EFFECTS OF VARIETY AND STORAGE METHODS OF CASSAVA PLANTING CUTTINGS ON ESTABLISHMENT AND EARLY GROWTH VIGOUR

\mathbf{BY}

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DEDICATION

I dedicate this work to my wife and son for their prayers and encouragement while was away from home for this study. I also dedicate it scale small scale famers who struggle to a get single meal per day.

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LIST OF ABBREVIATION AND ACRONYMS

% Percentage

ANOVA Analysis of variance

CGIAR Consultative Group on International Agricultural Research

cm Centimetre

Cr Storage reserves

CUDS Clamp under doable shade

FAO Food and Agriculture Organization of the United Nations

FAOSTAT Food and agriculture organization of the United Nations statistics division

GenStat General Statistics

HUOG Horizontal under open ground

HUS Horizontal under shade

K Potassium

Kg Kilogram

Lp Leaves and petioles

LSD Least Significant Difference

MAP Months after planting

N Nitrogen

°C Celsius degrees

OECD Organization for Economic Co-operation and Development

P Phosphorus

Rf Fibrous roots

RH Relative humidity

Rs Storage roots

Sb Stem and branches

USDA United State Department of Agriculture

VUS Vertical under shade

WAP Weeks after planting

WAS Weeks after storage

ABSTRACT

Storage of cassava planting materials has been a challenge due to properties of losing moisture and carbohydrates loss under storage for more than two months. Objective of the study was to contribute to effective storage of cassava cutting for improved crop establishment and food security. Two varieties of cassava cuttings 1 m long, Karembo & KME4 were stored for four months under four different storage methods in two locations Kabete and KARLO Kiboko. The storage methods were clamp and double shade (CUDS), horizontal under shade (HUS), vertical under shade (VUS) and the control horizontal under open ground (HOUG). In each storage method hitag 2 xsense data loggers were installed to record data on temperature and RH. Percentage carbohydrate, moisture content (MC), 100% dry cuttings (DC) and cuttings dried to 25% or more of its stored length but not 100% were measured at intervals of 4 weeks (0, 4, 8, 12 and 16). Data were subjected to analysis of variance (ANOVA) using GenStat and mean separated using LSD. CUDS in both locations had low temperature and higher RH 18.78 °C, 72.07% in Kabete, respectively and 24.99 °C, 60.13% in Kiboko, respectively. This could explain why the storage methods performed better than the rest. The higher temperature and lower RH recorded under control (HUOG) at Kabete 21.13 °C, 61.89% respectively and 28 °C, 40.91 % at Kiboko respectively further supports this argument. The results showed less desiccation to stored cuttings in CUDS than those stored in HUOG. The moisture loss in CUDS was from 70.16 % -56.69 % while that of HUOG dropped from 70.16 % to 27.26 %, 8 weeks after storage (WAS). Also the results showed that temperature and RH have effects on carbohydrate loss of stored cuttings. In Kiboko stored cuttings lost more carbohydrate than cuttings stored in Kabete with difference in loss of 0.99 (LSD = 0.18). The results have proven that safe storage of cassava planting material is affected by plant related factors such as cultivar as well as environmental conditions such as temperature, RH and radiation.

Sampled cuttings from each storage methods were taken to field in the same locations to evaluate their sprouting ability, number of primary shoots formation, number of leaves, rate of leaf formation and early growth vigour at 8 WAP. From stored cuttings 10 cm from each end was discarded and the remaining 80 cm was cut into 20 cm cuttings having 4-7 nodes each. The trial was split plot design in RCBD with main plot as storage method and sub plots were varieties replicated three times. The sprouting test was done at interval of 4 weeks (0, 4, 8 12 and 16 weeks after storage). The cuttings were planted at 60° slanting position and irrigated three days per week to maintain field capacity soil moisture levels. Data were subjected to analysis of variance (ANOVA) using GenStat and means separated by LSD. The results showed that storage methods, variety and duration of storage were highly significant (p > 0.01) between treatments. The results also showed significant differences in storability between varieties KME4 and Karembo and number of primary shoots per plant (ANPS). Kabete had 1.60 ANPS compared to Kiboko with 1.04. This implies temperature influences carbohydrate loss in stored cuttings and it affects early growth vigour of cassava sprouts from the planted cuttings. Also results shown that number of leaf formation per day was higher in Kiboko than Kabete.

From this study cassava planting material were sensitive to environmental conditions especially temperature and RH, during storage. Thus, optimum temperature and relative humidity should be factored in cassava cuttings storage to avoid increased death of stored cuttings. Where possible cassava cuttings should be plated immediately or few days after harvest to avoid loss of carbohydrate and moisture which occurs in storage particularly when stored for more than 8 weeks as it affects early growth vigour and number of primary shoots which has an effect on the final crop production.

Keywords: carbohydrate, cassava cuttings, cassava planting materials, moisture, storage methods

CHAPTER ONE

1.0 INTRODUCTION

Cassava (*Manihot esculenta* Cruntz) is a tropical, perennial woody plant with height ranging from 1 m to 4 m. It is traditionally grown on low fertile soil for their starchy roots, with no or little application of inputs. Although cassava is a perennial crop, the roots can be harvested from 6 months after planting (MAP) to 24 MAP depending on climatic conditions and cultivar (Alves, 2002). In humid low land tropics, it can be harvested 6-7 MAP while in prolonged cold and drought farmers harvest cassava after 18- 24 MAP (Alves, 2002). Cassava belongs to the order malpighiales, family euphorbiaceae, genus manihot and species *esculenta* Crantz. It has sub species namely *M. esculenta* Crantz ssp. *flabellifolia* (Pohl) Cifferi and *M. esculenta* Crantz ssp. *Peruviana*. Between the three subspecies of cassava, *Manihot esculenta* ssp. *esculenta* is the cultivated strain while *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana* are wild species (Chavarriaga-Aguirre and Halsey, 2005; Alvis, 2002; OECD, 2014). Other close relative of *Manihot esculenta* ssp. *esculenta* is *M. pruinosa*, those three close relative to cultivated cassava are capable of interbreeding. Studies indicate that the cultivated species originated from the South Brazilian Amazon (FAO, 2013).

Cassava is one of the leading food and feed crops in the world. According to USDA (2003) cassava ranks fourth among staple crops, with a global production of about 160 million tons. Alves, (2002) suggested that cassava ranks number six of the most important source of calories in human diet. The crop is grown in over 90 countries and in the tropical developing countries, it is the most important source of calories after maize and rice. It is staple food for half a billion people in Africa, Asia and Latin America (CGIAR, 2015; Halsey *et al.*, 2007). The starchy root of cassava is mostly used as food by small farmers in developing countries (Halsey *et al.*, 2007).

Moreover, cassava has become an industrial crop, where it provides raw material, for example, in the production of ethanol and starch for industrial uses (Banito *et al.*, 2010; FAO, 2013). As such increased food demand due to the rapidly growing population as well as demand for raw material for the industrial processing has led to an expansion of cassava cultivation in many African countries (Banito *et al.*, 2010) which has led to increased cassava production globally (Figure 1). According to Vietnam Ministry of Industry and Trade report (2014) the export of cassava and cassava products reached 3.1 million tons with a turnover of \$ 1.1 billion, 25.7% up in volume and 18.6% in value. Vietnam produces cassava yield of 17.6 t ha⁻¹ and currently is among the 10 highest producing countries in the world. Production potential of cassava is 80 t ha⁻¹ as compared to world average production of 12.8 t ha⁻¹ (FAO, 2013).

The world cassava production was approximated as 277 million tons in 2013 (Figure 1), while in Africa it was 91 million tons, contributing to 51% of the world production, in the same year Asia and America produced 32% and 17% (Figure 2) of the world production respectively (FAOSTAT,

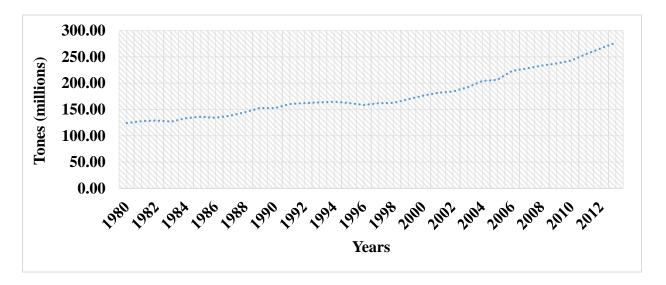


Figure 1: Production of cassava globally from 1980-2013

Source: FAOSTAT, 2015; downloaded on Tuesday Mar 31 2015 at 21:15:34

According to FAO, (2009) demand for food continues to grow as a result of population increase from 7 billion to 9 billion. The projection indicated increased food production by 70% from 2005/7 to 2050 (FAO, 2009). The highest growth was projected in Sub-Saharan Africa of +144% (FAO, 2009). Africa's growing demand for food has been met increasingly by imports from the global market (World Bank, 2012) even when Africa continent has enormous potential, to feed itself and eliminate hunger and food insecurity, as well as become major player in global food markets. Cassava has ability to increase production from the present world average of 12.8 to 80 t ha⁻¹. According to Alexandratos and Bruinsma, (2012) cassava has more non-food uses like feed and production of biofuel.

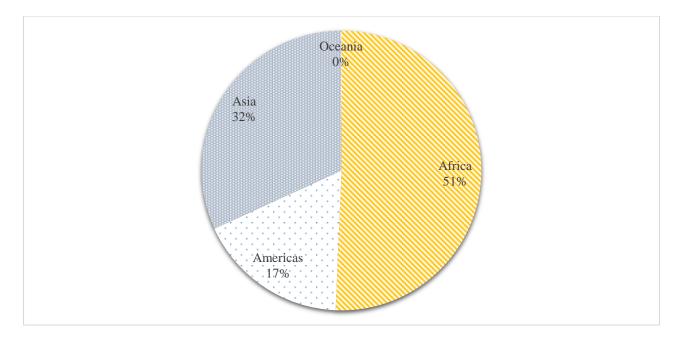


Figure 2: Production of cassava by region from 1980 - 2013

Source: FAOSTAT, 2015, Date downloaded: Tuesday March 31 2015, at 21:15:34

Cassava differs from the other major root crops in that its edible roots do not undergo dormancy and do not have a natural function in the preservation of the planting material during the dry season (Lancaster and Coursey, 1984). *Manihot esculenta* Cruntz fits well for cultivation by

smallholder farmers in low fertile soils, low or no input application and with low or unpredictable rainfall. Thus the crop indirectly presents a relief to subsistence farmers faced with high economic, political, and environmental difficulties (Ekwe and Njoku, 2011). Also, the plant is highly tolerant to acid soils, and forms symbiotic association with soil fungi that help its roots to absorb phosphorus and micronutrients (FAO, 2013). The cyanide in leaves are removed through cooking and breeding program are making varieties with low content of cyanide. As a self-defence mechanism against herbivores, it produces two glycosides in its leaves which, when digested, produce highly toxic hydrogen cyanide (FAO, 2013). Due to its efficient use of water and soil nutrients, and tolerance to sporadic pest attacks, farmers, use none or few inputs, and harvest reasonable yields where other crops fail (FAO, 2013).

1.1 Statement of the problem and justification

Cassava production depends on a supply of quality stem cuttings at the onset of rains. One disadvantage with vegetatively propagated crops is that, diseases can build up over several generations of propagation which results in drastic yield reduction over the growth seasons (FAO, 2013). FAO (2010) through strategic plan 2010-2015 for cassava diseases in Central, East and Southern Africa (CaCESA) showed that there is desperate need for clean cassava planting material of improved cassava varieties.

Cassava under subsistence agriculture is harvested piecemeal over a period of one year or more (FAO, 2013). Cassava is mainly grown as a staple subsistence crop in the developing countries. In the tropics it grown using vegetative propagation system. Thus, research in the agronomy, and genetic improvement of the crop and seed quality has been neglected by scientists and commercial institutions in industrialized countries (Halsey *et al.*, 2007). Consequently, the multiplication rate of planting materials is very low (6-10 cuttings per stem per year) compared to

grain crops propagated using true seeds. In addition, cassava stem cuttings are bulky and highly perishable as they dry up within a few days (Otoo, 1996). These reasons cause cassava cuttings to be very expensive as compared to true seeds (Table 1). As a result, farmers don't have alternative than using the cuttings that are secondary output from fields intended for tuber production (FAO,2010).

Table 1: Price of cassava cuttings as compared to price of maize seeds ha⁻¹

Crop	Area (Ha)	Quantity of seed	Price (Ksh)	Total
Cassava cuttings (30 cm)	1	10,000 cuttings	3	30,000.0
Maize	1	25 kg	110	2,750.00

Source: National Cereals and Produce Board of Kenya Date downloaded: Tuesday March 31 2015.

In cassava production, problem of stem storage arises when harvest and subsequent plantings are separated by time for several months due to drought, low temperature or floods (Leihner, 1982). The quality of the stems cuttings can be highly affected, especially under conditions of prolonged storage. The time lag between the initial harvesting of cuttings and their transportation to their destination planting sites range from one day to two weeks. This duration is influenced by the availability of harvesting labour to efficiently cut and package planting material, the availability of transport, and the financial capacity of the farmer (Table 2). The harvesting and post-harvest handling of the stem cuttings may expose them to moisture loss, pests and disease consequently affecting sprouting, rooting and growth vigour (CIAT, 2007). In areas with long dry or cold seasons stem storage of cassava is a challenge to the farmers. Thus, lack of quality, enough and timely planting material is one of the most important limiting factor for cassava production worldwide (Velásquez, 2006).

Table 2: Rate of loss of cassava cuttings in Burundi from cuttings harvesting to their planting 20 days' rate.

Partner	Area Planted	Cuttings Received	Loss at Reception	Percentage Loss at Reception	% sprouting
	(Ha)		P		
BDD Bubanza	30	300,000	166,000	55.3%	88
Caritus Belgique	10	100,000	43,181	43.2%	83
BDD Ngozi	10	100,000	20,500	20.5%	90
BDD Muyiga	10	100,000	34,000	34.0%	68
CRS Kirundo	48	480,000	71,000	14.8%	90
BDD Ruyigi	38	380,000	24,000	6.3%	93
Total	146	1,460,000	358,681	24.6%	85.3

Source: Crop Crisis Control Project (C3P) brief 4, 2007

1.2 Objective

1.2.1 Overall objective

The objective of this study was to contribute to efficient storage of cassava planting materials and establishment of cassava crop for improved food security

1.2.2 Specific Objectives

- To determine the effect of storage methods on carbohydrate and moisture reduction in cassava planting material.
- To determine effect of storage methods and varieties of planting material to establishment and growth vigour.

1.3 Hypothesis

- Carbohydrate and moisture reduction of cassava planting material are not influenced by storage methods.
- Different cassava varieties under different storage methods of planting cuttings do not respond differently on establishment and growth vigour.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General description

Cassava (*Manihot esculenta* Crantz) is among the 100 species of trees, shrubs and herbs of the genus Manihot, (FAO, 2013). It has palmate leaves bearing 3 to 9 lobes (Plate 1) and covered with a shiny, waxy epidermis (OECD, 2014).



Plate 1: Cassava plant having (a) 5 and (b) 7 lobes leaves

It is an amphidiploid or sequential allopolyploids with 2n = 36 chromosomes, and undergoes cross pollination (El-Sharkawy, 2004). The crop is cultivated in an area of over 13 million hectares and used as a staple food and animal feed in tropical and sub-tropical Africa, Asia and Latin America within 30° N and 30° S, and about 70% of the production is found in Africa and Asia (El-Sharkawy, 2004). The edible part is the storage roots and its dry matter contain more than 80% starch but with very low protein content (El-Sharkawy, 2004). The plant is grown from stem cuttings and adventitious roots are developed that arise from basal cut of the stem cutting, sometimes roots develop from the bud buried in the soil (Alves, 2010).

