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TOWARDS IMPROVEMENT OF UGANDAN CASSAVA GERMPLASM FOR DROUGHT TOLERANCE

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A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) in Plant Breeding and Biotechnology of Makerere University

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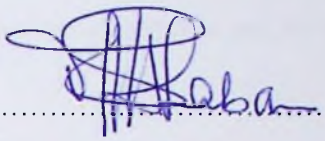
DECLARATION

I, Turyagyenda Laban Frank, declare that the research reported in this thesis is my original work and the thesis has not been submitted for any degree or examination at any other University. The thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons or referenced literature.



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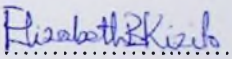

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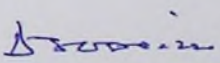

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DEDICATION

This thesis is dedicated to my parents Mr. Eliezer E. Mabuye (deceased) and Mrs. Dina Mabuye for their support and encouragement especially during the earlier stages of my education development.

To my wife Sharon Turyagyenda, our daughters Desire Nimusiima and Diana Tumusiime and our son Daniel Turyagyenda for their support, patience and for hard times they went through during my study period.

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EXECUTIVE SUMMARY

Cassava is staple food crop with potential to improve household food and income security for over 800 million people in tropical countries. However, cassava's survival and ability to produce reasonable yields under drought conditions is drastically affected. This calls for research to select and develop cassava cultivars that are capable of yielding better in water constrained environments. Genetic improvement of cassava requires clear understanding of physiological and morphological responses of the crop to drought stress, and genetic diversity among genotypes. In addition, identification of gene sources and genes involved in drought tolerance would enhance incorporation of biotechnology in cassava improvement. This research was carried out to evaluate Ugandan cassava germplasm for drought tolerance, determine genetic diversity that exists within the germplasm, and identify candidate drought tolerance genes in cassava. A total of 66 cassava genotypes were used in this study. Replicated field screening trials for cassava genotypes were established in Buliisa, Nakasongola and Kabanyolo. Genetic diversity assessment among the cassava genotypes was evaluated using 26 SSR markers, while gene expression studies of ten genes were undertaken using Real Time PCR under stressed and well watered conditions in a tolerant and a susceptible genotype.

Results from field trials indicated significant difference ($p<0.05$) among genotypes in response to drought stress. Genotypes MM96/0686, Magana, Yellow, TME204, Nyamutukura, MH97/2961, Akena and NASE12 were least affected by drought, while Bao, Nyalanda, Egabu, Icilicili and Rwaburaru were more susceptible. Genetic diversity analysis revealed high genetic diversity ($He=0.667$) among the cassava genotypes grown in Uganda, suggesting sufficient genetic base that can benefit cassava breeding. Genetic diversity assessment also indicated that landraces have unique alleles and therefore, including them in hybridization could provide highly heterogeneous base populations for selection. Furthermore, this study identified four (*MeALDH, MeZFP, MeMSD and MeRD28*) drought tolerant associated genes that can be used as gene-based markers for drought tolerance breeding. However, detailed studies to biologically validate these genes need to be undertaken.

Contrary to the general belief that cassava is tolerant to water stress, this study demonstrated that severe water stress is detrimental to cassava growth and productivity, and highlighted several drought tolerant and susceptible cassava genotypes. The study also revealed high genetic diversity among the cassava genotypes grown in Uganda, suggesting that genetic diversity and diversification

of cassava has been maintained over years of clonal propagation and human selection. Furthermore, as of this writing, this is the first report on gene expression in cassava using Real Time PCR and the candidate drought tolerance genes identified in this study provides the most important step towards improvement of cassava for drought tolerance through genetic engineering. Moreover, gene expression data were collected under water stressed conditions that closely resemble the actual field conditions, making it the first study of its kind and thus the methodologies developed will guide scientists interested in similar studies either in cassava or other crop species. Overall, information and materials developed from this research will significantly contribute towards improvement of cassava for drought tolerance.

CHAPTER 1: GENERAL INTRODUCTION

1.1 BACKGROUND

Cassava (*Manihot esculenta* Crantz) is a perennial crop native to tropical America (Olsen and Schaal, 2001). The crop along with maize, wheat and rice constitute the most important sources of energy in the diet of many people living in the tropical countries where it is a staple for over 800 million people (Burns *et al.*, 2010; Perez *et al.*, 2011). Its carbohydrate production is about 40% higher than rice and 25% than maize (Tonukari, 2004) making it the cheapest source of calories for human and animal feeding. The annual world crop production is estimated at 241 million tonnes (Bull *et al.*, 2011), of which the majority (over 85%) is produced by African and Asian small-scale farmers. Cassava is mainly grown for its starchy roots though different parts of the cassava plant are used for different purposes. The starchy roots can be kept in the field until farmers need to harvest it, allowing piece meal harvests (Iglesias *et al.*, 1997).

About 70% of world cassava root production is used for human consumption and the remaining is used for animal feed and industrial products, such as starch, glucose and alcohol (El-Sharkawy, 2004). The young leaves of cassava are also consumed as a vegetable in many African countries (Lancaster and Brooks, 1983; Hahn, 1988; Fregene *et al.*, 2000) to provide proteins, calcium, iron and vitamins, therefore supplementing predominantly starchy diets in poor communities. Among the root and tuber crops of the tropics, cassava is the most favoured root crop because the starchy roots provide more returns per unit input than any other staple crop (Akoroda, 1995; Fregene et al., 2000). The crop can also tolerate considerable amount of drought stress, compared to other staples, with no specific sensitive growth stage, except for crop establishment stage (Perez *et al.*, 2011). Its adaptability to various cropping systems, ability to do well in diverse environments with minimal inputs, and diversity in uses (Fregene *et al.*, 2000) makes cassava an ideal food security crop particularly in marginal areas where drought prone crops such as grain crops would perish.

In Uganda, cassava is the second after bananas in terms of area occupied, total production and per capita consumption, respectively (Otim-Nape and Zziwa, 1990). It is the leading food security base with annual production of 5,179,000 metric tonnes (FAOSTAT, 2010). The crop is mainly grown by resource poor small scale farmers using traditional methods, with little or no inputs. Cassava is propagated vegetatively by cuttings of about 20-30 cm long. Farmers may obtain the planting materials from their own fields, neighbours and relatives or sometimes from abandoned

fields. From abandoned fields, some farmers may unknowingly collect volunteer seedlings. Indeed, frequent use of volunteer seedlings by small scale farmers has been reported (Elias *et al.*, 2000; Kizito, 2006) and this can increase cassava's diversity.

Although cassava is a perennial crop, after full development of the canopy, root growth gradually decreases and ultimately reaches a point when maximum or near maximum yield is obtained, a point sometimes referred to as maturity period (Vries, 1985). The storage roots can be harvested any time from 6-24 months after planting depending on cultivar and growing conditions (Ngendahayo and Dixon, 2001). When cassava is harvested too early, it often leads to reduction in yield, while delayed harvest leads to development of woody and fibrous storage roots, and thus reduction in starch content (Ngendahayo and Dixon, 2001; Benesi, 2005). Nevertheless, cassava offers the advantage of a flexible harvesting date, allowing farmers to keep the roots in the ground until needed or allowing piecemeal harvesting due to roots' high perishability.

Despite its importance as food security crop and source of income to resource poor farmers living in marginal areas, cassava production is constrained by biotic and abiotic constraints. The major diseases affecting cassava production are Cassava Brown Streak Disease (CBSD) (Hillocks *et al.*, 2001), Cassava Bacterial Blight (Lozano, 1986) and Cassava Mosaic Disease (Akano *et al.*, 2002). The most important abiotic factors affecting cassava yields include drought stress and acidic soils (Anderson *et al.*, 2004). In addition, postharvest physiological deterioration (PPD) rapidly renders the roots unpalatable and unmarketable (Reilly *et al.*, 2007). Limited availability and adoption of improved cassava genotypes, lack of suitable genotypes for some geographic areas due to unreliable rainfall and limited land are other factors that further constrain cassava production (Kiwanuka and Kintu, 2004).

Research to develop genotypes resistant to different stresses has been undertaken and indeed, progress through breeding has been achieved at various cassava breeding programmes worldwide. For instance, molecular markers tightly associated with CMD resistance gene (*CMD2*) have been used in marker assisted breeding for CMD resistance (Akano *et al.*, 2002). Breeding for tolerance to cassava post harvest physiological deterioration has also been recently reported (Morante *et al.*, 2010). However, little progress has been made to develop cassava genotypes tolerant to drought. Drought is one of the major abiotic stresses limiting crop productivity worldwide (Saini and Westgate, 2000; Prasad *et al.*, 2008) and it has been reported that cassava production is drastically

affected by drought (Anderson *et al.*, 2004). Worse still is the fact that in Africa, cassava growth cycle in marginal areas is often interrupted by 3-6 months of drought resulting in poor growth, development and low economic yield (Pardales *et al.*, 2001; Bakayoko *et al.*, 2009).

With the current global warming effects, it is very likely that drought will become the major environmental factor affecting cassava production and production of other crops (Funk *et al.*, 2005; WWF, 2006). It has already been reported that rainfall in eastern and southern Africa has declined by approximately 15% during the last 30 years due to severe and frequent droughts (Funk *et al.*, 2008). Yet the characteristic unfavourable terrain in marginal areas where cassava is mostly grown makes irrigation unaffordable to resource poor farmers. Therefore to improve cassava yields and productivity and avoid losses due to drought, breeders should target improved performance under water stress by identifying and developing cassava genotypes well adapted to marginal agro-ecological zones.

Breeding for drought tolerance in cassava, though in its infancy, has had several challenges. First, there is insufficient knowledge of varietal variability among cassava germplasm grown in different countries in response to this complex trait. For guided crosses within respective country breeding programmes, genetic diversity or variability among cassava populations maintained on farmers' fields or in a breeding population should be well understood (Beeching *et al.*, 1993; Moyib *et al.*, 2007). This is especially important where there is continuous germplasm exchange between different ethnic groups and farmers and where traditional farming is practiced. For instance, in Uganda, farmers have preferences for different cassava genotypes among different ethnic groups. Farmers in northern parts of the country prefer growing bitter genotypes while central and western regions prefer growing sweet types. Farmers select and name their cassava according to complex values based on morphology, food, social and commercial interests. Thus genotype names may differ among regions, among communities and even among families. Their preferences determine the degree to which genotypes will be adopted or change between human generations, evolve over generations or stay the same (Kizito, 2006).

The second challenge in cassava drought stress breeding is the crop's high genotype by environment (G x E) interaction (Ceballos *et al.*, 2004). Genotype by environment (G x E) interaction is among the factors that can indirectly affect the response to selection and, in general, the efficiency of breeding programs (Ceccarelli, 1996). It determines if a genotype is widely

adapted for an entire range of environmental conditions or separate genotypes must be selected for different sub-environments. Breeding plans therefore may focus on the G X E interaction to select the best genotype for target environments. Breeding experiments involving G x E interactions are normally tested over a wide range of diverse environments (e.g. locations, years, growing seasons) and may involve a large number of genotypes thus necessitating specific experimental designs (Stroup, 1990) making the venture costly. Moreover, cassava has a long growth cycle, requiring between eight and twelve years to develop a new cassava variety (Raji *et al.*, 2009; Fregene *et al.*, 2001).

The third challenge in cassava breeding for drought stress is the lack of genomic resources to facilitate the selection process. Drought response is influenced by environmental factors and therefore phenotypic markers may not always be reliable in selection in a breeding programme. Drought is a complex trait controlled by many genes and identification of the genomic regions that contain genes (QTLs) associated with drought cannot be done based only on conventional phenotypic evaluation (Collard *et al.*, 2005). Collard *et al.* (2005) reported that the development and application of molecular markers could be a breakthrough in marker assisted selection (MAS). Unlike conventional phenotype based alternatives, molecular markers are stable and detectable in all tissues regardless of growth, differentiation, development, or defence status of the cell and are not confounded by the environment, pleiotropic and epistatic effects (Agarwal *et al.*, 2008). Therefore marker assisted breeding (MAB) offers a possibility of efficiency and precision in introgression of desired traits. Thus, through this approach, drought tolerant genes can be identified and transferred to drought susceptible genotypes through MAB. On the other hand, successful production of transgenic cultivars relies on identification of useful genes and their successful transfer to the host cultivar. Indeed, several drought tolerant genes have been identified in other species including arabidopsis, medicago, maize and rice (Bruce *et al.*, 2002; Nguyen *et al.*, 2004). However, in cassava, genes associated with drought tolerance have not yet been identified and genes identified in other species have not been evaluated for their expression in cassava.

Systematic cassava breeding can contribute to meeting of Millennium Development Goals (MDGs) to reduce the proportion of people who suffer from extreme poverty and hunger by half, before the year 2015 (Goal 1) and this can be achieved without damaging the environment (Goal 7) (UN 2010). Breeding and distributing high-yielding and disease-resistant crops varieties is one of the critical strategies to achieve MDGs. Many agronomically hostile or degraded environments

require major scientific interventions to become productive agricultural systems (Timmer, 2003). Secondly many cropping systems use large quantities of chemical inputs, such as herbicides, pesticides and fertilizers to increase food productivity. Although increased food production and productivity using external agro-inputs contributes to reducing hunger and poverty, agro-inputs such as herbicides and fertilizers damage the environment and can be unhealthy for people. Besides, they are inaccessible to many subsistence farmers who produce the bulk of food in developing countries. Biotechnology offers the potential to reduce the need for these inputs in economically and environmentally sustainable ways (MDG 7).

Breeding programmes can successfully produce drought tolerant cassava genotypes if they are guided by information on varietal responses to drought and if appropriate biotechnology techniques are employed. Genetic variability in response to drought if well characterised and utilised would result in appreciable progress in production of drought tolerant genotypes. El-Sharkawy, (2007) reported that there is variability among cassava genotypes in response to water stress with some genotypes having high levels of drought tolerance, while others being highly susceptible. Until now genetic response to water stress of cassava genotypes in Uganda had not been evaluated. The evaluation of the cassava germplasm should be accompanied by their characterization or genetic diversity assessment. Knowledge about germplasm diversity and genetic relationships among breeding materials is an invaluable aid in crop improvement strategies.

A number of methods are currently available for analysis of genetic diversity among genotypes, breeding lines and populations. These methods have heavily relied on pedigree data, morphological data, agronomic performance data, biochemical data, and more recently molecular (DNA-based) data (Mohammadi and Prasanna, 2003). Analysis of genetic relationships in crop species serves as a platform for stratified sampling of breeding populations. In breeding, genetic diversity finds its invaluable applications in (i) analysis of genetic variability in cultivars (Smith, 1984; Cox *et al.*, 1986), (ii) identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell, 1998), and (iii) introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al.*, 1998). An understanding of genetic relationships among pure lines can be particularly useful in planning crosses, assigning lines to specific heterotic groups, and for precise identification with respect to plant varietal protection (Hallauer and Miranda, 1988). Fregene *et al.*,

(2001) reported that there is insufficient knowledge of genetic diversity among cassava germplasm worldwide.

For successful breeding of a trait with high G X E influence such as drought tolerance, it is also important to identify candidate drought tolerance genes and understand their expression under water stress conditions. This is an important step in the improvement of cassava through biotechnological approaches, particularly, the use of transgenic approach. Limited information on candidate drought tolerance genes in cassava holds back progress in molecular breeding of this trait. This is one of the gaps that this research sought to address.

1.2 PROBLEM STATEMENT

Most climate-change studies indicate an expansion of arid zones on our planet (Funk *et al.*, 2005; WWF, 2006). As a result of global warming and climate variability, water will become increasingly scarce for rain-fed agriculture and drought stress will become the major environmental factor affecting crop production. In fact it has already been reported that during the last 30 years, rainfall in eastern and southern Africa has declined by 15% due to severe and frequent droughts (Funk *et al.*, 2008). This has had a negative impact on food security in the region resulting in many households becoming food insecure. For example, in 2008 alone, over 14,622,000 people suffered from food insecurity due to drought in the Horn of Africa alone (Djibouti, Ethiopia, Kenya, Somalia and Uganda) and these people depended on World Food Program (WFP) for relief food (OCHA, 2008). As such, the United Nations recognized that there is need to lay strategies to reduce the proportion of people suffering from hunger and set up eight millennium development goals, the first one being "reduction of the number of people suffering from extreme poverty and hunger by half by 2015 (UN 2010). However, increased agriculture production and productivity to meet this MDG 1 can be regressed by climate variability and subsequent drought effects.

It is estimated that current food production will need to double by 2050 if food security for global population is to be met (Baulcombe *et al.*, 2009). With population growth and global climatic changes, there is a lot of pressure on the available arable land (Baulcombe *et al.*, 2009; Challinor *et al.*, 2007). As a result, agricultural production, throughout the world and particularly in the developing countries, will steadily move into semi-arid zones where rainfall and soil conditions are unfavourable for crop productivity. Cassava is a crop with potential to improve food security

and income for households living in marginal areas due to its ability to adapt easily in such areas. It is important to note that, the yield potential of cassava is drastically reduced by drought (Anderson *et al.*, 2004) especially during establishment, although there is variability in cassava genotypes in response to drought. Drought results in poor growth and development, and low economic yield in cassava (Pardales *et al.*, 2001; Bakayoko *et al.*, 2009).

Since most marginal areas in Africa, where cassava is mostly grown, are characterised by unfavourable (hilly) terrain, irrigation is unaffordable to resource-poor farmers. In Africa, agriculture is in the hands of resource poor farmers who cannot afford irrigation costs. This has negative impact on agriculture as most farmers will continue to depend on unreliable rain-fed agriculture. The most logical strategy to improve crop productivity in drought prone areas is through breeding for drought tolerance. Therefore to improve cassava yields and productivity and avoid losses due to drought, breeders should target improved resistance to water stress by identifying and developing cassava genotypes that can yield well in marginal agro-ecological zones.

Breeding for drought tolerance in cassava requires clear understanding of the morphological and genetic/molecular basis of drought tolerance, which is lacking. In Uganda, at the National Cassava Breeding Programme, there is limited information on genetic diversity among cassava germplasm to enable breeders to select suitable parental genotypes to cross to generate and/or maintain adequate genetic diversity for selection. There is also limited information on varietal response to drought of Ugandan cassava germplasm making it difficult for breeders to identify gene sources for drought tolerance breeding. Elsewhere, transgenic approaches to drought tolerance are showing encouraging results. For this to be undertaken in cassava, information on candidate genes associated with drought tolerance is necessary. Unfortunately, this is limited in cassava. In crops where this information is available, for instance in maize (Bruce *et al.*, 2002), rice (Degenkolbe *et al.*, 2009) and wheat (Abebe *et al.*, 2003), breeding for improved resistance to water stress has had progress.

1.3 RATIONALE

Water for agriculture is increasingly becoming scarce for rain-fed agriculture (Funk *et al.*, 2005; WWF, 2006). In cassava production, the adverse effects of drought ultimately affect the livelihood of many households that depend more or less entirely on cassava for their food and

income. Cassava is a staple and food security crop for more than 800 million people in sub-Saharan Africa (Burns *et al.*, 2010; Perez *et al.*, 2011). In Uganda alone, per capita consumption of cassava is over 132 kg, accounting about 11% of the caloric intake and is the second most important staple food crop after banana (Haggblade and Dewina, 2010). Furthermore, 40% of cassava production goes to markets, providing income to farming households and other stakeholders involved in cassava value chain, including local traders, processors and exporters (USAID, 2010). It also provides employment to many Ugandans both on farm and in industries (animal feed, starch, biscuits, bread, brewing, plywood, paperboard and textile industries). For example, cassava demand as an industrial raw material is estimated at 46,744 tonnes of fresh cassava per annum (USAID, 2010). Besides, Uganda is a food basket for eastern and central Africa, especially Rwanda, Democratic republic of Congo and South Sudan. In year 2007 alone, Uganda exported 20,506 metric tonnes of cassava (worth US$ 1.9 million) to these countries (USAID, 2010). Therefore research on its improvement is likely to have great impact on food security and wellbeing of not only Ugandans, but also to the whole eastern and central African region. This research was carried out firstly to identify drought tolerant genotypes that can be directly used by communities in drought prone areas where moisture availability is a limiting factor. The drought tolerance genotypes are also invaluable resource for breeders for drought tolerance studies.

Farmers in drought prone areas suffer from crop yield losses when rainfall amounts are inadequate for cassava establishment and development. Although there is varietal variability in cassava in response to drought, farmers have their preferred genotypes that they treasure and keep whether tolerant or susceptible to drought stress. It is therefore important that breeders improve farmer preferred genotypes for drought tolerance. The second objective of this study aimed at evaluating both elite and cassava landraces for their genetic diversity to provide information to breeders about their uniqueness, relatedness and potential for utilization in cassava breeding for drought tolerance. Drought tolerance is a complex trait under control of multiple genes, and thus may require advanced breeding methods such as genetic engineering which involves transfer of suitable tolerant genes to susceptible genotypes. The third objective of this research aimed at identification of candidate drought tolerance genes that can be utilised in molecular breeding for drought tolerance in cassava. The information would improve understanding of drought tolerance mechanisms for effective use of genetic and genomic approaches to breed cassava with improved

water use efficiency. Candidate drought tolerance genes are relevant for breeders in national, regional and international cassava breeding programs to apply biotechnology approaches.

1.4 ABOUT THIS THESIS

The aim of the research described in this thesis was to provide information that will contribute towards improvement of cassava's drought tolerance. The research had three specific objectives, to;

1. Evaluate cassava genotypes grown in Uganda for drought tolerance,
2. Determine the level of genetic diversity among Ugandan cassava germplasm,
3. Identify candidate drought tolerance genes in cassava and evaluate their expression under water stress conditions.

The research consisted of both field trials and laboratory studies. The first objective was carried out in Uganda under field conditions while the second and the third objectives were carried out at Biosciences eastern and central Africa (BecA), Nairobi Kenya. These objectives are addressed in the experimental research described in chapters 4-6.

Evaluation of Ugandan cassava germplasm for drought tolerance

Chapter 4 addresses research carried out in the field in three locations, two of which were in drought prone districts of Buliisa and Nakasongola. The third field trial was established at Kabanyolo which receives optimum rainfall to act as reference. This objective was based on the hypothesis that there is varietal variability among cassava genotypes in response to drought stress as established in previous studies (El-Sharkawy, 2007). The objective was achieved through field trials at the three locations for two seasons, which were conducted between October 2008 and October 2010.

Genetic diversity among cassava genotypes in Uganda

The study addressing this topic is described in chapter 5. The objective was based on the hypothesis that there is wide genetic diversity among cassava genotypes under traditional farming system (Elias *et al.*, 2001a). The rationale of this objective was based on the fact that information on genetic diversity among the breeding populations is an important requirement for crop improvement to guide selection for crossing and/or formation of heterotic groups. It was considered necessary that after screening landraces for drought tolerance in the field (Chapter 4),

breeders would need information on how genetic diverse are the genotypes to foster breeding for drought tolerance. This objective was achieved through genetic diversity assessment using single sequence repeat (SSR) markers. This work was undertaken in the laboratory at BecA, Nairobi.

Identification of candidate drought related and drought responsive genes in cassava

The experiment to address this objective is described in detail in chapter 6. The objective was based on the hypothesis that differences in response to drought stress among cassava genotypes are a result of differences in drought stress-induced regulatory and functional genes. One drought tolerant genotype and one susceptible genotype identified through field evaluation trials described in chapter 4 were evaluated under water stress conditions in a screen house at BecA. Drought tolerance genes that have been identified and biologically validated in other plants including crop species were evaluated for their expression in the two cassava genotypes in response to water stress, in order to identify candidate drought tolerance genes. The rationale of this objective was based on fact that, successful breeding for complex traits like drought tolerance requires MAB to offer accurate and timely introgression of desired traits, and that this depends on identification of suitable genes. This objective was achieved through gene expression studies carried out in the laboratory at BecA, Nairobi, using Real-Time PCR.

1.5 OUTLINE OF THIS THESIS

This thesis is made up of nine main sections as shown below:

1. Summary
2. Chapter 1: General introduction
3. Chapter 2: Literature review
4. Chapter 3: Materials and methods
5. Chapter 4: Evaluation of Ugandan cassava germplasm for drought tolerance
6. Chapter 5: Genetic diversity among cassava landraces in Uganda
7. Chapter 6: Identification of candidate drought tolerance genes in cassava
8. Chapter 7: General discussion, conclusions and recommendations
9. References

Chapters 4-6 are written in IMRAD format that include Introduction, Materials and methods, Results, and Discussion, following Makerere University Directorate of Research and Graduate Training guidelines for the format of Research Proposals, Research Reports, Thesis and Dissertations of 2011.

CHAPTER 2: LITERATURE REVIEW

2.1 TAXONOMY OF CASSAVA

Cassava *(Manihot esculenta)* Crantz belongs to family *Euphorbiaceae.* The family is characterised by latex production (Nassar, 2002; Hershey, 2005). Of the 98 *Manihot* species, cassava is the only species that is widely cultivated for food production (Rogers and Appan, 1973; Onwuene, 1978). Several studies have revealed that all cassava species have a diploid genome (2n=2x=36) (Rogers and Appan, 1973; Onwuene, 1978; Nassar, 2002). Indeed the diploid nature of cassava is supported by empirical studies conducted by Jos and Nair, (1979) on the meiotic behaviour of cassava. They observed regular 18 bivalent formation of the chromosomes typical of diploids. Nevertheless, spontaneous triploids (3n) and tetraploids (4n) which differ from diploid plants in vigour, leaf shape and leaf size, have also been reported (Hahn *et al.*, 1980). Cassava is reported to be highly heterozygous (Fregene *et al.*, 2001) and the heterozygosity levels of different cassava genotypes have been further confirmed from diversity studies using isozymes (Lefevre and Charrier, 1993), AFLP and SSRs (Sanchez, *et al.*, 1999; Fregene *et al.*, 2000; Fregene *et al.*, 2003; Turyagyenda *et al.*, 2012).

2.2 ORIGIN AND DOMESTICATION OF CASSAVA

It is believed that cultivated cassava had wild progenitors located in Brazil (Olsen, 2004). This idea has been supported by studies using single nucleotide polymorphisms (SNPs) and simple sequence repeat (SSR) markers (Olsen and Schaal, 2001; Olsen, 2004). The domestication of cassava has also been confirmed to have started from Brazil (Schaal *et al.*, 2006). Domestication was based on farmers' preferred traits such as large root size, rapid growth rate, large number and big size of stems and the ability to propagate through stem cuttings (Olsen and Schaal, 2001; Olsen, 2004; Schaal *et al.*, 2006).

Brazil is home to approximately 80 *Manihot* species. Species that exhibit the most primitive characters such as dioecious inflorescences in *M. stipularis, M. pusilla* and *M. longipetiolata* and non-lobed and sessile leaves in *M. stricta, M. purpureocostata* and *M. salicifolia* are confined to Brazil and it is for this reason that Brazil is considered the primary centre of diversity of cassava (Nassar, 2002). It is from Brazil that cassava was carried by Indians during their migrations to Mexico during the 11th Century. In Mexico there was widespread hybridization between the cultivated species and wild ones resulting in new species and creating a secondary centre of

diversity (Nassar, 2002). Cassava was introduced in Africa, by Portuguese during their navigation in 16th Century, first in West Africa, central Africa and the Congo basin and later to East Africa through Madagascar and Zanzibar (Janssens, 2001).

2.3 BOTANICAL DESCRIPTORS OF CASSAVA

Cassava is a woody shrub which is botanically perennial, though in agriculture, it is regarded as annual or biennial as farmers usually harvest its edible roots during the first or second year (Onwuene, 1978; Hahn *et al.,* 1990). The plant height of a mature cultivated cassava usually ranges from one to four metres (Onwuene, 1978; Hershey, 2005), although cultivars with height exceeding 12 meters have been reported (Rogers and Appan, 1973). The wild shrub cassava trees may grow to a height of 20 meters (Nassar, 2000). The branching height in cassava is variable; some branching at a height of as low as 20 cm from the ground while others may branch near the apex or may never branch at all. Stem colour varies from gray or brown to reddish. Stems normally branch into dichotomous or trichotomous and the branching points frequently exhibit a terminal inflorescence (Nassar, 2000). Cassava has simple leaves, with 3-9 leaf lobes arranged spirally around the stem. A plant may have two or three different leaves, a condition known as foliar polymorphism. Older leaves are shed leaving prominent leaf scars. The shedding of leaves varies with tolerance to water stress; the more tolerant genotypes retaining most of their leaves, "stay green" while less tolerant genotypes shedding most of their leaves (Lenis *et al.*, 2006).

Flower production in cassava, like in any other flowering plant, is influenced by environmental factors, such as photoperiod and temperature. Flowers develop in axils between the branches and therefore genotypes that don't branch do not produce flowers. Even in genotypes that branch, there is variation in flowering ability. Some genotypes flower frequently and regularly, while others rarely flower or do not flower at all. Cassava is monoecious with both male and female flowers found on the same plant. The male flowers develop near the tip, while female flowers develop closer to the base of the inflorescence (Ekanayake *et al.*, 1997). To enhance cross pollination, the male and female flowers mature at different times, a condition known as protagyny. The female flowers usually mature one to two weeks earlier than the male flowers (Janssens, 2001). Insect pollination especially by honey bees is common (Hahn *et al.*, 1979). Cassava is conventionally propagated by stem cuttings, thereby maintaining a genotype. However, propagation by seed is also possible but is mainly used for breeding purposes (Onwuene, 1978).

Cassava plant contains latex and poisonous cyanogenic glucoside that consists of 95% Linamarin and 5% lotaustralin (Koch *et al.*, 1992; Conn, 1994). When exposed to air or water, cyanogenic glucoside is decomposed to hydrocyanic acid (HCN). The decomposition is catalysed by enzyme *linamarase*, which naturally occurs in cassava. *Linamarase* also acts on the glucosides when the cells are ruptured. HCN in cassava tissues has been medically proven to be a potential health hazard for consumers if the plant is inadequately processed (Tylleskär *et al.*, 1992; McMahon *et al.*, 1995). Leaves have the highest concentrations of cyanogenic glucosides than roots (McMahon *et al.*, 1995) and depending on the concentration level, cassava may be described as bitter or sweet. So often, sweet cultivars produce less than 50 mg of cyanogenic glucosides per kg of fresh roots, while bitter ones produce cyanogenic glucosides above this level (Ceballos *et al.*, 2004). Cassava genotypes with high cyanogenic glucoside content (>1000 mg/kg dry weight HCN) are said to be toxic, while cassava with low content of cyanogenic glucosides (<300 mg/kg dry weight HCN) are said to be safe for consumption without processing (Iglesias *et al.*, 2002). Sun drying or boiling eliminates the risk of poisoning due cyanogenic glucosides (Janssens, 2001). The bitter and the sweet types are botanically similar and are not considered as different species.

Roots are the main storage organs in cassava. Cassava is mainly propagated from stem cuttings which produce adventitious roots at the base of the cuttings. When cassava plants are propagated by seed, the seed first develop tap root system. The adventitious roots from stem cuttings and tap root of seedlings develop into fibrous root systems which within 30-60 days increase in diameter to become storage roots. However, not all fibrous roots develop into storage roots. Only few fibrous roots (three-ten roots) depending on variety develop into storage roots, while the rest remain thin and serve to absorb water and nutrients for the plant (Alves, 2002). These storage roots swell with starch to a length of 30-80 cm and diameter of 5-10 cm and may weigh 1-4 kg (Ekanayake *et al.*, 1997; Janssens, 2001). The cassava storage root has three distinct tissues; the inner edible parenchyma (85% of total dry weight), the middle peel (11-20%) and the outer layer (periderm) (3% of the total dry weight of the root). Root size and shape depend on cultivar and environmental conditions (Alves, 2002). The pulp, the peel and the periderm colour are cultivar dependent.

2.4 ECONOMIC IMPORTANCE OF CASSAVA

Cassava is mainly grown for its starchy roots though all parts of the cassava plant are useful. The starchy roots are consumed in a wide range of forms in different parts of Africa, either fresh or

processed (Nweke, 1994). Roots from bitter cultivars need to be processed before consumption, while roots from sweet cultivars can be eaten fresh either roasted or boiled (Kapinga *et al.*, 1997; Cardoso *et al.*, 1998; Chiwona-Karltun *et al.*, 1998). Cassava storage roots can be harvested anytime from 6 to 24 months after planting, and can be left in the ground as a food reserve for household food security in times of famine and drought (Asafu-Agyei and Osafo, 2001). In Uganda cassava is considered a major contributor to food security and a crop with potential of improving livelihoods of the low resource farmers (Otim-Nape and Zziwa, 1990). Cassava is particularly important in areas where food supply is constantly threatened by environmental constraints such as drought and pest outbreaks because of its ability to grow under conditions considered as sub-optimal for the majority of food crops.

The leaves are an important vegetable; rich in protein, minerals and vitamins comparing favourably with other green vegetables. This qualifies cassava leaves as good protein sources and therefore can supplement predominantly starchy diets in poor communities, especially in the dry season when other vegetables are in short supply (Lancaster and Brooks, 1983; Onwuene, 1978; FAO, 1993; Nweke, 1994; IITA, 2001). Cassava leaf vegetables are most preferred in Democratic Republic of Congo, Gabon, Central African Republic, Angola, Sierra Leone, Rwanda and Liberia. In Democratic Republic of Congo, Central African Republic and Sudan, the cassava leaf vegetables are called "sakasaka" or "pondu", "Kizaka" in Angola, "Mathapa" in Mozambique, "Chigwada" in Malawi, "Chombo" or "Ngwada" in Zambia, "Gweri" in Cameroon, "Kisanby" in Tanzania, "Cassada leaves" in Sierra Leone, "Banankou boulou nan" in Mali, "Mafe haako bantare" in Guinea, and "Isombe" in Rwanda (Hahn, 1992).

Other than food, cassava has commercial uses and can be utilized to improve household income. It has been reported that cassava's role as a traditional human food is changing to becoming an efficient industrial and/or income crop (Nweke, 2009; Benesi, 2005). The starch extracted from cassava roots can be used for diverse food and non-food uses. The stem cuttings are commercial planting materials (Alves, 2002) and therefore household income can be improved through selling cassava cuttings as planting materials. This increasing benefit of cassava has contributed to its widespread popularity.

2.5 OPTIMUM ENVIRONMENTAL CONDITIONS FOR CASSAVA GROWTH

Cassava grows best in areas with a mean air temperature of 25-29°C, and a soil temperature of about 30°C. Below 10°C the cassava growth is inhibited, while at higher temperatures, starch production in the leaves is slow, reducing the amount of cassava produced. Cassava grows better in sunny and wet climate (Janssens, 2001), though its high adaptability makes it possible to be grown in relatively drier areas. The growth and productivity of cassava is also reduced by shades and therefore, the crop should be cultivated in areas where there is full sunlight. Cassava productivity is high at an altitude of 1,800 metres above sea level.

The crop is hardy and can tolerate a wide range of soils. However, good soil is one of the most important factors required for high cassava yields. Cassava grows better in deep well drained sand-clay soils but its growth is affected by hydromorphic or too sandy soils (Janssens, 2001). High fertility and excessive nitrogen limit tuberization (Janssens 2001). Cassava requires soil pH between 4.5 and 6.5. Limestone should be incorporated into more acidic soils at 3 to 4 months before planting, using a rate of 2 to 4 tons per hectare, depending on the level of acidity.

Cassava thrives best when rainfall is well distributed throughout the growing period. Precipitation of 1,000-1,500 mm per year is required for optimum performance of cassava. Due to its well established root system, cassava is tolerant to dry or seasonally dry conditions, with rainfall as low as 500 mm, and thus its species mainly occur in dry or seasonally dry conditions, though a few species can be found in rain forests (Rogers and Appan, 1973). Cassava does not tolerate flooding and freezing conditions (Kizito, 2006). Excessive water will cause the tubers to rot, producing unmarketable tubers that deteriorate rapidly. In drought prone areas it loses its leaves to conserve moisture, producing new leaves when rains resume (Kizito, 2006). Leaf shedding has been associated with sensitivity to water stress (Lenis *et al.*, 2006).

Under optimum growth conditions the crop reaches maturity in about 9 months but can take longer than 18 months to produce a crop under adverse conditions such as cool or dry weather (Kizito, 2006). The exact time in terms of months after planting, when it is best to harvest cassava, also depends on the cultivar, varying from seven months after planting (MAP) to 24 MAP (Onwuene, 1978). Cassava can grow indefinitely, alternating periods of vegetative growth, storage of carbohydrates in the roots and even periods of almost dormancy, brought about by severe climatic conditions such as low temperatures or prolonged water deficit (Alves, 2002).

This makes cassava adaptable to variations in relief, soils and cropping systems within the same agro-ecological zone.

2.6 CASSAVA PRODUCTION CONSTRAINTS

Cassava production is greatly constrained by both abiotic and biotic factors. The common biotic constraints include pests like cassava green mite (*Mononychellus tanajoa)* (Skovgard *et al.*, 1993), cassava mealy bug *(Phenacoccus manihoti)* (Boher and Verdier, 1994), and diseases including cassava bacterial blight (Boher and Verdier, 1994), cassava brown streak disease (CBSD) (Hillocks *et al.*, 2001) and cassava mosaic disease (CMD) (Patil and Fauquet, 2009). The abiotic factors constraining cassava production include drought stress, acid soil and cold stress (Anderson *et al.*, 2004).

2.7 CASSAVA BREEDING AND BREEDING OBJECTIVES

Cassava breeding started as early as its domestication process. Domestication process involved selections by subsistence farmers, and this marked the beginning of cassava breeding. Farmers at that time selected cassava varieties with large root size, rapid growth rate, large number and big size of stems and the ability to propagate through stem cuttings (Jennings, 1979). Furthermore farmers have continuously undertaken individual plant selections (i.e., for shorter stature, higher root dry matter content (DMC), disease resistance, early bulking, and preferred culinary root qualities) (Kawuki *et al.*, 2011). However, the more rigorous scientific breeding only began in the early twentieth century. Two international agricultural centres, International Institute of Tropical Agriculture (IITA) and International Centre for Tropical Agriculture (CIAT), in partnership with the national agricultural research systems (NARS), have championed cassava breeding in Africa, Asia and Latin America since late 1960s (Kawano, 2003; Ceballos et al., 2004). The breeding objectives in these International, local and regional centres have been guided by the prevailing local/national needs, regional and or global mandates.

2.7.1 Conventional breeding

Scientific cassava breeding is not as old as in other crops and, therefore, the divergence between landraces and improved germplasm is not as wide as in crops with a more extensive breeding history (Ceballos *et al.*, 2004). Hallauer and Miranda, (1988) revealed that little progress has been made to use general combining ability as a criterion of parental selection. Parental lines are

selected based mainly on their *per se* performance. Crossing can be done manually through controlled pollinations to produce full-sib families or through polycross nurseries (open pollination) resulting in half-sib families (Ceballos *et al.*, 2004). Conventional breeding in cassava has resulted into the production of improved genotypes. For example, in 1982, an improved hybrid with higher protein content (5% compared to 1.5% in typical cassava root) was developed in Brazil by Nassar and Ortiz (2010). They further reported that cassava breeders in Brazil have since then developed highly productive cassava genotypes containing up to 50 times as much beta-carotene as regular cassava.

