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Towards Identifying the Physiological and Molecular Basis of Drought Tolerance in Cassava (*Manihot esculenta* Crantz)

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Towards Identifying the Physiological and Molecular Basis of Drought Tolerance in Cassava (*Manihot esculenta* Crantz)

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Dedication:

To
My husband Murori,
and children Makena and Muthomi

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Acronyms and Abbreviations

Abbreviation	Description
ABA	Abscisic acid
ABI	Applied Biosystems
AGB	above-ground fresh-biomass
ANOVA	Analysis of variance
BecA	Biosciences for eastern and central Africa
BMZ	German Federal Ministry for Economic Cooperation and Development
bp	base pair
CBSD	cassava brown streak disease
CE	capillary electrophoresis
CEC	Cation Exchange Capacity
CIAT	International Center for Tropical Agriculture
cM	centiMorgan
CMD	cassava mosaic disease
COSCA	Collaborative Study of Cassava in Africa
CP	cross pollinated
DAP	days after planting
DDPSC	Donald Danforth Plant Science Center
DM	dry matter content
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylene Diamine Tetra-acetic Acid
Embrapa	Brazilian Agricultural Research Corporation
ESSR	Expressed Simple Sequence Repeat
ETH	Swiss Federal Institute of Technology Zurich
GAUG	Georg-August University Goettingen
GCP	Generation Challenge Program
GDP	Gross Domestic Product
HI	harvest index
HLS	height of leafless stem

Abbreviation	Description
HPS	height of primary stem
HSS	height of secondary stem
IITA	International Institute of Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute
KARI	Kenya Agricultural Research Institute
LG	linkage group
LL	length of expanded leaf
LOD	logarithm of odds
LR	leaf retention
LW	width of expanded leaf
MAB	marker-assisted breeding
NARS	National Research Systems
NBL	number of branching levels
NPK	nitrogen phosphorous potassium
NPS	number of primary stems
NSR	number of storage roots
PCR	Polymerase Chain Reaction
QTL	quantitative trait loci
REC	recombination frequency
rfu	relative fluorescent units
SD	stem diameter
SRFW	storage root fresh weight
SSA	Sub-saharan Africa
SSR	Simple Sequence Repeat
TBE	Tris Borate EDTA
TE	Tris EDTA
Wa	weight in air
Ww	weight in water
ZIL	Swiss Center for International Agriculture

1. General Introduction

1.1. Background

In the 21st century, the world population is increasing at a high rate. The population is faced with a crisis that defines human development and links today and tomorrow. This crisis is climate change. Sub-Saharan African (SSA) countries, which are more prominent in increasing human population, are more vulnerable to climate change. This is because, they are located in the hot tropical regions, and they highly depend on climate-sensitive sectors such as agriculture, forestry and tourism. In addition, SSA countries not only have high poverty rates, but also limited financial, institutional and human capacity to adapt to climate change (Thomas and Twyman, 2005).

Agriculture is the backbone of most countries in Africa. More than eighty percent of agriculture in SSA is rain-fed. The sector contributes about 30 percent of the Gross Domestic Product (GDP) and 30 percent of the total export value. Ninety five percent of the population depends on agriculture for its livelihood (Kaushik, 2008). In the past years, many African countries have experienced erratic droughts and declines in water supply. These have aggravated food shortages on the continent. Some countries like Kenya have declared food shortage a national disaster. Recent prediction estimates that, by the year 2050, at least one in every 4 people is likely to live in a water-deficient area (UNFPA, 1999; FAO, 2004).

Water-stress occurs ubiquitously during the growing season of many plants, and has intense negative impacts on agricultural productivity. For example, in maize a mild drought of 4 days at the flowering and silking phase of development can result in up to a 50% decrease in grain yield (Wang et al., 2005). In order to take the right turn towards a more sustainable food security situation in Africa, dramatic yield increases in the large regions susceptible to drought need to be ensured (Figure 1.1). Genetic enhancement of crops for drought tolerance appears to represent the best and most cost-effective route for ensuring sustainable and increased crop yields in the harsh SSA climate, where timing and amount of rain is often unreliable. Such genetic enhancement can be achieved by applying plant breeding techniques together with biotechnology methods. To utilize such techniques fully, there is a need to

understand the molecular and physiological basis of drought tolerance and susceptibility.

In order to improve the understanding of drought tolerance mechanisms in cassava (*Manihot esculenta* Crantz), one of the most important drought-tolerant crops, a multi-disciplinary project, funded by the Generation Challenge Program (GCP) and the German Federal Ministry for Economic Cooperation and Development (BMZ), ''Identifying the physiological and genetic traits that make cassava one of the most drought tolerant crops'' was initiated. The project was implemented by several research institutions in collaboration with universities (Figure 1.2). The research presented here has been undertaken within this project.

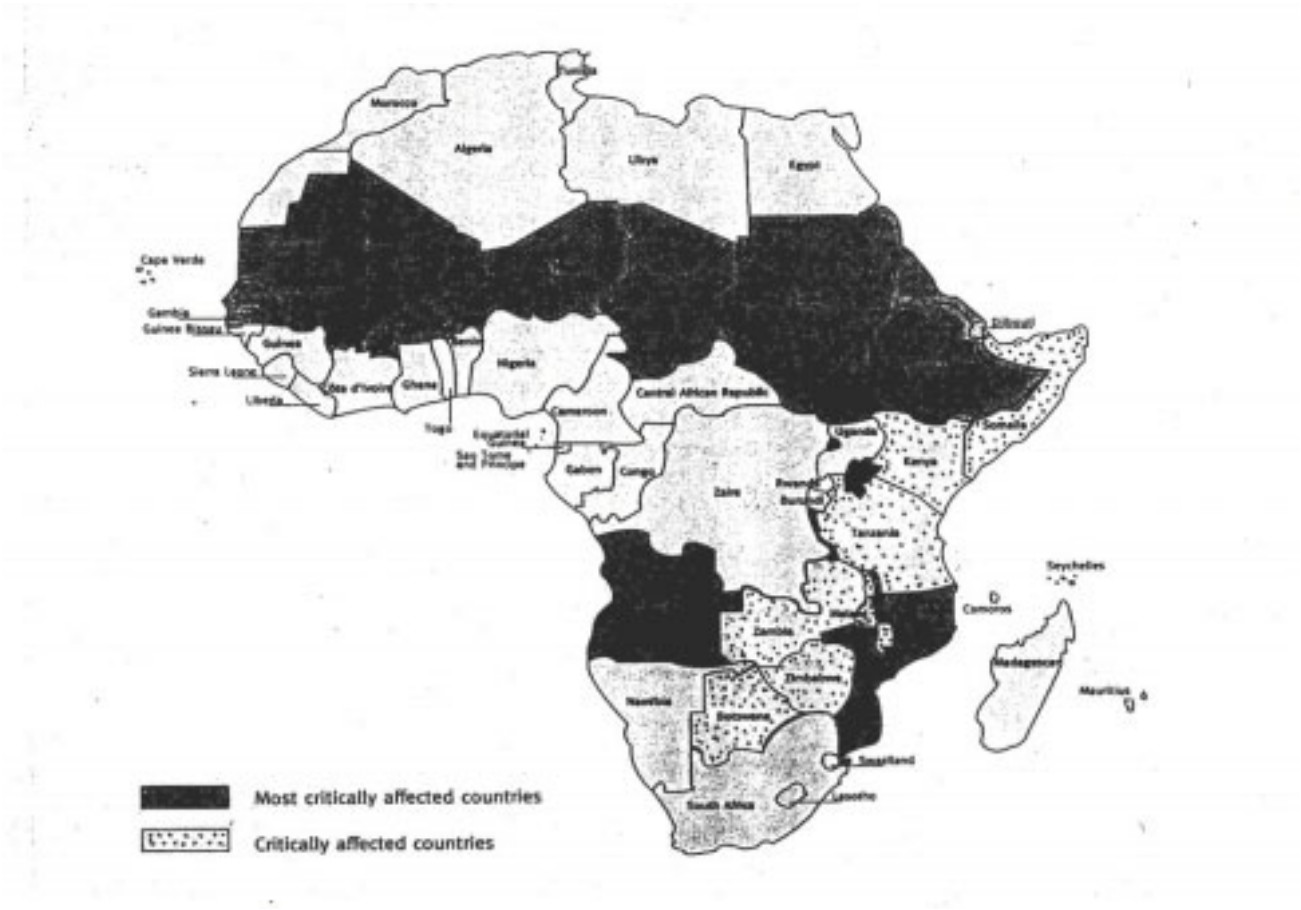

Figure 1.1. Countries affected by drought in Africa.
Source: Moustafa et al. (2002)