During the first two months, the cassava plant mainly develops shoots (stems and leaves) and a fine root system. The development of storage roots begins with secondary growth in several fibrous roots which range from 3 to over 15 storage roots plant ⁻¹, depending on cultivar type and growth stage. The starch deposition occurs at about 25–40 days after planting in many cultivars (El-Sharkawy, 2004)

2.2 Reproductive biology

The cassava plant is monoecious and bears separate male and female flowers on the same plant (Halsey *et al.*, 2007; Chavarriaga-Aguirre and Halsey, 2005). The time interval from planting to flowering depends on the specific genotype and environmental conditions, and may vary from 1 to more than 24 months (Halsey *et al.*, 2007, Chavarriaga-Aguirre and Halsey, 2005). Nunekpeku *et al.*, (2013) observed that onset of flowering generally occurred between 2–4 months after planting. Male and female flowers are borne on the same branched panicle (Plate 2). The flowers are small, with the male flower being about 0.5 cm in diameter and the female flower slightly larger (Halsey, 2005) (Plate 3). A flower bud typically forms where the plant branches, so that more-highly-branched genotypes flower more than the sparsely branched (OECD, 2014). According to OECD (2014) the influence of environment to flowering particular genotype is high, it can happen the plant may flower at one location, while in other location will not flower, a particular clone may not flower at all across all environment, sometimes in other environment produce aborted flowers, or produce numerous flowers and set seed in another environment.



Plate 2: (a) an open staminate flower ready for pollination (b) fertilized pistillate flower and has formed fruit already.

The female flowers open for approximately one day, and the stigma is receptive throughout that time (Alves, 2002). Fertilization occurs 8 to 19 hours after pollination (OECD, 2014). Cassava has protogyny (Plates 3), pistillate flowers opening one to two weeks before the staminate flowers on the same inflorescence (Alves, 2002; OECD, 2014).

Cassava plant has more than one inflorescence and thus different flowers in the same plant open at a time and self-pollination occur. The major pollination agent in cassava are insect, thus it results to high outcrossing that leads to a high heterozygous F1 plants. Female flowers are ready for pollination 15 days after floral initiation. An indication of receptivity is the presence of a drop of nectar within the flower (Halsey et al., 2008).



a) Female flowers



b) Male flower

Plate 3: Cassava male and female flowers

2.3 Seed characteristics

Developing seeds are viable 2 months after pollination, and the fruit becomes mature about 3 months after pollination (Chavarriaga-Aguirre and Halsey, 2005; Halsey *et al.*, 2007). Seed production and viability are variable, depending largely on the vigour and number of flowers borne by the parent plant (Halsey *et al.*, 2007). Cassava seed is subject to a dormancy period of various lengths, depending on the genotype. Seeds falling to the soil become dormant, forming seed banks from which plants may germinate (OECD, 2014). Seeds can remain viable when stored under ambient conditions for up to one year, although germination percentages may decline substantially after six months (OECD, 2014).

Botanical seed is not typically used for commercial propagation. Genetically, any particular cassava genotype is extremely heterogeneous, and propagation from sexual seed results in wide and unpredictable diversity of phenotypes (Chavarriaga-Aguirre and Halsey, 2005). Establishment and survival of seedlings of different parents ranged between 33.5 and 53.7% when seeds were sown in situ compared to 90.0% establishment among clones (Rajendran *et al.*,

2005). Competition from weeds and unfavourable soil conditions during early stage of seed germination resulted in poor establishment and survival of seedlings (Rajendran *et al.*, 2005). Propagation of cassava is typically accomplished through vegetative cuttings in order to preserve the known characteristics of favoured lines.

2.4 Vegetative propagation

Cassava has the tendency for natural inter-varietal and interspecific hybridization, thus cassava varieties are effectively preserved through vegetative propagation (Chavarriaga-Aguirre and Halsey, 2005). Propagation of cassava through seed is feasible even if it is highly heterozygous, but no viable seed propagation system is yet available (Leihner, 2002). The main constraint for vegetative propagation of cassava is the rapid loss of viability of stems under storage, due to difficulties protecting the voluminous planting stems from bad weather, insect and non-insect pests and diseases, desiccation, bruising and peeling (Rajendran *et al.*, 2005; OECD, 2014). Traditionally, cassava is propagated vegetatively using 15-30 cm cassava stakes with 7-8 viable nodes. Cassava planting had multiplication rate of 1:10 after about 6-12 months relatively less than propagation rate of commercial crops propagated through seed (Leihner, 2002). Sprouting of cassava stakes and early growth of the plantlets from stakes depends on endogenous nutrients stored in the stems, rather than on soil nutrients, so early growth vigour is determined by the quality of the cuttings and not soil nutrient (El-Sharkawy, 2004; OECD, 2014)

In some countries like Nigeria they have developed the technology for rapid multiplication of cassava cutting. This technology has advantage of rapidly multiplying cassava stems within short time period thereby enabling farmers to have access to the improved varieties. The technology uses small cutting with two nodes. This system produces 12,000 to 24,000 commercial stake after one year (Leihner, 2002). The quality of planting stem depends on stem age, number of node per

cutting, thickness and size of cutting (Bridgemohan and Bridgemohan, 2014). Too old stakes will have less food as most of its part is more lignified while too young stakes will have more water hence easy to lose water. However, older stems have longer stems and have more buds per stem, which result to more planting setts per plant (Howeler, 2002). Stem cuttings from lower and middle part of the stem of cassava have higher sprouting ability than those taken from upper part of stem. The stakes sprouting, verified by Howeler, (2002) that it has effect from method and length of storage after harvesting. Research shows that the sprouting percentage decreases with increase in the length of storage but the rate of decrease depends on method of storage.

2.5 Quality of cassava stem cuttings

Factors affecting quality of cassava planting material are: - age of stem, stem diameter, number of nodes and health of stem. It is essential to take into mind these factors for the high percentage sprouting and vigorous plants that will be capable of producing a good number of roots.

2.5.1 Age of stem cuttings

The best age of stem cuttings is between 8 and 18 months. Cuttings from older part of cassava stem give better yield than cuttings from younger parts because the mature part has enough food reserve for supporting sprouting before the plants start photosynthesis (Ekanayake *et al.*, 1997). Cuttings from green parts are susceptible to pathogens and insect pests as compared to mature part so sprouting percentage become low. But also, immature stems cannot be stored for a long time, because they dehydrate rapidly (FAO, 2010). Stem cuttings from too old plants are lignified, and contain only small amounts of nutrients for sprouting which results to weak sprouting. One practical way of knowing whether a stem is sufficiently mature is to determine the relationship between the diameter of the pith and the stem cutting in a transversal cut (FAO, 2010; MRDP2, 2015) (Figure 3). If the diameter of the pith is equal to or less than 50 percent of

the diameter of the stem, it is sufficiently mature to be used for propagation (FAO, 2010; MRDP2, 2015)

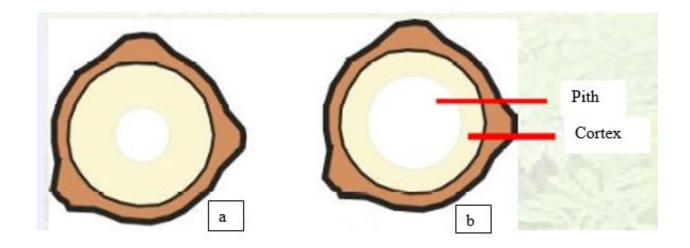


Figure 3: Transversal cut of cassava stem showing (a) good planting material (b) poor planting material depending on the diameter and pith size

Source: MRDP2, 2015

2.5.2 Stem diameter

Practically any part of the cassava stem can be used for propagation, but thin stems have less food reserve to support the sprout and early growth (Ekanayake *et al.*, 1997). Thin stems have little nutrients and moisture. Its sprouts are weak, and plants produce only few and small tuberous roots (Ekanayake *et al.*, 1997).

2.5.3 Number of nodes

For the best sprouting the planting cuttings of 20-30 cm long with 5-7 nodes is advisable (Ekanayake *et al.*, 1997). Cuttings with length of 18-25 cm but with similar number of nodes are advisable FAO, 2010). Shoots and roots develop from the nodes (Ekanayake *et al.*, 1997). You may obtain a plant from a small cutting with only 1 node, but the possibility of sprouting is low.

Cuttings with 1-3 nodes do not sprout well because of small amounts of nutrients especially when soil moisture is limited (IITA, 1990). Longer stakes, with 8–10 buds, have a better chance of conserving their potential viability, but require more planting material per unit area which is expensive to the farmers. Long cuttings have been reported to give higher yields than short ones, because they have more buried nodes that produce more stems and normally results to higher yields (IITA, 1990).

2.5.4 Health of stems

The epidermis and buds of stem cuttings may be bruised or damaged by friction causing wounds during preparation, transportation, storage and planting. Each wound is a potential site of entry for micro-organisms that cause rot during storage or after planting (IITA, 1990).

2.5.5 Original position of planting material

Cuttings from the mid-section of the cassava stems usually perform better than those at the top or the bottom sections (CIAT, 2011). This variation in performance of the plant depend on the physiological status of the vegetative cutting and maturity. This can result to experimental errors and undesirable variation in experiments (CIAT, 2011).

2.6 Storage of cuttings

Cassava planting material may have to be stored several months when the growing environment is not conducive i.e. dry, cool or flooded (Leihner, 2002). During storage the stem gradually deteriorate and eventually lose their viability. Physiological deterioration is determined by observing respiration and dehydration levels of the stems (Leihner, 2002). Cassava stem contain living tissues which continue to metabolize during storage thus depleting soluble carbohydrates (Leihner, 2002; Moyo, 2004). Sprouting and vigour of cassava seedling mostly depend on sufficient carbohydrate reserves and mineral elements essential for the initial phase of the

regeneration and the development of both roots and leaves (El-Sharkawy, 2004). Selection of well-developed and nourished mature and health stems from mother plant and right storage conditions are the first steps towards minimizing deterioration storage effects (Leihner, 2002). Cassava stems dehydrate during storage, particularly when stored in open air, exposed to the sun (Leihner, 1986). The rate of moisture loss is determined by plant related factors such as moisture content of the stake and degree of lignification at harvest as well as environmental factors such as radiation, temperature and relative humidity (Leihner, 1986). Also moisture loss in storage is higher in short stems than long stems. Drastic drop in sprouting percentage occurs when the stem moisture falls below 60%, thus moisture in cassava cutting under storage should be above 60% if the viability is to be maintained. Also, long stems 50 -100 cm should be treated with fungicide and insecticide before storage and kept in a shaded place with RH of 70-80% and moderate ambient temperature of 20-23°C. Excessive heat and direct sun accelerate the metabolic activities and dehydration (Leihner, 2002).

2.7 Storage methods of cassava cuttings

According to Howeler, (2002), long storage (1.5 - 2 months) cassava cuttings can be stored in bundles upright under a shaded tree to retain the sprouting at 80% or more. Some farmers store cassava cuttings horizontally under shade (Nayar *et al.*, 2002) but with this method, they can lose up to 60 % of planting materials. For long storage more than 5 months, vertical storage under shade with lower end (2-3 cm) buried in the soil and wetting the soil is the best method for storage in hot and dry weather. Other method used in research of storing cassava by Nayar *et al.*, (2002) was zero energy cool chamber (ZECC) which show less drying of cassava stem than cuttings stored under tree shade. In cool temperature, cuttings can be stored in underground tunnel (Ekanayake *et al.*, 1997). The storage starts by placing the layer of dry straw and then

cuttings arranged in layers and covered on top with another layer of straw 1 cm thickness and then covered with soil 1-2 cm thickness. Tunnel/clamp storage has been used to store potatoes using low cost technology that can he designed using locally available materials for ventilation and insulation. This method also can be used to store cassava cuttings. Simple clamp uses a wooden ventilation box and clean grass or straw for insulation (Lisinska, and Leszczynsk, 1989). The grass was protecting cuttings from too high heat or too low temperature by acting as insulator of temperature from external environment (Lisinska and Leszczynsk, 1989).

2.8 Carbohydrate content of cassava stem

The content of carbohydrate of cassava stem varies with variety. According to Zhul *et al.*, (2015) contents of carbohydrate of variety SC205 popular variety found in China was 30 % with 15 % extractable starch using simple water based methods. Pooja and Padmaja, (2015) have shown that using dry samples cassava stem contains 15% of carbohydrate content. Wood plants uses carbohydrate for growth and maintenance. Significant carbohydrate is used for maintenance respiration during dormant period when temperature is low (Kozlowsk, 1992). He also added that during storage, some carbohydrate is depleted by maintenance respiration while protein and lipids were not affected regardless of the length of storage. The rate of respiration varies with physiological activities and temperature. Maximum respiration occurs when the leaves emerges from bud (Kozlowsk, 1992).

2.9 Pathological effect on stored cassava stems

When cassava stakes are stored, a number of pathogens and insects start to infest them, causing deterioration of the stem and bud. This reduces both vigour and viability of planting materials. Microbial deterioration can be reduced by chemical treatment before storage (Leihner, 1986). Pathogens and insect infection occur either from carryover or from new infection from soil borne

pathogens and insect irrespective of measures to control them (Leihner, 2002). Treatment before storage of cassava stakes reduce the infestation and infection. Stakes stored without treatment led to increased number of infestation and infection of the stems (Leihner, 1986). In the non-treated stakes common systemic fungi observed were *Diplodia spp* while *Fusarium spp*, *Diplodia spp*, *Colletotricum spp* and *Aspergillus spp*. infested the external part of cuttings. In treated stakes the systemic fungi were not found while the external colonizers occurred similar to the untreated stems (Leihner, 1986).

Fungus infection is a serious problem in pregermination or pre-sprouting nursery operations. In most farms, soot fungus reduces sprouting ability, vigour and in severe cases damage or delay the emergence of new buds (Eke-Okoro, 2010). *Colletotrichum gloesporioides f.sp. manihotis* causes Anthracnose in cassava and is mainly transmitted through infected planting materials (Fokunang *et al.*, 2004). Other disease causing problem in stored cassava cutting is glomerella stem rot which is caused by *Colletotrichum spp.* (Hillocks and Wydra, 2002).

2.10 Cassava nutritional requirement

Cassava is known to be among crops well adapted to poor or degraded soils because of its tolerance to low pH, high levels of exchangeable aluminium (Al) and low concentrations of phosphorus (P) in the soil (Howeler, 2002; FAO, 2013). It has been shown that cassava can tolerate low pH up to < 4 which is not the case with tomatoes, maize or wheat (Islam *et al.*, 1980; Table 3) Sprouting, number of primary stems are contributed by cuttings nutrients and carbohydrate (FAO, 2013). However, soil nutrient doesn't have any contribution on early performance of cassava plant.

Table 3: Approximate classification of soil according to nutritional requirement of cassava

Soil parameter	Very low	Low	Medium	High	Very high
рН	<3.5	3.5-4.5	4.5-7	7-8	>8
Organic matter (%)	<1.0	1.0-2.0	2.0-4.0	>4.0	
Al saturation (%)			<75	75-85	>85
Salinity (Ms cm ⁻¹)			< 0.5	0.5-1.0	>1.0
Na saturation (%)			<2	2-10	>10
$P(\mu gg^{-1})$	<2	2-4	4-15	>15	
K (meq 100 ⁻¹ g)	< 0.10	0.10-0.15	0.15-0.025	>0.25	

Where; pH = pH is a measure of how acidic/basic water, Al = aluminium, Na = sodium, P = phosphorus, K = Potassium

Source: Howeler, 2002.

2.11 Growth and development

The cassava plant has two distinct growth phases, the first growth phase (from planting to 8 weeks) involves the production of stems and leaves and the thin and thick root systems (Figure 4). During this phase, the cassava tuber begins to form. The second growth phase (8–72 weeks) involves the rapid growth of the stems and leaves, as well as bulking of the cassava tuber formed in the first phase (Titus *et al.*, 2011). The second phase is an exponential phase during which root bulking occurs (Figure 4). In cassava, it has been demonstrated that the relationship between storage root mass and total plant mass is a linear one (Gray, 2000). This linearity shows that storage root bulking rate kept pace with the rate of crop growth. The true leaves start to expand around 30 DAP, that time the photosynthesis process starts to contribute to plant growth. Before 30 DAP, shoot and root growth depends on the carbohydrate and minerals stored in the stem cuttings (OECD, 2014).

