. The knowledge of seed vigor of seedling enables farmers to make decisions on the suitability ofthe seed for immediate planting or prolonged storage.

There arises variation between seeds and their respective vigor from varied factors of which affect the seed. The arising variations are as a result of seed lots maturity at the time of harvest, the pattern of growth of the plant and the post-harvest handling. Despite seed vigor determination by the conditions of the environment during its maturity both the pre-harvest and post-harvest storage and handling may equally influence it (Nerson, 2007). Various factors in the environments onto which the plant is subjected to as it grows affect the end product in relation to its germination and vigor. The moisture

content of the seedling vigor affects the speed with which the seedling emerges evidenced by seedling emergence being faster at a moisture content of less than 20% during harvest. Cotton seeds are grown in areas in which the temperatures are high with lower rainfall subsequently yield seedling vigor that is of poor quality, and the yields seem very low (Hansen *et al.,* 2013).

A correlation exists between the rate of seedling germination and its vigor (Rajjou, *et al.,* 2012; Ranal 2006). Cotton growth and emergence at the early stages correlate with yields being higher and seedling survival being greater (Wanjura *et al.,* 1969). The speed with which seed germinates is an aspect of vigor that is of great importance and has been used to measure seedling vigor in rice.

2.6.8 Seed Length and Width

The hulled grain length determines the seed longevity and hence is the most important dimension of the grain. The hulled grain width gives the maximum dimension for the rough rice kernel. There is always a variation in both the width and length of rice and on some occasion's variations on genotype resulting from the varying length of the pedicel and awn (IRRI, 2009). The varieties of rice grain are classified as long, medium or short based on the dimension of the kernel ratio. As a result of the dimension and type of the rice kernel being of importance to the rice processors as well as the millers, the two characteristics are put into consideration during new genotype breeding. Basing on the length of the hulled grain it is classified as either being short, medium, long or very long of length variation below 7.5mm, 7.5-9mm, 9-10mm and above 10mm respectively.

Likewise based on the width the hulled grain is classified as being slender, semi-long, semi-spherical or spherical with width variation.

2.6.9 Thousand (1,000) Seed Weight

This is a measure of the purity of varieties of hulled grains based on their weight which is equally of importance in Genotype identification. Small seeds have very low seedling vigor. They also tend have mechanical complications that makes it difficult to harvest as more force is required to detach the husks and the hull. Consequently, making crop cultivation is problematic. However, natural selection is favored because of numerous association with seed output per plant being large, with maturity being very rapid and the geographical distribution. This characteristic is of importance in the calculation of the rates of seedling and losses from harvest (Anon, 2007, Vanangamudi *et al.*, 1987).

2.6.10 Milling Characteristics

The quality of milling is an aspect of a rice grain that is of great importance (Bergman *et al.*, 2006). The separation of the edible rice from the glumes through milling involves removal of the hull from the rough rice yielding the brown rice using a huller, or equally a wooden pestle and mortar can be used as the huller. Likewise, watermills too are used in various areas in Africa and Asia (Li, 2003).

The process of milling involves the separation of the hull from the paddy. What follows is the removal of the bran from the external parts of the brown rice. The process requires the application of abrasive mill that ought to produce whole grains. However, sometimes the mill breaks the grains due to mechanical challenges as a result of the shape and size

of the grains (Li, 2003). The separation of the intact kernels from the broken ones is the last step in the milling of rice. This is done using screens made for use on long, medium or short varieties in whole grain production (IRRI, 2009). The cost of the broken grains tends to be relatively cheaper compared to whole grain. Seed varieties that tend to have more quantity production of broken grains equally tend to produce very few whole grains resulting in a reduction in profit generation sales.

To ensure proper quality of milled rice, there is need to use paddy of good quality with mature kernels that are uniform in both their shape and size, fishers that are either empty or with half-filled grains and equally not contaminated with weed seeds or stones (IRRI, 2009). Crop management tends to affect the paddy quality which consequently influences the quality of rice that is milled. The rice milling quality is further modified by the cracks in the paddy grain all of which are due to weathering stress that they are subjected to when harvesting. When harvesting is done late, drying that isn't to the optimal expected standard and the stress it is subjected to during processing (Mahadevappa and Nandisha, 1987).

The content of moisture in the paddy should range between 10-14%, its purity should be high. Equally, there should be cleaning, andproper maintenance of the rice mills and its operators should be skilled (IRRI, 2013). The quality of milled rice is majorly affected by the content of moisture in that either extremes of the moisture content that is, either too low or too high result in both the whole rice and milling recovery declining. Value addition is achieved in the rice through milling, and this prolongs the shelf-life of the

grains as well as ensuring that the grains have the qualities desired by consumers. Milling mainly aims at providing complete removal of the germs and the colored bran as possible (Pantindol, 2000). Bran quantity left on the grain surface of milling is known as the degree of milling (IRRI, 2009).

2.6.10.1Milling degree

This is the measure of the bran percentage that is removed from the brown rice kernel, and this consequently has an influence on the color of the rice and equally the cooking behavior of the rice grain. For example, the un-milled rice fails to cook well as it poorly absorbs water (IIRR, 2009). There is the improved rate of water absorption up to almost 25% degree of milling with a negligible effect on overall quality. The degree of milling equally affects the nutrient quantity as most of the micro-nutrient found on the layers that are at the periphery tend to be removed when the degree of milling is very high.

Various factors affect the milling degree. The hardness of the grain reduces the degree of milling significantly. The physical dimensions of the grains such as the shape and size also determine the milling degree. Other factors include the ridges on the grain surface, the bran thickness and the efficiency of the milling. The degree of milling equally influences the milling recovery and consumer acceptance as well (Farooq *et al.*, 2005).

2.6.10.2 Milling recovery

This is the milled rice percentage obtained from the paddy sample after the process of milling and is mathematically obtained through the division of polished rice recovered by used sample paddy weight (IRRI, 2009). The maximum total yield of milled of milled paddy varies between 65% and 75% (Thompson *et al*.,1990).

2.6.10.3 Head or whole rice

The basis of quality milling appraisal highly depend on the rice head. Additionally, the total rice obtained after milling is done also dictates the quality that results. The head grain weight in the rice lot is the percentage of the head with the indication of maximum head yield ranging from 55 to 65 percent.

2.6.10.4 Grain quality

From the research done there are at least 1200 varieties of rice grain worldwide Li (2003). Nguyen (2001) showed in his report that the uniqueness in types are caused by form and structure if the plants and grains, maturity in development, how it spreads its branches, its way of production and how it adapts to biotic and abiotic. Slaton *et al*. (2000) state that rice market is set into three categories, i.e., long-grain, medium-grain, and short-grain. The type of grain must be of the size and shape specification set for that type for it to qualify in that particular market. Therefore, size and shape of the grain are among the first qualification of rice quality that breeders follow is coming up new type to release for economic production (Mutters, 1998). All the match standard must be met for consideration for launch. Therefore, size and shape standards must meet the minimum requirements. The weight also should be at par with the international standards (Mutters, 1998; Anon, 2007).

In its early stages of development, close viewing and examination of the grain are done to confirm that its structure is conforming to that of other grains that are commercially acceptable. Close visual examination, aided by appropriate equipment, is necessary to ascertain the grain quality. The observer must proof that the sample meets the

configurations in the commercially acceptable variety standards of grain types. Existing faults, e.g., flared shape, sharp ends, excess germs among others which can affect milled rice must be removedin theearly stage of development since this will reduce the yield and the general output (Mutters, 1998).

On farmers, Juliano (1993) says thequality of the grain is determined in the seed planted, and the grain consumed, without moisture, microbial wear and decay. Rice traders are looking no moisture rice and high yield rice with high integrity genotype. Outside qualities and genotype name are the main determinants of the market, while physio-chemical attributes determine consumption quality. Martinez *et al*. (2005) sayproduction of rice is also affected by consumers demand better quality. Various rice quality of rice is mostly determined by the price of the product in the market and also how it is accepted. When the consumer does not like the physio-chemical attributes and the physical appearance the product may lose its value in the market (Anon., 2007). Hammermeister (2008), indicated that once you know the science of a single grain you will automatically know about the grain quality, the quality still applies whether it is used for feed or human consumption. IRRI (2009) states further that quality of a rice grain is not dependent on its own but it is also affected but the environment of crop production, harvesting and machine it is handled with.

Irshad (2001) placed rice qualities into three categories (1) physical characteristics, e.g., shape, size, milling and moisture content. (2) physio-chemical characteristics analysis,e.g., Protein content, amylose content, expansion volume, cooking time and gel

constituency and (3) organoleptic quality of the cooked rice, e.g., taste smell or aroma, stickiness, hardness, consistency and color. The quality characteristics can be categorized into 3 broad areas. The first group in dependent on the physical features which include moisture content, shape, size, and milling. The second category relies on the the analysis of physio-chemical traits of rice including amylose content, protein content, gel consistency, volume of expansion of cooked rice, and cooking time. The third group depends on the organoleptic quality of cooked rice which include color, aroma, hardness, stickiness, and consistency. In this research,the length of the grain, seed length and width and milling characteristics are the physical properties of milled rice grain.

2.6.10.5 Grain dimension

Width and length of a rice grain are crucial characteristics that determine their classes. Rice is with reason classified into three categories depending on three physical qualities, i.e., weight shape and length. Length measure of rice grain into the most significant dimension, while two of the three dimensions determine the shape, i.e., Width, length, and thickness. Richman *et al.* (2006) categorized rice grain already milled in length-width ratio. Belsnio (1980) managed to identify the grain categories by measuring the length of the grain as a whole then he classified them according to the whole length as extra-long of 7 mm or more long of about 6 mm and shorter milled rice of anout5 mm or more but shorter than 6 mm. Many people have attempted to modify the categories into better names like medium slender, long slender though it cannot be justified since there is no big difference between the long and long slander and medium and medium slander, (Belsnio, 1980).

2.6.10.6 Thousand (1,000) grain weight

This is the measure that provide crucial information about the density of the grain. Different grain hasuniquedensity,and they also retain moisture differently and even cook in different ways (Richman *et al*., 2006). The moisture content of rice kernels, weather condition and the soil type in which it has grown always determines the weight of rice.

2.6.11 Rice production in Kenya

In 2007 rice yield was 42bags /haas compared to today where it has declined to 29bags /ha. Rice production in Kenya is mostly carried out using schemes. The large scale schemes that have supplied the locals with rice in the country include Bunyala Scheme in the Western part of Kenya, West Kano and Ahero in Nyanza, and Mwea Scheme in the Coastal regions. The areas of rice production cover a total of 13000 ha. Most rice consumes in Kenya are produced in irrigated land while some are produced in rainfed conditions. Rice consumption is at 300000 tonnes compared to annual production of 45000 to 80000 tonnes which therefore leads to importation which was costs Kenya around 7 billion annually. Increased rice production in Kenya will, thus, lead to increased food security and household incomes,and it will reduce the amount spent on rice importation. Rice consumption per capita in Kenya is approximated to be 10-18 kg per capita per year (Africa rice) rice tops in annual consumption at 12% as compared to wheat (4%) and maize at (1%) (MoA, 2009).

Poor qualities of seed, lack of information, poor infrastructure, poor storage facilities and ashortage of skilled labor are significant challenges to rice production. The Kenyan government is trying to develop infrastructure especially seed storage facilities, cool store

for bulk seeds, freezes for seed storage, increasing research centers for seed multiplication, adequate areas for varieties evaluation, good laboratories for seed analysis, seed processing equipment,and rice harvesting machines. Inventions such as proper utilization of post-harvest technology, use of improved cultural methods, improved harvesting, timing and post-harvest handling techniques will help minimize the cost of production.

2.6.12 Effect of Storage time and environment on seed quality

According to Chirchir *et al.,* (2016), the scale used in finding the period of storage is the "relative storability index" classification. The index has been developed through coordinated research over a long period under standard conditions of temperature and relative humidity. Previous research has also indicated that the period a seed takes to remain in storage is affected by its initial quality, moisturea content, relative humidity, temperature, and gaseous exchanges in the environment it is stored (Mbofung, *et al.,* 2013). The length of time that a specific seed takes in storage is genetically determined though the prevailing conditions may lengthen or shorten this period. The storage preservation substances are genetically ingrained within the seed (Delouche, 2016). The seed remains of good quality within the predetermined period. Beyond this stage, the seed deteriorates. Seed deterioration is defined as irreversibledamage to the viability qualities. The process can only be slowed to avoid huge losses (Delouche, 2016).

Best storage environment has controlled conditions such that for a 1% decrease in seed moisture content, the storage of seed is doubled; in a 5-degree decrease in storage temperature, it doubles the storage of a seed (Harrington and Gould 2015.) For more than two years now these rules have been applied in food storage (Walters, 1998).Temperature

and humidity must be control carefully. When temperature rises and the relative humidity is maintained, the seeds are likely to decay at a high rate (Timóteo and Marcos-Filho, 2013). Hence, the storage environment must have a standard balance of these two properties. Seeds subjected to unstable temperature decay faster than those in a stable climate (Fenner, 2012).

2.6.13 Yield components

Yield formation closely linked with physiological traits is vital. Takai *et al*. (2006) stated that it is essential to increase size and radiation use efficiencies during grain filling. Ying *et al*. (1998) indicate that further improvement in rice production potential depends more on the ability to increase biomass production than on that of growing harvest index (HI).

2.6.14 Effect of storage temperature and environmental factors

Temperature and the moisture contents of seeds are the key contributors to variation in the condition of stored seeds. When the low temperature is maintained it reduces the enzyme activity in breathing process and of decline speed in the viability of orthodox seeds during storage, (Timóteo and Marcos-Filho, 2013). The decay cannot be avoided but can be reduced in during storage under favorable environment (Santos *et al*., 2004). Decay of seeds is caused by storage under high temperature and relative humidity by promoting degenerative changes like the restructuring and sudden loss of integrity in the cell membrane system and activities of the tissues. This is majorly caused by lipid peroxidation due to increased oxygen (Alscher *et al*., 2002).

The degree of seed dormancy is affected by various factors. Investigation of these factors shows that the domancy may stop whenever significant adjustment occur. The factors

include light quality, moisture content (water), photoperiod, temperature, and seed nutrient content (Hilhorst, 2007; Fenner, 2012; Bewley *et al.*, 2012).

2.6.15 Rice juvenile characters

Recently, it has been found that plants develop in phases each with different growth patterns (Sylvester *et al.,* 2001). Recognition of the heterochrony in the development of individual organs such as leaves is essential (Asai, *et al.,* 2002; Jones, 1999). However, the juvenile-adult-reproductive phase is important since it changes the characteristics of the whole plant.

2.6.16 Molecular Markers

Molecular markers, which express themselves as genetic markers, are applied in the study of molecular biology and biotechnology. In the study, they are used in the identification of the specific sequence of DNA in a pool of unknown DNA. A molecular marker is specific to a particular location in the genome. They mainly include restricted fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNA (RAPD) and simple request repeats (SRRs) also known as microsatellites (Roviglioni, *et al*., 2000; Bhat, 2006). The markers are found on genetic and physical maps (Temnykh *et al.,* 2000; Temnykh *et al.,* 2001; McCouch *et al.,* 2002) for amplification of rice.

The initial step in the process is taking a sample of an individual's DNA which is used as the“reference sample”. The buccal swab is the most efficient and widely used method of obtaining the reference samples since the risk of the sampleis significantly minimized.

Sometimes the process may be impossible to use due to circumstances such as where legalities are involved, as in the case where a court order is required and cannot be obtained.In such instances, other methods are allowed. The substitute methods can be used to obtain a sample of body fluids such as blood, saliva, semen or fluid or tissue from personal items such as toothbrush, razor. More so, the samples can be obtained from stored samples such as banked sperm or biopsy tissue. An indication of an individual's profile can be obtained from samples of biological / blood relative or even human remains that had already been profiled (McKie, 2009).

A reference sample is always used for the analysis of each of the created DNA profiles. Various techniques have been developed to assist in the process. The next step is the comparison of the samples against the developed DNA sample to determine a genetic match (Glenn and Schable 2005). Afterwards, the matched profile can be further analyzed to verify the common pattern which can later be marked as the resultant mark (McKies 2009).

Notably, Mullis *et al*. (1986) developed a Polymerase Chain reaction to enhance the process of amplifying the specific portions of the DNA strands during analysis and observed that the reactions could amplify the samples to almost indefinite form. To date, the technique is still used to advance technological development in line with DNA study. The process takes place in a manner similar to the biological process of DNA replication. However, in this case, the replication is confined within a specific DNA sequence of interest. Mullis PCR technique has enabled researchers to make remarkable advances in

carrying out DNA profiling, thus enabling them to recover information even from distorted or small initial samples (Primrose and Twyman, 2013).

The PCR technique focusses on a small region of the DNA and amplifies it to the required dimensions. The process involves denaturing of the whole DNA sample and portioning into separate individual polynucleotide strands. The initial step is facilitated by heating under preconditioned temperature. Two oligonucleotide DNA primers carry out the division of the neighboring opposite sites of the DNA strands. The process is done carefully to ensure the normal enzymatic extension of the active sites and terminals of each of the primers automatically lead to the next. Replication enzymes are employed within the reaction framework. These enzymes should not be denatured and hence must be resilient and tolerant to high temperatures. An example of such an enzyme is thermostable tag polymerase. The enzymes catalyze the regeneration process leading to the production of two new duplicates of the initial sequence. The process is done repeatedly and hence an exponentially growing number of copies of the DNA sample of interest are produced. The instruments used for thermal cycling are commercially available from relevant manufacturers and dealers. The exponential process can produce up to a million-fold or more of the initial DNA strand within two hours or even less (McCouch *et al.,* 2002).

Genetic diversity is an inherited variation among and between populations, created, activated and maintained by evolution (Demol, *et al,* 2001). Plant characterization is indispensable tool for selecting varieties or lines based on agronomical, morphological, genetic or physiological characters (Ndour, 1998). To separate genotypes characterization

helps to evaluate the phenotypic diversity through agro morphological properties (Bajracharya *et al*., 2006).

2.6.16.1 Microsatellites in rice

Microsatellites refer to the in tandem and repeated nucleotide units ranging from 1 to 6 of the base pairs and the alleles always have a clear difference in the number of the repeating units. They are co-dominant and have the characteristic polymorphic markers corresponding to the DNA strands. For rice genome, the microsatellite markers are genetically marked and cover them wholly. The pattern exhibited shows that within every 16 to 20 cm, there is one microsatellite (Thomson *et al.*, 2003). The coverage enabled by the genome enhances the ability of the microsatellites to anchor the PCR markers which are randomly produced. Examples of such markers include AFLPs which occupy known regions of the rice genome. They make it economical easier to produce genetic maps of the same rice genomes (McCoouch *et al.*, 2002). Additionally, the rice microsatellites utilize the gene-tagging and marker-assisted characteristics (Thomson *et al.*, 2003; McCouch *et al.*, 2002). They are polymorphic between and within the rice varieties,and thorough analysis is required to ascertain the dominating pattern (Akagi *et al.*, 1996; Panaud *et al.*, 1996; Olufowote *et al.*, 1997). If a high level of polymorphism is required, microsatellites of greater informativeness are useful in developing the unique DNA profiles of the predetermined rice genotype (Muhammad *et al.*, 2009).

2.6.17 Constraints to Rice Production

Every industry has specific challenges that inhibit optimum performance. Rice production faces an equal share of such challenges in the different regions where large

and small-scale production is done. The major constraints include poor irrigation, insufficient supply of water, inadequate credit and loans for farmers, low or poor seed quality and varieties, unprecedented climatic changes and poor paddy prices. Moreover, rice farmers have weak and few associations that can champion for their welfare to the relevant authorities and policymakers. The milling capacity is also still low (Africa Rice Centre, 2009). In Kenya, farmers have suffered due to lack of proper rice certification scheme which makes it difficult to address almost all the above-mentioned challenges. The other global problem is the numerous diseases that affect the crop. For instance, rice blast, caused by *Magnaporthe grisea* which is fungal, is the most significant of the diseases that affect the cultivation of rice. Rice ragged stunt (vector: BPH), *tungro* (vector: Nephotettix spp) and the sheath blight are some of the deadly disease that make farmers make big losses to curb them since they have deadly effects on the crop. *Cochliobolus miyabeanus* and ascomycete fungus are the agents that make rice to have brown spot disease (Mew, 2004).

Rice crops are infected by several nematode species causing diseases such as the White tip disease (*Aphelenchoide bessei*), Ufra (*Ditylechus dipsaci*) and the root-knotdisease (*Meloidogyne graminicola*). The upland areas are mostly affected by *Pratylenchus spp* nematode species. Rice root nematode (*Hirscmaniella oryzae*) which can migrate, tend to destroy the rice if in higher inoculum levels completely. In addition to the parasites being obligate, they decrease the plant vigor and increase the susceptibility of the plants to diseases and pests. *Eudicot Striga hermonthica* is the main parasitic weed in rice despite being of local importance for rice (Mew *et al.*, 2004).

2.6.18 Genetic diversity of rice

Thousands of valuable allelic variations of traits of economic significance remain unutilized in nearly all crop plants. These can be discovered and effectively used to meet the existing and emerging challenges that threaten world food security. Sadly, this genetic wealth is being eroded due to neglect and over-exploitation. Developmental activities and exploitive land-use planning are destroying natural habitats, and modern varieties are replacing native species and landraces, resulting in a reduction of varietal diversity. Major crop species (rice, wheat and millet) suffered the most during the green revolution. In order to successfully meet future food requirements, it is necessary to manage the continuing genetic erosion and address the issues of genetic conservation and optimum utilization of what remains of the genetic diversity of important crop plants.

2.6.18.1 New rice varieties

Earth Institute has researched with an objective of increasing the productivity of rice. This has consequently resulted in the production of New Rice for Africa (NERICA) which can withstand the low input with the harsh conditions for growth that are characteristic of agriculture in Africa (Wopereis *et al,* 2008; Kijima *et al.,* 2011). Research is being conducted in China with the hope of developing perennial rice to improve food security and sustainability. Many genes have been identified and cloned by scientists in the *gibberellin* signaling pathway such as GAI1 (Gibberellin Insensitive) and SLR1 (Slender Rice). If the gibberellin signaling is disrupted,the growth of the stem is in turn significantly reduced. Shorter plants being naturally more stable have the photosynthetic investment of the stem cut. The assimilates are redirected into the process of grain production in form of commercially acceptable chemical fertilizers. These rice

varieties can increase their yield two or three times if nitrogenous fertilizers are available accompanied by intensive management of the crop (Welch *et al.,* 2010).

2.6.18.2 Golden rice

Since rice kernels lack vitamin A, people who consume the rice to obtain their calories risk suffering from a deficiency in vitamin A. To overcome this deficiency, genetically engineered rice that produces *beta-carotene,* vitamin A precursor, has been developed by German and Swiss researchers. The *beta-carotene*, the vitamin A precursor, is responsible for turning the white processed rice into a golden color thus the name "golden rice." Upon consumption of the rice by humans, the *beta-carotene*is converted to vitamin A. Despite the fact that some strains of rice tend to produce beta-carotene in their hull there hasn't been any non-genetically engineered strains that produce beta-carotene in the kernel despite thousands of the strandsbeing tested. More efforts aimed at improving on the quality and quantity of nutrients in the golden rice are underway with the International Rice Research Institute (IRRI) attempting to develop and evaluate the Golden Rice in the hope of addressing the Vitamin A deficiency (Brooks, 2013).

2.6.18.3 Flood-tolerant rice

Most rice-growing farmers majorly in South and South East Asia are faced with the challenge of flooding which annually affects 20 million hectares. Constant flooding that exceeds a week affects the various standard rice varieties since the prolonged flooding does not allow the plants gain access to the requirements such as essential gas exchanges and sunlight resulting in the plant's inability to recover. There have been massive losses

in yields in the past as a result of flooding with rice crops valued at $ 65 million lost in the Philippines in 2006.

Swarna Sub1 Genotype of rice has been developed to curb this hazard through*Marker – assisted* selection. The Swarna Sub1 can withstand more extended flooding period of about 14 days under the flooded plain. This Genotype of Rice's ability to submerge below the flooded plain is due to the presence of the Sub1 gene from the Indian cultivar FR13A into Cultivar Swarna which is vulnerable to flood.

2.6.18.4 Drought tolerant rice

Rice production is significantly affected by drought which is one of the environmental stresses that rice is subjected to. Rice production in the South and South East Asia is most of the times at risk as the drought affects 19-23 hectares. Conventional commercial rice varieties can be severely affected when subjected to drought condition with insufficient water which is necessary to enable them to obtain the required level of nutrients from the soil. The drought has resulted in losses of up to 40% that has affected some parts in India with annual losses of about $ 800 million. The International Rice Research Institute (IRRI) has been in the forefront in researching and developing rice varieties which are tolerant and resistant to drought. Farmers in Nepal and some parts of Philippines are already using the Sookha variety; an example of the drought resistant variety 5411 has also been developed and made available to farmers who live in regions that receive very little rainfall (Sinha, and Tripathi, 2016).