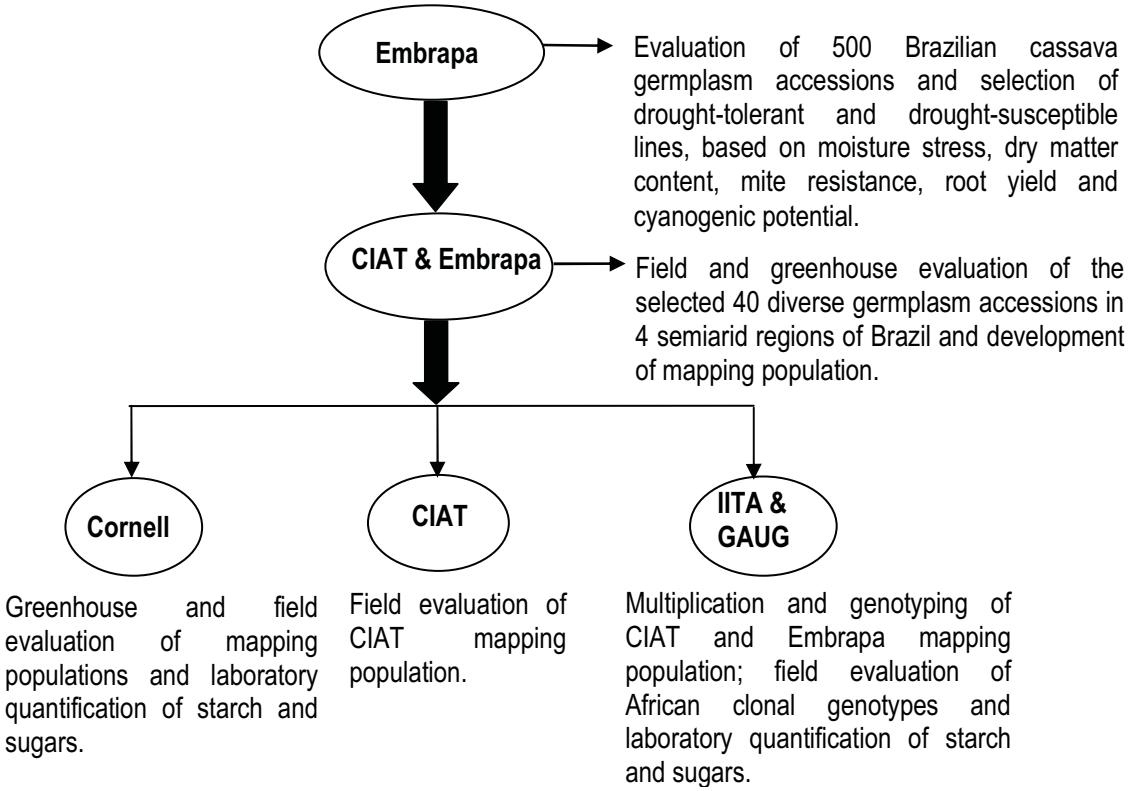

Figure 1.2. Research institutions and universities involved in the Generation Challenge Program (GCP) and the German Federal Ministry for Economic Cooperation and Development (BMZ) funded project ‘‘Identifying the physiological and genetic traits that make cassava one of the most drought tolerant crops” and their roles. Brazilian Agricultural Research Corporation (Embrapa); International Center for Tropical Agriculture (CIAT); International Institute of Tropical Agriculture (IITA); Georg-August-University Goettingen (GAUG); Cornell University (Cornell).

In Africa, cassava is one of the most important staple foods in the human diet, and it is cultivated in areas considered marginal for other crops. Thus, the objective of the present research, as part of the GCP/BMZ project was to improve understanding on the molecular and physiological basis of drought tolerance in cassava.

1.2. Importance of cassava

Cassava is the fourth most important food source of carbohydrates after rice, sugarcane and maize for over 700 million people in developing countries of the tropics and sub-tropics (Balagopalan, 2002; Fregene and Puonti-Kaerlas, 2002; El-

Sharkawy, 2003). It plays an important role in food security because it can be used in diverse ways. In Africa, the leaves are consumed as a green vegetable, and provide protein and vitamins A, B and C. About 38% of the sweet cassava cultivars produced are peeled and eaten raw or boiled (Table 1.1). These cultivars have low cyanogenic glycoside content. Those that have high cyanogenic glycoside content are processed and cooked before consumption (Balagopalan, 2002). Another 51% is processed and used in diverse ways. The flour is used in partial substitution for wheat flour (Almazan, 1990), as a base in canned foods, ice cream, wafers, biscuits, chips, cakes, doughnuts, breads and confectionary (Balagopalan, 2002). The leaves, stems and roots are fed to animals. The high energy value of cassava makes it a good source of carbohydrate in animal diets (Omole and Eshiet, 1992). In Asia and parts of Latin America, cassava is used commercially for the production of animal feed. Cassava starch is utilized both in food and non-food applications such as baby formulas, pharmaceuticals, paper manufacturing and textile industries (Fregene et al., 1997; Balagopalan, 2002). Other industrial uses of the tuber are in the production of alcohols and manufacture of adhesives. Cassava has been reported to have anticancer properties. Genes isolated from the plant have been exploited to eradicate brain tumours in laboratory rats (Cortés et al., 2002).

Table 1.1. World utilization patterns of cassava. Figures are in percentage of total production (Cock, 1985).

	Human food		Animal				
Region	Fresh	Processed	feed	Starch	Export	Waste	Stock
World	31	34	11	5	7	10	1
Africa	38	51	1	<1	<1	9	<1
America	18	24	33	10	<1	14	<1
Asia	34	22	3	9	23	6	4

1.3. Cassava: Origin, distribution and production

Vavilov (1951) placed the origin of cassava in the Brazilian-Bolivian region. He proposed that the centers of diversity were the places of origin of cultivated plants. The crop was probably domesticated in the southern rim of the Amazon basin in Brazil from wild *M. esculenta* populations (Cock, 1985; Olsen and Schaal, 2001). The process of cassava domestication involved selection for root size, growth habit, number of stems, and the ability to clonally propagate through stem cuttings (Kizito, 2006).

The first mention of cassava cultivation in Africa was in 1558. Cassava was introduced into Africa and Asia by Portuguese travelers in the 15th century. The crop's introduction to East Africa has been postulated between 1760 and 1861 (Théberge, 1985; Carter et al., 1992; 1993). Today, cassava is grown in all African countries south of the Sahara and North of river Limpopo (Hillocks et al., 2002). Although cassava is native to the Americas, Africa produces substantially more cassava than the rest of the world combined (FAO, 1997). Nweke et al. (2002) revealed that, between 1961 and 1999, total cassava production in Africa nearly tripled from 33 million tonnes per year between 1961 and 1965 to 87 million tonnes per year between 1995 and 1999, in contrast to the more moderate increases in Asia and Latin America. A survey conducted by Collaborative Study of Cassava in Africa (COSCA) concluded that the main reason for this increase in cultivation was a response to famine, hunger and drought. This confirms the value of cassava as a security crop (Hillocks et al., 2002).