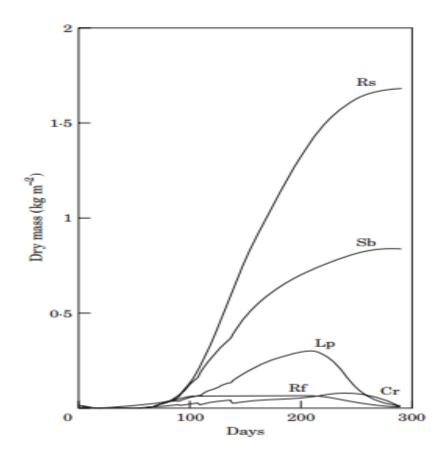


Figure 4: The dynamics of biomass allocation between storage roots (Rs), stems and branches (Sb), leaves and petioles (Lp), fibrous roots (Rf), and storage reserves (Cr).

Source: Gray, 2000

2.12 Water requirement for cassava plant establishment and early growth

Cassava is very sensitive to soil water deficit during the first three months after planting (FAO, 2013), but it has no critical period when water is required for flowering and seed formation. According to FAO (2013) stakes will be able to sprout and grow well when the temperature is above 15°C and the soil moisture content is at least 30 percent of field capacity. Water stress during sprouting and early growth reduce significantly the growth of roots and shoots which will result in subsequence reduction of growth of storage roots (Sunitha *et al.*, 2013).

CHAPTER THREE

3.0 EFFECT OF STORAGE METHODS ON CARBOHYDRATE AND MOISTURE REDUCTION IN CASSAVA PLANTING MATERIALS.

3.1 Abstract

Storage of cassava planting materials has been a challenge due to properties of moisture and carbohydrates loss under storage for more than two months. Objective of the study was to determine the effect of storage methods on carbohydrate and moisture reduction of cassava cuttings. Two varieties of cassava cuttings 1 m long, Karembo & KME4 were stored for four months under four different storage methods in two locations Kabete and Kiboko. The storage methods were clamp and double shade (CUDS), horizontal under shade (HUS), vertical under shade (VUS) and the control horizontal under open ground (HOUG). In each storage method hitag 2 xsense data loggers were installed to record data on temperature and RH. Percentage carbohydrate, moisture content (MC), 100% dry cuttings (DC) and cuttings dried to 25% or more of its stored length but not 100% were measured at intervals of 0, 4, 8, 12 and 16 weeks respectively. Data were subjected to analysis of variance (ANOVA) using GenStat version 13 and means separated using LSD. CUDS in both locations had low temperature and higher RH 18.78 °C, 72.07% in Kabete respectively and 24.99 °C, 60.13% in Kiboko respectively. This temperature and RH could explain why the storage methods performed better than the rest. Temperature and RH recorded under control (HUOG) at Kabete were 21.13 °C, 61.89% respectively and 28 °C, 40.91 % at Kiboko respectively further support this argument. The results showed less desiccation for materials stored in CUDS than those stored in HUOG. The moisture loss in CUDS was from 70.16 % - 56.69 % while that of HUOG dropped from 70.16 % to 27.26 % within 8 weeks after planting. Also the results showed that temperature and RH have effects on carbohydrate loss of stored cuttings. Kiboko stored cuttings lost more carbohydrate than cuttings stored in Kabete with difference in loss of 0.99 (LSD = 0.18). The results have proven that safe storage of cassava planting material is affected by plant related factors such as cultivar as well as environmental conditions such as temperature, RH and radiation.

Keywords: carbohydrate, cassava cuttings, cassava planting materials, moisture, storage methods

3.2 Introduction

Cassava contributes to food security and livelihood to majority of small scale farmers in semiarid areas. It is also a source of raw materials to more than 1000 microprocessors and traders around the world (Balagopalan, 2002). Cassava is a source of carbohydrate in Africa after maize and rice, it is estimated that 70 million people consume more than 500 Kcal per day while more than 500 million consume 100 Kcal per day (Aerni, 2005). The ability of cassava to grow in marginal land as well as flexibility in harvest of tuber when needed make it the best crop of choice for most poor farmers. Worldwide cassava production increased from 163 MT in 1980 to 270 MT in 2013.A lot of effort has been put to increase cassava production to cater for food, energy and animal feeds requirements in Africa but planting material has been a challenge to most farmers. Most farmers use planting materials from previous crop which, normally have disease infection as well as low nutrients as crop was not managed as seed production rather than food production (Ogero *et al.*, 2012).

Cassava planting materials require storage especially when climatic condition such as floods, drought and low temperature or delayed land preparation and other factors. However, use of fresh planting materials is preferable than stored cuttings (Leihner, 1983; Lozano *et al.*, 1977) as it has been observed that the longer the duration cassava planting materials are stored, deteriorate their sprouting. Sprouting ability depend on storage conditions (Oka *et al.*, 1987) as well as other

factors like temperature and moisture of field. Leihner (1986) reported that cassava stems loose carbohydrate reserves during storage mainly in form of total carbohydrate and reducing sugars. More lignified cuttings contain small amount of food reserved for shoots development during sprouting (Lozano *et al.*, 1977). Despite good storage conditions, long storage durations bring about some losses in moisture, carbohydrates, and nutrients, which would partially account for reduced early vigour (Leihner, 1982). Cassava cuttings dehydrate when stored. The rate of moisture loss is high when the cuttings are stored in open air and exposed to sun (Leihner, 1982). Moisture loss on planting material are influenced by plant factors (level of lignification and moisture content at harvest) and environmental factors (radiation, humidity, temperature and wind speed) (Leihner, 1982).

When harvesting and planting are separated in time, a farmer can decide to leave some portion of crop as seed for next planting. But this can cause pests carryover and cause big loss to small scale farmers (Leihner, 1982). Also where land is unavailable, storage of planting materials is inevitable. Objective of the study was to determine the effect of storage methods on carbohydrate and moisture reduction of cassava cuttings.

3.3 Materials and methods

3.3.1 Description of sites

The experiment was conducted in two sites namely University of Nairobi Kabete Campus and KALRO Kiboko. Kabete is situated about 15 km to the west of Nairobi city and lies at 1° 15′S latitude and 36° 44′E longitude and at altitude of 1930 m above sea level (masl) (Onyango *et al.*, 2012). Kabete has a bimodal distribution of rainfall, with long rains from early March to late May and the short rains from October to December (Onyango *et al.*, 2012). The mean annual

temperature is 18 °C and total annual rainfall ranging between 700-1500mm (Wasonga *et al.*, 2015).

The second site was KALRO - Kiboko which lies within longitudes 37°.43 212′ E and latitudes 2°.12 933′S, and 821.7 m above sea level in Makueni County, 187 km east of Nairobi, Kenya (Kivuva *et al.*, 2015). The location receives between 545 mm and 629 mm of rainfall coming in two seasons. The long rains season is between April and May while the short rains season is between October and January. The mean annual temperature is 22.6°C, where by the mean annual maximum temperature is 28.6°C and mean annual minimum temperature is 16.5°C (the Kenya gazette, 2010).

3.3.2 Source of Cassava stem cuttings

The stem cuttings comprised varieties Karembo and KME4 which were obtained from KARLO Thika. The materials were selected on basis of the diseases free and high yielding of the varieties. KME4 has maturity of 8 – 10 months, fresh tuber yield is 38 t ha⁻¹, resistance to cassava mosaic virus and cassava brown streak. Karembo mature in 8 months and fresh tuber yield range from 50 -70 t ha⁻¹, it has great tolerance to cassava mosaic virus and cassava brown streak virus (Kenfap services limited, 2013).

3.3.3 Experimental design

The design was split plot in randomized complete block design (RCBD) (Petrenko, 2014). The main plot being storage methods (with 4 levels) sub plot being varieties (2 levels). The storage duration was in 5 terms (0, 4, 8, 12, and 16 weeks after storage). Each main plot had 30 cuttings while the sub plot had 15 cassava cuttings from single variety each having 100 cm length with diameter range of 1.5 cm - 3.4 cm. These 15 cuttings were tagged numbers 1-15. Cassava cuttings were stored in four different storage methods namely; clamp under double shade

(CUDS) (Plate 5), horizontal in open air under shade on the soil (HUS), vertical in open air under shade on soil (VUS) (Sales and Leihner, 1980; Plate 6) and horizontal under open ground with no shade on the soil (HUOG) (control or farmer's way of storing cassava cuttings when waiting transport or planting) (Plate 4). The shade was made by simple wooden poles and grass thatch (Plates 4 and 6). Three hitag 2 xsense loggers were installed to monitor temperature and relative humidity in clamp, under shade and under direct sun light. Two cuttings per storage method were sampled to form 6 cuttings. The 6 cuttings were cut into 20 cm cuttings after removing 10 cm from each end. The cuttings were mixed together and some were sampled for moisture and carbohydrate analysis.



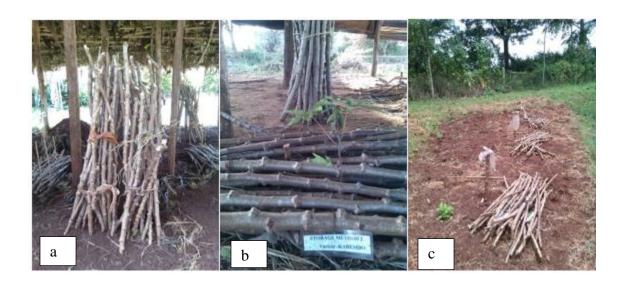


Plate 4: (a) Simple shade and (b) different storage methods under shade.



Plate 5: Clamp under double shade storage method.

Where; (a) clamp structure frame, (b) arranged cuttings for storage (c) clamp storage method covered by grass 1 cm thick and then 0.06 m³ of soil to cover 1 cm thick.



Plates 6: Different storage methods

Where; (a) Vertical under shade with lower end of cassava touching the soil, (b) horizontal under shade, (c) cuttings stored horizontal in open ground under direct sun light

3.3.4 Carbohydrate determination

In each storage method six samples of 20 cm each were taken to the laboratory for carbohydrate tests. The sample cuttings ware cut at the middle and both sides of the cut were grated to obtain composite sample. The samples were dried to constant weight and put in container (Plate 7). Total saccharides in samples was estimated by the anthrone method which is a simple calorimetric method with relative insensitivity to interference from other cellular components (Clegg, 1956; Ravi and Suryakumari, 2005).



Plate 7: Sample for carbohydrate determination

1 gram from sample were taken in duplicate and transferred to graduated 100 ml beaker. Then 10 ml of distilled water were added and then stirred thoroughly to dispense the sample (Plates 8).

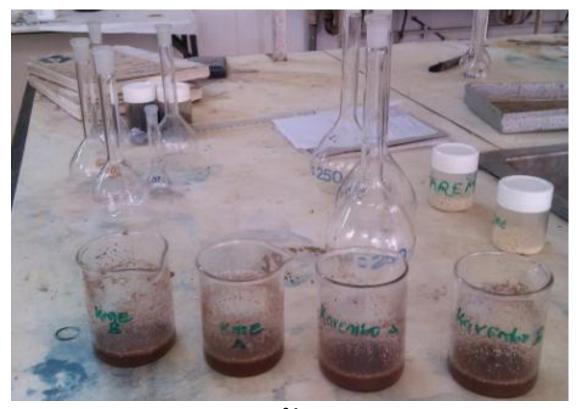


Plate 8: Carbohydrate extraction from samples

From 10 ml of sample suspensions 13 ml of 52% perchloric acid were added in order to solubilize starch in samples (Rose, 1991). The suspensions were stirred for 20 minutes and then diluted to 100 ml. Then the suspensions were filtered to 250 ml flask and the solution were diluted to the mark to form a stock solution (Clegg, 1956). From the stock solution 10 ml was drawn and diluted to 100 ml. 1 ml of the diluted sample, standard sample and blank were pipetted into individual test tubes, then in each test tube 5 ml of anthrone reagent in concentrated sulphuric acid was added. The reactions in this process were, concentrated H₂SO₄ catalyses the dehydration of sugars to form furfural (from pentose's) or hydroxy methyl furfural (from hexoses). Adding anthrone into the sample give condensation product with bluish or green coloured (Figure 5).

Figure 5: Chemical reaction in carbohydrate determination

The test tubes then were transferred to boiling water for exactly 12 minutes then cooled to room temperature. From the test tubes the solutions were transferred to glass cuvettes to read absorbance at 630 nm wave length (Clegg, 1956).

The formula used to calculate the concentration of carbohydrate in the samples was: -

$$\frac{\text{Absorbance of sample}}{\text{Concentration of sample}} = \frac{\text{Absorbance of standard}}{\text{Concentration of standard}}$$
 (1)

3.3.5 Determination of moisture loss from stored cassava stem cuttings

The moisture content was determined by constant temperature oven method (ISTA, 2015). From the composite sample made, 2 g of sample were measured in duplicate into moisture dishes (Plates 9) and put to oven at 103 °C for more than 17 hrs to obtain constant weight.



Plate 9: (a) cut cuttings for samples and (b) shredded sample in moisture dishes for moisture determination

Calculations and expression of results using constant temperature oven methods were calculated for each replicate in three decimal places using the following formula

$$\frac{\text{Loss of weight x 100}}{\text{Intinial weight}} = \frac{\text{M2-M3 x 100}}{\text{M2-M1}} \text{ (ISTA, 2015)}$$
 (2)

Where: M_1 is weight (in grams) of empty container; M_2 is weight (in grams) of container + sample before drying; M_3 is weight in grams of container + dry sample

3.4 Data analysis

The data were subjected to analysis of variance (ANOVA) (Kroonenberg and van Eeuwijk, 1998;Cohen and Brooke, 2004;Cheng & Shao, 2006; Smith, 2006) to determine the difference between storage methods and varieties. GenStat 13th Edition (64-bit) SP2 were used. Means were separated using least significant difference (LSD) at $p \leq 0.05$ (Kivua *et al.*, 2015). The assumptions were that the population was normally distributed, samples were independent, variance of population was equal and group of samples was equal.

Complete model used was: -

 $Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkl}$ (Kroonenberg and van Eeuwijk, 1998; Smith,2006)

Where μ is the general mean of the population α_I , β_j , γ_k is mean of storage methods, mean of varieties and mean of duration of storage, as main effects while $(\alpha\beta)_{ij}$, $(\alpha\gamma)_{ik}$, $(\beta\gamma)_{jk}$ are corresponding two-way interaction effect and $(\alpha\beta\gamma)_{ijk}$ three-way interaction effect. ϵ_{ijkl} represent the expected error (Kroonenberg and van Eeuwijk, 1998). The computed data composed percentage dry cuttings more than 25% of its storage length but not 100% (% DC >25% SL), Percentage dry cuttings 100% of its storage length (% DC), percentage moisture of stored cuttings (% MC) and carbohydrate content of stored cuttings sampled at specific duration according to equations 2, 3,4 and 5.

% DC > 25% SL =
$$\frac{DC > 25 \text{ cm}}{TNC} \times 100$$
 (3)

Where; % DC >25 % SL sampled cuttings with >25 cm of its stored length drying percentage but less than 100; DC >25 cm = sample cuttings with >25 cm of its stored length dry; and TNC= total number of cuttings at a given time.

$$\% DC = \frac{DC}{TNC} \times 100 \tag{4}$$

Where; % DC = cuttings sample 100 % of its stored length dried; DC= total dried cuttings samples; and TNC= total number of cuttings at a given time

% carbohydrate =
$$\frac{25 \times b}{a \times w}$$
 (5)

Where; b = absorbance of diluted sample; a = absorbance of dilute standard sample; and w = absorbance weight of sample (g).