The deeper rooting 1 (DRO 1) was successfully inserted into the commercial rice Genotype IR64 from the rice Genotype *Kinandang Patong* from the Philippine upland. This resulted in far deeper root system in the plants which enables the plants to derive the

necessary nutrients in times of drought. It has been possible by accessing the soil layers that lie very deep which were exhibited by 10% yield drop of the IR64 + DRO1 rice when subjected to moderate drought condition unlike the 60% for IR64 Genotype that is unmodified (Uga *et al.*, 2011).

2.6.19 Varieties in Kenya

Table 2.2 Varieties of irrigated rice and their characteristics

Variety	Height in cm	Maturity days	Yield t/ha	Cooking quality	RYMW	Blast
"Basmati 217"	118	122	4.6	Very good	Resistant	Susceptible
"Basmati 370"	118	122	5.3	Very good	Resistant	Susceptible
"IR 2035-25-2"	86.2	128	5.5	Good	Moderately susceptible	Moderately resistant
"IR 2793-80-1"	89	142	6.4	Good	Susceptible	
"BW 96"	68	135	9.0	Fair	Susceptible	Moderately resistant
"UP 254"	84.2	124	6.4	Good	Moderately susceptible	Moderately resistant
"AD 9246"	78.2	128	5.1	Good	Moderately resistant	Moderately susceptible
"IR 19090"	96.6	122	5.8	Good	Moderately susceptible	Moderately resistant

Varieties for lowland (swampy) zones	Varieties for upland (dry land) zones
"Ci cong Ai"	"Dourado Precose"
"TGR 78"	"2051 A 233/79"
"IR 2793-80-1"	"TGR 94"
"BW 196"	"WAB 181-18"
"WaBis 675"	"Nam ROO"
	"NERICA 1", "NERICA 4", "NERICA 10", "NERICA 11"

2.6.20 Rice Varieties in Tanzania

Variety	Optimal production altitutde	**Grain Yield**	**Special Attributes**
Supa	0-400 m	1.5-3.5 t/ha	moderately resistant to RYMV and sheath rot.
IR 54	400-600m	4.0-7.0 t/ha;	moderately resistant to bacterial blight and sheath rot
IR 22	400-1000 m	6.6-8.0 t/ha	days to maturity: 120-13"5; resistant to bacterial blight.
Katrin	400-1000 m	6.6-8.0 t/ha;	very low panicle shattering.
Dakawa	400-1000 m	3.5-5.2 t/ha	none-photoperiod sensitive; resistant to lodging except under high N levels; easy to thresh.
TXD	0-400 m	4.8-7.0 t/ha;	moderately resistant to sheath rot, blast and RYMV.
TXD	0-400 m	2.8-6.5t/ha	moderately resistant to sheath rot, blast and RYMV.
SARO 5	0-600 m	4.0-6.5t/ha	susceptible to RYMV and sheath rot. Adapted to rain-fed lowlands and irrigated ecosystems.
Kalalu	-	2-3t/ha	resistant to RYMV and blast.
Mwangaza	-	2-3t/ha	resistant to RYMV and blast.

CHAPTER THREE:MATERIALS AND METHODS

3.1 Experimental site

This study was carried out at KALRO in Kiambu county and KALRO Mwea in Kirinyaga, through field and laboratory experiments.

3.1.1 Kenya Agricultural Research Institute (KALRO) Mwea

The Kenya Agricultural and Livestock Research Organization in Mwea is located in Central province Kirinyaga County in Kenya. It lies at latitude 37”13’E and 37”30’E longitude 0”32’S and 0”46’S and is approximately 1,175m above sea level (Nyangau *et al*., 2914). The area receives a bimodal rainfall and total precipitation of 439mm annually with mean temperature of 27.4-19.2 degrees celcious. The soils are classified as alluvial soils and are well drained type. KALRO Mwea is located in Kirinyaga County in Kenya near Kadongu village. The county can be divided into three ecological zones; the lowland areas that fall between 1158metres to 2000 metres above sea level. The midland areas that lie between 2000 metres to 3400 metres above sea level and the highland comprising areas of falling between 3400 metres to 5380 metres above sea level. The county has a tropical climate and an equatorial rainfall pattern. The county has two rainy seasons, the long rains which average 2,146 mm and occur between the months of March to May and the short rains which average 1,212 mm and occur between the months of

The area receives uneven rainfall and total monthly precipitation of 151mm with mean temperature of 15.6°c -28.6°c. The soils are classified as black cotton soils and are poorly drained type. (www.kirinyaga.go.ke).

Table 3.1: Temperature in Mwea, Kirinyaga County in 2016

month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Okt	Nov	Dec
mm	23	25	62	203	40	7	2	5	9	78	201	74
°C	21.3	22.3	22.9	22.5	21.8	20.4	19.6	20.0	21.4	22.7	22.1	21.0
°C (min)	12.4	13.0	13.9	14.1	13.7	12.4	11.8	12.0	12.6	13.8	14.0	12.8
°C (max)	30.2	31.6	32.0	30.9	29.9	28.5	27.5	28.0	30.3	31.6	30.3	29.3
°F	70.3	72.1	73.2	72.5	71.2	68.7	67.3	68.0	70.5	72.9	71.8	69.8
°F (min)	54.3	55.4	57.0	57.4	56.7	54.3	53.2	53.6	54.7	56.8	57.2	55.0
°F (max)	86.4	88.9	89.6	87.6	85.8	83.3	81.5	82.4	86.5	88.9	86.5	84.7

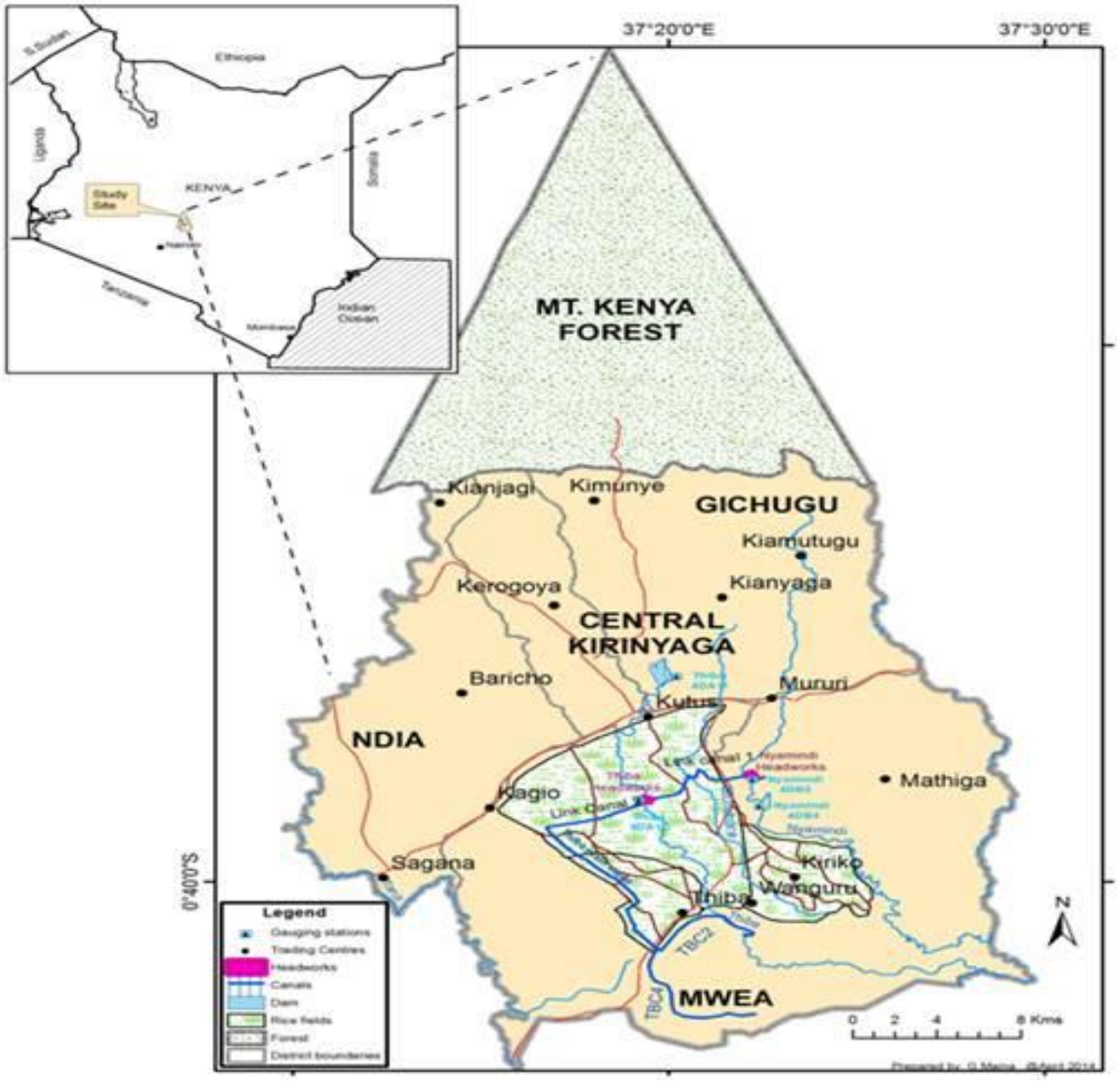

Figure 3.1: Map showing study site in Kirinyaga County
Source: www.google.ae/search

3.1.2 Juja Kiambu County

Kiambu County is divided into four broad topographical zones viz, Upper Highland, Lower Highland, Upper Midland and Lower Midland Zone. The area lies between 1,500-1,800 metres above sea level. The upper midland zone lies between 1,300-1,500 metres above sea level and it covers mostly parts of Juja and other constituencies with the

exception of Lari. The lower midland zone partly covers Thika Town, Limuru and Kikuyu constituencies. The area lies between 1,200-1,360 metres above sea level. The county is covered by three broad categories of soils which are: high level upland soils, plateau soils and volcanic footbridges soils. These soils are of varying fertility levels with soils from high-level uplands, which are from volcanic rocks, being very fertile. Most parts of the county are covered by soils from volcanic footbridges, red to dark brown friable clays, which are suited for cash crops like coffee, tea and pyrethrum. However, parts of Thika Town, Ruiru, Juja and Lari constituencies are covered by shallow soils, which are poorly drained, and these areas are characterized by low rainfall.

3.1.3 Ecological conditions

Water in the county is from two principal sources- surface and sub-surface. About 90 percent of the county‘s water resource comprises of both surface water resources and ground water potential. The county is divided into several sub-catchments areas. The first one is Nairobi River Sub-catchment, second one is Kamiti and Ruiru Rivers Sub-catchment, third one is the Aberdare plateau the fourth is Chania River and its tributaries.

3.1.4 Climatic conditions

The county experiences bi-modal type of rainfall. The long rains fall between Mid-March and the short rains between Mid-October to November. The annual rainfall varies with altitude, with higher areas receiving as high as 2,000 mm and lower areas of Thika Town constituency receiving as low as 600 mm. The average rainfall received by the county is 1,200 mm.

The area's average temperature is 26 ^{o}C with temperatures ranging from 7^{o}C in the upper highlands, to 34 ^{o}C in the lower midland zone found partly in Thika Town constituency..

Table 3.2: Climate weather data Juja in 2016

month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Okt	Nov	Dec
mm	36	40	90	175	110	27	12	14	15	57	148	75
°C	20.0	20.6	21.0	20.9	19.9	18.3	17.3	17.7	19.2	20.5	19.9	19.6
°C (min)	12.4	12.3	13.5	14.6	13.8	12.0	11.3	11.5	12.0	13.3	13.8	13.1
°C (max)	27.6	28.9	28.6	27.2	26.0	24.7	23.4	24.0	26.5	27.7	26.1	26.1
°F	68.0	69.1	69.8	69.6	67.8	64.9	63.1	63.9	66.6	68.9	67.8	67.3
°F (min)	54.3	54.1	56.3	58.3	56.8	53.6	52.3	52.7	53.6	55.9	56.8	55.6
°F (max)	81.7	84.0	83.5	81.0	78.8	76.5	74.1	75.2	79.7	81.9	79.0	79.0

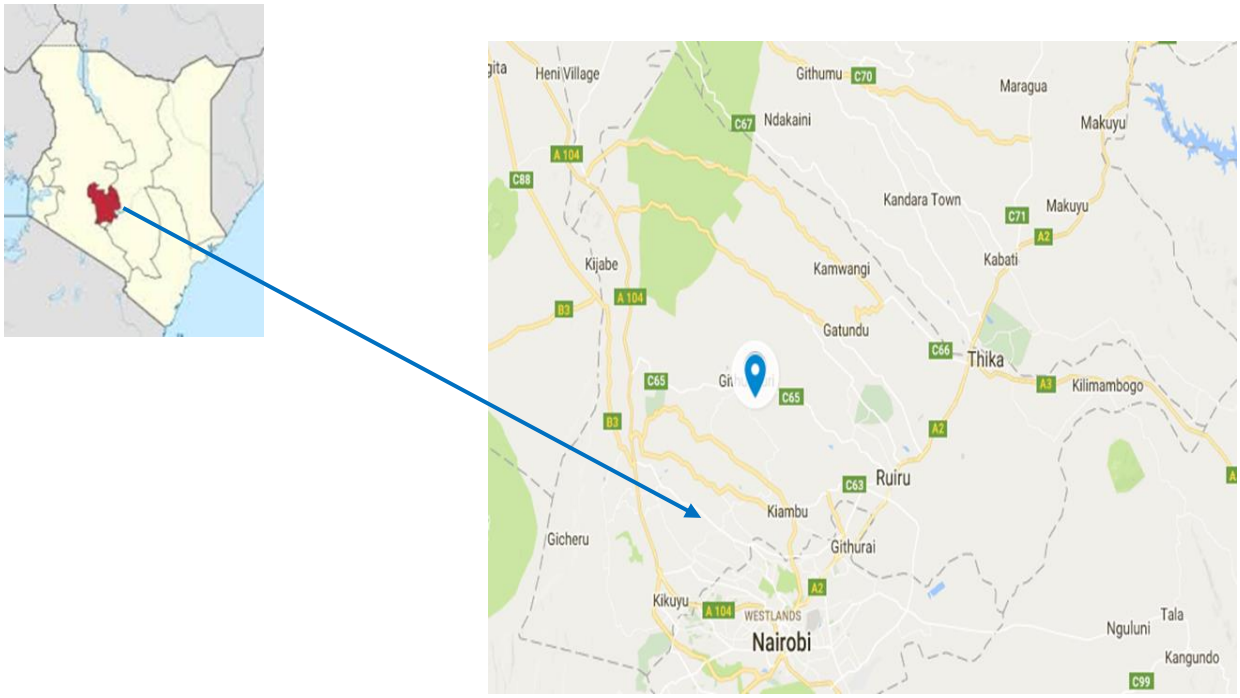

Figure 3.2: Map of Kenya and location of Kiambu County

3.2 Seed collection

Seeds from several known and released rice varieties in Kenya were collected from KALRO and MIAD research stations and farmers markets in the two sites. Randomly selected accessions from a total of 18 varieties were studied. Freshly harvested seeds obtained from the final seed production field experiment were used as standard checks.

3.3 Accessions used in the study

Sr.no Genotype Source Attribute 1 IR 2793 Kenya Improved variety 2 BS 217 Kenya Improved variety 3 BS 370 Kenya Improved variety 4 BW 196 Kenya Improved variety 5 ITA 310 Kenya Improved variety 6 Red Afaa Tanzania Landrace 7 IR 54 Tanzania

Table 3.3: Profiles **of rice varieties used in the study.**

	Accession	**Source**	Brief Description
1	Basmati 370	KALRO Mwea	Long and slender kernels
2	Basmati 217	KALROMwea	Long and Slender kernels
3	NERICA 1	KALRO(Mwea)	
4	NERICA 4	KALRO (Mwea)	
5	Red Afaa	KALRO (Mwea)	Landrace, Short grain
6	Kilombero	KALRO (Mwea	Landrace, medium grain
7	Dourado Precose	KALRO(Mwea)	
8	Uzungu	Africa seed company	
9	Mzungu	Farmer stocks, Mwea	
10	Kahogo	Traders stock, Mwea	Landrace, Medium grain
11	Japan 54	Traders	
12	Japan 64	Traders	
13	Saro 5(TXD)	KALRO	Short grain, bold kernels
14	Supasaro	KALRO	
15	IRO5N2	KALRO	
16	IR2793	KALRO	Short grain, bold kernel
17	BAS 196	KALRO	
18	ITA310	KALRO	Medium to short grain

Improved variety 8 Kilombero Tanzania Landrace 9 IR 64 Tanzania Improved variety 10 Kahogo Tanzania Landrace 11 Saro 5 Tanzania Improved variety 12 Wahiwahi Tanzania Landrace 13 Supa Tanzania Landrace Basmati 370 is an improved Kenyan variety with short grains

3.4 Kenya Bureau of Standards (KEBS)

This is located at South C estate, Nairobi county. Kenya Bureau of Standards (KEBS) is located at Kenya Bureau of Standards along Popo Road, Off Mombasa Road, Nairobi. Kenya

3.5 To determine the quality of selected rice genotypes

3.3.1 Seed quality tests

The 10 rice accessories were subjected to seed quality tests which were done at Jomo Kenyatta University of Agriculture and Technology laboratory in Juja. Aspects of seed quality to be tested included seed purity, viability, seed vigor and seed dormancy tests.

3.3.2 Seed purity experiments

The main aim of this was to determine the percentage of seed in each sample. Each of the 8 seed lots were sampled and mixed well and weighed into 90kg lots using a weighing balance. The seeds from each sample were placed on an open surface for visual observation. Sorting was done in order to separate pure seeds, foreign seeds, and soil and dust particles. Each group was then weighed to obtain a ratio of the pure seed to the other seed/materials in the samples.

3.3.3 Germination tests

Each of the 8 seed lots were sampled and subjected to sgermination tests. A sample of 400 seeds per genotype was drawn from the pure seed sample and seeds placed in four germination trays containing 25cm sterilized sand representing four replicates**.** The days to coleorhiza ans coleoptile formation were recorded. Germinated seeds were counted and data recorded fro 7, 14 and 21 days. Seeds germinated after 21 days are categorized as normal while ungerminated are abnormal (AOSA 2007). Seed were considered germinated if the radicle (coleoptile) and the plumule (coleorhiza) are visible.

Results are expressed as percentage by number.

Germination rate is the average number of seeds that germinate over the time period.

Germination (%) = G / X * 100

Where: G = number of normal germinated seedling

X = number of seed sown (excluding empty and infested

3.3.4 Seed vigour experiments/seed ageing

Seed vigor was evaluated using the Accelerated Ageing(AA) test. The test was performed according to the AOSA (2005) Seed Vigor Testing handbook. One hundred seeds per genotype per replication per treatment were placed in a single layer on wire mesh in a 10 by 10 by 4 cm acrylic box containing 40 mL of distilled water. Lids were placed over boxes, which were then placed inside an AA chamber at a temperature of 45°C and a RH of approximately 98% for 24, 48 and 72 h. After the aging period, the seeds were removed from the chamber and planted on moistened sand in germination trays and

containing 2.5 cm of moistened sand. Measurements were taken for the days to coleoptile and coleorhiza formation.Germination counts were taken for 7, 14 and 21days (Marcos-Filho, 2015).

Plate 1: seeds within acrylic box in an ageing chamber. **Plate 2;** Sample of standard germination tests after ageing

3.3.5 Seedling vigor experiments

The 8 seed lots were sampled again and 50 seeds from each lot were subjected to vigor tests. The seeds were planted in germination pots in a greenhouse at normal temperature and light conditions. Each set was replicated four times. This was noted by observing the emergence dates, shoot height every 7 days.

3.3.6 Data analysis

The obtained data was subjected to analysis of variance and the significance determined at the 0.05 level by the F test. Regression analysis was carried out using the GENSTAT

version 7. Regression analysis was used to describe the relationship between the percentage of normal seedlings and the inert matter.

3.4 Determining the effect of storage time on seed growth, quality

3.4.1 Seed production and storage

Twenty one day old seedlings were transplanted to JKUAT paddy field in in a series of lots [per month from February 2015 to July 2015. Subsequently, harvesting was undertaken from August 2015 to January 2016 as each lot matured.Hand harvesting of rice panicles was done and sun dryng to ensure maximum of 14% moisture content. They were stored in gunny bags at room temperature, about 21°C, the relative humidity was also uncontrolled for 1, 2, 3.4.5.6 months respectively depending on the harvesting time.

3.4.2 Determining moisture content of seeds

To obtain initial and final moist content 3 petridishes per genotype with samples of 100 seeds per lot were wighed and oven dried using long method103°C for 72h (ISTA, 2012). Initial and final weights were substracted and converted into percentage to obtain the moisture content. The moisture contentwas calculated using the formula below

$$\text{Moisture content (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

where,
W1 = weight of container with lid;
W2 = weight of container with lid and sample before drying; and
W3 = weight of container with lid and sample after drying.

$$\%\,\text{Moisture Content} = \frac{\text{Weight of fresh seeds} - \text{Weight of dry seeds}}{\text{Weight of fresh seeds}} \times 100\%$$

3.4.3 Experimental design and layout for field experiment

The field was laid out in a randomized complete block design (RCBD). Hundred varieties were replicated three times with each genotype was sown in a plot size of 3.0m x 2.0m with spacing of 20cm x 20cm. The total area covered by the 90 small plots was 1,200m^2.

3.4.4 Data collection

3.4.4.1 Leaf length and width

The length and width of a randomly selected leaf from 10 plants from each plot was measured using vernier calipers from one end to the other of each and this was recorded (Rickman *et al*., 2006).

3.4.4.2 Grain width

The length and width of randomly selected grains from 10 plants from each plot was measured using vernier calipers from one end to the other of each and this was recorded (Rickman *et al*., 2006).

3.4.4.3 Seed germination

Germination tests were conducted using sand as the growing medium in accordance with ISTA (2007) procedure. One hundred seeds replicated four times per Genotype were sown in two trays. (ISTA, 2007) Both normal and abnormal seedlings were examined and counted during the inspection on the 7^{th}, 14^{th} and 21^{st} day to determine the germination percentage (Rickman *et al*., 2006).

3.4.4.4 Seedling height

Three heights of sampled from 10 plants per plot were measured using a meter rule, at transplanting, I and 2 months after transplant and at maturity.

3.4.4.5 Number of spikes

At maturity, the number of spikes per sample from 10 plants per plot was counted and noted.

3.4.4.6 Number of days to flowering

The number of days to flowering was counted from 10 plants per plot was manually counted and noted.

3.5 Statistical analysis

Data collected was analyzed using GenStat statistical package to generate analysis of variance (ANOVA) and means separated by Fishers's unprotected and LSD at 5%. The means were separated using Least significantdifferences (LSD) at 5% level.

3.6 Effect of storage time and genotype on yield and yield components

3.6.1 Experimental design and treatment coding

A completely random block design with 3 replications was used and plot area was $6m^2$.The arrangement was a 5by6 factorial design including 5 varieties and 6 storage levels. The plot sizes were 3m× 2m. Each of the 3 blocks had 30 plots giving a total of 90 experimental plots the spacing was 20cm within rows and 20cm between rows, the total plant population was 100 per plot.

Table 3.4 Coding of the treatments

Code	Treatments
Storage time (Months)	
M1	1 month old seeds
M2	2 month old seeds
M3	3 month old seeds
M4	4 month old seeds
M5	5 month old seeds
M6	6 month old seeds
Genotypes	
V1	J54
V2	J64
V3	B370
V4	B217
V5	Pishori

3.6.2 Nursery and Transplanting

The seeds were soaked in water for two days prior to sowing to aid in breaking seed dormancy, Seeds were first planted in a nursery according to best practices and were sown on January 25 and transplanted on February 20 for a season at hill space of 20 cm×20 cm with one plant per hill.

These seedlings were transplanted in experimental blocks and various juvenile growth characters were observed. Juvenile characters include number of leaves per plant, height, days to flowering, number of tillers per plant, yield as detailed in section 3.4.4. The seedlings transplanted in a randomized complete block design replicated thrice. The plots were hand weeded and kept weed free up to harvesting.

3.6.3 Temperature and rainfall variation

Average ambient seed storage temperature at the JKUAT University laboratory was 21.3°C (± 6.3) ranging from a high of near 30°C to a low of about 7°C. Mean humidity for ambient storage was 73.7% (± 8.3) ranging from a high near 90% to a low near 30%. All commercial and local seed lots for all species were stored in the same ambient storage and exposed to the same ambient conditions.

3.6.4 Planting in Mwea and Kiambu

Seedlings were grown in two locations in Randomized Complete Block Design (RCBD) with 3 replications Trials were evaluated at least weekly during the growing season, and data collected for the following traits; seedling vigor, tillering characteristics, days to 50% flowering date, plant height at maturity, number of grains, total above ground biomass, grain weight,harvest index. Measurements were taken on leaf dimensions were recorded by measuring blade length, blade width at the widest point. 10 plants were sampled.

3.6.5 Data collection and measurement of yield characteristics

Data was taken for each environment on plant height, number of tillers per hill, number of days to 1st heading and when 50% of the crop had headed. The days to maturity and flowering as well as yield and yield components will be recorded. The yield for each lot was calculated and used for analysis. Plots were maintained in a weed-free condition with soil moisture and nutrient supply considered non-limiting.