1.4. Biology of cassava

Cassava belongs to the genus *Manihot* in the family Euphorbiaceae, subfamily Crotonoideae and tribe Manihotae. It is the only cultivated species in this genus producing tuberous roots (Chiwona-Karltun, 2001). Rogers and Appan (1973) recognized 98 *Manihot* species of herbs, trees and shrubs. Cassava is a perennial woody shrub with the mature plant height ranging from 1-4 m depending on genotypes and environment (Osiru et al., 1996). Onwueme (1978) and IITA (1990) have reported some dwarf varieties that attained less than 1 m height.

Cassava has simple-lobed leaves. The lobes range from 3 to 11 and they have palmated veins. The crop is monoecious, i.e. having the male (pistillate) and female (staminate) flowers located on the same plant. The female flowers open 10-14 days before the male ones. This enhances cross pollination and the seeds produced are genetically heterozygous (Ng and Ng, 2002). Self fertilization has been reported in instances where the male and female flowers on different branches of the same genotype open at the same time (Mahungu and Kanju, 1997; Alves, 2002; Jennings and Iglesias, 2002). The fruit is round and winged with three seeds. The fruit naturally splits explosively, 70-95 days after pollination, ejecting the seeds at some distance (Onwueme, 1978; Osiru et al., 1996). The seeds are ovoid and they germinate about 16 days after release (Alves, 2002).

Cassava is generally propagated from stem cuttings. The stem is woody, cylindrical with alternating nodes and internodes. In breeding experiments and under natural conditions, seed propagation is common (Alves, 2002; Halsey et al., 2008). Cassava forms adventitious roots from the basal cut surface of the stock in one week. These roots develop to form a fibrous root system in 30-60 days. Between 5 and 20 of the fibrous roots swell due to cambium activity and starch accumulation to produce storage roots. The fully developed cassava storage root has a periderm (bark), a cortex (peel) and a parenchyma, the latter being the edible part. It contains starch, which makes up about 85% of the total root mass (Wheatley and Chuzel, 1995). The other fibrous roots remain thin and continue to help in water and nutrients absorption.

Cassava has a diploid genome with a chromosome number of 2n = 36. Some triploid (3n = 54) and tetraploid (4n = 72) genotypes have also been reported. Triploids have been shown to grow and yield better than tetraploid and diploid plants (Hahn et al., 1990).

1.5. Cassava ecology and physiology

Cassava is grown in Africa, Asia and Latin America between latitudes 30ºN and 30ºS. The crop requires a mean temperature greater than 18ºC, although some varieties have been reported to grow in areas with annual mean temperatures below 16ºC, albeit it does not put up with freezing conditions. Cassava tolerates a soil pH range from 4 to 9 (Howeler, 1978; 2002). It is usually cultivated in areas considered marginal for other

crops with soils of low fertility and annual rainfall of less than 600 mm as in the semiarid tropics (De Tafur et al., 1997) to more than 1000 mm in the sub-humid and humid tropics (Pellet and El-Sharkawy, 2001). In many of these regions, rainfall distribution is not homogeneous, and there are prolonged periods of drought during the crop cycle. Because of its inherent tolerance to stressful environments, it is considered a contributor to food security against famine, requiring minimal inputs. This makes it an essential crop for drought-prone areas of the tropical and sub-tropical Africa, Asia and Latin America.

In cassava, many traits have been associated with drought tolerance, such as leaf gaseous exchange, leaf retention, osmotic adjustment, accumulation of specific low molecular weight proteins, abscisic acid (ABA), and accumulation and utilization of non-structural carbohydrates. But, it is also known that genotypic variation in drought tolerance exists. For example, in 1992, cassava accessions in Petrolina (northeast Brazil) suffered from a more severe drought than normal, with total annual rainfall of less than 200 mm and, despite this harsh environment, a large number of accessions persisted and produced from 13 to 18 t ha^{-1} fresh roots, while some failed (El-Sharkawy, 2007).

1.5.1. Sensitivity of cassava stomata

Stomata have an inherent ability to respond to changes in the water status of the plant and the atmosphere (Alves, 2002). Cassava maintains a high stomata conductance and keeps internal CO_2 concentration high when water is available. The stomata remain partly closed when water becomes limiting with no changes in leaf water potential. This prevents the leaves from desiccation (El-Sharkawy, 2003). In addition, cassava stomata are located mainly on the lower surface of the leaf (abaxial) except in 2% of the 1500 germplasm accessions studied that had stomata on their adaxial surface (El-Sharkawy et al., 1985; El-Sharkawy and Cock, 1987b).

1.5.2. Leaf retention (stay-green) and changes in leaf expansion rates

Prolonged retention of cassava leaves has been recognized as a key trait to increasing both root yield root quality. Cassava leaves remain photosynthetically active under stressed conditions. The leaves are also capable of partially recovering, once water becomes available. This represents an important mechanism of saving the biomass

invested in leaf formation (El-Sharkawy, 2003). In addition, when there is low air humidity, the cassava leaf area is reduced due to decreasing cell proliferation and modifications of photosynthetic pathway to maintain high photosynthetic activity. This is rapidly reversed following the recovery from stress (Alves and Setter, 2004). This "stay-green'' characteristic allows subsistence farmers to continuously harvest the leaves and is also instrumental for increasing root yield.

1.5.3. Osmotic adjustment

Osmotic adjustment is recognized as an effective component of drought resistance in many crops (Kramer and Boyer, 1995). It involves the accumulation of osmotically active solutes in a cell in response to a fall in water potential of the cell's environment. This helps in maintaining turgor and its dependent processes during water-limiting episodes. As a consequence, the stomata remain partially open for CO_2 assimilation to take place. This results in cell enlargement and plant growth at high water stress conditions (Alves, 2002).

1.5.4. Accumulation of specific low molecular weight proteins

The amount of proteins that accumulate during plant cell dehydration cannot be underestimated. Many of these known families of such proteins are LEA proteins, named after their initial observation as 'Late Embryogenesis Abundant' during cotton embryo development (Close et al., 1993; Dure III, 1993). Their accumulation confers osmoprotection to cellular membrane and protein systems.

1.5.5. Abscisic acid (ABA) accumulation

Environmental stress has been shown to stimulate the biosynthesis and release of the phytohormone ABA in plants. This hormone regulates essential physiological and developmental processes in plants as well as imposed adaptive responses to environmental stress (Zeevaart and Creelman, 1988). In addition to controlling the opening and closing of the stomata, ABA promotes distinctive developmental changes that assist plants cope with water deficit (Alves and Setter, 2000). These include restriction of shoot growth and leaf area expansion (Lecoeur et al., 1995), stimulation of root extension (Sharp et al., 1994), and accumulation of osmotically active solutes (LaRosa et al., 1987). In response to water deficit, cassava leaves rapidly accumulate ABA and, correspondingly, halt leaf expansion growth (Alves and Setter, 2000).

1.5.6. Accumulation and utilization of non-structural carbohydrate reserves

Research in cassava and other crops demonstrated that, when water is limited, plants close their stomata and limit photosynthesis. Starch is the most important form of carbon reserve in plants (Martin and Smith, 1995). Therefore, an important coping mechanism for plants is the ability to mobilize stored carbohydrates to provide a source of substrate for metabolism and osmolyte synthesis (Blum, 1998). Cassava, with its thick woody tissues, amasses abundant starch reserves in its stem, leaves and roots that are mobilized during stress, and this contributes to drought tolerance.