Conventional breeding has also been essential in development of pest and disease resistance in cassava. For example cassava mosaic resistant materials were produced in Tanzania through crossing cassava with its wild relative *M. Glaziovii* (Nassar and Ortiz, 2010). Through further hybridization of cassava with *M. Glaziovii* and its hybrids, researchers at the International Institute of Tropical Agriculture (IITA) in Nigeria produced more mosaic-resistant cassava variety that gave rise to a family of mosaic- virus-resistant genotypes that are now cultivated in sub-Saharan Africa (Nassar and Ortiz, 2010). Another important achievement of conventional breeding is the utilization of wild cassava in the discovery of a durable resistance to mealy bug in *M. glaziovii* and transference of its genes to common cassava (Nassar, 1997).

2.7.2 Marker Assisted Selection (MAS).

Genetic improvement of this important crop can be made more efficient through the use of easily assayable molecular markers through marker assisted selection (Blair *et al.*, 2007). This would enable precise identification of genotypes without the confounding effect of the environment, thereby increasing heritability. Application of MAS in cassava breeding also contributes to efficient reduction of large breeding populations at the seedling stage based upon "minimum selection criteria", given the length of the growing cycle of cassava and the expense involved in the evaluation process (Blair *et al.*, 2007). With the use of genetic markers, breeding is more precise, thereby eliminating several backcrosses and time consuming phenotypic (visual) evaluations. The selection of progeny based on genomic breeding values derived from molecular marker data has been reported to substantially increase the rate of genetic gain, especially if the number of cycles of evaluation or generations can be reduced (Meuwissen *et al.*, 2001).

Pilot studies on application of marker assisted breeding in cassava have reported enormous progress for different important traits including pest and disease resistance. For example, molecular markers for cassava mosaic disease resistance gene (*CMD2*) have been developed and applied in breeding for CMD resistance (Akano *et al.*, 2002). Nevertheless, although, a molecular map of cassava has been developed (Mba et al. 2001), the heterozygous nature of cassava complicates work to improve the existing molecular map and to implement marker-assisted selection (Cach *et al.*, 2006).

2.7.3 The need for genetic diversity in cassava

Cassava genetic improvement follows the basic breeding scheme that starts with the assembly and evaluation of a broad germplasm base, followed by production of new recombinant genotypes derived from selected elite clones. For many years of cassava cultivation, farmers have used genetic variation to select preferred genotypes of the crop resulting in high genetic diversity on their farms. For successful breeding, screening and evaluation of genetic variability of available germplasm is critical (Beeching *et al.*, 1993; Moyib *et al.*, 2007). Genetic diversity within the crop reduces its vulnerability to biotic and abiotic stresses, ensures long term selection gain and promotes rational use of genetic resources (Martin *et al.*, 1991; Smith and Smith, 1992; Messmer *et al.*, 1993; Barrett and Kidwell, 1998). Genetic diversity is thus resourceful to geneticists, breeders and taxonomists (Prince *et al.*, 1995). In populations, the genetic composition and genetic diversity are derived from wild progenitors and influenced by evolutionary processes such as mutation, recombination, genetic drift, migration, natural selection and human selection (Hedrick, 1999). Frankel *et al.* (1995) defined genetic diversity as the product of interplay of biotic factors, physical environment, artificial and plant characters such as size, mating system, mutation, migration and dispersal.

Genetic improvement of cassava for abiotic and biotic tolerance is the major strategy for cassava breeders. However, this strategy requires a precise understanding of the genetic structure and variation underlying cassava varietal differences in a number of important traits. It is particularly important to understand genetic relationships to guide the planning of crosses, assigning of lines to specific heterotic groups, and for precise identification with respect to plant varietal protection (Hallauer and Miranda, 1988; Manosh *et al.*, 2008). Selection of divergent parents increases chances of producing progenies with heterosis since it is a function of heterozygosity (Manosh *et al.*, 2008). Positive correlation between genetic diversity and heterosis, for seed yield has been

reported in cultivated sunflower (Cheres *et al.*, 2000) and tuber yield in potato (Manosh *et al.*, 2008). Diversity assessment can also lead to discovery of new genotypes and identification of new combinations with maximum genetic variability for further selection and introgression of desirable genes (Mohammadi and Prasanna 2003).

The interaction of various factors such as biotic stress (particularly pests and diseases), artificial selection at farm level, environmental factors and plant characteristics such as mating system, dispersal, spontaneous hybridisation leading to volunteer seedlings, the heterozygous nature and vegetative propagation of cassava have contributed to shaping the genetic structure in cassava (Frankel *et al.*, 1995; Elias *et al.*, 2001a; Fregene *et al.*, 2003). It is also reported that polyploidy and apomixis may have contributed to the evolution and genetic diversity of cassava and related species (Nassar, 2002). Therefore, evaluating genetic diversity and further characterizing cassava populations is an invaluable asset in the genetic improvement strategies of cassava.

2.7.4 Assessment of genetic diversity in cassava

The commonly used approach to infer genetic diversity among populations and genotypes is through the use of selectable and neutral genetic markers. Traditional identification of plants is based on selectable or morphological traits recorded in the field during plant growth. In cassava, morphological and agronomic markers have been used for divergence and characterisation studies and phylogenetic relationships (Lefevre and Charrier, 1993; Perera *et al.*, 2000; Elias *et al.*, 2001a; Benesi *et al.*, 2006). The commonly morphological traits used to classify *Manihot* species include hairiness of unexpanded apical leaves, colours of unexpanded apical leaves, mature leaf colour, leaf vein colour, flowers and seeds, leaf shape and size, mature stem colour, tip shoot colour, petioles length and colour, phyllotaxi, leaf lobe shape, number of leaf lobes, petiole colour, plant and first branch height, and root peduncle lengths, root shape, root surface, inner root skin, root pulp and root position (Elias *et al.*, 2001a; Alves, 2002; Benesi, 2002). Phenotypic markers are still playing an important role in conventional plant breeding as well as in identification of specific markers and quantitative trait loci (QTLs) (Fregene *et al.*, 2000; Akano *et al.*, 2002; Mkumbira, 2002).

However, evaluations of genetic diversity among germplasm using morphological traits has several setbacks. The evaluation is lengthy and costly (Patterson and Weatherup, 1984) and the traits are influenced by environmental conditions and therefore may be less accurate in genetic

distance estimation (Jorge, 1995; Simwambana *et al.*, 1996). The morphological traits must also be assessed during the fixed vegetative phase of the crop development and sometimes requires growing the plants to full maturity prior to identification (Chakravarthi and Naravaneni; 2006). Morphological and agronomical traits respond to selection pressure and therefore change after several years of natural and/or artificial selection hence ambiguous with limited use for cultivar identification (Tanksley *et al.*, 1989; Yong *et al.*, 2009). They are also difficult to analyze because they do not have the simple genetic control assumed by genetic models (Liu and Furnier, 1993). In addition, different farming practices and utilization of some morphological parts such as the picking of cassava leaves for use as vegetable food causes morphological changes of the cassava plant making scoring inaccurate (Onwuene, 1978; Simwambana *et al.*, 1996). Therefore, based on several reports on weaknesses of use of selectable markers, it is imperative, where possible, to employ alternative better approaches to access genetic diversity.

The neutral genetic (DNA based, molecular) markers that reveal polymorphisms at DNA-level have been proposed as the appropriate markers to assess genetic diversity. The development of molecular marker techniques has revolutionised genetic studies in cassava and other crop species because they can accurately infer genetic diversity among populations and genotypes (Chakravarthi and Naravaneni, 2006; Raji *et al.*, 2009). They are not influenced by the environment and it is possible to accurately locate QTL, identify and characterise genotypes at molecular level, conduct MAS in early stages of the crop cycle and apply DNA fingerprinting to crop species (Fregene *et al.*, 1994; Colombo *et al.*, 2000; Elias *et al.*, 2000; Gupta and Varshney, 2000). Molecular or neutral markers are more informative, superior to selectable methods of genotyping and give rise to a higher number of polymorphisms (Tanksley *et al.*, 1989; Messmer *et al.*, 1993; Karp *et al.*, 1997; Chakravarthi and Naravaneni, 2006; Raji *et al.*, 2009). DNA markers have been successfully used in cassava to understand phylogenetic relationship within the cassava genus (Roa *et al.*, 1997; Olsen and Schaal, 1999), assessment of genetic diversity (Beeching *et al.*, 1993; Mkumbira *et al.*, 2003; Elias *et al.*, 2001a; Kizito, 2006), development of genetic maps and identification of quantitative loci (QTL) for traits of importance (Fregene *et al.*, 1997; Jorge *et al.*, 2001; Okogbenin and Fregene, 2002; Lokko *et al.*, 2007).

A variety of molecular markers have been used in different crop species for assessment of genetic diversity (Semagn, *et al.*, 2006). Those which have been used to study genetic diversity in cassava include; RFLPs (Beeching *et al.*, 1993), RAPDs (Mignouna and Dixon, 1997; Asante and Offei,

2003), AFLP markers (Fregene *et al.*, 2000; Raji *et al.*, 2009;) and SSR markers (Kawuki *et al.*, 2009; Sree Lehka *et al.*, 2010; Montero-Rojas *et al.*, 2011). SSR markers remain competitive because of being multi-allelic, highly polymorphic, co-dominant and highly reproducible and provide rich genetic information with good genome coverage (Kawuki *et al.*, 2009; Sree Lehka *et al.*, 2010). SSR markers are also cheap and amenable to most breeding procedures and thus, applicable in public breeding programmes which may not afford expensive diversity assessment techniques. Microsatellite markers for various crops, including maize (Taramino and Tingley, 1996), soybean (Devos *et al.*, 1995), barley (Russell *et al.*, 1997), potato (McGregor *et al.*, 2000) and cassava (Chavarriaga-Aguirre *et al.*, 1998; Mba *et al.*, 2001) have been developed.

Several studies have described the use of SSR markers in different crops including cassava. SSR markers have been used in genetic mapping, usually integrating them onto existing RFLP framework maps (Roder *et al.* 1998; Liu *et al.*, 1996; Taramino and Tingey, 1996; Wu and Tanksley, 1993; Bell and Ecker, 1994). The discovery, inheritance and variability of fourteen GA repeats have been described for cassava (Chavariaga-Aguirre *et. al.*,. 1998). Some of these SSR markers were used to evaluate the genetic diversity of the core collection of about 600 genotypes of the cassava world germplasm bank at CIAT (Chavarriaga-Aguirre *et al.*, 1999). The technique has been used to identify markers linked to disease resistance such as CMD and CBB (Akano *et al.*, 2002). However, while genomic SSR markers are mostly neutral, genic SSR markers from EST's or cDNAs may retain some adaptive roles and may be affected by natural and/or artificial selection (Yong *et al.*, 2009). Secondary, if no SSR markers are available, development of SSR markers is time consuming, expensive, need DNA sequence information and specific primers (Kulembeka, 2010).

2.8 DROUGHT STRESS

Drought is one of the major abiotic stresses limiting crop productivity worldwide, with devastating economical and sociological impact, and plays a major role in determining the distribution of plant species across different types of environments (Kramer and Boyer, 1995; Saini and Westgate, 2000; Prasad *et al.*, 2008). Boyer (1982) defined drought as a period of below normal precipitation that limits plant productivity in a natural or agricultural system. It influences various plant physiological processes resulting in depressed growth, development and economic yield (Pardales and Esquibel, 1996). The stress component that defines drought is a decrease in the availability of soil water that can be quantified as a decrease in water potential (Verslues *et al.*,

2006). In the field, drought can cause a number of plant stresses including temperature, light and nutrient stresses (Verslues *et al.*, 2006). In addition to its direct effects on yield, drought can also reduce the potential beneficial effects of improved crop management practices such as fertilizer application or pest and disease management.

Cassava, like any other plant is affected by drought stress, though its capacity to withstand significant periods of water stress is relatively higher than other crops, especially cereals. The effects of drought stress are more pronounced during the earlier stages of cassava growth and development (El-Sharkawy, 1993; Okogbenin *et al.*, 2003; Bergantin *et al.*, 2004). Previous research has indicated that water stress is one of the most limiting growth factors to economic yield of cassava and production of the crop is often unstable when the rainfall pattern is fluctuating or when the genotype planted is sensitive to water stress (Pardales and Esquibel, 1996; Pardales *et al.*, 2001; Bergantin *et al.*, 2004). Water stress can result in 22-60% yield loss in cassava resulting in food insecurity and loss of income to households that derive their livelihood from cassava growing. The adverse effects of drought stress on cassava yields are a result of reduced dry matter allocation to storage roots and increased acceleration of leaf senescence, leading to a decrease in canopy size and loss in photosynthesis (Rivero *et al.*, 2007; Lahai and Ekanayake, 2009). It has been postulated that water stress diminishes photosynthetic carbon fixation (Okogbenin *et al.*, 2011) and this may affect starch partitioning in storage roots. Also it is probable that the starch reserves in root storage could be depleted through respiration during prolonged water stress.

Climate models have indicated that drought episodes will become more frequent because of the long-term effects of global warming (Funk *et al.*, 2005; WWF, 2006; Rivero *et al.*, 2007). Verslues *et al.* (2006) reported that because of increasing scarcity of water resources and competition for them, irrigation is not a practical option for alleviating drought in most of the rain-fed areas. Therefore drought management strategies need to focus on maximizing extraction of available soil moisture and the efficiency of its use in crop establishment, growth, biomass and grain yield. These range from changes in traditional management and agronomic practices to the use of marker-assisted selection for the improvement of drought-related traits and the development of transgenic crops with enhanced tolerance of drought and improved water use efficiency that would minimize drought-related losses and ensure food production for the growing global population.

2.8.1 Drought stress response mechanisms in plants

Plants respond differently to drought stress. A decrease in water potential (Ψ) or decreased free energy of the water makes it more difficult for the plant to take up water (Verslues *et al.*, 2006). This in turn elicits a range of responses that allow the plant to avoid water loss, allow water uptake to continue at reduced Ψ or allow the plant to tolerate reduced tissue water content. The plants can therefore resist drought by low water potential avoidance, dehydration avoidance or by dehydration tolerance (Figure 1)

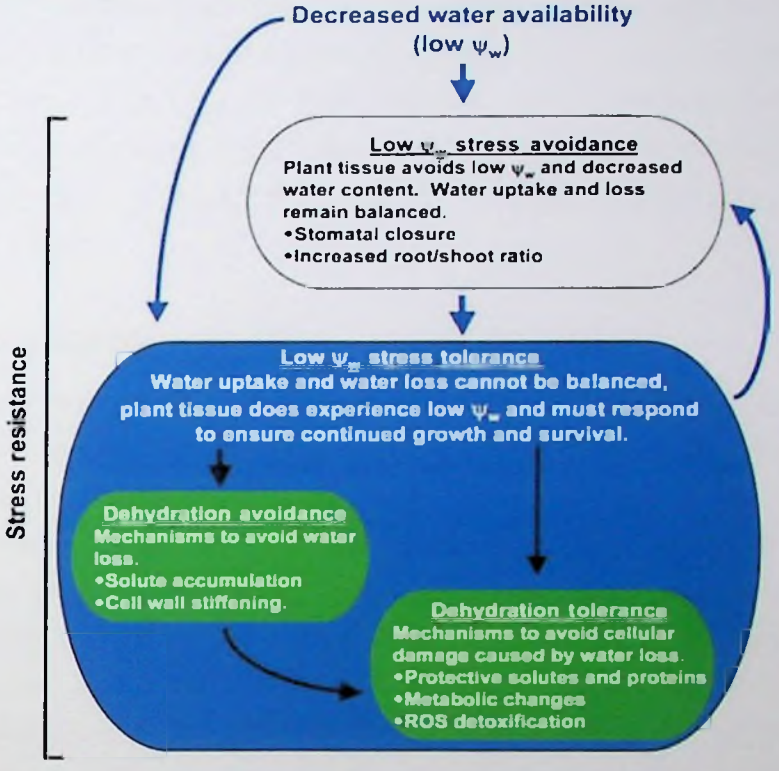

Figure 1: Drought tolerance mechanisms in plants as described by Verslues et al., 2006. ROS=Reactive oxygen species

2.8.1.1 Low water potential avoidance

The plant's first response to moisture stress is low Ψ avoidance, in which case tissue Ψ and water content are maintained close to the unstressed level (Verslues *et al.*, 2006). Verslues *et al.* (2006) reported that plants can avoid low Ψ by increasing water uptake or limiting water loss such that the rates of water loss and water uptake remain balanced. Under short term stress, the balance can be maintained by stomatal closure, but under longer term stress, changes in root and shoot growth, leading to an increased root/shoot ratio, tissue water storage capacity, and cuticle thickness and

water permeability are also important (Verslues *et al.*, 2006). Increased root growth to maximize water uptake is one of the most important traits associated with increased low Ψ avoidance.

It has been reported that with mild water stress or water stress of a limited duration, low Ψ avoidance mechanisms by themselves can be sufficient to maintain plant performance (Kramer and Boyer, 1995). With minimum stress conditions, modifications such as increased root growth or decreased stomatal conductance have the potential to increase crop productivity (Verslues *et al.*, 2006). However, stomatal closure results in loss of photosynthesis caused by reduced stomatal CO_2 uptake or a shift of resources into root growth at the expense of photosynthetic and reproductive tissue (Verslues *et al.*, 2006). Besides, low Ψ avoidance mechanisms do not themselves offer any protection from the effects of low water potential if the stress becomes more severe and the plant is no longer able to maintain a balance between water uptake and loss (Verslues *et al.*, 2006).

2.8.1.2 Dehydration avoidance

Under prolonged drought stress, plants may respond through dehydration avoidance mechanisms. Dehydration avoidance is defined as the plant capacity to sustain high plant water status or cellular hydration under the effect of drought (Blum, 2005). The main mechanisms of dehydration avoidance are osmotic adjustment (OA) and cell wall hardening (Blum, 2005; Verslues *et al.*, 2006). Osmotic adjustment refers to the active accumulation of additional solutes in response to low water potential (Verslues *et al.*, 2006). Blum (2005) reported that OA helps to maintain higher leaf relative water content (RWC) at low leaf water potential and thus helps to sustain growth while the plant is meeting transpirational demand by reducing its leaf water potential. The accumulated solutes should not interfere with cellular function and therefore plants accumulate one or more types of compatible solutes, such as proline or glycine betaine, which can accumulate to high levels without interfering with metabolism (Yancey *et al.*, 1982; Verslues *et al.*, 2006). Osmotic adjustment and accumulation of compatible solutes can be an important factor in drought tolerance in the field (Kramer and Boyer, 1995; Morgan, 1984, 1991), and engineering of increased synthesis of compatible solutes is one approach that has been taken to increase abiotic stress tolerance in plants (Apse and Blumwald, 2002; Bohnert and Shen, 1999). Nevertheless, dehydration avoidance through increased accumulation of compatible solutes can be energy and resource intensive for the plant, and, in cases of severe stress where soil water content is largely depleted, may have only a small effect on water uptake (Kramer and Boyer, 1995). Besides

dehydration avoidance through cell wall hardening results in a rigid cell wall and loss of turgor which prevents any further expansion of the cell and is largely confined to non-growing tissues (Verslues *et al.*, 2006).

2.8.1.3 Dehydration tolerance

The plant can also respond to drought stress through dehydration tolerance. Dehydration tolerance is the second defence line after dehydration avoidance and is defined as the relative capacity to sustain or conserve plant function in a dehydrated state (Blum, 2005). During severe water stress, plants employ different mechanisms to tolerate reduced water content. The most extreme examples of dehydration tolerance are 'desiccation-tolerant' or "resurrection plants" that can recover from a fully air-dried state (Oliver *et al.*, 2000; Vicre *et al.*, 2004; Blum, 2005). When fully dehydrated, these plants enter into a metabolically dormant state similar to seed dormancy. However, most crop plants lack the ability to enter a dormant state to tolerate complete desiccation and instead attempt to tolerate lesser degrees of water loss while maintaining metabolic activity (Verslues *et al.*, 2006).

Dehydration tolerance in crop plants only exists in the seed embryo, but the plant loses its tolerance once the seed embryo germinates (Blum, 2005). Dehydration tolerance mechanisms protect cellular structure from the effects of dehydration and include protective proteins such as dehydrins and other late-embryogenesis abundant (LEA) proteins (Verslues *et al.*, 2006). These accumulate in response to decrease in tissue water content and act as chaperones to protect protein and membrane structure (Bravo *et al.*, 2003; Hara *et al.*, 2001). Other components of dehydration tolerance include accumulation of compatible osmolytes (also known as osmotic adjustment, OA), reactive oxygen species (ROS) detoxication, stem reserve utilization for grain filling under drought stress and non-senescence ('delayed senescence' or 'stay-green') (Blum, 2005; Verslues *et al.*, 2006).

2.8.2 Drought tolerance genes

Plants respond and adapt to moisture stress, by triggering a wide variety of plant responses, including alterations in gene expression (Seki *et al.*, 2002; Umezawa *et al.*, 2006). It is the genes with altered expression that are believed to be involved in the pathways of plant responses to drought (Guo *et al.*, 2009). Through high-throughput microarray and Real time PCR studies, a number of these candidate genes that respond to drought stress at the transcriptional level have

been reported (Seki *et al.*, 2002; Liu and Baird, 2004; Hazen *et al.*, 2005; Cattivelli *et al.*, 2007; Shinozaki and Yamaguchi- Shinozaki, 2007; Talame *et al.*, 2007; Guo *et al.*, 2009). Some of these genes have been reported to play important roles in protecting plants from drought stress through different ways such as stress perception and signal transduction (Zhang *et al.*, 2004; Umezawa *et al.*, 2006). Putative drought-tolerance genes have also been used in engineering plants with improved drought stress tolerance (Pellegrineschi *et al.*, 2002; 2004; Abebe *et al.*, 2003; Umezawa *et al.*, 2006). Gou *et al.* (2009), performed gene expression experiments using drought-tolerant and drought-sensitive barley genotypes to compare differences in transcription levels between drought-tolerant and drought-sensitive genotypes under drought-stress conditions. They (Guo *et al.*, 2009), were able to separate drought-tolerance-related genes from drought-responsive genes. Some of the genes that have been reported relevant to drought tolerance are shown in Table 1.

Table 1: Genes that have been reported to respond to drought in plants

Genes	Function/name	Mechanism of action	References
DREBs/CBFs/ABF3	Stress induced transcription factors	Enhanced expression of downstream stress related genes confers drought/cold/salt tolerance. Constitutively over expression can lead to stunting growth.	Oh et al. (2005), Ito et al. (2006)
SNAC1	Stress induced transcription factor	Expression reduces water loss increasing stomatal sensitivity to ABA	Hu et al. (2006)
RD28	A. thaliana Water channel	It protects desiccated cells by transporting small molecules across cell membranes	Yamaguchi-Shinozaki et al. (1992)
P5CS d	Pyrroline-5-carboxylate synthetase	Enhanced accumulation of proline leads to increased osmotolerance	Kavi Kishor et al. (1995), Zhu et al. (1998)
mtlD	Mannitol-1-phosphate dehydrogenase	Mannitol accumulation leads to increased osmotolerance	Abebe et al. (2003)
GF14λ	14-3-3 protein	Lines over expressing GF14λ have a ''stay green'' phenotype improved water stress tolerance and higher photosynthetic rates under water deficit conditions	Yan et al. (2004)
NADP-Me	NADP-malic enzyme	The over expression decreased stomatal conductance and improves WUE	Laporte et al. (2002)

Gene expression studies or candidate gene approach has been successfully undertaken in many plants including *Arabidopsis, Medicago* and Maize (Bell *et al.*, 2001; Bruce *et al.*, 2002; Nguyen *et al.*, 2004). Unfortunately cassava is not sufficiently close phylogenetically to any of these species to take advantage of these studies through comparative genomics. No literature is available reporting about gene expression studies to identify candidate genes under drought

conditions in cassava. Drought tolerance candidate genes identified in other species have also not been evaluated for their expression in cassava under different water stress conditions.

Candidate genes can be identified via comparisons of gene expression profiles from plant tissues in response to stress. Real time PCR, DNA microarray and differential display analysis are some of the major methods employed in identifying candidate genes in crops (Lievens *et al.*, 2001). Real time PCR represents a powerful tool for the detection and quantification of mRNA and is widely and increasingly used because of its high sensitivity, good reproducibility, and wide dynamic quantification range (Pfaffl and Hageleit, 2001).

2.8.3 Drought stress tolerance in cassava

There is wide variation among cassava genotypes in response to drought stress and its components, reported elsewhere, with some having high levels of drought tolerance, while others are susceptible to drought stress (El-Sharkawy, 2007). Drought tolerant genotypes may employ any of the above mentioned mechanisms (low water potential avoidance, dehydration avoidance and dehydration tolerance) to survive in water stress conditions (Raheem and Chukwuma, 2001; Ch'avez *et al.*, 2005; Lenis *et al.*, 2006). In Uganda, some cassava genotypes have been postulated by both researchers and farmers to be drought tolerant, but there is limited empirical data. Nevertheless, cassava is generally believed to be less sensitive to water stress compared with grain crops and can withstand 4-6 months of dry season and almost never fails due to drought especially when drought appears after its full establishment in the soil (Pardales and Esquibel, 1996; El-Sharkawy, 2006; Lenis *et al.*, 2006; Perez *et al.*, 2011).

Several physiological, morphological and biochemical traits associated with drought tolerance in cassava have been reported (Mutegi-Murori, 2009). These include leaf gaseous exchange, accumulation and utilization of compatible osmolytes, leaf retention (Lenis *et al.*, 2006; Ssemakula and Dixon 2007; Mutegi-Murori, 2009; Subere *at al.*, 2009), among others. Genotypes with drought tolerance associated traits are particularly important since cultivation of cassava continues to expand to non traditional agricultural areas including semi-arid and marginal areas due to intensifying pressure on prime agricultural land (El-Sharkawy, 1993; Aina *et al.*, 2007; Mutegi-Murori, 2009). Ekanayake *et al.* (1996) emphasised the need for identifying and developing cassava genotypes that are well adapted to marginal areas with emphasis on drought tolerance. Though cassava is hardy to water stress conditions, severe drought results in adverse effects on its yield as result of its effects on plant phenology, phasic development and growth,

assimilate partitioning, plant reproduction process and root development (Agili and Pardales, 1999).

It suffices to note that the reported studies on drought tolerance in cassava have been carried out on morphological and physiological traits (Lenis *et al.,* 2006; Ssemakula and Dixon 2007; Mutegi-Murori, 2009; Subere *et al.,* 2009). Reports on cassava drought tolerance at molecular level are rare. This is the gap that this study seeks to fill. This study includes an analysis of drought tolerance at molecular level and this will in turn provide information on the molecular basis of drought tolerance in cassava

2.8.4 Breeding for drought tolerance in cassava

Although cassava can relatively tolerate drought stress, it can be genetically improved to enhance its productivity in drought prone environments. Breeding for drought tolerance in cassava has been substantially based on classical approach until recent (Okogbenin 2013). However, of recent, genetic improvement for drought adaptation in cassava has been enhanced by the increasing volume of genomic resources that are now available to breeding programmes. This modern breeding essentially entails combining genetic and genomic resources with good phenotyping protocols to efficiently breed for drought adapted varieties with speed and genetic again (Okogbenin 2013). Drought tolerance is a complex multigenic trait and thus, breeding for this trait is expensive due to the need for highly replicated phenotyping trials over several environments. This justifies the quest for a marker assisted breeding (MAB) approach that increases precision of selection and reduces the requirement for phenotyping. Many countries, including African national and regional breeding programs have initiated MAB strategy for drought tolerance breeding in cassava (Okogbenin et al. 2013). The most common objectives for breeding for drought tolerance in several cassava breeding programmes include: characterization of germplasm for tolerance to extended water shortages, either natural or imposed, and to low fertility soils; characterization of germplasm for vigour under early drought; study of leaf photosynthetic potential in relation to productivity under various edaphoclimatic conditions; and identification of other plant traits that might be of use in cassava improvement. Selection of parental materials for tolerance to water stress and infertile soils has resulted in breeding improved germplasm adapted to both stress environments.

The rigorous breeding of cassava for drought tolerance has been undertaken in several countries. For example, in Nigeria, the National Root Crops Research Institute (NRCRI) has initiated

massive screening of elite germplasm to identify genotypes that can be hybridized and recombined to develop an array of genotypes adapted to the various needs of drought-prone areas. The selection of progenies is done at drought-prone sites and this is critical for the identification of genotypes that perform well under water stress (Okogbenin, 2013). In Brazil, the International Fund for Agricultural Development (IFAD) supported a long-term project for selection of promising genotypes in different semi-arid environments in North eastern Brazil. IFAD later supported distribution of the elite germplasm throughout Africa (Okogbenin 2013). It is important to note that the effects of diseases such as cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) can mask the cassava's response to drought and therefore, prior to screening for drought tolerance, the resistance to these diseases should be incorporated (Okogbenin, 2013), especially, in Eastern and Southern Africa, where these diseases are prevalent.

The most cost-effective way of evaluating drought tolerance in cassava is to use well-designed and planned field experiments to determine relevant physiological traits (Okogbenin 2013). Cassava requires three months of rainfall or irrigation for establishment, after which, tolerance to drought can be measured effectively. Therefore, the ideal sites for field trials should be sites in semi-arid regions or drought prone sites with optimum rainfall distributed over three months of the year. In Africa, IITA and national programmes such sites include Hombolo in Tanzania, Kiboko and Kibwezi in Kenya and Minjibir (Kano State) in Nigeria for the evaluation of drought tolerance (Okogbenin 2013).

The conventional traits measured in drought adaptation studies of cassava include; number of primary stems, number of branching levels, height of primary and secondary stems, leaf retention using two methods (percentage method or visual score; and leaf scar method), height of leafless stem, length and width of fully expanded leaf lobe, carbohydrate content of leaves, stems, and petiole, stomatal conductance, osmotic adjustment (OA), ABA content of leaves and stems, above-ground biomass, length and number of fibrous root system, storage root fresh weight, number of storage roots, stem diameter, storage root dry matter (percentage) and storage root starch content (percentage) (Okogbenin, 2013). Though a good number of the conventional traits used to assess drought adaptation are available and several drought studies have been carried out in other countries, limited research has been done in Uganda on cassava for drought tolerance. This research sought to address this gap especially limited knowledge of molecular basis of drought tolerance in cassava.

CHAPTER 3: GENERAL MATERIALS AND METHODS

3.1 SCREENING LOCAL GERMPLASM FOR DROUGHT TOLERANCE

Materials

Forty six different cassava genotypes were collected from 15 districts in Uganda (Luwero, Mukono, Wakiso, Busia, Mbale, Palisa, Soroti, Tororo, Dokolo, Kaberamaido, Lira, Buliisa, Hoima, Kibale and Masindi) and these were used in this study. The genotypes included thirteen improved genotypes released by cassava breeding programme of National Crops Resources Research Institute (NaCRRI) of National Agriculture Research Organization (NARO) based at Namulonge. The genotypes used in this study are shown in Table 2.

Table 2: Cassava genotypes used in field screening for drought tolerance

Genotype	Type	Genotype	Type
Akena	Elite	Luderudu	Landrace
Bukalasa	Elite	Lugbara	Landrace
MM96/0686	Elite	Maburu	Landrace
MH97/2961	Elite	Magana	Landrace
NASE1	Elite	Mercury	Landrace
NASE11	Elite	Mufumbachai	Landrace
NASE12	Elite	Musita	Landrace
NASE2	Elite	Namukoni	Landrace
NASE3	Elite	Namulalu	Landrace
NASE9	Elite	Njule	Landrace
Omongole	Elite	Nyakakwa	Landrace
TME14	Elite	Nyalanda	Landrace
TME204	Elite	Nyamutukura	Landrace
Aladoalado	Landrace	Nyapamitu	Landrace
Bao	Landrace	Nyaraboke	Landrace
Buganda	Landrace	Nyarare	Landrace
Ditu	Landrace	Pilipili	Landrace
Icilicili	Landrace	Rugogoma	Landrace
Egabu	Landrace	Rwaburaru	Landrace
Gwaranda	Landrace	Ryahorore	Landrace
Kabwa	Landrace	Tongolo	Landrace
Kidimo	Landrace	Tongolo2	Landrace
Kwatamumpale	Landrace	Yellow	Landrace

Site

The genotypes were evaluated in the field in two drought prone sites in Buliisa and Nakasongola districts. Buliisa district is found in mid-western Uganda, while Nakasongola is found in central Uganda. For reference, similar field trial was established at Makerere University Agricultural Research Institute Kabanyolo (MAURIK) which receives high rainfall. The rainfall characteristics

of the three sites for a period of ten years are shown in Figure 2. From the available data, it is apparent that during the last 10 years, Buliisa received rainfall ≤800 mm, Nakasongola received rainfall ≤1400, and Kabanyolo received between 1200-1600 mm of rainfall.

Figure 2: Rainfall distribution for the three study sites over a period of ten years

Source: Meteological unit at NaRL -NARO-Kawanda. Courtesy of Dr. Komutunga Everlyne

To examine cassava response to drought, data were taken on; fresh root yield (FRY), harvest index (HI), root starch content (RSC), number of roots (NR), leaf retention (LR), root dry matter content (DMC), plant height (PH), vigour of sprouted seedlings (VG) and above ground biomass (AGB). Detailed methodology is described in chapter 4.

3.2 GENETIC DIVERSITY ASSESSMENT

A total of 66 genotypes were used for genetic diversity assessment. These genotypes included 46 used in field screening for drought tolerance in section 3.1 above, 12 genotypes whose DNA had been preserved at BecA and eight genotypes collected earlier together with those used for field screening in section 3.1 but later discarded because they succumbed to cassava brown streak and cassava mosaic virus diseases. The genotypes were grouped according to the regions of Uganda, from where they were collected, i.e. Central (Luwero, Mukono, Wakiso), Eastern (Busia, Mbale, Palisa, Soroti, Tororo), Northern (Dokolo, Kaberamaido, Lira) and Western (Buliisa, Hoima,

Kibale, Masindi). Leaf samples for DNA extraction were collected after four months of establishment and DNA extracted from the leaf sample in the laboratory at National Crop Resources Research Institute (NaCRRI) Namulonge Uganda. DNA extraction followed the previously described method (Dellaporta *et al.*, 1983). The DNA extracted was dissolved in low salt TE and transferred to BecA laboratories for diversity assessment. Genetic diversity was assessed with SSR markers. Detailed methodologies are discussed in chapter 5.

3.3 IDENTIFICATION OF CANDIDATE DROUGHT TOLERANCE GENES

Plant materials

Two cassava genotypes, a drought tolerant MM96/0686 and susceptible Nyalanda were used in this study. These genotypes were selected basing on their response to drought stress in the field where MM96/0686 was found to be tolerant and Nyalanda to be susceptible (Turyagyenda *et al.*, 2013). MH96/0868 is an improved cassava genotype obtained from cassava breeding program at the National Crop Resources Research Institute (Uganda), while Nyalanda is a landrace obtained from farmers' fields in Masindi district in western Uganda.

Cassava cuttings of the two genotypes were transferred to BecA, Nairobi and grown in 20 litre plastic buckets in a glasshouse. The transfer of plant and DNA materials to Kenya followed phytosanitary and quarantine procedure of both Uganda and Kenya. After establishment and under controlled glasshouse conditions, a half number of the genotypes were exposed to water stress for a total of 10 days by withholding water and the other half were maintained under well watered conditions (control). After 10 days of stress, total RNA was extracted from leaf samples of the genotypes from each treatment using Concert Plant RNA Reagent (Catalogue number 12322-012; Invitrogen) following manufacture's protocol.

The total RNA was converted to cDNA using GoScript Reverse transcriptase system (Promega, USA) following the manufacturer's instructions. The cDNA generated from cassava under water stress and control was used to evaluate expression of genes that have been reported to confer drought tolerance in other plants using Real Time PCR (Seki *et al.*, 2002; Shinozaki and Yamaguchi- Shinozaki, 2007; Talame *et al.*, 2007; Guo *et al.*, 2009).The Real Time PCR reactions were normalised with cassava actin gene as reference (Guo *et al.*, 2009; Yang *et al.*, 2011). Detailed methodology is described in chapter 6.

CHAPTER 4: SCREENING LOCAL CASSAVA GENOTYPES FOR DROUGHT TOLERANCE

Major sections of this Chapter were published in:

Turyagyenda F. Laban, Elizabeth B. Kizito, Yona Baguma and David Osiru 2013. Evaluation of Ugandan cassava germplasm for drought tolerance. *International Journal of Agriculture and Crop Sciences. IJACS/2013/5-3/212-226*

4.1 INTRODUCTION

Cassava constitutes the most important source of energy in the diet of people living in the tropical countries where it is a staple for over 800 million people (Burns *et al.*, 2010; Perez *et al.*, 2011). The annual world crop production is estimated at 241 million tonnes (Bull *et al.*, 2011) of which majority come from small-scale farmers in African and Asia. The crop has many advantages over other crops, including better adaptability to diverse and poor soil conditions and wide flexibility in planting and harvesting times (Sakai *et al.* 1994). In Africa, cultivation of cassava is often in hands of resource poor farmers in marginal areas where soil and climatic conditions are less favourable (El-Sharkawy 1993). In fact, expansion of cassava cultivation in semi-arid and marginal areas has continued to increase due to limited arable land (El-Sharkawy, 1993; Aina *et al.*, 2007; Mutegi-Murori, 2009). In these areas, unfavourable hilly terrain makes irrigation unaffordable to resource poor farmers, forcing them to depend on rainfall for agriculture, whose distribution and amounts have become unreliable and unpredictable. It is noteworthy to mention that rainfall in eastern and southern Africa has declined by approximately 15% in the last 30 years, and droughts have become more frequent and severe than ever (Funk *et al.*, 2008).

Although cassava is well-known for its capacity to withstand significant periods of water stress, its growth and productivity is generally constrained by moisture stress, especially, during the earlier stages of growth (El-Sharkawy, 1993; Okogbenin *et al.*, 2003; Bergantin *et al.*, 2004.; Perez *et al.*, 2011). According to Bakayoko *et al.* (2009), drought is a major abiotic constraint to cassava production in Africa. Cassava plants which undergo water stress in the first six months after planting produce low yields (Santisopasri *et al.*, 2001). Earlier studies by Pardales and Esquibel (1996) and Agili and Pardales (1999) reported that cassava is sensitive to soil water deficiency during the first 3 months of growth, resulting in significantly reduced growth and development of both the roots and shoots, which in turn affects the succeeding development of the plant including tuber development. When the rainfall pattern is fluctuating or when the genotypes planted are sensitive to water stress, production of cassava is often unstable (Bergantin *et al.*, 2004).