3.5 Results

3.4.1 Weather data during the experiment duration

The mean temperatures were 24.12 °C and 12.53 °C in Kiboko and Kabete respectively (Figure 6 a and b). Rainfall in Kiboko was negligible while in Kabete was around 5.67 mm. RH were around 82% and 65 % in Kiboko and Kabete respectively. Data where obtained from ICRISAT Kiboko and Kabete meteorological stations (Figure 6)

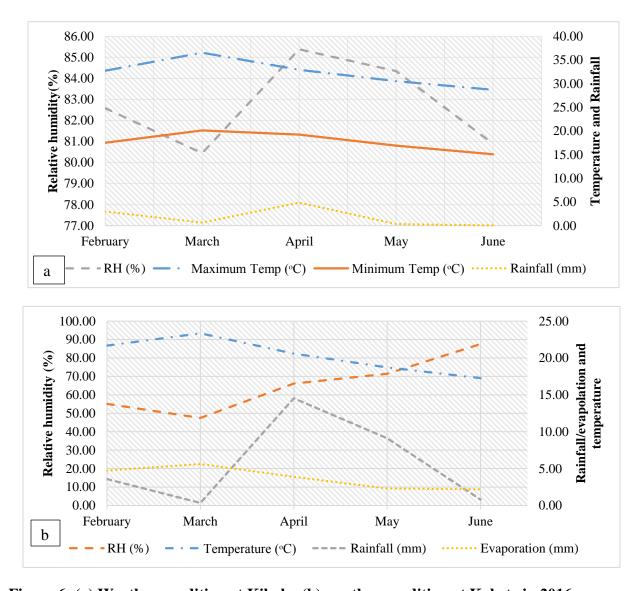


Figure 6: (a) Weather condition at Kiboko (b) weather condition at Kabete in 2016

Data recorded by hitag 2 xsense data loggers (Table 4) were different for each storage methods. Minimum temperature was recorded in CUDS and highest was under HUOG in both locations. Graphical presentation of RH and temperature are shown in annex 4 - 9.

Table 4: Temperature and relative humidity in storage methods season

Site	Storage method	Min T	Max T	Mean T	Min RH	Max RH	Mean RH
Kabete	CUDS	12.25	24.5	18.78	38.00	100.00	72.05
Kabete	HUS &VUS	11.00	29.50	19.16	20.00	100.00	66.23
Kabete	HUOG	9.00	39.50	21.13	20.00	100.00	61.89
Kiboko	CUDS	17.50	32.75	24.99	20.00	92.00	60.13
Kiboko	HUS&VUS	14.50	40.00	25.42	20.00	100.00	56.66
Kiboko	HUOG	12.75	45.50	28.00	20.00	100.00	40.91

Where; T = temperature (°C), RH= relative humidity, Max = maximum, Min = minimum, CUDS

= clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade and HUOG = horizontal in under open ground

3.4.2 The percentage of cuttings that had more than 25% of its storage length dried but less than 100% (% DC >25% SL)

The results showed highly significant difference (p < 0.001) among storage methods as well as varieties (Figure 7). Among storage methods, CUDS had the minimum increase of % DC >25% SL, at 4 and 8 weeks after storage in Kabete. Highest percentages (more than 50%) were found in horizontal under open ground with no shade on the soil only 4 weeks after storage (Figure 7; Table 6).

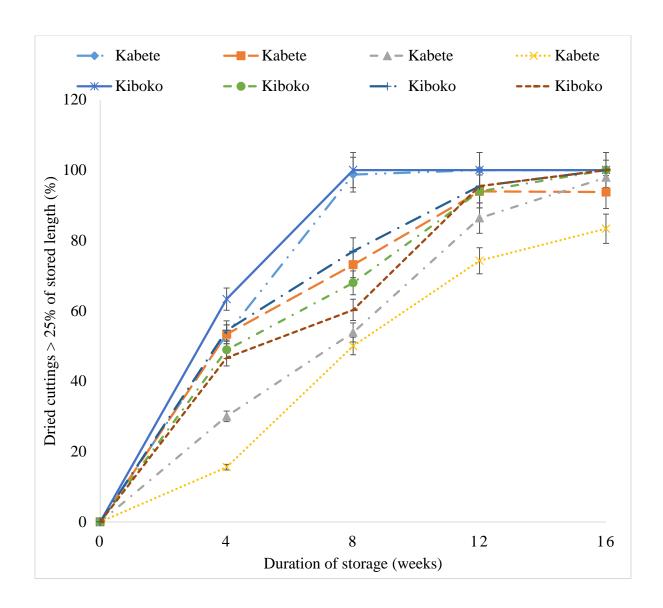


Figure 7: Rate of drying of cuttings under different storage methods in Kabete and Kiboko
Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under

shade HUOG = horizontal in under open ground and error bar represent LSD at 0.05

CUDS performed better in all location and almost in all parameters which can be attributed to low mean temperature of 18.78 °C and 24.99 °C in Kabete and Kiboko respectively. Low mean temperature and medium RH reduced the desiccation of stored cuttings. The maximum temperature was recorded by temperature data logger's in horizontal under open ground on soil which was 45.50°C in Kiboko. The maximum temperatures were recorded around 11:00 hours to

17 hours. Also the results showed that CUDS had high average relative humidity of 72.05% and 60.13% in Kabete and Kiboko respectively while lowest mean relative humidity were recorded in horizontal storage under open ground 61.89% and 40.91% Kabete and Kiboko respectively. This justify that cuttings stored in HUOG will lose more water than cuttings stored in CUDS. Low relative humidity means cuttings will lose water to surrounding. High evaporation of 5.62 mm was recorded at Kabete when average rainfall was 0.35 mm and also at the same time low RH was recorded.

The results also showed that the performance of HUS at Kabete when the temperature was low was better than VUS. But under high temperature VUS at Kiboko the lower end of cassava cuttings touching the soil did better than HUS. The mean temperature under shade was 19.16 °C and 25.42 °C, at Kabete and Kiboko respectively (Figure 7). While the maximum was 29.50 °C and 40.00 °C, Kabete and kiboko respectively. Relative humidity was 66.23% and 56.66% Kabete and Kiboko respectively. (Table 4).

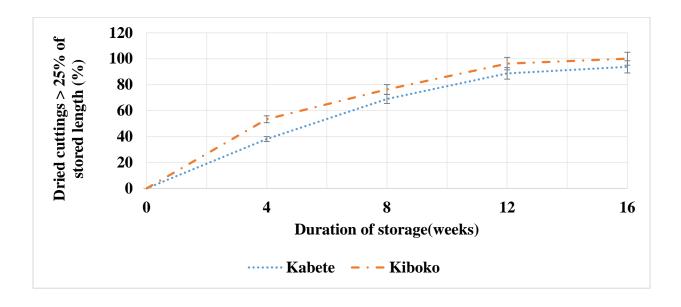


Figure 8: Rate of dehydration of cassava cuttings at Kabete and Kiboko for a duration of 16 weeks after storage

KME4 showed the best storability as compared to Karembo by having low % DC >25% SL relative to that of Karembo (Figure 9).

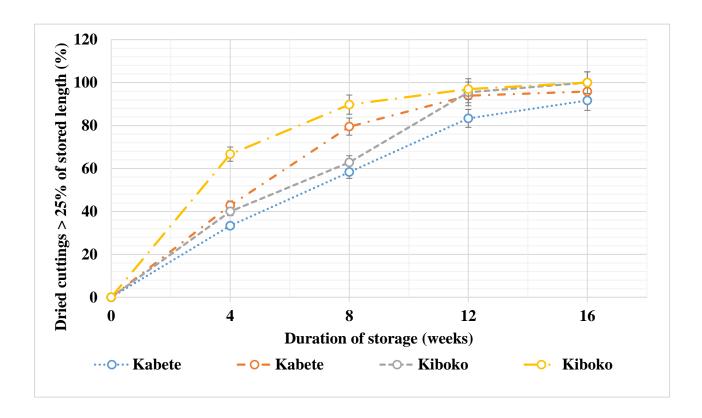


Figure 9: Increase in percentage dried cutting of different variety 25% of its stored length in different duration of storage and locations

3.4.3 Percentage cuttings dried 100% of its stored length (%DC)

There was significant difference among storage methods at p < 0.01 as well as varieties (Figure 10; Table 5). CUDS had lowest average dry cuttings of 34.40 % as compared to other storage methods. The highest was under HUOG with 52.56 % for whole period of storability test. Also KME4 had less dried cuttings 32.14 % as compared to 47.15 % of Karembo. Duration of storage was highly significant at p < 0.001 (Figure 9). The results showed as weeks of storage advanced the percentage of dried cuttings was increased from 0 % to 100 % depending on storage methods and cuttings variety (Table 6). The rate of increase of dried cuttings was high in HUOG and low

at CUDS in the first 4 weeks. But also the rate was high in Karembo variety and less in KME4 under the same storage methods.

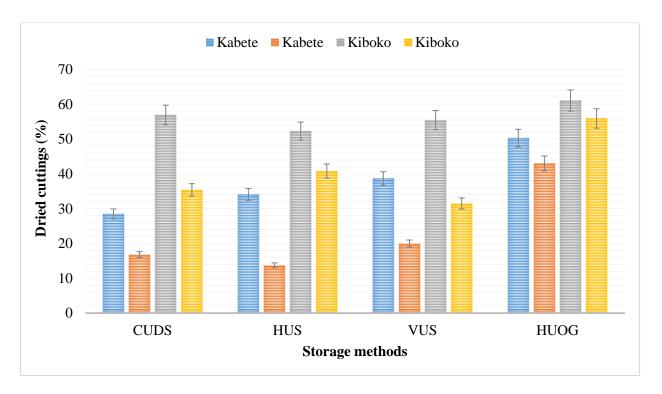


Figure 10: Effects of storage methods and variety on cassava cuttings drying in storage

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade, HUOG = horizontal in under open ground and error bar are standard errors

Varieties performed differently in different locations. Storage was better in Kabete than in Kiboko due difference in RH, and temperature. The rate of drying of cuttings was high in Kiboko than Kabete. Average relative humidity measured by Meteorological station Kabete was 60.04% and temperature was 23.54 °C as compared to average relative humidity measured by ICRISAT Kiboko field station of 83.91% and mean temperature of 33.18 °C measured from February to July 2016. Variety KME4 performed better in both sites than Karembo (Figure 10).

KME4 stored in Kiboko was performed better than Karembo stored in Kabete regardless of the difference in temperature and relative humidity.

3.4.4 Percentage moisture of stored cuttings (MC %)

Moisture content of stored cuttings at different storage duration showed highly significant difference among sites (p < 0.001; Figure 11). The mean of moisture content of stored cuttings at 0 weeks of storage was 70.16 % both in Kabete and Kiboko. After 16 weeks of storage moisture content reduced to 14.23 % at Kiboko and 39.70 % at Kabete depending on methods of storage (Figure 11). Thus, the results showed that the rate of moisture content loss was influenced by environmental conditions of particular location (Figure 11). The rate of dehydration in Kiboko was higher as compared to Kabete. Also results have shown that at 8 WAS in Kabete there was an increase in moisture content of stored cuttings then from 12 WAS started decreasing (Figure 11).

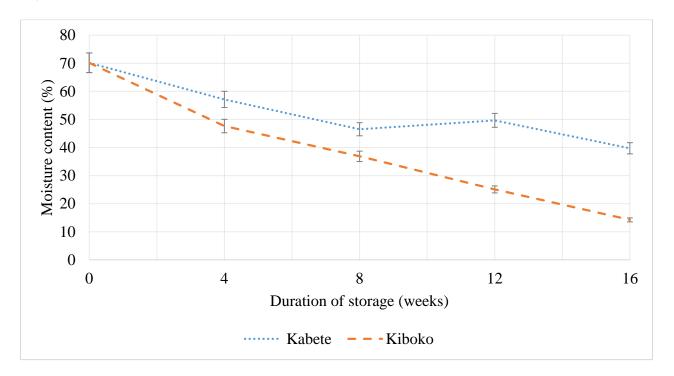


Figure 11: General percentage moisture content of cuttings with reference to duration of storage in two locations.

Moisture reduction from stored cuttings in both Kabete and Kiboko site and dehydration of cuttings stored under CUDS was lower as compared to other storage methods while under HUOG dehydration of cuttings was highest (Figure 12). Thus, the best storage methods were CUDS followed by VUS and then HUS.

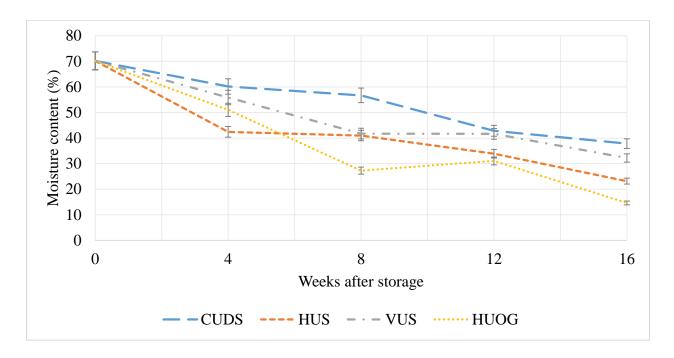


Figure 12: Rate of moisture reduction of different storage methods

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade and HUOG = horizontal in under open ground

Rate of dehydration of stored cuttings depends on varieties (Figure 13). Whereby, the rate of moisture loss of Karembo was higher compared to KME 4 CUDS performed better in all duration of storage from week 0 to week 16 after storage for stored cuttings.

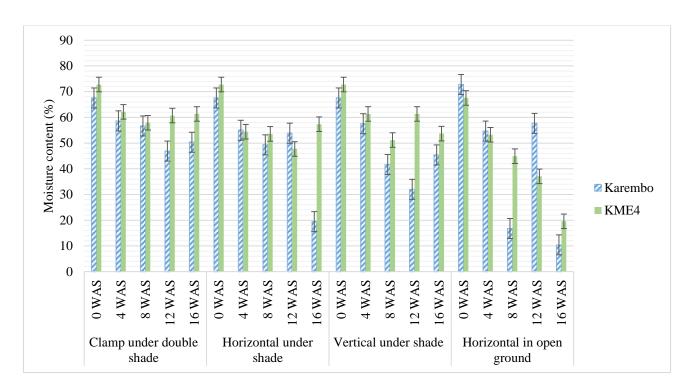


Figure 13: Percent moisture content of different cassava variety cuttings at different duration of storage.

3.4.5 Carbohydrate content of stored cuttings

The amount of carbohydrate in cuttings during storage differed significantly among locations at p < 0.001 (Figure 14). The results indicate that cuttings stored in Kiboko lost more carbohydrate than cuttings stored in Kabete 8 weeks after storage to 16 weeks. Duration of storage showed significant difference between weeks after storage at p > 0.001. The results showed that the more farmer store cuttings for long duration the more cuttings consume carbohydrate for maintenance (Figure 14).

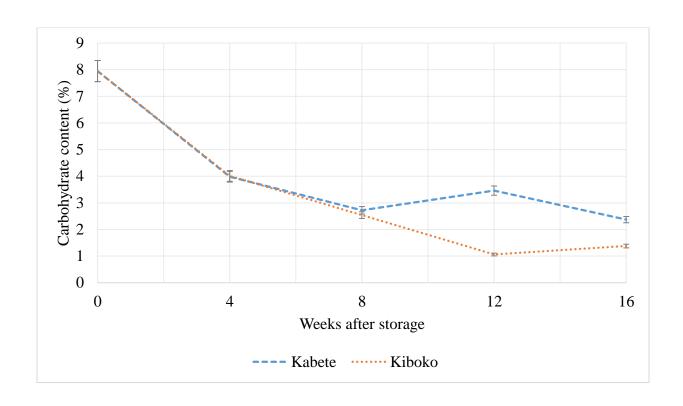


Figure 14: Average carbohydrate content reduction of cuttings with reference to duration of storage. The bars represent LSD at 0.05

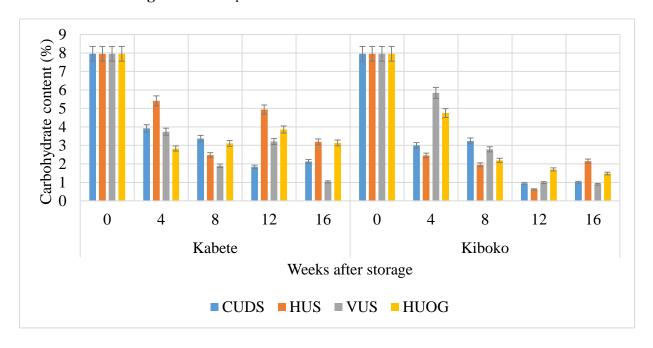


Figure 15: Average carbohydrate content of stored cuttings with reference to duration of storage, location and storage methods

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade and HUOG = horizontal under open ground. The bars represent LSD at 0.05

Also the results showed highly significant difference among varieties at p < 0.001. This mean the consumption of carbohydrate during storage differ with varieties. The interaction between location and variety was non-significant at p < 0.05. The interaction between duration of storage and variety was highly significant at p < 0.001. The results showed that the longer cassava cuttings stored the more they lost carbohydrate even when the moisture content of cuttings are maintained (Figure 16). The results showed that environmental conditions, storage methods, duration of storage and variety have contribution in carbohydrate consumption during storage.