1.6. Mechanisms of drought tolerance in plants

Plant growth, productivity and distribution are affected by both abiotic and biotic factors. The abiotic factors include drought, freezing, poor soils and salinity; the former being the most prevalent. Plants have developed varied adaptive strategies to cope with these stresses. Drought tolerance in wild species is usually defined in terms of survival, while in cultivated crops, it is in terms of productivity (Passioura, 1983). Tolerance is the variation in yield between stress and non-stress environments (Rosielle and Hamblin, 1981) or the relative yield of an accession as compared to other accessions subjected to the same drought stress (Hall, 1993; Gebeyehu, 2006). Fischer and Maurer (1978) have defined tolerance as the reduction of the decline in yield caused by stress compared to yield under non-stress environment. Although the mechanisms of maintaining plant growth and development in water-stressed environments are complex, plants generally use three strategies to survive drought environments. These are drought escape, dehydration avoidance and dehydration tolerance (Blum, 1998; 2005). Dehydration tolerance and dehydration avoidance have been noted as the two major mechanisms of drought resistance in higher plants (Babu et al., 1999). Although, in cassava, various genotypes use different or, a combination of physiological mechanisms to deal with drought. These are escape (by early bulking and maturity), avoidance (by deep fibrous root system and stomatal closure) and tolerance (plasticity in vegetative growth, remobilization of substrates for growth and abscisic acid accumulation) (Ekayanake, 1998; Okogbenin et al., 2003).

1.6.1. Drought escape

Drought escape is associated with short cycle crops, which flower early or have short growth duration. This type of drought survival mechanism is advantageous especially in environments with terminal and predictable drought and where physical or chemical barriers inhibit the growth of roots (Blum, 1998). Nevertheless, late flowering in plants can be beneficial in escaping early-season drought especially where drought is followed by rains (Ludlow and Muchow, 1990; Ludlow, 1992). In general, this mechanism allows plants to grow and complete their life cycle before soil moisture becomes limiting.

1.6.2. Dehydration avoidance

Dehydration avoidance is the ability of a plant to retain reasonably high amounts of water under soil or atmospheric water-stress, either through reduction of water loss and/or maintaining proper water uptake (Blum, 1998). Jones and Zur (1984) recognized two types of dehydration avoiders. These are 'water savers' or plants that avoid dehydration through reduced transpiration, and 'water spenders' or plants that use means other than transpiration to conserve water. Features that enable plants to avoid dehydration include a vigorous, deep and extensive root system, mucilaginous, narrow and hairy leaves, osmotic adjustment to lower the osmotic potential, and/or modified and limited number of stomata to reduce water loss (Hsiao et al., 1973; 1976; Acevedo et al., 1979). These mechanisms allow plants to maintain a positive tissue-water relation even under limited soil moisture conditions.

1.6.3. Dehydration tolerance

Dehydration tolerance is the ability of a plant to continue with its metabolic processes and maintain growth at a low water potential. This happens when tissues are no longer protected by avoidance mechanisms during high dehydration levels. Stem reserve mobilization is one of the dehydration tolerance processes in plants as it tends to proceed at levels of water deficit sufficient to inhibit photosynthesis. For example, in cereals, it has been shown that grain growth is partially supported by translocated plant reserves stored mainly in the stem during pre-anthesis growth stages (Santiveri et al., 2004). These reserves provide a source for grain filling when water-stress

occurs. The mechanisms of drought tolerance function at the tissue or cellular level to stabilize and protect the cellular and metabolic integrity (Tuinstra et al., 1997).

1.7. Cassava breeding and biotechnology

Plant breeding is the art and science of manipulating the genetics of plants, followed by selection of resulting plants that most closely approach the desired combination of characters, for the economic and social utility to man. It is one of the most ancient forms of agricultural activities where, although the early plant domesticators (hunters and gatherers) had no concept of genes or their manipulation, they selected superior plants with improved productivity suited for their environmental conditions. Breeding methods are grouped into four distinct categories according to the reproduction type of the resulting cultivars: lineal cultivars with self fertilization, population cultivars with cross fertilization, hybrid cultivars with controlled crossing between the parents, and clonal cultivars with vegetative propagation (Schnell, 1982; Bond and Poulsen, 1983; Pochard et al., 1992 as cited by Ghaouti, 2007). Plant breeding is an important approach needed to sustain food production for the long-term future.

Cassava is a clonal crop and, despite it being a major food crop, its scientific breeding began only around 1937 (Kizito, 2006). After formation of the International Institute of Tropical Agriculture (IITA) in Nigeria and the International Center for Tropical Agriculture (CIAT) in Colombia in the early 1970's, significant progress has been made. These two international centers collaborate with National Research Systems (NARS) to study the crop in depth. The objectives are centered on yield increase, improving root quality, and multiple pest and disease resistance. Despite the progress achieved, the breeding process has been slow and inefficient as compared to other crops. This is because of the long breeding cycle (9-18 months), low seed yield per pollination (a maximum of three seeds per cross), and the heterozygous nature of the parents and progenies evaluated (CIAT, 2003; Ceballos et al., 2004). The heterozygous nature allows a considerable genetic load of deleterious or undesirable alleles to persist in populations, masks allelic differences in segregating populations, and also, it creates difficulties in transferring desirable traits from one genotype to another (Ceballos et al., 2004; Setter and Fregene, 2007). This makes the breeding process lengthy with no assurance of release and adoption of a new variety. Given the difficulties of conventional breeding in cassava, molecular DNA markers, could be a

boost for breeders. These markers can accelerate the process of crop improvement through selection and transfer of traits of interest, especially, those that are difficult to phenotype in large scale trials (Crouch and Serraj, 2002; Setter and Fregene, 2007).

Molecular markers both biochemical and DNA are sequence variants that can readily be detected and whose inheritance can be monitored (Farooq and Azam, 2002; Kumar et al., 2009). They are detectable in all tissues and not affected by environmental conditions. Different marker systems have been developed in the last two decades, however, simple sequence repeats (SSR's), also known as microsatellites, are most effective in detecting polymophisms in cassava (Weising et al., 2005). SSR's are hyper-variable tandem repeats of DNA motifs 2-5 bases long, common in eukaryotic and prokaryotic genomes (Zhu et al., 2001). They are widely distributed in higher plants. The variation comes from differences in the number of repeat units originating from errors in copying of DNA during replication. SSR's are preferable because they are simple to implement in most laboratories, easy to analyse and fast to obtain results, amenable for high throughput marker genotyping, polymorphic, and they are co-dominant markers, which allows to identify heterozygotes through them (Senior and Heun, 1993; Akkaya et al., 1995; Lelley et al., 2000).

The availability of molecular DNA markers represent the most significant advance in breeding and have greatly contributed to cassava improvement and genetics in the development of genetic maps, identification of quantitative trait loci (QTL) for some important traits (Fregene et al., 1997; Cortés et al., 2002; Okogbenin and Fregene, 2003), in the assessment of genetic diversity, taxonomical studies and confirmation of ploidy levels (Fregene et al., 2001; Fregene et al., 2003; Mkumbira et al., 2003).

1.8. Rationale of the study

Cassava is a major staple in the tropics of Africa. It is cultivated in areas considered marginal for many other crops. These areas are characterized by low soil fertility and low annual rainfall. It is the cheapest source of food calories providing a major source of energy for nearly two out of every five Africans (Nweke, 2004). Cassava appeals to low-income households because it can be "banked" in the soil as a food reserve source from 8 to 36 months following planting. Therefore, it serves as a buffer against uncertainties of small farm life. The HIV/AIDS epidemic has also weakened the labor

force in many rural areas in Africa where agriculture is dominated by women (Bryson, 1981; Barany et al., 2001). Cassava is popular with these women farmers because of its flexible requirements in terms of planting, weeding, and harvesting (Nweke et al., 2002). In Africa, yields are only 8-10 tones per hectare, on average, approximately half of those achieved in Asia and Latin America (FAO, 2003). Drought is an important constraint to production in semi-arid regions of southern and eastern Africa and in the marginal areas bordering the Sahel (Moustafa et al., 2002).