Plate 3: Rice planting in JKUAT farm

3.6.6 Thousand (1000) grain weight

Eight replicates each of 100 rice grains was randomly counted and each sample was weighed in grams based on the IISTA (2007) procedures. To get 1000 grain weight. The 100 seed weight of the samples was obtained and multiplied by 10.

$$100 \text{ seed weight} = \frac{1000}{\text{seeds per kg}} \times 100$$

3.6.7 Biomass

Above groung biomass was harvested and weighed after sun dryng, and this was recorded as Biomass.

3.6.8 Panicle length

A vernier caliper was used to measure the length of panicles after the harvesting and threshing.

3.7 Data analysis

Analysis of variance was carried out on variables including seedling vigor plant height, tiller number, flag leaf length. The data was subjected to Analysis of Variance (ANOVA) using GenStat statistical software version 13.The significance of mean value and LSD at 5% significance level of the characters collected was done.

3.8 Establishing relatedness among rice varieties using DNA markers

3.8.1 Genetic variability of rice based on simple sequence repeats (SSRs) markers

3.8.1.1 Plant materials

Eighteen rice genotypes including landraces and improved lines, were used in this studybetween August and December (Table 3.1). A sample of every genotype was derived from seeds collected individually from 2 weeks oId plants. Seeds were grown and maintained under conditions in the greenhouse of KEBS. Leaves for DNA extraction were randomly chosen and stored in zip locks and frozen at -20 awaiting DNA analysis. DNA was extracted according to the method of Djè *et al*. (2000).Leaves were harvested, and then stored in zip locks until DNA isolation.

Table 3.5 Accessions used in the study

	Accession	Source
1	Basmati 370	KARI
2	Basmati 217	KARI
3	NERICA 1	KARI(Mwea and Kibos)
4	NERICA 4	KALRO (Mwea and Kibos)
5	Red Afaa	KALRO (Mwea and Kibos)
6	Kilombero	KALRO (Mwea and Kibos)
7	Dourado Precose	KALRO(Mwea and Kibos)
8	Uzungu	Africa seed company Africa seed company
9	Mzungu	Farmer stocks, Mwea
10	Kahogo	Traders stock, Mwea
11	Japan 54	Traders
12	Japan 64	Traders
13	Saro 5(TXD)	KALRO
14	Supasaro	KALRO
15	IRO5N2	KALRO
16	IR2793	KALRO
17	BAS 196	KALRO
18	ITA310	KALRO

Juvenile tissues have ability to release more DNA since they have higher cell number per weight and fewer quantities of polysaccharides and polyphenols hence lowered chances of contaminants when extracting DNA.Cetyl Trimethyl Ammonium Bromide (CTAB) procedure by Mace *et al*., (2003) was used to extract DNA. Leaves of about 25mg were cut from young plants and placed in 50 ml falcon tubes. Into each tube, 450 µl of preheated extraction buffer containing 100 mM TrisñHCl (pH 8), 1.4 M NaCl, 20 mM EDTA, CTAB (2.5% w/v), mercaptoethanol (3% v/v) was added. Upon covering the falcons with strip caps, samples were placed in a GenoGrinder for 20 minutes at a speed of 1500 resolutions per minute. Incubation was done at 65 °C for 1 hour with frequent mixing to disperse lumps. To the samples, 450 µl of chloroform - isoamyl alcohol (24:1 v/v) was put and centrifuged at 4000 rpm for 15 minutes at 24 °C. A clear aqueous phase

was obtained and poured into new falcon tubes. Again, 450 µl isopropanol was added and incubated at -20 °C for 2 hours. The mixture was then centrifuged for 20 minutes at 4 °C and 3500 rpm to precipitate the DNA. The supernatant was decanted and pellets left to dry for 30 minutes. To each sample, 200 µl low salt TE (10 mM tris, 0.1 mM EDTA (pH 8)) and 3 µl RNase A (10 mg/ml) was added before incubating for 30 minutes at 37 °C. Two hundred µl chloroform - isoamyl - alcohol was added to each sample and centrifuged at 24 °C for 15 minutes. To each sample, 315 µl ethanol - sodium - acetate solution was added, then placed at -20 °C overnight. The samples were centrifuged at 4 °C for 15 minutes before decanting the supernatant from each sample. The pellets were then washed with 100 µl of 70% ethanol. The samples were centrifuged for 5 minutes before draining the ethanol. The pellets were air dried then resuspended in 100 µl of low salt TE buffer and stored at 4 °C (Mace *et al*., 2003).

A composition was made of 3 µl genomic DNA with 5 µl loading dye per sample. The quality of genomic DNA was assessed by loading the mixture into agarose (0.8%) gel in 0.5 x TBE buffer containing 40 mM Tris - acetate and 1 mM EDTA (pH 8.0). Electrophoresis was done at 100 volts for 2 hours and the gels photographed. Quality of the DNA was assessed using gel documentation transilluminator (UV Tech). The DNA was quantified at 260 and 280 nm using a spectrophotometer nanodrop 2000 thermoscientific. The DNA samples were diluted to final concentration of 10 ng/Ïl and stored at -20oC for Polymerase Chain Reaction (PCR) DNA amplification.

3.8.2 Polymerase chain reaction amplifications

A total of 5 primers were used in the SSR analysis. Primers were obtained and DNA amplification performed in a Thermal Cycler were performed in 60 µl reaction mixture composed of of 1 x PCR buffer, 2mM MgCl2, 0.16 mM dNTPs, 0.16pmol fluorescent dye, 0.04pmol forward and reverse primers and units Taq polymerase. Primers of known sequence were used in amplification of the 18 samples (Billot *et al*., 2012). 10 µl of the reaction mixture was put in three well plate which were loaded in the programmable PCR thermal cycler machine (GeneAmpÆ PCR system 9700, Applied BioSystems) for amplification. The amplification was carried out using the following profile as developed by Folkertsma *et al*., (2005): 1 cycle of initial denaturation at 94 °C for 15 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50 °C for 1 minute and elongation at 72 °C for 2 minutes. This was followed by 1 cycle of final elongation at 72 °C for 20 minutes and holding time ∞ at 4 °C. The PCR product was loaded on 2% agarose gel in TAE buffer containing 40 mM Tris-acetate, 1 mM EDTA at a pH of 8.0. The gel was stained with gel red (0.5 µl/ml) and DNA fragments visualized by illumination device with UV light. A 500bp ladder was used. Amplification was scored by noting presence of one or two sharp bands within the size range of upto 500 bp

3.9 Data analysis

Genetic distance was obtained by first scoring SSR bands in each accession for presence (1) or absence (0) (Pejic *et al*., 1998). In order to describe population structure and variability among populations, the nonparametric Analysis of Molecular Variance (AMOVA) was done to assess variation and structure of the population. A phylogenetic tree /dendrogram was constructed to explain the relationsbetween accessions and Pair

wise similarity values calculated with a similarity coefficient. The similarity matrix was converted into dendrogram using UPGMA with a FORTRAN/Darwin program RAPDPLOT (Black, 1993).

The bands were scored for 0 of 1 for their presence or absence in each genotype, (Pejic *et al*., 1998; (Fernandez, *et al.,* 2002). Nei's genetic diversity (Nei, 1973) was computed from the binary data for all pair wise combinations of rice genotypes. Polymorphism information content (PIC) for each SSR primer set was determined as described in (Agrama and Tuinstra, 2003; Senior *et al*. 1998).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1Determining the quality of selected rice genotypes in Kenya

4.1.1 Effect of genotype on Coleorhiza and coleoptile formation

The genotype had a significant effect ($p \leq 0.05$) on number of days to both coleorhiza and coleoptile formation among rice genotype ($p \leq 0.05$) (Table 4.1). Similarly, the ageing time and interaction between rice genotypes and ageing time had a significant effect ($p \leq 0.05$) on the coleoptile and coleorhiza formation (Fig 4.1).

Table 4.1 Mean effect of Genotype on Germination

Geno type	Coleorhiza formation	Coleoptile formation	Germination % at 7 days	Germination % at 14 days	Germination % at 21 days
B217	8.75d	9.81c	33.69a	52.44a	56.62a
Uzungu	10.19bc	11.25b	21.88c	27.94e	34.44ef
Supasaro	10.13bc	11.19b	24.69bc	31.69de	37.31de
B370	9.94bc	11.00b	28.94ab	41.31b	48.94b
Pishori	10.44b	11.50b	26.44bc	34.81cd	43.94bc
J54	8.88d	10.19c	29.12ab	37.62bc	41.94cd
Mzungu	11.19a	12.44a	22.00c	27.62e	31.31f
J64	9.75c	10.94b	20.56c	31.5de	35.81ef
LSD	**0.399**	**0.425**	**3.742**	**3.653**	**3.405**
p value	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**

**Means with the same letters within a column are not significantly different (LSD α =0.05)*

The ageing time had a significant effect on coleorhiza and coleoptile formation and germination percentage, with 72hours having the highest number of days to coleorhiza and coleoptile formation, while 0 hours of ageing showing the earliest formation of these parts. At 0hrs, or standard germination, the germination percentage was highest (Table 4.2).

Table 4.2 Mean effect of ageing time on germination

Ageing Time	Coleorhiza formation	Coleoptile formation	Germination % at 7 days	Germination % at 14 days	Germination % at 21 days
0hrs	3.34d	4.38d	73.28a	75.72a	80.66a
24hrs	6.59c	7.91c	30.38b	37.5b	44.06b
48hrs	13.31b	14.38b	0c	24c	30c
72hrs	16.38a	17.5a	0c	5.25	10.44d
LSD	**0.282**	**0.301**	**2.646**	**2.583**	**2.408**
p value	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**

*Means with the same letters in the same column are not significantly different*LSD α=0.05

According to findings in this experiment, there was a negative relationship between seed vigour and ageing time when rice varieties and lines were subjected to 45 degrees temperature. Seed vigor reduced with an increase in ageing time. The control experiment, at 0 hours/normal germination generally gave the earliest number of days to coleoptile formation, and highest germination percent at 7,14 and 21 days. In fact after 48 and 72 hours of treatment, the mean germination at day 7 was zero, meaning there was no coleoptile or coleoptile that had formed at this time.

In general, genotype B217 depicted the earliest mean number of days to coleorhiza formation, coleoptile formation and germination percentage across all the treatments. In this set up, the genotype significantly ($p \leq 0.05$) affected the days to coleorhiza formation such that there were differences across the genotypes. The genotype Basmati 217 showed earliest mean days to coleorhiza formation while Mzungu showed latest coleorhiza formation (Figure 4.1).

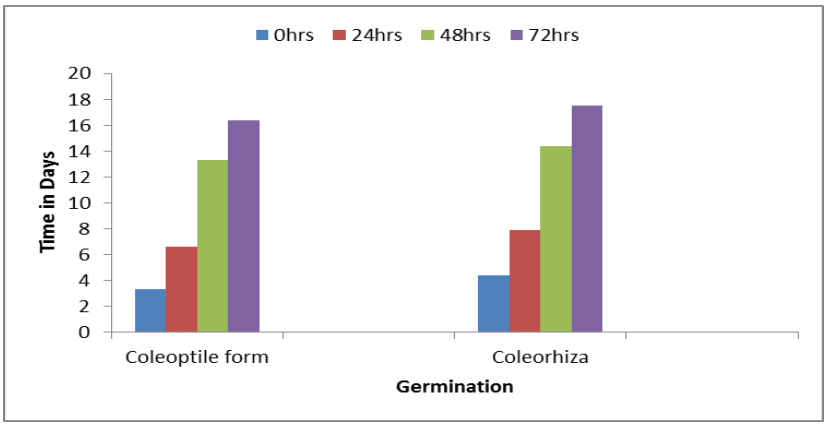

Figure 4.1: Effect of ageing time on days to coleoptile and coleorhiza formation

The genotype significantly affected the germination percentage after 7 days such that there were differences across the varieties ($p \leq 0.05$). The highest mean germination percentage was 33.69 while the lowest was 20.56.The genotype Basmati 370 had the highest mean percentage mean of seeds that had germinated, by day 7 while Line Mzungu had the lowest ($p \leq 0.05$). The genotype significantly affected the germination percentage after 14 days such that there were differences across the varieties ($p \leq 0.05$). The highest mean germination percentage was 52,44 while the lowest was 27.62.The genotype Basmati 270 had the highest mean percentage of seeds that had germinated by day 14,while Mzungu had the lowest (Fig 4.1).

Ageing time had a significant effect on the percentage germination after 21 days ($p \leq 0.05$) as shown in table 4.2 above, with the highest percentage being 80.6% .and the lowest being 10.44%. Zero ageing time had the highest mean percentage while 72 hours had the lowest percentage of seeds that had germinated by 21 days (Figure 4.2). In general the number of seeds that germinated increased with the increase in age, and

observations made on day 21 gave the highest percentages. Mzungu took longest meannumber of days to coleorhiza formation (11.19), which was significantly higher than the rest of the genotypes. Genotype B217 had the earliest means time taken to form coleorhizae (Fig 4.4).

The ageing time had a significant effect on the coleoptile formation at 7, 14 and 21 of seed treatment ($p \leq 0.05$) (Figure 4.2). Seeds treated for 0hrs had the earliest, while 72 hours had the latest coleoptile formation. This means the vigour was successfully reducing with reducing number of hours in treatment. Genotype B217 was significantly leading in the earlest time to coleoptile formation while Mzungu had the latest. Pishori, Uzungu, Supasaro, B370 and J54 being not significantly different among each other (Fig 4.5). In the 7^{th} day of the trial there were seeds that had been treated for 48 and 72 hours that had not germinated. The highest germination was in seeds not treated/ normal germination in 0hrs followed by those treated for 24 hours.

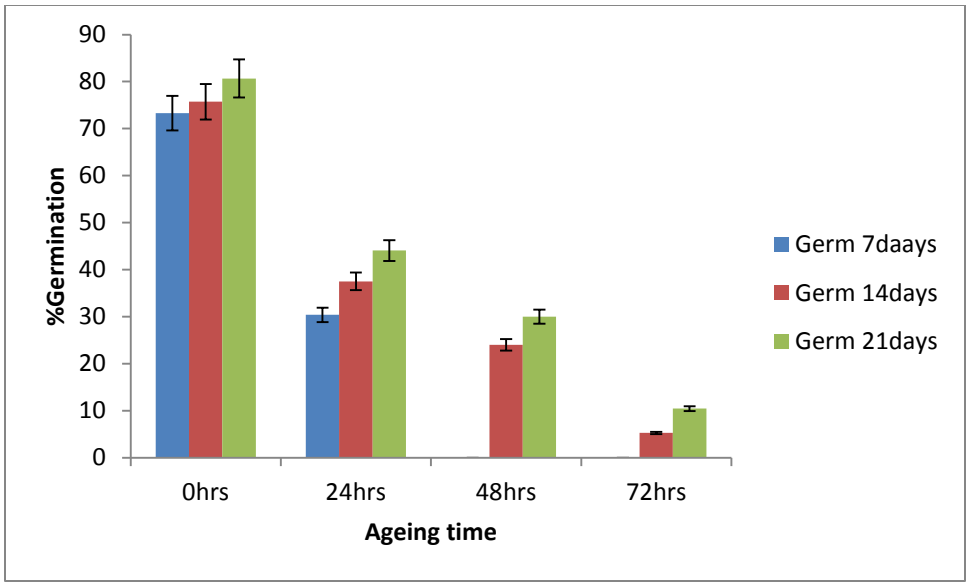

Figure 4.2: Effect of ageing time on percentage germination at 7,14 and 21 days

B217 was leading in germination percentage across the treatments while the lowest germination percent was noted in J64, Uzungu and Mzung (Figure 4.3

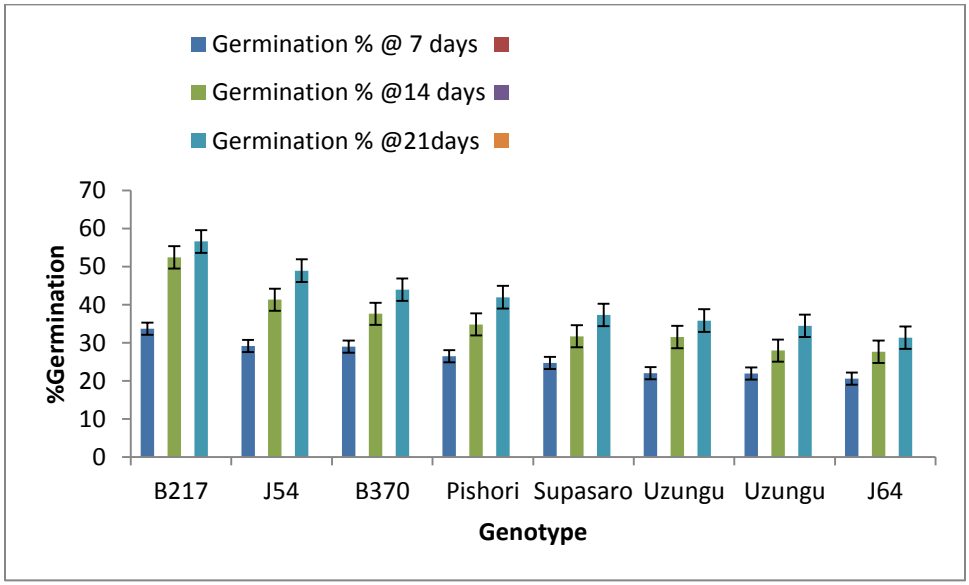

Figure 4.3: Effect of genotype on germination percentage at 7, 14 and 21 days

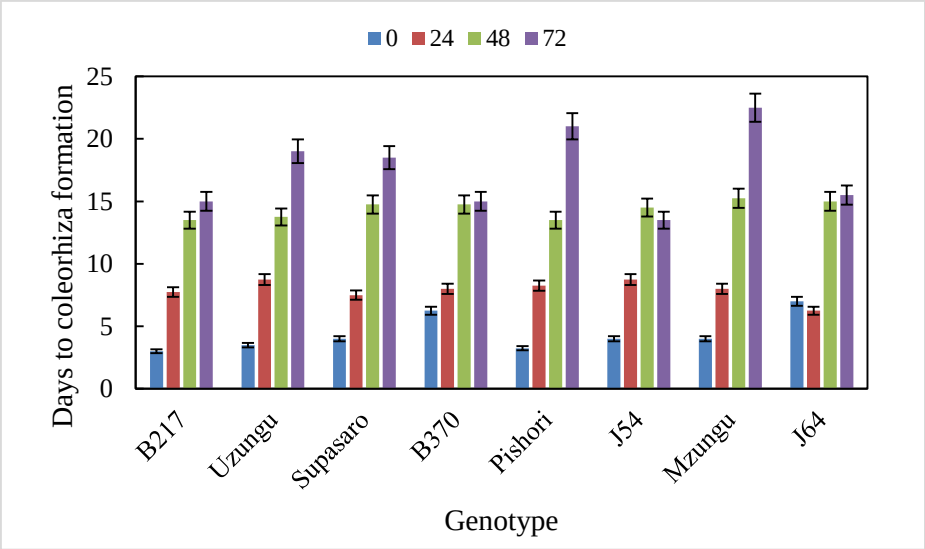

Figure 4.4: Effect of interaction between genotype and storage time on coleorhiza formation

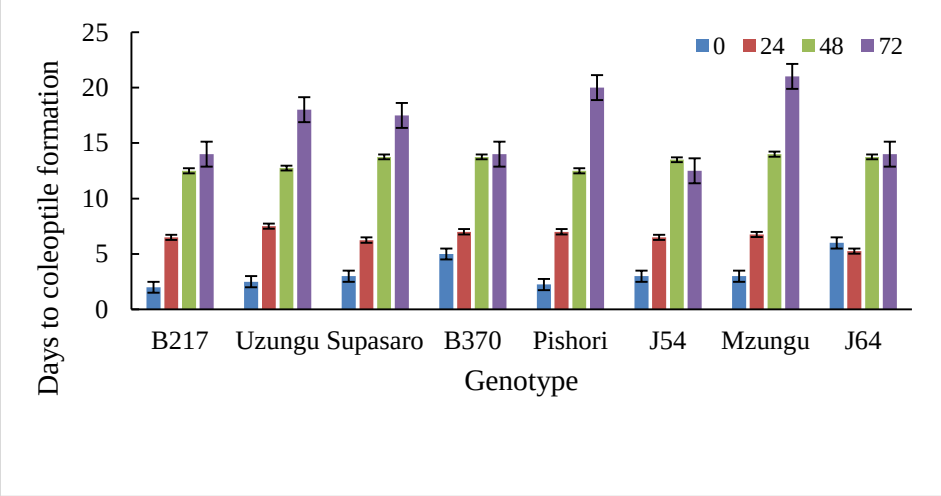

Figure 4.5: Effect of interaction between genotype and ageing time on the number of days to coleoptile formation

Results show only two bars, indicating that when percentage germination was measured at 7 days, there was no germination in treatment 3 and 4, 48 and 72 hours respectively in most of the varieties(Fig 4.6).

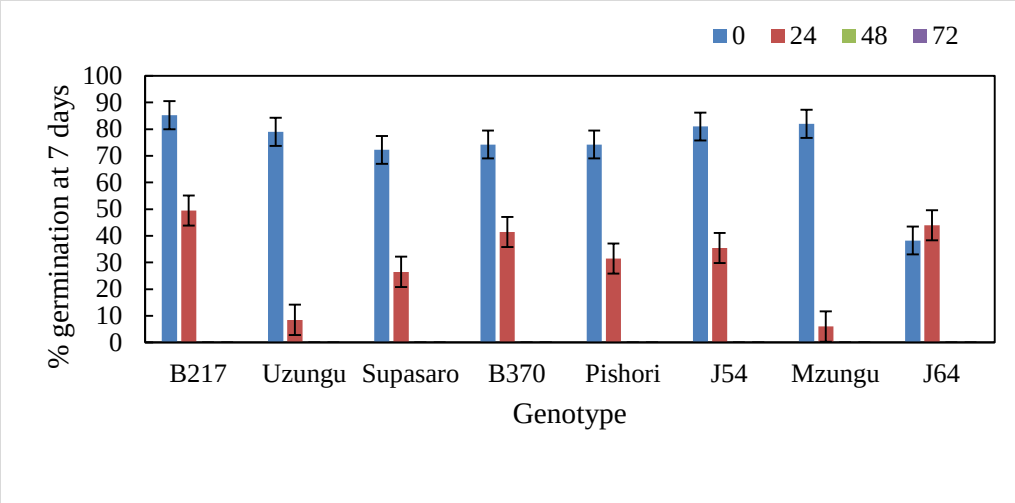

Figure 4.6: Effect of genotype on germination at 7 days

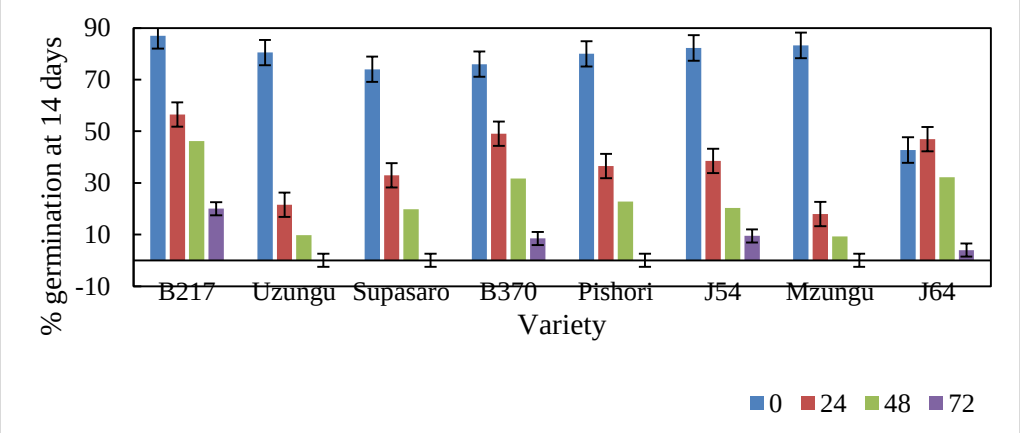

Figure 4.7: Effect of genotype on percent germination at 14 days

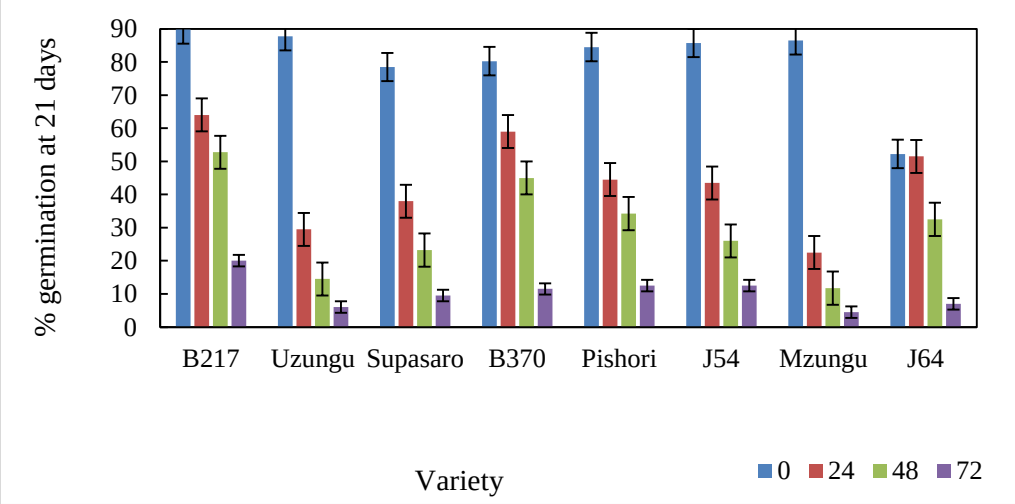

Figure 4.8: Effect of genotype on percentage germination at 21 days

Table 4.3: Effect of **genotype on seed quality parameters**

	% germination 7 days	Germination index	Length	Seedling vigor index	% broken seeds	% discolored seeds	Emergence (days)	Inert material	Normal seed
B217	85.3a	12.2a	2.68c	228.1bc	7ab	3.0b	2.3d	0.23bc	99.67bc
Uzungu	79.0a	11.3a	2.48d	195.6c	5.8b	1.8b	3.8bc	0.18bc	99.75b
Supasaro	72.3a	10.3a	1.70f	96.9d	2.8b	3b	5.5a	0.19bc	99.75b
B370	74.3a	10.6a	3.93b	291.4ab	0.0b	0b	2d	0.02c	99.98a
Pishori	74.3a	9.9a	2.13e	157.4cd	25a	10b	2.5d	0.33b	99.33d
J54	81.0a	11.6a	2.43d	196.3c	0.5b	0.8b	3cd	0.04c	99.95a
Mzungu	82.0a	11.7a	4.25a	348.7a	25a	25a	2d	1.38a	98.15e
J64	38.3b	5.5b	2.23e	84.8d	7ab	0.5b	4.3b	0.3b	99.62c
LSD	**13.77**	**2.19**	**0.1**	**48.53**	**0.11**	**0.06**	**0.61**	**0.13**	**0.07**
p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Values followed by the same letters within the column are not significantly different(LSD α =0

There was a significant effect of the genotype on percentage germination at 7 days (p (p≤0.05 for germination index, seedling length, seedling vigor index, broken seeds, discoloured seeds emergence, inert material, and normal seed (Fig 4.8, Table 4.3).