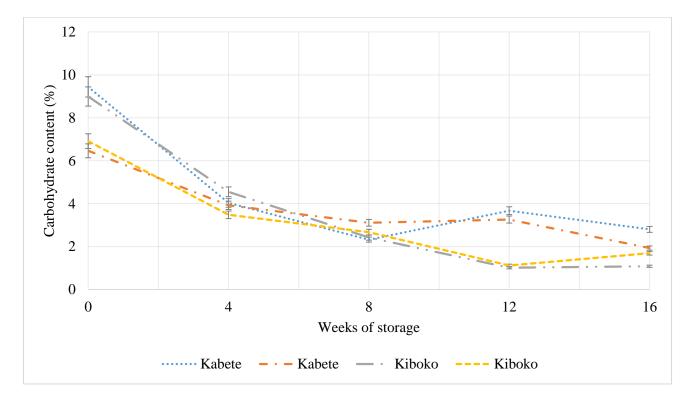


Figure 16: Percentage carbohydrate reduction of different varieties with reference to duration of storage and location

Table 5: Mean square of location, storage methods, variety and duration of storage of cassava planting materials and their significance.

Source of variation	d.f.	%DC25%SL	%_DC	% Carbohydrate	% Moisture
Location	1	3193.1*	19500.2***	29.831***	11492.33***
Residual	4	413.2	148.3	0.244	0.92
WAS	4	76354.7***	60959.7***	295.957***	12955.59***
Location x WAS	4	354.4*	3854.9***	12.865***	1437.68***
Residual	16	115.8	141	0.277	6.21
SM	3	3717***	4485.3***	2.123 ^{NS}	2564.61***
Location x SM	3	908.3***	523.5***	9.246***	464.51***
WAS x SM	12	722.9***	933.4***	4.191***	377.96***
Location x WAS x SM	12	189.9 ^{NS}	704.4***	4.35***	317.48***
Residual	60	102.2	63.6	0.772	46.35
V	1	6057.2***	13527.2***	19.996***	3862.09***
Location x V	1	56.8 NS	15.8 ^{NS}	1.135 ^{NS}	53.74^{NS}
WAS.V	4	1320.5***	2900.2 ***	16.194 ***	269.25**
SM. V	3	864.9**	602.7*	2.488 NS	362.18**
Location x WAS x V	4	308.2^{NS}	135.8 ^{NS}	3.049 *	101.67 ^{NS}
Location x SM x V	3	168.6 ^{NS}	254.9 ^{NS}	3.129*	23.06^{NS}
WAS x SM x V	12	238.6 ^{NS}	296.8*	3.965***	253.58***
Residual	92	149.7	131	1.098	73.74
Total	239				

^{* =} Significant at 0.05 probability level (p < 0.05), **= Significant at 0.01 probability level (p < 0.01), *** = significant at 0.001 probability level (p < 0.001), NS = non-significant, V=Variety, %DC25%SL = Percentage dry cuttings 25% or more of its stored length, %DC= Percentage dry cuttings 100% of its stored length, WAS= Weeks after storage, SM=Storage methods and V= variety.

Table 6: Effects of storage methods, duration of storage and variety of cassava planting materials on drying, desiccation and carbohydrate computation during storage.

Treatment	Variety	Duration of storage (Weeks)	% DC 25% OL	% DC	% CHO	% MC
Clamp under double shade	Karembo	0	0.00	0.00	9.44	67.55
		4	47.78	0.00	3.13	59.76
		8	79.49	75.64	3.94	57.94
		12	87.88	71.17	0.84	32.71
		16	93.75	66.67	1.52	29.66
	KME4	0	0.00	0.00	6.46	72.76
		4	14.44	0.00	3.79	60.61
		8	30.77	24.83	2.67	55.44
		12	81.82	41.06	1.94	52.97
		16	89.58	64.58	1.62	45.99
Horizontal under shade	Karembo	0	0.00	0.00	8.99	67.55
		4	52.22	4.89	5.03	39.05
		8	76.92	56.41	1.5	31.37
		12	96.97	77.58	2.77	33.37
		16	100.00	77.08	2.42	13.74
	KME4	0	0.00	0.00	6.91	72.76
		4	32.22	0.00	2.84	45.83
		8	53.85	30.77	2.93	50.61
		12	84.85	36.85	2.79	34.41
		16	97.92	68.75	2.92	32.58
Vertical under shade	Karembo	0	0.00	0.00	9.44	67.55
		4	61.11	16.83	4.98	50.81
		8	82.05	59.63	2.5	34.76
		12	96.97	85.91	3.06	28.67
		16	97.92	72.92	1.08	28.24
	KME4	0	0.00	0.00	6.46	72.76
		4	41.11	1.11	4.6	60.89
		8	58.97	19.03	2.2	48.73

Treatment	Variety	Duration of storage (Weeks)	% DC 25% OL	% DC	% CHO	% MC
		12	90.91	43.94	1.15	54.59
		16	95.83	64.58	0.85	36.2
Horizontal in open ground	Karembo	0	0.00	0.00	8.99	72.76
		4	57.78	7.78	4	52.27
		8	100.00	80.77	1.55	21.14
		12	100.00	93.94	2.68	36.49
		16	100.00	95.83	2.75	8.48
	KME4	0	0.00	0.00	6.91	67.55
		4	58.89	0.00	3.57	49.82
		8	98.72	58.97	3.75	33.37
		12	100.00	92.42	2.87	25.63
		16	100.00	95.83	1.85	20.83
Grand mean			61.52	39.64	3.74	45.71
LSD 5% Location			7.29	4.37	0.18	0.344
LSD 5% Duration of storage			4.66	5.14	0.23	1.078
LSD 5% Storage methods			3.69	2.91	0.32	2.486
LSD 5% Variety			3.14	2.93	0.27	2.202
LSD 5% Location x WAS			8.21	7.19	0.31	1.382
LSD 5% Location x SM			7.57	4.98	0.41	3.055
LSD 5% Location x Variety			8.36	7.44	0.30	2.215
LSD 5% WAS x SM			7.17	4.64	0.66	4.914
LSD 5% WAS x SM x Variety			12.88	11.80	1.07	8.461
CV			19.90	28.90	28.00	18.8

[%] DC 25% OL = Percentage dry cuttings > 25 % of its stored length, % DC = Percentage dry cuttings 100% of its stored length, % CHO=

Percentage carbohydrate content of cuttings, % MC= percentage moisture content of cuttings, WAS = weeks after storage, SM = storage method

3.6 Discussion

Cassava plant establishment depends on quality planting materials. Cassava is vegetatively propagated and its cuttings are expensive as compared to true seed of other crops like maize. If 25% of the original length of cassava is dried during storage, only 75% of stored cuttings is available for planting. Similar results were obtained by Leihner, (2002). Differences among the storage methods were observed in results as highly significant in stem dehydration and moisture loss. The carbohydrate loss was non-significant at p < 5% among storage methods but location of storage was significant meaning environmental factors had contribution in rate of carbohydrate consumption in stored cuttings. Kiboko being hotter than Kabete had higher respiration rate than Kabete as the rate increases because the heat speeds up the reactions which means kinetic energy become higher.

CUDS was shown to lose less moisture content. At the end of storage, the cuttings had an average of 53.54% moisture content as compared to control which had an average of 38.83%. This result may be attributed to differences in temperature and relative humidity among storage methods. Ratanawaraha *et al.*, (2000) argued that storage under shade is better than under full sunlight. Direct sunlight containing radiation energy to drive water away which will accelerate the rate of moisture loss. The maximum temperature of 45 °C and 39.5 °C were recorded in the control at Kiboko and Kabete respectively. From the results it showed some increase in carbohydrate as well as moisture content of the stored cuttings at 12 WAS. This can be attributed to rainfall received in March to April. The rainfall stimulated some stored cuttings to sprout and form leaves which were contributing to photosynthesis. But the general trend was decreasing in moisture and carbohydrate of stored cuttings. Similar results were obtained by Leihner, (2002) who showed that storing planting material under inadequate condition can cause cassava stake to

loss 70% of its sprouting ability if they were stored for 15 days at 24 °C. The difference in moisture lost is determined by cultivar, plant related factor such as degree of lignification at harvesting time and length of stored cuttings as well as environmental factors such as temperature, RH, radiation and wind speed. Ravi and Suryakumari, (2005) when working on the novel technique to increase the shelf life of cassava planting materials found similar factors affecting rate of moisture loss. The cuttings stored using HUOG in both sites lost moisture from 70.16 % to 27.26% in just 8 weeks of storage probably due to exposure solar radiation and wind (Kinama et al., 2005). Kinama et al., 2005 found that when soil doesn't have cover it loses more moisture through evaporation than when it contains cover crop or mulch. When there is high evaporation than rainfall results to high loss of moisture content of planting materials. It also explains why cuttings stored in Kiboko dehydrated faster than that stored in Kabete because evaporation in Kiboko is higher than in Kabete. Pilbeam, et al., (1994) showed that evaporation in Kiboko is around 131.2 mm – 224.9 mm from year 1991 – 1992. When stored cuttings are in open ground without cover or shade will lose more moisture content than when under shade and covered like in CUDS. According to Leihner, (1982) cassava stored planting materials lose moisture faster in shorter stems than in long stems as the loss of moisture was recorded to start from two cut ends of stem cuttings increasing to the middle of the stem. This resulted in some cuttings losing its viability from 25 % of its length to 100 %. It was recorded that only at 4 WAS moisture loss ranged between 31.11 % to 58.33% depending on method of storage and location of storage. The loss of moisture was significantly different between varieties Karembo dehydrated more than KME4 stored in the same environment. Pérez, et al., (2011) argued that cassava variety have different capacity of withstanding storage duration from harvesting to planting. This difference influences crop establishment and yield. The range of moisture content after 16 weeks of storage was from 56.16 % - 43.27 % for KME4 and 49.09 % - 34.30 % for Karembo depending on site of experiment. Kiboko is a hot area with mean temperature of 25 °C and Kabete of 22 °C. This can explain the difference of moisture loss among the sites. In clamp storage in Kiboko the mean temperature recorded was almost similar (25.22 °C and 25.82 °C) perhaps due to hot air movement across the ventilation of clamp which was meant to reduce the RH in storage to control storage sprouting. This can explain the difference in performance of the clamp under double shade between Kabete and Kiboko. Thus Kabete site is good environment for storage of cassava cuttings as compared to Kiboko because of low temperature and medium RH which does not trigger sprouting in storage or make stored cuttings to desiccate.

The results showed high significant differences between location and variety in carbohydrate loss of stored planting materials. The results showed significant loss of during the first 4 weeks after storage then after the loss decreased. Ravi and Suryakumari, (2005) found that carbohydrate content of stored cuttings decreases significantly in one month after storage. There after the change in carbohydrate content was less as compared to first four weeks. The high rate of loss of carbohydrate can be due to fact that of high moisture content of cuttings in first 4 weeks of storage which results to normal metabolic activities. As weeks of storage advanced the decrease in moisture content of stored cuttings limits metabolic rate. But other scenario can be due to stress of wounding of stored cuttings hence plant will be struggling to heal the wound caused in harvesting. The carbohydrate observed at 0 week of storage was 9.21% and 6.68 % for Karembo and KME4 respectively. According to Kozlowsk, (1991) carbohydrate consumption during storage can be due to maintenance respiration to keep planting material alive. Leihner, (2002) reported that physiological deterioration of cassava planting materials is linked with two main factors namely respiration and dehydration. He further said the respiration will be accelerated when cuttings are stored in hot environment than being stored in dry and cool environment. This can explain why the decrease in carbohydrate in Kiboko site was high as compared to Kabete. But also the results showed us that cuttings stored in HUOG lost less carbohydrate than other methods, it can be due to the fact that when cuttings lose high amount of moisture the maintenance respiration also will reduce or stop. Oka *et al.*, (1987) found that respiration rate of stored cuttings increases soon after harvest of planting materials then after decrease before increase at slow rate again. This indicates that, variety and storage methods should be considered when a farmer wants to store the cassava cuttings with reference to a certain duration.

CHAPTER FOUR

4.0 EFFECT OF STORAGE METHODS AND VARIETIES OF CASSAVA PLANTING MATERIALS ON ESTABLISHMENT AND GROWTH VIGOUR.

4.1 Abstract

Cassava plant establishment depends on quality of planting materials. Early growth vigour depends on carbohydrate and nutritional content of planting materials (cuttings). This experiment was done to determine the effects of carbohydrate and moisture content of planting materials after storage for maximum of 16 weeks on crop establishment and early growth vigour. Planting materials were sampled from each storage method and taken to field in the same locations to evaluate their sprouting ability, number of primary shoots formation, number of leaves, rate of leaf formation and early growth vigour at 8 weeks after planting (WAP). From stored cuttings 10 cm from each end was discarded and the remaining 80 cm was cut into 20 cm cuttings having 4-7 nodes each. The trial was split plot design in RCBD with main plot as storage method and sub plots were varieties replicated three times. The sprouting test was done at interval of 0, 4, 8 12 and 16 weeks after storage respectively. The cuttings were planted at 60° slanting position and irrigated with 1000 ml to 1500 ml of water per plant (Bridgemohan and Bridgemohan, 2014) three days per week to maintain field capacity soil moisture levels. Data were subjected to analysis of variance (ANOVA) using GenStat and means separated by LSD. The results showed that storage methods, variety and duration of storage had significant at p > 0.01 among treatments applied. The results also showed significant differences in storability between varieties KME4 and Karembo. Sprouting percentage at Kabete was 54.73 % while in Kiboko had 37.78 %. The results also showed that Kabete had 1.60 number of primary shoots per plant (NPSP⁻¹) compared to 1.04 of Kiboko. This implies temperature influences carbohydrate loss in stored cuttings and it affect formation of primary shoots and early growth vigour of cassava sprouts from the planted cuttings. The rate of leaf formation at Kiboko was higher as compared to Kabete which could have been contributed by difference in temperature between locations. Thus, temperature and relative humidity should be considered in cassava cuttings storage to avoid increased death of stored cuttings. Where possible cassava cuttings should be planted immediately or few days after harvest to avoid moisture and carbohydrate loss that occur during storage duration as well as losing planting materials. In case of storage cassava cuttings, they should be stored in clamp under double shade methods

The objective of the study was to determine the effect of variety and storage methods of cassava cuttings in crop establishment and early growth vigour.

4.2 Introduction

Cassava stand establishment require good planting materials. The planting stakes of cassava are said to be good if they are of right stem age which is between 8 – 18 months, right stem diameter meaning the diameter of pith is equal to or less than 50% of the total diameter of the cutting (FAO 2013; CIAT, 1984), adequate number of nodes meaning cuttings of 20-25 cm should have 5-7 number of nodes (Penh, 2015).

Good planting materials come from plants grown from fertile soil or well fertilized soil. Fertile soil or fertilized soil will provide enough food for new sprout hence vigour and high yield (Penh, 2015; Leihner, 1983). Number of nodes per cuttings vary with length of inter nodes. The higher inter node length means the less nodes per cuttings of 20-25 cm. The length of internodes varies with response to genotype, plant age and environmental factors (Penh, 2015). This meaning that the cassava stand establishment vary according to genotype. The shoots which develop from cuttings depends on length of cuttings, mother plant, bud dormancy, genotype and environmental conditions (CIAT, 1987). Early crop establishment depend on nutritional status of planting

material (Leihner, 1983). The study done by Leihner, (1983) also proved that the cuttings with good nutritional content at crop establishment produce good stand during early growth and yields higher than cuttings with poor nutrition. Good quality planting material is essential for obtaining good yields.