Currently, there is limited information on the physiological and molecular mechanisms that make some cassava accessions more drought-tolerant than others (El-Sharkawy, 2007). This is probably due to the fact that breeders and scientists are unsure of the key physiological traits to measure, in addition to yield characteristics, for drought-tolerance evaluation (Jenks et al., 2007). In addition, cassava is a complex crop to breed using conventional methods. It is traditionally a vegetatively propagated crop through stem cuttings, and seed production is extremely low (Iglesias et al., 2008). This is a serious limitation to plant breeding, which relies on recombination during crossing in order to achieve any progress. In addition, its phenology is highly influenced by the environment, affecting time to flowering (Whyte, 1987; Halsey et al., 2008). Cassava suffers quickly from inbreeding depression and has a high degree of heterozygosity (González et al., 1998; Lopez et al., 2005). For these reasons, it is extremely difficult, time-consuming and expensive to combine an array of preferred characteristics, both agronomic and organoleptic. Biotechnology tools can play a major role in increasing the accuracy and efficiency of cassava breeding through marker-assisted breeding (MAB). This calls for the need to understand the physiological and molecular drought tolerance mechanisms in cassava.

1.9. Objectives of the study

The ultimate goal of the project was to identify the physiological and genetic traits that make cassava one of the most drought-tolerant crops. The present study was conducted within the framework of the Generation Challenge Program (GCP) and the German Federal Ministry for Economic Co-operation and Development (BMZ) funded project with the specific objectives:

- to develop a protocol for hardening and rapid micro-propagation of cassava plantlets under local conditions;
- to identify some of the main physiological and metabolic attributes that contribute to drought tolerance in cassava;
- to identify drought-tolerant and drought-susceptible cassava germplasm from Africa;
- to evaluate the CIAT and Embrapa mapping populations using molecular markers;
- to perform linkage analysis to determine the map distance of molecular markers.

1.10. Thesis outline

This introductory chapter will be followed by chapter 2 describing a successful protocol that has been developed to acclimatize and rapidly micro-propagate tissue culture cassava plantlets under local conditions. An attempt is made to describe the step by step procedure from when the plantlets were received until they were ready to go to the field. Chapter 3 deals with the agronomic and morphological evaluation of contrasting African cassava germplasm accessions under water-stressed and well-watered conditions in Kiboko, Kenya. Chapter 4 focuses on laboratory results of carbohydrate, protein and phytohormone quantification of the African cassava germplasm. In chapter 5, genomic and expressed sequence tag (EST) derived SSR markers (ESSR) are utilized for the genotyping and linkage mapping of the CIAT mapping population. In chapter 6, the main findings of the study are highlighted and summarized.

2. Hardening of Cassava *In Vitro* Plantlets and Rapid Micro-propagation of Cassava Plants Through Nodal Cuttings

Abstract

Cassava has become suitable for food security and economic development in unfavoured areas of the tropics. Lack of good quality planting material, in larger quantities and at the right time is one of the most important constraints limiting expansion of cassava production in Africa. Plant tissue culture technology has been successfully used to propagate cassava and other plant species like sugarcane, bananas and sweet potato. This has facilitated international exchange of clean clones, conservation of germplasm, and it has also helped alleviate cassava's multiplication constraints at farm level in developed countries. However, in Africa hardening of cassava in vitro plantlets and production of massive plants is a major drawback because this technology is capital-, labor- and energy-intensive. This study describes a successful protocol for hardening and rapid micro-propagation of cassava plantlets under local Kenyan conditions using nodal cuttings, vermiculite, sterile soil and improvised humidity chambers. A total of 1173 plants from 31 putative drought-tolerant and drought-susceptible germplasm accessions were acclimatized using the developed protocol as compared to 722 plants obtained with the use of sub-culturing technique. Overall increase after 210 days with a rate of 13.8 for direct and 8.5 for in-direct micro-propagation were observed. The protocol was also cheaper in terms of consumables as compared to the tissue culture/in-direct method of micro-propagation.

Key words: Cassava; direct micro-propagation; hardening; in-direct micro-propagation; *in-vitro*; Kenya; *Manihot esculenta*; nodal cuttings; tissue culture

2.1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important staple food crops in Africa. In Democratic Republic of Congo (DRC), Ghana, Mozambique, Nigeria, Tanzania and Uganda, it is the number one staple crop (Nweke et al., 2002). Its importance as a food crop in Africa becomes obvious when its annual per-capita consumption is compared to the rest of the world. Whereas the world average annual cassava consumption was 17 kg/capita in 2001, Africa's annual consumption was above 80 kg/capita. Latin America's consumption decreased by half over the past 30 years to slightly more than 20 kg/capita in 2002 (Aerni, 2006). Its productivity, drought and acid soil tolerance, and its ability to grow on marginal soils with minimum inputs makes it a vitally important crop to some of the world's low-income food-deficient countries and a significant famine reserve crop (Cock, 1985; Xia et al., 2005).

Cassava has a high yield potential. According to FAO, 172 million tonnes of cassava were produced worldwide in 2000, of which Africa produced 54%, Asia 28%, while Latin America and the Caribbean produced 19% (Manyong et al., 2004). In Africa, average yields are only 8-10 tons per hectare compared to potential yields of over 80 tonnes under ideal conditions (Taylor and Fauquet, 1997). The gap between the actual and potential yields on farmers fields is around 8-fold. This is a clear indication that the highest potential of cassava production is far from being reached, although when compared to maize, sorghum and rice in environments with no production constraints, cassava can match or exceed the energy production per hectare of these crops (Vries et al., 1967).

Cassava roots are the major portion of economic product in Africa, which are consumed as human food after varying degrees of processing. In addition, they are increasingly being used as a potential substitute for maize in feed concentrates and for wheat in bakery goods. The variety of cassava starch cannot be under-estimated in addition to its role in the production of glue, paper and biodegradable plastics. The roots also serve as a source of cash income for small-holder farmers (Bottema and Henry, 1990; Escobar et al., 2006). Cassava storage roots do not function as propagules like other tuberous roots. The roots serve as a repository of photosynthate

and, thus, they help the plant to recover from defoliation after drought episodes (Han et al., 2001).

Cassava plants are conventionally propagated through stem cuttings. Although this system is commonly used, diseases often accumulate in the propagules resulting in infected stands and reduced yields. Other challenges include high perishability, as cuttings dry up within a few days, high handling and transport costs, low propagation rates compared to grain crops, and inconvenient weight and bulk of the material (Escobar et al., 2006). A collaborative research conducted in 2003 by IITA, the Swiss Federal Institute of Technology (ETH Zurich), the Donald Danforth Plant Science Center (DDPSC, USA), CIAT, the Brazilian Agricultural Research Corporation (Embrapa, Brazil), representatives from local universities, farmer organizations, and multinational companies sponsored by the Swiss Center for International Agriculture (ZIL) revealed that lack of clean planting stakes was the most important constraint facing subsistence farmers in Africa (Figure 2.1).

Tissue culture has been effectively used to eliminate viruses and other systemic diseases from elite cassava vegetative materials (Jorge et al., 2000). This has allowed exchange and conservation of rejuvenated propagation materials, which have higher yields than the same varieties propagated for successive years in the field (Kassianof, 1992). However, one of the major limitations for a wider adoption of this technique in developing countries is the unavailability of a procedure for hardening and multiplication of the tissue culture plantlets before final transplanting in the production sites. Although reports are available on *in vitro* hardening of cassava in the developed world, the protocols are difficult and expensive to implement in developing countries since the technology is capital-, labor- and energy-intensive (IAEA, 2004).

Even though labor is cheap in developing countries, the resources of trained personnel and equipment are often not readily available. In addition, electricity and clean water are costly especially with the plight of climate change and global warming. It is, therefore, necessary to have a low-cost technique for acclimatization and rapid micro-propagation of tissue culture plantlets suited for developing countries.

The present study was conducted within the framework of the project "Identifying the physiological and genetic traits that make cassava one of the most drought-tolerant

crops" implemented since 2005 by the Brazilian Agricultural Research Corporation (Embrapa); the International Center for Tropical Agriculture (CIAT); the International Institute of Tropical Agriculture (IITA); Cornell University, USA and University of Goettingen, Germany.