Correlation analyses

There was a strong positive correlation between number of days to coleorhiza formation, coleoptile formation and the hours of treatment (r=0.936, r=0.935) (Figure 4.9). While there was a negative correlation between germination percentages at 7, 14 and 21 days and the hours of treatment (r=-0.873, r=-0.882 and r=-0.888) respectively (Figur 4.11). There was a strong positive correlation between coleorhiza and coleoptile formation across the treatments (r=0.998).

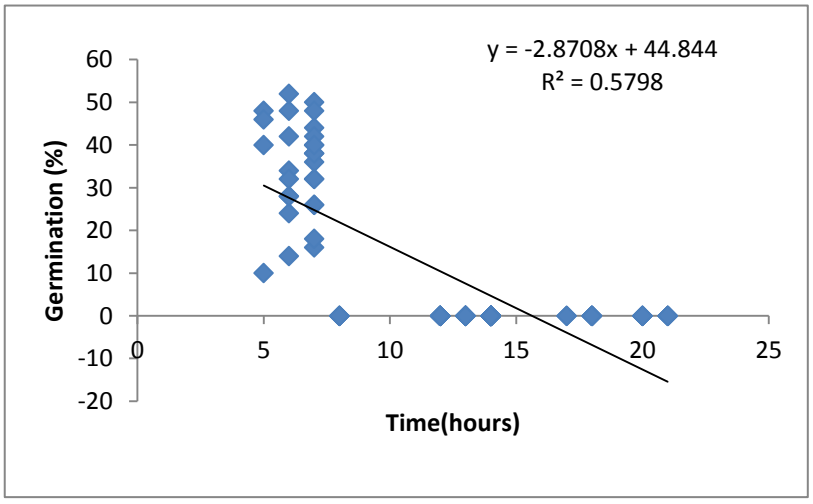

Figure 4.9: Relationship between storage time and germination

The results indicated that there was a positive correlation between genotype and the number of days to coleorhiza formation. This indicates that there were differences or changes in the time of coleorhiza formation with the changes in varieties (Fig 4.10).

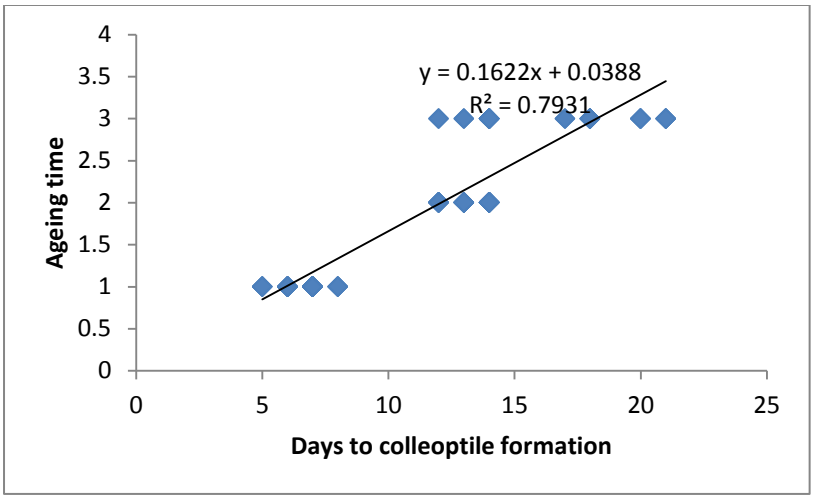

Figure 4.10: Relationship between ageing time and coleoptile formation

The results also indicate a positive correlation between the number of days to coleorhiza formation and the number of days to coleoptile formation.

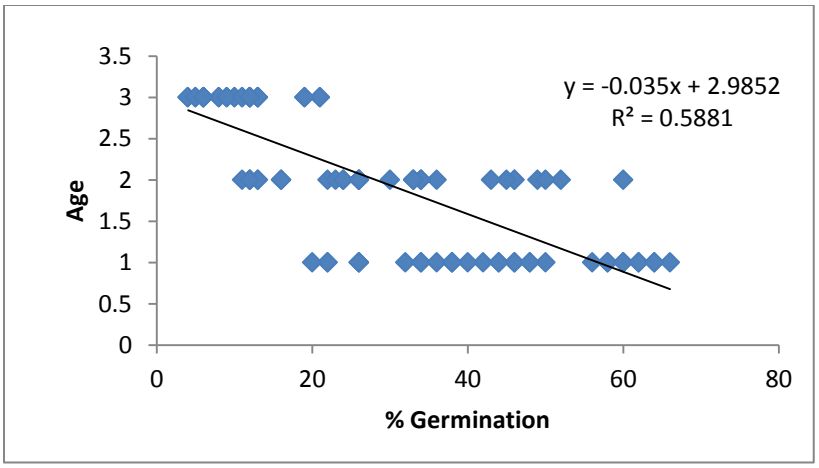

Figure 4.11: Relationship between ageing time and germination

Also noted was a negative correlation between the percentage germination at 7 days and genotype, coleorhiza formation and coleoptile formation (r=0.864, r=0.870). The germination percentage at 14 days also had a negative correlation with genotype, coleorhiza and coleoptile formation. Similarly at 21 days of age the germination percentage had negative correlation with genotype, coleorhiza and coleoptile formation.

The percentage germination after 7 days had a positive correlation with both germination percentages at 14 and 21 days. Germination percentage at 14 days also had appositive correlation to germination at 7 and 21 days of age (Fig.4.11). The germination percentages at 21 days also indicated a positive correlation with germination percentages at both 7 and 14 days of age. Generally, there was an increase in germination percentage over time (Table 4.4).

Table 4.4: Correlation between ageing time and germination

	Hours of treatment	**Coleopti le form**	**Coleorhi za form**	**Germinati on 7 days**	**Germinati on 14 days**	**Germinati on 21 days**
Hours of treatment	1					
Colleptille form	.936**	1				
Colleoptira form	.935**	.998**	1			
Germinati on 7 days	-.873**	-.859**	-.861**	1		
Germinati on 14 days	-.882**	-.864**	-.870**	.939**	1	
Germinati on 21 days	-.888**	-.857**	-.863**	.930**	.991**	1

Discussion

The observations from this study reveal that seed viability under accelerated ageing conditions declines with the ageing time. According to Sun *et al*. (2007) seed vigor is a key aspect of seed quality; it predicts possible seed germination, seed longevity, seedling growth and ability to withstand adverse conditions. Foolad *et al*. (2007) describes seeds with strong vigor can highly raise the speed and uniformity of seed germination and the

final percentage of germination, and lead to perfect field emergence, good crop performance, and even high yield under different conditions.

Seed vigor weighs the level of damage piling when viability reduces which is a factor of moisture and temperature. When this damage is in excess, the seed may die. The present study has shown that prolonged duration of higher temperatures results in reduction of seed viability higher temperatures other than promoting stress may cause protein denaturation and seed death. These results agree with those from study which was carried out on treated maize that was aged for 45 degrees and treated. The maize had significant decrease in percentage germination/viability when stressed for 72 hours.

The present study has also confirmed that the genotype has significant effect on seed vigor. This agrees with the findings of Sun *et al*. (2007) who found that aspects such as temperature and genotype influence viability. The presentation of any phenotype or observed character in a crop is a factor of both the genotype and the environment. Generally, certain genotypes are able to withstand harsh environmental conditions better than others as revealed in the study. Also as the seed ages, it may acquire moisture from the environment which may encourage high pathogenic activity. This moisture may also increase incidences of microorganisms such as bacteria and fungi that enhances rotting and final death of seed A study carried out in Corn to assess the effect of ageing at high temperatures confirmed that environment with hight relative humidity and temperature gave reduced germination rates (Sun *et al*., 2007).

Further observations from the present study revealed that storage duration or the time taken to store seeds has a negative correlation to the viability of rice seeds. The seeds that were aged for 24, 48 and 72 hours had a decrease in viability. However, it was worth noting that seeds stored for a longer time may be given more time to improve on the percentage germination since their speed of germination is generally low. It was therefore noted that although at 7 days after sowing a number had not germinated, when observations were made after 14 and 21 days, the performance was improved. Studies on germination and vigor could therefore be extended further than 7 days to check performance. The accelerated aging (AA) test provides valuable information on storage and seedling field emergence potentials. The seeds are hydrated to a specific level when exposed to relatively high temperature (40 to 45^{o}C) and humidity (around 100 % Relative Humidity - RH) Following this aging treatment, seeds are subjected to a germination test and higher vigor seed lots tolerate this aging condition better than lower vigor seed lots and produce a higher percentage of normal seedlings

When seeds have strong vigour, they will have excellent emergence and uniform germination giving increased yield even in diverse environments. Further advantages of vigour testing are that high vigour enables uniform nurseries and fields. High temperature and relative humidity within storage of seeds promotes deterioration by promoting degenerative changes like destabilization in enzyme function, destructuring and final loss of integrity of the cell membranes system, due to lipid peroxidation because of increased reactive oxygen species (Alscher *et al*., 2002). For most crops, seeds produced in warmer temperatures are generally less dormant at maturity than those developed at cooler temperatures, as described for *Beta vulgaris*, *Lactuca sativa*, *Amaranthus retroflexus*,

wild oat (*Avena fatua*) Fenner, 1991), wheat (Biddulph *et al.*, 2007), lettuce (Contreras *et al.*, 2009), weedy rice (Gu *et al.*, 2006), and *Arabidopsis* (Donohue *et al.*, 2008; Kendall *et al.*, 2011; Kendall and Penfield, 2012; Huang *et al.*, 2012). When seeds are subjected to high temperatures within a short period of time and high relative humidity, they undergo an ageing process (Bewley and Black, 2012). AA tests have been used to test the ability of soybeans to emerge, and the physiological ability (Torres *et al*., 2004). Accelerated ageing tests help in projecting the growth in the field, but also survival and behavior in storage. This assists gratly in checking viability (Bewley and Black 2012).

Seed purity is a key factor that is influenced mainly by seed handling procedures such as harvesting, transportation, dryng, storage ans processing. A pure and clean seed lot results in a healthy field lot. For example, Thompson *et al.*, (1990) suggested that when the broken grain is found to be above 75% of the whole kernel percentage, then the whole of that rice sample is considered broken.

4.2 Effect of storage time and genotype on growth of rice

4.2.1 Effect of storage time and genotype on germination percentage in Mwea

The storage time had a significant effect on the percentage germination ($P \leq 0.05$). Seeds planted after 5 months of storage gave the highest mean germination percentage (80.6) while seeds planted after 6 months of storage gave the lowest germination percentage (71.67) (Table 4.4). There was a significant effect of the genotype on the percent germination ($P \leq 0.05$). Genotype pishori gave the highest mean germination percentage (79.7%) while genotype B370 gave the lowest germination percentage (70%) across the storage period (Table 4.5).

There was a significant interraction between months and genotype on percentage germination ($P \leq 0.05$). Genotype pishori stored for 5 months had the highest percentage germination (93.3) while genotype B370 stored for 2 months had the lowest germination percentage (40.67). For the 2 and 5 months storage period, genotype pishori had the highest germination (Table 4.6). However in 3 and 4 months of storage, the germination for pishori was not leading for instance at 1 month, both pishori and J64 had highest germination. This showed that there was interaction between storage time and genotype (Figure 4.12).

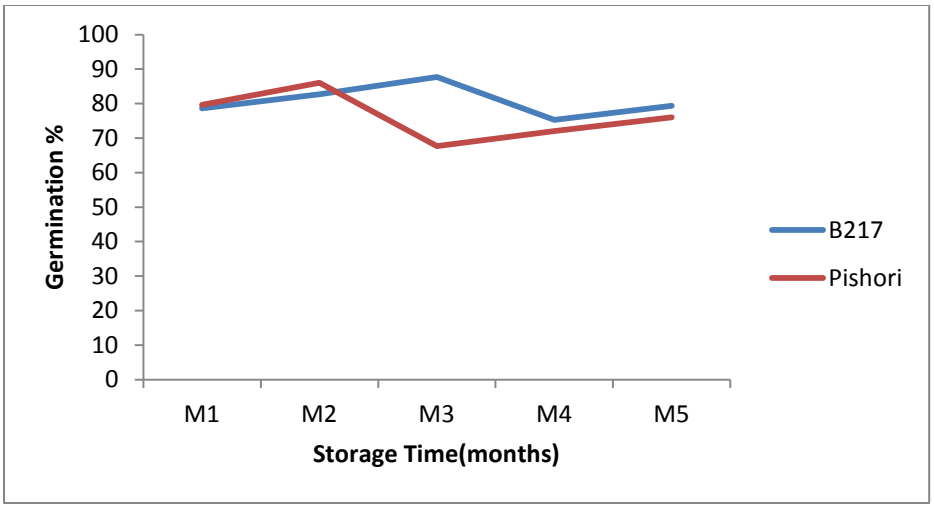

Figure 4.12: Mean effect of interaction between storage time and genotype on germination at Mwea

4.2.2 Effect of storage time and genotype on seedling vigor index at Mwea

The storage time had a significant effect on the seedling vigor index (P ≤0.05). Seeds planted after 4 months of storage gave the highest mean seedling vigor index (673.6) while seeds planted after 2 months of storage gave the lowest seedling vigor index (514.7). The genotype had a significant effect on the seedling vigor index (P≤0.05). Genotype pishori gave the highest mean seedling vigor index (645) while genotype J64 gave the lowest seedling vigor index (558).

There was a significant interaction between storage time and genotype on seedling vigor index (P ≤0.05). Genetype B370 stored for 4 months (M3V4) gave the highest seedling vigor index (789) while pishori stored for 4 months M5V4 gave the lowest seedling vigor index (268). Genotype B370 had the highest SVI when stored for 2 and 3 months but did not lead in the 1st, 2nd and 5th month. This showed that there was interaction between the genotypes and storage time (Figure 4.13)

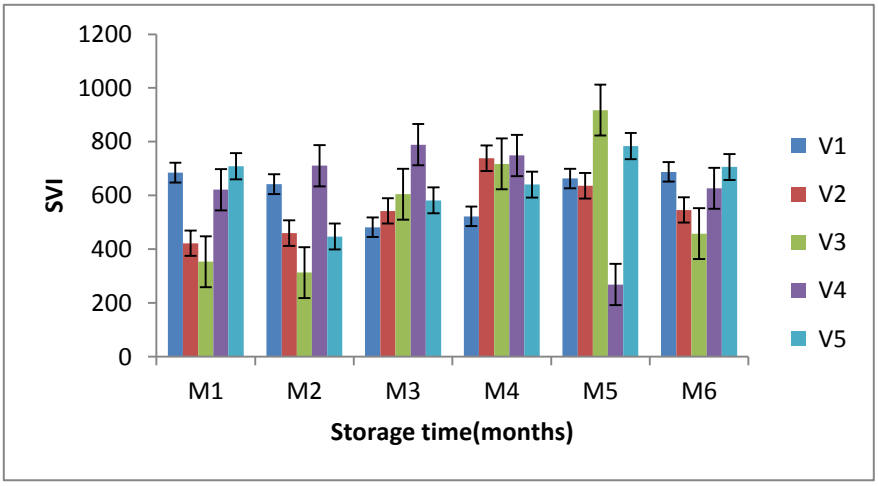

Figure 4.13: Effect of genotype and storage time on seedling vigor index at Mwea

4.2.3 Effect of storage time and genotype on height at transplanting at Mwea

The storage time had a significant effect on the height of plants measured at transplanting stage (P≤0.05). The tallest plants were obtained from seeds planted after 6 months of storage (24.4) while the shortest heights were obtained after 4 months of storage (16.8). The genotype had a significant effect on the height of plants measured at transplanting stage (P≤0.05).The tallest means were obtained from seeds of pishori of storage (24.4cm) while the shortest heights were obtained from seeds of J64 (19.6).

There was a significant interaction between genotype and storage time on height at transplant (P≤0.05). The interaction between genotype and storage time had a significant effect on the time of transplanting. The tallest plants were obtained from B370 stored for 6 months M6V3 (26.5) while pishori stored for 3 months was the shortest M3V5 (14.2). While genotype B370 obtained the tallest height at 6 months of storage, it did not lead in the 1st, 2nd 3$^{rd, 4th}$ and 5th months of storage.

The storage time had a significant effect on the height measured at 1 month after trans-planting (P≤0.05). The tallest plants were obtained from M4 (73.3) while the shortest were obtained from M2 (58.4). The genotype had a significant effect on the height measured at 1 month after transplanting (P ≤0.05). The tallest plants were obtained from genotype B217 (73.3cm) while the shortest plants were obtained from B370 (63cm).

There was a significant interaction between storage time and genotype on the height measured at 1 month after transplanting (P≤0.05). The tallest plants were obtained from B217 stored for 3 months treatment combination M4V3 (87.7cm) while the shortest plants were obtained from J64 stored for 1 months (48.7cm). Although genotype B217 obtained the tallest plants at 3 months of storage, it has lower heights in the other months, thus confirming the interaction.

The storage time had a significant effect on the height measured at 2 month after transplanting (P≤0.05). The tallest plants height was obtained from M5 (66.3) while the shortest plants were obtained from M3 (55.2).

The genotype had no significant effect on the height measured at 2 months after transplanting (P≥0.05). There was a significant interaction between storage time and genotype on the height measured at 2 month after transplanting (P≤0.05). The tallest plants height was obtained from B217 stored for 3 months, M4V3 (87.7cm) while the shortest plantsheight was obtained from J64 stored for 1 month (48.7cm)(Table 4.7).

The storage time had a significant effect on the height measured at maturity (P ≤0.05). The tallest plants height was obtained from M4 (72.3) while the shortest plantsheight was obtained from M6 (65.5).

The genotype had a significant effect on the height measured at maturity (P ≤0.05).The tallest plants height was obtained from genotype V4 B217, (73.3cm) while the shortest plants height was obtained from B370, (65.6cm).

There was a significant interaction between storage time and genotype on the height measured at maturity (P ≤0.05) (Table 4.7). The tallest plants height was obtained from treatment combination B217 stored for 3 months, M4V3 (91.9cm) while the shortest plants height was obtained from J64 stored for 1 month M3V1 (58.2).

4.2.4 Effect of storage time and genotype on panicle length at Mwea

The storage time had no significant effect on the panicle length (P≥0.05) . Genotype had no significanton panicle length (P≥0.05). There was no significant interaction between genotype and storage time on the panicle height (P≥0.05).

4.2.5 Effect of storage time and genotype on number of days to flowering at Mwea

The storage time had a significant effect on the number of days to flowering (P ≤0.05). Seeds planted after 3 months of storage flowered early (58.99) while those planted after 6 months of storage flowered latest (94.76) as shown in table 4.5. However the effect of genotype on number of days to flowering was not significant (P≥0.05)(Table 4.6). There was a significant interraction between storage time and genotype on the number of days taken to flowering of the plants (P≤0.05). J64 stored for 3 months M3V1 exhibited the earliest number of days to flowering (57) while M4V3 gave the latest number of days to flowering (97.87).Figure 4.14

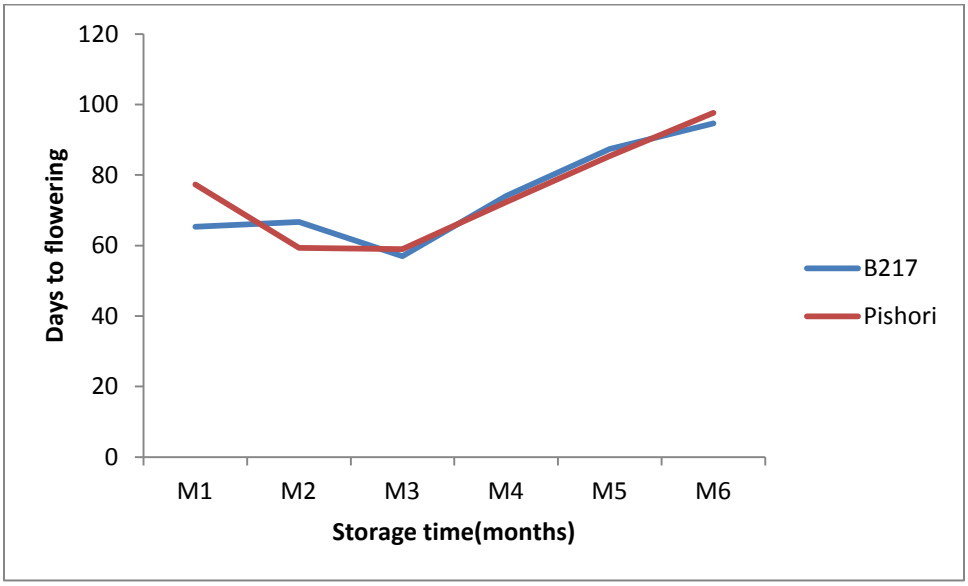

Figure 4.14: Interraction of genotype and storage interaction on flowering at Mwea

Table 4.5: Effects of Storage time on growth parameters of Rice at Mwea

Storage time	Germ.	Height 1(cm)	Height 2(cm)	Height 3(cm)	Length (cm)	Seed VI	DTF	No. of Tillers	Leaf length (cm)	Leaf Width
M1	73bc	16.75e	63.05bc	64.06bc	7.52d	558.4d	72.0c	7.9c	29.95	0.95
M2	73.93bc	19.09d	58.36c	63.23ab	7.04d	514.7e	64.5d	8.7bc	31.56	0.95
M3	73.47bc	17.21e	56.87c	57.15c	8.14bc	600.0c	58.9e	11.4a	28.42	0.93
M4	75.93ab	20.15c	71.19a	72.3a	8.59b	673.6a	73.0c	9.7abc	25.23	0.94
M5	71.67c	21.35b	68.78ab	66.3a	9.2a	653.9b	81.9b	8.9bc	30.06	0.95
M6	78.4a	24.38a	64.1bc	66.5bc	8.1c	605c	94.7a	10.2ab	30.7	0.95
P value	**0.001**	**0.001**	**0.01**	**0.001**	**0.001**	**0.01**	**0.001**	**0.004**	**0.26**	**0.90**
LSD	**3.6**	**0.99**	**7.54**	**5.2**	**0.52**	**16.59**	**2.7**	**1.8**	**5.52**	**0.06**

Values followed by the same letters within the column are not significantly different (LSD α =0.05)M-Month

Table 4.6: Effect of genotype on growth parameters at Mwea

Genotype	Growth parameters					
	Germnation(%)	1000GW	GN	BM2	Days to Flowering	FullGW
J54	73.7	33.6	1133	87	72.4	72.4
J64	76.3	31.7	993	77	75.2	75.2
B370	70	31.6	906	74.9	74.2	74.2
B217	72.4	33.8	989	75.5	73.8	73.8
Pishori	79.7	31.9	1013	79.1	75.2	75.2
P value	≤.001	0.865	0.226	0.564	8.63	0.968
LSD	9.23	5.45	192.5	16.06	0.97	0.863

Values followed by the same letters within the column are not significantly different (LSD α =0.05. V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

4.2.6 Effect of storage time and genotype on number of spikes at Mwea

The time of storage had no significant effect on the number of spikes produced by the rice genotypes (P≥0.05). The type of genotype had no significant effect (P≥0.05) on the number of spikes that were produced by the plants at maturity (Table 4.5). The interaction between storage time and genotype had a significant effect on the number of spikes produced by the rice plants at maturity (P≤0.05).Table 4.6

4.2.7 Effect of storage time and genotype leaf length at Mwea

There was no significant effect of the genotype, storage time or genotype x storage time interaction on the length of leaves at maturity(P≥0.05).