With increasing water scarcity due to climate change, combined with limited arable land, major efforts and strategies in agriculture should be geared towards sustaining crop production and productivity under limited water resources and marginal land conditions in order to meet increasing food demands. Burns *et al.* (2010) reported that the greatest challenge in agriculture is producing enough food, in a sustainable manner, to meet the needs of an increasing global

population. In this respect, cassava deserves particular attention because many of the world's poorest and most food insecure households are highly dependent on this crop as a contributing, if not the principal, source of food, nutrition, and cash income (Alexandratos, 1995). Millions of farmers, processors and traders earn their livelihood from cassava (Bergantin *et al.*, 2004). There is thus an urgent need to identify and develop cassava genotypes well adapted to semi-arid marginal areas, with special emphasis on drought tolerance, for maximization of productivity in drought-prone areas. This is important for improvement of food security and alleviation of poverty among the resource-poor farmers living in these areas. This is in line with Millennium Development Goal number one (MDG1), which calls for the world to reduce by half the proportion of people who suffer from extreme poverty and hunger, before the year 2015. Cassava is an important engine for growth in many countries if its production, diversification, and commercial uses are improved or developed (Bergantin *et al.*, 2004).

Elsewhere, varietal variability among cassava genotypes in response to water stress has been reported (El-Sharkawy, 2007). This variability can be exploited to identify drought tolerant genotypes and to improve cassava's tolerance to drought. However, information regarding genetic variability for drought tolerance among different cassava genotypes in Uganda is not well established. This information is an important resource to help cassava breeders to accurately identify drought tolerant genotypes to act as gene sources for breeding. Also traits associated with drought stress tolerance among the drought tolerant genotypes enables breeders to prioritise traits to work on when designing trials for breeding drought tolerant cassava. This study evaluated improved and landrace cassava genotypes for their tolerance to drought under different field moisture regimes in the seasonally dry districts of Uganda, in order to; 1) identify drought tolerant cassava genotypes for direct utilization by farmers living in semi-arid agro-ecological zones of Uganda, and by breeders in breeding programs; 2) assess the genotype by environment (G x E) effects of key traits associated with drought in cassava for rational distribution to different agro-ecological zones.

4.2 MATERIALS AND METHODS

4.2.1 The study site

The genotypes were evaluated in the fields in two drought prone sites in Buliisa and Nakasongola, and one site at Kabanyolo, which receives normal rainfall as reference. The total annual rain fall

in these districts during the experiment period was 1,581 mm of rainfall in Nakasongola, 1,052 mm in Buliisa and 2,448 mm in Kabanyolo (reference). The distribution of rainfall during the experimental period is presented in Figure 3.

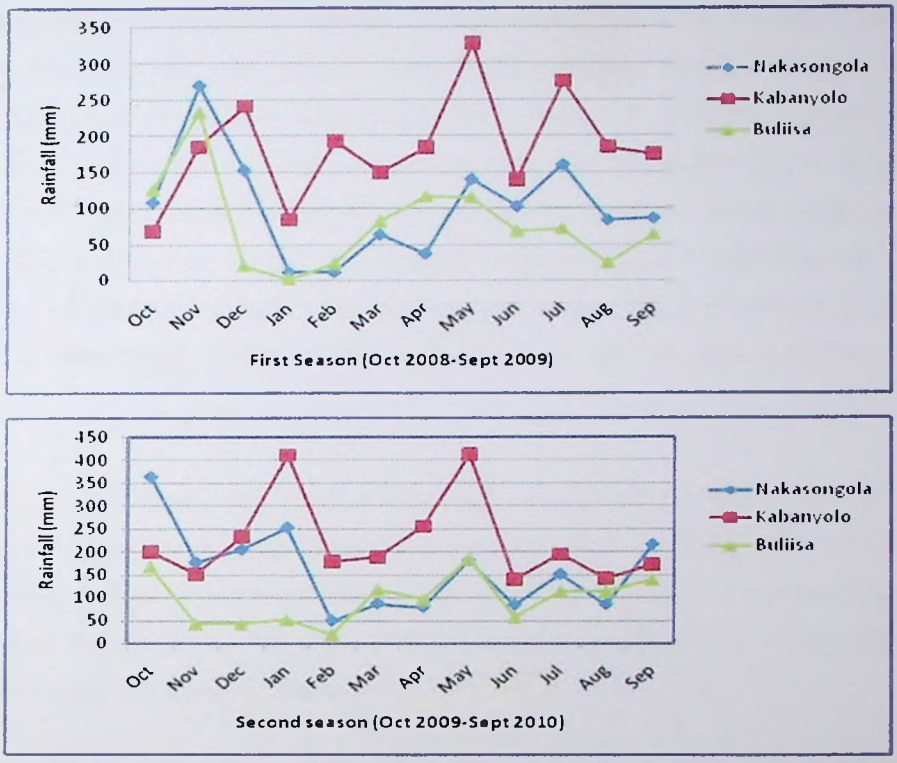

Figure 3: Rainfall distribution at experimental sites during experimental period

4.2.2 Plant materials and experimental design

Forty six cassava genotypes collected from 15 districts in Uganda were used in this study. The genotypes included 13 improved genotypes released by cassava breeding programme of National Crops Resources Research Institute (NACRRI) of National Agriculture Research Organization (NARO) based at Namulonge.

Cassava cuttings of uniform length (20-30 cm) were planted in a randomised complete block design (RCBD) with two replications of 10 plants of each genotype. The plant spacing used was 1 m x 1m between plants and 2 metres between plots. A plot was made up of two rows of 5 plants each for each genotype. Two cropping seasons were planted, the first season in October 2008 and

the second season in October, 2009. Field evaluation plots were kept weed-free by regular weeding with hand hoe, and neither pesticides nor fertilizers were applied.

4.2.3 Data collection

To examine cassava response to drought, the following traits were measured; fresh root yield (FRY), harvest index (HI), root starch content (RSC), number of roots (NR), leaf retention (LR), root dry matter content (DMC), plant height (PH), vigour of sprouted young plants (VG) and above ground biomass (AGB). Data were taken from the four middle plants for each genotype (the fresh root yield calculated from the area covered by these four plants up to the next plants). The evaluated plants were tagged with coloured ribbons for easy identification. LR and plant height were measured at 6 months after planting, while FRY, HI, RSC, NR, DMC and AGB were measured at harvesting. Details on the assessment of each of these variables are described thereafter.

HI: This is a ratio of harvestable yield to the total biomass yield on fresh weight basis. At harvest, four plants per genotype were uprooted and harvestable roots carefully collected from each. For each genotype, weight of the roots and the above ground biomass (stems, branches and leaves) were weighed separately. HI was computed as a ratio of weight of the harvestable roots to total biomass (root and above ground biomass)

$$HI = \frac{Wr}{Wr+Wab} \qquad Where\ W_r = Weight\ of\ roots,\ W_{ab} = weight\ of\ above\ ground$$

DMC and RSC: DMC and RSC were estimated using specific gravity methodology (Kawano *et al.*, 1987), as reported by Chavez *et al.* (2005). Briefly a sample of approximately 2-5 kg of roots was prepared from pool of roots for each genotype by cleaning to ensure that the roots are free from soil and other debris. The samples were put in perforated gunny bag and weighed in air using a spring balance (W_A) kg. The same samples in the same bag were weighed with the roots submerged in water (W_W) kg. DMC and RSC were estimated using the formulae:

$$DMC = \left(\frac{WA}{WA-WW} X158.3\right) - 142 \text{ and } RSC = \left(\frac{WA}{WA-WW} X112.1\right) - 106.4$$

LR: Leaf retention was visually estimated visually according to Lenis *et al.* (2006).

NR: The number of roots was determined from four plants of each genotype. Harvestable roots from each plant were counted and recorded.

PH: The plant height was determined on the primary stem from the ground using a measuring stick calibrated in centimetres. The measurements were done six months after planting.

VG: Data on vigour was collected for the first planting (2008) because drought set in early during crop establishment stage. Vigour data was collected to determine genotypes that were more sensitive to early drought. The vigour was scored on a sale of 0-3 (0=dead, 1=drying, 2 =wilting, 3=healthy) (Fig. 4). No further data were collected from first planting as most of the plants in drought prone sites died within the two months.

4.2.4 Data analysis

The data collected were subjected to different statistical analyses using GenStat Ver. 14.1 (GenStat, 2011). Mixed model was used with replications and environments/locations being treated as random effects while genotypes as fixed effects.

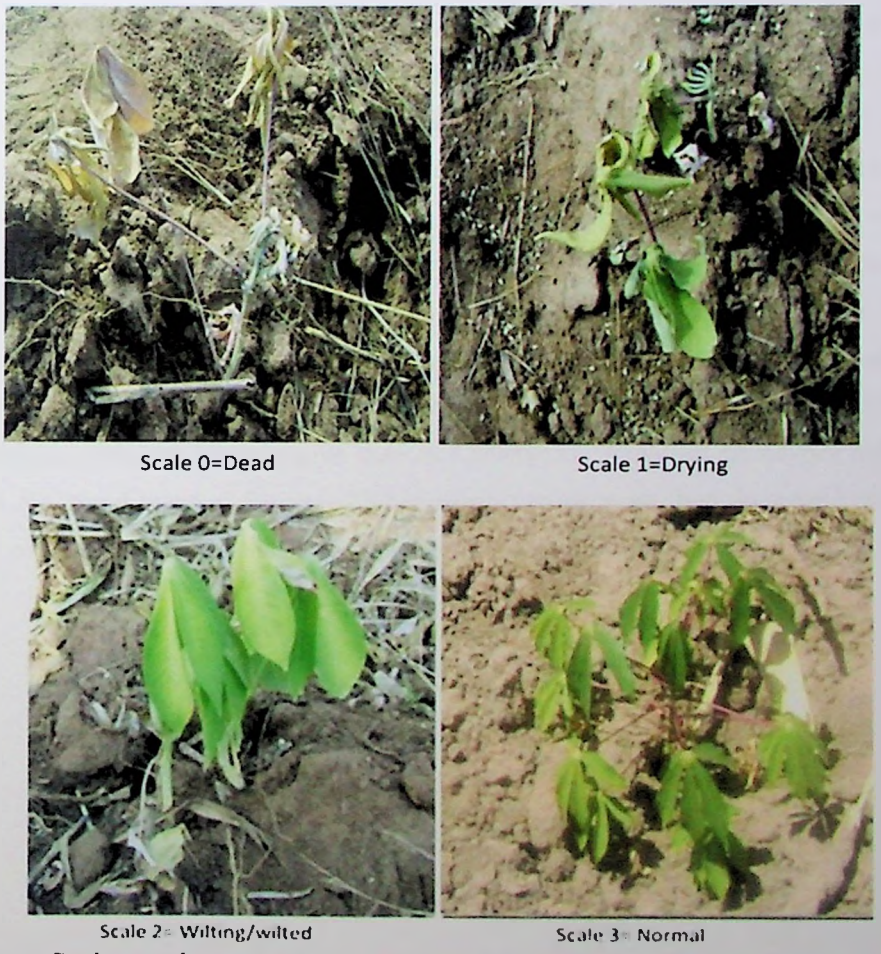

Figure 4: Scales used to score vigour performance of genotypes in response to drought stress

4.3 RESULTS

4.3.1 Response of selected clones to drought stress in 2008 growing season

Forty six genotypes were planted in 2008 planting season. All planted cuttings at the three sites sprouted. However, in drought prone districts of Buliisa and Nakasongola, most cassava plantlets that had sprouted succumbed to early drought during three months of establishment and dried up. Similarly, in Kabanyolo drought started within three months and the performance of genotypes was generally poor. Results on the vigour performance/survival are presented in Table 3. No further data were collected from 2008 planting as most of the plants in drought prone sites died within three months. The rate at which genotypes died in response to drought was different, some dying in large numbers and earlier than others.

Buliisa was most hit by drought with mean of 85.27% loss in vigour performance of genotypes when compared with vigour performance of the same genotypes grown at Kabanyolo, which received relatively high amounts of rainfall. In Nakasongola, drought stress resulted in mean decline of 44.19% in vigour performance (Table 3). On average, genotypes in Buliisa and Nakasongola had a score of less than one indicating that they either had died or were drying. In Kabanyolo, despite early drought stress, some genotypes remained healthy with a score of more than 2 with overall mean of 1.29. Eight genotypes performed better in drought prone districts than under normal rainfall at Kabanyolo in response to severe drought stress. The cassava clone 192/0067 (also referred to as Akena) performed better in both Buliisa and Nakasongola than Kabanyolo. Yellow, Magana, MH97/2961, Mufumbachai, Ryahorore, NASE11 and Nyaraboke performed better in Nakasongola than in Kabanyolo. Nineteen genotypes had mean vigour more than the grand mean at Buliisa, 23 had mean vigour more than the grand mean at Nakasongola, and while 23 genotypes had mean vigour more than the grand mean vigour at Kabanyolo (Table 3).

Table 3: Vigour performance of 46 cassava genotypes at three different locations in Uganda

Genotype	Kabanyolo	Buliisa	% difference	Nakasongola	% difference
Akena (192/0067)	0.19	0.20	-5.26	0.40	-110.53
AladoAlado	0.50	0.08	84.00	0.35	30.00
Bao	1.50	0.07	95.33	0.22	85.33
Buganda	2.19	0.40	81.74	0.35	84.02
Bukalasa	1.25	0.35	72.00	0.87	30.40
Ditu	2.00	0.35	82.50	0.60	70.00
Icilecili	1.09	0.12	88.99	0.35	67.89
Egabu	1.06	0.05	95.28	0.45	57.55
Gwaranda	1.81	0.45	75.14	1.17	35.36
Kabwa	1.66	0.37	77.71	0.60	63.86
Kidimo	2.16	0.20	90.74	1.00	53.70
Kwatamumpale	2.59	0.22	91.51	1.62	37.45
Luderudu	0.78	0.05	93.59	0.77	1.28
Lugbara	1.44	0.00	100	0.97	32.64
Maburu	1.41	0.02	98.58	0.92	34.75
Magana	0.94	0.20	78.72	1.00	-6.38
Mercury	1.12	0.05	95.54	0.72	35.71
MM96/0686	1.91	0.10	94.76	1.17	38.74
MH97/2961	1.55	0.42	72.90	1.78	-14.84
MufumbaChai	0.69	0.20	71.01	0.78	-13.04
Musita	1.44	0.00	100	0.75	47.92
Namukoni	1.56	0.05	96.79	0.60	61.54
Namulalu	0.59	0.02	96.61	0.27	54.24
NASE1	0.97	0.47	51.55	0.32	67.01
NASE11	0.31	0.05	83.87	0.57	-83.87
NASE12	1.53	0.57	62.75	0.52	66.01
NASE2	0.56	0.13	76.79	0.35	37.50
NASE3	0.37	0.08	78.38	0.37	0.00
NASE9	0.87	0.05	94.25	0.57	34.48
Njule	1.19	0.39	67.23	0.47	60.50
Nyakakwa	1.25	0.20	84.00	0.95	24.00
Nyalanda	1.63	0.07	95.71	0.35	78.53
Nyamutukura	1.91	0.15	92.15	0.75	60.73
Nyapamitu	2.41	0.02	99.17	0.45	81.33
Nyaraboke	0.06	0.00	100	0.27	-350
Nyarare	1.50	0.10	93.33	0.62	58.67
Omongole(192/0056)	1.09	0.17	84.40	0.87	20.18
Pilipili	0.87	0.13	85.06	0.80	8.05
Rugogoma	1.63	0.05	96.93	0.87	46.63
Rwaburaru	2.19	0.07	96.80	0.55	74.89
Ryahorore	0.62	0.32	48.39	0.85	-37.10
TME14	1.69	0.32	81.07	1.53	9.47
TME204	1.16	0.87	25.00	0.72	37.93
Tongolo	2.13	0.12	94.37	1.18	44.60
Tongolo2	1.41	0.27	80.85	0.60	57.45
Yellow	0.56	0.17	69.64	0.95	-69.64
Mean at sites	***1.29***	***0.19***	***85.27***	***0.72***	***44.19***
SE=0.81					

*Percent difference=percent difference between the reference site and the treatment site=(R-T)/R*100) where Kabanyolo (KAB) was regarded as reference (R) and Buliisa (BUL) and Nakasongola (NAK) as treatment sites (T)*

The performance of genotypes averaged for the drought prone districts was compared to the performance of their counterparts at Kabanyolo and results are presented in Table 4. Drought stress resulted in 65.3% reduction in vigour performance. Twenty one genotypes had decline more than 65.3% (the general mean decline) in their performance in response to drought stress (Table 4). The five most outstanding genotypes in each of the drought sites were; TME204, NASE12, NASE1, Gwaranda and MH97/2961 in Buliisa, and MH97/2961, Kwatamumpale, TME14, Tongolo and MM96/0686 in Nakasongola. Akena, Yellow, Mufumbachai, MH97/2961 and Ryahorore genotypes were most stable to water stress across the three sites.

Table 4: Vigour performance of 46 cassava genotypes for two different environments in Uganda

Genotype	Stress*	Reference	% difference	Genotype	Stress	Reference	% difference
Akena	0.30	0.19	-57.89	NASE1	0.40	0.97	58.76
AladoAlado	0.21	0.50	58.00	NASE11	0.31	0.31	0.00
Bao	0.15	1.50	90.00	NASE12	0.55	1.53	64.05
Buganda	0.37	2.19	83.11	NASE2	0.24	0.56	57.14
Bukalasa	0.61	1.25	51.20	NASE3	0.22	0.37	40.54
Ditu	0.47	2.00	76.50	NASE9	0.31	0.87	64.37
Icilicili	0.24	1.09	77.98	Njule	0.44	1.19	63.03
Egabu	0.25	1.06	76.42	Nyakakwa	0.57	1.25	54.40
Gwaranda	0.81	1.81	55.25	Nyalanda	0.21	1.63	87.12
Kabwa	0.49	1.66	70.48	Nyamutukura	0.45	1.91	76.44
Kidimo	0.60	2.16	72.22	Nyapamitu	0.24	2.41	90.04
Kwatamumpale	0.92	2.59	64.48	Nyaraboke	0.14	0.06	-133.33
Luderudu	0.41	0.78	47.44	Nyarare	0.36	1.50	76.00
Lugbara	0.49	1.44	65.97	Omongole	0.52	1.09	52.29
Maburu	0.47	1.41	66.67	Pilipili	0.46	0.87	47.13
Magana	0.60	0.94	36.17	Rugogoma	0.46	1.63	71.78
Mercury	0.39	1.12	65.18	Rwaburaru	0.31	2.19	85.84
MM96/0686	0.64	1.91	66.49	Ryahorore	0.59	0.62	4.84
MH97/2961	1.10	1.55	29.03	TME14	0.92	1.69	45.56
MufumbaChai	0.55	0.69	20.29	TME204	0.80	1.16	31.03
Musita	0.37	1.44	74.31	Tongolo	0.65	2.13	69.48
Namukoni	0.32	1.56	79.49	Tongolo2	0.44	1.41	68.79
Namulalu	0.15	0.59	74.58	Yellow	0.56	0.56	0.00
				Mean	***0.47***	***1.37***	***65.29***
				LSD	***0.55***	***0.24***	

**averaged over two moisture stress environments; Percent difference=percent difference between the reference site and the treatment site=(R-T)/R*100) where Kabanyolo (KAB) was regarded as reference (R) and Buliisa (BUL) and Nakasongola (NAK) as treatment sites (T)*

4.3.2 Response of selected clones to drought stress in 2009 growing season

The forty six cassava genotypes were replanted for 2009. Mean squares (MS) indicated that genotype and location effects were highly significant ($P \leq 0.05$) for all the parameters evaluated at the three locations (Table 5). At the three locations, genotype x location was also highly significant except for AGB. Because, Kabanyolo was expected to have different weather conditions from the other two drought prone sites (Buliisa and Nakasongola), data from

Kabanyolo was removed and data from the two drought prone sites were analysed separately to validate genotype by location effects at the drought prone districts (Table 6). The results from this analysis showed that genotypes performed significantly different at the two sites, and only four parameters showed significant differences for location (vigour, number of roots, above ground biomass and leaf retention) and genotype-by-location (vigour, number of roots, Harvest index, leaf retention and plant height) (Table 6). Results further showed that water stress had significant effects on both the vegetative and yield parameters of cassava (Table 7). For example, water stress resulted in mean decline of 22.34% in HI, 37.04% in yield, 19.43% in number of roots, 16.56% in DMC, and 20.81% in root starch content (Table 7).

Table 5: Analysis of variance (ANOVA) for selected agronomic traits of forty six cassava genotypes under contrasting water stress conditions at three locations

	Mean squares			
SOV	**Genotype (df=45)**	**Location (df=2)**	**Genotype x Location (df=90)**	**error**
VG[a]	8.92***	482.17***	4.7***	0.66
HI	0.10***	0.97***	0.02***	0.01
NR	52.61***	191.11***	5.66***	3.68
DMC	271.19***	1390.27***	120.23***	87.04
RSC	135.99***	697.19***	60.29***	43.65
FRY	436.05***	2341.99***	49.30***	35.22
AGB	243.92***	612.74***	48.29	49.65
LR	576.70***	45313.10***	292.60***	158.50
PH	7923.70***	87903.10***	2640.30***	893.70

*[a]Data collected from 2008 planting; HI= harvest index, RSC =root starch content, NR= number of roots, LR= leaf retention, DMC= root dry matter content, PH= plant height, VG=Vigour of sprouted seedlings and AGB=above ground biomass ***significant difference at P≤0.05;*

Table 6: Analysis of variance (ANOVA) for selected agronomic traits of forty six cassava genotypes at the two drought prone sites (Buliisa and Nakasongola)

SOV	**Genotype (df=45)**	**Location (df=1)[b]**	**Genotype x Location (df=45)**	**error**
VG[a]	3.71***	262.66***	2.73***	0.51
HI	0.10***	0.05	0.02***	0.02
NR	40.03***	136.28***	4.73	3.67
DMC	230.27***	14.31	99.95	73.44
RSC	115.48***	7.18	50.12	36.83
FRY	375.41***	43.21	29.38	27.22
AGB	182.23***	590.72***	55.83	44.32
LR	557.10***	26527.30***	252.20***	159.00
PH	4965.20***	206.70	3652.90***	856.00

*[a]Data collected from 2008 planting. [b]Kabanyolo excluded from analysis as it was regarded as wet environment with different environmental conditions from the other two sites) HI= harvest index, RSC =root starch content, NR= number of roots, LR= leaf retention, DMC= root dry matter content, PH= plant height, VG=Vigour of sprouted seedlings and AGB=above ground biomass, ***significant difference at P≤0.05*

Harvest Index

Harvest Index showed significant variation among genotypes in response to water stress. The mean HI at Buliisa, Kabanyolo and Nakasongola, respectively were 0.38, 0.47 and 0.35. There were significant reductions for HI in Buliisa and Nakasongola relative to Kabanyolo. Nakasongola was most affected with mean decline of 25.53% while Buliisa had mean decline of 19.15% (Table 7). Overall, water stress resulted in mean decline of 22.34%. Eight genotypes (NASE9, NASE1, TME14, TME204, NASE2, MM96/0686 and Buganda) had least percentage reductions in HI in response to drought stress (Table 8).

Fresh Root Yield

Fresh root yield was significantly different amongst genotypes and across the three locations (Table 6). Mean yields were 13.93 t/ha at Kabanyolo, 8.5 t/ha at Buliisa and 9.04 t/ha at Nakasongola. However, fresh root yield was not significantly different among the two drought prone sites (Table 6). Mean yield performance of genotypes declined by 39% in Buliisa and 35% in Nakasongola when compared with their performance at Kabanyolo (Table 7). Relative to normal rainfall environment, there was a decline of 37% in yield performance of genotypes in drought areas. The effect of drought was least for genotypes; TME204, NASE9, TME14, Akena, NASE1, Yellow, MM96/0686 and NASE2 (Table 8). Pilipili, Mercury and Nyalanda, all of them landraces, were most affected with a decline of 81%, 80% and 79% in fresh root yield, respectively.

Table 7: Means and percentage difference for selected agronomic traits of cassava genotypes under contrasting water stress conditions

Site	VG[a]	*% Diff*	AGB	*% Diff*	HI	*% Diff*	FRY	*% Diff*	NR	*% Diff*
KAB	1.30		15.23		0.47		13.93		5.97	
BUL	0.20	*84.91*	12.00	*21.21*	0.38	*19.15*	8.50	*38.98*	4.31	*27.81*
NAK	0.73	*43.76*	14.28	*6.24*	0.35	*25.53*	9.04	*35.10*	5.31	*11.06*
LSD (5%)	***0.06***		***1.24***		***0.02***		***1.04***		***0.32***	

Table 7: cont'd

Site	DMC	*% Diff*	RSC	*% Diff*	LR	*% Diff*	PH	*% Diff*
KAB	40.52		22.85		67.69		170.30	
BUL	33.52	*17.28*	17.90	*21.66*	43.92	*35.12*	141.40	*16.97*
NAK	34.08	*15.89*	18.29	*19.96*	56.65	*16.31*	142.60	*16.27*
LSD (5%)	***2.72***		***1.93***		***1.92***		***4.55***	

*LSD=least significant difference at P≤0.05. % Diff=Percent difference between the reference site and the treatment site=(R-T)/R*100) where Kabanyolo (KAB) was regarded as reference (R) and Buliisa (BUL) and Nakasongola (NAK) as treatment sites (T). FRY= fresh root yield=), HI= harvest index, RSC =root starch content, NR= number of roots, LR= leaf retention, DMC= root dry matter content, PH= plant height. VG=Vigour of sprouted seedlings and AGB=above ground biomass*

Number of roots

Significant differences were observed among the genotypes and locations for number of roots. There was also significant genotype by location effect (G x L) on the root number at the three locations (Table 5). When data was analysed for the two drought prone districts, there was no significant genotype-by-location effect (Table 6). The highest mean number of roots was recorded at Kabanyolo site with a mean of 5.97 roots per plant, while Buliisa and Nakasongola recorded a mean of 4.31 and 5.31 roots per plant, respectively. There was a decline in number of roots per plant by 27.81% for Buliisa and 11.06% for Nakasongola relative to number of roots per plant for Kabanyolo, the less-stressed environment. Overall, drought resulted in a decline of 19.43% in number of roots when the performance of genotypes at the two drought sites was averaged and compared with reference site, Kabanyolo. The root number was less affected by drought in; Rwaburaru, Magana, NASE12, NASE11, Kidimo and MM96/0686, with less than 1% decline (Table 8). The highest decline (53.3%) in number of roots due to drought was recorded in Nyalanda. Across locations, MM96/0686 had the highest number (10) of roots followed by landrace Yellow (9) while Kwatamumpale had the least mean number of roots (3) across locations.

Above ground Biomass

Fresh shoot yield or above ground biomass (AGB) was significantly different among the genotypes. However, there was no G x L effects for AGB when analysed for both the three sites and the two drought prone sites (Table 5 and 6). The mean AGB was 12 t/ha for Buliisa, 15.23 t/ha for Kabanyolo and 14.28 t/ha for Nakasongola. Twelve genotypes (Rwaburaru, Tongolo2, TME204, NASE3, Ryahorore, Yellow, Akena, NASE11, Nyapamitu, Omongole, Nyakakwa and Namulalu) performed slightly better in drought prone districts than in normal rainfall district. However, other than Akena and NASE11, overall performance both in dry and normal rainfall environment was low. The ten genotypes with high mean shoot yields, across sites included MM96/0686, NASE9, Buganda, Akena, NASE12, NASE11, MH97/2961, Nyamutukura, NASE1 and NASE2.

Root dry matter content

Results on DMC showed significant differences amongst genotypes, locations and G x L effects for the three locations (Table 5). For the two drought prone sites, location and genotype-by-

location effects were not significant (Table 6). The mean DMC was 33.5% for Buliisa, 40.5% for Kabanyolo and 34.1% for Nakasongola. Relative to the reference location, Kabanyolo, mean root dry matter content decreased by 17.3% for Buliisa and 15.9 % for Nakasongola. The overall effect of drought on DMC was low (16.58%). Four genotypes; NASE2, MM96/0686, MH97/2961 and Nyamutukura had stable and high DMC in both dry and normal rainfall sites. Nyamutukura is a local genotype, while the rest are improved genotypes.

Root starch content

Starch production varied among genotypes, locations and genotype-by-location for the three locations, though location and genotype-by-location effects were not significant among the two drought prone sites. The mean starch production was highest in Kabanyolo (22.85%) and lowest at Buliisa (17.90%). Relative to Kabanyolo, there was a decrease in root starch content of 21.66% for Buliisa and 19.96% for Nakasongola. Overall, drought stress reduced starch content by 20.81%. Like for DMC, four genotypes; NASE2, MM96/0686, MH97/2961 and Nyamutukura had stable and high starch content in both dry and normal rainfall sites (Table 8).

Plant height

Plant height differed significantly among genotypes and locations (Table 5 & 6). Average plant height for all genotypes at the three locations were; 170.3 cm, 141.4 cm and 142.6 cm for Kabanyolo, Buliisa and Nakasongola, respectively. Genotype-by-location effects were also significant. Compared to Kabanyolo, drought stress reduced plant height by 16.97% and 16.27% for Buliisa and Nakasongola, respectively. Genotypes; TME204, Njule, NASE12, Nyamutukura, TME14, Bao, Yellow, Rugogoma and Nyakakwa, were more stable with less than 6% decline in plant height in the drought environment.

Leaf retention

The highest mean leaf retention was recorded at Kabanyolo (67.69%) and the least in Buliisa (43.92%) while in Nakasongola leaf retention was 56.65%. Relative to normal rainfall site at Kabanyolo, there was a decline of 35.12% and 16.31% in leaf retention for Buliisa and Nakasongola, respectively. Genotypes, Location and Genotype-by-location effects were all significant (Table 5 and 6). Genotypes; Nyakakwa, NASE12, TME204, Nyamutukura, TME14, MH97/2961, MM96/0686 and Njule were the most stable across locations (Table 8).

Table 8: Means of traits measured for 46 genotypes for water stressed environments as compared to reference site

Variety	*Above ground biomass*		*Harvest Index*		*Fresh Root yield (t/ha)*		*Number of root*		*Dry Matter content*		*Root starch content*		*Leaf retention*		*Plant height*	
	*Stress**	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*
Akena	21.08	19.17	0.42	0.46	15.33	16.06	6.67	7.83	37.85	34.93	20.96	18.90	60.10	74.73	166.12	188.00
Aladoalado	11.39	15.67	0.38	0.51	7.03	16.83	4.33	5.50	20.72	38.18	8.83	21.20	58.45	77.47	135.63	177.63
Bao	11.17	19.67	0.31	0.47	5.09	19.33	3.08	6.50	39.90	40.74	22.41	23.01	34.90	70.43	159.46	166.88
Buganda	16.88	24.22	0.40	0.40	11.97	17.11	2.75	5.50	34.35	28.32	18.48	14.21	40.76	74.40	142.31	161.88
Bukalasa	12.29	13.33	0.27	0.48	4.88	11.67	3.17	4.33	26.26	35.70	12.75	19.44	43.06	71.58	138.87	204.17
Ditu	11.19	11.61	0.33	0.36	5.81	6.89	3.83	4.33	30.79	48.13	15.96	28.24	52.38	70.72	141.07	165.63
Icilicili	9.00	16.83	0.28	0.45	4.24	16.44	3.92	6.33	26.45	45.29	12.89	26.23	42.96	80.86	142.07	207.50
Egabu	9.63	10.75	0.26	0.38	2.30	8.58	3.00	3.67	27.18	38.96	13.40	21.74	50.31	73.82	125.71	189.00
Gwaranda	16.64	16.83	0.26	0.46	6.28	13.89	3.17	5.67	36.70	28.31	20.15	14.21	50.74	75.49	149.85	196.88
Kabwa	11.81	14.60	0.35	0.46	6.89	12.27	3.42	4.50	36.48	42.91	19.99	24.54	44.82	65.85	152.60	182.50
Kidimo	8.97	12.17	0.26	0.48	2.93	12.78	3.58	3.50	28.35	40.27	14.24	22.67	46.99	65.65	137.73	192.50
Kwatamumpale	6.88	11.11	0.27	0.39	2.42	7.06	2.08	4.00	23.60	46.95	10.87	27.41	53.81	69.98	123.23	153.75
Luderudu	9.82	13.67	0.29	0.40	4.09	10.72	3.50	4.33	29.32	40.60	14.92	22.91	54.41	68.59	144.23	163.13
Lugbara	8.96	12.72	0.32	0.38	3.92	7.56	3.50	4.33	32.89	42.83	17.45	24.48	47.09	75.06	142.85	172.50
Maburu	9.67	13.61	0.31	0.39	4.64	8.67	3.08	6.67	27.31	36.68	13.50	20.13	46.68	60.32	165.08	200.75
Magana	15.28	16.28	0.52	0.56	17.58	19.94	7.58	5.67	36.63	50.10	20.10	29.63	58.44	72.68	153.13	184.75
Mercury	9.89	21.89	0.30	0.48	3.89	19.44	3.92	4.83	37.24	50.73	20.53	30.08	50.35	62.67	164.81	210.63
MM96/0686	22.50	24.94	0.48	0.48	21.00	22.39	9.92	10.00	54.81	56.16	32.97	33.93	63.91	72.65	135.31	164.13
MH97/2961	18.06	20.22	0.51	0.52	19.42	22.28	7.67	9.83	46.85	51.10	27.33	30.34	54.79	62.04	146.60	173.88
Mufumbachai	17.22	17.50	0.31	0.43	8.97	15.28	5.42	7.50	30.54	41.74	15.78	23.72	55.52	69.02	137.67	184.17
Musita	11.61	13.06	0.40	0.53	8.33	15.39	6.58	7.33	38.29	53.17	21.27	31.81	49.42	66.22	125.50	160.63
Namukoni	11.03	14.28	0.40	0.56	7.43	16.67	5.00	5.33	33.77	44.88	18.07	25.94	48.20	65.84	112.13	154.14
Namulalu	14.73	14.50	0.36	0.50	7.64	14.50	3.75	4.67	27.50	31.07	13.63	16.16	53.31	64.67	140.15	158.75

Table 8 cont'd

Variety	*Above ground Biomass*		*Harvest Index*		*Fresh Root yield (t/Ha*		*Number of root*		*Dry Matter content*		*Root starch content*		*Leaf retention*		*Plant height*	
	*Stress**	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*	*Stress*	*Ref*
NASE1	15.61	20.78	0.54	0.46	17.79	18.67	5.25	6.50	31.75	32.98	16.64	17.51	50.61	72.55	135.63	164.38
NASE11	19.91	18.47	0.34	0.54	10.91	22.53	5.75	4.67	35.22	33.62	19.10	17.96	50.05	67.89	138.29	153.88
NASE12	19.19	19.28	0.46	0.58	16.86	23.33	8.75	6.67	34.35	47.67	18.48	27.91	53.92	58.11	121.15	121.00
NASE2	15.97	19.61	0.48	0.45	15.36	14.67	6.67	8.17	49.42	57.19	29.15	34.66	52.27	63.05	118.92	137.38
NASE3	15.89	13.47	0.49	0.59	14.89	19.33	6.50	8.33	36.82	42.93	20.23	24.56	51.59	61.04	144.38	174.25
NASE9	20.83	24.56	0.45	0.38	16.11	13.72	4.58	6.00	30.68	38.46	15.89	21.39	53.11	68.45	168.21	184.38
Njule	14.31	14.56	0.22	0.44	2.92	11.78	3.67	4.67	27.35	21.06	13.52	9.07	52.32	59.69	137.69	132.88
Nyakakwa	13.22	13.00	0.38	0.51	6.59	13.94	6.33	7.33	33.82	66.81	18.11	41.47	55.93	58.96	152.92	162.13
Nyalanda	5.94	14.17	0.30	0.47	2.69	12.78	3.58	7.67	24.28	43.25	11.35	24.78	44.56	74.34	159.81	181.88
Nyamutukura	18.63	18.67	0.47	0.53	14.79	19.87	4.83	4.83	45.27	49.10	26.22	28.93	58.51	65.50	177.00	179.00
Nyapamitu	8.41	8.00	0.25	0.43	2.20	6.67	3.67	5.00	34.75	36.03	18.76	19.67	51.63	61.60	146.25	190.00
Nyaraboke	9.67	11.00	0.24	0.40	2.89	7.56	4.08	4.33	33.06	36.64	17.57	20.11	48.51	68.37	131.75	165.50
Nyarare	10.91	11.11	0.36	0.48	6.70	10.78	3.50	5.00	27.88	26.22	13.90	12.72	49.33	73.94	133.15	154.38
Omongole	10.89	10.44	0.33	0.40	4.81	8.22	6.08	7.33	29.71	43.09	15.20	24.67	42.55	57.66	84.94	116.88
Pilipili	9.64	11.44	0.25	0.47	2.06	10.56	3.25	5.50	50.57	39.32	29.97	22.00	50.13	67.02	140.50	175.00
Rugogoma	10.09	12.07	0.30	0.52	4.76	11.87	2.67	4.83	21.45	36.09	9.34	19.71	51.45	61.24	149.36	157.67
Rwaburaru	15.42	11.94	0.23	0.38	4.33	6.50	4.08	2.67	42.04	25.08	23.93	11.92	47.57	64.48	131.36	185.50
Ryahorore	9.78	8.39	0.30	0.53	4.15	9.39	4.58	4.83	41.37	30.89	23.45	16.03	55.52	79.89	126.85	157.50
TME14	16.22	17.00	0.50	0.46	15.72	15.00	5.92	8.17	34.16	23.24	18.35	10.61	57.67	65.02	130.44	132.88
TME204	15.92	13.44	0.55	0.52	19.56	14.56	7.83	8.33	31.53	48.79	16.49	28.71	59.78	66.47	169.06	156.25
Tongolo	9.86	11.39	0.33	0.44	6.36	8.50	3.67	4.17	23.77	32.08	10.99	16.87	39.85	53.84	127.44	144.13
Tongolo2	12.73	10.44	0.30	0.35	5.50	6.83	4.75	7.17	32.23	50.19	16.98	29.70	42.50	54.67	193.13	243.25
Yellow	14.44	12.94	0.51	0.56	16.00	16.89	8.25	10.00	39.60	35.31	22.20	19.16	60.68	78.34	150.00	158.13
Mean	***13.24***	***15.10***	***0.36***	***0.47***	***8.61***	***13.82***	***4.81***	***5.96***	***33.80***	***40.52***	***18.09***	***22.85***	***50.90***	***67.67***	***142.40***	***170.48***
CV	***50.99***	***51.04***	***34.57***	***24.85***	***59.35***	***51.40***	***41.72***	***32.19***	***26.73***	***26.38***	***35.37***	***33.12***	***28.33***	***18.55***	***22.85***	***18.25***
Se	***6.83***	***7.72***	***0.13***	***0.12***	***5.24***	***7.09***	***2.01***	***1.92***	***9.04***	***10.69***	***6.40***	***7.57***	***14.43***	***12.55***	***32.51***	***31.06***

Stress= mean performance in stressed environments (average performance for Buliisa and Nakasongola); Ref= reference site (Kabanyolo)

4.4 DISCUSSION

The overall objective of this study was to screen cassava genotypes commonly grown in Uganda, for drought tolerance. This objective was achieved through undertaking field based evaluations in two drought prone sites, Nakasongola and Buliisa, and at Kabanyolo, which receives normal rainfall, as a reference site. During the study period, Nakasongola and Buliisa received less rainfall compared to Kabanyolo in Wakiso district. Therefore, differences in performance of the genotypes at different locations can reasonably be attributed to rainfall differentials among sites. Genotypes differed significantly in all the nine traits studied suggesting that the cassava genotypes evaluated has adequate genetic variability. El-Sharkawy, (2007) reported similar results when he subjected cassava genotypes to water stress. El-Sharkawy's study established that some genotypes had high levels of drought tolerance, while others were susceptible. In addition, genotypic effects were dominant compared to location and genotype-by-location effects suggesting a strong genetic basis for the phenotypic differences observed among the genotypes. The dominant genotypic effects further indicate that selection of desirable characters among these genotypes would lead to significant progress in cassava drought improvement schemes.