The main objective of the present study was:

- to develop a protocol for hardening and rapid micro-propagation of cassava plantlets under local, low-cost conditions.

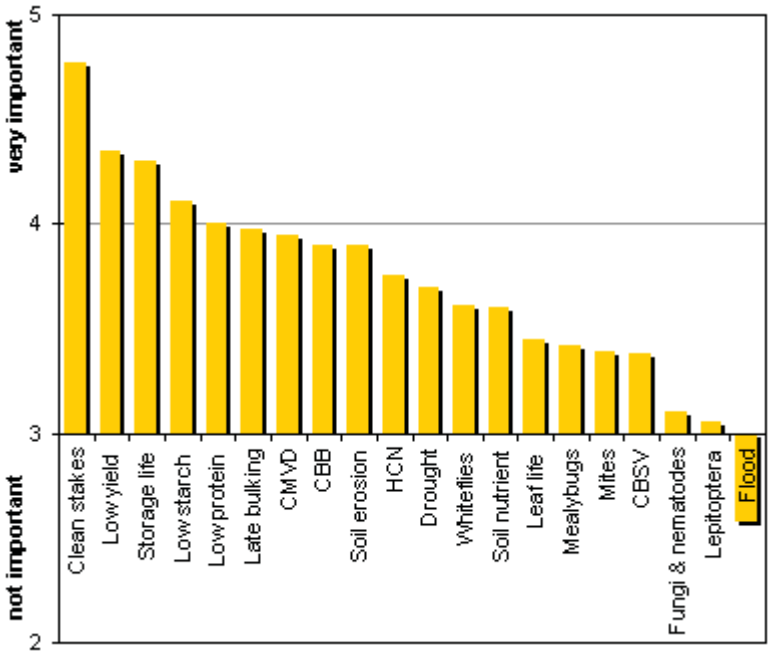

Figure 2.1. Average ratings of the importance of problems in cassava subsistence agriculture in Africa, as assessed in 2003 by researchers from International Institute of Tropical Agriculture (IITA, Nigeria), the Swiss Federal Institute of Technology (ETH, Switzerland), the Donald Danforth Plant Science Center (DDPSC, USA), International Centre for Tropical Agriculture (CIAT, Colombia), the Brazilian Agricultural Research Corporation (Embrapa, Brazil), representatives from local universities, farmer organizations, and multinational companies, on a scale from 1 (not important) to 5 (very important) (Adopted from Aerni, 2006).

2.2. Materials and methods

In vitro plants of 31 putative drought-tolerant and drought-susceptible African cassava germplasm accessions were obtained from IITA, Nigeria (Table 2.1). The plantlets were delivered in polystyrene boxes at Kenya Agricultural Research Institute (KARI, Nairobi). Upon arrival, the plantlets were removed from the boxes (Figure 2.2a). They were counted, genotypes confirmed and kept in the growth room for three days to recover since they had spent one week in the dark during transportation and clearance. On the fourth day, the individuals of every genotype were divided into two, the ones to be sub-cultured to act as a backup (in-direct micro-propagation), and also to compare the multiplication rate with the rapid micro-propagation, and the ones to be hardened and multiplied without sub-culturing (direct micro-propagation) (Table 2.1).

2.2.1. Sub-culturing (in-direct micro-propagation)

The plastic tape that had sealed the bottles was removed. The bottle neck with the plantlets was passed over an open flame and opened aseptically. The plantlet was picked from the culture jar and placed on a Petri-dish with the aid of a sterile forceps. The leaves were chopped off and the stem was cut into small pieces of about 3-4 cm each having at least two nodes. The nodal explants were placed in Kilner jars containing 50 ml basic semisolid culture medium (Murashige and Skoog, 1962) with 2% sucrose and solidified with 2.5% phytagel at pH 5.8 before autoclaving at 121ºC for 20 min. at 15 psi. The bottles were capped with tops and plastic tape. The cultures were kept in the tissue growth room at 27ºC in a 16-h photoperiod. The plantlets were sub-cultured after 2 months. In the 4th month, the plantlets were acclimatized in the green-house.

2.2.2. Transplanting and hardening (direct micro-propagation)

Perforated plastic pots (3" x 4" gauge 100) were filled with sterile, medium-grade vermiculite to three quarters their volume. Vermiculite is cheap and locally available and it promotes maximum root growth since it is well aerated and retains moisture and nutrients. Soil was not used since it gets compact after watering and, hence, damages the absorbent hairs, root cap and roots. The plastic pots containing vermiculite were placed in plastic trays. Plastic tape and bottle caps were removed from the bottles. A spatula was used to disturb the semisolid media taking care not to

damage the roots. The bottles were tapped gently at the bottom in an inclined position so as to extract the plant. Forceps and tweezers were not used so as not to damage the stem. The plantlets were pulled out of the bottle and thoroughly but carefully washed with running tap water to remove adhering medium completely. The plantlets were placed in the perforated pots with vermiculite and labeled (Figure 2.2b). The roots and the stem were handled carefully to prevent physical or physiological damage. The pots were placed on a trough with 1 cm level water. To conserve moisture and maintain a high humidity, each plant was covered with a transparent polythene bag (9" x 13" gauge 300) after pre-spraying with 0.2% Diethane M45 (Mancozeb, 80%; Manganese, 16%; Zinc, 2%; Ethylene bisdithiocarbamate, 62%) fungicide solution and tied with a rubber band at the base (Figure 2.2c). The pots containing the plantlets were not moved during the first month. Watering was done every week through the base of the trough. The temperature in the green-house was maintained between 25ºC and 30ºC throughout the acclimatization period. On the 21st day, one corner of the transparent bag was cut open to enable the plantlets adapt to the micro-environment of the green-house. The other corner was cut on the 24th day. The whole polythene paper was completely removed on the 28th day (Figure 2.2d). During this step, the plantlets were protected from strong dehydrating winds by restricting entrance to the green-house. Between day 30 and 40, the plantlets were transplanted into plastic bags (5" x 8" gauge 100) containing sterile soil (forest soil: sand: humus 45:30:25), since they required more nutrients and space for growth and development (Figure 2.2e and f). A water soluble foliar feed (NPK, 19:19:19) was applied every week up to 90 days at a rate of 1% (1 g l^{-1} of water).

2.2.3. Rapid micro-propagation

After 3 months, each plant of the hardened plants was cut with a sterilised surgical blade in a slanting position into small pieces containing at least two nodes. The cuttings were planted in polythene bags (5" x 8") containing sterile soil and were well labelled (Figure 2.2g). The plant parts were well watered and then covered with a humidified transparent polythene bag (9" x 13") and tied with rubber bands (Figure 2.2h). They were kept in the green-house under high humidity at temperature between 25ºC and 30ºC. On the 7th day, one corner of the polythene bag was chopped off to enable the plants to adapt to the micro-environment of the greenhouse. On the 10th day, the other corner of the covering polythene bag was also chopped off and plants

were maintained that way for 7 more days. On the 14^{th} day, the polythene bag was removed completely. The materials were allowed to grow in the green-house for 1 month and the rapid micro-propagation procedure was repeated again (Figure 2.2i). The plantlets were taken to the field for establishment after 90 days (Figure 2.2i, 2.2j).

2.3. Results and discussion

All 31 putative drought-tolerant and drought-susceptible cassava germplasm accessions evaluated in this study responded well to both hardening and rapid micro-propagation. The number of explants obtained varied among the accessions due to the variation in the number and quality of the starting materials (Table 2.1), however, the increase rates did not vary accordingly (Figure 2.3, Table 2.1). The plantlets formed using both the direct and in-direct methods of micro-propagation were generally strong and healthy. The number of plantlets obtained via direct micro-propagation were higher than that of the in-direct method of micro-propagation. However, no clear genotype relationship could be observed.