4.2.8 Effect of storage time and genotype leaf width at Mwea

There was no significant effect of the genotype on the width of leaves at maturity (P≥0.05).

Table 4.7: Effects of interaction of storage time and genotype on growth at Mwea

Treatment	Germination (%)	SeedlingVigor1ndex	Harvest Index	Height (cm)	Days to flowering	Length (cm)
M1 V1	79.67cdef	685.1fg	17.2ghi	68.5efgh	73.33fghi	8.6cdef
M1 V2	66ij	422.4o	16.0hij	66.8fghi	76.33efg	6.4jkl
M1 V3	61j	353.8p	15.8ij	64.1fghi	65.33klmn	5.8lm
M1 V4	78.67cdef	621.5ij	16.9hi	73.3cdef	67.67ijkl	7.9defgh
M1 V5	79.67cdef	709ef	17.8fghi	73.5cdef	77.33ef	8.9abcde
M2 V1	82.33bcde	642.2hi	21.1bcde	68.1efgh	68hijkl	7.8efgh
M2 V2	78defg	460.2n	17.7fghi	82.5abc	66klm	5.9klm
M2 V3	40.67k	313.1q	16.4hij	66.6fghi	66.67jklm	7.7fghi
M2 V4	82.67bcde	710.9ef	18.1fghi	79.2bcd	62.67lmno	8.6cdef
M2 V5	86abc	447.2no	22.1bc	58.5i	59.33no	5.2m
M3 V1	73fghi	481.8n	18.2fgh	58.2i	60.67mno	6.6ijkl
M3 V2	68.67hij	542.5m	16.4hij	63.9ghi	59.0o	7.9defgh
M3 V3	70.33ghi	604.9jk	18.1fghi	76.9cde	57.0o	8.6cdef
M3 V4	87.67ab	789b	19.2efg	81.4bc	58.67o	9.0abcd
M3 V5	67.67hij	581.9kl	14.2j	59.6hi	59.0o	8.6cdef
M4 V1	66.33hij	522.1m	17.4ghi	66.9fghi	70.67ghijk	8.7bcdef
M4 V2	83.67bcde	738.7de	18.1fgh	63.6ghi	75fg	8.1defgh
M4 V3	77.33defg	717.5def	21.0cde	91.8a	74fgh	8.2defg
M4 V4	78.33cdef	748.8cd	22.37bc	68.9efgh	73fghi	9.0abcd
M4 V5	74fgh	640.8hij	21.9bcd	70.2defg	72.33fghij	8.9abcde
M5 V1	67.67hij	663.1gh	22.0bc	69.7efg	70.67ghijk	9.8ab
M5 V2	76.67efg	636.3hij	19.2efg	86.7ab	81.33de	8.3defg
M5 V3	92.67a	917.4a	19.8def	67.0fghi	87.33cd	9.9a
M5 V4	28l	268.8r	22.5bc	67.2fghi	85d	9.6abc
M5 V5	93.33a	784bc	23.3b	68.8efgh	85.33cd	8.4def
M6 V1	73fghi	688fg	23.3b	67.5fghi	91.33bc	8.7bcdef
M6 V2	84.67bcd	546.1lm	23.1bc	61ghi	93.67ab	7.2ghij
M6 V3	78defg	458.2no	26.5a	65.4fghi	94.67ab	7.0hijk
M6 V4	79cdef	626.7hij	25.7a	70.2defg	96ab	8.5cdef
M6 V5	77.33defg	706.1ef	23.3b	63.2ghi	97.67a	8.9abcde
P value	**0. 001**	**0.001**	**0.01**	**0.01**	**0. 001**	**0.001**
LSD	**7.998**	**37.1**	**2.23**	**9.4**	**6.082**	**1.17**

Values followed by different letters within the column are significantly different (P<0.05)
M=Month, Key: V1=J54, V2-J64, V3=B217, V4=B370, V5=Pishori, M=month

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4.2.9 Effect of storage time and genotype on germination percentage at JKUAT

The time of storage had no significant effect ($P \geq 0.05$) on the percentage germination while genotype had a significant effect ($P \leq 0.05$) on the germination (Table 4.10). There was a significant ($P < 0.05$) interaction between months and genotype on the percentage germination. Genotype pishori stored for 5 months M5V5 gave the highest percentage germination (93.33) while genotype B217 stored for 5 months M5V4 gave the lowest germination percentage (28). Although pishori had the highest germination when stored for 5 months, it did not lead at all in 1,2,3,4 and 6 months storage period. Different genotypes led in different months thus depicting an interaction (Fig 4.15).

4.2.10 Effect of storage time and genotype on seedling vigor index at JKUAT

The storage time had a significant effect on the seedling vigor index ($P \leq 0.05$). Seeds planted after 5 months of storage gave the highest mean seedling vigor index (616) while seeds planted after 1 months of storage gave the lowest seedling vigor index (463.9). The genotype had a significant effect on the seedling vigor index ($P \leq 0.05$). Genotype J64 gave the highest mean seedling vigor index (622.16) while genotype B370 gave the lowest seedling vigor index (484.6)

The interaction between months and genotype had a significant effect on the seedling vigor index ($P \leq 0.05$). Genotype J54 stored for 4 months M4V1 gave the highest seedling vigor index (759.5) while genotype B217 stored for 5 months M5V4 gave the lowest

seedling vigor index (249.2). J54 had the highest seedling vigor index when stored for 1 and 6 months, but did not lead in the rest of the months.

4.2.11 Effect of storage time and genotype on height at transplanting at JKUAT

The storage time had no significant effect on the height of plants measured at transplanting stage. The genotype had no significant effect ($P \geq 0.05$)) on the height of plants measured at transplanting stage. The interaction between genotype and storage time was not significant ($P > 0.05$) on the height at transplanting.

The storage time had a significant effect ($P \leq 0.05$) on the height measured at 1 month after transplanting. The tallest plants height was obtained from M4 (66.3) while the lowest height was obtained from M2 (56.87).

The genotype had a significant effect ($P \leq 0.05$) on the height measured at 1 month after transplanting. The tallest plants height was obtained from genotype V4 (B217-67.4cm) while the lowest height was obtained from V3 (B370-63cm)(Table 4.9)

There was a significant ($P \leq 0.05$) interaction between storage time and genotype on the height measured at 1 month after transplanting. The tallest plant height was obtained from genotype B217 stored for 4 months M4, V4 (87.7cm) while the shortest was obtained from J54 stored for 2 months M2V1 (48.7cm). The storage time had a significant effect ($P \leq 0.05$) on the height measured at maturity. The tallest plants were obtained from M5 (68.19) while the lowest height was obtained from M3 (55.15).

The genotype had a significant effect on the height measured at maturity ($P < 0.05$). The tallest plants was obtained from genotype B217-62cm) while the lowest height was

obtained from J54 -60.29cm). There was a significant interaction ($P \leq 0.05$) between storage time and genotype on the height measured at maturity. The tallest plant were obtained from genotype J64 stored for 1 months M1V2 (88.4cm) while the shortest was obtained from J54 stored for a three months M3V1 (57.6cm).

4.2.12 Effect of storage time and genotype on panicle length at JKUAT

The effect of storage time on the panicle length was not significant ($P \geq 0.05$) . However; the effect of genotype on panicle length was not significant ($P \geq 0.05$). There was no significant effect ($P \geq 0.05$) of the treatment combinations interaction between genotype and storage time on the panicle height.

4.2.13 Effect of storage time and genotype on number of days to flowering at JKUAT

The storage time has a significant effect ($P \leq 0.05$) on the number of days to flowering (4.8). Seeds planted after 3 months of storage flowered earliest (58.87) while those planted after 6 months of storage flowered latest (94.67). However the effect of genotype on number of days to flowering was not significant (Table 4.10).

There was a significant effect ($P \leq 0.05$) of treatment combination on the number of days taken to flowering of the plants. Genotype B370 stored for 3 months M3V3 exhibited the earliest number of days to flowering (63) while genotype V5 stored for 6 months M6V5 gave the latest number of days to flowering (103.2.)(Table 4.11)

Table 4.8: Mean effect of storage time on growth parameters of rice in JKUAT

Storage time	Germ %	Height (cm) Transplant	Height 2(cm)	Height Maturity	Length (cm)	Seed VI	Days to Flowering
M1	74.8ab	18.83cd	57.91	73.69	6.09e	463.9c	94.7a
M2	77.2a	16.95d	62.67	70.79	6.1e	471.5c	81.9b
M3	75.93ab	19.59bc	61	67.64	7.48c	564.3b	73.0c
M4	76.8a	21.54ab	62.02	70.13	8.42b	646.0a	72.0c
M5	72.07b	20.22bc	57.23	70.08	8.62a	616.3a	64.5d
M6	77.13a	22.5a	62.76	70.3	7.1d	548.6b	58.9e
P Value	**0.014**	**0.001**	**0.5**	**0.5**	**0.001**	**0.001**	**0.001**
LSD	**4.2**	**2.78**	**7.44**	**6.3**	**0.13**	**32.9**	**2.7**

Values followed by different letters within the column are significantly different (P<0.05)
M=Month

Table 4.9: Mean effects of storage time on yield of rice in JKUAT.

Storage time	Grain Filling	AVG PNH	Grain No	Biomass	Harvest Index	Grain Width	1000GW	Yield (t/ha)
M1	5.693	20.05	482.6	59.9c	27.59ab	0.9	32.6b	7.3abc
M2	5.913	20.01	598.3	72.03abc	30.23ab	0.9	36.13b	4.6a
M3	5.4	18.82	465.1	82.34a	24.41b	0.9	38.8b	9.4c
M4	6.253	19.05	579.5	63.61bc	34.03ab	0.9	35.47b	7.9bc
M5	5.067	18.34	573.7	80.9ab	36.79a	0.9	50a	4.ab
M6	5.02	19.55	509.9	69.58abc	30.14ab	0.9	40.6ab	4.8ab
P Value	**0.3**	**0.6**	**0.5**	**0.008**	**0.03**	**0.60**	**0.01**	**0.012**
LSD	**1.2**	**2.6**	**69.4**	**17.8**	**10.34**	**0.09**	**9.5**	**2.2**

Values followed by different letters within the column are significantly different (P<0.05)
M=Month

Table 4.10: Mean effect of genotype on growth parameters in JKUAT

Genotypes	Growth Parameters							
	% Germination	Seedling VI	Leaf Length (cm)	Leaf width (cm)	No. of Tillers	Days to flowering	Panicle Length (cm)	Grain Filling
J54	73.7ab	614abc	31.21	0.95	8.7	72.4	18.21	5.93
J64	76.3ab	558a	29.5	0.95	10.1	75.2	18.98	5.32
B370	70.0a	561ab	29.7	0.94	9.5	74.2	19.98	5.96
B217	72.4ab	628c	27.57	0.95	9.2	73.8	19.05	5.04
Pishori	79.7b	645d	28.62	0.93	9.8	75.2	20.31	5.54
P value	**<0.001**	**<0.001**	**0.261**	**0.11**	**0.5**	**0.15**	**0.35**	**0.465**
LSD	**3.9**	**29.9**	**4.9**	**0.05**	**1.62**	**2.4**	**2.27**	**1.14**

Means with the same letters in the same column are not significantly different LSD α=0.05, V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

4.2.14 Effect of storage time and genotype on number of spikes at JKUAT

The time of storage had no significant effect on the number of spikes produced by the rice genotypes ($P \geq 0.05$). The type of genotype had no significant effect on the number of spikes that were produced by the plants at maturity ($P \geq 0.05$). There was a significant interaction between storage time and genotype on the number of spikes produced by the rice plants at maturity ($P \leq 0.05$). It is important to note that when applied separately, treatments, genotype and storage time did not have a significant effect. However, in interraction the effect was significant as a result of interaction between the two treatments.Treatment combination pishori stored for 1 month M5V1 had the highest number of spikes (7.6) while treatment combination J64 stored for 4 months M2V4 had the lowest mean number of spikes.

4.2.15 Effect of storage time and genotype leaf length

There was no significant effect of the treatment on the length of leaves at maturity ($P \geq 0.05$).

4.2.16 Effect of storage time and genotype leaf width

There was no significant effect of the treatment on the width of leaves at maturity ($P \geq 0.05$).

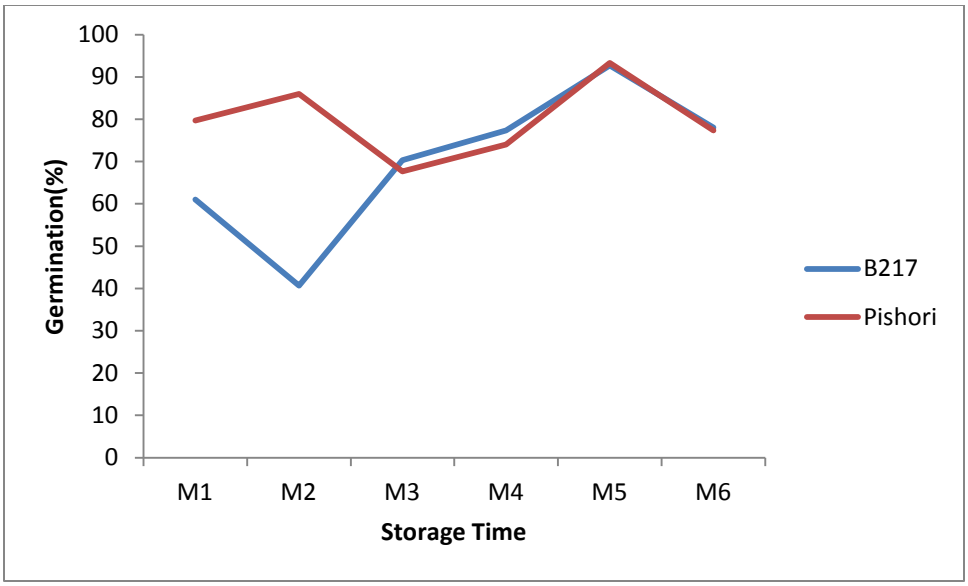


Figure 4.15: Effect of Interraction between genotype and storage time on germination Mwea

Table 4.11: Effects of interaction between storage time and genotype on growth in JKUAT

Treatments	Germ%	Length(cm)	SV1	Days to flowering	Height Transplant(cm)	Height 1month(cm)	Hmaturity (cm)
M1 V1	82.33bcef	6.8kl	557.2efgh	73.33ghi	19.13	55.37	74.07bcdef
M1 V2	66.0kl	4.6o	303.1l m	76.33fgh	18.2	60.27	88.4a
M1 V3	67.33jk	4.6o	305.7lm	65.33klm	19.87	57.07	67.5cdefg
M1 V4	78.67cdefgh	6.9k	548.1fgh	67.67ijkl	20.3	61.3	73.6bcdef
M1 V5	79.67cdefg	7.6ij	605.5cdef	77.33fg	16.67	55.53	64.9defg
M2 V1	82.67bcdef	6.4m	529.0ghi	68ijkl	17.43	57.93	68.9cdefg
M2 V2	78defgh	5.3n	415.9jk	66klm	16.03	57.91	65.1defg
M2 V3	56.67l	6.6lm	374.1kl	66.67jkl	16.7	71.27	75.3abcdef
M2 V4	82.67bcdef	7.6ij	628.3cde	62.67lmn	17.93	62.7	73.8bcdef
M2 V5	86abcd	4.8o	410.3jk	59.33n	16.63	63.53	70.7cdefg
M3 V1	84.67abcde	5.5n	462.7ij	60.67mn	21.6	59.43	68.6cdefg
M3 V2	71.67ghijk	8.4def	599.9cdefg	59n	17.6	59.53	64.7defg
M3 V3	68ijk	7.3j	498.3hi	57n	17	64.9	64.9defg
M3 V4	87.67abc	8.1fg	710.1ab	58.67n	19.65	61.1	70.9cdefg
M3 V5	67.67jk	8.1fg	550.4fgh	59n	22.1	60.03	69.0cdefg
M4 V1	89.33ab	8.5cde	759.5a	70.67hijkl	21.5	60.43	66.5defg
M4 V2	77.67defgh	7.5j	582.5defg	75gh	20.1	57.23	75.0abcde
M4 V3	69.67hijk	8.7bc	608.7cdef	74gh	20.4	58.47	65..4defg
M4 V4	75.33efghijk	8.7bc	655.4bcd	73ghi	21.5	68.23	76.7abcd
M4 V5	72ghijk	8.7bc	624.0cde	72.33ghij	24.2	65.73	67.0defg
M5 V1	79.67cdefg	9.5a	756.8a	70.67hijk	17.4	57.7	62.2efg
M5 V2	76.67defgji	8.1fg	621.0cdef	81.33ef	18	60.47	75.7abcde
M5 V3	82.67 bcdef	8.8b	726.4ab	87.33cd	21	59.9	73.6bcdef
M5 V4	28m	8.9b	249.2m	85de	22.8	51.77	81.2abc
M5 V5	93.33a	7.8hi	728.0ab	85.33de	21.9	56.33	57.7g
M6 V1	77.33defghi	8.6bcd	667.5bc	91.33bc	23.6	64.27	63.9defg
M6 V2	79.67cdefg	5.3n	422.2jk	93.67ab	21.55	63.61	60.8fg
M6 V3	73.33fghijk	5.4n	394.5jk	94.67ab	21.4	55.53	86.2ab
M6 V4	79.33cdefg	8.3ef	658.5bc	96ab	22.05	65.77	73.7bcdef
M6 V5	76efghij	7.9gh	600.4cdefg	97.67a	23.9	64.63	66.8defg
P value	0.001	0.001	0.001	0.01	0.07	0.9	0.02
LSD	9.6	0.3	73.5	5.98	5.1	21.3	14

Means followed by the same letters within the column are not significantly different (LSD α *=0.05*, V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

Discussion

In Mwea, the storage time had a significant effect on the germination percentage, seed vigor index, heights at transplanting, one month, two months after transplanting and height at maturity. It also had a significant effect on the days to flowering. The seeds planted after 3 and 4 months of storage scored highest in terms of seedling vigor index (M3V4) height 1 month after transplanting (M4V3) height at maturity (M4V3), days to flowering (M3V3).

The effect of genotype on germination, seedling vigor index and height at transplanting, after 1, 2 months and at maturity was significant.The interaction between genotype and storage period had a significant effect on the germination, seedling vigor index, heights at transplanting, months after planting and at maturity. It also had a significant effect on the number of days to flowering and number of spikes. Genotype, pishori scored the highest percentage germination, seedling vigor index, height at transplanting, pishori also had the highest panicle length, although this was not significant. For the interactions, the combination, The seeds planted after 3 and 4 months of storage scored highest in terms of seedling vigor index(B217 stored for 4 months,M3V4),height 1 month after transplanting (B370 stored for 3 months, M4V3) height at maturity(B370 stored for 4 months,M4V3), days to flowering (B370 stored for 3 months, M3V3).

At JKUAT, the storage time significant effect on the germination percentage, seed vigor index, heights at one month, two months after transplanting and height at maturity. It also had a significant effect on the days to flowering. The seeds planted after 4 months of storage scored highest in terms of seedling vigor index, height at transplanting, height at

maturity. Seeds planted after 5 months of storage gave the best seedling vigor index, height at 3 months after planting and maturity.

The interaction between genotype and storage period had a significant effect on the germination, seedling vigor index, heights at transplanting, 1 and 2 months after planting and at maturity. Seeds stored for 4 months had the highest seedling vigor index and height at 1 month after transplanting. These results agree with the findings of a study on alfalfa that indicated that although the ability to germinate may be retained, but the percentage germination may be reduced over time. According to their results, seed performance increased until about 3 to 4 months then finally decreased with seed age or storage period.

These results also agree with Abebaw, (2013) on an experiment on the effect of storage and varieties on quality, growth and yield of Tef Eragrotis Tef (Zucc) also confirmed that seed vigour index and seedling length were affected both by Genotype and seed storage period. The change of temperature, photoperiod, or nutrient or drought stress, during seed development, maturation, and after dispersal, may strongly affect seed performance (Donohue, 2009). Glaucia *et al.* (2009), in an experiment assessing the storage of sorghum seeds harvested at different moisture levels found that as harvest proceeded with greater moisture contents, the physiologic quality of these seeds decreased; seed physiologic quality decreased significantly. Sun *et al*. (2007) found that seed genetics, production, and storage environment are major influencers of seed longevity, viability, and vigor. In another study which was done in five-year storage period in several cereals, germinability level was decreased 38 % m average for all cereals (Julijo and Vlado, 1997).

The study continued by reporting that the germination potential of seeds after accelerated aging was highly correlated with seed survival in storage under a Genotype of conditions for up to 3 years. Thus, in addition to predicting field performance, the accelerated aging test is also a predictor of seed deterioration during storage (Delouche and Baskin, 2016; Tekrony *et al*., 1993). In yet another study the influence of moisture content (12.1-13.0%) on wheat seed. Viability was investigated by Desmarcheher *et al.* (1993). No significant effect on quality and viability.This is in contrast to results from the present study.The change of temperature, photoperiod, or nutrient or drought stress, during seed development, maturation, and after dispersal, may strongly affect seed performance (Donohue, 2009).

Sun *et al*. (2007) defined seed vigor as a quantitative trait that is affected by many factors, and that vigor is measured through individual traits among which are germination, seedling length, root length, seedling fresh weight, and seed longevity.The results observed in this study reinforce those of Corte *et al*. (2010) and Nakada *et al*. (2010), who noted an increase in lipid peroxidation with increasing deterioration of the seeds. Thus, the reduced activity of catalase can make the seeds more sensitive to the effects of free radicals and enhance peroxide formation in cells, making the seeds more subject to loss of viability.

In several studies, significant relationships have been observed between loss of viability and decreased activity of this enzyme. In wheat seeds artificially aged, Ganguli and Sen-Mandi (1993) showed that the enzyme α-AM was synthesized in reduced rates by aleurone layer. According to the authors, deteriorative changes may occur in the aleurone layer during aging (Timóteo and Marcos-Filho, 2013). These changes may determine the

decrease in amylase production which in turn affects the germination. This was verified through vigor tests. Where the seed performance was reduced during storage.

In natural environments dormancy of dry seeds can be released by storage of the seeds for several months at mild temperatures after-ripening or by cold stratification, which is a low-temperature treatment of imbibed seeds (Bewley *et al.*, 2012). In a study on different corn types, observed that different genotypes manifest differentiation in quality when stored in the same environment. Similar findings, in which seeds of tomato (*Solanum lycopersicum* L.), dandelion (*Taraxacum officinale* F. H. Wigg.), onion (*Allium cepa* L.), and eggplant (*Solanum melongena* L.) stored in environments with fluctuating relative humidity for periods longer than 12 weeks rapidly lost their viability(Bewley and Black, 1994).

One of the characteristics of varieties with a wide adaptation is that the seed of these varieties has good physiological characteristics and is still suitable for planting after long storage under not too favorable conditions (Govindarasu *et al*. 2000). Mohapatra *et al*. (2011) and Chau and Bhargava (1993) observed that the time of flowering and the position of the spikelet in the rachis affected the quality and proportion of high-density grains. Kumar and Mishra, (2014) suggests that membrane degradation has is key factor when ageing in most seeds (Varghese and Rai 2005). A major challenge in present agriculture is loss of viability of stored seedIn modern agriculture seed.

The present study confirms that the effect of genotype on performance of crop cannot be underscored since this affects ageing of the seed. Different seed genotypes perform differently when stored over time (Varghese and Rai, 2005). Krishnaveni (1984) and

German *et al*. (1993) while working on maize reported significant reduction in germination, root length, viability, dry matter reduction and vigour index with response to period of ageing. Similar results were also found by Singh *et al*. (2003), Varghese and Rai (2005), Kumar and Rai (2007), and Kumar and Rai (2009). Rajić *et al*. (2005) obtained similar results for sugar beet seed. The germination energy and germination increased significantly over the period of six months after harvest, while Tatić *et al*. (2008) concluded that, beside the storage period, the method of storage has a significant influence on seed quality.The achieved values of the genotype × storage period interaction indicated that the seed germination rate was lowest after a year of storage for three hybrids but highest as storage period went on. The effects of genotype, storage period and chemical treatment on sunflower seed germination were statistically highly significant (Mrda *et. al.*, 2010).