The 2008 season provided information on the effect of severe drought during crop establishment stages. The planted cassava stakes had sprouted, but due to severe drought before full establishment, most of the genotypes died especially in drought prone districts. The data taken on vigour on all the plants indicated that genotypes differed in the rate at which drought caused death. For instance, some genotypes kept on growing to three months after planting, while others died early after sprouting. The information generated is important because drought effects in cassava have been reported to be severe during early stages of establishment than in later stages of development (Pardales and Esquibel, 1996; Perez *et al.*, 2011). Genotypes whose plants died early after sprouting in drought prone areas but had high vigour at Kabanyolo may be regarded as susceptible, while those that survived in all the sites or died later in the prone districts may be putative drought tolerant. Based on this description, genotypes TME14, MM96/0686, Kwamtamumpare, Gwaranda, MH97/2961, TME204, Tongolo, Bukalasa and Magana are classified as candidate drought tolerant genotypes. Among these, TME14, MH97/2961, TME204 and MM96/0686 are improved genotypes from breeding programmes while the rest are local genotypes. Bao, Nyalanda, Egabu, Icilicili, and Rwaburaru (all local genotypes) are here regarded as drought susceptible.

Genotype, location and genotype-by-location effects showed highly significant mean squares ($P<0.01$) for all the parameters evaluated, except for above ground biomass when all the three sites were included in the analysis. These reflect genotypic differences towards adaptation to different agro-ecological zones and tolerance to water stress. Rainfall has been reported as the critical factor in defining agro-ecological zones (Aina *et al.*, 2007). The significance of genotype-by-location effects suggests that genotypes may be selected for specific adaptation to different environments. Based on this, rational distribution of genotypes to areas with different rainfall regimes or different rainfall seasons is possible. Farmers living in marginal agro-ecological zones would be guided by tolerance of genotypes to erratic rainfall conditions, while farmers in agro-ecological zones with optimum growth conditions would be guided by factors such as high yield and tolerance to pests and diseases. The less adapted genotypes in drought-prone areas may be useful in areas with optimum rainfall or areas where drought stress is not critical. However, when data from the two drought prone sites were analysed separately, location effects were significant for only four parameters (vigour, number of roots, above ground biomass, and leaf retention), while genotype-by-location effects were significant for vigour, number of roots, Harvest index, leaf retention and plant height). This suggests that the significant differences for location and genotype-by-location among the other parameters can be attributed to Kabanyolo since it received more rainfall than the other two sites.

This study revealed that HI was different among genotypes and the three locations. Harvest Index is ability to convert biomass into economic yield (Mutegi-Murori, 2009) and therefore reflects the partitioning efficiency of dry matter towards economic root production. Mutegi-Murori (2009) in her thesis, suggested that selections based on HI are stable across evaluation stages making HI an important trait in cassava breeding because it truly represents genotype's yield potential. Kawano, (1990) reported that true genetic progress in cassava can be achieved through utilization of HI. In this study, genotype effects were dominant indicating that the primary effect of the HI differences amongst the genotypes is attributable to genetic effects. Okogbenin *et al.* (2003) evaluated cassava genotypes in Nigeria and reported that HI was not significantly different among locations with different water stress levels. All these studies indicate that differences in HI among genotypes are largely attributed to genetic effects and thus, HI is an important trait in the selection of genotypes under water stress conditions.

Contrary to results obtained by Okogbenin *et al.* (2003), in this study HI was significantly reduced by drought. With a few exceptions, lower HI was recorded in Buliisa and Nakasongola and high HI recorded at Kabanyolo. This suggests that poor growth conditions in drought stressed Buliisa and Nakasongola affected root bulking and hence resulted in poor assimilate partitioning. Similar observations were made by Cach *et al.* (2006), who reported highly significant genotype-by-environment effects. It does suffice to note that indirect selection for yield through HI is more effective than direct selection for yield itself especially in early evaluation and selection stages (Kawano *et al.*, 1998). Recently, Perez *et al.* (2011) established that heritability for HI was significantly higher than for fresh root yield justifying the importance of including HI as a selection criterion.

Cassava, under favourable conditions is highly productive as shown in this study with high mean storage root yield of 13.9 t/ha in Kabanyolo, which was above the reported average yield of 12.7 t/ha for Uganda, 12.4 t/ha for Africa and 10.2 t/ha for world (FAOSTAT, 2010). The yield obtained in this study is within the range reported by similar studies on cassava. El-Sharkawy (1993) reported cassava yields of 8-16 t/ha of fresh roots with landraces in marginal areas without application of agrochemicals. Burns *et al.* (2010) reported that African subsistence farmers achieve yields of 8–10 tonnes of fresh roots per hectare over a 12–18 month crop cycle. In Indonesia and Thailand yield of 18–23 t/ha has been recorded (Burns *et al.*, 2010). The results obtained from this study and elsewhere indicate that the yield potential of 90 tonnes per hectare reported in Colombia under ideal conditions (El-Sharkawy, 1993), are yet to be achieved. The yield of 13.9 t/ha at Kabanyolo was still below the cassava's production potential and this may be attributed to reduced rainfall a few months towards harvesting.

Yield stability is more important and requires genotypes that do not produce high yield only in favourable conditions but can also produce high yields under water stress conditions (Mutegi-Murori, 2009). There was a decline by 37.04% in total fresh yield by water stress. Okogbenin *et al.*, (2003) reported a decline of 22% in root yield when both Nigerian and IITA improved genotypes were exposed to water stress. Connor and Palta (1981) reported that water stress after 1-5 MAP led to reduction in storage root yield by 32-60%. The critical period for water deficient in cassava is 1-5 MAP, which coincides with the stages of root initiation and tuberization (Connor and Palta, 1981; Aina *et al.*, 2007). The effect of moisture stress was more pronounced on root yield parameters than shoot growth, indicating that the mechanisms through which cassava

tolerates drought stress leads to temporary halt in the partitioning of assimilate for root bulking and thus adversely affecting economic yield. Though cassava can survive conditions of moisture stress where other crops could not (Fukai and Hammer, 1987), unfavourable moisture conditions adversely affect its economic yield.

The above ground biomass (AGB) production is important in the production of animal feed and planting materials. AGB is especially important in areas where animal feed supply is critical during the dry season to supply forage and where planting materials are scarce. Moisture stress in this study reduced AGB by 13.72% relative to normal rainfall environment. Different reports indicate similar trend of the effect of water stress on AGB. Okogbenin *et al.* (2003) reported a decline of 37% in shoot growth in Nigeria. There was no G x L effect on AGB suggesting that the trait is mostly affected by moisture at the test locations. Previously, work by Connor et al. (1981) suggests that vigorous genotypes produce better under stress than less vigorous types. In this study, AGB could have been underestimated because fallen leaves were not included in its determination as it was difficult to determine which leaves fell from which plant/genotype in the field trial.

The average DMC was highest at Kabanyolo with mean of 40.52%. Dry matter production and partitioning is an important root yield parameter in cassava and thus considered to be an important selection criterion in breeding programmes for enhanced yield (Lahai and Ekanayake 2009; Aigbe and Remison 2010b). Total dry matter production provides a good estimate of the degree of adaptation of a genotype to the environment in which it is grown (Kamara *et al.*, 2003). In cassava, foliage and storage roots develop simultaneously (Cock, 1984; Mutegi-Murori, 2009). This creates competition for photosynthetic assimilates between roots and foliage. For this reason, appropriate partitioning of dry matter is important. An ideal cultivar would be one that has rapid shoot growth to ensure full photosynthetic structures before storage root initiation. This would reduce internal competition for assimilates. DMC values obtained in this study are within the range that has been reported elsewhere. Both Raji *et al.* (2007) and Akinwale *et al.* (2009), reported average DMC ranging from 30-39% using Nigerian germplasm. Westby (2002) reported that DMC in cassava varies from 20-45% depending on variety and environmental conditions and health of the plant. The magnitude of genotype-by-location interaction was much smaller than that of the genotype effect suggesting that genetic effects override environmental effects in influencing DMC partitioning.

Dry matter content was high in storage roots of MM96/0686, NASE2, Nyamutukura and MH97/2961, suggesting higher root shoot ratio for the genotypes. These genotypes also recorded high fresh root yields. Thus more photosynthate was preferentially allocated to storage roots of MH96/0686, NASE2, Nyamutukura and MH97/2961 to produce high root yields in all locations. Similar results have been reported by Lahai *et al.* (1999) who found higher root yields for cultivars that allocated higher proportion of DMC to storage roots than those that accumulated higher DMC in stems. In general, moisture stress reduced DMC by 16.58%. Similar reports on the effects of water stress on DMC are available. Bakayoko *et al.* (2009) reported that percentage root dry matter content is high when the water stress does not exceed one month's period in the first 6 months of planting indicating the importance of rainfall especially, during the early development stage. Lahai and Ekanayake (2009) reported that drought stress reduced dry matter allocation to storage roots and increased its partitioning to rootstocks, fibrous roots and stems.

Just like for DMC, similar reductions have been observed for cassava starch content following drought stress. Starch content in cassava roots is important especially for cassava processed products such as starch flour and powder (Aigbe and Remison 2010a). Starch is also an important raw material for a number of industries including textiles, paper, adhesives, pharmaceuticals and food (Aigbe and Remison 2010a). It has been postulated that water stress diminishes photosynthetic carbon fixation (Okogbenin *et al.*, 2011) and this may affect starch partitioning in storage roots. It is also probable that the starch reserves in storage roots could be depleted through respiration during prolonged water stress.

Water stress resulted in 25.71% decline in LR relative to that achieved at reference Kabanyolo. This is attributed to shedding of leaves by plants to minimise water loss through transpiration. Rivero *et al.* (2007) reported that drought accelerates leaf senescence, leading to a decrease in canopy size, loss in photosynthesis and reduced yields. Therefore genotypes that retain more leaves and thus high photosynthesis during stress are desirable. Leaf retention has been reported as one of the desired traits in achieving high yields in crops under limited moisture (Lenis *et al.*, 2006). Lenis *et al.* (2006) reported that cassava genotypes with greater leaf longevity produced high total fresh biomass and a 33% higher root dry matter compared to drought susceptible genotypes. Cultivars with high LR or stay green trait are potentially drought tolerant and therefore may be suitable genotypes for marginal areas where rainfall is unreliable. In this study, genotypes;

Nyakakwa, NASE12, TME204, Nyamutukura, TME14, MH97/2961, MM96/0686 and Njule had least reductions in LR across locations and therefore may be putative drought tolerant.

It is desirable to breed and select for high leaf retention when developing genotypes adapted to dry areas. Rivero *et al.* (2007) were able to enhance drought tolerance by delaying drought-induced leaf senescence in tobacco through transformation using *isopentenyl transferase* gene. The suppression of drought-induced leaf senescence resulted in outstanding drought tolerance with vigorous growth and high yield of transgenic plants after a long drought period that killed the control plants. Production of drought-tolerant crops able to grow under restricted water regimes without reduction of yield would minimize drought-related losses and ensure food production in water-limited lands (Rivero *et al.*, 2007).

Drought stress resulted in stunted growth in cassava as estimated by plant height. Relative to reference site (Kabanyolo), plant height was reduced by 16.62% under water stressed environments. This decline is lower than 41% reported by Aina *et al.* (2007) in Nigeria and 62.05% reported by Bergantin *et al.* (2004) in Philippine. Bergantin *et al.* (2004) reported that drought generally renders cassava plants stunted in their height and leaf production when compared with their well-watered counterparts. Pardales and Esquibel (1996) and Agili and Pardales (1999) earlier showed that shoot development in cassava is highly suppressed when juvenile plants are exposed to prolonged limited soil water conditions. However, the relationship between plant height and drought has not been well established.

4.5 CONCLUSIONS AND RECOMMENDATIONS

It is evident from the data in preceding sections, that water stress adversely affects cassava growth and productivity. It is therefore important that cassava breeding efforts be aimed at production of drought tolerant genotypes if cassava cultivation is to be successfully extended to the increasing arid /marginal areas. The study also revealed large genetic variability among cassava genotypes in response to drought. This variability can be effectively utilised in breeding to achieve appreciable progress in production of drought tolerant genotypes. The information generated from this study is important for breeders for selecting parents with traits associated with drought tolerance.

However, before undertaking breeding, it is important that the information on the genetics of the drought associated traits be known. It is recommended that studies to understand genetic inheritance of drought tolerance traits be undertaken. Genotypes MM96/0686, Magana, Yellow, TME14, NASE1, NASE2, TME204, Nyamutukura, MH97/2961 and NASE12 performed better across locations for important drought tolerance traits (HI, LR, FRY and NR) and these are recommended as potential parental lines for hybridization. Harvest index and leaf retention are associated with drought avoidance mechanisms. The farmers' preferred landraces Nyalanda, Icilicili, Bao and Pilipili which are susceptible to drought and would require genetic improvement.

CHAPTER 5: GENETIC DIVERSITY AMONG CASSAVA GENOTYPES IN UGANDA

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5.1 INTRODUCTION

Evolution of cassava as a domesticated crop within Africa is not well documented despite its wide adaptability. There are many reports on landraces of cassava in sub-Saharan Africa but with limited studies on the genetic relatedness between landraces and elite genotypes. Since the introduction of the crop to Africa from Latin America by Arab and European traders, asexual propagation and human selection for resistance and adaptability to biotic and abiotic constraints are expected to have reduced cassava's genetic diversity (Fregene *et al.*, 2003). However, comparable levels of genetic diversity observed in cassava landraces from Africa and several Neo-tropical countries (Brazil, Colombia, Peru, Venezuela, Guatemala, Mexico and Argentina) (Fregene *et al.*, 2003), suggest that further diversification of the crop may have occurred within Africa. Cassava is an out-crossing crop and can result in production of volunteer seedlings which are subjected to natural and/or human selection and thus modifying diversity (Elias *et al.*, 2001b; Kizito *et al.*, 2005; Montero-Rojas *et al.*, 2011). Besides, traditional farming practice, which involves growing several genotypes of the crop in a single field (Ferguson *et al.*, 2011), tends to maintain or even increases genetic diversity by promoting gene flow through hybridisation (Elias *et al.*, 2001b; Resende *et al.*, 2004).

Traditional cassava farmers prefer a diversifying rather than a directional selection. They keep even low yielding or abiotic/biotic stress susceptible genotypes at low frequencies instead of discarding them (Elias *et al.*, 2001b). There is thus high genetic diversity among crop populations maintained on farmers' fields. Human selection of many different genotypes for diverse attributes such as farmer preferred agronomic and quality traits, resistance to pests and diseases, desirable plant architecture and other adaptation characteristics has been a key process in maintaining genetic diversity among cassava landraces (Raji *et al.*, 2007). Many years of farmers' selection results in diverse landraces with genes required for adaptation to biotic and abiotic stresses in higher frequencies. Landraces are therefore valuable for plant breeding because of their co-adapted gene complexes with tolerance to diseases and adaptation to specific ecological conditions. For example, a major dominant gene, *CMD2*, which confers resistance to cassava mosaic disease, has been reported in Nigerian landraces (Akano *et al.*, 2002). African cassava landraces have also been reported to possess farmer preferred agronomic and food quality characteristics that could be utilized for root quality and productivity improvement but their use for cassava improvement has been limited (Raji *et al.*, 2007). Including adapted landraces in

breeding schemes broadens the genetic base of breeding programme and improves chances of producing progeny that meet farmers' needs.

Continuous germplasm exchange between different farmer groups and farmers, through formal and informal distribution channels, makes pedigree information limited and unreliable (Ajmone-Marsan *et al.*, 1992; Schut *et al.*, 1997; Mignouna *et al.*, 1998). It is thus important to assess agro-morphological and genetic diversity of cassava germplasm used by farmers. A prerequisite for genetic improvement of cassava is knowledge of the extent of genetic variation present between cultivars (Beeching *et al.*, 1993; Moyib *et al.*, 2007). Information on genetic diversity guides selection of divergent parents to broaden genetic base of a breeding population and thus leading to production of progeny with heterosis. This is helpful because heterosis is a function of heterozygosity and therefore parental diversity would maximise heterotic combination (Manosh *et al.*, 2008). Positive correlation between genetic diversity and heterosis, for seed yield has been reported in cultivated sunflower (Cheres *et al.*, 2000) and tuber yield in potato (Manosh *et al.*, 2008). Diversity assessment can also lead to discovery of new genotypes and identification of new combinations with maximum genetic variability for further selection and introgression of desirable genes (Mohammadi and Prasanna 2003).

Selectable and neutral genetic markers are commonly used to assess genetic diversity among populations and genotypes. Selectable markers (morphological and agronomical traits) respond to selection pressure and change after several years of natural and/or artificial selection (Yong *et al.*, 2009). On the other hand neutral genetic markers are least subjected to selection pressure and can accurately infer genetic diversity among populations and genotypes (Chakravarthi and Naravaneni, 2006; Raji *et al.*, 2009). Several neutral genetic markers have been used to study genetic diversity in cassava; RFLPs (Beeching *et al.*, 1993), RAPDs (Mignouna and Dixon, 1997; Asante and Offei, 2003), AFLP markers (Fregene *et al.*, 2000; Raji *et al.*, 2009;) and SSR markers (Kawuki *et al.*, 2009, Sree Lehka *et al.*, 2010, Montero-Rojas *et al.*, 2011). However, SSR markers remain competitive because of being multi-allelic, highly polymorphic, co-dominant and highly reproducible and provide rich genetic information with good genome coverage (Kawuki *et al.*, 2009, Sree Lehka *et al.*, 2010). The SSR markers are also affordable and amenable to most breeding procedures and thus applicable in public breeding programs which may not be able to afford expensive diversity assessment techniques. The objective of this study was to assess genetic diversity of cassava landraces and elite genotypes grown in Uganda on farmers' fields using genomic SSR markers.

5.2 MATERIALS AND METHODS

5.2.1 Plant material

A total of 66 genotypes were used for diversity assessment. Fifty four DNA samples were obtained from cassava genotypes (thirty nine landraces and fifteen elite genotypes) planted at Kabanyolo and the rest 12 were accessed at BecA laboratories where they had been preserved at -80°C. The 66 genotypes were grouped according to the regions of Uganda from where they were collected; Central (Luwero, Mukono, Wakiso), Eastern (Busia, Mbale, Palisa, Soroti, Tororo), Northern (Dokolo, Kaberamaido, Lira) and Western (Buliisa, Hoima, Kibale, Masindi) (Table 9).

Table 9: The type and region of origin of cassava genotypes used in genetic diversity assessment

Genotype*	Type	Region	Genotype	Type	Region
Bamunanika	Landrace	Central	TME14	Elite	Eastern
Kabwa	Landrace	Central	TME204	Elite	Eastern
MM96/0686	Elite	Central	Yellow	Landrace	Eastern
MH97/2961	Elite	Central	ApacApac	Landrace	Northern
MM96/4271	Elite	Central	Bao	Landrace	Northern
Mubende	Landrace	Central	Nyaraboke	Landrace	Northern
Muwangazi	Landrace	Central	Bukalasa	Elite	Western
Nakati	Landrace	Central	Deruderu	Landrace	Western
NASE11	Elite	Central	Gwaranda	Landrace	Western
NASE9	Elite	Central	Hoima1	Landrace	Western
Njule	Landrace	Central	Hoima2	Landrace	Western
Ebwantereka	Landrace	Central	Hoima3	Landrace	Western
Akena (192/0067)	Elite	Eastern	Kakumiro	Landrace	Western
Aladoalado	Landrace	Eastern	Kidimo	Landrace	Western
Buganda	Landrace	Eastern	Kwatamumpale	Landrace	Western
Ditu	Landrace	Eastern	Lugbara	Landrace	Western
EgabuK	Landrace	Eastern	Ryahorore	Landrace	Western
EgabuS	Landrace	Eastern	Maburu	Landrace	Western
Icilicili	Landrace	Eastern	Masindi1	Landrace	Western
Luderudu	Landrace	Eastern	Masindi2	Landrace	Western
Magana	Landrace	Eastern	Masindi3	Landrace	Western
Mercury	Landrace	Eastern	Mukalasa	Elite	Western
Mufumbachai	Landrace	Eastern	Nyakakwa	Landrace	Western
Musita	Landrace	Eastern	Nyalanda	Landrace	Western
Nabunanyuza	Landrace	Eastern	Nyamutukura	Landrace	Western
Namukoni	Landrace	Eastern	Nyapamitu	Landrace	Western
Namulalu	Landrace	Eastern	Nyarare	Landrace	Western
NASE1	Elite	Eastern	Rugogoma	Landrace	Western
NASE12	Elite	Eastern	Rujumba	Landrace	Western
NASE3	Elite	Eastern	Rwaburaru	Landrace	Western
Omongole (192/0056)	Elite	Eastern	TimTim	Landrace	Western
Pilipili	Landrace	Eastern	Tongolo	Landrace	Western
Serere	Elite	Eastern	Tongolo2	Landrace	Western

**includes 12 landraces whose DNA had been preserved at BecA.*

5.2.2 DNA extraction

Young fully developed leaves were collected from each genotype planted at Kabanyolo for DNA extraction after four months of field establishment. DNA was extracted using standard procedures according to Dellaporta *et al.* (1983). Briefly, freshly harvested young leaf (0.3 g) of each genotype was ground in liquid nitrogen using a pestle and a mortar. The fine powder was transferred to 1.5 ml eppendorf tube using a frozen spatula. Eight hundred micro litres (800 µl) of preheated (65°C) extraction buffer and 50 µl of 20% SDS solution were added to each tube and the mixture homogenized for 30 seconds by intermittent inversion. The tubes were incubated at 65°C for 15 minutes with intermittent inversions and incubated at room temperature for 5 min. Proteins and polysaccharides were precipitated by adding 250 µl of ice-cold 5M potassium acetate and mixed by inverting the tubes 5-8 times. The tubes were placed on ice for 20 minutes and centrifuged at 13,250 relative centrifugal force (rcf) in eppendorf centrifuge 5418 (Germany) for 10 minutes. The supernatant was transferred to a new eppendorf tube and 500 µl of ice-cold iso-propanol added and mixed by inverting gently 8-10 times to precipitate crude DNA. The mixture was incubated at -20°C for 30 minutes and centrifuged at 13,250 rcf for 10 minutes. The supernatant was poured off and last drops of isopropanol were removed by placing eppendorf face down on paper towels for 30 minutes. After draining off isopropanol, 200 µl low salt TE was added to each sample followed by 3µl RNAse A (10 mg ml-1) (Cat.EN0531 Fermentas) and incubated at 37°C for 30 min. Two hundred micro litres of phenol:chloroform:isoamylalcohol (25:24:1) was added to each sample and inverted twice to mix and centrifuged at 13,250 rcf for 10 minutes. The upper layer was transferred to fresh eppendorf tube and 200 µl chloroform:isoamyl alcohol (24:1) added. The tubes were inverted twice to mix and centrifuged at 13,250 rcf for 10 minutes. The upper aqueous layer (approximately 200 µl) was transferred to fresh tubes.

To purify DNA, 500 µl ethanol: 3 M sodium acetate solution (30:1.5) was added to each sample and incubated for 5 minutes at -20°C. The samples were centrifuged at 13,250 rcf for 5 minutes, the supernatant was decanted and the pellet washed with 200 µl 70% ethanol. The samples were centrifuged at 13,250 rcf for 5 minutes, the supernatant decanted and DNA pellet air dried for 1 hr by placing the tubes face down on paper towels. The pellet was re-suspended in 100 µl low-salt TE buffer and stored at 4°C. The concentration and purity of 66 DNA samples were checked by NanoDrop UV spectrophotometry at A260 and A280, while the integrity of DNA was analysed by

1.5% agarose gel electrophoresis in TBE buffer stained with ethidium bromide (Figure 5). The DNA samples were standardised to 50 ng/µl before PCR analysis.

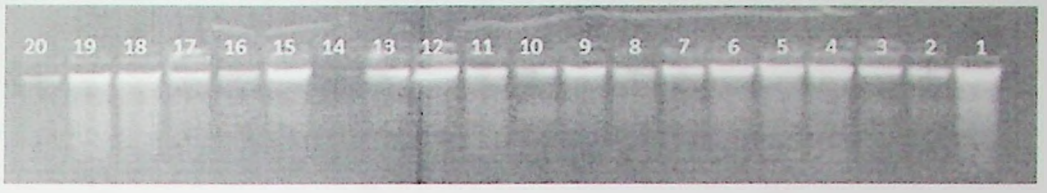

Figure 5: An example of agarose gel electrophoresis to check the DNA integrity for twenty samples

5.2.3 Polymerase chain reaction (PCR)

Polymerase chain reaction was carried out in a GeneAmp® PCR system 9700 thermal Cycler (Applied Biosystems, USA) using 26 primers listed in Table 10. Ten microlitres of PCR reaction mix included 50 ng/µl of DNA; 1X buffer (10 mM Tris-HCL pH 8.0, 1 mM EDTA pH 8.0), 0.2 mM dNTPs, 2 mM MgCl2; 0.08 pmole each of forward and reverse primers and 0.375 µl Taq polymerase (5U/ µl) (BioLabs New England). PCR conditions included an initial denaturation of 3 min at 95 °C, followed by 35 cycles of 30 seconds at 95 °C; 1 min at the annealing temperature defined for each primer used (Table 10) and 1 min at 72 °C. The final extension included 30 min at 72 °C and final hold at 4°C.

Table 10: SSR markers used to assess genetic diversity in 66 cassava genotypes

Name	Type of repeat	Left primer (Forward 5' - 3')	Right primer (Reverse 5' - 3')	Annealing temperature (°C)	Product size
SSRY5	GA(38)	TGATGAAATTCAAAGCACCA	CGCCTACCACTGCCATAAAC	55	173
SSRY9	GT(15)	ACAATTCATCATGAGTCATCAACT	CCGTTATTGTTCCTGGTCCT	55	278
SSRY12	CA(19)	AACTGTCAAACCATTCTACTTGC	GCCAGCAAGGTTTGCTACAT	55	266
SSRY19	CT(8)CA(18)	TGTAAGGCATTCCAAGAATTATCA	TCTCCTGTGAAAAGTGCATGA	55	214
SSRY21	GA(26)	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA	55	192
SSRY38	CA(17)	GGCTGTTCGTGATCCTTATTAAC	GTAGTTGAGAAAACTTTGCATGAG	55	122
SSRY51	CT(11)CG CT(11) CA(18)	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT	55	298
SSRY52	GT(19)	GCCAGCAAGGTTTGCTACAT	AACTGTCAAACCATTCTACTTGC	55	266
SSRY59	CA(20)	GCAATGCAGTGAACCATCTTT	CGTTTGTCCTTTCTGATGTTC	55	158
SSRY63	GA(16)	TCAGAATCATCTACCTTGGCA	AAGACAATCATTTTGTGCTCCA	55	290
SSRY64	CT(13)CG CT(6)	CGACAAGTCGTATATGTAGTATTCACG	GCAGAGGTGGCTAACGAGAC	55	194
SSRY69	CT(18)ATT AT(2) CTTTCTT CTTT(2)CCTTCT	CGATCTCAGTCGATACCCAAG	CACTCCGTTGCAGGCATTA	55	239
SSRY100	CT(17)TT CT(7) CCCT	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	55	210
SSRY102	GT(11)	TTGGCTGCTTTCACTAATGC	TTGAACACGTTGAACAACCA	55	179
SSRY110	GT(12)	TTGAGTGGTGAATGCGAAAG	AGTGCCACCTTGAAAGAGCA	55	247
SSRY135	CT(16)	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	45	253
SSRY147	GA(16)	GTACATCACCACCAACGGGC	AGAGCGGTGGGGCGAAGAGC	45	113
SSRY148	GA(21)	GGCTTCATCATGGAAAAACC	CAATGCTTTACGGAAGAGCC	45	114
SSRY151	GA(126)	AGTGGAAATAAGCCATGTGATG	CCCATAATTGATGCCAGGTT	45	182
SSRY155	GA(136)	CGTTGATAAAGTGGAAAGAGCA	ACTCCACTCCCGATGCTCGC	55	158
SSRY161	CT(11)TT CT(21)CA(19)	AAGGAACACCTCTCCTAGAATCA	CCAGCTGTATGTTGAGTGAGC	55	220
SSRY169	GA(19)A(3)GAA(2)	ACAGCTCTAAAAACTGCAGCC	AACGTAGGCCCTAACTAACCC	55	100
SSRY171	TA(5)CATA GATA(8) GC GA(23)GTGA(2)	ACTGTGCCAAAATAGCCAAATAGT	TCATGAGTGTGGGATGTTTTTATG	55	291
SSRY181	GA(22)G(3)C GA(3) GGAA GA(4)	GGTAGATCTGGATCGAGGAGG	CAATCGAAACCGACGATACA	55	199
SSRY182	CA(17)N(31)GAGG GA(8)	GGAATTCTTTGCTTATGATGCC	TTCCTTTACAATTCTGGACGC	55	253
NS911		TGTTGTTCAGACGATGTCCAA	TTGAAGCAGTTATGAACCGT	50	127

Accessed from IITA-Nairobi, courtesy of Morag Ferguson

The PCR products were checked for amplification on 1.5% agarose gel electrophoresis stained with ethidium bromide in 1X TBE buffer at 80 V for 1 hour and visualised on trans-UV and photographed in UVP DIGIDOC-IT system (UVP BioImaging systems, USA). PCR products with high quality amplifications (high quality single bands) (Figure 6) were subjected to capillary electrophoresis with ABI 3730 DNA genetic analyzer for fragment segregation and allele calls (recorded as peaks) were made using GENEMAPPER software v.3.7 (Applied Biosystems) (Figure 7).

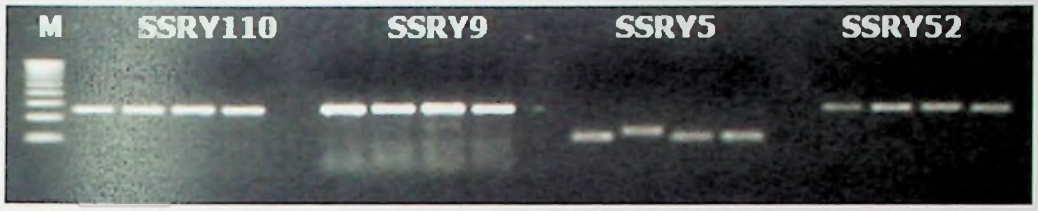

Figure 6: A gel picture showing polymorphism in four cassava genotypes by four SSR markers M=Marker ladder.

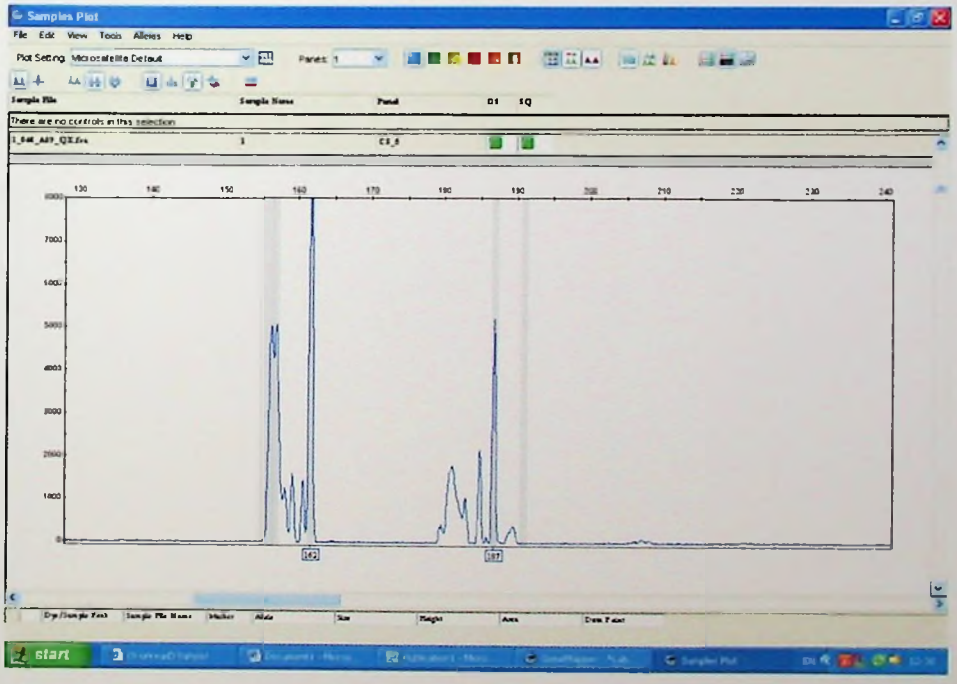

Figure 7: An example Allele peaks generated by GeneMpapper scored for analysis for SSR9. This Figure shows a heterozygous sample with two alleles (162/187)

5.3 DATA ANALYSIS

The genotypic data generated by GeneMapper were analysed for genetic diversity parameters, including number of alleles per locus, allelic frequency, percent of polymorphic loci, observed heterozygosity, genetic differentiation and gene diversity (expected heterozygosity) obtained per locus and per group of genotypes (landraces and elite), using Power marker software (Liu and Muse, 2005), GenAlEx (Peakall and Smouse, 2006) and Darwin (Perrier and Jacquemound-Collet, 2006). Cluster analysis for the genotypes was performed on the similarity matrix using the neighbour-joining algorithm (Nei, 1973) and the results displayed as a dendrogram.

5.4 RESULTS

Genetic diversity parameters were assessed with 26 SSR markers across all the cassava genotypes (landraces and elite genotypes n=66) and the results are presented in Table 11. All the SSR markers were highly polymorphic as indicated by the PIC values. A total of 154 polymorphic alleles across the groups and regions were observed. The number of alleles across loci and groups ranged between three and 11 with average number of alleles of 5.9. SSRY100 marker recorded the highest number of alleles (11) followed by SSRY69 with 10 alleles (Table 11). The number of alleles in landraces ranged from 2 alleles in SSRY155 to 10 alleles with SSRY100 and SSRY69 with mean number of alleles across all loci of 5.8 (Table 13). In elite genotypes, the number of alleles ranged between 2 alleles (SSRY147 and SSRY5) and 8 alleles (SSRY64 and SSRY100) with average number of alleles across loci of 4.5.

Table 11: Genetic diversity parameters averaged across all groups and loci for 66 genotypes

Marker	# of Alleles	#Unique alleles*	% alleles unique	He	Ho	PIC	F	Fst
SSRY9	7	1	0.143	0.649	0.606	0.620	0.074	0.007
SSRY102	3	0	0.000	0.574	0.424	0.484	0.268	0.076
SSRY169	6	2	0.333	0.626	0.364	0.591	0.425	0.011
SSRY51	4	0	0.000	0.740	0.485	0.691	0.351	0.025
SSRY64	9	1	0.111	0.842	0.909	0.822	-0.072	0.018
SSRY135	8	2	0.250	0.766	0.803	0.727	-0.041	0.018
SSRY148	6	2	0.333	0.717	0.985	0.667	-0.367	0.007
SSRY63	5	1	0.200	0.594	0.516	0.531	0.140	0.035
SSRY182	9	4	0.444	0.653	0.576	0.597	0.126	0.058
SSRY19	9	3	0.333	0.726	0.727	0.697	0.005	0.008
SSRY69	10	4	0.400	0.803	0.818	0.775	-0.011	0.041
NS911	5	0	0.000	0.664	0.846	0.623	-0.268	0.008
SSRY161	6	1	0.167	0.761	0.708	0.723	0.077	0.025
SSRY110	7	0	0.000	0.612	0.848	0.565	-0.379	0.030
SSRY52	4	0	0.000	0.574	0.600	0.520	-0.037	0.018
SSRY151	4	1	0.250	0.631	0.982	0.560	-0.550	0.020
SSRY155	3	0	0.000	0.477	0.615	0.377	-0.284	0.105
SSRY12	5	1	0.200	0.640	0.769	0.597	-0.195	0.013
SSRY21	7	3	0.429	0.778	0.937	0.743	-0.196	0.017
SSRY38	5	1	0.200	0.715	0.906	0.663	-0.260	0.002
SSRY147	3	1	0.333	0.507	0.970	0.386	-0.912	0.001
SSRY5	3	1	0.333	0.544	0.952	0.439	-0.745	0.002
SSRY181	5	1	0.200	0.684	0.800	0.627	-0.162	0.023
SSRY100	11	3	0.273	0.805	0.515	0.781	0.367	0.035
SSRY171	4	1	0.250	0.673	0.953	0.604	-0.409	0.003
SSRY59	6	3	0.500	0.575	0.273	0.484	0.531	0.046
Mean	**5.923**	**1.423**	**0.240**	**0.667**	**0.726**	**0.611**	**-0.082**	**0.025**

He=expected heterozygosity, Ho=observed heterozygosity, Unique alleles=Alleles only in landraces*

Of the 154 alleles revealed by 26 SSR markers across loci and groups, 41 (26.6%) were unique alleles either occurring only in landraces or in elite genotypes. Thirty seven (90.2%) of the unique alleles occurred only in landraces (accounting for 24% of total alleles) and four (9.8%) occurred

only in elite genotypes. SSRY182 and SSRY69 recorded the highest number (4) of unique alleles. Most of these unique alleles were 'rare' with allele frequencies of less than 0.05 (Table 12). Genotypes from northern Uganda had no unique alleles, while genotypes collected from eastern Uganda had higher number of unique alleles not presented in genotypes from other regions (Table 12), but these differences are most likely a reflection of sample size than inherently more unique genotypes.