Losses up to 13.7% and 10.6% were observed in direct and in-direct hardening, respectively. Direct hardening had higher losses, which was due to the feeble stems and roots of the imported materials and the fact that they had stayed in the dark for one week during shipping and clearance. The plantlets that were sub-cultured first were stronger, and also the bottles used were large and, hence, more nutrients were available as compared to materials micro-propagated directly from IITA. This lead to a low percentage of plants being lost.

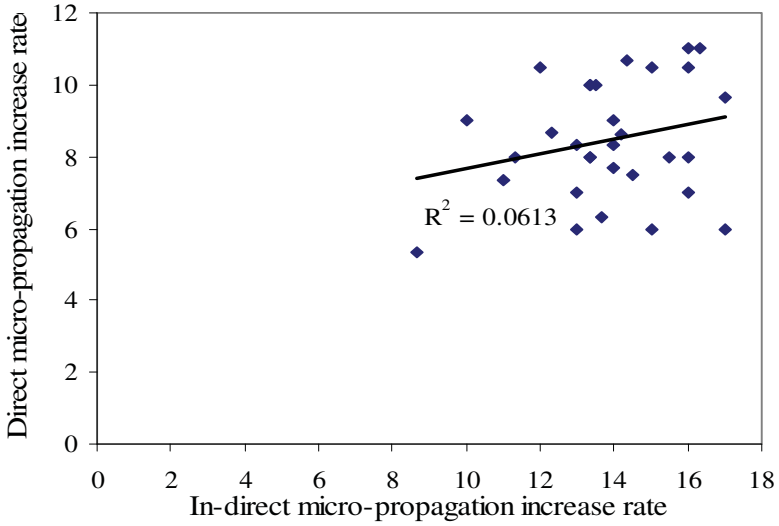

Figure 2.3. Direct and in-direct micro-propagation increase rates of putative drought-tolerant and drought-susceptible African cassava germplasm accessions micro-propagated at Kenya Agricultural Research Institute (KARI), Nairobi.

Table 2.1. Putative drought-tolerant and drought-susceptible African cassava germplasm accessions obtained from the International Institute of Tropical Agriculture (IITA), Nigeria and the total number (no.) of explants obtained through in-direct micro-propagation (sub-culturing) and direct micro-propagation techniques and the respective multiplication rates (= final no. of plants / initial no. of plants); (CBSD, cassava brown streak disease).

Accession	Known	Indirect micro-propagation			Direct micro-propagation		
identifier	characteristic	Day 0	Day 210	Rate	Day 0	Day 210	Rate
TME 4	CBSD resistant	2	21	10.5	2	30	15.0
96/1089A	CBSD resistant	3	22	7.3	3	33	11.0
TME 117	CBSD resistant	3	27	9.0	3	42	14.0
96/1569	CBSD resistant	3	21	7.0	3	48	16.0
I92/0057	Mild drought-resistant	3	24	8.0	3	40	13.3
TME 7	Mild drought-resistant	3	33	11.0	3	48	16.0
92B/00061	Drought resistant	3	29	9.7	3	51	17.0
I92/0326	Drought resistant	2	12	6.0	2	34	17.0
I95/0104	Drought susceptible	3	16	5.3	3	26	8.7
I30572	Drought susceptible	3	24	8.0	3	40	13.3
14(2)1425	Drought susceptible	3	25	8.3	3	39	13.0
97/4779	Drought susceptible	3	21	7.0	3	39	13.0
94/0026	Stay green	2	16	8.0	2	31	15.5
95/0166	Stay green	3	19	6.3	3	41	13.7
95/0289	Stay green	3	26	8.7	3	37	12.3
96/0160	Stay green	3	30	10.0	3	40	13.3
96/0596	Stay green	1	6	6.0	1	13	13.0
96/1087	Stay green	5	43	8.6	5	71	14.2
96/1708	Stay green	3	30	10.0	3	40	13.3
97/2205	Stay green	3	24	8.0	3	48	16.0
97/3200	Stay green	3	23	7.7	3	42	14.0
98/0581	Stay green	2	15	7.5	2	29	14.5
99/0204	Stay green	3	27	9.0	3	30	10.0
I91/02312	Stay green	3	25	8.3	3	42	14.0
I91/02327	Stay green	2	21	10.5	2	24	12.0
I91/1934	Stay green	3	33	11.0	3	49	16.3
M98/0068	Stay green	2	20	10.0	2	27	13.5
94/0020	Stay green	3	24	8.0	3	34	11.3
01/0090	Stay green	3	32	10.7	3	43	14.3
I91B/00462	Stay green	2	12	6.0	2	30	15.0
01/0014	Stay green	2	21	10.5	2	32	16.0
Total		85	722		85	1,173	
Mean		2.7	23.3	8.5	2.7	37.8	13.8

In vitro propagation via nodal cuttings has the potential to produce thousands of plants and cuttings within a year. Using conventional micro-propagation technique, a mature cassava plant will give between 10-30 normal-sized stem cuttings for planting after one year (Smith et al., 1986). We observed that, a propagation system based on

two-node cuttings (direct method) was a practical and effective method of propagation in a developing country with limited technical expertise and facilities as compared to the use of media. Propagation through sub-culturing (in-direct method) was more than 3 times as expensive as through nodal cuttings in terms of consumables (Table 2.2) and time, which could not be quantified in this study. Directly sub-cultured plantlets required daily care to ensure that there was no contamination. Although the cost of Kilner jars, which accounted for 47% of the total expenditure, could be reduced by substituting them with jam jars, whose use resulted in a lot of contamination because the caps were slightly loose, and they also could not withstand autoclaving.

Nevertheless, propagation through nodal cuttings (direct method) reduced the time frames for propagation and, by containing multiplication where phytosanitary conditions are better, the development and dissemination of disease-free clones should be enhanced. Also, because the system is green-house based, environmental conditions can be controlled and, hence, the optimum time of planting may perhaps be controlled. Plant establishment was successful upon transfer to soil.

2.4. Conclusions

Tissue culture techniques are indispensable as tools for biotechnology transfer and for germplasm conservation. Micro-propagation through tissue culture (in-direct) in standard conditions is the most widely used, although the frequent transfer makes the technique costly and increases the risks of contamination. Besides, positive selection during sub-culturing could be a source of morphological, cytological and genotypic variation (Rout et al., 1998). Rapid micro-propagation through nodal cuttings (direct) offers an alternative to enhanced rates of multiplication over more conventional methods like the use of stem cuttings. Conventional methods are slow and as interest in cassava research grows, it becomes increasingly more important to develop techniques for the rapid multiplication and distribution of new cultivars, or disease-free material of established cultivars. Breeding programmes would also benefit from this method for rapidly multiplying new lines for field trials and evaluation, thereby shortening the time required for the release of a new cultivar.

Table 2.2. Cost comparison of direct and in-direct methods of micro-propagating putative drought-tolerant and drought-susceptible cassava germplasm accessions at Kenya Agricultural Research Institute (KARI), Nairobi, Kenya. The costs are for 1000 plantlets each.

Item description	Quantity	Cost (US$)
Direct micro-propagation		
Plastic bags (3" x 4") G100	10 pkts	30
Plastic bags (5" x 8") G100	10 pkts	36
Humidifier (9" x 13") G300	10 pkts	50
Vermiculite	5 sacks	50
Rubber bands	1000	12
Labels	1000	14
Polythene sheeting	10 m	43
Sterile soil	50 kilos	36
Trays	100	50
Casual labor	1 person	170
Total direct micro-propagation		491
In-direct micro-propagation		
Jik and Teepol	1 vial	3
Cassava tissue culture media	25 litres	420
1000 ml beaker	1	50
Erlenmeyer flasks set	1	40
Surgical blades and forceps	1 set	70
pH meter buffer solution	1 set	40
Kilner jar containers	100	720
Distilled water	25 litres	10
Casual labor	1 person	170
Total in-direct micro-propagation		1,523

(a) Plantlets removed from box and kept in the growth room

(b) Plantlets put in vermiculite before covering with humidity bags

(c) Trays and plastic bags acting as humidity chambers

(d) Transplanted plants after the humidity bags were completely removed

Figure 2.2. A step by step protocol for hardening and rapid micro-propagation of cassava germplasm accessions through nodal cuttings. Photos a to j.