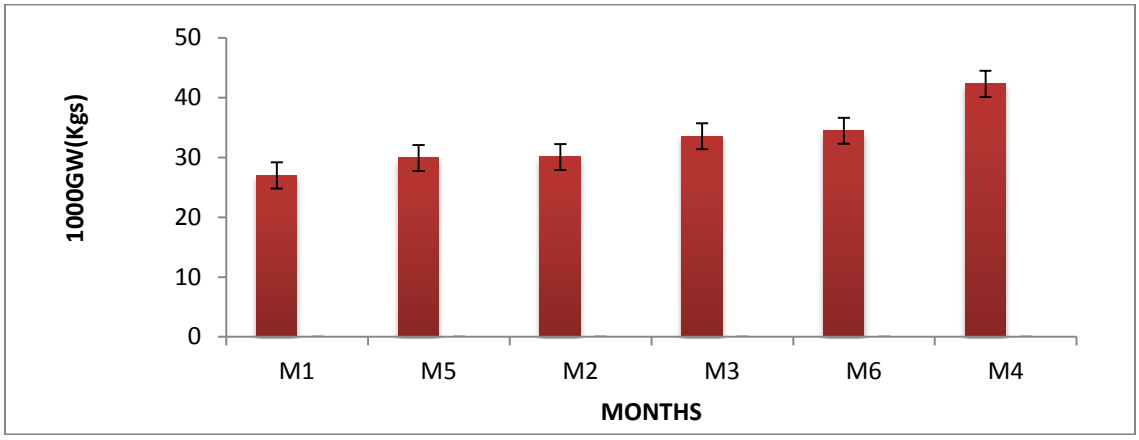
Marinković (2008), who observed significant differences in germination rate during sunflower seed storage, stated that the genotype itself influences the storability too. Along this line, Đukanović (1999) concluded that the genotype is the most important factor that affects the storage characteristics and their changes in corn inbredlines and hybrids. The analysis revealed that in general the effect of the genotype was the most pronounced with a very prominent contribution of the genetic backgroup of the genotypes. Also the effect of the parental environment was very significant.Seed deterioration is associated with loss of viability during storage results in decreased early growth of roots and shoots and in increased variability of growth between plants. This early inhibition of growth-rate does not persist and there is some evidence that, under

normal agricultural conditions, initial low rates of growth may be compensated at later stages of development.

4.3 Effect of storage time and genotype on the yield and yield components of rice Mwea

4.3.1 Effect of storage time and genotype on 1000 grain weight

The storage time had a significant effect of the storage period on 1000grain weight (P≤0.05). This means that there were differences across the genotypes (Figure 4.16). The highest grain weight was obtained from seeds stored for 3 months (38.94) while the lowest weight was obtained from one month old seeds (27). The genotype had no significant effect (P≥0.05) on the 1000grain weight (Table 4.12). There was no significant interaction between storage time and genotype (P≥0.05) (Table 4.13).



Key: V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

Figure 4.16: Effect of storage time on 1000 grain weight:

Storage time had a significant effect (P≤0.05) on the biomass**.** The application of different storage period of rice results in variation of the biomass obtained from the harvested crop. The highest biomass was obtained from seeds planted after 5 months of

storage (80.9), while the lowest weight was obtained from seeds planted after 1 month of storage (61.8)

However the genotype had no significant effect on the biomass obtained ($P \geq 0.05$), thus the five genotypes had a similar effect on the amount of biomass obtained from the harvested plants. The interaction between storage time and biomass had no significant effect ($P \geq 0.05$) on the biomass.

4.4.2 Effect of storage time and genotype on Grain weight

The time of storage had a significant effect on the weight of grains obtained after harvest ($P \leq 0.05$).The highest storage was obtained from seeds stored for 5 months (40.8) while the lowest was obtained from seeds planted after 1 month of storage (26.16).

The genotype did not have a significant effect on the grain weight obtained after harvesting. There was no significant interaction between genotype and storage time on grain weight ($P \geq 0.05$).

4.3.3 Effect of storage time and genotype on grain width

The storage time had no significant effect ($P \geq 0.05$) of storage time on the grain width. All the seeds stored gave an almost similar width with the minimum being 0.9cm. There was no significant effect ($P \geq 0.05$) of genotype on the full grain weight. All the varieties had a similar mean grain width.There was no significant interaction ($P \geq 0.05$) between storage time and genotype on grain width.

4.3.4 Effect of storage time and genotype on Yield

The storage time had a significant effect (P≤0.05) on the yield (Fig 4.12). The seeds stored for 3 months gave the highest yield (9.394) while seeds stored for 2 months gave the lowest yield (4.6).

The genotype had no significant effect (P≤0.05) on the yield. The effect of interaction between storage time and genotype on yield was significant (Table 4.13). The highest full grain weight was obtained from combination of pishori stored for 3 months M3V5 while B370 stored for 2 months M2V3 had the lowest yield.

Table 4.12: Mean effect of Storage time on yield of Rice in Mwea

Storage time	Grain Filling	AVG PNH	Grain Number	Biomass	Harvest Index	Grain Width	1000GW	Yield (t/ha)
M1	5.69	20.05	965	61.8	43.7	0.9	27d	7.3ab
M2	5.91	20.01	1085	74.00	44.5	0.9	30.08cd	4.6b
M3	5.4	18.82	930	83.9	45	0.9	38.94a	9.4a
M4	6.25	19.05	1026	63.7	49.5	0.9	29.9cd	7.8a
M5	5.07	18.34	1014	80.9	47.9	0.9	36.24ab	4.8b
M6	5.02	19.55	1020	69.6	49.6	0.9	33.07bc	4.8b
P value	**0.3**	**0.7**	**0.8**	**0.06**	**0.8**	**0.6**	**5.52**	**0.006**
LSD	**1.3**	**2.5**	**209.8**	**16.95**	**11.2**	**0.1**	**0.001**	**2.9**

Means with the same letters in the same column are not significantly different LSD α=0.05, Key: V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

Plate 4: Harvesting of rice in Mwea plots

Table 4.13: Effects of interaction of storage time and genotype on yield at Mwea

Treatments	Biomass	Panicle Height	Grain Weight	Grain No.	Grain filling	1000 %GW	Yield (t/ha)
M1 V1	76.0	17.86	24.7	1412	5.33	24.68	0.23
M1 V2	70.6	19.57	27.6	472	5.53	27.64	0.23
M1 V3	76.8	18.76	26.5	1022	6.4	26.52	0.20
M1 V4	59.2	18.54	27	929	3.93	26.98	0.22
M1 V5	66.7	25.55	29.2	991	7.27	29.2	0.21
M2 V1	89.2	17.88	30.2	1173	5.8	30.17	0.20
M2 V2	76.7	18.99	25.5	1029	5.6	25.45	0.20
M2 V3	80.7	25.69	27.9	1129	6.73	27.91	0.20
M2 V4	74.4	18.75	34.1	1027	5.27	34.13	0.21
M2 V5	94.2	18.75	32.7	1064	6.17	32.73	0.20
M3 V1	99.1	17.92	41.8	1115	5.93	41.75	0.18
M3 V2	87.9	19.61	33.5	955	4.87	33.47	0.21
M3 V3	68.5	18.78	32.2	641	6.13	32.21	0.23
M3 V4	115.8	19.56	46.8	856	5.53	46.84	0.20
M3 V5	117.1	18.21	40.4	1083	4.53	40.44	0.21
M4 V1	78.9	19.55	31.4	1069	5.6	31.42	0.19
M4 V2	81	17.96	32.9	1182	7.53	32.87	0.19
M4 V3	63.5	19.44	30.9	1019	5	30.86	0.20
M4 V4	50	18.52	29.1	992	7.53	29.06	0.18
M4 V5	55.5	19.8	25.3	866	5.6	25.28	0.21
M5 V1	88.8	17.03	36.7	1014	7.6	36.74	0.18
M5 V2	79.3	18.66	37.9	1245	3.2	37.9	0.19
M5 V3	93	17.91	40.1	744	7.27	40.12	0.21
M5 V4	77	18.85	33.4	1004	3.07	33.36	0.20
M5 V5	68.1	19.25	33.1	1064	4.2	33.1	0.21
M6 V1	90.4	19	36.9	1015	5.3	36.91	0.19
M6 V2	66.6	19.11	33.1	1075	5.2	33.1	0.19
M6 V3	67.1	19.28	31.8	877	4.2	31.77	0.21
M6 V4	76.6	20.08	32.6	1125	4.93	32.63	0.20
M6 V5	73.1	20.27	30.9	1007	5.47	30.94	0.21
P value	**0.583**	**0.57**	**0.798**	**0.275**	**0.093**	**0.7**	**0.9**
LSD	**37**	**5.64**	**12.35**	**469.1**	**2.851**	**12.35**	**0.05**

Values followed by different letters within the column are significantly different (P<0.05)
M=Month, Key: V1=J54, V2-J64, V3=B217, V4=B370, V5=Pishori, M=month

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4.3.6 Effect of storage time and genotype on 1000 grain weight

The storage time had a significant effect (P≤0.05) on the 1000grain weight. This means that there were differences across the treatments. The 1000 highest grain weight was obtained from M3 (50kg) while the lowest weight was obtained from one month old seeds (32).The genotype had no significant effect on the 1000grain weight. There was a significant interaction between storage time and genotype on 1000 grain weight (Table 4.17). The highest 1000 grain weight was obtained from B217 stored for 3 months (69kg)while the lowest was obtained from pishori stored for 5 months (40.05kg)

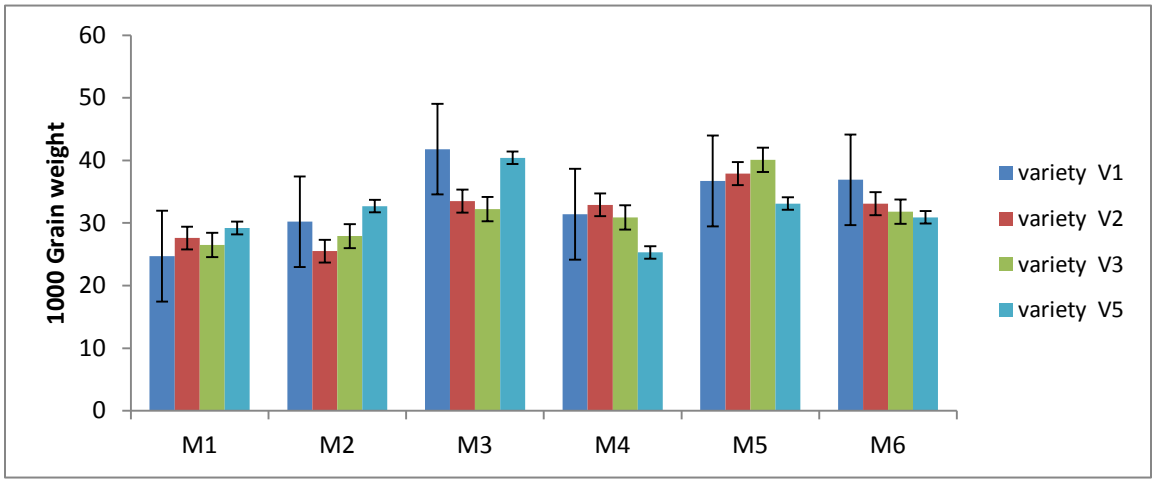


Figure 4.17: Effect of interaction between genotype and genotype on 1000 grain weight

4.3.7 Effect of time of storage and genotype on biomass

Time of storage had a significant effect on the biomass (P≤0.05), meaning the application of different storage period of rice results in variation of the biomass obtained from the harvested crop (Table 4.14). The highest biomass was obtained from seeds planted after 3 months of storage (82.34), while the highest was obtained from seeds planted after 1

month of storage (69.9). However the genotype had no significant effect on the biomass (Table 4.15). There was a significant interaction between storage time and biomass (Table 4.16). The highest biomass was obtained from genotype B217 stored for 3 months (94.39) while lowest was from seeds stored for 1 month.

Table 4.14: Effects of storage time on yield of rice in JKUAT

Storage time	Grain Filling	AVG PNH	GN	BM	HI	Grain Width	1000GW	Yield(t/ha)
M1	5.693	20.05	482.6	59.9c	27.59ab	0.9	32.6b	7.3abc
M2	5.913	20.01	598.3	72.03abc	30.23ab	0.9	36.13b	4.6a
M3	5.4	18.82	465.1	82.34a	24.41b	0.9	38.8b	9.4c
M4	6.253	19.05	579.5	63.61bc	34.03ab	0.9	35.47b	7.9bc
M5	5.067	18.34	573.7	80.9ab	36.79a	0.9	50a	4.ab
M6	5.02	19.55	509.9	69.58abc	30.14ab	0.9	40.6ab	4.8ab
P Value	**0.3**	**0.6**	**0.5**	**0.008**	**0.03**	**0.60**	**0.01**	**0.012**
LSD	**1.2**	**2.6**	**69.4**	**17.8**	**10.34**	**0.09**	**9.5**	**2.2**

Means with the same letters in the same column are not significantly different LSD α=0.05, V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

Table 4.15: Effects of genotype on yield of Rice at JKUAT

Genotype	Grain Filling	AVG PNH	GN	BM	HI	Grain Width	1000GW	Yield(t/ha
V1	5.93	18.21	668.8a	77.23	36.31	0.89	40.28	6.33
V2	5.32	18.98	496.5b	72.98	26.07	0.93	36.56	4.91
V3	5.96	19.98	452.8b	66.88	27.7	0.92	38.67	5.13
V4	5.04	19.05	494.4b	69.77	28.47	0.90	37.89	7.32
V5	5.54	20.31	561.8ab	70.1	34.11	0.86	41.28	7.88
P Value	**0.48**	**0.36**	**0.05**	**0.07**	**0.20**	**0.40**	**0.8**	**0.28**
LSD	**1.19**	**2.28**	**154.6**	**16.2**	**10.7**	**0.07**	**8.6**	**3.24**

Means with the same letters in the same column are not significantly different LSD α=0.05, V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

Table 4.16: Interraction of storage time and genotypes on biomass

Storage time	Genotypes				
	V1	V2	V3	V4	V5
M1	54.0bcde	64.7abcde	70.0abcde	47.1e	63.8abcde
M2	80.7abcde	66.0abcde	61.9abcde	71.3abcde	80.2abcde
M3	87.0abcd	76.1abcde	60.4abcde	94.4a	93.7ab
M4	74.5abcde	86.6abcde	53.4cde	49.5de	54.1bcde
M5	86.1abcde	83.5abcde	89.6abc	81.4abcde	63.8abcde
M6	81.1abcde	60.9abcde	65.9abcde	75.0abcde	65.0abcde
P value			**0.008**		
LSD			**39.8**		

Means with the same letters in the same column are not significantly different LSD α=0.05, Key: V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

4.3.8 Effect of storage time and genotype on grain weight at JKUAT

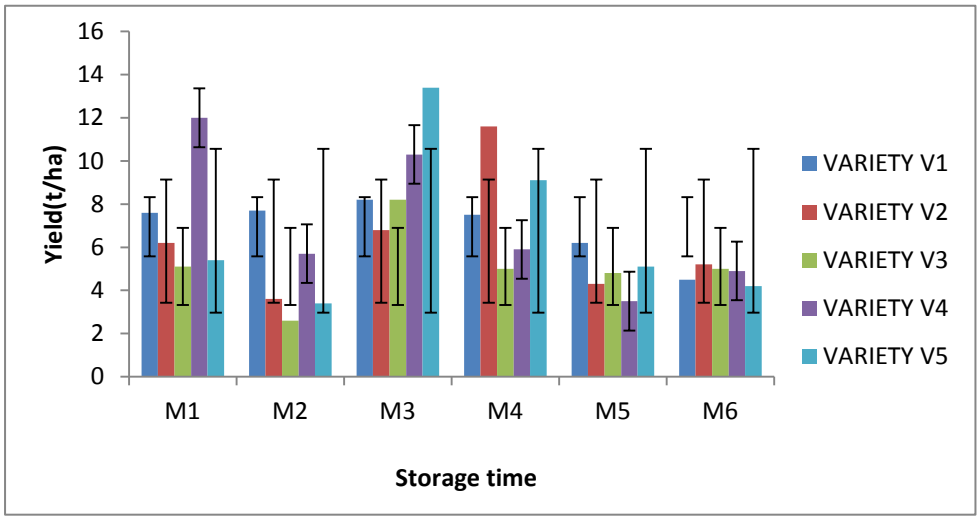
The time of storage had no significant effect (P≤0.05) on the weight of grains obtained after harvest. The genotype had a significant effect on the grain weight obtained after harvesting. Genotype V1 (J54) had the highest mean grain weight (26.1) while genotype V3 (B370) had the lowest grain weight (16.9). There was no significant Interaction between genotype and storage time on grain weight (P≥0.05).

4.3.10 Effect of storage time and genotype on grain width at JKUAT

The storage time had no significant effect of on the grain width. The genotype had no significant effect on the full grain width. There was no significant interaction between storage time and genotype on grain width (P≥0.05).

4.3.11 Effect of storage time and genotype on yield at JKUAT

The storage time had a significant effect (P≤0.05) of on the yield. The seeds stored for 3 months gave the highest yield (9.394) while seeds stored for 2 months gave the lowest yield (4.592). The genotype had no significant effect on the yield. There was a significant interaction between storage time and genotype on full grain yield(Fig.4.18). The highest full grain weight was obtained from combination of B217 stored for 3 months M3V4 while J64 stored for 1 month treatment combination had the lowest full grain weight.(Table 4.17)(Fig. 4.21)



Key: V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

Figure 4.18: Effect of storage time and genotype on yield

Table 4.17: Mean effects of interaction between storage time and genotype on yield in JKUAT

	Biomass	Grain numbe	Grain number	Grain filling	1000% GW	Yield (t/ha)
M1V1	53.98bcde	706abcd	706abcd	5.3abcde	30.7c	7.6bc
M1V2	64.68abcde	236f	236f	5.5abcde	33.3c	7.9abc
M1V3	70.04abcde	511abcdef	511abcdef	6.4abc	32.3c	5.1c
M1V4	47.05e	464.7bcdef	464.7bcdef	3.93.9	33.0c	15.5a
M1V5	63.76abcde	495.3abcdef	495.3abcdef	7.3ab	33.7c	5.4c
M2V1	80.72abcde	866.7a	866.7a	5.8abcde	37.0bc	9.1abc
M2V2	65.98abcde	514.7abcdef	514.7abcdef	5.6abcde	32.0c	3.6c
M2V3	61.91abcde	564.7abcdef	564.7abcdef	6.7abc	32.3c	2.6c
M2V4	71.29abcde	513.7abcdef	513.7abcdef	5.3abcde	40.3bc	2.7c
M2V5	80.24abcde	532abcdef	532abcdef	6.2abc	39.0bc	3.4c
M3 V1	87.02abcd	557.7abcdef	557.7abcdef	5.9abcd	44.7bc	3.3c
M3V2	76.13abcde	477.7bcdef	477.7bcdef	4.9abcde	35.0bc	5.8bc
M3V3	60.44abcde	320.7ef	320.7ef	6.1abc	36.7bc	8.2abc
M3V4	94.39a	428cdef	428cdef	5.5abcde	40.0bc	9.7abc
M3V5	93.73ab	541.7abcdef	541.7abcdef	4.5bcde	37.7bc	13.4ab
M4V1	74.46abcde	534.7abcdef	534.7abcdef	5.6abcde	35.7bc	4.2c
M4V2	86.58abcde	591abcdef	591abcdef	7.5a	37.0bc	2.7c
M4V3	53.44cde	509.7abcdef	509.7abcdef	5.0abcde	36.7bc	5.0c
M4V4	49.49de	496abcdef	496abcdef	7.5a	36.3bc	4.3c
M4V5	54.08bcde	766.3abc	766.3abc	5.6abcde	31.7c	9.1abc
M5V1	86.09abcde	840.3ab	840.3ab	7.6a	42.0bc	9.1abc
M5V2	83.55abcde	622.3abcde	622.3abcde	3.2de	44.3bc	4.3c
M5V3	89.58abc	372def	372def	7.3ab	55.7ab	4.8c
M5V4	81.44abcde	502abcdef	502abcdef	3.1e	39.0bc	3.5c
M5V5	63.82abcde	532abcdef	532abcdef	4.2cd	69.0a	8.4abc
M6V1	81.1abcde	507.3abcdef	507.3abcdef	5.3abcde	51.7abc	4.5c
M6V2	60.95abcde	537.3abcdef	537.3abcdef	5.2abcde	37.7bc	5.2c
M6V3	65.87abcde	438.7cdef	438.7cdef	4.2cde	38.3bc	5.0c
M6V4	74.98abcde	562.3abcdef	562.3abcdef	4.9abcde	38.7bc	8.2abc
M6V5	64.99abcde	503.7abcdef	503.7abcdef	5.5abcde	36.7bc	7.5bc
P alue	**0.81**	**0.82**	**0.82**	**0.1**	**0.05**	**0.2**
LSD	**39.8**	**378.8**	**378.8**	**2.8**	**21.3**	**7.6**

*Means followed by the same letters within the column are not significantly different (LSD α =0.05)*Key: V1=J54, V2-J64, V3=B370, V4=B217, V5=Pishori, M=month

Further Interractions

There was a significant interaction (P≤0.05) between storage time and genotype on germination in mwea and jkuat. Comparison between B217 and pishori indicated an interaction. Similarly, there was a significant interaction between storage type and genotype on the seedling vigor index and yield in JKUAT. There was a significant interaction between storage time and Days to flowering (Fig. 4.20), storage time and (Fig 4.21).

There was a significant interraction brtween storage time and seedling vigor index on the genotype (Fig 4.22). Further interractions are depicted in Figure 4.23, 4.24, 4.25, 4.26 which depict a variation in performance of genotypes from one storage time to another or from one ageing time to another. This confirms presence of interraction.

Ensuring appropriate germplasm and genotype maintenance, facilitating production, distribution and marketing of good quality seeds will help farmers' access credit and high-quality inputs. Providing adequate supply and marketing of high-qualityinput, Ensuring affordable loans to farmers.