Cassava are usually harvested in dormant period in between two rain season when the root reach better commercial quality with maximum production and starch content of roots (Leihner,1980). So when stakes are harvested at this season they need to be stored for next planting season.

Moisture loss of cuttings during storage has proven to have strong influence on stake viability and vigour (Leihner, 1983). But also it may have influence on some biochemical properties of cuttings that has influence on sprouting and nutrition of stored cuttings (Leihner, 1983). Reabsorption of moisture from the environment is possible when cuttings are submerged in water but it will absorb very small quantity and only if the cuttings had not lost water to critical level. According to Leihner, (1983) critical moisture level of cassava planting materials in which sprouting below that will be reduced drastically was found to be 50%.

The minimum mean temperature for growth of cassava is 17 °C (Cock, 2011). The absolute minimum should not go below 10 °C because at this temperature and below sprouting of cuttings is delayed and may fail completely (Adeyemo, 2009; Cock, 2011).

4.3 Materials and methods

4.2.1 Site description

The experiment was conducted in two sites namely University of Nairobi Kabete Campus and KARLO Kiboko. Kabete is situated about 15 km to the west of Nairobi city and lies at 1° 15′S latitude and 36° 44′E longitude and at altitude of 1930 m above sea level (masl) (Onyango *et al.*, 2012). Kabete has a bimodal distribution of rainfall, with long rains from early March to late May and the short rains from October to December (Onyango *et al.*, 2012) and total annual rainfall

ranging between 700-1500mm (Wasonga *et al.*,2015). The mean annual temperature is 18 °C. The soils in Kabete are characterized as deep, well drained, dark reddish-brown to dark brown, friable clay (Onyango *et al.*, 2012). The soil is classified as a humid Nitisol (Karuku, *et al.*, 2012).

The second site was KARLO-Kiboko which lies within longitudes 37°.43 212′ E and latitudes 2°.12 933′S, and 821.7 m above sea level in Makueni County, 187 km east of Nairobi, Kenya (Kivuva *et al.*, 2015). The location receives between 545 and 629 mm of rainfall coming in two seasons. The long rains season is between April and May while the short rains season is between October and January. The mean annual temperature is 22.6°C, while the annual maximum temperature is 28.6°C and annual minimum temperature is16.5°C. The soils are well drained, Fluvisols, Ferralsols, and Luvisols with soil pH of about 7.9 (CIMMYT, 2013). Soil analysis showed pH of experimental site was 5.85 (H₂O), 5.45 (Cacl) and 5.25 (H₂O), 4.50 (Cacl) Kiboko and Kabete respectively (Table 7).

Table 7: Soil analysis data for basic nutrients

Site	% N	P (ppm)	K Cmol (+)/kg	% Organic Carbon	pH Cacl	pH (H ₂ O)
Kabete	0.29	8.05	1.10	2.26	4.50	5.25
Kiboko	0.10	14.15	1.33	0.97	5.45	5.85
Sufficient range	5.1 - 5.8	4 -15	0.15 - 0.025	2.0 - 4.0	4.5-7	4.5-7

4.2.2 Source of cassava cuttings

Two cuttings per storage method were sampled to form 6 cuttings. Then 10 cm were removed from each end. Remaining part were cut into 20 cm cuttings. The cuttings were mixed together and sampled for sprouting test. The diameter of cuttings ranged from 1.5 cm to 3.4 cm (Table 8).

Table 8: Characteristic of cuttings before planting

Variety	Minimum diameter (cm)	Maximum diameter (cm)	Average diameter (cm)
Karembo	1.50	3.00	1.98
KME4	1.51	3.40	2.14

4.2.3 Experimental design

The trial was a split plot design in RCBD (Okoli *et al*, 2010) with main plot as storage methods and sub plots being varieties (Figure 17), replicated three times. The sprouting Test was done at interval of 0, 4, 8, 12 and 16 weeks after storage. The spacing between cuttings was 1 m. Sub plot having varieties had six plants for data collection. Planted cuttings had 20 cm each having 4 -7 nodes. The cuttings were planted at 60° slanting position and irrigated three days per week to maintain moisture at field capacity (Bridgemohan and Bridgemohan, 2014). Weed control was done manually using hand hoe every 4 weeks. The sprouting percentage of cuttings was scored from week 3 to week 8. Number of leaves per plant and primary shoots were measured from 5 WAP – 8 WAP (Ekanayake, 1996).

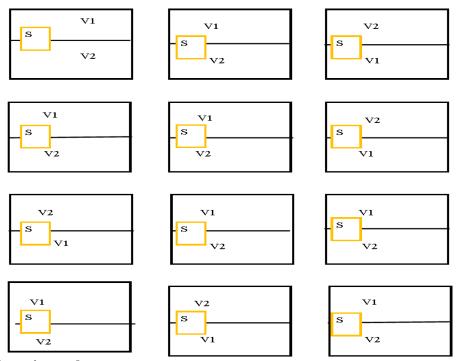


Figure 17: Experiment layout

Where; S = storage methods and V1 and V2 = varieties

4.4 Data analysis

Data was subjected to ANOVA to determine the differences among treatments and locations using GenStat (Payne., 2012) and means were separated using least significant difference (LSD) at $p \le 0.005$. Complete split plot model used for analysis was:-

 $\label{eq:Yijkl} Yijkl = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\beta\gamma)_{ik} + (\alpha\gamma)_{jk} + (\beta\gamma\alpha)_{ijk} + \epsilon_{ijkl} \quad \text{(Kroonenberg and van Eeuwijk,}$ 1998; Smith, 2006)

Where Yijkl is perfomance of stored cuttings, μ is the general mean of the population αi , βj , and γk is mean of storage methods, mean of varieties and mean of duration of storage, as main effects while $(\alpha \beta)_{ij}$, $(\beta \gamma)_{ik}$ and $(\alpha \gamma)_{jk}$ are corresponding two-way interaction effect and $(\beta \gamma \alpha)_{ijk}$ three-way interaction effect and ϵ_{ijkl} represent the expected error (Kroonenberg and van Eeuwijk, 1998).

The data taken were sprouting percentage, number of primary stems per plant, number of leaves per plant, leaves formation per day and vigour. The vigour score was based on scale 0 = not germinated, 1 = very poor vigour, 3 = poor vigour, 5 = intermediate vigour, 7 = vigorous and 9 = highly vigorous (Ekanayake, 1996).

4.5 Results

4.4.1 Weather data during the experiment duration

The mean temperatures were 31.34 °C and 20.32 °C in Kiboko and Kabete respectively. Rainfall in Kiboko was negligible while in Kabete it was 5.67 mm. RH were around 82% and 67 % in Kiboko and Kabete respectively (Figure 18). The data were obtained from ICRISAT Kiboko and Kabete meteorological stations from February to July 2016 in both locations. The RH in Kiboko being higher than Kabete can be to Kiboko is near the Indian Ocean.

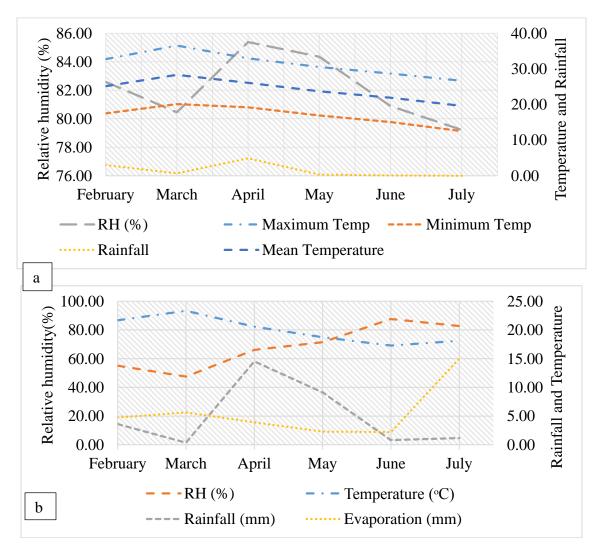


Figure 18: Average Temperature, RH, rainfall and evaporation in 2016(a) Kiboko (b)

Kabete

4.4.2 Sprouting percentage

The results of location of storage, duration of storage, storage methods and variety showed highly significant differences within treatments at p < 0.001 (Table 9). The results further showed that cuttings stored in Kabete had higher average sprouting percentage of 54.73% as compared to those stored in Kiboko with 37.78% percentage for the entire experimental duration (Table 9). Also the results showed that sprouting of cuttings at 0 week of storage had highest sprouting percentage of 81.96 as compared to 16.67 percentage of sprouting after 16 weeks after storage (Table 9). CUDS had highest average sprouting percentage of 75.57 and 42.22 in Kabete and Kiboko respectively and the lowest was HUOG with 34.44 % both in Kabete and Kiboko (Table 9). KME4 performed better by having average of 55.83 % sprouting compared to 36.67 % sprouting of Karembo (Table 9).

Table 9: Sprouting percentage of stored cuttings from 0 week to 16 weeks of storage in different storage methods and location

		Kabete					Kiboko			
WAS	CUDS	HUS	VUS	HUOG	Mean	CUDS	HUS	VUS	HUOG	Mean
0	94.5	97.22	88.94	86.11	91.69	61.11	75.00	80.56	72.22	72.22
4	86.11	41.67	66.67	83.33	69.45	69.44	58.33	80.56	58.33	66.67
8	88.89	50.00	61.11	2.78	50.70	52.78	25.00	13.89	41.67	33.34
12	44.44	44.44	33.33	0.00	30.55	27.78	16.67	13.89	0.00	14.59
16	63.89	13.89	47.22	0.00	31.25	0.00	2.78	5.56	0.00	2.09
Mean	75.57	49.44	59.45	34.44	54.725	42.22	35.56	38.89	34.44	37.78
LSD 0.05										6.40
CV										26 .80

Where; WAS = weeks after storage, CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade and HUOG = horizontal under open ground, CV= coefficient of variation and LSD = Least significant different.

CUDS in Kabete had sprouting of 75.57% while in the Kiboko had 42.22 % (Table 9). KME4 performed better by having average sprouting percentage of 63.61 in Kabete and 48.06 in Kiboko than 45.84 Kabete and 27.50 in Kiboko of Karembo (Figure 19). KME 4 showed to have high percentage sprouting at 16 weeks after storage of 24.31 as compared to 9.03 of Karembo.

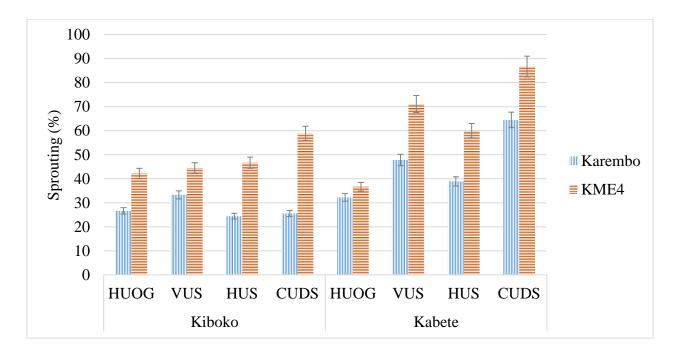


Figure 19: Sprouting percentage of different cassava varieties from different storage methods and Location

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade and HUOG = horizontal under open ground.

4.4.3 Number of primary shoots per plant (NPSP⁻¹)

Number of primary shoots showed significant differences between location at p > 0.01. The trial at Kabete had 1.60 shoots as compared to 1.04 at Kiboko in 8 MAP. The results showed that duration of storage, storage methods and variety were significantly different at p > 0.001. Among duration of storage, zero weeks of storage (harvested and planted without storage) had an average of 1.95 NPSP⁻¹ which decreased gradually as weeks of storage advanced up to 0.82 NPSP⁻¹ in 16

WAS. The average number of primary stems in CUDS was 1.64 followed by HUS (1.43), VUS (1.29) and the last was HUOG (0.93) (LSD = 0.24) (Table 10). Between varieties KME4 had 1.58 NPSP^{-1} as compared to 1.07 of Karembo (LSD = 0.16).

The results showed Kabete had 2.12 NPSP⁻¹ as compared to 1.78 in Kiboko from zero week that decreased gradually up to 1.52 at Kabete and 0.12 at Kiboko by 16 WAS. As duration of storage was advancing the average number of primary shoots was decreasing depending on storage methods as well as location of storage (Table 10).

There was significance difference in performance of varieties in different locations on same storage methods (Table 10). Variety KME4 and Karembo under CUDS at Kabete had 2.20 and 1.76 NPSP⁻¹ respectively while at Kiboko CUDS had 1.68 and 0.90 NPSP⁻¹ KME4 and Karembo respectively (Figure 20).

Table 10: Average number of primary shoots per specified duration of storage, location and storage method

		Kabete	e				Kibok	0			
WAS	CUDS	HUS	VUS	HUOG	Mean	CUDS	HUS	VUS	HUOG	Mean	Overall mean
0	2.31	1.92	2.11	2.15	2.12	1.9	1.46	1.66	2.12	1.79	1.95
4	1.88	1.56	1.98	2.02	1.86	1.89	1.61	1.62	1.88	1.75	1.81
8	1.86	1.71	1.68	0	1.31	1.8	0.97	0.53	1.09	1.10	1.21
12	2.08	1.26	1.5	0	1.21	0.85	0.5	0.39	0	0.44	0.82
16	1.85	1.75	2.46	0	1.52	0	0.17	0.33	0	0.13	0.82
Mean	2	1.64	1.95	0.83	1.61	1.29	0.94	0.91	1.02	1.04	1.32
$LSD_{0.05}$											0.26
CV											34.8

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade, HUOG = horizontal under open ground and WAS = weeks after storage

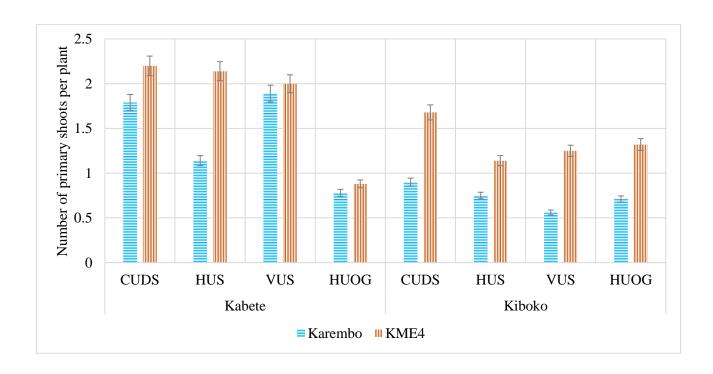


Figure 20: Number of primary shoots per plant with reference to storage methods, location and varieties.

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade and HUOG = horizontal under open ground.

4.4.4 Number of leaves per plant (NLP⁻¹)

The number of leaves per plant 8 WAP shows how plants are vigorous and established. In the study the number of leaves per plant at 8 WAP did not differ significantly between locations of experiment at p < 0.05. Duration of storage, storage methods and variety significantly differed among treatments at p < 0.001. The results showed that cassava cuttings planted soon or few days after harvest had 5.32 leaves by 8 the WAP while plants established from cuttings that were stored for 16 weeks had 4.16 leaves at 8 WAP. Further, the results showed that CUDS had 10.27 leaves while other storage methods had $8.32 - 4.16 \text{ NLP}^{-1}$ at 8 WAP. Varieties KME4 had 10.30 while Karembo had 5.00 (LSD = 1.52).

The results showed that at 0 week in both Kabete and Kiboko the number of leaves were 5.04 and 5.61 respectively 8 WAP which increased with increasing weeks of storage to 11.85 and 18.97 at Kabete and kiboko respectively at 8 WAS then started decreasing as WAS advanced. The highest number of leaves 31.08 were observed in variety KME4 under CUDS 8 WAS (Figure 21). At 16 WAS plants established from the cuttings had 1.54 and 8.32 leaves per plant at Kiboko and Kabete respectively. The number of leaves per plant for CUDS were 9.27 and 11.27 in Kabete and kiboko respectively and the lowest were observed at HUOG, 1.61 and 6.72 Kabete and Kiboko respectively.