The average expected heterozygosity (gene diversity) (H_e) across all the groups and loci ranged from 0.477 in SSRY155 to 0.842 in SSRY64 with an average of 0.667, while observed heterozygosity ranged from 0.273 to 0.985 in SSRY59 and SSRY148, respectively, with an average of 0.726. Polymorphic information content (PIC) of loci across the groups was highest in SSRY64 (0.822) and lowest in SSRY155 (0.377) with average of 0.611(Table11).

Gene diversity among landraces was high ranging from 0.490 (SSRY155) to 0.834 (SSRY64) with a mean of 0.661. Landraces also had higher observed heterozygosity, ranging between 0.294 in SSRY59 and 0.980 in SSRY148, with mean of 0.729, and higher PIC ranging between 0.370 in SSRY155 and 0.813 in SSRY64 with mean 0.604. Among elite genotypes, gene diversity ranged from 0.291 (SSRY155) to 0.824 (SSRY64) with average of 0.637 while observed heterozygosity ranged from 0.2 in SSRY59 to 1.0 in SSRY148, SSRY151 and SSR5 with average of 0.720. The average PIC in elite genotypes was 0.585 (Table 13).

Table 12: Allele frequencies of unique alleles by group (elite and landrace), and regions in Uganda from where the genotypes were collected

Unique alleles by group				Unique alleles by regions				
Marker	Allele	Elite (n=15)	Landrace (n=51)	Marker	Allele	Central (n=12)	Eastern (n=24)	Western (n=27)
SSRY135	258	0.067		SSRY64	189	0.042		
SSRY182	229	0.067		SSRY135	258	0.083		
SSRY155	153	0.067		SSRY182	219	0.042		
SSRY100	241	0.033		SSRY38	72	0.042		
SSRY9	261		0.029	SSRY9	258		0.104	
SSRY169	83		0.088	SSRY135	247		0.021	
SSRY169	99		0.020	SSRY148	111		0.021	
SSRY64	189		0.010	SSRY182	220		0.021	
SSRY135	247		0.010	SSRY19	208		0.021	
SSRY135	255		0.010	SSRY69	220		0.021	
SSRY148	109		0.020	SSRY69	230		0.021	
SSRY148	111		0.010	SSRY110	243		0.063	
SSRY63	282		0.010	SSRY151	216		0.031	
SSRY182	219		0.010	SSRY155	153		0.042	
SSRY182	220		0.010	SSRY21	187		0.065	
SSRY182	226		0.049	SSRY100	218		0.083	
SSRY182	234		0.020	SSRY100	241		0.021	
SSRY19	208		0.010	SSRY59	156		0.021	
SSRY19	213		0.020	SSRY59	163		0.021	
SSRY19	214		0.010	SSRY135	255			0.019
SSRY69	214		0.010	SSRY148	109			0.037
SSRY69	220		0.010	SSRY63	282			0.019
SSRY69	230		0.010	SSRY182	234			0.037
SSRY69	238		0.010	SSRY19	214			0.019
SSRY161	216		0.020	SSRY69	214			0.019
SSRY151	216		0.011	SSRY69	238			0.019
SSRY12	263		0.100	SSRY161	216			0.038
SSRY21	187		0.031	SSRY110	251			0.037
SSRY21	191		0.021	SSRY147	111			0.019
SSRY21	193		0.052	SSRY181	188			0.037
SSRY38	72		0.010	SSRY100	237			0.037
SSRY147	111		0.010	SSRY171	289			0.037
SSRY5	105		0.057	SSRY59	142			0.019
SSRY181	188		0.020					
SSRY100	185		0.029					
SSRY100	202		0.039					
SSRY100	218		0.039					
SSRY171	289		0.020					
SSRY59	142		0.010					
SSRY59	156		0.010					
SSRY59	163		0.010					
Total alleles		4	37			4	15	14

Central=Luwero, Mukono, Wakiso, Eastern =Busia, Mbale, Palisa, Soroti, Tororo, Northern =Dokolo, Kaberamaido, Lira and Western =Buliisa, Hoima, Kibale, Masindi districts; Unique alleles=alleles exclusively present one group

Table 13: Comparison of genetic diversity parameters averaged across loci and individuals within group and region

Group /region	Number of Alleles	Gene Diversity (He)	Heterozygosity (Ho)	PIC
Group				
Landrace (n=51)	5.769	0.661	0.729	0.604
Elite (n=15)	4.500	0.637	0.720	0.585
Region				
Central (n=12)	4.423	0.641	0.703	0.582
Eastern (n=24)	5.038	0.668	0.714	0.615
Northern (n=3)	3.038	0.571	0.782	0.503
Western (n=27)	5.038	0.653	0.740	0.593
Across groups/regions (n=66)	5.923	0.667	0.726	0.611

Gene diversity was high in genotypes collected from Eastern Uganda and was least in genotypes collected from Northern Uganda. The genetic differentiation between the two groups (landraces and elite) as estimated by *Fst* (theta) averaged over all loci, was low (mean=0.025). For regions, the *Fst* values between genotypes collected from Central and Eastern regions was 0.012, Central and Northern was 0.048, Central and Western 0.012, Eastern and Northern 0.045, Eastern and Western 0.013, and between Northern and Western *Fst* was 0.035. The sample sizes used for different groups and regions were different and this could have affected the results.

Principal coordinate analysis (PCoA) was calculated from dissimilarity coefficients and is graphically presented in Figure 8. The coordinates were calculated for the two first axes with positive Eigen values. The two axes accounted for 43.62% of the total variation with the first axis (PCoA1) accounting for 23.56% and second (PCoA2) accounting for 20.06%. PoCA1 roughly separated landraces from elites with only Mukalasa, NASE1 and Bukalasa being on the side of landraces. The PCoA2 put 12 (80%) of elite genotypes on the upper cluster. The three elite genotypes (Akena, NASE11 and Mukalasa) that clustered with landraces in lower cluster may indicate that these genotypes may have similar allele frequencies as landraces in the lower cluster. The PCoA however, showed loose clustering of both the elite and landraces because elite genotypes clustered together with some landraces indicating that they share some alleles.

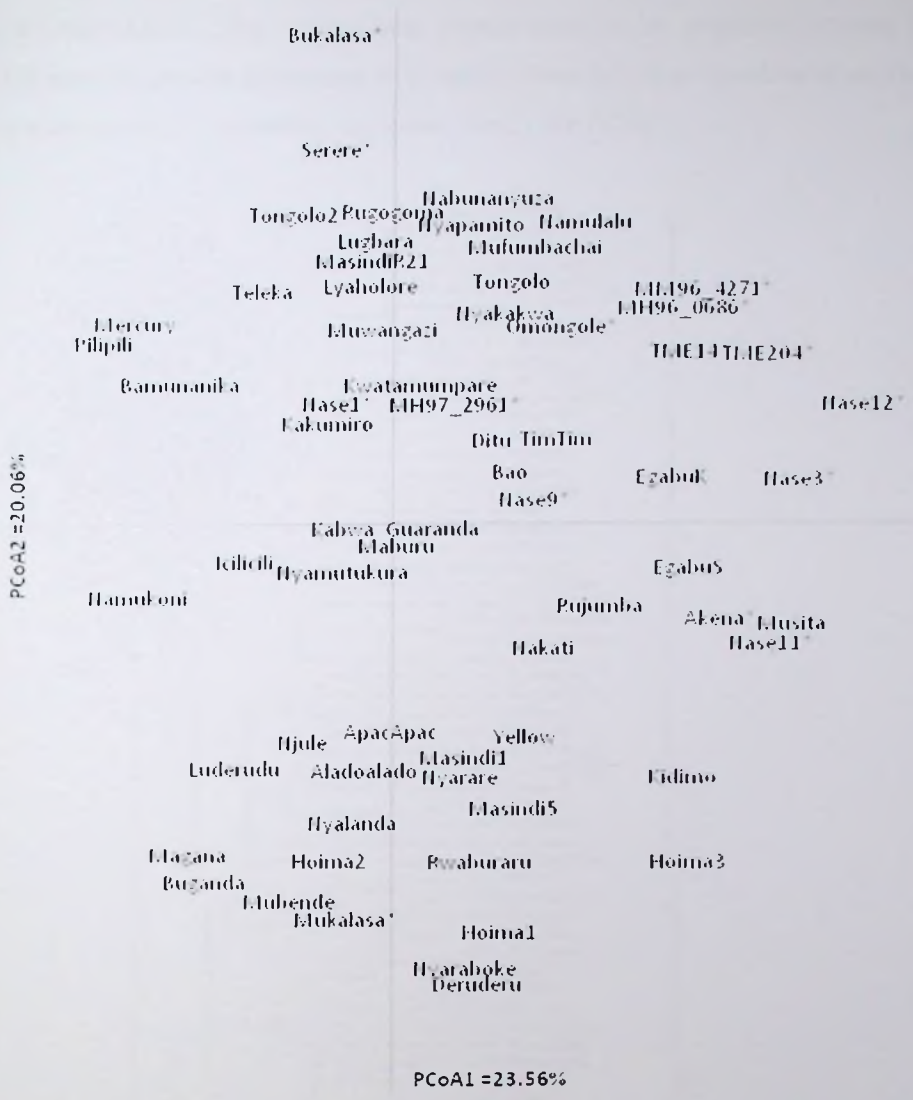

Figure 8: Principal coordinates analysis from dissimilarity coefficients of 66 cassava genotypes. Elite genotypes are marked with Asterisks

The dendrogram was constructed using the neighbour joining method (NJ) (Nei, 1973) and separated the 66 genotypes into four major clusters (Fig. 9). All elite genotypes were included in cluster 1 (C1). The clusters C2, C3 and C4 contained only landraces. Clustering of genotypes did not reflect regions from where they were collected neither did it discriminate the elite genotypes from landraces. The dendrogram showed strong relationship between Nyarare and unknown Masindi1 (which were actually duplicates), Ditu and TimTim, Nyalanda and Icilicili, and Aladoalado and Njule. The dissimilarity coefficients ranged from 0.029 in Masindi3/Lugbara to

0.598 in Icilicil/NASE11. The results from cluster analysis by neighbour joining method (NJ) (Nei, 1973) were in general agreement with results from principal coordinates analysis at a level consistent with percent of variability accounted for by the PCoA.

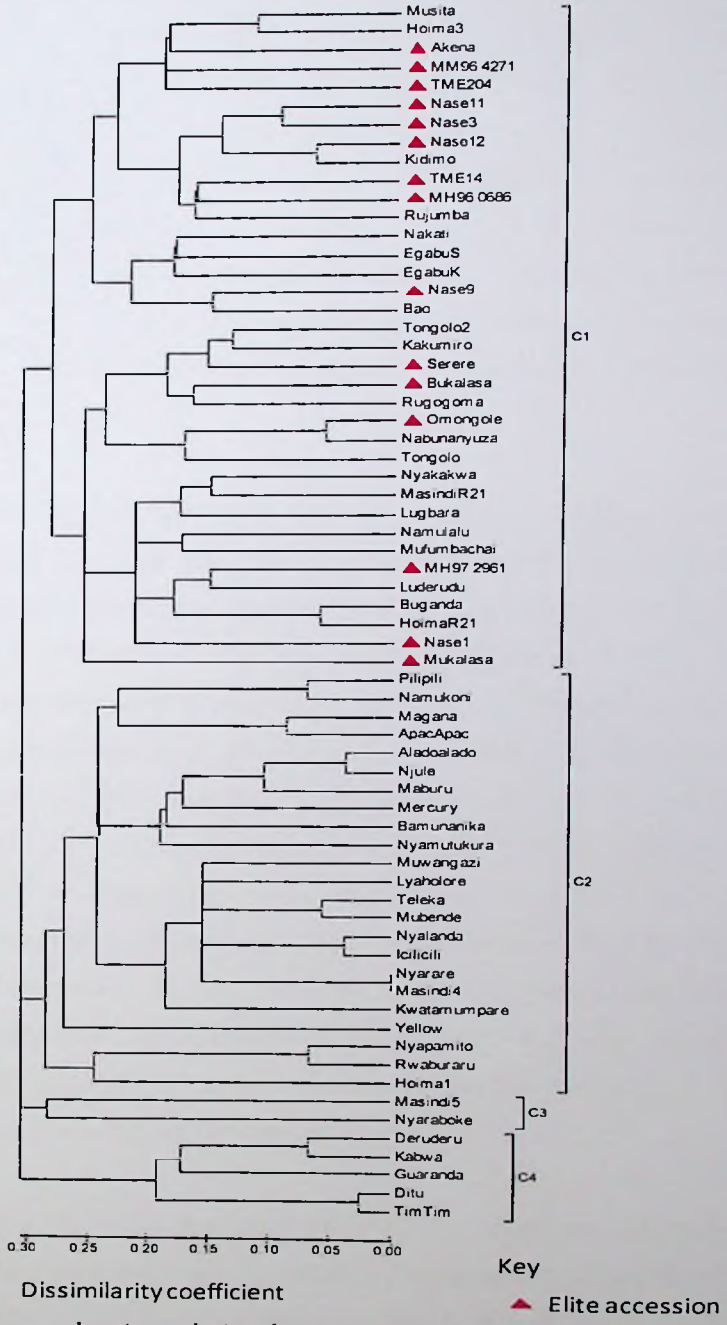

Figure 9: Dendrogram showing relationships among cassava genotypes

5.5 DISCUSSION

Genetic diversity and differentiation among Ugandan landraces and elite cassava genotypes was assessed with 26 SSR markers. Twenty six SSR markers are considered adequate to give reliable results on genetic diversity among and within populations of cassava (Fregene *et al.*, 2003). Kalinowski (2005) demonstrated that reliable estimates of genetic diversity can be obtained from less than 20 individuals per population with 16 polymorphic loci. Nei (1978) had earlier indicated that the reliability of genetic diversity results depends more on the number of loci than on the number of individuals sampled. Moyib *et al.* (2007) obtained comparable results when using between five and 16 polymorphic SSR markers with Nigerian cultivated cassava suggesting that application of few number of polymorphic SSR markers is possible for genetic variation studies in cassava. However in this study, the estimates of genetic differentiation varied widely at different loci (ranging between 0.01 and 0.105) suggesting that assessment of SSR diversity may require more than 16 SSR markers.

The SSR markers showed high mean PIC (61.1%) demonstrating their ability to discriminate between individual genotypes. The higher the PIC of the marker, the more informative the marker is. In both landraces and elite genotypes, SSRY64 was the most informative marker (PIC = 82.2%) while SSRY155 (PIC = 37.7%) was the least informative. The PIC obtained in this study is within the range of the previous studies in cassava using SSR markers. Sree Lekha *et al.* (2010) obtained an extremely high mean percentage polymorphism (88.89%) among the Indian cassava while Adebola *et al.* (2009) reported slightly lower mean PIC (55%) with genotypes collected from different African countries. Kawuki *et al.* (2009) reported PIC ranging from 35.8% to 75.9% with average of 57.1% when using cassava germplasm from Africa, Asia and America. The high levels of PIC obtained in these studies suggest that polymorphic SSR markers in cassava are generally very informative. The SSR markers provide greater discrimination among genotypes making them appropriate for diversity assessments. The high PIC average value of SSRs is derived from their multi-allelism, rapid mutation rate and low probability of being affected by the narrowing influence of selection (Kawuki *et al.*, 2009).

Cassava genotypes showed high mean number (5.923) alleles per polymorphic locus across the genotypes. The high number of alleles obtained is in agreement with a recent study conducted on cassava in Puerto Rico by Montero-Rojas *et al.* (2011) who found an average of 7.15 alleles per locus ranging between 2 and 14 alleles per locus. Fregene *et al.* (2003) reported average number

of alleles of 6.0 for cassava landraces from Colombia and 5.2 for Brazilian cassava. Kawuki *et al.* (2009), working with three different populations, from Asia, Africa and American cassava reported number of alleles ranging between three and eleven. Similarly, Kizito *et al.* (2005) using landraces from Ugandan reported average of alleles of 5.229 per SSR locus. Therefore, the number of alleles among cassava genotypes from Uganda is comparable to that from Puerto Rico, Asia, Colombia and Brazil.

Both expected (H_e) and observed heterozygosity (H_o) averaged across the landraces and elite genotypes were high, 0.667 and 0.726, respectively. The expected heterozygosity (H_e) indicates that the probability that two randomly selected alleles from a genotype of germplasm in Uganda are different is 66.7%. Expected heterozygosity also known as "gene diversity" was introduced by Nei (1978) to explain the probability that two alleles arbitrarily selected are different. Similar high observed and expected heterozygosity in cassava has been reported. Raji *et al.* (2009) using cassava germplasm from various countries in Africa reported average values of H_e and H_o of 0.630 and 0.730, respectively. Montero-Rojas *et al.* (2011) reported H_e of 0.709 and H_o of 0.671 with Puerto Rico cassava germplasm. However, the average values of H_e obtained in this study were higher than obtained by Kizito *et al.* (2005) of 0.532 among Ugandan landraces and Kawuki *et al.* (2009) of 0.553, 0.556 and 0.615 for Asian, African and American cassava germplasm, respectively. These marginal differences are most likely due to differences in genotypes assayed in the studies. Cassava is clonally propagated by cuttings, and this together with human selection for varied traits and environment over time, is expected to have reduced its genetic diversity (Fregene *et al.*, 2003). However, the high diversity obtained in this study is an indication that genetic diversity and diversification of the crop has been maintained. The out-crossing and heterozygous nature of cassava combined with the use of volunteer seedlings as new genotypes maintains considerable genetic diversity in cassava (Fregene *et al.*, 2003; Raji *et al.*, 2009). High genetic diversity is desirable because it increases fitness and therefore, reduces the likelihood of local extinction (Futuyma, 2005).

Heterozygosity was slightly higher in landraces than elite genotypes. Similarly, the number of alleles in landraces was higher than in elite genotypes. High heterozygosity means high genetic variability suggesting that the landraces were slightly more variable than the elite germplasm. The relatively lower genetic variability within the elite cultivars is a direct reflection of sharing of most of the alleles among themselves suggesting comparatively narrow genetic base. This may be

attributed to limited number of germplasm resources available to cassava breeders during the initial development of elite cultivars. In addition breeding preferences for selected traits of economic importance and the vegetative propagation nature of cassava (Raji *et al.*, 2009) may be responsible for low genetic diversity observed in elite genotypes. The high genetic diversity among landraces can be attributed to spontaneous recombination and farmer selection from volunteer seedlings as new genotypes, a practice common in the traditional agricultural practice of slash and burn (Fregene *et al.*, 2003; Kizito *et al.*, 2005). Genetic diversity among landraces in a particular geographical region is also affected by different cultural traditions, inter-ethnic contacts and economic pressures (Mignouna and Dixon, 1997; Emperaire *et al.*, 2001; Raji *et al.*, 2007). The differences in heterozygosity obtained between landraces and elite groups may also have resulted from usage of different sample sizes (n) for different groups. For example, 15 samples were used for elite and 51 samples for landrace. Nevertheless, since many alleles were unique to the landraces, breeding programmes can benefit from including these genetic diverse landraces in breeding programmes to broaden or widen genetic base.

The genetic differentiation between landraces and elite cassava genotypes estimated by *Fst* averaged across all loci, was generally low (*Fst*= 0.025). Genetic differentiation is estimated from the formula; $F_{ST} = F_{IT}-F_{IS}/(1-F_{IS})$; Where F_{ST} is a measure of the genetic differentiation over subpopulations (always positive), F_{IS} is measure of the deviation from Hardy-Weinberg equilibrium within subpopulations and F_{IT} is a measure of the deviation from Hardy-Weinberg equilibrium in the total population (Wright, 1978). The F_{ST} values ranging from 0-0.05 reveal low genetic differentiation, 0.05-0.15 moderate genetic differentiation, 0.15-0.25 high genetic differentiation, 0.25 or more-highest levels of genetic differentiation i.e. the populations are independent. The low overall genetic differentiation may indicate that there is some random mating and interbreeding among the groups or at least among their ancestors. Likewise, the negative coefficient of inbreeding obtained in this study is an indication that there is limited or no inbreeding within landraces and elite genotypes. Assortative mating, which results in excess heterozygosity, could also contribute to the negative inbreeding coefficients within the groups and low genetic differentiation between the groups (Hedrick, 1999).

The *Fst* among genotypes from different regions were also low ranging from 0.048 between genotypes collected from central and northern regions to 0.012 between genotypes collected from central and eastern, and from central and western regions. The low differentiation between

genotypes in central, eastern and western regions may indicate that farmers in these regions frequently exchange genotypes among themselves. The slightly higher difference between the northern region and others could be simply a function of the low number of genotypes from the north, but could also indicate less exchange between it and other regions. Lesser exchange could be attributed to the fact that farmers in northern Uganda prefer growing bitter types of cassava (cassava suitable for processing), while farmers from other regions of Uganda prefer sweet types (cssava types suitable for fresh boil and eat). This differentiation should be confirmed in a further study using a large sample size from the different regions.

The genetic differentiation between genotypes from northern region and central region may suggest that genotypes from these regions could be used as a basis for the developing heterotic pools (Fregene *et al.*, 2003). Heterosis is expressed when there are differences in allele frequencies (genetic diversity) between populations (Falconer and Mackay, 1996). Nevertheless, to confirm heterotic potential, progeny from various crosses need to be evaluated (Hallauer and Miranda, 1988). The study also revealed a wide range of coefficient of genetic similarity from 0.029 to 0.598 indicating that it is possible to identify different parental combinations with maximum genetic variability for introgression of desirable genes from diverse landraces into the available elite genetic base (Smith, 1984; Mohammadi and Prasanna, 2003). Magoon and Krishnan (1977), and Cowen and Frey (1987) reported that parents should be genetically diverse to provide allelic variation that can be used to create new favourable gene combinations and perhaps exploit heterosis.

The SSR markers revealed that thirty (58.8%) of the landraces had 37 unique alleles that were not present among the elite genotypes. The unique alleles that were present only in landraces accounted for 24% of the total alleles across landraces and elite genotypes. This unique pattern of allele frequencies in the landraces justifies their use in cassava improvement and/or germplasm conservation for future use. The unique alleles might be associated with traits in landraces that are not present in elite genotypes. Though not quantified in this study, landraces so often have superior curlinary root qualities as compared to elite cassava genotypes. Nyalanda and Icilicili had the highest number of loci with unique alleles, suggesting that these genotypes may have many unique traits and their origins are different from the rest especially the elite materials. Including landraces with unique alleles in breeding is likely to increase the chances of producing progeny

with farmer preferred traits. Use of landraces to introgress tolerance to cassava mosaic disease has already been reported (Akano *et al.*, 2002).

The clustering revealed duplication among landraces. Farmers in different districts gave different genotypes similar names or vice versa (two different genotypes were given the same name i.e. Tongolo and Egabu). The unintended misnaming by farmers is due to morphological similarities or perceived differences, use and/or trait characteristic. For example the two "Tongolo" genotypes are bitter types and resemble each other in most morphological features except that "Tongolo2" has taller stems than Tongolo. Also in Masindi, a genotype "Ryahorore" locally translated as "taste for yourself" is a name given to the genotype due its good taste. Thus, this name can be applied or referred to more than one variety owing to the good taste. Several authors (Mignouna and Dixon, 1997; Mignouna *et al.*, 1998; Raji *et al.*, 2007) reported that farmers frequently exchange planting materials among themselves over a wide geographic range and that different ethnic groups assign different vernacular names to similar genotypes or similar names to different genotypes.

5.6 CONCLUSIONS AND RECOMMENDATIONS

The study revealed that a number of landraces have unique alleles not represented in elite germplasm used in Ugandan cassava breeding programme. These alleles could be associated with the farmer preferred traits or may suggest that the landraces have genes for adaptation to particular areas. Some landraces such as Magana and Nyamutukura showed drought tolerance in the field (Turyagyenda *et al.*, 2013) and because they have unique alleles, not present in improved genotypes, their inclusion in breeding program would increase chances of producing drought tolerant progeny with farmer preferred traits. Inclusion of such unique landraces in cassava breeding programmes would increase chances of producing farmer preferred and or better adapted cassava genotypes.

Cassava is generally expected to have low genetic diversity because it is clonally propagated by cuttings, and human selection over years for varied traits and environments over time is expected to have reduced cassava's diversity (Fregene *et al.*, 2003). However, the high diversity obtained in this study is an indication that genetic diversity and diversification of the crop has been maintained. As reported elsewhere, (Fregene *et al.*, 2003; Raji *et al.*, 2009), the out-crossing and heterozygous nature of cassava combined with the use of volunteer seedlings as new genotypes could be responsible for maintaining considerable genetic diversity in Ugandan cassava. The high genetic diversity revealed in this study implies a high amount of additive genetic variance upon which progress in plant breeding depends. The study also revealed limited levels of differentiation between genotypes from different regions of the country. Nevertheless, the observed genetic differentiation among genotypes from different regions provides an opportunity for the establishment of heterotic pools within in a breeding programme. The possibility of establishing heterotic pools should be explored further using an increased sample size.

CHAPTER 6: IDENTIFICATION OF CANDIDATE DROUGHT RELATED AND DROUGHT RESPONSIVE GENES IN CASSAVA

Major sections of this Chapter were published in:

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6.1 INTRODUCTION

Drought is one of the major abiotic stresses affecting plant growth and reducing crop productivity and accounts for approximately 70% of the crop yield loss (Bray *et al.*, 2000). The continuous increase of world population, ever increasing deterioration of arable land, scarcity of water, and global climate changes all underscore the importance of developing stress-tolerant crops. Because severe drought has negative effects on crop yield and can results in severe food insecurity, engineering drought resistant crops has become a demanding mission for crop scientists (Benze *et al.*, 2007). This is particularly important for resource-poor farmers who cannot afford irrigation costs. Although conventional breeding for drought tolerance has achieved successes in crops such as maize (Hoisington *et al.*, 1996), rice (Zhang *et al.*, 2006), and wheat (Zhao *et al.*, 2000), a big gap still remains between the current resistance levels and what is needed for most of the major crops such as cassava.

Plants respond to drought by inducing different mechanisms to perceive and transmit the stress signals to cellular machinery which in turn activate adaptive responses, such as accumulation of metabolites and drought related proteins (Thomashow 1999; Xiong *et al.*, 2002; Ramachandra-Reddy *et al.*, 2004). Through high throughput microarray and real time PCR studies, a number of genes that respond to drought stress at the transcriptional level have been reported (Seki *et al.*, 2002; Shinozaki and Yamaguchi- Shinozaki, 2007; Talame *et al.*, 2007; Guo *et al.*, 2009). Some of these genes have been biologically validated and have been found to protect plants from desiccation through stress perception, signal transduction, transcriptional regulatory networks in cellular responses, or tolerance to dehydration (Wang *et al.*, 2005; Umezawa *et al.*, 2006). For example, *Arabidopsis farnesyltransferase* (*ERA1*) regulates ABA sensing and drought tolerance (Wang *et al.*, 2005). Drought stress-induced regulatory and functional genes have been used to improve stress tolerance through genetic engineering. For example *Vigna aconitifolia pyrroline-5-carboxylate synthetase* (P5CS) has been used to engineer drought tolerant rice and *manganese superoxide dismutase* (*MnSOD*) for drought tolerant alfalfa (Bartels and Sunkar, 2005).

Genetic improvement of drought tolerance has been quite slow because the trait is a complex and is influenced by coordinated expression of a network of genes (Thomashow 1999; Xiong *et al.*, 2002) and affected by a large number of environmental, anatomical, physiological, biophysical, biochemical and developmental factors (Soltis and Soltis 2003). Drought tolerance is controlled by multiple genes, making its study more difficult (Vinocur and Altman, 2005). Nevertheless with

rapid development of functional genomics and biotechnology, new opportunities are available to improve the crop's tolerance to drought. Already, several reports suggesting that increased expression of drought stress related genes could improve drought tolerance in crops are available (Xu *et al.*, 1996; Zhang *et al.*, 2001; Garg *et al.*, 2002; Hu *et al.*, 2006).

The reported molecular work on drought tolerance has been done mostly on a non-crop modal plant, *Arabidopsis thaliana*, (Umezawa *et al.*, 2006) and cereals such as maize (Bruce *et al.*, 2002). These studies need to be extended to important crops like cassava. Cassava is a potential food security crop as well as income source for most resource poor households living in marginal areas in tropical countries. However, this crop is affected by drought especially when drought appears in its early stages of growth and development (Pardales *et al.*, 2001; Okogbenin *et al.*, 2003; Bergantin *et al.*, 2004; Perez *et al.*, 2011). Conventional breeding for drought tolerance in cassava has been hindered by genotype by environment (G X E) interaction, long life cycle (Fregene *et al.*, 2001; Ceballos *et al.*, 2004) and limited seed production. Marker sssisted breeding (MAB) offers a possibility of efficiency and precision in introgression of desired traits. However, this requires a deeper understanding of the possible physiological mechanisms available for water stress tolerance and the identification of suitable genes for introgression into cassava genotypes to improve their drought tolerance. In Cassava, information on genes associated with drought tolerance is limited. This study aimed at identifying candidate drought tolerance genes in cassava as a step towards improvement of cassava for drought tolerance through molecular breeding.

6.2 MATERIALS AND METHODS

6.2.1 Treatments

Two cassava genotypes, drought-tolerant MM96/0686 and -susceptible Nyalanda, were used in this study. MH96/0868 is an improved cassava variety obtained from cassava breeding program at NACRRI, Uganda, while Nyalanda is a landrace obtained from farmers' fields in the Masindi district in western Uganda (1° 40' 28" North, 31° 42' 54" East). Previous field studies evaluating 46 cassava genotypes in Uganda, indicated that MM96/0686 was tolerant to drought and had high Harvest Index, Dry matter content, starch content, root yield and leaf retention under water stress compared to other genotypes, while Nyalanda was among the genotypes adversely affected by water stress and was significantly different from MM96/0686 in these phenotypes under drought stress (Chapter 4 of this thesis). Based on the field data, these two genotypes were selected for

detailed gene expression studies that were performed in a glasshouse. The glasshouse conditions during the day were set at 25-30° C, a condition adopted from previous studies (Alfredo and Setter 2004) with night time temperatures typically ranging between 15-20°C, and humidity typically at approximately 50-80%.

Cassava cuttings (30 cm in length) of the two genotypes were grown in 20 litre plastic buckets of similar shape and colour in a randomised complete block design (RCBD) replicated three times. Similar shapes were used to prevent differences in evaporation surfaces and similar colour to prevent colour effects. Before planting, each bucket was filled with 20 kg of sterilised soil (Forest soil: river sand: ballast at 4:2:1 (v/v/v) respectively). One plant cutting was vertically placed in the soil in the middle of each bucket. To identify the effect of water stress on gene expression, plants were exposed to water stress by withholding water. A similar number of plants per genotype were well watered to act as the control. Three plants per genotype were planted for each replication for each treatment, making a total of nine plants per treatment. Before application of treatments, all the plants were watered with one litre of water every two days until 60 days after planting (DAP). After 60 DAP, stress treatment plants were subjected to gradual water stress conditions for a total of 10 days by withholding water to a soil moisture content (SMC) of around 50% for five days and then to about 25% for five days. Gradual moisture stress was applied to mimic natural field drought conditions. Control plants were maintained at SMC of $\geq$75% by applying one litre of water every two days. The soil moisture content was monitored with portable moisture meter (Delta systems, UK).

6.2.2 Assessment of morphological and physiological response to drought stress

During the water stress treatment period, physiological and morphological traits were measured, including leaf wilting, stomatal conductance, leaf relative water content (RWC), leaf retention and plant height. Leaf wilting was scored on scale of 1 to 3 modified from Bettina *et al.* (2007) (1 = no wilting; 2 = minimal wilting, where the plant showed leaf wilting only during hot hours from which the leaves recovered from wilting; and 3= severe wilting, where wilting leaves did not recover from wilting). For easy scoring, scales 2 and 3 of Bettina *et al.* (2007) were combined to indicate minimal wilting, and 4 and 5 to indicate severe wilting; the stress treatment did not go to the level of killing plants, and therefore level 6 of Bettina *et al.* (2007), i.e. death, was eliminated. The method of visual scoring of wilting is flexible as long as specific wilting categories are defined appropriately (Bettina *et al.*, 2007). At 10 days of drought stress, just before leaf sample

collection, measurements were conducted for stomatal conductance on the third fully expanded leaf using AP4 Porometer (Delta-T Devices, England) and RWC on the fourth fully expanded leaf, following the procedure of Degenkolbe *et al.* (2009). Leaf retention was visually estimated according to Lenis *et al.* (2006). Plant height was measured with a tape measure and taken from the base of the plant to its tip. The number of leaves with over 50% functional area was counted at 10 days of stress for both genotypes under drought dtress and control conditions.

6.2.3 Leaf harvesting and RNA extraction

At 10 days of stress, the third fully expanded leaf was separately collected from three plants per genotype from each replication for each treatment. Leaf samples were collected between 12:00-12:30 pm. To avoid taking material from the elongation zone at the base of the leaf blade or senescent tissue at the tip of the leaves, leaf samples were harvested from the middle section of the blades of fully expanded green leaves (Degenkolbe *et al.*, 2009). The leaf samples were collected in eppendorf tubes, immediately put in liquid nitrogen and subsequently stored at -80°C until RNA extraction. Total RNA was extracted from the leaf samples (three biological replicates per genotype per treatment) using Concert Plant RNA Reagent (catalogue number 12322-012; Invitrogen) following manufacturer's protocol. The concentration of RNA from each sample was determined by UV spectrophotometry at A260 using NanoDrop ND-1000 (Nano Drop technologies USA), while the integrity of total RNA was analysed on 1.5% 1x TBE (tris-borate-EDTA) agarose gel electrophoresis (visualized with ethidium bromide) after denaturation in 1x FDE (90% v/v formamide, 10% v/v 10×TBE buffer, 0.5% w/v bromphenol blue, 25 mM EDTA) at 65°C for five minutes and snap cooling on ice (Figure 10).

DNA contamination was removed using RNase-free DNAseI (Fermentas cat. EN0521) following the manufacturer's protocol. From each biological replicate, two reverse transcription reactions (two technical replications) were prepared. One microgram (1 µg) of total RNA was converted to cDNA by GoScript Reverse transcriptase system (Promega, USA) following the manufacturer's instructions with minor modifications mainly in the concentration of the tempelate used. Briefly, 1 µg of total RNA was mixed with 10 pmol random hexamer oligonucleotides and Ribonuclease-free water added to the final volume of 10 µl. The mixture was incubated at 75°C for 5 minutes. Then 1µl of GoScript reverse transcriptase (Promega), 0.5 µl of RNasin RNase inhibitor (Promega), 4 µl of 5x PCR buffer, 1 µl of 10Mm dNTP and 1.2µl (25 mM) $MgCl_2$ were added to the mixture on ice. Ribonuclease-free water was added to the final volume of 20 µl and the

mixture incubated at the annealing temperature of 25°C for 5 minutes. Extension was carried out at 42°C for 60 minutes and the reaction inactivated at 70°C for 15 minutes. For each sample, two control reactions were included throughout this process; one without reverse transcriptase and one without RNA template. The resultant cDNA was stored at -20°C until usage no more than four weeks after cDNA synthesis.

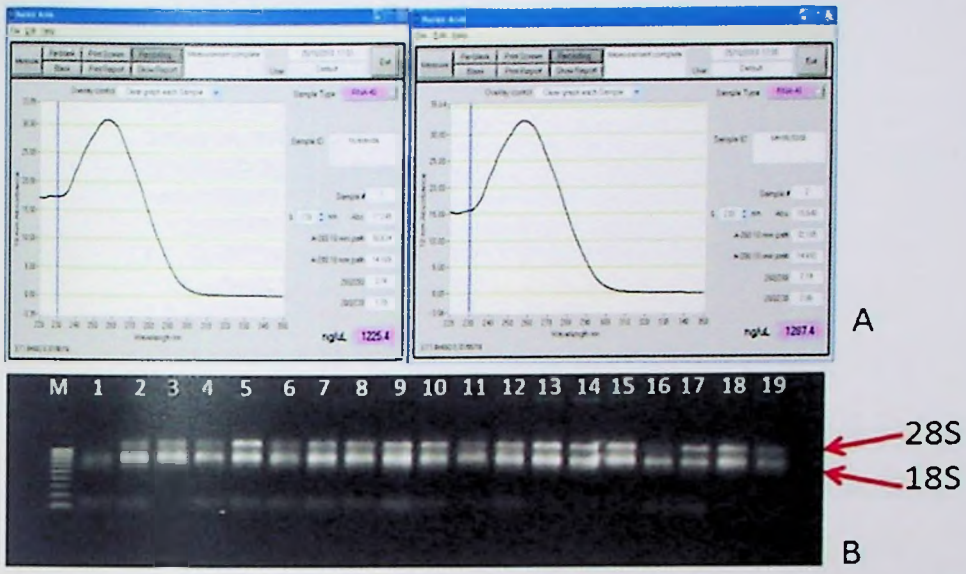

Figure 10: RNA quality check A) with Nanodrop, B) with agarose gel electrophoresis.

Two µg of total RNA from 19 samples (1-19) were run on a 1% denaturing agarose gel (B). The 18S and 28S ribosomal RNA bands were clearly visible in the intact RNA sample after short time run. M= DNA size marker

6.2.4 Gene identification and PCR optimization

A literature survey was conducted to identify genes that have been functionally validated to play roles in drought tolerance in plants. From the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/), cDNA sequences of these genes were obtained and used to identify cassava orthologs through BLAST searches of the cassava genome data base (http://www.phytozome.net). This resulted in identification of gene orthologs in cassava. When the query sequence was highly similar to several cassava genes (i.e., in a gene family) a multiple alignment of the highly similar cassava orthologs was conducted with EBI clustalw2 tool (http://www.ebi.ac.uk/Tools/clustalw2/index.html). Only orthologs with high scores, percentage nucleotide identity to the gene of interest above 60% and high query coverage of the query sequence were considered for clustering. Primers suitable to Real Time PCR (amplifying a 233-

332 bp product) and specific to the gene of interest (i.e., full length coding sequence exhibiting highest similarity to the query sequence; through maximum specificity at 3' end of the primer) were manually designed (Figure 11) to amplify the identified cassava orthologs. The designed primers ranged from between 19-25 nucleotides (Table 14).