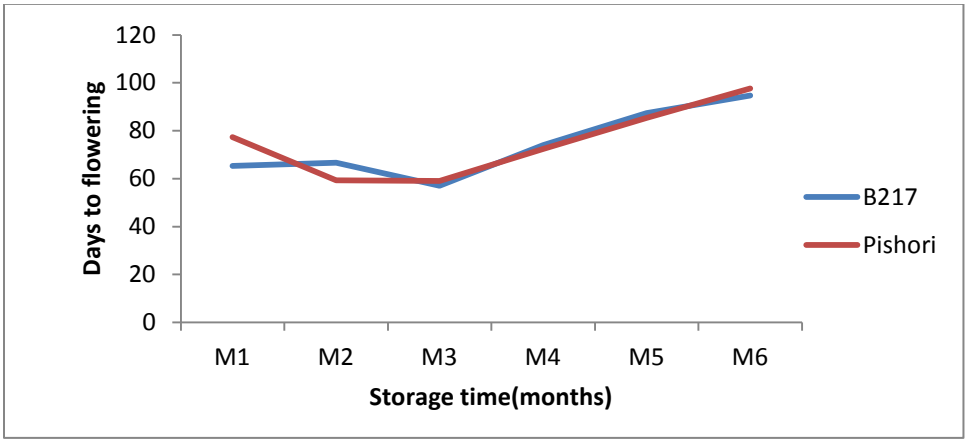


Figure 4.20: Effect of interractions between storage time and genotype on flowering (Mwea)

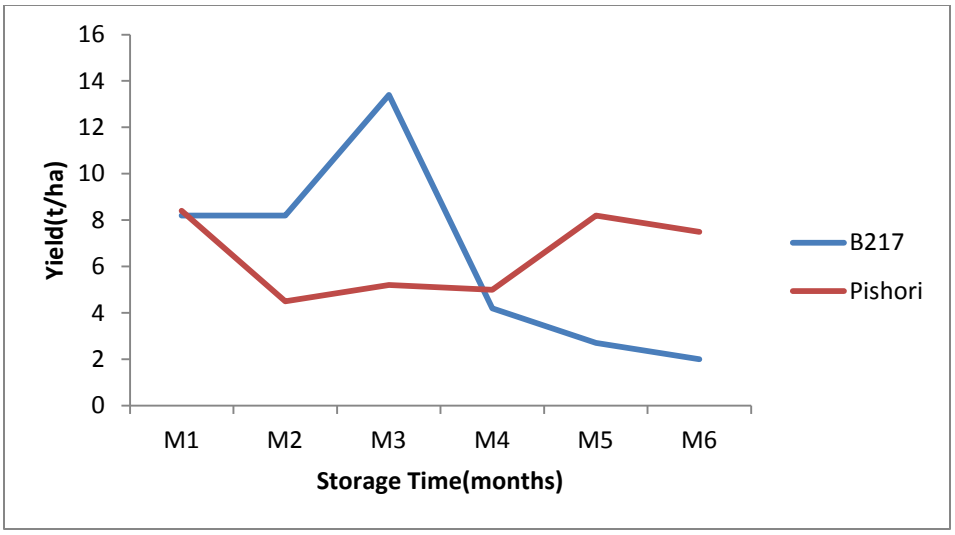


Table 4.21: Effect of interractions between storage time and genotype on yield (JKUAT)

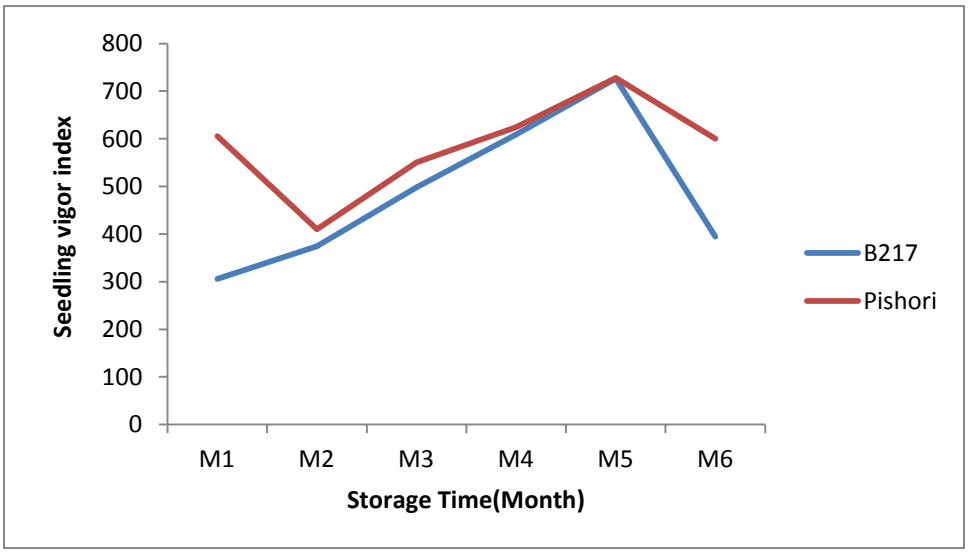
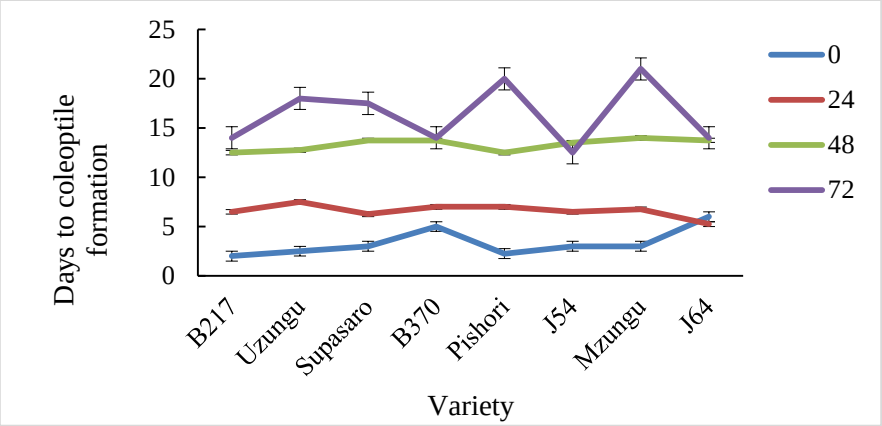
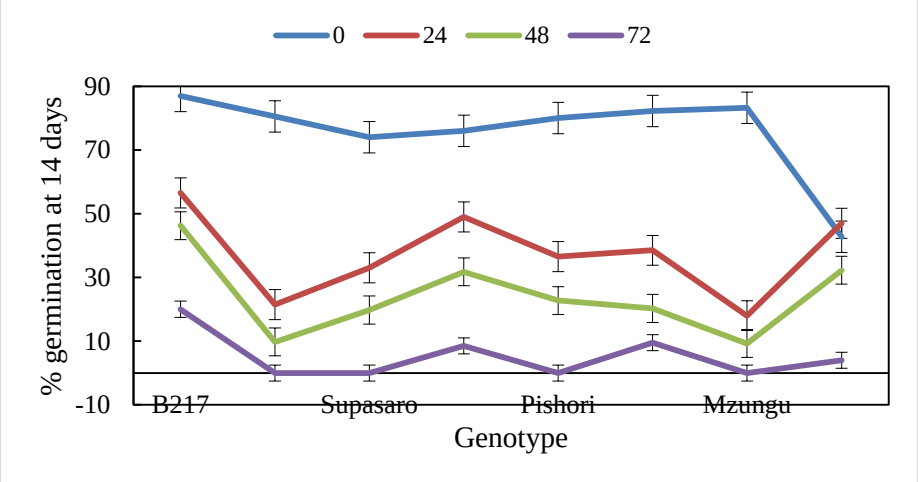


Figure 4.22: Effect of interractions between storage time and genotype on seedling vigor index (JKUAT)



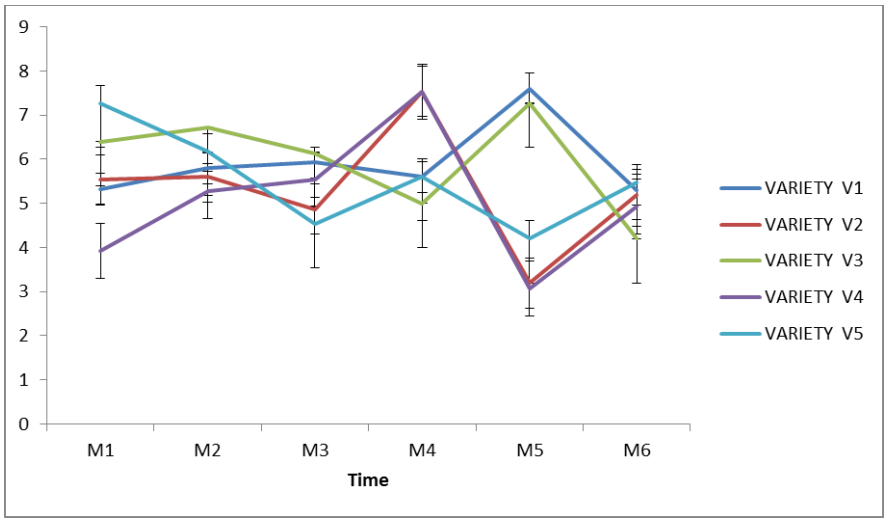
Key: V1=J54, V2-J64, V3=B217, V4=B370, V5=Pishori, M=month

Figure 4.23: Interraction of ageing time and genotype on coleoptile formation



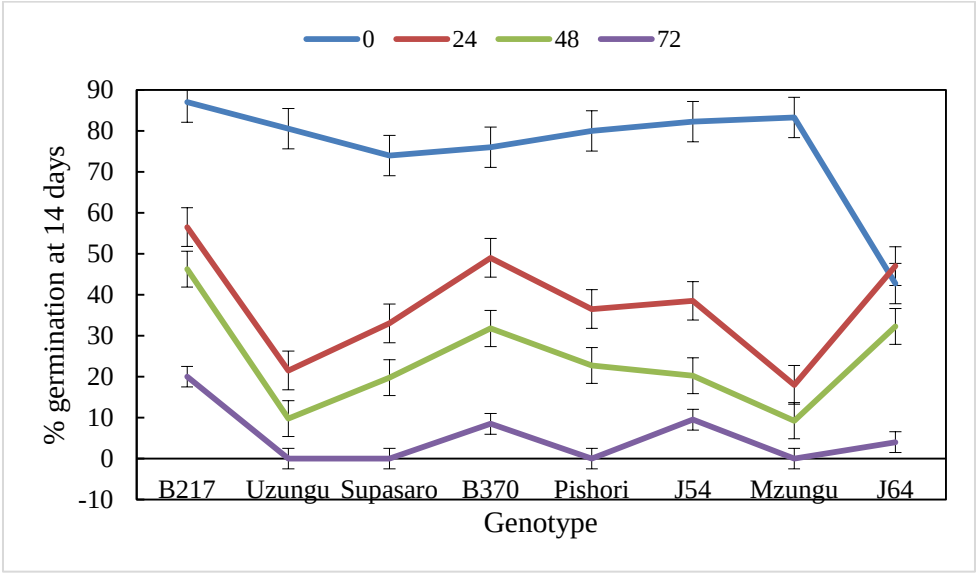
Key: V1=J54, V2-J64, V3=B217, V4=B370, V5=Pishori, M=month

Figure 4.24:Interraction of ageing time and genotype on germination



Key: V1=J54, V2-J64, V3=B217, V4=B370, V5=Pishori, M=month

Figure 4.25: Interraction of ageing time and genotype on seedling vigour index



Key: V1=J54, V2-J64, V3=B217, V4=B370, V5=Pishori, M=month

Figure 4.26:Interraction of ageing time and genotype on germination

Discussion

The effect of genotype on biomass was not significant as shown in the present study.This is in contrast to Song et.al, 2002 who through an experiment, found that there were difference between the cultivars in the relative proportion of biomass accumulated during grain filling. Term longevity is considered as both a biological and an economic category. Economic longevity, which defines the period during which the seed preserves the technological and market values, is of primary importance for agricultural production (Dokić *et al*., 2008). Viability, a period during which seed can be used for sowing and production depends on its genetic constitution and genotype (Tomić *et al*., 1998).

Storage conditions and the period of storage have large influence on the quality of sunflower seed. The goal of storing is to provide optimum preservation of physiological and physical characteristics of seed, while poor storage conditionscan lead to loss of seed viability (Đukanović and Sabovljević, 2001). Indicators of seed vigor such as germination energy, germination, and field emergence) determine directly the number of plants per unit area, which is one of the three basic components of yield in the world of plants. In both mwea and JKUAT, the effect of storage time on 1000 grain weight, biomass and yield was significant. The effect of interaction between storage type and genotype was significant with B217 stored for three months leading in biomass and yield. These results agree with another study, where the initial seed viability was less than 50% but the yields were not significantly affected. But the deterioration during storage reduced seed viability to below 50% and therefore affected the yields negatively.

One of the factors that influence seed quality is the activities around harvesting such as timing and handling. During harvesting, its recommended that one considers performing the activity nearer to physiological maturity, yet it's the time when the water contained is highest for most cereal crops. For instance, the water may be at 30% and use of mechanization during harvesting may result in serious damages and excessive lossess (Lacerda *et al*., 2003; Marcos-Filho, 2005). This may call for slight delays in harvesting to allow dryng. The challenge is that the more the seeds are mature the further the rate of deterioration. There may also be harsh weather which lowers both quality and quantity of yields (Marcos-Filho, 2005). Some farmers resort to applying dessicants or selective herbicides to try and fasten maturity in order to allow early harvesting (Lacerda *et al*., 2003). This process may result in effects on quality.

The effect of genotype on full grain weight was significant in Mwea. These findings from the present study confirmed the earlier findings of Chang (1985) and Ellis *et al*. (1991) on varietal differences in seed longevity of rice who worked on Upland Japonica.and lowland japonica. They found great varietal differences with respect to survival among the two cultivars. One could relate the variation in seed performance to variation in level of phenolic content and the associated antioxidative defence system.

The variation in performance of genotypes could be attributed to some types containing greater levels of amylopectin, normally referred to as glutinous rice, or having no glutin, or high amylose content rice. Further tests may be needed to check the glutin content of the rice. This is because Juliano *et al*., 1993 suggested that glutinous genotypes loose

their viability quicker than non-glutinous rice. One of the findings in this study is that rice genotypes may need some maturity period to pass prior to sowing.

This is supported by a study in soybeans by Mbofung, (2012) indicating an immature seed or seed that has endured weathering conditions in the field may not store well. Warm and wet conditions are known to increase infection by some fungi especially Phomopsis longicolla on soybean (Spears *et al*., 1997). Findings from the present study also reveal that there are differences in genotypic performance of seeds. This agrees with a claim that the chemical composition of the seed determines the optimum seed storage ability, which varies among varieties, among species, among cultivars and among tissue types in the individual seed (Vertucci and Roos, 1990).

4.3.12 Correlating juvenile and yield components

There was a positive and significant correlation between the biomass and 1000 grain weight. The correlation between biomass and 1000grain weight was strong, $r=0.8346$, $n=8$, $P<.001$, this shows that these are the most important traits associated with 1000 grain weight. Biomass is the total weight of a plant which in the present study, was measured as above ground dry biomass. From these results, a unit increase in biomass results in an increase in the 1000 grain weight(Fig. 4.27).

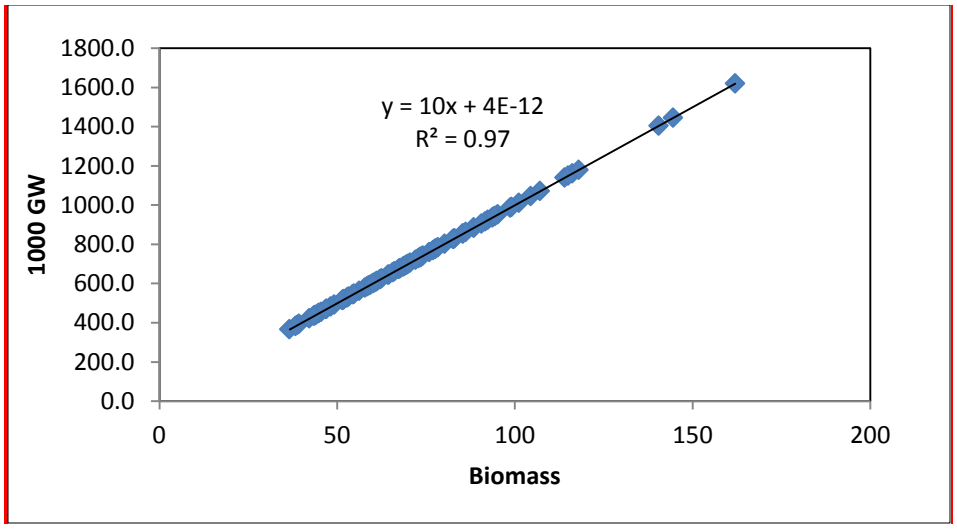


Figure 4.27: Correlation between biomass and 1000 grain weight

Seed storage tests are important and necessary in order to obtain clearer picture on the actual performance of rice in field conditions. The findings of this study depict that the storage time is a key factor affecting vigor of most seeds. It is therefore necessary that seeds should not be stored in extremely high temperature conditions as this increases the levels of deterioration and therefore reduces actual viability of the seeds. The present research has shown that there is actual variation in vigor of seeds of different varieties which results in variation of field performance later on. The need to improve irrigated rice varieties is important since irrigated rice produced in Kenya now count for 95% Kimani *et al*., (2011), compared to 5% is produced under rainfed conditions (NIB, 2008). Several inherent or genetic factors of the seed such as hybrid vigor, hard-seededness, susceptibility to seed damage, and chemical composition can influence the seed vigor and ultimately, viability (Copeland and McDonald, 2001).

There was a positive and significant correlation between the panicle height and 1000 grain weight. The correlation between the two variables was strong, r=0.8346, n=8, P<.001. There was a positive and significant correlation between the harvest index and 1000 grain weight. The correlation between the two variables was strong, r=0.8346, n=8, P<.001(Fig. 4.29)

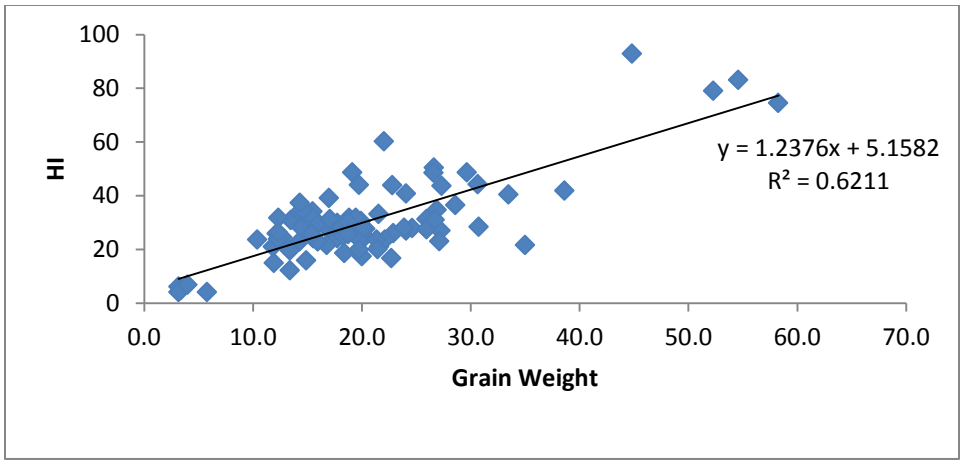


Figure 4.29: Correlation between Harvest Index and 1000 grain weight

There was a positive and significant correlation between the seedling vigor index and seed length (Fig. 4.30). The correlation between the two variables was strong, r=0.8346, n=8, P<.001

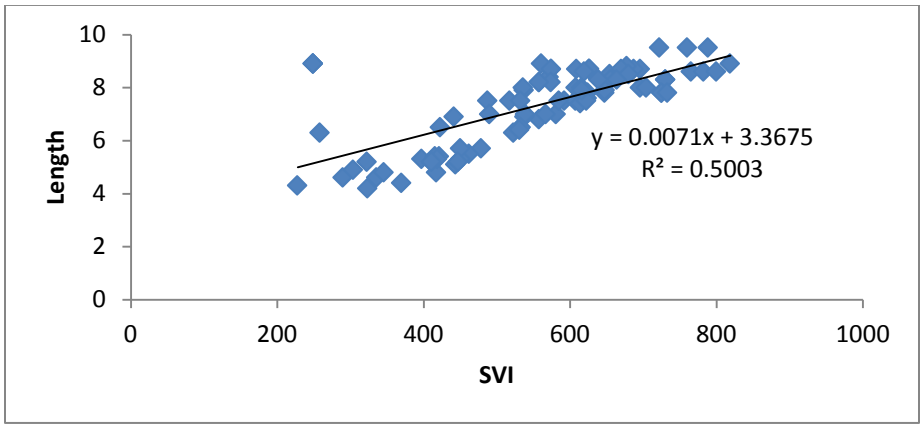


Figure 4.30: Correlations between seedling vigor index and length(Jkuat)

There was a positive and significant correlation between germination and seedling vigor index (Fig 4.31). The correlation between the two variables was strong, r=0.8346, n=8, P<.001

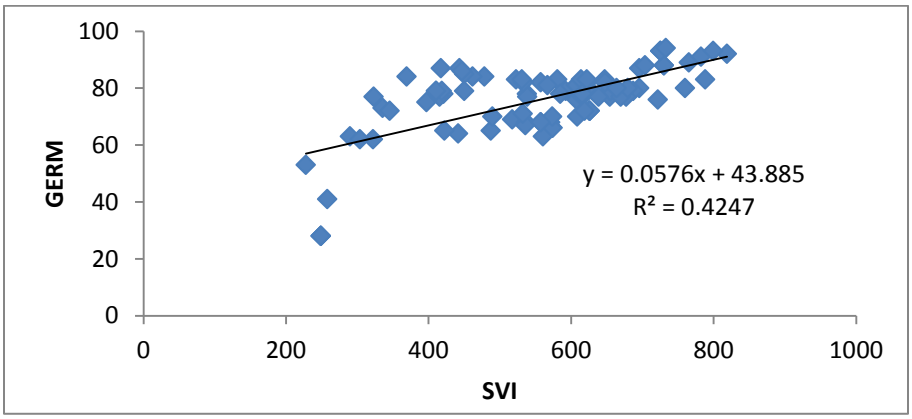


Figure 4.31:Correlation between seedling vigor index and germination

There was a positive and significant correlation between the height at 3 months and days to flowering. The correlation between the two variables was strong, r=0.8346, n=8, P<.001(Fig 4.32).

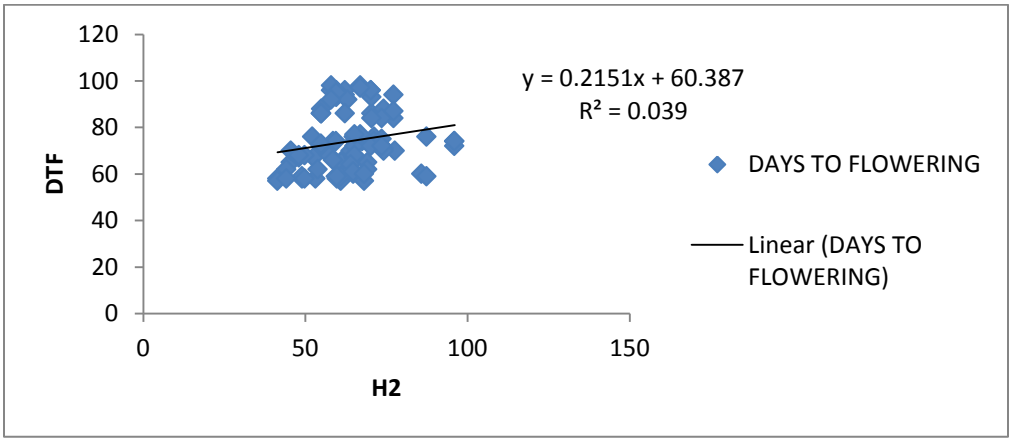


Figure 4.32: Correlation between height 1 month after delivery and days to flowering

There was a positive and significant correlation between the harvest index and 1000 grain weight(Fig 4.33). The correlation between the two variables was strong, r=0.8346, n=8, P<.001

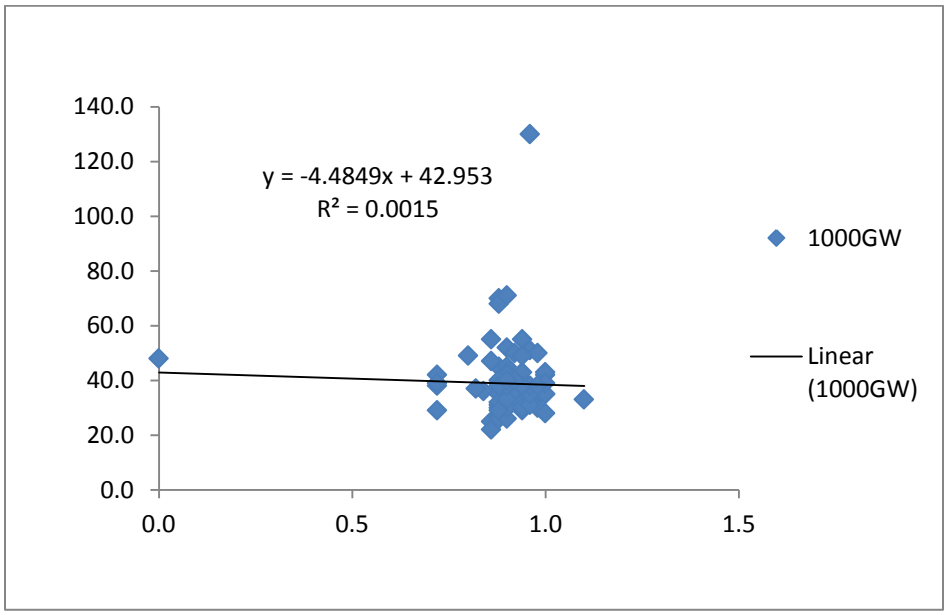


Figure 4.33: Relationship between 1000 grain weight and germination

There was a positive and significant correlation between the biomass and 1000 grain weight. The correlation between biomass and 1000grain weight was strong, r=0.8346, n=8, P<.001, this shows that these are the most important traits associated with 1000 grain weight. There was a positive and significant correlation between the panicle height and 1000 grain weight. The correlation between the two variables was strong, r=0.8346, n=8, P<.001.

Abebaw (2013) showed that seed vigor, growth and yield component traits had positive genotypic correlations ranged from 0.11 to 0.74 of which eight traits showed strong and significant associations and only three traits had negative but non-significant correlations. The investigations showed that germinability at all investigated cereals' seeds were m negative correlation with storage longevity. Seed quality also affects the rate and uniformity of emergence, and on the dynamics of initial plant growth (Mrđa *et al.,* 2010).

4.4 Fingerprinting and studyng relationships between genotypes.

The phylogenetic tree obtained classified the rice genotypes under study into three major clusters I, II and III. In sub cluster two genotypes Durado precise and Pishori were identical with a bootstrap value of 91%. In Cluster I Kenyan genotype B217 clustered with Tanzanian landraces Kahogo and Red afaa.

Major cluster 1 had Durado Precose, Pishori, B217, Kahogo and Red Afaa with Durado precise and pishori grouped together with highest similarity. Similar case of identical genotypes were observed in major cluster II. Mzungu and Ner1 genotypes were identical varieties with a bootstrap value of 66% while Supa saro and ITA 310 were also clustered as identical genotypes with a 53% bootstrap values. Major cluster 11 had Mzungu, Nerica 1, Supasaro, IITA 310, J54 and J64. Generally, major cluster II had the lowest bootstrap value of 10% in the phylogenetic tree.

In major cluster 111, the genotypes found were TXD (SARO5), IR0592, Uzungu, BAS196, IR2193, NER1 and NER10. Major cluster III consisted of two identical genotypes Ner11 and Ner10 in sub cluster IIIb with a bootstrap value of 62%. Tanzanian genotypes Saro 5 clustered with international genotypes as shown in sub cluster IIIa. The bootstrap value included in this phylogenetic tree showed the confidence limits of each clustering (Figure 4.34).