Variety KME4 had 8.53 and 12. 08 NLP⁻¹in Kabete and Kiboko respectively while Karembo had 5.72 and 4.28 NLP⁻¹ in Kabete and Kiboko respectively.

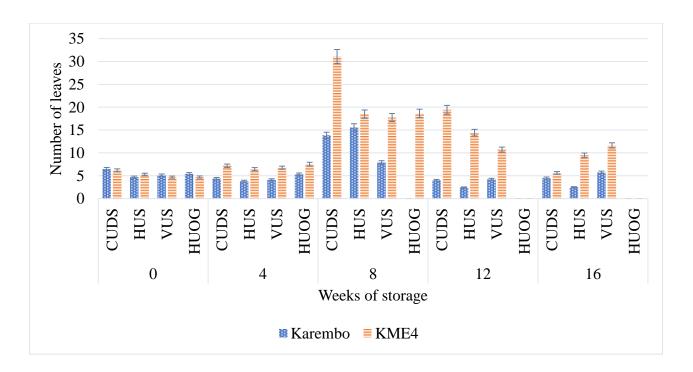


Figure 21: Number of leaves per plant from different varieties under different storage methods and duration of storage

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade, HUOG = horizontal under open ground and bar represent standard error

4.4.5 Rate of leaves formation per day (Lfd⁻¹)

There was significant difference between location at p < 0.05, where the rate of leaf formation per day was, 0.60 and 0.39 for Kiboko and Kabete respectively. Duration of storage, storage methods and variety were significantly different at p < 0.01 among treatments applied. The highest leaf formation rate (0.81) was observed at 8 WAS and lowest rate (0.09) at 16 WAS (LSD = 0.12). The rate of leaf formation at 0 WAS was 0.76 per day. Variety KME4 had 0.60 while Karembo had 0.38 rate of leaf formation per day (LSD = 0.07). CUDS had 0.61 followed by HUS (0.49), VUS (0.48) and the last was HUOG (0.40) rate of leaf formation (LSD = 0.12).

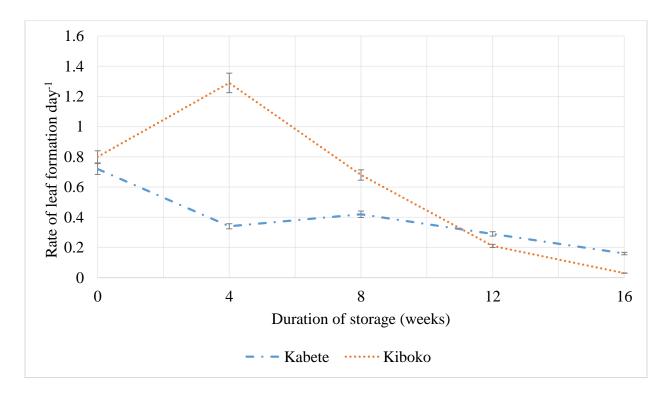


Figure 22: Rate of leaf formation of cassava plant at different duration of storage and location

Increased leaf formation was observed at Kiboko from 0 WAS to 4 WAS as compared to Kabete (Figure 22), then after reduced as weeks of storage advanced. The results showed that KME4

performed better in Kiboko by having rate of leaf formation of 0.77 as compared to 0.44 at Kabete. While variety Karembo had lower rate (0.43) in Kiboko and 0.33 in Kabete (Figure 23).

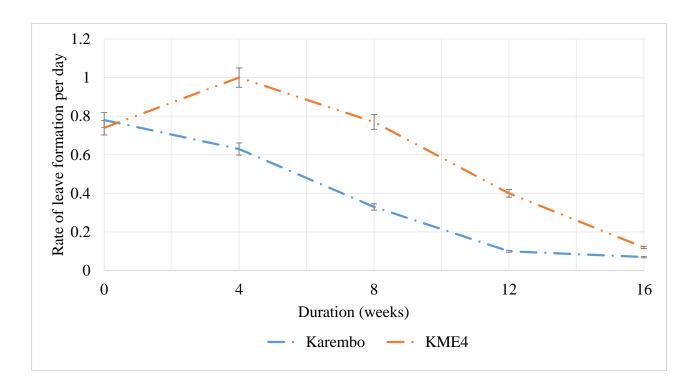


Figure 23: Rate of leaf formation on establishment of different variety of cassava cuttings stored for different duration. bar represent standard error

4.4.6 Plant vigour

Plant vigour did not significantly differ between locations but differed significantly among duration of storage, storage methods as well as variety at p < 0.05. The growth vigour was 4.33 at 0 WAS and 0.94 at 16 WAS (Table 11). The average vigour between two sites was high at 4 WAS. CUDS had average of growth vigour of 3.7 between Kiboko and Kabete and the average lowest growth vigour of 2.35 between two sites was observed at HUOG (Table 11). Variety KME4 was more vigourous than Karembo by having difference of 1.05 (LSD = 0.34 (Table 13). The results showed that at 8 WAS the vigour of cuttings in both sites reduced drastically. At Kabete 8 WAS, HUOG had 0 growth vigour meaning that they didn't sprout (Figure 24)

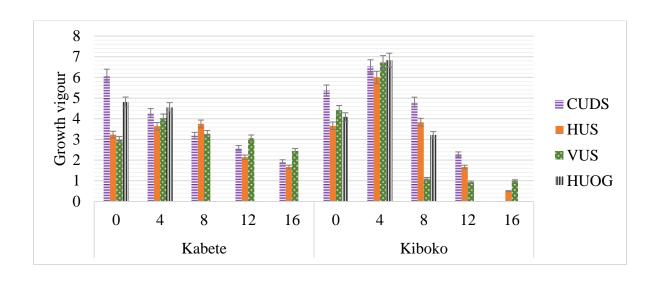


Figure 24: Average vigour of cassava plants from cuttings stored in different storage methods, locations and duration of storage.

Growth vigour at Kiboko and Kabete was 4.38 and 4.28 respectively at 0 WAS decresed as duration of storage advanced to 0.37 at Kiboko and 1.51 Kabete at 16 WAS. The highest growth vigour (6.52) was recorded at Kiboko at 4 WAS while at Kabete it was 4.12. Variety KME4 had growth vigour of 3.91 and 3.18 at Kiboko and Kabete respectively while Karembo had 2.39 and 2.58 at Kiboko and Kabete respectively (Table 14). The growth vigour of cassava decreased as duration of storage advanced (Figure 25)

Table 11: Average plant vigour of cassava plant from different storage methods and duration of storage.

	CUDS	HUS	VUS	HOUG	Mean
0 WAS	5.72	3.44	3.71	4.45	4.33
4 WAS	5.40	4.82	5.38	5.69	5.32
8 WAS	3.99	3.79	2.18	1.61	2.89
12 WAS	2.43	1.90	2.00	0.00	1.58
16 WAS	0.96	1.08	1.72	0.00	0.94
Mean	3.70	3.01	3.00	2.35	3.01
LSD					0.57
CV					36.30

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade, HUOG = horizontal in under open ground and WAS = weeks after storage

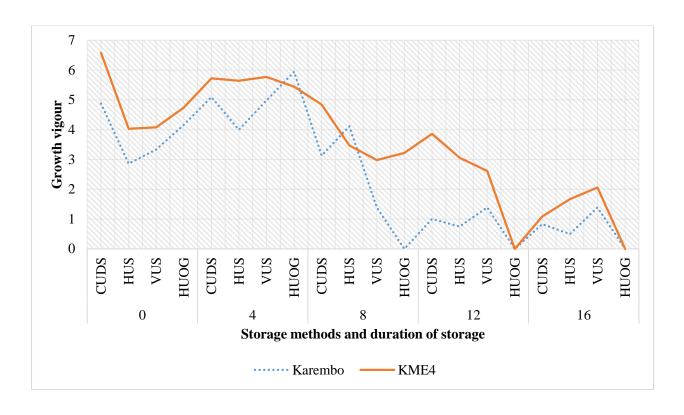


Figure 25: Growth vigour of cassava plants under different storage methods, duration of storage and varieties.

Where; CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade and HUOG = horizontal in under open ground

Table 12: The sprouting percentage, number of leaves, number of primary shoots, rate of leaf formation, sprouting percentage and growth vigour of cuttings stored for different duration.

Duration of storage	Sprouting %	NLP ⁻¹	NPSP ⁻¹	RLFD ⁻¹	Vigour
0 WAS	81.96	5.323	1.955	0.7605	4.331
4 WAS	68.06	5.697	1.805	0.8138	5.322
8 WAS	42.01	15.407	1.206	0.5502	2.894
12 WAS	22.57	6.907	0.822	0.2467	1.583
16 WAS	16.67	4.932	0.821	0.0918	0.942
LSD5%	6.923	2.399	0.25	0.119	0.721
CV	12.20	25.60	15.40	19.7	19.50

WAS = weeks after storage, NLP⁻¹ = number of leaves per plant, NPSP⁻¹ = number of primary shoots per plant, RLFD⁻¹= rate of leaf formation per day.

Table 13: The sprouting percentage, number of leaves plant ⁻¹, number of primary shoot, rate of leaf formation day ⁻¹ and growth vigour of cuttings stored in different storage methods.

Storage methods	Sprouting %	NLP ⁻¹	NPSP ⁻¹	RLFD ⁻¹	Vigour
CUDS	58.89	10.270	1.642	0.6083	3.702
HUS	42.50	8.317	1.427	0.4909	3.008
VUS	49.17	7.861	1.291	0.4759	2.997
HOUG	34.44	4.165	0.926	0.3952	2.351
LSD5%	6.403	2.73	0.238	0.115	0.566
CV	26.80	69.10	34.80	45.30	36.70

Where; WAS = weeks after storage, NLP⁻¹ = number of leaves per plant, NPSP⁻¹ = number of primary shoots per plant, RLFD⁻¹ = rate of leaf formation per day.

Table 14: The sprouting percentage, number of leaves per plant, number of primary shoot, rate of leaf formation day ⁻¹ and growth vigour of different varieties of cuttings

Variety	Sprouting %	NLP ⁻¹	NPSP ⁻¹	RLFD ⁻¹	Vigour
Karembo	36.67	5.00	1.07	0.38	2.49
KME4	55.83	10.30	1.58	0.6	3.54
LSD5%	5.03	1.52	0.16	0.07	0.33
CV	42.40	77.70	47.50	58.30	43.70

Where; WAS = weeks after storage, NLP⁻¹ = number of leaves per plant, NPSP⁻¹ = number of primary shoots per plant, RLFD⁻¹ = rate of leaf formation per day.

4.6 Discussion

The study results have shown that cassava crop establishment depends on variety and storage condition of the cuttings planted. KME4 has shown to have better storability than Karembo. Variety KME4 stored in Kabete in CUDS storage methods for 16 weeks still had sprouting of 94.44% as compared to 33.33% of Karembo under similar conditions. This may be due to genetic variability among the varieties. Similar results were obtained by Oka et al., (1987). He was storing two cultivars of cassava and found that one cultivar dehydrated more than the other which contributed to reduction in sprouting of planted cuttings. Also the causes of this variability among cultivars might be physiological differences among stem structure from one cultivar to another. Nassar et al., (2010) found differences in collenchyma and internal parenchyma among cultivars of cassava and such differences may be the reason for differences in storability of KME4 and Karembo. But also this difference in storability indicate that when selecting planting materials for storage, it requires knowledge of the characteristics of cultivar. The results also indicated that there was significant influence of environmental factors in storability and sprouting of cassava. Kabete had average temperature of 20.32 °C and 65.55 % RH while Kiboko had 31.34 °C and 82.15 RH. This difference can be major contributor to performance of cassava storability and establishment. The results showed sprouting and crop establishment depends mostly on initial moisture content of cuttings. In Kiboko cuttings lost moisture content at high rate due to high average temperature to the extent that they lost viability and vigour in shorter period than in Kabete. This can be due to fact that in coastal Kenya the potential evaporation is always higher than rainfall except in May, April and November where rainfall is high (Kibe et al., 1981). But also According to Kinama, et al., (2005), water loss from plant surface depends on RH, wind speed, radiation and temperature.

The number of primary shoots from the cuttings depends on carbohydrate and nutrient composition of planting materials. The results showed significant differences between location. This can be due to difference in loss of carbohydrate between cuttings stored in Kabete and Kiboko.Ravi and Suryakumar, (2005); Oka *et al.*, (1987) reported cassava having low early growth vigour which resulted to reduction in production for cuttings which lost carbohydrate during storage. Also similar results were observed in planting materials stored in different storage methods, CUDS had low temperature and RH around 70% which resulted to reduction in rate of moisture and carbohydrate loss hence more primary shoots and number of leaves than cuttings stored in different methods and environment with high temperature.

Additionally, the results showed that the rate of leaf formation per day was significant between locations. Kiboko had higher rate of 0.60 per day as compared to 0.39 per day in Kabete. Akparobi *et al.*, (2000) found simiral results. The rate of leaf formation in low temperature is less compared to environment with temperature mean around 30 °C. In Kabete the mean of 20 °C and the minimum temperature of 12.7 °C around June had influenced low growth vigour in cassava plants. The results also showed significant difference among storage methods, where CUDS had planting materials more vigorous than other methods. This might be due to less carbohydrate and reduced moisture loss during storage than other methods. Similar results have been reported by Hobman *et al.*,(1987). This is further illustratted by poor stand establisment in Kiboko from 4 WAS to 16 WAS. In all parameters the optimum perfomance was observed at 4 WAS then after reduced as weeks of storage advanced as explained by reduced carbohydrate due to metabolism and reduction of moisture content of stored cuttings.

CHAPTER FIVE

5.0 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General discussion

The results of this study of storage methods, duration of storage and variety of cassava planting materials and how they responded to moisture, carbohydrate content, drying of cuttings as well as crop establishment (sprouting %, ANL, ANPS, RLF/day) and vigour showed significant treatments effects. In both objectives CUDS, performed better than other storage methods. CUDS had minimum rate of moisture and carbohydrate loss than other storage methods. Similar results were obtained by Kozlowsk (1991) and Leihner, (2002). They found that if cassava cuttings were stored under low temperature and RH around 70%, there is reduction in the rate of carbohydrate and moisture loss. Also number of cuttings that dried more than 25% of its stored length or 100% of its stored length were recorded less in cuttings under CUDS than in other three storage methods. This can be explained by radiation in other three storage methods to be higher than in CUDS hence results to lose moisture from each end of cuttings. When cuttings have bruises in epidermis of cuttings they lose more moisture as it will be losing from two cut ends and in bruised epidermis. The highest cuttings death was observed in cuttings stored under HUOG. This can be due to differences in temperature and RH observed during the experiment as HUOG were exposed direct to radiation and wind. Also, it was observed that cassava planting materials left on open ground under direct sun light lost moisture at high rate than those stored under shade. Additionally, planting materials stored under shade but with high temperature greater than 20 °C had higher rate of carbohydrate loss due to increased rate of respiration of the stored cuttings for survival. Ravi and Suryakumari, (2005); Zhu, et al., (2015) found when cassava cuttings stored in high temperature the rate of carbohydrate loss is high also. The results also showed clear significant differences between variety in sprouting percentage, early growth vigour, rate of leaf formation per day, leaves number per plant and number of primary shoots per plant, showing KME4 performed better than Karembo in storability. This could be due to genetic difference among the varieties.

Additionally, the results showed that CUDS was the only storage method with 94.44 % sprouting for cuttings stored at Kabete for 16 weeks while, cuttings stored in Kiboko had 0 %. This shows clearly the influence of temperature and RH in storage of cassava planting materials. Kinama *et al.*, (2005) found that the rate of soil evaporation is high in soil with no cover than in mulched soil. The high rate of evaporation affecting the soil will affect cuttings stored on open ground under direct sunlight and wind movement as well. In CUDS shade reduced sunlight rays and clamp insulate cuttings from too high or too low temperature and maintained RH around 60 % - 70 %. In general, the sprouting at 4 weeks after storage was 68.06 %. Thus harvesting of cuttings should be done after other operations are completed (land preparation and transportation arrangements). KME4 has shown that it has the ability to sprout in the field than Karembo. KME4 had sprouting mean of 86.11 % as compared to 77.81 % of Karembo at 0 WAS. As weeks of storage advanced Karembo performed worse in Kiboko as they were losing moisture at high rate as compared to Karembo in Kabete.