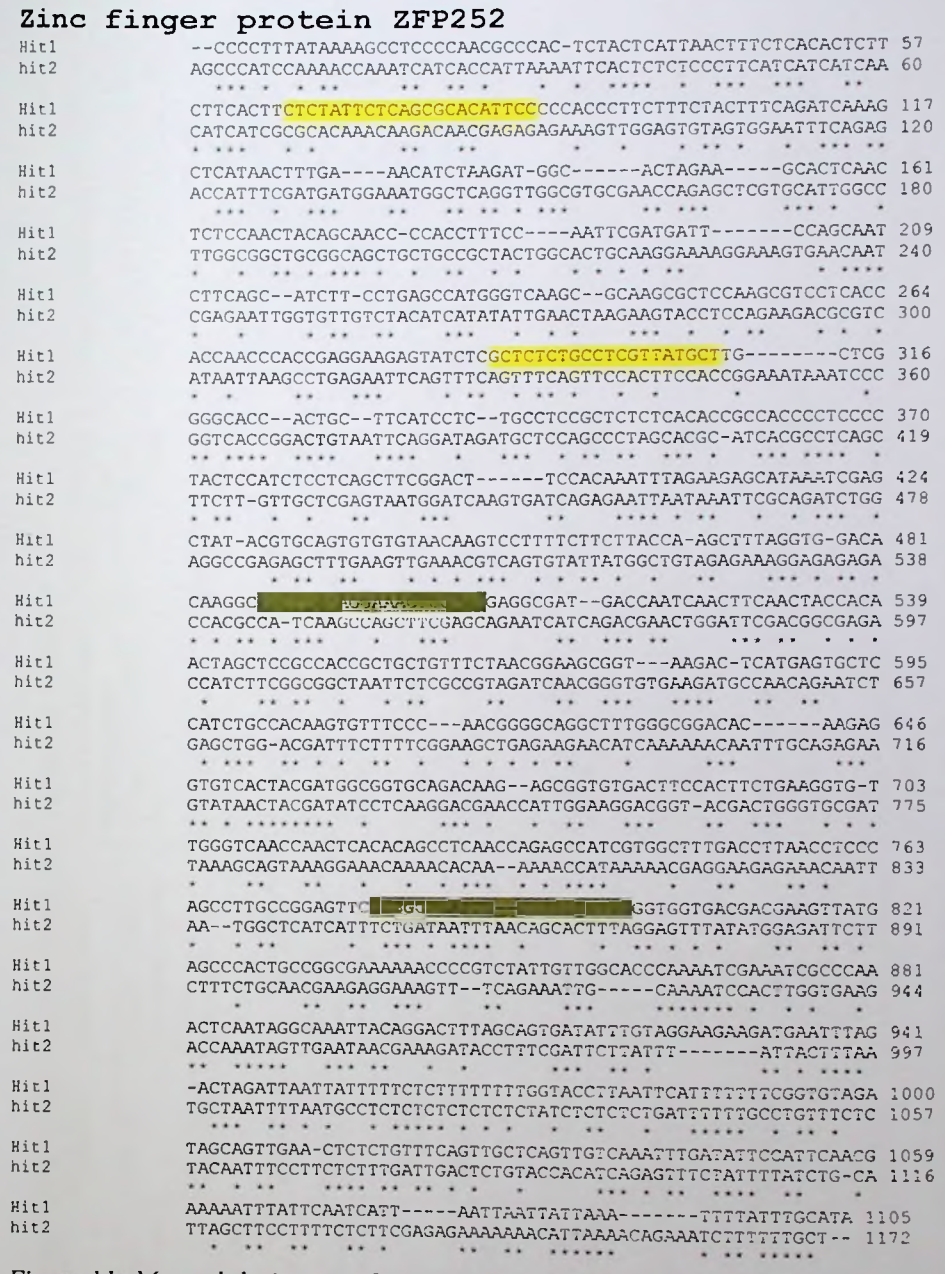

Figure 11: Manual designing of primers after clustalw2 alignment of different cassava orthologs. Designed primers highlighted

Table 14: Primers designed for 10 genes used in gene expression analysis

Gene name	Genotype	Mode of action	*Cassava ortholog used to design primers*	e-value	Primer ID	Primer sequence (5'- 3')	Length	Expected size (bp)
Oryzasativa Japonica Group zinc finger protein ZFP252	AY219847	Osmotic adjustment through proline and sugars	*cassava4.1_014662m.g (MeZFP)*	0	ZFP1F	CTC TAT TCT CAG CGC ACA TTC C	22	245
					ZFP1R	AGC ATA ACG AGG CAG AGA GC	20	
Arabidopsis thaliana amino acid transporter family II protein	NM_129684	Not known	*cassava4.1_007924m.g (MeATTF)*	2.7e-36	ATTF1F	GTG GAA CTT TCT CCT CTC AGC A	22	300
					ATTF1R	GCG TTA AAC TAC ATC CAT GGG C	22	
Arabidopsis thaliana ALDH7B4	NM_104287	Antioxidant/ROS scavenging	*cassava4.1_014540m.g (MeALDH)*	5.9e-43	ALDH1F	GGA TGG AAT GCA TGC ATT GCA CTG	24	263
					ALDH1R	CTG ATT CAC TGT TTG TTG CAC CAT C	25	
Pisumsativum manganese superoxide dismutase	U30841	Antioxidant/ROS scavenging/detoxicati on	*cassava4.1_015272m.g (MeMSD)*	4.8e-40	MSD1F	ATG AAT GCA GAA GGT GCT GCA	21	269
					MSD1R	GAA GGG CAT TCT TTG GCA TAC	21	
Arabidopsis thaliana GER3 (GERMIN 3)	NM_122070	No role	*cassava4.1_016243m.g (MeGE3)*	2.4e-51	GE31F	CGC TTG CAA GAA ACC TGC AG	20	254
					GE31R	TGA ACC CAG CAC AGA TAG AC	20	
Arabidopsis thaliana GBF3 (G-BOX BINDING FACTOR 3);	NM 180118	Transcription factor and regulate alcohol dehydrogenase (Adh) via ABA	*cassava4.1_008459m.g (MeGBF3)*	1.9e-18	GBF32F	TGC ATC AAC TGT TGG GTG CG	20	244
					GBF32R	ACC CAG AGC CAT GAG AAG GCT	21	
Arabidopsis thaliana 14-3-3 protein GF14 lambda (GRF6)	AF145298	Signalling factor/Delay leaf senescence (stay green trait)	*cassava4.1_014556m.g (MeGF14)*	1.3e-104	GF141F	AGC ACG CTT CTC TCT CTC TC	20	261
					GF141R	AGG AAA CGA TCC TCC AAG CG	20	
Arabidopsis thaliana RD28	NM_129274	Turgor responsive /transport of small molecules across membranes	*cassava4.1_013192m.g (MeRD28)*	7.6e-64	RD282F	TGC ACT GCT GGT ATC TCA GG	20	237
					RD282R	GAT CTC AGC TCC CAA TCC AG	20	
Arabidopsis thaliana MYC2	NM_102998	Transcription factor and regulate ABA dependant RD22 and ADH1	*cassava4.1_002918m.g (MeMYC2*	1.1e-40	MYC21F	AGC GTC TCC AGA CCT TGA TC	20	233
					MYC21R	AGT GGG ACC TGA GAT CAG C	19	
Vigna aconitifolia pyrroline-5-carboxylate synthetase	M92276.1	Osmotic adjustment	*cassava4.1_002381m.g (MeP5CS)*	1.4e-78	VAP1F	AGA CGT TAA GCG TAT CGT TG	20	332
					VAP1R	CAA GAA GTT GAG CTG ATG TC	20	

[a]*The cassava orthologs in brackets were assigned gene names starting with "Me" for "Manihot esculenta*

The primers were optimized for target gene specificity with endpoint PCR using cDNA from cassava. The PCR conditions were as follows: initial denaturation at 95°C for 3 minutes; denaturation at 94°C for 30 seconds, annealing at different temperatures ranging from 50-60°C for 1 minute, extension at 72°C for 2 minutes for 35 cycles; with final extension of 72°C for 15 minutes. The PCR products were visualized on 2% 1X TBE agarose gel electrophoresis, with ethidium bromide and run at 90 volts for 45 minutes. All designed primers (100%) amplified cDNA to the expected product with a single band at annealing temperature of 55°C on conventional PCR system and thus this temperature was selected as the appropriate annealing temperature for Real Time PCR.

6.2.5 Real Time PCR

Real time PCR was performed on 7500HT standard Real-Time PCR system (ABI-PRISM®, USA) using SYBR Green JumpStart Taq Ready Mix (Sigma USA). The Real Time PCR was run on three biological replicates for each treatment for each genotype. For every biological replicate, duplicate reactions were run. The 20 µl reaction volume consisted of the following: 10 µl of 2x SYBR Green I ready mix, 0.02 µl of passive reference dye, 1 µl (10 Pmol) each of forward and reverse gene specific primer, 2 µl of temperate cDNA (50 ng) and 5.98 µl water. The PCR conditions were as follows: initial denaturation at 94°C for 2 min; 40 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 1 minute, and extension at 60°C for 30 seconds. The dissociation curve analysis was carried out at the default setting of 7500HT Real-Time PCR system (ABI-PRISM®, USA) to confirm the specificity of each reaction. A subset of the amplification products were also run on a 1X TBE agarose gel, stained with EtBr to ensure that each primer pair had one specific product (Figure 12).

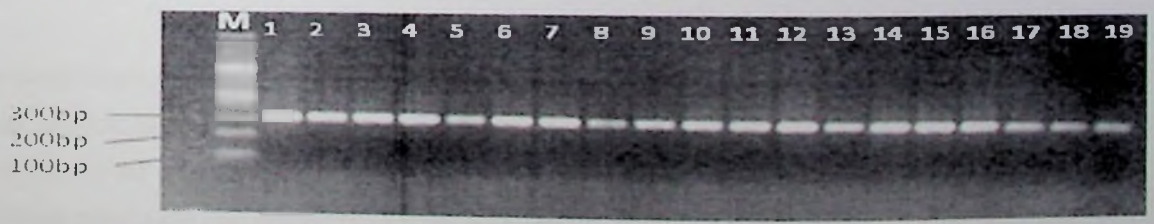

Figure 12: Sample gel picture of independent RT-PCR products to determine specificity of primers.

Specificity of Manihot esculenta amino acid transporter family II protein (MeATTF) primer pairs (expected product size 300 bp). M= DNA ladder, samples 1-19 are cassava cDNA samples

The Real Time PCR reactions were normalised with cassava *actin* gene (F: TGCAGACCGTATGAGCAAG; R: CACCCTTGGAAATCCACATC) as reference (Guo *et al.*, 2009; Yang *et al.*, 2011). The actin gene was evaluated and found to be stably expressed in both well-watered and water-stressed treatments for both genotypes (Figure 13).

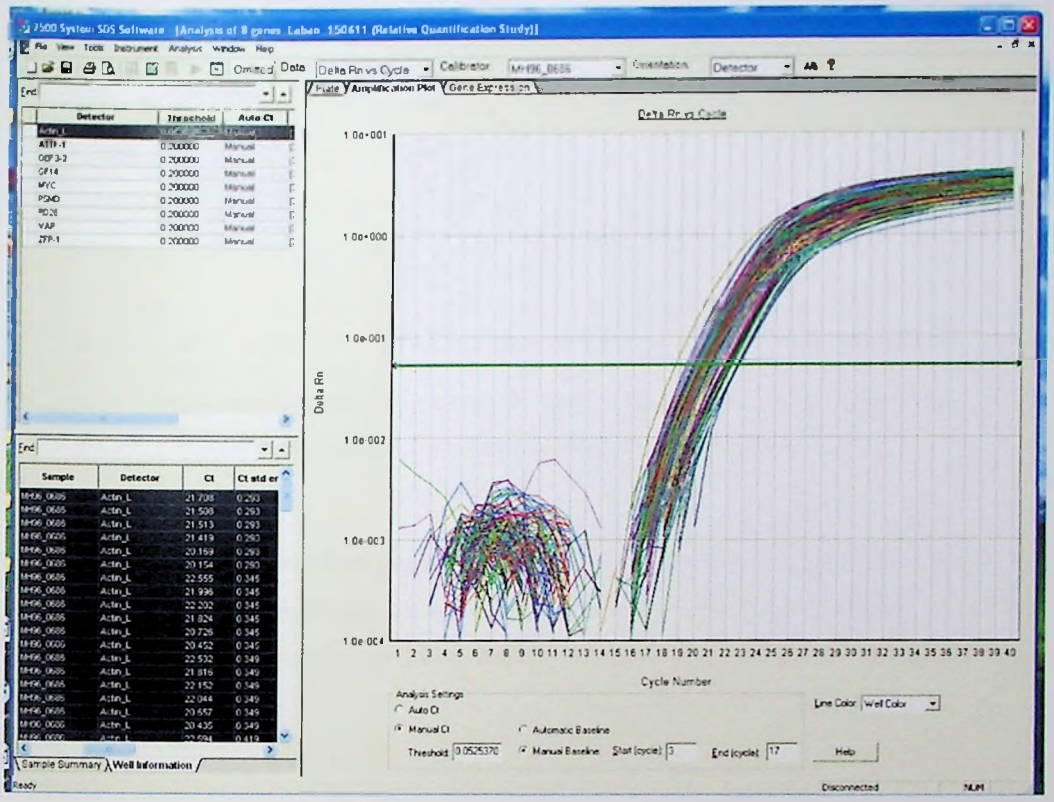

Figure 13: Co-expression of actin gene (reference gene) with other eight target genes for the genotypes under well watered and water stressed conditions

The amplification efficiency of the primers was determined by performing Real Time PCR on serial dilution in the range of 1:2; 1:4, 1:8; 1:16; 1:32 and 1:64 from the pooled cDNA of all samples. All the primers amplified the genes with approximately the same efficiency as that of the reference gene *actin* and ranged from 1.96-2.01 (Figure 14).

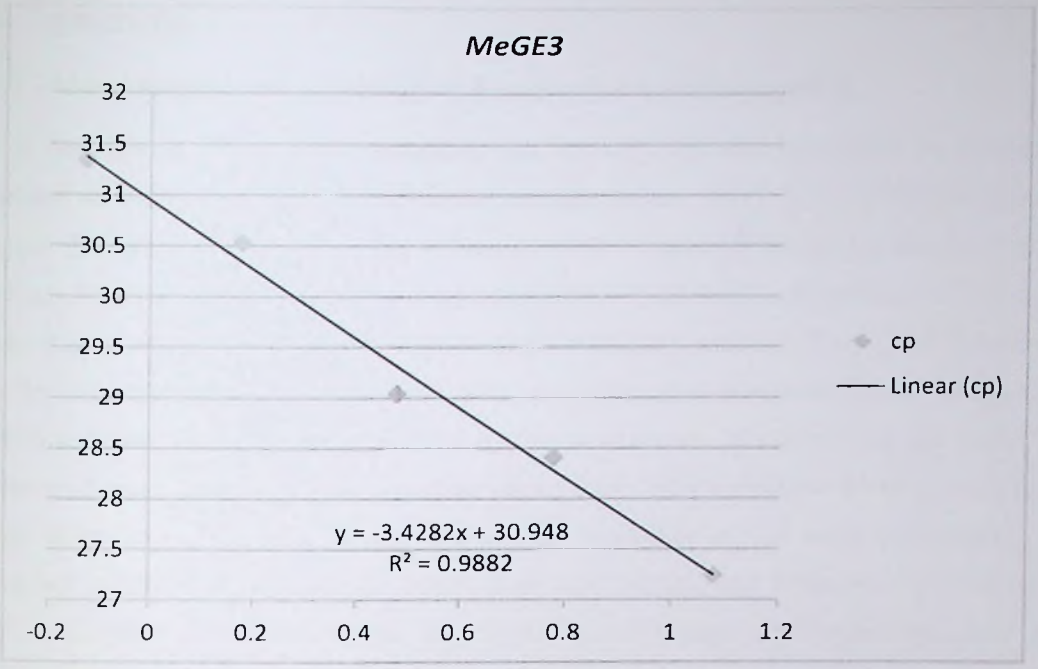

Figure 14: Determining expression efficiency for gene MeGE3 gene, using the formula $E=10^{(-1/slope)}$. *The efficiency of 1.96 was obtained. Cp=crossing point or threshold point*

The ΔΔCT method of relative gene quantification was used to calculate differential expression of genes in the two genotypes under drought-stressed conditions relative to genotypes under control/well-watered conditions using Relative Expression Software Tool (REST) (Pfaffl *et al.*, 2002).

6.3 RESULTS

6.3.1 Morphological and physiological drought stress characteristics

At T_0 (the day at which stress treatment was started), the two genotypes were healthy and exhibited no readily observable symptoms of drought stress. At 10 days of reduced soil moisture content, the two genotypes exhibited different levels of drought stress: the drought susceptible genotype Nyalanda showed severe wilting symptoms on the leaves, including mild chlorosis and death of many of the lower leaves compared to the drought tolerant genotype MM96/0686 (Figure 15). At 10 days of water stress, the mean SMC in stressed plants was significantly lower (28.80 ± 1.08) than in well-watered plants (83.00 ± 2.45). At this time, Nyalanda had lost over 37.8% of its leaves through shedding due to moisture stress, while MM96/0686 retained most of its leaves ("stay green" trait) (Figure 15 and Table 15). Nyalanda leaves were permanently wilted, compared to MM96/0686 genotype where slight leaf wilting was limited to hot hours (~1200-1500 hrs), after which the leaves recovered from wilting. In MM96/0686, the stomata conductance was also more than two times lower than in Nyalanda.

Figure 15: Effect of water stress on tolerant (MM96/0686) and susceptible (Nyalanda) genotypes. In control plants (A) all genotypes showed no wilting symptoms while at 10 days of gradually applied drought stress (B), MM96/0686 (row of pots on the left) was not much affected by water stress, while Nyalanda (row of pots on the right) exhibited severe wilting symptoms.

Table 15: Physiological and morphological response of the two genotypes at 10 days of moisture stress.

*Physiological and morphological drought stress-related traits were measured on three plants per replication for each treatment (stressed and control) at 10 days of water stress (just before leaf sample collection for qRT-PCR). All values are means at P≤0.05. (ns= not significant; **significant at P ≤0.05; SBG= Significance between genotypes (columns); significance between treatments is shown in rows).*

Cultivar	Conductance *mmol $m^{-2}S^{-1}$*		Leaf retention (%)		Relative water content (%)		Number of Leaves		Plant height (cm)^{ns}	
	Control	Stressed	Control	Stressed	Control	Stressed	Control	Stressed	Control	Stressed
MM96/0686	350.0±37.21	168.9±200**	76.88±3.65	76.33±2.33ns	97.3±1.12	95.4±2.92ns	26.78±1.27	24.89±1.50ns	52.22±2.86	52.11±2.64ns
Nyalanda	492.5±43.0	355±21.2**	63.33±4.22	51.25±2.48**	94.95±1.37	81.3±2.92ns	21.50±1.56	13.37±1.59**	48.67±3.51	47.50±2.80ns
SBG	**	**	**	**	*ns*	**	**	**	*ns*	*ns*

6.3.2 Cassava drought stress gene PCR assay validation

The results on gene expression data of the two genotypes in response to moisture stress are presented in Table 16. Of the ten genes previously confirmed to have a functional role in drought stress that were selected for this study, seven are candidate drought response genes in *Arabidopsis thaliana*, one in *Vigna aconitifolia*, one in *Pisum sativum* and one in *Oryza sativa*. The expression of all the ten genes responded to water stress some in both Nyalanda and MM96/0686, while others in one of the two genotypes. Nine of the genes were up-regulated and one gene (*MeGE3*) was down regulated. In Nyalanda, six genes were differentially expressed in response to water stress, five of which (*MeATTF*, *MeGBF3*, *MeGF14*, *MeP5CS* and *MeMYC2*) were up-regulated and one of which was down-regulated (*MeGE3*). Four genes (*MeALDH*, *MeMSD*, *MeRD28* and *MeZFP*) were not differentially expressed in response to moisture stress in Nyalanda. For the drought tolerant genotype, MM96/0686, seven genes were differentially expressed of which six genes (*MeALDH*, *MeATTF*, *MeGBF3*, *MeMSD*, *MeRD28* and *MeZFP*) were significantly up-regulated by moisture stress and one gene *MeGE3* was down-regulated by stress. Three genes (*MeGF14*, *MeMYC2* and *MeP5CS*) were not differentially expressed in drought tolerant (Table 16).

Table 16: Effect of water stress on mRNA levels in drought tolerant and drought susceptible genotypes after 10 days of stress

GENOTYPE	Gene	Expression	Std. Error	95% C.I.	Probability	Result
MM96/0686 Stressed against well watered	*MeALDH*	2.815	1.818 - 4.183	1.327 - 6.112	0.000	Up regulated
	MeATTF	3.245	1.444 - 9.582	1.069 - 11.530	0.000	Up regulated
	MeGBF3	3.241	2.221 - 5.467	1.688 - 9.982	0.000	Up regulated
	MeGE3	0.317	0.181 - 0.647	0.097 - 0.988	0.006	Down regulated
	MeGF14	*1.303*	*0.963 - 1.768*	*0.844 - 2.344*	*0.095*	*NS*
	MeMYC2	*1.350*	*0.718 - 2.196*	*0.608 - 3.336*	*0.204*	*NS*
	MeMSD	3.148	2.316- 4.431	1.897-6.394	0.001	Up regulated
	MeRD28	1.511	1.062 - 1.998	0.852 - 2.153	0.013	Up regulated
	MeP5CS	*1.425*	*0.686 - 3.784*	*0.384 - 4.745*	*0.301*	*NS*
	MeZFP	4.043	2.869 - 5.828	2.164 - 8.014	0.000	Up regulated
NYALANDA Stressed against well watered	*MeALDH*	*2.160*	*1.003 - 5.120*	*0.464 - 7.983*	*0.056*	*NS*
	MeATTF	2.671	1.902 - 3.812	1.393 - 4.954	0.001	Up regulated
	MeGBF3	1.875	1.161 - 3.028	0.733 - 3.979	0.018	Up regulated
	MeGE3	0.205	0.057 - 0.671	0.034 - 0.941	0.000	Down regulated
	MeGF14	2.285	1.094 - 6.336	0.826 - 18.602	0.031	Up regulated
	MeMYC2	2.201	1.391 - 3.371	0.976 - 4.183	0.006	Up regulated
	MeMSD	*1.506*	*0.983 - 2.255*	*0.815 - 4.129*	*0.063*	*NS*
	MeRD28	*1.128*	*0.994 - 1.286*	*0.875 - 1.381*	*0.059*	*NS*
	MeP5CS	1.662	1.125 - 2.414	0.977 - 3.496	0.007	Up regulated
	MeZFP	*1.578*	*0.806 - 3.368*	*0.525 - 5.198*	*0.159*	*NS*

C.I =confidence interval at 95%; Expression=Fold change in expression of a gene relative to control; NS=non significant

Most of the drought response genes exhibited different regulation during drought stress responses of the two genotypes. Three genes (*MeATTF*, *MeGBF3* and *MeGE3*) did have similar expression patterns in both tolerant and susceptible genotypes, where *MeATTF* and *MeGBF3* were up-regulated and *MeGE3* was down-regulated during drought stress. The remaining seven genes exhibited different expression patterns in drought tolerant and drought susceptible genotypes. Four of the genes; *MeZFP*, *MeALDH*, *MeMSD* and *MeRD28* were exclusively up-regulated in drought tolerant genotype and the three genes (*MeGF14*, *MeMYC2* and *MeP5CS*) were exclusively up-regulated in drought susceptible genotype (Figure 16).

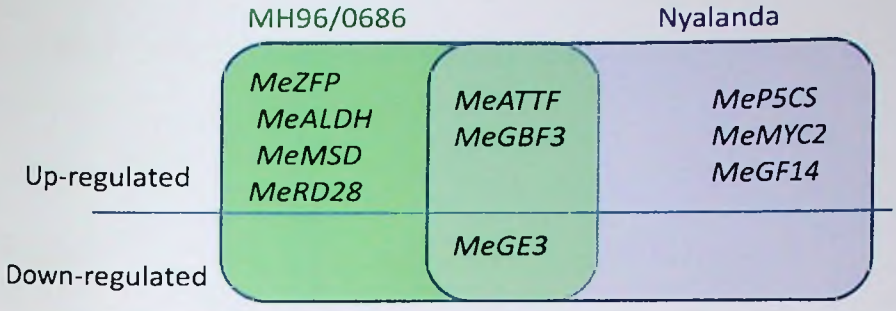

Figure 16: Differential gene expression in two contrasting cassava genotypes in response to water stress

Given that the expression of the 10 previously identified plant drought-tolerance genes is responsive to drought in cassava, the next question was whether their expression can provide insight into the basis of the relative drought tolerance and susceptibility of the two genotypes; genes expressed at higher levels in MM96/0686 compared with Nyalanda are candidates for conferring drought tolerance. For this, two comparisons between genotypes were made; relative expression levels under non-stressed conditions (basal expression levels) and relative expression levels under drought stress. To determine the relative expression levels under non stressed conditions, baseline expression levels were compared under well-watered conditions for the two genotypes (Table 17). Even in the absence of drought stress, three genes exhibit different expression levels in the two genotypes. *MeGF14* and *MeMYC2* were expressed at approximately twice the level in MM96/0686 (DT) compared with Nyalanda (DS). Alternatively, *MeMSD* was expressed at approximately half the level in DT compared with DS.

The relative gene expression levels under drought stress were next compared between the tolerant and susceptible genotypes (Table 18). Under drought stress, 7 of the 10 genes were expressed at significantly higher levels in the tolerant genotype compared with the susceptible genotype. This included four that had been identified as exclusively up-regulated by drought stress in tolerant MM96/0686 (*MeALDH, MeZFP, MeRD28* and *MeMSD*), two that were up-regulated in both genotypes (*MeGBF3, MeATTF*), and one that was exclusively up-regulated in susceptible Nyalanda (*MeP5C5*; however, not up-regulated enough to surpass expression in DT under drought stress). This also included the one gene down-regulated by drought stress in both genotypes (*MeGE3*).

Table 17: Differential gene expression in drought tolerant and drought susceptible under well-watered conditions

Gene	Expression (fold difference in baseline expression levels)	Std. Error	95% C.I.	Probability (P=0.05)	Relative significant difference (DT vs. DS)
MeALDH	1.379	0.598 - 2.832	0.370 - 4.163	0.319	NS
MeATTF	1.679	0.531 - 4.431	0.384 - 6.376	0.211	NS
MeGBF3	1.094	0.570 - 1.789	0.272 - 2.591	0.746	NS
MeGE3	1.959	0.501 - 6.700	0.270 - 10.729	0.221	NS
MeGF14	*2.332*	*1.202 - 5.717*	*0.824 - 17.293*	*0.013*	*Up regulation*
MeMYC2	*1.924*	*1.118 - 3.617*	*0.741 - 4.594*	*0.019*	*Up regulation*
MeMSD	*0.584*	*0.429 - 0.837*	*0.366 - 1.282*	*0.014*	*Down regulation*
MeRD28	1.120	0.990 - 1.297	0.867 - 1.409	0.075	NS
MeP5CS	1.934	0.746 - 3.786	0.657 - 6.197	0.054	NS
MeZFP	0.858	0.504 - 1.471	0.317 - 2.258	0.539	NS

Table 18: Differential gene expression in drought tolerant and drought susceptible under water stress conditions

Gene	Fold higher in MM96/0686 (compared to Nyalanda)	Std. Error	95% C.I.	Probability (P=0.05)	Result
MeALDH	1.797	1.038 - 3.258	0.644 - 4.879	0.040	Up regulation
MeATTF	2.040	1.585 - 2.669	1.377 - 3.090	0.000	Up regulation
MeGBF3	*1.890*	*1.379 – 2.579*	*1.099 -3.128*	*0.000*	Up regulation
MeGE3	3.033	1.762 - 5.233	1.122 – 8.913	0.000	Up regulation
MeGF14	1.330	0.842- 1.921	0.669 - 2.521	0.123	NS
MeMYC2	1.180	0.767 - 1.775	0.634 - 2.532	0.350	NS
MeMSD	1.221	0.800 - 1.664	0.589 – 3.109	0.326	NS
MeRD28	1.501	1.065 – 1.966	0.866 - 2.133	0.017	Up regulation
MeP5CS	1.659	0.992 - 2.709	0.712 - 3.197	0.040	Up regulation
MeZFP	2.197	1.115 - 3.590	0.815- 3.900	0.017	Up regulation

6.4 DISCUSSION

Cassava represents an essential crop for increasing food security in sub-Saharan Africa and other food-insecure regions. Unfortunately, drought presents a formidable challenge towards attainment of this goal for resource-poor farmers. This study focused on the morphological, physiological and molecular characterization of a drought-tolerant improved variety and drought-susceptible landrace from Uganda. The results confirm the drought response status of these genotypes, as already suggested by field data. The molecular component of this study focused on genes previously confirmed to be functionally involved in drought tolerance/susceptibility from other studies, in other species. The primary criterion to select genes to include in this study was based on the confirmed functional role of the genes in drought tolerance in other species in order to increase chances of inclusion of functional drought response genes in cassava. Furthermore, this study has established molecular tools that can be used to further characterize and breed for drought tolerance in cassava through inclusion of gene expression-based phenotyping. These approaches could find application for identification of quantitative trait loci (QTLs) to guide selection of the best genotypes during the breeding program.

Field observations in Uganda had previously identified MM96/0686 and Nyalanda as exhibiting drought tolerance and susceptibility, respectively. The greenhouse trial conducted in this study confirmed this, with drought tolerant and drought susceptible exhibiting disparate responses to progressive application of severe drought stress. Drought tolerant genotype exhibited little drought stress at 10 days of water stress, while drought susceptible exhibited severe drought stress symptoms, especially in lower leaves, many of which had wilted and dried out.

Physiological and morphological data indicated that stomata conductance in drought tolerant, MM96/0686 was significantly lower than in drought susceptible Nyalanda). Reduced stomatal conductance is an effective adaptive response associated with drought tolerance in plants (Heschel and Riginos, 2005). Stomatal conductance was approximately twice as high in Nyalanda as in MM96/0686, indicating that MM96/0686 is more effective at water use efficiency (WUE) than Nyalanda. Lower stomata conductance is an indication of reduced water loss through stomata for increased water use efficiency. Wajid *et al.* (2011) also reported that cultivars susceptible to water stress have higher stomatal conductance and transpiration rate than drought tolerant ones. Relative water content was slightly but significantly different between the two genotypes, confirming that the drought susceptible leaves that were subjected to analysis were in fact drought stressed. The

fact that MM96/0686 exhibited higher RWC than Nyalanda but its stomatal conductance (thus transpiration rate/water loss) remained much lower than in Nyalanda further confirms that MM96/0686 survives better than Nyalanda in drought stress conditions. Leaf retention was lower in drought susceptible than in drought tolerant, and number of leaves was higher in drought tolerant than drought susceptible, reflecting the stressed state of drought susceptible compared to drought tolerant. The improved genotype, MM96/0686 has been reported to have a stay green trait, a trait associated with drought tolerance (Baguma Yona, personal communication). Besides, after 10 days of water stress, Nyalanda had showed severe wilting, while MM96/0686 leaves showed minimal wilting symptoms. The results thus indicated that the two genotypes consistently showed significant contrasts in key drought-tolerance parameters, and so were appropriate plant materials for gene expression study.

After physiological and morphological characterization of the two genotypes' drought responses, molecular analysis was conducted for known drought-related plant genes. In this study we examined the expression of ten genes in response to water stress. Data was collected at the end of water stress period when the soil moisture content was 28.80 ± 1.08 in water stressed treatment and 83.00 ± 2.45 in well watered plants. This soil moisture content was comparable to 25% that was reported by Aina *et al.* (2007) to represent severe moisture stress in cassava. At this moisture content, the water stressed drought susceptible plants showed severe wilting symptoms. It was at this point that data was collected.

Gene expression was determined with high confidence because the two genotypes used in the study exhibited highly disparate levels of susceptibility to drought stress as reflected in physiological and morphological responses to water stress. In addition, using gradual water deficit to mimic natural drought conditions, places this study in a context that more closely resembles typical field conditions during water stress. Most gene expression studies used single genotype (either drought tolerant or susceptible) without comparing the expression of genes between drought tolerant and susceptible genotypes. Single genotype approach is limited to identifying genes responsive to drought but cannot differentiate between drought-tolerance-adaptive genes that enhance drought tolerance in plants and drought-responsive genes. Therefore our study provides accurate information on drought adaptive and drought responsive genes. As of this writing, this is the first report on gene expression in cassava using Real Time PCR and the differentially expressed genes identified in this study should provide valuable insight into

molecular mechanisms of drought tolerance in cassava. Importantly, the information generated will help cassava breeders and other scientists to understand how different cassava genotypes respond to drought stress and how drought tolerant genotypes adapt to moisture-stress environments.

The differential expression of the ten genes in this study confirms that they respond to water stress in cassava. Plants respond and adapt to moisture stress by triggering a wide variety of plant responses, including alterations in gene expression (Seki *et al.*, 2002; Umezawa *et al.*, 2006). It is the genes with altered expression that are probably involved in the pathways of plant responses to drought (Guo *et al.*, 2009). Depending on their expression to water stress in different genotypes, the genes were categorised into four groups. The first group consisted of four genes, *MeALDH, MeZFP, MeRD28 and MeMSD* that were exclusively up-regulated in drought tolerant genotype and not expressed in drought susceptible genotype. The second group consisted of three genes, *MeP5CS, MeMYC2 and MeGF14* that were exclusively up-regulated in drought susceptible genotype and not in tolerant genotype. The third group had two genes *MeGBF3* and *MeATTF* that were up-regulated in both tolerant and susceptible genotypes, while the fourth group had one gene *MeGE3* that was down-regulated by water stress treatment in both drought tolerant genotype (MM96/0686) and drought susceptible Nyalanda.

6.4.1 Genes exclusively up-regulated by moisture stress in drought tolerant genotype

Four genes, *MeALDH, MeZFP, MeRD28 and MeMSD* were exclusively up-regulated by water stress in the drought tolerant genotype and were not expressed in drought susceptible genotype. Under well-watered/control conditions, *MeALDH, MeZFP* and *MeRD28* were not differentially expressed between drought tolerant and drought susceptible. This acting as a baseline, it means that the up-regulation of these genes in drought tolerant was due to water stress. On the other hand, the expression *MeMSD* was lower in drought tolerant than in drought susceptible in control treatment (well watered) but its expression in drought tolerant in response to stress was 2-fold higher than in drought susceptible indicating that water stress resulted in higher expression of this gene in drought tolerant genotype compared to drought susceptible genotype. Therefore these four genes *MeALDH, MeZFP, MeRD28 and MeMSD* might be involved in drought adaptation in cassava. Guo *et al.*, 2009, reported that to identify genes responsible for drought tolerance requires genotypes with similar genetic backgrounds but with contrasting drought tolerance. He further indicated that developing such near isogenic lines requires several years of backcrossing

and selection. They (Guo *et al.*, 2009) suggested an alternative method of identifying common genes that are differentially expressed between drought-resistant genotypes with different genetic backgrounds and drought-sensitive genotypes under drought conditions to link candidate genes to drought tolerance. Nevertheless since their gene expression under control conditions as a baseline was not different, their differential expression in drought tolerant variety under drought conditions may be linked to their role played in drought tolerance.

Among the four genes, *MeMSD* codes for manganese superoxide dismutase (*MnSOD*) enzyme that plays role in oxidative stress tolerance in plants. Over expression of superoxide dismutase (*SOD*) enzymes has been reported to increase oxidative stress tolerance in different transgenic plants compared to their wild types (Van Breusegem *et al.*, 1999; Sen Gupta *et al.*, 1993; Basu *et al.*, 2001; Wang *et al.*, 2005). For example Sen Gupta *et al.* (1993) reported that a 3-fold increase in total pea Cu/ZnSOD activity resulted in significant increase in resistance to MV-induced membrane damage in transgenic tobacco. Basu *et al.* (2001) showed that a 1.5-2.5-fold increase in total SOD activity in transgenic *Brassica napus* plants transformed with wheat *MnSOD* increased oxidative stress resistance compared with wild type controls. Wang *et al.* (2005) reported that a 1.4-fold increase in total SOD activity in the *MnSOD* transgenic rice plants was enough to increase oxidative stress resistance and drought tolerance when the gene was fused with a chloroplast transit peptide sequence in order to target the *MnSOD* to the chloroplast. The 3.148 fold increase obtained in this study in drought tolerant genotype indicates a level that is adequate to associate this gene with drought tolerance in cassava. *Superoxide dismutase* enzymes are involved in scavenging Reactive Oxygen Species (ROS) that are produced in plants during water stress (Price *et al.*, 1989; Kendall and McKersie, 1989; McKersie *et al.*, 1993, Fryer *et al.*, 2002, Hernandez *et al.*, 1995) and so this gene could be involved in ROS scavenging in cassava.

The gene *MeZFP* that encodes for Zinc finger protein (ZFP252) was also exclusively expressed in drought tolerant genotype. Zinc finger protein has been reported to confer drought tolerance in plants by maintaining cell membrane integrity during water stress (Xu *et al.*, 2008). Xu *et al.* (2008) reported that the relative electrolyte leakage, an indicator of membrane injury (Morsy *et al.*, 2005), was lower in *Oryza sativa* ZFP252 transformed rice plants than in wild type and *Oryza sativa ZFP252* knock out transformed plants under drought stress. This suggests that *ZFP252* protects plants from stress by maintaining cell membrane integrity. The transformed rice plants also had higher free proline contents and soluble sugars than non transgenic and *ZFP252*-knock

out transgenic rice plants (Xu *et al.*, 2008). The findings by Xu *et al.* (2008) suggest that enhanced stress tolerance of *ZFP252*-transgenic plants might partially be through activation of proline synthesis and proline transport pathways by *ZFP252* in rice under salt and drought stresses. In this study *MeZFP* was over expressed by 4-fold, exclusively in drought tolerant genotype. It is therefore possible that this gene is among the genes that enhance drought tolerance in cassava through increasing free osmoprotectant proline and soluble sugars.

MeRD28 encodes for *responsive to desiccation* gene (*RD28*). This gene was increased by 1.5-fold by water stress, exclusively in drought tolerant cassava genotype suggesting that it has a role to play in enhancement of drought tolerance in cassava. Daniels *et al.* (1994) reported that *RD28* is a turgor responsive mercury resistant plasma membrane aquaporin found in plasma membranes of all plant tissues except seeds. Earlier studies by Yamaguchi-Shinozaki *et al.* (1992) reported that *RD28* enhances drought tolerance through ABA-independent pathway. It protects desiccated cells by transporting small molecules across cell membranes. It is likely that this gene enhances the cells' desiccation tolerance in drought tolerant cassava.

The fourth gene that was exclusively expressed in drought tolerant variety is a *MeALDH* that encodes for *aldehyde dehydrogenase ALDH7B4*. It was over-expressed by 2.8-fold and therefore may be involved in enhancement of drought tolerance in cassava. The findings in this study are in agreement with studies by Kotchoni *et al.* (2006) who reported that transgenic Arabidopsis thaliana plants with increased amounts of *ALDH7B4* were more tolerant to dehydration and salt stress than wild type plants. Kotchoni *et al.* (2006) further reported that over-expression *ALDH7B4* gene in transgenic plants reduced the level of lipid peroxidation under drought and salt stress suggesting that the gene confer tolerance to both osmotic and oxidative stress in Arabidopsis thaliana through ROS (H_2O_2) scavenging and reduction of lipid peroxidation. This gene had also been reported to be induced by pathogens (Zimmermann *et al.*, 2004) and therefore might be a multi-stress responsive gene. Over-expression by 2.8-fold and exclusive up-regulation of this gene in drought tolerant cassava is an indication that the gene may be involved in enhancement of drought tolerance in cassava, probably through ROS scavenging and reduced lipid peroxidation.