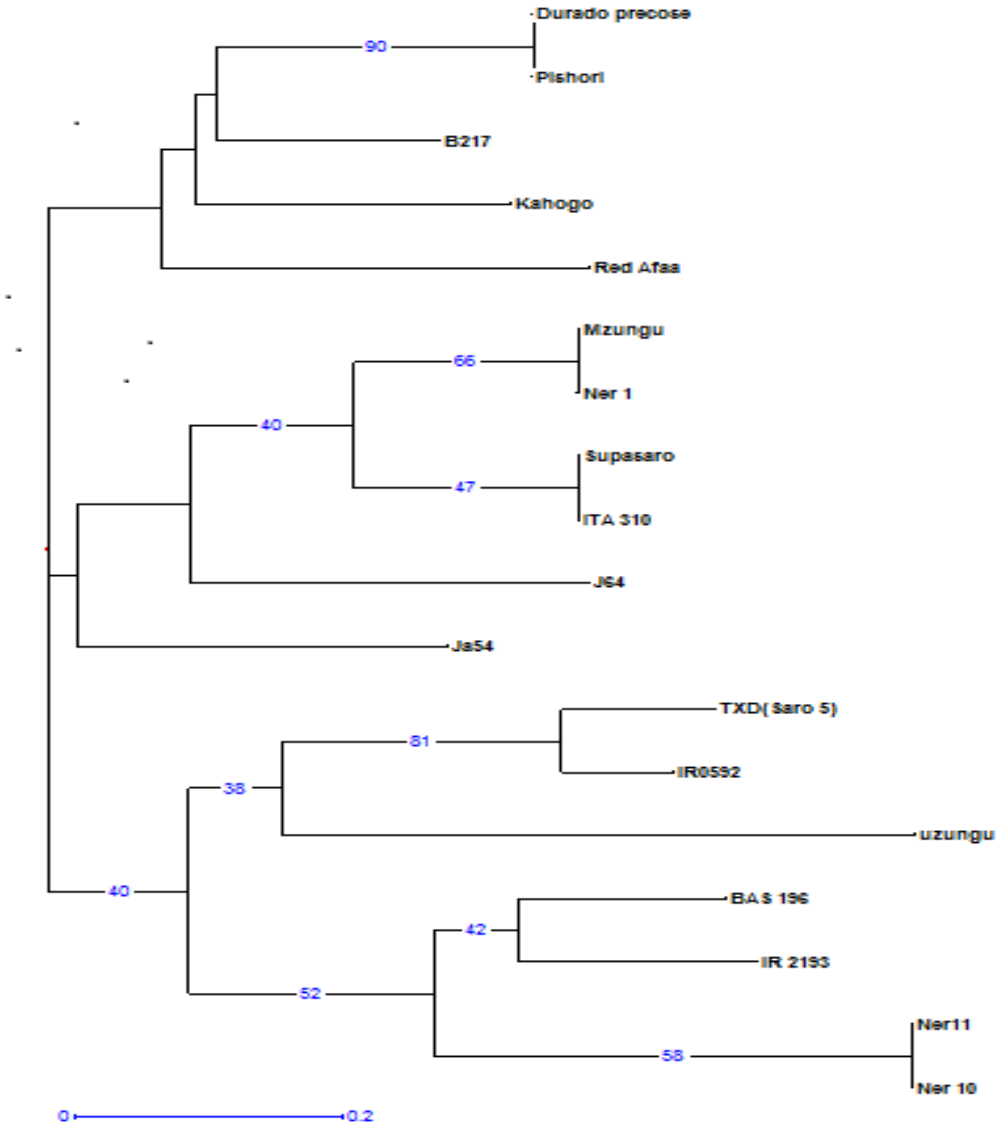


Figure 4.34: Phylogenetic tree showing distance between genotypes

Dissimilarity matrix based on Jaccard's presence/absence index was obtained as shown in table the genetic dissimilarity ranged from 1.0 to 0.2 the highest dissimilarity index of 1.0 was mostly observed between genotype Uzungu against all the other rice genotypes under study. Similar observation was noted between Ner10 and Ner11 genotypes against all the other rice genotypes under study. Least dissimilarity index of 0.2 was observed between IR0592 against TDX (Saro 5) genotypes. In addition dissimilarity index of 0.0 was observed ITA 310 and Supasaro, Ner 10 and Ner 11, Pishori and Durado precise, Ner1 and Mzungu. This dissimilarity index of 0.0 observed showed that the genotypes were molecular replicates (Table 4.18).

Table 4.18: Dissimilarity matrix showing relationship between genotypes

	Uzungu	Ner 1	IR0592	TXD(Saro 5)	Pishori	Durado precose	Red Afaa	Ja54	B217	J64	Kahogo	IR 2193	BAS 196	Ner 10	Ner11	ITA 310	Supasaro
Uzungu	1																
Ner 1	1.00																
IR0592	0.75	0.60															
TXD(Saro 5)	0.80	0.67	0.20														
Pishori	1.00	0.80	0.83	0.86													
Durado precise	1.00	0.80	0.83	0.86	0.00												
Red Afaa	1.00	0.83	1.00	1.00	0.60	0.60											
Ja54	1.00	0.60	0.67	0.71	0.60	0.60	0.67										
B217	1.00	0.67	0.71	0.75	0.40	0.40	0.50	0.71									
J64	1.00	0.67	1.00	1.00	0.67	0.67	0.75	0.75	0.80								
Kahogo	1.00	0.80	0.83	0.86	0.50	0.50	0.60	0.60	0.40	0.67							
IR 2193	1.00	1.00	0.80	0.83	0.75	0.75	1.00	0.80	0.83	1.00	0.75						
BAS 196	1.00	0.80	0.60	0.67	0.80	0.80	1.00	0.83	0.67	1.00	0.80	0.33					
Ner 10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.67				
Ner11	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.67	0.00			
ITA 310	1.00	0.33	0.80	0.83	0.75	0.75	0.80	0.50	0.83	0.50	0.75	1.00	1.00	1.00	1.00		
Supasaro	1.00	0.33	0.80	0.83	0.75	0.75	0.80	0.50	0.83	0.50	0.75	1.00	1.00	1.00	1.00	0.00	
Mzungu	1.00	0.00	0.60	0.67	0.80	0.80	0.83	0.60	0.67	0.67	0.80	1.00	0.80	1.00	1.00	0.33	0.33

4.4.1 Fingerprinting of genotypes using SSR markers

DNA fingerprints were obtained by coding the amplified fragments either 0 or 1.There were 50 polymorphic bands in total gotten from the 5 pairs of SSR primers. Some of the genotypes could be identified as unique using SSR primers. This shows that SSR primers can be used to clearly distinguish the rice genotypes Genotypes TXD and B217 were identified by 4 out of the 5 markers (Table 4.19).

The present study found that SSRs can be used to fingerprint rice DNA and establish the level of relatedness within a population. SSR markers have an advantage over other previously used markers such as Snps due to their cost effectiveness. Successful differentiation of cotton has been achieved using SSR markers (Liu *et.al*, 2006). Genetic diversity studies on Soybean have also been carried out using SSRs to identify soybean species (Gao *et al*). Past studies have found SSRs as useful in crops like cotton (Liu *et al.*, 2006).

Table 4.19: Fingerprinting genotypes using SSR markers

Genotype	Region	Marker 1			Marker 2		Marker 3			Marker 4		Marker 5	TOTAL
		100	150	200	100	150	100	150	250	100	150	100	
Uzungu	TZ	0	0	0	0	0	0	1	0	0	0	0	1
Ner 1	KE	1	0	0	1	0	1	0	0	0	0	0	3
IR0592	KE	1	0	0	1	0	0	1	0	0	0	1	4
TXD(Saro 5)	TZ	1	0	0	1	0	0	1	0	1	0	1	5
Pishori	KE	0	0	0	0	1	1	0	0	0	0	1	3
Durado precise	KE	0	0	0	0	1	1	0	0	0	0	1	3
Red Afaa	TZ	0	0	1	0	1	1	0	0	0	1	0	4
Ja54	INT	0	0	1	1	0	1	0	0	0	0	1	2
B217	KE	1	0	0	0	1	1	0	0	0	1	1	5
J64	INT	0	0	0	0	0	1	0	0	0	0	0	1
Kahogo	TZ	0	0	0	0	0	1	0	0	0	1	1	3
IR 2193	INT	0	0	0	0	0	0	0	1	0	0	1	2
BAS 196	KE	1	0	0	0	0	0	0	1	0	0	1	3
Ner 10	KE	0	0	0	0	0	0	0	1	0	0	0	1
Ner11	KE	0	0	0	0	0	0	0	1	0	0	0	1
ITA 310	INT	0	0	0	1	0	1	0	0	0	0	0	2
Supasaro	TZ	0	0	0	1	0	1	0	0	0	0	0	2
Mzungu	TZ	1	0	0	1	0	1	0	0	0	0	0	3
TOTAL		6	0	2	7	4	11	3	4	1	3	9	50

Five polymorphic markers used in this study showed a total of 11 alleles across the loci of the 18 rice genotype's studied. Alleles per locus ranged from 1 allele in RM 1 and 2 alleles in the other 4 SSR markers used with a cumulative average of 1.8 alleles. Gene diversity a parameter used to show expected heterozygosity ranged from 0.191 in RM 261 to 0.5 in RM 22 with an average of 0.336 across the 5 microsatellite markers used. Observed heterozygosity showed a value of 0.0 across all the markers used on the other hand inbreeding coefficient value of 1.0 was consistent across all the markers used. Polymorphic information content a parameter that shows informativeness of a marker ranged from 0.169 in RM 261 to 0.375 in RM 22 with an average of 0.268 across all the markers. Major allele frequency ranged from 0.889 in RM 261 to 0.5 in RM 22 with an average of 0.735 across the SSR markers used (Table 4.20).

Table 4.20: Allellic frequency and genetic diversity using SSR markers

Marker	Major Allele Frequency	Genotype No	Allele No	Gene Diversity	Heterozy gosity	PIC	F
RM 16	0.852	1.667	1.000	0.214	0.000	0.175	1.000
RM 459	0.694	2.000	2.000	0.410	0.000	0.324	1.000
RM 168	0.741	2.000	2.000	0.366	0.000	0.296	1.000
RM 261	0.889	2.000	2.000	0.191	0.000	0.169	1.000
RM 22	0.500	2.000	2.000	0.500	0.000	0.375	1.000
Mean	0.735	1.933	1.800	0.336	0.000	0.268	1.000

Table 4.21: Analysis of molecular variance

Source	Df	SS	MS	Est. Var.	%	P-value
Among Pops	2	4.375	2.188	0.071	4%	
Within Pops	15	26.625	1.775	1.775	96%	
Total	17	31.000		1.846	100%	<0.001

Analysis of molecular variance gives a summary on the distribution of variations among and within populations. The (Table 4.21) below shows that 96% ($P<0.001$) of the total variations observed are attributed to within populations while among population accounts for 4% ($P<0.001$) of the total variation observed.

4.4.2 Principle Coordinate analysis (PCoA)

Principle coordinate analysis (PCoA) was done to show the genetic dissimilarities amongst the rice genotypes studied. The first principal coordinate accounted for the highest variation observed at 39.18% while second principal coordinate accounted for 23.48% of the total variation observed. The two dimensional plot analyses genetic relationships using four quadrants. The first quadrant consisted of international Genotype J64. Also there was the ordination of Supasaro and ITA310 in the same location. In the second quadrant similar case of ordination of rice in same space is observed between Ner 10 and Ner 11. This quadrant also contains a Kenyan rice genotypes BAS 196 along with Uzungu from Tanzania and IR 2193. Quadrant three showed Durado precose and pishori both Kenyan varieties ordination in the same space. Red afaa, a Genotype from Tanzania ordinated far away from the rest of the rice varieties.Quadrant four showed ordination of two Tanzanian genotypes Mzungu and Ner 1 in the same space along with two other genotypes IR0592 and Saro 5(Fig 4.35).

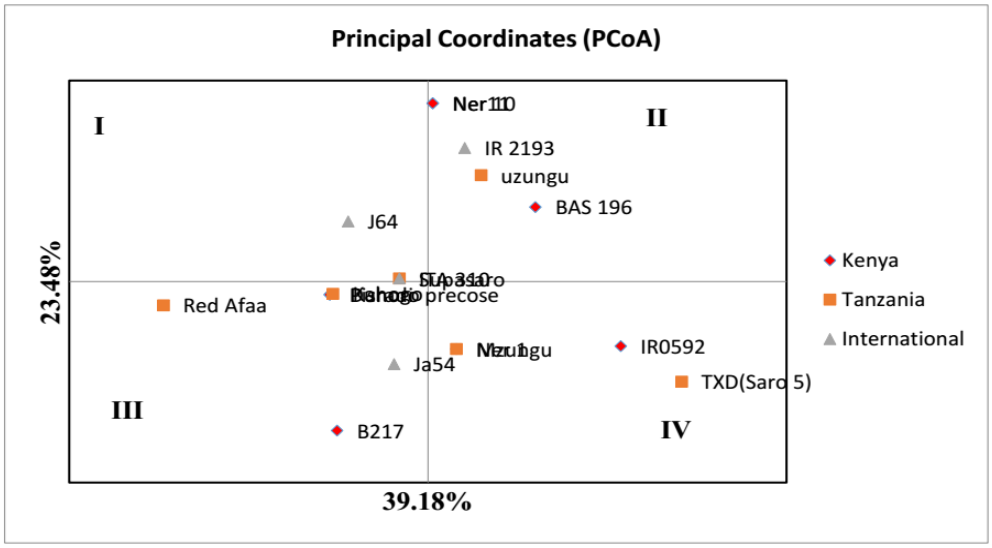


Figure 4.35: Principle coordinate analysis

Gene diversity is also referred to as expected heterozygosis. Rice genotypes studies showed relative heterozygosity. The value obtained was higher than an average of 0.358 that was obtained from a study on 300 rice accessions as reported by Chen *at al*. (2011). This value was also lower than 0.4181 obtained in diversity studies on selected Kenyan and Tanzanian rice based on gel consistency and alkali digestion (Chemutai *et al*., 2016). The low gene diversity value obtained is attributed to presence of a common gene pool amongst the rice varieties studied. Observed heterozygosity was lower than the expected heterozygosity this is attributed to the high inbreeding coefficient. This is evident by the inbreeding coefficient of 1 attained in this study. Similar conclusion has been reported by Musyoki *et al*. (2015). This is attributed to genetic improvement of rice using a common gene pool hence lowering the gene diversity.

The PIC is used in determination of allele diversity and it has variation between loci. Data obtained from PIC is used in the construction of a linkage map (Lapitan *et al*., 2007). This is divided into three categories; PIC values of the markers range between 0 and 1. PIC values greater than 0.5 are highly informative, ranging between 0.25 and 0.5 are relatively informative and less than 0.25 are less informative (Bostein, 1980). Based on this the most informative marker in this study was RM 22. Data on allele number reported in this study are less than an average of 6.3 alleles with a range of 2 to 11 alleles per locus reported in Pakistani Basmati and non-basmati varieties by Yu *et al*. (2003). Findings attained in this study varied from those reported in earlier studies given the different selection of rice genotypes and the use of a different set of SSR markers.

The high genetic dissimilarity index observed between *Ner 10*, *Ner 11* and *Uzungu* genotypes against all the other genotypes implies that these genotypes have an uncommon origin. These wide genetic variation can be attributed to genetic divergence that these genotypes have undergone in the course of crop improvement. For instance the *Ner 10* and *Ner 11* genotypes were developed through conventional hybridization (Kushwaha, 2016). Results showing no dissimilarity (0.00) have a common ancestry and can be referred to as genetic replicates. The low genetic dissimilarity observed can be attributed to possibility of introgression of similar traits during crop improvement hence presence of a common ancestry. This study adopted the neighbor joining method unlike the unweighted pair group method using arithmetic average (UPGMA) given it doesn't assume a rigid molecular clock and a similar evolutionary are neighbor joining phylogenetic tree generated revealed genetic relatedness based on the five microsatellite markers. The divergence observed by clustering of the rice genotypes into three major

clusters is indicative of genetic divergence. The long branches of the Ner 10, Ner 11 and Uzungu justifies the high dissimilarity index observed in the dissimilarity matrix. The long branched observed signifies that these genotypes have undergone much evolutionary change compared to other genotypes under study.

Bootstrap values give confidence limits to observed classification. Branches that has values more than 50% had the most statistically supportive values on the phylogenetic branch generated. Similar conclusions on acceptable values on the bootstrap values were reported in Pakistani rice genotypes by Pervaiz *et al*. (2010). AMOVA table was generated to account for the low bootstrap values that were observed in some of the phylogenetic branches. The low bootstrap values were as a result of the low redundancy in genetic dissimilarity data which means that there was a considerable variation in the rice genotypes studied. This is evident by the high within population variation. This means that within the Tanzanian, Kenyan and International rice genotypes there exist a high variation in each population compared to the low variation amongst these population (Boller, 2005). High within population variation is also elaborated by the high dissimilarity index values observed in the dissimilarity table. These finding are similar with observations made by Chemutai *et al*. (2016) in Kenyan and Tanzanian rice genotypes and in Indian teak (Ansari *et al*., 2012). The PCoA on the other hand complemented observations made in earlier visualizations of genetic structure. Genotypes that ordinated closer together had more similarity competed to those located far from each other. Based on this, principal coordinate 1 accounted highest variability compared to the second coordinate.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

Conclusion

There was variation in quality of genotypes with respect to germination and vigor (B370, 0hrs). Seed age affects the seed vigour and seed quality. The coleoptile and coleorhiza formation, germination percentage decreased with the ageing time with standard germination 0 hours showing highest performance while 72 hours had the lowest performance. Similarly there were differences in quality and purity across genotypes.

Seed storage time affects germination, seedling vigor index, plant height and 1000 grain weight , while genotype affects germination, seedling vigor index, days to flowering and interaction between storage time and genotype affected the days to flowering, 1000 grain weight, biomass and yield. The performance at 3 and 4 months of storage was leading in most parameters for both sites.

Seedling vigour which is a juvenile character had a significant correlation with 1000 grain weight. Height at transplanting had a positive correlation with grain number.which is an adult character. There are significant correlations between juvenile and yield components, which influences growth and productivity of crops.

The data indicated the occurrence of relatively high gene flow and elevated rates of admixture between cultivars grown in remote regions, probably favoured by local breeding activities. The results of this study significantly expand the current genetic

resources available for temperate varieties of rice, providing a valuable tool for future association mapping studies.

Uzungu genotype was the best in the fingerprinting since only one marker was able to identify it. It is therefore possible to identify a genotype using SSR markers successfully. We therefore reject the null hypothesis.

Recommendation

Accelerated ageing tests are useful in increasing the accuracy of predicted field emergence. The present research has shown that there is actual variation in vigor of seeds of different varieties which results in variation of field performance later on. The seed industries consider this test when screening genotypes for optimum field performance.

The study recommends that rice seeds should not be srored beyond 3 to 4 months as the viability decreases afterwards.

Juvenile characters such as seedling vigor and height at transplanting were found to be highly correlated with 1000 seed weight. I therefore recommend that farmers should effect agronomic practices that enhance seedling vigour and height at transplanting in order to realize optimal yields.

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APPENDICES

APPENDIX 1

Correlation Coefficients among agronomic traits recorded in Mwea

	1000GW	DAYS_TO_FLOWERING	GN	HEIGHT_2	HEIGHT_3	HI	Length	TOTAL_GN
AVG_PNH	-0.0865							
BM	0.4081***							
GERM	0.1806	-0.0545						
GW	0.5357	0.1102	0.5148					
GWIDTH	0.6081***	0.1694	-0.0612					
HEIGHT_1T	0.3305*	0.6341*	0.0089					
HEIGHT_3	0.0289	0.0393	0.1442	0.5155				
HEIGHT_M3	-0.0811	-0.0898	-0.0164	0.4007	**0.5183**			
HI2	0.1665	0.1743	0.6607	0.2358	**0.264**	**0.5249**		
LEAF_WIDTH	0.046	0.019	0.0079	0.3437	**0.312**	**0.1076**		
Length	0.1581	0.1411	0.051	0.4233	**0.2159**	**0.1621**		
SVI	0.2341**	0.0692	0.1061	0.2303	**0.1053**	**0.2182**	0.7073	
TOTAL_GN	-0.1066	-0.0179	0.6687	-0.0598	**0.0934**	**0.7893**	0.13	-
yy2	-0.1768	-0.2554	-0.2144	-0.0412	**-0.1581**	**-0.0055**	0.1007	0.088
	1	4	7	12	**13**	**15**	19	22

%1000GW DAYS_TO_FLOWERING GN HEIGHT_2 HEIGHT_3 HI Length TOTAL_GN

Correlation coefficients among agronomic traits recorded in JKUAT

		1000GW	DAYS_TO_FLOWERING	GERM	GN	GRAIN_FILLING	GW	HEIGHT_2	HEIGHT_3	HI	Length
%1000GW	1	-									
BM	3	0.4081									
GRAIN_FILLING	8	-0.0583	-0.1733	0.1433	-0.1613	-					
GW	9	0.5357	0.1102	0.1845	0.5148	-0.0969	-				
GWIDTH	10	0.6081	0.1694	0.0816	-0.0612	-0.1527	0.2556				
HEIGHT_1T	11	0.3305	0.6341	0.0298	0.0089	-0.0575	0.289				
HEIGHT_3	13	0.0289	0.0393	-0.0648	0.1442	-0.0624	0.1116	0.5155			
HEIGHT_M3	14	-0.0811	-0.0898	0.0095	-0.0164	-0.0765	-0.0356	0.4007	**0.5183**		
HI	15	0.2417	0.1161	0.1623	0.4973	-0.1412	0.7881	0.038	**0.1613**	-	
HI2	16	0.1665	0.1743	0.11	0.6607	-0.0531	0.4994	0.2358	**0.264**	**0.5249**	
NO_OF_TILERS	20	-0.0065	-0.0709	-0.0279	-0.0767	-0.0642	-0.1039	0.1122	**0.104**	**-0.1394**	0.0804
SVI	2	0.2341	0.0692	0.6517	0.1061	0.125	0.2514	0.2303	**0.1053**	**0.2182**	0.7073

	1											
TOTAL_GN	22	-0.1066	-0.0179	0.0942	0.6687	-0.0848	0.7687	-0.0598	**0.0934**	**0.7893**	0.13	
YIELD_YY	24	0.0836	0.0454	-0.0603	0.0561	0.0459	-0.4444	0.0731	**0.0697**	**-0.5493**	-0.1375	

Assessing Seed Vigor Characters of Selected Rice (*Oryza sativa* L.) Genotypes Using Accelerated ageing method

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Abstract
Rice (*Oryza sativa* L.) is an annual grass with the third highest world production after sugarcane and maize, However due to losses in vigor caused by poor seed storage, global consumption has surpassed production. Preservation of germplasm both in-situ and ex-situ is kea to the conservation of rice biodiversity. Seed vigor is a key element of seed quality and high vigor seeds gives uniform plants stand and higher yields per area Accelerated ageing tests enable testing the vigor of stored seeds by subjecting the seeds to a particular temperature and relative humidity over time and then performing standard germination tests. Viability of most seeds normally reduces with the storage period, storage temperature and relative humidity. There is limited information on the duration of storage for rice seeds. Accelerated aging is considered an excellent option as a vigor test when compared to seedling emergence and index of emergence speed because the shortest time of acquisition and efficient results Accelerated ageing tests at 0,24,48 and 72hrs(45^{o}C and 98%RH) were carried out JKUAT post-harvest laboratory using eight rice varieties' in four replications of100 seeds each. Data was collected on dates to sprouting dates, plumule and radical emergence and height. Data was analyzed using GE NSTAT statistical package. ANOVA and T tests at 5% significant level. Results showed that There was significant variation in both coleorhiza and coleoptile formation among rice varieties ($p<0.001$), treatments ($p<0.001$) and interaction between rice varieties and treatment (($p<0.001$). The difference between all treatments was significant with 72hours treatment having the highest number of days to coleorhiza and coleoptile formation. The present study has shown that prolonged duration of higher temperatures results in reduction of seed viability. The earliest coleorhiza formation was observed on day 2 and the latest on day 6.Results from this study will guide farmers and seed processors on considerations regarding storage period and storage temperature to ensure high quality seeds.

Key words: Rice; seed quality, accelerated ageing seed vigor,

Fingerprinting and Accessing Relatedness of Selected Rice (*Oryza sativa* L) Genotypes in Kenya

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Abstract: Rice is becoming an important food and cash crop in East Africa and is second to maize in terms of consumption. Genetic markers are very useful in managing germplasm and are greatly applied in biotechnology as breeding machinery in gene mapping and fingerprinting. Eighteen Rice accessions including landraces grown by the farmers and commercially released cultivars were used to assess the genetic diversity. Leaves were taken from two weeks old plants and the DNA extracted according to Mace et.al., 2003. PCR was done and DNA fragments visualized by illumination device with UV light. . SSR bands were scored as present (1) or absent (0) for each DNA sample, and used to compute the measures of genetic distance for all pairs of individuals. Analysis of Molecular Variance. Results indicated that the five polymorphic markers used in this study showed a total of 11 alleles across the loci of the 18 rice genotype's studied. . The expected heterozygosity ranged from 0.191 in RM 261 to 0.5 in RM 22 with an average of 0.336. Genotypes TXD and B217 were identified by the 5markers

Key words: DNA, Fingerprinting, Microsatellites, Fingerprinting, Rice

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