The results have shown that the number of shoots, rate of leaf formation per day and vigour is linked to food and nutrient stored in planting materials. Cassava cuttings at Kabete were planted in soil with 0.29% N while Kiboko soil had 0.1 % N but early growth vigour was high in Kiboko than in Kabete. Cock, (2011) reported that cassava growth is sensitive to temperature, it has effect on growth vigour of cassava, it affects sprouting, leaf formation and leaves number per plant. The optimum growth of cassava is around 25 °C – 30 °C but it can tolerate temperature as

low as 12 °C and as high as 40 °C. This explains why cassava crop grown at Kiboko was more vigorous than cassava crop grown in Kabete.

5.2 Conclusion

According to results obtained from this study storability of cassava depend on cultivar and environmental factors especially relative humidity, temperature, wind and radiation. It's better if cassava planting materials will be stored under shade and provide cover to insulate from high temperature and direct radiation. Prolonged storage should be avoided since it contributes to carbohydrate and moisture loss during storage. High temperature has influence in carbohydrate loss as it increases respiration rate of stored cuttings. Long term storage of cassava cuttings is possible using CUDS methods. But storability of cassava planting cuttings also influenced by cultivar genotype. Crop establishment and vigour is also influenced by storage conditions of planting materials and duration of storage. Long term storage of cassava planting material may work under low temperature and RH around 70%. However high RH will cause the stored cuttings to sprout which cause increased carbohydrate consumption of stored cuttings.

Also it was observed that the most important factor for cassava crop establishment and early growth vigour is the storage of cuttings under double shade which reduced temperature and protected cuttings from direct radiation. Also observation showed that crop establishment depends on variety genotype. For these reasons in case of commercial cuttings production they should consider parameter of storability to ensure maximum stand establishment. In hot environment duration of storage should be as short as possible to avoid poor stand establishment and early growth vigour which has high contribution to final yield of cassava crop. From the results KME4 was the best in terms of storability and early growth vigour than Karembo.

5.3 Recommendations

- Farmers may use CUDS methods of storage which preserve the planting material.

 However, to minimize cost and loss of planting material proper planning should be done before harvesting the cuttings as they tend to loss carbohydrate at high rate few weeks after harvest.
- For long duration storage under minimum temperature and RH around 70% is best while covering them from direct sun light and wind.
- Cassava which are grown for seed multiplication should be fertilized to ensure enough carbohydrate and nutrients to cuttings for longer storage and proper stand establishment.
- Cassava breeding program needs to develop varieties which withstand storability of cassava planting materials for longer duration.
- Further research on proper storage conditions of cassava planting materials especially the
 actual temperature and RH which will reduce moisture loss and reduce the carbohydrate
 consumption beyond the thresholds is recommended.
- The experiment was done using available materials that is grass thatch, soil, wooden poles
 which is available at vicinity of normal farmers. This reduce the cost of storage of cassava
 cuttings.

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APPENDICES

Appendix 1: Means of gradually dried, carbohydrate and moisture of cassava planting materials in different storage methods

Storage methods	% DC >25%	% DC 100%	% Carbohydrate	% Moisture
CUDS	52.55	34.40	3.54	53.54
HUS	59.49	35.23	3.91	42.88
VUS	62.49	36.39	3.63	48.32
HOUG	71.54	52.56	3.89	38.83
LSD5%	1.85	1.46	0.32	2.64
CV	11.20	14.20	16.60	11.10

% DC >25% OL = Percentage dry cuttings > 25 % of its stored length, % DC = Percentage dry cuttings 100% of its stored length, CUDS = clamp under double shade, HUS = horizontal under shade, VUS = vertical under shade and HUOG = horizontal under open ground.

Appendix 2: Mean of dried cuttings, moisture and carbohydrate content of cuttings in different storage duration and varieties.

Duration of storage	% DC >25%	% DC 100%	% Carbohydrate	% Moisture
0 WAS	0.00	0.00	7.949	70.16
4 WAS	45.69	3.83	3.993	53.32
8 WAS	72.60	50.76	2.629	41.67
12 WAS	92.42	67.86	2.264	37.35
16 WAS	96.87	75.78	1.876	26.96
LSD5%	2.20	2.42	0.23	1.56
CV	6.20	10.60	5.00	2.80

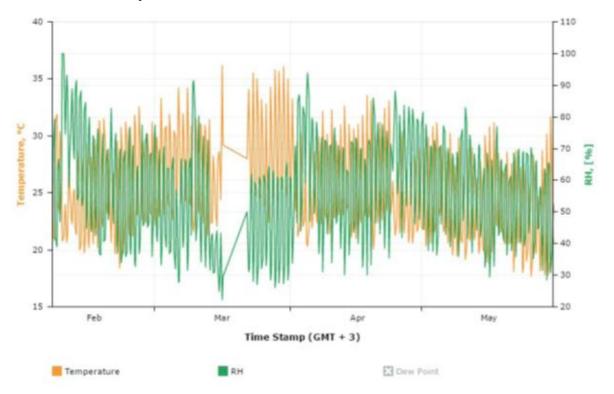
% DC 25% OL = Percentage dry cuttings > 25 % of its stored length, % DC = Percentage dry cuttings 100% of its stored length and WAS = weeks after storage.

Appendix 3: Mean of dried cuttings, Percentage carbohydrate and percentage moisture content of different varieties under different storage methods

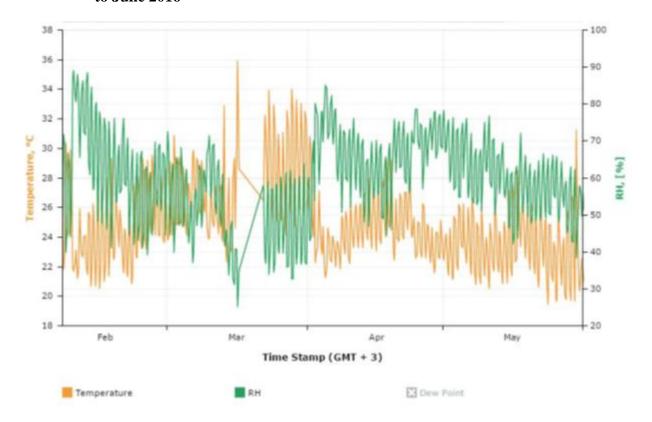
Variety	% DC >25%	% DC 100%	% Carbohydrate	% Moisture
Karembo	66.54	47.15	4.03	42
KME4	56.49	32.14	3.45	49.79
LSD5%	3.137	2.934	0.269	2.078
CV	19.90	28.9	28.00	17.70

% DC 25% \overline{OL} = Percentage dry cuttings > 25 % of its stored length, % DC = Percentage dry cuttings 100% of its stored length

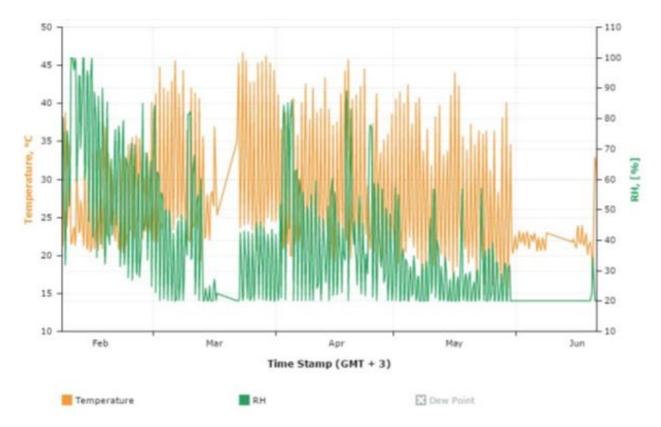
Appendix 4: RH and temperature from Xsense data loggers from HUS and VUS Kiboko February to June 2016.



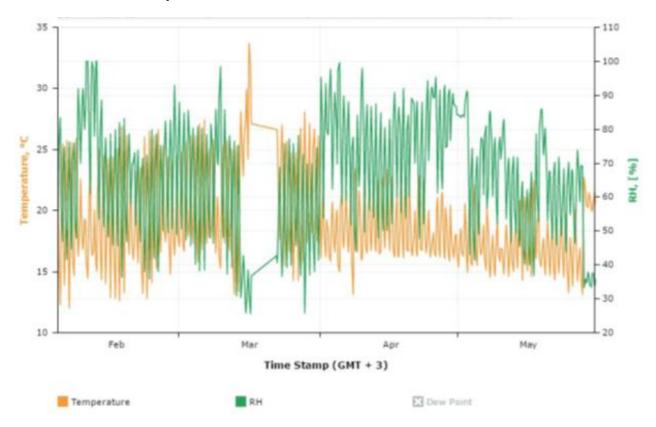
Appendix 5: RH and temperature from Xsense data loggers from CUDS Kiboko February to June 2016



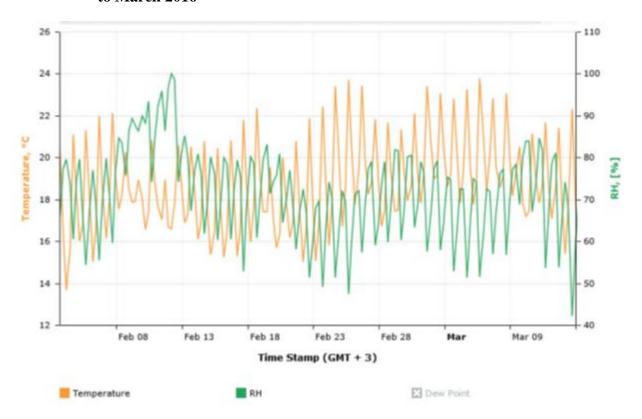
Appendix 6: RH and temperature from Xsense data loggers from HUOG Kiboko February to June 2016



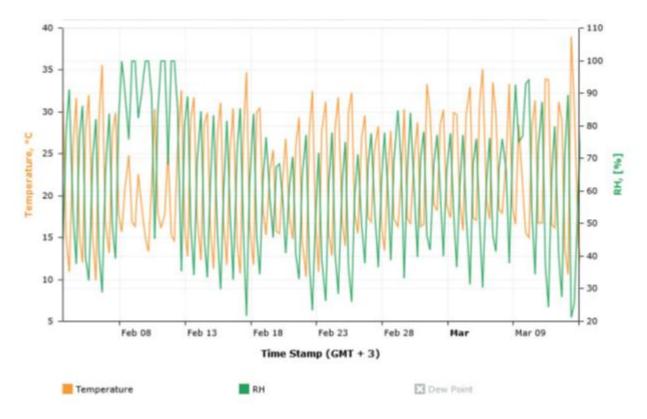
Appendix 7: RH and temperature from Xsense data loggers from HUS and VUS Kabete February to June 2016



Appendix 8: RH and temperature from xsense data loggers from CUDS Kabete February to March 2016



Appendix 9: RH and temperature from xsense data loggers from HUOG Kabete February to March 2016

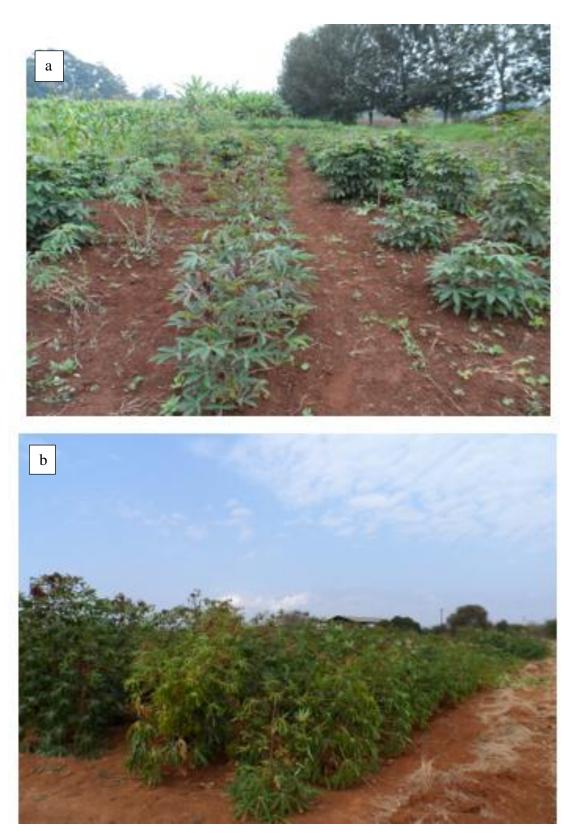


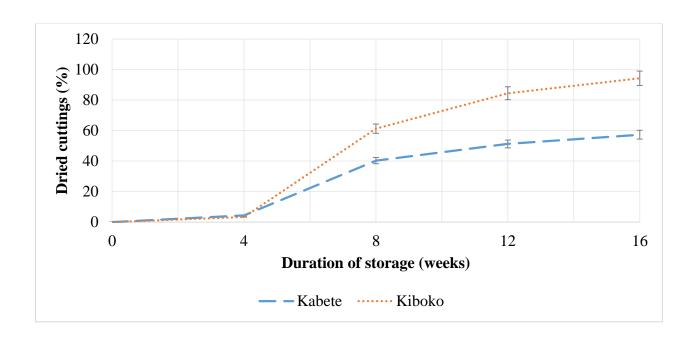
Appendix 10: (a) Shade used under CUDS, VUS and HUS in Kabete, (b) Opened CUDS for data collection and (c) cuttings stored in closer look.





Appendix 11: (a) Kabete experimental field and (b) is Kiboko experimental field for sprouting and early growth development





Appendix 12: Rate of drying of stored cuttings from Kabete and Kiboko as weeks of storage advanced.

Appendix 13: Mean square showing the effect of location of storage, duration of storage, storage methods and cuttings varieties of different traits and their level of significance

Source of variation	d.f.	Sprouting %	NLP ⁻¹	NPSP ⁻¹	RLFD ⁻¹	Vigour
Location	1	17238.2***	67.11NS	19.2107**	2.74334*	4.284NS
Residual	4	57.7	54.5	0.44	0.17881	2.569
WAS	4	38453.5***	928.01***	13.7768**	4.79236***	161.026***
Location. WAS	4	1072.8*	421.23***	3.3261**	2.28832***	23.049***
Residual	16	256	30.75	0.33	0.07512	2.778
SM	3	6437.1***	389.95***	5.4301***	0.46316**	18.267***
Location.SM	3	2892.7***	171.78*	4.1112***	0.18567NS	4.131NS
WAS.SM	12	1595.7***	114.05*	1.8454***	0.1643NS	8.139***
Location.WAS.SM	12	1554.1***	86.22NS	0.6583NS	0.1186NS	4.936*
Residual	60	307.4	55.86	0.42	0.09968	2.398
V	1	22028.8***	1686.99***	15.7599***	3.01571***	66.852***
Location.V	1	116.7NS	373.64**	0.6968NS	0.70354**	12.727**
WAS.V	4	1728.1**	294.8***	0.8532NS	0.50237***	2.779NS
SM. V	3	840.3NS	28.48NS	0.3944NS	0.04136NS	1.539NS
Location.WAS.V	4	697.1NS	201.49***	0.4559NS	0.52146***	1.865NS
Location.SM. V	3	458NS	49.29NS	1.1589*	0.06452NS	1.372NS
WAS.SM.V	12	738.4*	76.09*	0.584NS	0.09415NS	3.679*
Residual	92	384.7	35.35	0.39	0.08237	1.732
Total	239					

 NLP^{-1} = number of leaves per plant, $NPSP^{-1}$ =number of primary shoots per plant, $RLFD^{-1}$ = Rate of leaf formation per day, WAS = Weeks after storage, SM = storage methods, V = variety and d.f = Degree of freedom.