6.4.2 Genes up-regulated only in drought susceptible genotype

Three genes *MeMYC2, MeP5CS and MeGF14,* were exclusively up-regulated in drought susceptible genotype. These genes may therefore be regarded as general drought responsive genes. *MeMYC2* encodes for *MYC2* transcription factor. In Arabidopsis, *AtMYC2* regulates ABA-inducible drought responsive genes (*responsive to resiccation 22* or *RD22* and *alcohol dehydrogenase1* or *ADH1*) under drought stress conditions (Abe *et al.*, 2003). Recently, Yasunari *et al.* (2011) reported that *AtMYC2* is involved in ABA-mediated gene expression in Arabidopsis and is the key regulator of crosstalk in abiotic and biotic stress responses. Because it was not differentially expressed in drought tolerant cassava genotype in response to drought in this study, more research would be necessary to relate *MeMYC2* gene to enhancement of drought tolerance in cassava. Perhaps, this can be done by analyzing a panel of susceptible and tolerant genotypes. It is recommended that genes known to be regulated by *MYC2* transcription factor such as *RD22* and *ADH1* (Abe *et al.*, 2003) be investigated in cassava in order to ascertain the role of *MeMYC2* in drought tolerance.

MeP5CS was also exclusively up-regulated by water stress in drought sensitive genotype. *MeP5CS* encodes for pyrroline-5-carboxylate synthetase (*P5CS*). *P5CS* has been reported to be involved in drought tolerance through increased production of free proline, a compatible osmolyte in plants. Eliane *et al.* (2007) reported that after 15 days of water stress, the transgenic wheat plants transformed with *P5CS* had accumulated twice as much proline compared to non transformed plants yet in well-watered conditions, the concentration of free proline in transgenic and control plants was similar. They further reported that the level of lipid peroxidation measured in terms of malondialdehyde (MDA) content was 65% higher in control plants compared to transgenic plants. High lipid peroxidation indicates stress-induced damage at the cellular level as a result of increased ROS (Parvanova *et al.*, 2004; Shao *et al.*, 2005) and leads to cellular membrane rupture in water stressed plants (Hernandez *et al.*, 2000). It was not possible for this study to attribute drought tolerance in cassava to *MeP5CS* since it was not differentially expressed in drought tolerant genotype. In fact, slight expression levels for this gene are observed in both tolerant and susceptible genotypes, with marginally higher expression in Nyalanda. Further studies using several cassava genotypes that contrast each other in drought tolerance may give more insight on the role of *MeP5CS* in drought tolerance in cassava.

This study also could not associate *MeGF14* to enhancement of drought tolerance in cassava because it was not differentially expressed in drought tolerant genotype. *MeGF14* encodes for 14-3-3 protein GF14λ. Results from this research suggest that this gene could be a general water stress responsive gene in cassava. This is contrary to previous studies where this gene has been reported to confer drought tolerance in plants. Juqiang *et al.* (2004) reported that cotton lines that expressed GF14λ demonstrated a "stay-green" phenotype and improved water-stress tolerance. These lines wilted less and maintained higher photosynthesis than non-transgenic control plants under water-deficit conditions. In this study, the drought tolerant genotype exhibited a "stay green" phenotype, but *MeGF14* was not differentially expressed by water stress and therefore could not be associated with drought tolerance in cassava.

6.4.3 Genes up regulated by water stress in drought tolerant and susceptible genotypes

Two genes; *MeATTF* encoding for amino acid transporter family II protein and *MeGBF3* encoding for *GBF3* were up regulated by stress in both tolerant and susceptible genotypes. This suggests that these genes are constitutively expressed in both drought tolerant and drought susceptible genotypes. They may therefore be among the general drought responsive genes. GBF3 (*G-BOX BINDING FACTOR 3*), is one of the several G-box Binding factors (*GBFs*) which are basic leucine zipper (bZIP) proteins (Schindler *et al.*, 1992; Izawa *et al.*, 1993). In Arabidopsis, GBF3 is highly expressed in roots but not in leaves (Schindler *et al.*, 1992) and is believed to be involved in the regulation of *alcohol dehydrogenase* (*adh*) through ABA dependent pathway. The *adh* gene is responsive to cold and dehydration. On the other hand, *MeATTF* has been reported to be up-regulated by water stress (Bray, 2004, Guo *et al.*, 2009) though its actual function in drought tolerance was not well demonstrated. Although these two genes were constitutively expressed in both tolerant and susceptible genotypes, their expression in drought tolerant genotype was significantly higher than in drought susceptible genotypes, suggesting that they may have a role to play in drought tolerance. However, further studies using different genotypes and large sample size may give an insight to whether the two genes are associated with drought tolerance in cassava.

6.4.4 Genes down regulated by water stress

MeGE3 encoding for *GERMIN 3* (*GER3*) was down regulated by water stress treatment in both drought tolerant genotype (MM96/0686) and drought susceptible (Nyalanda) suggesting that

MeGE3 plays no role in drought tolerance in cassava. This is in agreement with Bray (2004) who reported that *Arabidopsis thaliana GER3* (Arabidopsis thaliana Germin like gene group 3) was repressed by drought stress in leaves of Arabidopsis thaliana. Germin proteins were first identified in wheat as genes that were expressed during germination (Lane *et al.*, 1991). The report by Bray, (2004) suggested that the possible role for Germin like genes is alteration of cell wall properties that control growth. It does suffice to note that under well watered conditions, most genes (70%) had similar expression levels in drought tolerant and susceptible genotype indicating that their differential abundance under moisture stress conditions was due to their differences in responding to stress in different genotypes.

6.5 CONCLUSIONS AND RECOMMENDATIONS

Ten genes responsive to water stress in cassava have been identified. Out of these, four genes *MeALDH, MeZFP, MeMSD* and *MeRD28* were exclusively up-regulated in drought tolerant variety and are therefore regarded to be associated with enhancement of drought tolerance cassava. Future studies to biologically validate these genes for their role in drought tolerance for possible utilization in genetic engineering are important. Earlier studies by Shinozaki and Yamaguchi-Shinozaki (2007) demonstrated the use of drought responsive genes in genetic engineering in Arabidopsis and other plant species. Equally important is the need to identify more genes associated with and/or responsive to drought in cassava. The differentially expressed genes found in this study should provide useful information for understanding cassava drought physiology and how different cassava genotypes adapt to drought-stress conditions.

CHAPTER 7: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

This study was implemented in two parts; i) field trials to assess response of genotypes to drought stress and ii) laboratory studies to assess genetic diversity and gene expression. Cassava varietal response to moisture was determined through three field screening trials. Two of these field trials were established in drought prone districts of Buliisa and Nakasongola in Uganda. These sites received less rainfall during the experimental periods. For reference purposes, a similar field trial was established at Kabanyolo in Wakiso district which received relatively high rainfall during the trial. Field evaluation of different genotypes under representative environments and in relevant cropping systems generates essential information on genotype/cultivar potential under natural conditions as well as their responses to specific limiting environmental factors (El-Sharkawy *et al.*, 1965; El-Sharkawy, 2006; Long *et al.*, 2006). Field evaluation is reported as the most valid ecosystem research in studying plant water relations and crop photosynthesis in relation to productivity (El-Sharkawy, 2006). Therefore the results reported in this thesis are comparable to expectations on the response of cassava genotypes to drought in Uganda. The information is important especially in Uganda where there has been limited scientific field research to support farmers and researchers perceptions of drought tolerance and susceptibility in cassava. Limited scientific research has been done on drought tolerance based on screen house trials which may not reflect the natural drought conditions in the field. It has been emphasized that the information generated from screen house trials is inadequate and not consistently reflective of the typical field conditions (Evans *et al.*, 1985; Kramer, 1981).

Most agronomic traits measured in the field screening of cassava genotypes for drought tolerance, including harvest index, dry matter content, number of storage roots per plant and leaf retention have been reported to be associated with drought tolerance (Lenis *et al.*, 2006; Subere at al., 2009; Okogbenin, 2013). The study showed that genotypes significantly differed for these traits suggesting that they differ in their response to drought stress. Similar observation had been made by El-Sharkawy, (2007). This varietal difference is important for breeders to select parental genotypes for guided crossing especially when dealing with complex quantitative traits including drought tolerance. A number of cassava genotypes were tolerant to water stress and these are important in two ways; i) they can be used as important gene sources for cassava breeding and ii) can be rationally distributed to farmers living in marginal agro-ecological zones where rainfall is inadequate.

Field screening trials also revealed significant G X E reflecting genotypic differences towards adaptation to different agro-ecological zones and tolerance to water stress. The significance of Genotype x location effects suggests that genotypes may be selected for specific adaptation to different environments. Based on this, policy makers, NGOs, government and institutions involved in distribution of cassava planting materials can be guided on which genotypes and ecological zones are adequate for promotion. Farmers living in agro-ecological zones where rainfall is erratic will be advised on genotypes with ability to tolerate limited water availability while farmers in agro-ecological zones with optimum growth conditions would be guided by factors such as high yield and tolerance to pests and diseases. Certainly, rainfall is the critical factor in defining agro-ecological zones (Aina *et al.*, 2007).

Contrary to the general belief that cassava is tolerant to water stress, it has been demonstrated in this thesis, that severe water stress is detrimental to cassava growth and productivity. When the drought stress appeared in the first three months after planting, most of the genotypes in drought prone districts wilted and/or died. This is in agreement with earlier findings that early drought stress within six months after planting adversely affects cassava growth and development (Santisopasri *et al.*, 2001). Although the effects of drought were different in different genotypes, the general observation was that the performance of all genotypes declined as a result of water stress. Therefore breeders should aim at developing drought tolerant cassava genotypes if expansion of cassava cultivation to arid areas, due to limited arable land, is to be successful.

With increased pressure on the arable land and with rapid increase in global population growth, more crop cultivation will extend to non-traditional agricultural land in order to meet global food demand. Well known that cassava is an important staple food for many households in the tropics, it is important that research considers improving of its water use efficiency so that effects of drought on its productivity are reduced. The high genetic variability among the cassava genotypes in response to drought revealed in this study is an indication that breeding for drought tolerance is possible since it is easy to select drought tolerant gene sources. However, heritability of drought tolerance among the cassava genotypes is not well understood and therefore studies to understand genetic inheritance of drought tolerance need to be undertaken.

This study also revealed that genotypes MM96/0686, Magana, Yellow, TME 204, Nyamutukura, MH97/2961 and NASE 12 were less sensitive to water stress compared to other genotypes. They performed better across locations for important drought tolerance agronomic traits (HI, LR, FRY and NR). These genotypes can therefore be important sources of genes for drought tolerance breeding. On the other hand, most of the landraces were sensitive to drought. Bao, Nyalanda, Egabu, Icilicili and Rwaburaru were the most hit. Therefore including these genotypes in breeding programs is paramount if adoption of the improved genotypes by farmers is to be enhanced. It is also recommended that molecular screening of putative drought tolerant genotypes reported in this study be carried out using the identified candidate drought tolerance genes. This will help to validate the field data and provide vital information for future breeding for drought tolerance in cassava.

Genetic gains can be made if genetic variation exists, traits are heritable, and when measurements can be precisely made. Efforts to attain these are underway for cassava. High genetic diversity observed among landraces in this study is an indication that cassava landraces in Uganda may be very useful sources of new and unique alleles for generation of breeding populations. Indeed in this study, it was revealed that landraces have unique alleles not represented in elite genotypes that are often used in cassava breeding program in Uganda. It is presumed that the unique alleles could be associated with farmer preferred traits in adapted landraces. This may explain why farmers keep landraces in their fields even when they are susceptible to diseases and other stresses due to their preferred qualities (such as culinary qualities) that may be lacking in elite genotypes.

The local genotypes Nyalanda and Icilicili had the highest number of loci with unique alleles. These genotypes were also observed to be sensitive to drought in the field. Personal observations on Nyalanda in farmers' fields during germplasm collection revealed that the genotype is also very susceptible to Cassava Mosaic Disease (CMD). Though the farmers are aware of these challenges to productivity of their genotypes, they tend to cling on them due their unique qualities. For example, farmers reported that Nyalanda is high yielding when conditions are optimum, has very good starch content and excellent culinary qualities. On the other hand, farmers reported that Icilicili genotype is excellent for flour production. Thus breeding interventions aimed at incorporating deficient traits in some of these varieties (i.e. pathogen/pest resistance, drought tolerance), would be desirable. Breeders should therefore include landraces in breeding schemes to increase chances of producing progeny with farmer preferred traits. The importance of using

landraces in improvement of cassava has been demonstrated through introgression of cassava mosaic disease tolerance in cassava (Lokko *et al.*, 2005).

Although the differentiation between genotypes collected from different regions was low and could have been affected by differences in sample sizes, the information generated indicates the possibility of developing heterotic pools for genotypes grown in the Northern region and those grown in Central, Western and Eastern regions of Uganda. If this possibility is confirmed by using large sample sizes, then it would help breeders to carry out guided crosses for improved genetic gain. Magoon and Krishnan (1977), and Cowen and Frey (1987) reported that parents from different heterotic pools provide allelic variation that can be used to create new favourable gene combinations and exploit heterosis.

Breeding for complex traits like drought tolerance requires high technological approaches. Private and public breeding programmes in different parts of the world have achieved substantial gains through conventional breeding. Maize is an excellent example that cassava can replicate (Bänziger *et al.*, 2006). Data presented in this thesis will contribute towards this goal, particulary for trait phenotyping. Moreover, this thesis provides information on genes that are involved in enhancement of drought tolerance in cassava. This is the first report on gene expression in cassava using Real Time PCR and the differentially expressed genes identified in this study should provide valuable insight into molecular mechanisms of drought tolerance in cassava. The thesis also provides information on the methodology that can be followed in determining candidate genes that may be involved in stress tolerance.

This research identified four candidate drought tolerance genes *MeALDH, MeZFP, MeMSD* and *MeRD28*. These genes are probably involved in drought stress tolerance because they were up regulated exclusively in drought tolerant genotype. However, it has been reported that not every up-regulated gene in response to stress has a role in adaptation. Instead some might be up-regulated because of the damage caused by the stress (Zhu, 2000). It has also been reported that adaptation to drought is a function of numerous genes and not a single gene (Ramanjulu and Bartels, 2002). For example, Seki *et al.* (2002) revealed that approximately 44 genes were up-regulated in response to dehydration in Arabidopsis, of which 30 were reported to be novel. Therefore; 1) the four genes identified in this study need to be biologically validated for their role in adaptation to drought stress. This would make a very important step towards understanding the

physiological responses to drought stress in cassava, 2) it is recommended that more research be conducted to indentify more genes associated with drought tolerance since it has been suggested that complex traits including dehydration tolerance are under control of many genes.

The identification of candidate drought tolerant genes was based on gene expression in response to 10 day water stress period. Assessments at many shorter intervals and extended period of stress (more than 10 days) may give more insight in molecular mechanisms of drought tolerance in cassava. The forte for this suggestion is based on the available evidence that some genes respond immediately (within seconds or minutes) while others take longer to respond (hours, days or even weeks) (Ramanjulu and Bartels, 2002). Probably the early responsive genes provide initial protection and amplification of signals while the genes that respond later are involved in adaptation to stress conditions. Such details could have been missed since data collection was done at 10 days of stress.

REFERENCES

Abe H, T. Urao, T. Ito, M. Seki, K. Shinozaki, K.Yamaguchi-Shinozaki. 2003. *Arabidopsis AtMYC2 (bHLH)* and *AtMYB2 (MYB)* function as transcriptional activators in abscisic acid signalling. Plant Cell, 15:63–78

Abebe, T., A.C. Guenzi, B. Martin, J.C. Cushman. 2003. Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. Plant Physiology. 131, 1748–1755.

Adebola A.J R, J. V. Anderson, O. A. Kolade, C. D. Ugwu, A. G.O. Dixon, I.L. Ingelbrecht. 2009. Gene-based microsatellites for cassava (*Manihot esculenta* Crantz): prevalence, polymorphisms, and cross-taxa utility. BMC Plant Biology, *9:118*

Agarwal M, N. Shrivastava, H. Padh. 2008. Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Reproduction, 27:617–631.

Agili S.M, J.R. Pardales. 1999. Influence of moisture and allelopathic regimes in the soil on development of cassava and mycorrhizal infection of its roots during establishment period. Philippine Journal of Crop Science, 22: 99-105.

Aigbe S. O, S. U. Remison. 2010a. The Influence of root rot on root starch content of cassava planted in different ecological Environments of Nigeria. Nigerian Annals of Natural Sciences, 10 (1): 60 – 70

Aigbe S. O, S.U. Remison. 2010b. Influence of root rot on dry matter partition of three cassava cultivars planted in different agro-ecological environments. Asian Journal of plant Pathology, 4(2):82-89.

Aina O.O, A.G.O Dixon, E.A Akinrinde. 2007. Effect of soil moisture stress on growth and yield of cassava in Nigeria. Pakistan Journal of Biological Sciences, 10(18): 3085-3090.

Ajmone-Marsan P, C. Livini, M.M. Messmer, A.E. Melchinger, M. Motto. 1992. Cluster analysis of RFLP data from related maize inbred lines of the BSSS and LSC heterotic groups and comparison with pedigree data. Euphytica, 60: 139–148.

Akano A.O, A.G.O. Dixon, C. Mba, E. Barrera, M. Fregene. 2002. Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. Theoretical and Applied Genetics, 105:521–525

Akinwale M.G, B.O Akinyele, A.G.O Dixon, A. C. Odiyi. 2009. Genetic variability among cassava genotypes in three agro-ecology zones of Nigeria. African Crop Science Conference Proceedings, 9:541–546.

Akoroda M.O. 1995. Alleviating hunger in Africa with root and tuber crops. African Journal of Root Tuber Crops, 1: 41-43.

Alexandratos N. 1995. World agriculture: towards 2010 an FAO study. Chichester, UK: Wiley.

Alfredo A. A. C, T. L. Setter. 2004. Response of Cassava Leaf Area Expansion to Water Deficit: Cell Proliferation, Cell Expansion and Delayed Development; Annals of Botany, 94: 605–613

Allem A. 1994. The origin of *Manihot esculenta* Crantz (Euphorbiaceae). Genetic Resources and Crop Evolution, 41: 133-150.

Alves A.A.C. 2002. Cassava botany and physiology. In: Cassava: Biology, Production and Utilization. (Eds. A.C. Belloti, J.M. Thresh and R.J. Hillocks). CAB International, Wallingford, Oxon, UK, pp 67-89.

Anderson J.V. D, M.A. Fregene, V. Jorge, C. Mba, C. Lopez, S. Restrpo, M. Soto, B. Piegu, V. Verdier, R. Cooke, J. Thome, P.D. Horvath. 2004. An EST resource for cassava and other species of Euphorbiacea. Plant Molecular Breeding, 56:527-539.

Apse M.P, E. Blumwald. 2002. Engineering salt tolerance in plants. Current Opinion in Biotechnology, 13, 146–150.

Asafu-Agyei J.N, D.M. Osafo. 2001. Dry matter production and distribution model in intercropped cassava. Ghana Journal of Agricultural Science, 34:27-37

Asante I.K, S.K. Offei. 2003. RAPD-based genetic diversity study of fifty cassava (*Manihot esculenta* Crantz) genotypes. Euphytica, 131: 113-119.

Bakayoko S., A. Tschannen, C. Nindjin, D. Dao, O. Girardin, A. Assa. 2009. Impact of water stress on fresh tuber yield and dry matter content of cassava (*Manihot esculenta* Crantz) in Côte d'Ivoire. African Journal of Agricultural Research, 4 (1):021-027

Bänziger, M., P.S. Setimela, D. Hodson, and B. Vivek. 2006. Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agric. Water Manage. 80:212–224.

Barrett B.A., K.K. Kidwell. 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Science. 38:1261–1271.

Bartels D, R. Sunkar. 2005. Drought and salt tolerance in plants. Critical Reviews in Plant Sciences, 24:23-58.

Basu U, A.G. Good, G.J. Taylor. 2001. Transgenic *Brassica napus* plants overexpressing aluminum-induced mitochondrial *maganese superoxide dismutase* cDNA are resistant to aluminium. Plant Cell Environment, 24:1269–78.

Baulcombe D, I. Crute, B. Davies, J. Dunwell, M. Gale, J. Jones, J. Pretty,W. Sutherland, C. Toulmin, N. Green. 2009. Reaping the Benefits: Science and the Sustainable Intensification of Global Agriculture; The Royal Society: London, UK. .

Beeching J.R, P. Marmey, M.C. Gavalda, M. Noirot, H.R. Haysom, M.A. Hughes A. Charrier. 1993. An assessment of genetic diversity within a collection of cassava (*Manihot esculenta* Crantz) germplasm using molecular markers. Annals of Botany. 72: 515–520.

Bell C.J, J.R. Ecker. 1994. Assignment of 30 microsatellite loci to the linkage map of Arabidopsis. Genomics, 19:137–144

Bell C.J, R.A Dixon, A.D. Farmer, R. Flores, J. Inman, R.A. Gonzales, M.J. Harrison, N.L. Paiva, A.D. Scott, J.W. Weller, G.D. May. 2001. The *Medicago* Genome Initiative: a model legume database. Nucleic Acids Research, 29: 114-117.

Benesi I.R.M. 2002. Native starch evaluation, and analysis of genetic distance using AFLP of elite cassava (*Manihot esculenta* Crantz) genotypes from Malawi. MSc Thesis in Plant Breeding, Department of Plant Sciences (Plant Breeding) faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa.

Benesi I.R.M. 2005. Characterisation of Malawian cassava germplasm for diversity, starch extraction and its native and modified properties. PhD thesis, University of the Free State, Bloemfontein, Republic of South Africa.

Benesi I.R.M., M.T. Labuschagne, A.G.O. Dixon, C.D. Viljoen, N. Mahungu. 2006. Genetic distance analysis of elite cassava (*Manihot esculenta* Crantz) genotypes in Malawi using morphological and AFLP marker technique. South African Journal of Plant and Soil, 23: 58-61.

Benze X, Y. Huang, N. Tang, L. Xiong. 2007. Over-expression of a LEA gene in rice improves drought resistance under the field conditions. Theoretical and Applied Genetics, 115:35–46. DOI 10.1007/s00122-007-0538-9

Bergantin V. R., A. Yamauchi, J. R. Pardales Jr, D. M. Bolatete Jr. 2004. Screening cassava genotypes for resistance to water deficit during crop establishment. Philippine Journal of Crop Science, 29(1):29-39

Bettina MJE, Melvin TT, Thomas AK. 2007. Visual assessment of wilting as a measure of leaf water potential and seedling drought survival. Journal of Tropical Ecology 23:497-500.

Blair M. W, M. A Fregene, S.E Beebe, H. Ceballos. 2007. Marker-assisted selection in common beans and cassava. In Marker-assisted selection: Current status and future perspectives in crops, livestock, forestry and fish. Guimarães E.P., J. Ruane, B.D. Scherf, A. Sonnino and J.D. Dargie (Ed). Available at http://www.fao.org/docrep/010/a1120e/a1120e00.htm accessed on 5th September, 2011

Blum A. 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research, 2005 (56): 1159–1168

Boher B, Verdier V. 1994. Cassava bacterial blight in Africa: the state of knowledge and implications for designing control strategies. African Crop Science Journal, 2:505–509

Bohnert, H. J, B. Shen. 1999. Transformation and compatible solutes. Scientia Horticulturae, 78: 237–260.

Boyer J.S .1982. Plant productivity and environment. Science, 218:443–448

Bravo L.A., J. Gallardo, A. Navarrete, N. Olave, J. Martinez, M. Alberdi, T.J. Close, L.J. Corcuera. 2003. Cryoprotective activity of a cold-induced dehydrin purified from barley. Physiologia Plantarum, 118:262–269.

Bray E.A, J. Bailey-Serres, E. Weretilnyk. 2000. Responses to abiotic stresses. In: Gruissem W, Buchannan B, Jones R (eds) Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, pp 1158–1249

Bray E.A. 2004. Genes commonly regulated by water-deficit stress in Arabidopsis thaliana. Journal of Experimental Botany, 55:2331–2241.

Bruce W.B., G.O. Edmeades, T.C. Barker. 2002. Molecular and physiological approaches to maize improvement for drought tolerance. Journal of Experimental Botany, 53:13–25.

Bull E. S, J. Ndunguru, W. Gruissem, J. R. Beeching, H.Vanderschuren. 2011. Cassava: constraints to production and the transfer of biotechnology to African laboratories. Plant Cell Reproduction, 30:779–787

Burns A, R. Gleadow, J. Cliff, A. Zacarias, T. Cavagnaro. 2010. Cassava: The drought, war and famine crop in a changing world. Sustainability, *2*: 3572-3607

Cach, N.T., J.I. Lens, J.C. Perez, N. Morante, F. Calle, H. Ceballos. 2006. Inheritance of useful traits in cassava grown in sub humid conditions. Plant Breeding, 125: 177-182.

Cardoso P, M. Ernesto, J. Cliff, J. Bradbury, 1998. Cyanogenic potential of cassava flour: field trial in Mozambique of a sample kit. International Journal of Food Science and Nutrition 49: 93-99.

Cattivelli L. F. R, F.W. Badeck, E. Mazzucotelli, A. M. Mastrangelo, E. Francia, C. Marèa, A. Tondelli, A. M. Stanca. 2007. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crops Research, 105:1-14

Ceballos, H., C.A. Iglesias, J.C. Perez, A.G.O. Dixon. 2004. Cassava Breeding: opportunities and challenges. Plant Molecular Biology, 56: 503-516

Ceccarelli S. 1996. Positive interpretation of genotype by environment interaction in relation to sustainability and biodiversity. In M. Cooper and G.L. Hammer, (Eds). Plant Adaptation and Crop Improvement. P. 467-486. Wallingford, UK, CABI.

Chakravarthi B. K, R. Naravaneni. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa.* L). African Journal of Biotechnology, 5 (9): 684-688

Challinor A, T. Wheeler, C. Garforth, P. Craufurd, A. Kassam. 2007. Assessing the vulnerability of food crop systems in Africa to climate change. Climatic Change 83: 831-399.

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

Chavarriaga-Aguirre P, M.M. Maya, M.W. Bonierbale, S. Kresovich, M.A. Fregene, J. Tohme, G. Kochert. 1998. Microsatellite in cassava (*Manihot esculenta* Crantz); discovery, inheritance and variability. Theoretical and Applied Genetics, 97:493-501.

Chavez A.L, T. Sanchez, G. Jaramillo, J.M. Bedoya, J. Echeverry, E.A. Bolanos H. Ceballos, C.A. Iglesias. 2005. Variation of quality traits in cassava roots evaluated in landraces and improved clones. Euphytica, 143:125–133

Cheres M. T, J. F. Miller, J. M Crane, S. J. Knapp. 2000. Genetic distance as a predictor of heterosis and hybrid performance within and between heterotic groups in sunflower. Theoretical and Applied Genetics, 100 (6), 889-894.

Chiwona-Karltun, L, J. Mkumbira, J. Saka, M. Bovin, N.M. Mahungu, H. Rosling. 1998. The importance of being bitter – a quantitative study on cassava cultivar performance in Malawi. Ecology of Food Science and Nutrition, 37: 219-245.

Cock J.H. 1984. Cassava. In: Goldworthy, F.R. and N.M. Fisher (Eds.); The Physiology of Tropical Crops, pp. 540-552. Wiley, Chichester, UK.

Collard B.C.Y, M.Z.Z. Jahufer, J.B. Brouwer, E.C.K. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142:169-196.

Colombo C, G. Second, A. Charrier. 2000. Diversity within American cassava germplasm based on RAPD markers. Genetic Molecular Biology, 23: 189-199.

Conn E. 1994. Cyanogenesis – a personal perspective. Acta Horticulturae, 375:31-43.

Connor D. J, J. Palta. 1981: Response of cassava to water shortage. III. Stomatal control of plant water status. Field Crops Research, 4:297-311.

Connor, D.J, J.H. Cock, and G.E. Parra. 1981. Response of cassava to water shortage. I. Growth and yield. Field Crops Research, 4:181–200.

Cowen N.M, K.J. Frey. 1987. Relationships between three measures of genetic distance and breeding behaviour in oats (*Avena sativa* L.). Genome, 29: 7–106.

Cox T.S, J.P. Murphy, D.M. Rodgers. 1986. Changes in genetic diversity in the red winter wheat regions of the United States. Proceedings of the National Academy of Sciences, 83:5583–5586.

Daniels J.M, T.E. Mirkov, M.J. Chrispeels. 1994. The plasma membrane of Arabidopsis thaliana contains a mercury-insensitive aquaporin that is a homolog of the tonoplast water channel protein TIP. Plant Physiology, 106: 1325-1333

Degenkolbe T., P. Thi Do, E. Zuther, D. Repsilber, D. Walther, D. K. Hincha, K. I. Ko¨hl. 2009. Expression profiling of rice cultivars differing in their tolerance to long-term drought stress. Plant Molecular Biology, 69:133–153.

Dellaporta S.J, J. Wood, J.B. Hicks. 1983. A plant DNA mini preparation. Version II. Plant Molecular Biology and Reproduction, 1:19–21.

Devos K.M, G.J. Bryan, A.J. Collins, P. Stephenson, R. Gale. 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theoretical and Applied Genetics, 90: 247-252.

Ekanayake I. J, A. G. O. Dixon, M. C. M. Porto. 1996. Performance of various cassava clones in the dry savanna region of Nigeria. In: Kurup G. T., M. S. Palaniswamy, V. R. Potty, G. Padmaja, S. Kabeerathamma, and S. V. Pillai (eds), Tropical Tuber Crops: Problems. Prospects and Future Strategies, pp. 207—215. Oxford & IBH Publishing Co., New Delhi. India.

Ekanayake, I.J., D.S.O. Osiru, M.C.M. Porto. 1997. Morphology of cassava. IITA Research Guide 55, pp. 33. IITA, Ibadan, Nigeria.

Eliane C. G.V, I. Schuster, M. Pileggi, C. A. Scapim, H.B. C. Molinari, C.J.M.Luiz, G. E. Vieira. 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. Journal of Plant Physiology, 164: 1367-1376

Elias M, D. McKey, O. Panaud, M.C. Anstett, T. Robert. 2001b. Traditional management of cassava morphological and genetic diversity by the Makushi Amerindians (Guyana, South America): Perspective for on-farm conservation of crop genetic resources. Euphytica, 120: 143-157.

Elias M. L. Penet, P. Vindry, D. MacKey, O. Panaud, T. Robert. 2001a. Unmanaged sexual reproduction and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (*Manihot esculenta* Crantz), in a traditional farming system. Molecular Ecology, 10: 1895–1907.

Elias M., O. Panaud, T. Robert. 2000. Assessment of genetic variability in a traditional cassava (*Manihot esculenta* Crantz) farming system, using AFLP markers. Heredity, 85: 219-230.

El-Sharkawy A. M. 2007. Physiological characteristics of cassava tolerance to prolonged drought in the tropics: Implications for breeding cultivars adapted to seasonally dry and semiarid environments. Brazilian Journal Plant Physiology, 19(4):257-286,

El-Sharkawy M.A, J.D Hesketh, H. Muramoto. 1965. Leaf photosynthetic rates and other growth characteristics among 26 species of *Gossypium*. Crop Science, 5:173-175

El-Sharkawy M.A, J.H. Cock. 1987. Response of cassava to water stress. Plant and Soil, 100:345–360.

El-Sharkawy M.A. 1993. Drought tolerant cassava for Africa, Asia and Latin America: breeding projects work to stabilize productivity without increasing pressures on limited natural resources. BioScience, 43:441–451.

El-Sharkawy M.A. 2004. Cassava biology and physiology. Plant Molecular Biology, 56:481–501

El-Sharkawy M.A. 2006. International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. Photosynthetica, 44:481–512

Emperaire L, F. Pinton, G. Second. 2001. Dinámica y manejo de la diversidad de las variedades de yuca del noroccidente amazónico (Brasil). Etnoecológica, 5:38-59 (Abstract in English).

Evans L.T, I.F. Wardlaw, R.W. King. 1985. Plants and environment: two decades of research at the Canberra phytotron. Botany Reviews, 51:203-272.

Falconer D. S, T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Essex, England

FAO. 1993. Project findings and recommendations. Technical report for project Ref. No. MLW/87/009 on strengthening cassava research and development in Malawi. Printed by UNDP and FAO, Rome. pp.29.

FAOSTAT. 2010. Food and Agriculture Organization of the United Nations. FAOSTAT. http://faostat.fao.org/ http://faostat.fao.org/ accessed on 23rd May, 2012

Ferguson, M. E., R. B. Jones, P. J. Bramel, C. Dominguez, C. Torre do Vale, J. Han. 2011. Post-flooding disaster crop diversity recovery: a case study of cowpea in Mozambique. Disasters, doi: 10.1111/j.1467-7717.2011.01242.x

Frankel O.H., A.H.D. Brown, J.J. Burdon. 1995. The conservation of plant biodiversity. pp. 299. Cambridge University Press.

Fregene M, A. Bernal, M. Duque, A.G.O. Dixon, J. Tohme. 2000. AFLP analysis of African cassava (*Manihot esculenta* Crantz) germplasm resistant to the cassava mosaic disease (CMD). Theoretical and Applied Genetics, 100: 678-685.

Fregene M, E. Okogbenin, C. Mba, F. Angel, M.C. Suarez, J. Guiterez, P. Chavarriaga, W. Roca M. Bonierbale, J. Tohme. 2001. Genome mapping in Cassava improvement: challenges, improvements and opportunities. Euphytica, 120: 159-165

Fregene M, F. Angel, R. Gomez, F. Rodriguez, P. Chavarriagga, W. Roca, J. Tohme, M. Bonierbale. 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). Theoretical and Applied Genetics, 95:431-441.

Fregene M. A., M. Suarez, J. Mkumbira , H. Kulembeka, E. Ndedya, A. Kulaya, S. Mitchel, U. Gullberg, H. Rosling, A. G. O. Dixon, R. Dean, S. Kresovich; 2003. Simple sequence repeat marker diversity in cassava landraces: genetic diversity and differentiation in an asexually propagated crop. Theoretical and Applied Genetics, 107:1083–1093.

Fregene M.A, J. Vargas, F. Angel, J. Tohme, R. Asiedu, M.O. Akoroda, W.M. Roca. 1994. Chloroplast DNA and nuclear ribosomal DNA variability in cassava (M. *esculenta* Crantz) and its wild relatives. Theoretical and Applied Genetics, 89: 719-727.

Fryer M.J, K. Oxborough, P.M. Mullineaux, N.R. Baker. 2002. Imaging of photo-oxidative stress responses in leaves. Journal of Experimental Botany, 53:1249–54.

Fukai S, G.L Hammer, 1987. A simulation model of the growth of the cassava crop and its use to estimate productivity in Northern Australia. Agricultural Systems, 23:237-257

Funk C, G. Senay, A. Asfaw, J. Verdin, J. Rowland, J. Michaelson, G. Eilerts, D. Korecha, R. Choularton. 2005. Recent drought tendencies in Ethiopia and equatorial-subtropical eastern Africa. Washington DC, *FEWS NET, 1: 1–13.*

Funk C, M.D. Dettinger, J.C. Michaelsen, J.P. Verdin, M. Brown, M. Barlow, A. Hoell. 2008. Warming of the Indian Ocean threatens eastern and southern African food security but could be mitigated by agricultural development. Proceedingds of National Academy of Science. USA, 105:11081-11086.

Futuyma D.J. 2005. Evolution. Sinauer Associates, Sunderland, Massachusetts. ISBN 0-87893-187-2

Garg A.K, J.K Kim, T.G Owens, A.P Ranwala, Y.D Choi, L.V Kochian, R.J. Wu 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proceedings of National Academy of Science, 99:15898–15903

Genstat. 2011. GENSTAT 14th Edition. Lawes Agricultural Trust.

Guo P, M. Baum, S. Grando, S. Ceccarelli, G. Bai, R. Li, M. K. , R. K. Varshney, A. Graner, J. Valkoun. 2009. Differentially expressed genes between drought-tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. Journal of Experimental Botany, 60(12): 3531–3544.

Gupta P.K, H.S. Balyan P.C. Sharma, B. Ramesh. 1996. Microsatellites in plants: a new class of molecular markers. Current Science, 70: 45–54.

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

Haggblade S, R. Dewina. 2010. Staple food prices in Uganda. Prepared for the Comesa policy seminar on "Variation in staple food prices: Causes, consequence, and policy options". Maputo, Mozambique, 25-26 January 2010 under the African Agricultural Marketing Project. http://aec.msu.edu/fs2/aamp/seminar_3/AAMP_Maputo_25_Uganda_ppr.pdf accessed on 23rd, July, 2012.

Hahn S, K. Bai, R. Asiedu. 1990. Tetraploids, triploids, and 2n pollen from diploid interspecific crosses with cassava. Theoretical and Applied Genetics, 79: 433-439.

Hahn S. K, E.R. Terry, K. Leuschener, I.O. Akobundu, C. Okali, R. Lal. 1979. Cassava improvement in Africa. Field Crops Research, 2:193-226.

Hahn S.K 1992. An overview of traditional processing and utilization of cassava in Africa. In: Cassava as Livestock Feed in Africa; Hahn S.K., L. Reynolds and G.N. Egbunike (Eds). Proceedings of the IITA/ILCA/University of Ibadan Workshop on the Potential Utilization of Cassava as Livestock Feed in Africa 14-18 November 1988 Ibadan, Nigeria

Hahn S.K, E.R. Terry, K. Leuschner. 1980. Breeding cassava for resistance to cassava mosaic disease. Euphytica, 29: 673-683.

Hahn, S. K. 1988. Overview of traditional processing and utilisation of cassava in Africa. In: Hahn, S.K., L. Reynolds and G.N. Egbunike (eds.). Proceedings of workshop of the potential utilisation of cassava as livestock feed in Africa, Ibadan, Nigeria. IITA/ILCA/Univ.pp.16-27.

Hallauer A.R, J.B. Miranda. 1988. Quantitative genetics in maize breeding. 2nd ed. Ames; Iowa State University Press, 1988. 468 p. ISBN 0-8138- 1522-3.

Hara M., S. Terashima, T. Kuboi. 2001. Characterization and cryoprotective activity of cold-responsive dehydrin from *Citrus unshiu*. Journal of Plant Physiology, 158, 1333–1339.

Hazen S.P, M.S. Pathan,A. Sanchez. 2005. Expression profiling of rice segregating for drought tolerance QTLs using a rice genome array. Functional Integrated Genomics, 5:104–116.

Hedrick P.W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution ,53:313-318

Hernandez J. A, E. Olmos, E.F. Corpas, F. Sevilla, L.A. Del Rio. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. Plant Science, 105:151–67.

Hernandez J.A, A. Jimenez, P.M. Mullineaux, F.Seviela. 2000. Tolerance of pea (Pisumsativum L.) to long-term salt stress is associated to induction of antioxidant defences. Plant Cell Environment, 23:853–62.

Hershey C.H. 2005. Cassava Genetic Improvement: Theory and Practice. CIAT/Rockefeller.

Heschel M.S, C. Riginos. 2005. Mechanisms of selection for drought stress tolerance and avoidance in Impatiens capensis (Balsaminacae). American Journal of Botany 92: 37–44.

Hillocks R.J, M.D Raya, K. Mtunda, H. Kiozia. 2001. Effects of cassava brown streak virus disease on yield and quality of cassava in Tanzania. Journal of Phytopathology, 149:389–394

Hoisington D, C. Jiang, M. Khairallah, J.M. Ribaut, M. Bohn, A. Melchinger, M. Willcox, D. Gonzalez-de-Leon. 1996. QTL for insect resistance and drought tolerance in tropical maize: prospects for marker assisted selection. Symposium of the Society of Experimental Biology, 50:39–44.

http://www.ebi.ac.uk/Tools/clustalw2/index.html (accessed in June 2010)

http://www.ncbi.nlm.nih.gov(accessed in June 2010)

http://www.phytozome.net(accessed in June 2010)

Hu H, M. Dai, J. Yao, B. Xiao, X. Li, Q. Zhang, L. Xiong. 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proceedings of the National Academy of Sciences 103 (35): 12987-12992

Iglesias C, T. Sanchez, H.H. Yeoh. 2002. Cyanogens and linamarase activities in storage roots of cassava plants from breeding programme. Journal of food composition and analysis, 15: 379-387.

Iglesias C.A, J. Mayer, A.L. Chavez, F. Calle. 1997. Genetic content in cassava roots. Euphytica, 94: 367–373.

IITA. 2001. Cassava. IITA/Crops. http://www.iita.org/crop/cassava

Ito Y, K. Katsura, K. Maruyama, T. Taji, M. Kobayashi, M. S Shinozaki. 2006. Functional analysis of rice *DREB1/CBF* in cold-responsive gene expression in transgenic rice. Plar

Izawa T, R. Foster, N.H. Chua. 1993. Plant bZIP protein DN Molecular Biology, 230: 1131-1144.

Janssens. M. 2001. Cassava, *Manihot esculenta* Crantz. In c Romain H. Raemaekers (Eds). Directorate General for Karmelietenstraat 15-Rue des petits Carmes 15, B-1000 E

Jennings, D.L. 1979. Cassava. In: Evolution of Crop plants. London, pp 81-84.

Jorge M.A.B. 1995. Germplasm collection and characterisatic Mozambique. Roots, 2: 1-4.

Jorge V, M. Fregene, C.M.Velez, M.C. Duque, J. Tohme, V. V resistance to Xanthomonas asonopodis pv. *Manihot*is ir Genetics, 102, 564-571.

Jos J.S, S.G. Nair. 1979. Pachytene pairing in relation to pollen Cytologia (Tokyo), 44:813-820.

Juqiang Y, C. He, J. Wang, Z. Mao, S. A. Holaday, R. D. Alle of the arabidopsis 14-3-3 protein GF14λ in cotton lead improves stress tolerance under moderate drought condi 1007–1014

Kalinowski S.T. 2005. Do polymorphic loci require large distances? Heredity, (94): 33-36

Kamara A.Y, A. Menkir, B. Badu-Apraku, O. Ibikunle. 2003. growth, yield and yield components of some maize Sciences, 141: 43-50.

Kapinga, R.N, N. Mlingi, H. Rosling. 1997. Reason for use of African Journal of Tropical Root Crops, 2:81-84.

Karp A. S, S. Kresovich, K.V. Bhat, W.G. Ayad, T. Hodg genetic resources conservation: a guide to the techr

International Plant Genetic Resources Institute, Rome, Italy. Available at URL http://198.93.227.125/publicat/techbull/TB2.pdf.

Kavi Kishor P.B, Z. Hong, G. H Miao, C. A.A. Hu, D.P.S. Verma, 1995. Overexpression of increases proline production and confers osmotolerance in transgenic plants. Plant Physiology, 108:1387-1394.

Kawano K, K.Narintaroporn, P. Narintaroporn, S. Sukarharm, A. Limsila, J. Limsila, D. Suparhan, V. Sawarat, W. Watanonta. 1998. Yield improvement in a multistage breeding program for cassava. Crop Science, 38: 325-332

Kawano K, W.M. F Goncalves, U. Cenpukdee. 1987. Genetic and environmental effects on dry matter content of cassava root. Crop Science, 27:69–74.

Kawano, K. 1990. Harvest index and evolution of major food crops cultivars in the tropics. Euphytica 46:195-202.

Kawano K. 2003. Thirty years of cassava breeding for productivitybiological and social factors for success. Crop Science, 43: 1325-1335.

Kawuki R.S, M. Ferguson, M.T. Labuschagne, L. Herselman, J. Orone, I. Ralimanana, M. Bidiaka, S. Lukombo, M.C. Kanyange, G. Gashaka, G. Mkamilo, J. Gethi, H. Obiero. 2011. Variation in qualitative and quantitative traits of cassava germplasm from selected national breeding programmes in sub-Saharan Africa. Field Crops Research 122:151–156

Kawuki S. R, M. Ferguson, M. Labuschagne, L. Herselman, D. J. Kim. 2009. Identification, characterisation and application of single nucleotide polymorphisms for diversity assessment in cassava (*Manihot esculenta* Crantz). Molecular Breeding, 23:669-684

Kendall E.J, McKersie B.D. 1989. Free radical and freezing injury to cell membranes of winter wheat. Plant Physiology, 76:86–94.

Kiwanuka S, J. and Kintu. 2004. Seed security in Uganda: current status, issues and challenges. Uganda Journal of Agricultural Sciences, 9: 17-22.

Kizito E, A. Bua, M. Fregene, T. Egwang, U. Gullberg, A. Westerberg. 2005. The effect of cassava mosaic disease on the genetic diversity of cassava in Uganda. Euphytica, 146: 45-54.

Kizito, E.B. 2006. Genetic and root growth studies in cassava (*Manihot esculenta* Crantz). Implications for breeding. PhD thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Koch B, V. Nielsen, B. Halkier, C. Olsen, B.L. Moller.1992. The biosynthesis of cyanogenic glycosides in seedlings of cassava (*Manihot esculenta* Crantz). Archives of Biochemistry and Biophysiology, 292: 141-150.

Kotchoni O. S, C. Kuhns, A. Ditzer, H.H. Kirch, D. Bartels. 2006. Over-expression of different *aldehyde dehydrogenase* genes in Arabidopsis thaliana confers tolerance to abiotic stress and protects plants against lipid peroxidation and oxidative stress. Plant, Cell and Environment, 2: 1033-1048.

Kramer P.J, J.S, Boyer. 1995. Water relations of plants and soils. San Diego: Academic Press, 1995. 495 p.

Kramer P.J. 1981. Carbondioxide concentration, photosynthesis, and dry matter production. BioScience, 31:29-33.

Kulembeka H. P. K. 2010. Genetic linkage mapping of field resistance to Cassava Brown Streak Disease in cassava (*Manihot esculenta* Crantz) Landraces from Tanzania. PhD Thesis, University of the Free State Bloemfontein, South Africa

Lahai M.T, J.B. George, I.J. Ekanayake. 1999. Cassava (*Manihot esculenta* Crantz) growth indices, root yield and its components in upland and inland valley ecologies of Sierra Leone. Journal of Agronomy and Crop Science, 182: 239-247.

Lahai T. M, I. J. Ekanayake. 2009. Accumulation and distribution of dry matter in relation to root yield of cassava under a fluctuating water table in inland valley ecology. African Journal of Biotechnology, 8 (19): 4895-4905

Lancaster P. A., J. E. Brooks. 1983. Cassava leaves as human food. Economic Botany, 37: 331-348.

Lane B.G, F. Bernier, E. Dratewka-Kos, R. Shafai, T.D. Kennedy, C. Pyne, J.R. Munro, T. Vaughan, D. Walters, Altomare. 1991. Homologies between members of the germin family in hexaploid wheat and similarities between these wheat germins and certain Physarumspherulins. Journal of Biological Chemistry, 266:10461–10469.

Laporte M, B. Shen, M.Tarczynski. 2002. Engineering for drought avoidance: expression of maize NADP-malic enzyme in tobacco results in altered stomatal function. Journal of Experimental Botany, 53:699-705.

Lefevre F. and Charrier A. 1993. Isozyme diversity within African Manihot germplasm. Euphytica 66: 73–80.

Lenis J.I, F. Calle, G. Jaramillo, J.C. Perez, H. Ceballos, J.H. Cock. 2006. Leaf retention and cassava productivity. Field Crops Research, 95:126-134.

Lievens S, S. Goormachtig, M. Holsters. 2001. A critical evaluation of differential display as a tool to identify genes involved in legume nodulation. Looking back and looking forward. Nucleic Acids Research, 29: 3459-3468

Liu K, S.V. Muse. 2005. PowerMarker: Integrated analysis environment for genetic marker data analysis. Bioinformatics, 21(9): 2128-2129.

Liu X, W.V. Baird. 2004. Identification of a novel gene, *HAABRC5*, from Helianthus annuus (*Asteraceae*) that is upregulated in response to drought, salinity, and abscisic acid. American Journal of Botany, 91: 184–191.

Liu Z, G.R. Furnier. 1993. Comparison of allozyme, RFLP and RAPD for revealing genetic variation within and between trembling aspen and big tooth aspen. Theoretical and Applied Genetics, 87: 97-105.

Liu Z.W, R.M. Biyashev, M.A.S. Maroof. 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theoretical and Applied Genetics, 93:869–876

Lokko Y, E.Y. Danquah, S.K. Offei, A.G.O. Dixon, M.A. Gedil. 2005. Molecular markers associated with a new source of resistance to the cassava mosaic disease. African Journal of Biotechnology, 49: 873-881.

Lokko Y, J.V. Anderson, S. Rudd, A. Raji, D. Horvath, M.A. Mikel, R. Kim, L. Liu, A. Hernandez, A.G.O. Dixon, I.L. Ingelbrecht. 2007. Characterization of an 18,166 EST dataset for cassava (*Manihot esculenta*) enriched for drought-responsive genes. Plant Cell Reproduction, 26:1605- 1618.

Long S.P, E.A. Ainsworth, A.D.B Leakey, J. Nösberger, D.R. Ort. 2006. Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentration. Science, 312:1918-1921.

Lozano J. C. 1986. Cassava bacterial blight: a manageable disease. Plant Disease Reporter, 70: 1089-1093.

Magoon M.L, R.K. Krishnan. 1977. Extending the frontiers of genetic improvement in cassava. In: Leaky C.L.A. (ed.), Proc. 3rd Symposium, ISTRC-AB, IITA, Ibadan Nigeria. Dec 2–9 1973, pp. 14–18.

Manosh K. B,. M. A.A. Mondal, M. Hossain, R. Islam. 2008. Utilization of genetic diversity and its association with heterosis for progeny selection in potato breeding programs. American-Eurasian Journal of Agriculture and Environmental Sciences, 3 (6): 882-887.

Martin J.M., T.K. Blake, E.A. Hockett. 1991. Diversity among North American spring barley cultivars based on coefficient of parentage. Crop Science, 31: 1131-1137.

Mba, R.E.C., P. Stephenson, K. Edwards, S. Melzer, J. Mkumbira, U. Gullberg, K. Apel, M. Gale, J. Tohme, M. Fregene. 2001. Simple sequence repeat (SSR) markers survey of the cassava

(*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava. Theoretical and Applied Genetics, 102: 21-31.

McGregor, C.E., C.A. Lambert, M.M. Greyling, J.H. Louw, L. Warnich. 2000. A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (Solanum tuberosum L.) germplasm. Euphytica, 113: 135-144.

McKersie B.D, Y. Chen, M. De Beus, S.R. Bowley, C. Bowler. 1993. Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (*Medicago sativa* L.). Plant Physiology, 103:1155–63.

McMahon, J.M., W.L.B. White, R.T. Sayre. 1995. Cyanogenesis in cassava (*Manihot esculenta* Crantz). Journal of Experimental Botany, 46:731-714.

Messmer M.M, A.E. Melchinger, R.G. Hermann, J. Boppenmair. 1993. Relationship among early European maize inbreds: II. Comparison of pedigree and RFLP data. Crop Science 33: 944-950.

Meuwissen T.H.E, B.J. Hayes, M.E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819–1829.

Mignouna H.D, A.G.O. Dixon. 1997. Genetic relationships among cassava clones with varying levels of resistance to the African cassava mosaic disease using RAPD markers. African Journal of Root and Tuber Crops, 2: 28-32.

Mignouna H.D, N.T.H. Ellis, M.R. Knox, R.Asiedu, Q.N. Ng. 1998. Analysis of genetic diversity in Guinea yams (*Discorea* spp.) using AFLP fingerprinting. Tropical Agriculture (Trinidad), 75: 224–229.

Mkumbira J. 2002. Cassava development for small scale farmers: approaches to breeding in Malawi. PhD Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden. Acta Universitatis Agriculturae Sueciae. Agraria 365. Tryck: SLU Service/Repro, Uppsala 2002.

Mkumbira, J., L. Chiwona-Karltun, U. Langercrantz, N. Mahungu, J. Saka, A. Mhone, M. Bokanga, , L. BrimerU. Gullberg, H. Rosling. 2003. Classification of cassava into 'bitter' and 'cool' in Malawi: From farmers' perception to characterization by molecular markers. Euphytica, 132:7-12.

Mohammadi S, B. Prasanna. 2003. Review and interpretation. Analysis of genetic diversity in crop plants. Crop Science, 43: 1235-1248.

Montero-Rojas, M., A.M. Correa, D. Siritunga. 2011. Molecular differentiation and diversity of cassava (*Manihot esculenta*) taken from 162 locations across Puerto Rico and assessed with microsatellite markers, AoB PLANTS 2011 plr010 doi:10.1093/aobpla/plr010

Morante N, T. Sanchez, H. Ceballos, F. Calle, J.C. Perez, C. Egesi, C.E. Cuambe, A.F. Escobar, D. Ortiz, A.L. Chavez, M. Fregene. 2010. Tolerance to post-harvest physiological deterioration in cassava roots. Crop Science, 50:1333–1338

Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. Annual Reviews of Plant Physiology, 35, 299–319.

Morgan, J.M. 1991. A gene controlling differences in osmoregulation in wheat. Australian Journal of Plant Physiology. 18, 249–257.

Morsy M.R, A.M. Almutairi, J Gibbons, S.J. Yun B.G. de Los Reyes. 2005. The *OsLti6* genes encoding low-molecular weight membrane proteins are differentially expressed in rice cultivars with contrasting sensitivity to low temperature, Gene, 344:171–180.

Moyib O, O. Odunola, A. Dixon. 2007. SSR markers reveal genetic variation between improved cassava cultivars and landraces within a collection of Nigerian cassava germplasm. African Journal of Biotechnology, 6: 2666-2674.

Mutegi-Murori-Murori R.W. 2009. Towards identifying the physiological and molecular basis of drought tolerance in cassava (*Manihot esculenta* Crantz). PhD Thesis. Georg-August University Gottingen. pp147

Nassar N, R. Ortiz. 2010. Breeding cassava to feed the poor. Scientific American 302:78–84

Nassar N.M.A. 1997. Development of cassava interespecific hybrids for savana (cerrado) conditions. Journal of Root Crops, 22:9-17.

Nassar, M.A. 2000. Wild cassava, *Manihot* spp.: Biology and potentialities for genetic improvement. Genetics and Molecular Research, 23 (1): 201-212

Nassar, M.A. 2002. Cassava, (*Manihot esculenta* Crantz), genetic resources: origin of the crop, its evolution and relationships with wild relatives. Genetics and Molecular Research, 1: 298-305.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of National Academy of Sciences, USA, 70:3321-3323.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

Ngendahayo M, A.G.D.O. Dixon, 2001. Effect of harvest on tuber yield, dry matter, starch and harvest index of cassava in two ecological zones in Nigeria. In: Akoroda, M.O. and J.M. Ngeve (eds.). Root Crops in the Twenty first Century. Proceedings of the Seventh Triannual Symposium of the International society for Tropical Root Crops- Africa Branch (ISTRC-

AB) held at Centre International des Conférences, Cotonou, Bénin 11-17 October 1998. pp.661-667.

Nguyen, T.T., N. Klueva, V. Chamareck, A. Aarti, G. Magpantay, A.C. Millena, M.S. Pathan. H.T. Nguyen. 2004. Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. Molecular Genetics and Genomics, 272: 35–46.

Nweke F. 2009. Controlling Cassava Mosaic Virus and Cassava Mealybug in Sub-Saharan Africa. IFPRI Discussion Paper 00912. 2020 Vision Initiative. International Food Policy Research Institute (IFPRI). http://www.ifpri.org/sites/default/files/[illegible]) accessed on 12[th] April, 2012.

Nweke F.I. 1994. Farm level practices relevant to cassava plant protection. African Crop Science Journal, 2: 563-582.

OCHA. 2008. Eastern Africa preparedness and response to drought and impact of soaring food Prices. Summary report presented to the regional humanitarian partnership team (RHPT) and Donors, Nairobi. Accessed on 25[th] July, 2012 at http://www.google.co.kr/url?sa=t&rct=j&q=impact%20[illegible]20in%20east%20africa&source=web&cd=3&[illegible]un.org%2FOchaLinkClick.aspx%3Flink%3D[illegible]QXc04CYBQ&usg=AFQjCNGW1OOORIHeRStGuETF3X[illegible]

Oh S.J, S.I. Song, Y.S. Kim, H.J. Jang, S.Y. Kim, M. Kim, Y.K. Kim, B.H. Nahm, . J.K. Kim. 2005. Arabidopsis *CBF3/DREB1A* and *ABF3* in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiology, 138: 341–351.

Okogbenin E, T. Setter, M. Ferguson, R. Mutegi. H. Ceballos. B. Olasanmi and M. Fregene. 2013. Phenotypic approaches to drought in cassava: review. Frontiers in physiology volume 4. article 90: doi:10.3389/fphys.2013.00093

Okogbenin E, I.J. Ekanayake, M.C.M. Porto. 2003. Genotypic variability in adaptation responses of selected clones of cassava to drought stress in the Sudan savanna zone of Nigeria. Journal of Agronomy and Crop Science, 189, 376-389.

Okogbenin E, M. Fregene. 2002. Genetic analysis and QTL mapping of early root bulking in an F1 population of non-inbred parents in cassava (*Manihot esculenta* Crantz). Theoretical and Applied Genetics, 106: 58-66.

Okogbenin E, T.L. Setter, M. Ferguson, R. Mutegi-Murori, A.C. Alves, H. Ceballos, M. Fregene. 2011. Phenotyping cassava for adaptation to drought. In: Monneveux P, J-M Ribaut, (eds). Drought Phenotyping in Crops: From Theory to Practice. CIMMYT/Generation Challenge Programme, Mexico City, pp 381-400.

Oliver M.J, Z. Tuba, B.D. Mishler. 2000. The evolution of vegetative desiccation tolerance in land plants. Plant Ecology, 151, 85–100.

Olsen K.M, B.A. Schaal. 1999. Evidence on the origin of cassava: phylogeography of *Manihot esculenta*. Proceedings of the National Academy of Sciences (USA), 96: 5586-5591.

Olsen K.M, B.A. Schaal. 2001. Microsatellite variation in cassava (*Manihot esculenta*, Crantz) and its wild relatives: Further evidence for a southern Amanzonian origin of domestication. American Journal of Botany, 88:131–142.

Olsen, K.M. 2004. SNPs, SSRs and inferences on cassava's origin. Plant Molecular Biology, 56: 517-526.

Onwuene I.C. 1978. The Tropical Tuber Crops: Yams, Cassava, Sweetpotato, Cocoyams. John Wiley, Sons Ltd. pp 234.

Otim-Nape, G.W, S. Zziwa. 1990. Cassava as a major staple food crop in Uganda. Working Paper No. 1. Collaborative Study of Cassava in Africa. Namulonge Research station, Kampala, Uganda.

Pardales J.R Jr, A. Yamauchi, D.V. Belmonte Jr, C.B Esquibel. 2001. Dynamics of root development in root crops in relation to the prevailing moisture stress in the soil. In Proceedings of the Sixth Symposium of the International Society of Root Research, Nagoya, Japan, pp 72-73

Pardales J.R Jr, C.B Esquibel. 1996. Effect of drought during the establishment period on the root system development of cassava. Japanese Journal of Crop Science, 65(1): 93-97

Parvanova D, S. Ivanov, T. Konstantinova, E. Karanov, A. Atanassov, T. Tsvetkov. 2004. Transgenic tobacco plants accumulating osmolytes show reduced oxidative damage under freezing stress. Plant Physiology and Biochemestry, 42:57–63.

Patil B.L, C.M. Fauquet. 2009. Cassava mosaic geminiviruses: actual knowledge and perspectives. Molecular Plant Pathology, 10:685–701

Patterson H.D, S.T.C. Weatherup. 1984. Statistical criteria for distinctiveness between genotypes of herbage crops. Journal of Agronomy and Science, 102: 59-68.

Peakall R, P. E Smouse. 2006. GENALEX 6. Genetic analysis in Excel. Population genetic software for teaching and Research. Molecular Ecology Notes, 6:288-295.

Pellegrineschi A, L.M. Noguera, B. Skovmand, R.M. Brito, L. Velazquez, M.M. Salgado, R. Hernandez, M. Warburton, D. Hoisington. 2002. Identification of highly transformable wheat genotypes for mass production of fertile transgenic plants. Genome, 45:421–430.

Pellegrineschi A, M. Reynolds M. Pacheco R.M. Brito, R. Almeraya, K. Yamaguchi-Shinozaki, D. Hoisington. 2004. Stress-induced expression in wheat of the Arabidopsis thaliana *DREB1A* gene delays water stress symptoms under greenhouse conditions. Genome. 47:493–500.

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

Perez J. C, J. I. Lenis, F. Calle, N. Morante, T. Sanchez, D. Debouck, H. Ceballos. 2011. Genetic variability of root peel thickness and its influence in extractable starch from cassava (*Manihot esculenta* Crantz) roots. Plant Breeding, doi:10.1111/j.1439-0523.2011.01873.x: pg 1-6

Perrier X, J.P. Jacquemound-Collet. 2006. DARwin software. http://darwin.cirad.fr/darwin

Pfaffl M.W, M. Hageleit. 2001. Validities of mRNA quantification using recombinant RNA and recombinant DNA external calibration curves in real-time RT-PCR. Biotechnology Letter. 23:275- 282.

Pfaffl M.W., G.W. Horgan, L. Dempfle. 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research 30:e36

Prasad P.V.V, S.A Staggenborg, Z. Ristic. 2008. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In: Response of Crops to Limited Water: Understanding and Modeling Water Stress Effects on Plant Growth Processes (pp 301–355). Ahuja LR, Reddy VR, Saseendran SA & Yu Q (eds). Advances in Agricultural Systems Modeling Series 1. American Society of Agronomy: Madison, WI, USA.

Price A.H, N. Atherton, G.A.F Hendry. 1989. Plants under drought stress generate activated oxygen. Free Radical Research Communication, 8:61-66.

Prince J.P, V.K. Lackney, C. Angeles, J.R. Blauth, M.M. Kyle. 1995. A survey of DNA polymorphism within the genus Capsicum and fingerprinting of pepper cultivars. Genome, 38: 224-231.

Raheem D, C. Chukwuma. 2001: Foods from cassava and their relevance to Nigeria and other African countries. Agriculture and Human Values, 18:383-390.

Raji A.A., A.G.O. Dixon T.A.O. Ladeinde. 2007. Agronomic traits and tuber quality attributes of farmer grown cassava (*Manihot esculenta*) landraces in Nigeria. Journal of Tropical Agriculture, 45, 9–13.

Raji A.A.J, I. Fawole M. Gedil, A.G.O. Dixon. 2009. Genetic differentiation analysis of African cassava (*Manihot esculenta*) landraces and elite germplasm using amplified fragment length polymorphism and simple sequence repeat markers. Annals of Applied Biology, 155:187–199.

Ramachandra-Reddy A, K.V. Chaitanya, M. Vivekanandan. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology, 161:1189-1202.

Ramanjulu S, D. Bartels. 2002. Drought and desiccation-induced modulation of gene expression in plants. Plant, Cell and Environment, 25: 141–151

Reilly K, D. Bernal, D. F. Cortes, R. Gomez-Vasquez, J. Tohme, J. R. Beeching. 2007. Towards identifying the full set of genes expressed during cassava post-harvest physiological deterioration. Plant Molecular Biology, 64: 187-203.

Resende A, P. Filho, M. Machado. 2004. Esterase polymorphism marking cultivars of *Manihot esculenta*, Crantz. Brazilian Archives of Biology Technology, 47: 347–353.

Rivero R. M, M. Kojima, A. Gepstein, H. Sakakibara, R. Mittler, S. Gepstein, E. Blumwald. 2007. Delayed leaf senescence induces extreme drought tolerance in a flowering plant Proceedings of National Academy of Sciences USA, 104(49): 19631–19636.

Roa, A.C, M.M. Maya, M.C. Duque, J. Tohme, A.C. Allem, M.W. Bonierbale. 1997. AFLP analysis of relationships among cassava and other *Manihot* species. Theoretical and Applied Genetics, 95:741-750.

Roder M.S, V. Korzun, K. Wendehake, J. Plaschke, M.H Tixier, P. Leroy, M.W. Ganal. 1998. A microsatellite map of wheat. Genetics, 149:2007–2023

Rogers D.J, S.G. Appan. 1973. *Manihot* and *Manihotoides* (*Euphorbiaceae*), a computer assisted study. Flora Neotropica, Monograph No. 13, pp. 272. Hafner Press, New York.

Russell R.J., J.D. Fuller, M. Macaulay, B.G. Hats, A. Jahoor, W. Powell, R. Waugh, 1997. Direct comparison of levels of variation among barley genotypes detected by RFLPs, AFLPs, SSRs and RAPDs. Theoretical and Applied Genetics, 95: 714-722.

Saini H.S, M.E. Westgate. 2000. Reproductive development in grain crops during drought. Advances in Agronomy, 68: 59–96.

Sakai T, Y. Nakagawa-Murata, I. Uritani, E. S. Data. 1994: Occurrence and characteristics of stress metabolites in cassava roots. In: I. Uritani, V. V. Garcia, and E. M. T. Mendoza (eds), Postharvest Biochemistry of Plant Food Materials in the Tropics, pp. 95-110. Japan Science Society Press, Tokyo.

Sanchez G, S. Restrepo, M. Dugue, M. Fregene, M. Bonierbale, V. Verdier. 1999. AFLP assessment of genetic variability in cassava genotypes (*Manihot esculenta* Crantz) resistant and susceptible to cassava bacterial blight (CBB). Genome, 42: 163-172.

Santisopasri V, K. Kurotjanawong, S. Chotineeranat, K. Piyachomkwan, K. Sriroth, C.G. Oates 2001. Impact of water stress on yield and quality of cassava starch. Industrial Crops and Products, 13:115-29.

Schaal B.A, K.M. Olsen, L.J.C.B. Carvalho. 2006. Evolution, domestication and agrobiodiversity in tropical crop cassava. In: Motley, T.J., N. Zerega and H. Cross (Eds.); Darwin's harvest: New approaches to the origins, evolution and conservation of crops, pp. 269-283. Colombian University Press, New York.

Schindler U, A.E. Menkens, H. Beckmann, J.R. Ecker, A.R. Cashmore. 1992. Heterodimerization between light-regulated and ubiquitously expressed Arabidopsis thaliana GEF bZIP proteins. Embo Journal, 4:1261-1273.

Schut J.W, X. Qi, P. Stam. 1997. Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. Theoretical and Applied Genetics, 95: 1161–1168.

Seki M, M. Narusaka, H. Abe, M.Kasuga, K.Yamaguchi-Shinozaki, P. Carninci, Y.Hayashizaki, K. Shinozaki. 2002. Monitoring the expression pattern of Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell, 13, 61–72.

Semagn K, Å. Bjørnstad, M.N Ndjiondjop. 2006. An overview of molecular marker methods for plants. African Journal of Biotechnology, 25: 2540-2569.

Sen Gupta A, R.P Webb, A.S Holaday, R.D Allen. 1993. Over-expression of *superoxide dismutase* protects plants from oxidative stress. Plant Physiology, 103:1067–73.

Shao H.B, Z.S Liang, M.A Shao, B.C. Wang. 2005. Changes of anti-oxidative enzymes and membrane peroxidation for soil water deficits among 10 wheat genotypes at seedling stage. Colloids Surf B Biointerfaces, 10;42(2):107-13.

Shinozaki K, K. Yamaguchi-Shinozaki. 2007. Gene networks involved in drought stress response and tolerance: Journal of Experimental Botany, 58(2): 221–227

Simwambana, M.S.C., S.K. Hahn, T.U. Ferguson. 1996. Morphological changes in cassava as affected by leaf vegetable picking. Roots, 3: 5-7.

Skovgard H, J. Tomkiewicz, G. Nachman, M. Munster-Swendsen. 1993. The effect of the cassava green mite *Mononychellus tanajoa* on the growth and yield of cassava *Manihot esculenta* in a seasonally dry area in Kenya. Experimental and Applied Acarology, 17:41–58

Smith J.S.C, O.S. Smith. 1992. Fingerprinting of crop genotypes. Advances in Agronomy 47: 85-140

Smith J.S.C. 1984. Genetic variability within U.S. hybrid maize: Multivariate analysis of isozyme data. Crop Science, 24: 1041-1046.

Soltis D.E, P.S. Soltis. 2003. The role of phylogenetics in comparative genetics. Plant Physiology. 132:1790–1800

Sree Lekha S, S.V. Pillai, J. S. Kumar.2010: Molecular genotyping of Indian cassava cultivars using SSR markers: Advanced Environmental Biology. 4(2): 224-233.

Ssemakula G, A. Dixon. 2007. Genotype X environment interaction, stability and agronomic performance of carotenoid-rich cassava clones. Scientific Research and Essay, 2 (9), pp. 390-399,

Stroup W. W. 1990. Nearest neighbor adjusted best linear unbiased prediction in field experiments. Proceedings of the 1990 Kansas State University Conference on Applied Statistics in Agriculture, 169-184.

Subere J. O.Q, D. Bolatete, R. Bergantin, A. Pardales, J. J. Belmonte, A. Mariscal, R.Sebidos, A. Yamauchi. 2009. Genotypic variation in responses of cassava (*Manihot esculenta Crantz*) to drought and rewatering: Root system development. Plant Production Science, 12(4): 462-474

Talame V, N.Z. Ozturk, H.J. Bohnert, R. Tuberosa. 2007. Barley transcript profiles under dehydration shock and drought stress treatments: a comparative analysis. Journal of Experimental Botany, 58:229–240.

Tanksley, S.D., N.D. Young, A.H. Paterson, M.W. Bonierbale. 1989. RFLP mapping in plant breeding: New tools for an old science. Biotechnology, 7: 257-264.

Taramino G, S. Tingley, 1996. SSR for germplasm analysis and mapping in maize. Genome. 39: 277-287.

Thomashow M.F. 1999. Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annual Review of Plant Biology. 50:571-599

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

Timmer C. P. 2003. Biotechnology and food systems in developing countries. Journal of Nutrition. 133(11): 3319-3322

Tonukari N.J. 2004. Cassava and the future of starch. Electronic Journal of Biotechnology http://www.ejbiotechnology.info/content/vol7/issue1/issues/2/. ISSN: 0717-3458. (accessed on 03 March 2009).

Turyagyenda F. L, E.B. Kizito, Y. Baguma, D. Osiru. 2013. Evaluation of Ugandan cassava germplasm for drought tolerance. International Journal of Agriculture and Crop Sciences. IJACS/2013/5-3/212-226

Turyagyenda L.F, E.B. Kizito, M.E. Ferguson, Y. Baguma, J.W. Harvey, P. Gibson, B.W. Wanjala and D.S.O. Osiru. 2012. Genetic Diversity among farmer-preferred cassava landraces in Uganda. African Crop Science Journal, 20: 15-30

Tylleskär T, R. Cooke M. Banea, N. Poulter, N. Bokanga, H. Rosling. 1992. Cassava cyanogens and konzo, an upper motor neuron disease found in Africa. Lancet, 339, 208- 211

Umezawa T, M. Fujita, Y. Fujita, K. Yamaguchi-Shinozaki, K. Shinozaki. 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Current Opinion in Biotechnology, 17: 113–122.

United Nations. 2010. Millennium development goals. UN Department of Public Information, Rome. http://www.un.org/millenniumgoals. Accessed Novembr, 2011

USAID, United States Agency for International Development. 2010. Market assessment and baseline study of staple foods. Country Report-Uganda. Accessed at http://pdf.usaid.gov/pdf_docs/PNADW642.pdf on 23rd, July, 2012.

Van Breusegem F, L. Slooten, J. Stassart, T. Moens, J. Botterman, M. Van Motagu, D. Inze. 1999. Overproduction of Arabidopsis thaliana *FeSOD* confers oxidative stress tolerance to transgenic maize. Plant Cell Physiology, 40:515–23.

Verslues P. E, M. Agarwal, S. Katiyar-Agarwal, J. Zhu, J.K. Zhu. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant Journal, 45: 523–539

Vicre M, J.M. Farrant, A. Driouich. 2004. Insights into the cellular mechanisms of desiccation tolerance among angiosperm resurrection plant species. Plant Cell Environment, 27, 1329–1340.

Vinocur B, A. Altman. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Current Opinion in Biotechnology, 16(2): 123–132.

Vries De C.A., 1985. Optimum harvest time of cassava (Manihot esculenta Crantz). Abstract on Tropical Agriculture, 10: 9-14.

Wajid A.J, J.B, Muhammad, M.B, Kumbhar, N.U, Khan, M.I, Kerio. 2011. Effect of water stress on physiological and yield parameters at anthesis stage in elite spring wheat cultivars. Sarhad Journal of Agriculture 27(1): 59-65.

Wang Fang-Zheng, Q. B. Wang, S.Y. Kwon, S.S. Kwak, W.A. Su. 2005. Enhanced drought tolerance of transgenic rice plants expressing a pea *manganese superoxide dismutase*. Journal Plant Physiology, 162:465-472.

Wang Z.Y, J.L. Weber, G. Zhong, S.D. Tanksley, 1994. Survey of plant short tandem DNA repeats. Theoretical and Applied Genetics, 88: 1-6.

Westby A. 2002. Cassava utilization, storage and small-scale processing. In: Cassava: biology, production and utilization (Hillocks RJ, Thresh JM and Bellotti AC, eds). CABI Publishing, New York, USA, pp 281–300.

World Wide Fund for Nature (WWF). 2006. Climate Change Impacts on East Africa. A Review of the Scientific Literature. Accessed April 2012 at http://www.wwf.or.jp/activities/lib/pdf/climate/environment/east_africa_climate_change_impacts_final.pdf

Wright S. 1978. Evolution and the genetics of population, variability within and among natural populations. The University of Chicago Press, Chicago.

Wu K.S, S.D. Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. Molecular Genetics and Genomics, 241:225-235.

Xiong L, K.S Schumaker, J.K Zhu. 2002. Cell signaling during cold, drought, and salt stress. Plant Cell 14: 165–183

Xu D, X. Duan, B. Wang, B. Hong, T.H.D Ho, R.Wu. 1996. Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiology, 110: 249-257.

Xu D.Q, J. Huang, S.Q. Guo, X. Yang, Y.M. Bao, H.J. Tang, H.S. Zhang. 2008. Over-expression of a TFIIIA-type zinc finger protein gene *ZFP252* enhances drought and salt tolerance in rice (*Oryza sativa* L.). FEBS Letters, 582:1037-1043.

Yamaguchi-Shinozaki K, M. Koizumi, S. Urao, K. Shinozaki. 1992. Molecular cloning and characterization of 9 cDNAs for genes that are responsive to desiccation in Arabidopsis thaliana: Sequence analysis of one cDNA clone that encodes a putative transmembrane channel protein. Plant Cell Physiology, 33(3): 217-224.

Yan J. C. H, J. Wang, Z. Mao, S. A. Holaday, R. D. Allen, H. Zhang. 2004. Overexpression of the Arabidopsis 14-3-3 Protein *GF14λ* in cotton leads to a "stay-green" phenotype and improves

stress tolerance under moderate drought conditions. Plant Cell Physiology, 45(8): 1007–1014

Yancey, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus, G.N. Somero. 1982. Living with water stress: evolution of osmolyte systems. Science, 217, 1214–1222.

Yang J, A. Dong, P. D. Zhang. 2011. Expression profiling of cassava storage roots reveals an active process of glycolysis/gluconeogenesis. Journal of Integrated Plant Biology, 53(3):193–211.

Yasunari F, M. Fujita, K. Shinozaki, K.Yamaguchi-Shinozaki. 2011. ABA-mediated transcriptional regulation in response to osmotic stress in plants. Journal of Plant Research. 124 (4):509-525.

Yong J. P, J. K. Lee, N.S. Kim. 2009. Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops. Molecules, 14: 4546-4569.

Zhang H.X, J.N. Hodson, J.P. Williams, E. Blumwald 2001. Engineering salt-tolerant *Brassica* plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. Proceedings of National Academy of Sciences, 98:12832–12836

Zhang J.Z, R.A Creelman, J.K. Zhu. 2004. From laboratory to field: Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. Plant Physiology, 135:615-621.

Zhang X, S. Zhou, Y. Fu, Z. Su, X. Wang, C. Sun. 2006. Identification of a drought tolerant introgression line derived from Dongxiang Common Wild Rice (*O. rufipogon Griff.*). Plant Molecular Biology, 62:247-259

Zhao S.H, F.Z . Wang, L. Lu, H.Y. Zhang, X.Y. Zhang. 2000. Breeding and selection of drought resistant and salt tolerant wheat variety Cang 6001. Acta Agriculturae Boreali-Sinica, 15:113–117

Zhu J.K, J. Liu, L. Xiong. 1998. Genetic analysis of salt tolerance in Arabidopsis: evidence for a critical role of potassium nutrition. Plant Cell, 10: 1181–1191.

Zhu J.K. 2000. Genetic analysis of plant salt tolerance using Arabidopsis. Plant Physiology, 124, 941-948.

Zimmermann P, N. Hirsch-Hoffmann, L. Henning, W. Gruissem. 2004. Genevestigator. Arabidopsis microarray database and analysis toolbox. Plant Physiology, 136:2621–2632.