Figure 2.2. continued

(e) Plantlets freshly transplanted from vermiculite to bigger pots with soil

(f) Established plants in pots before rapid micro-propagation

(g) Nodal explants for rapid micro-propagation

(h) Nodal explants covered with humidity bags

(i) Established plantlets in the greenhouse ready for transfer to the field

(j) An established field at KARI, Kiboko Research Station in Makindu, Eastern Kenya with hardened and rapidly micro-propagated plants

3. Agronomic and Morphological Evaluation of Contrasting Cassava Germplasm Accessions under Drought Stress at Kiboko, Kenya

Abstract

Cassava is the most important root crop in the tropics and sub-tropics especially in sub-Saharan Africa. It is a rustic crop and can produce under conditions of erratic rainfall and impoverished soils, where few other crops survive. To improve the understanding on agro-morphological attributes that contribute to cassava drought tolerance, a study was conducted with 31 putative drought-tolerant and drought-susceptible African germplasm accessions at Kiboko Research Station, eastern Kenya. The site was at the Ministry of Agriculture, characterized by Acri-orthic Ferralsol soil. Accessions were evaluated for eight agro-morphological traits at different stress phases (120, 150, 180 and 210 days after planting),, and six yield parameters at final harvest (210 days after planting) under both well-watered and water-stressed conditions to determine their response to moisture stress. Analysis of variance was carried out for all agronomic and morphological traits and broad sense heritability estimated. ANOVA results showed genotypic differences in all traits assessed except harvest index. Variation was observed between treatments at different stress phases for almost all traits. Genotype and genotype by environment interaction had different levels of influence on trait expression. There was low to intermediate broad sense heritabilities of most traits assessed except harvest index and dry matter content which had almost no genetic effects. It is important that field trials be conducted in several locations for at least two seasons. Considering the relationship between traits, yield parameters were positively correlated with morphological traits. For instance, genotypic ability for leaf retention, which is an important trait related to cassava performance, was highly correlated with root fresh weight, number of storage roots, above-ground fresh biomass and dry matter content across genotypes.

Keywords: agro-morphological; cassava; drought; evaluation; Kenya; *Manihot esculenta*; stress

3.1. Introduction

Cassava (*Manihot esculenta* Crantz) is widely cultivated in Africa, Asia and Latin America. It is grown from sea level up to altitudes of 2000 m asl. near the equator in a wide range of environments. The crop is highly productive in favorable conditions, but also produces reasonably well in marginal areas (Cock, 1985; El-Sharkawy and Cock, 1987a). It ranks sixth among crops as a source of calories in the human diet worldwide (Setter and Fregene, 2007). It is a major source of carbohydrates in the tropics and neotropics, providing a cheap source of dietary starch for over 700 million people in these regions. These areas have many subsistence farmers who lack the resources to purchase and apply agro-chemicals on a regular basis and, hence, utilize low-fertility and stress-prone soils (Taylor et al., 2004). An estimated 70 million people in the tropics obtain more than 500 cal/day from cassava, whereas more than 500 million obtain more than 100 cal/day from this crop (Cock, 1985; Kawano, 2003; Ojulong et al., 2008). The metabolizable energy of dry cassava (3500 to 4000 kcal g^{-1}) compares well to that of maize flour (Kawano, 2003).

Despite cassava being native to the Amazon region, Africa produces more than the rest of the world combined. In Africa, the production has increased more than threefold between 1980 and 2005 (Nhassico et al., 2008). This has been attributed to a 70% increase in the area of land cultivated as opposed to an increase in yield per hectare. In addition, the population in African countries has more than doubled within this time frame as compared to a 1.5 increase worldwide. Also, the adult HIV prevalence has increased to 6.1% in Africa as compared to 1.0% worldwide (UNAIDS, 2006), leaving a weakened labor force. These households under stress from HIV/AIDS have switched from high-input to low-input farming systems that involve cassava (FAO, 2008). Although there has been a 33% increase in total production of cassava in Africa, the yield per hectare has declined (from 1.2% to 0.6%) over the last two decades (IITA, 1997; Hillocks, 2002).

In Africa, cassava yields are approximately 10 tons fresh roots per hectare. This is half of those obtained in Asia and Latin America and 6 times less than the maximum yields obtained in experimental fields in a 12 month growing season (Hershey, 1987).

Nevertheless, since in Africa, cassava is grown with minimal or no external inputs, these yields compare favourably with other basic energy crops.

Cassava production in Africa is constrained by a number of biotic and abiotic factors, the former being pests and diseases. These include both indigenous pests and severe exotic ones, introduced due to the crop's intensified cultivation; the most devastating ones in recent years are cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Due to the crop's vegetative nature of propagation, small-scale farmers acquire planting materials from their neighbors, during travel, or as volunteer plants left in fallow (Mkumbira et al., 2003). This leads to pest and disease accumulation and dissemination. Main abiotic factors are problematic soils, freezing and drought (Ludlow and Muchow, 1990). Among these, drought is the most prevalent environmental factor limiting the crop's productivity, growth and survival (Saini and Westgate, 1999; Prasad and Staggenborg, 2008). Although precise crop losses due to drought are difficult to estimate, complete crop failure has been reported (Bohnert and Jensen, 1996).

Cassava is better adapted to water-limiting environments than other crops. This has been shown by its ability to produce a yield even under adverse edaphic and atmospheric conditions. It is commonly cultivated in areas receiving less than 800 mm of rainfall per year with a dry season of 4–6 months (Alves and Setter, 2004). This attribute is of great importance as the demand for food and fresh water supplies increases due to world population growth and climate change (Khush, 1999; Gleick, 2003).

Drought stress can occur at any stage during a crop's life cycle. Tolerance to drought is the phenotypic expression of a number of agronomic, morphological and physiological characteristics that act together to bring about a concerted response to drought in plants resulting in improved yield (crops), or survival and production of offspring. Within cassava germplasm maintained in several generations, a wide variation for tolerance to prolonged drought has been identified (CIAT and Embrapa, 1996; 1999; El-Sharkawy, 2007). In Africa, some cassava accessions have been recognized as having tolerance to water stress, although there is limited, if any, systematic data available relating to this germplasm. This is mainly due to the fact that breeders are unsure of the traits to assess for cassava drought tolerance

evaluation. This stems from a fundamental lack of knowledge concerning the mechanisms that contribute to drought tolerance in cassava.

In cassava, major agro-morphological traits have frequently been studied with the aim of understanding various aspects of its productivity. These include plant height, harvest index, dry matter content, storage roots per plant, root characteristics and weight, shoot weight and leaf retention (Lenis et al., 2006; Ssemakula and Dixon, 2007; Eke-Okoro et al., 2008; Subere et al., 2009). Nevertheless, no systematic evaluation has been performed in Kenya for these traits in putative drought-tolerant and drought-susceptible African cassava germplasm accessions. Therefore, there is a need to evaluate African accessions to determine the agronomic and morphological characteristics that make certain accessions drought-tolerant, as water increasingly becomes a rare commodity and cassava cultivation continues to expand into non-traditional areas such as the semi-arid tropics.

The present study was conducted within the framework of the project "Identifying the physiological and genetic traits that make cassava one of the most drought-tolerant crops" implemented since 2005 by the Brazilian Agricultural Research Corporation (Embrapa); the International Center for Tropical Agriculture (CIAT); the International Institute of Tropical Agriculture (IITA); Cornell University, USA and University of Goettingen, Germany.

Main objectives of the present study were:

- to determine the most important agronomic and morphological attributes that are related to drought tolerance in cassava;
- to identify drought-tolerant and drought-susceptible cassava germplasm from a selection of African germplasm accessions.

3.2. Materials and methods

3.2.1. Study site

Agronomic and morphological evaluations were conducted at the experimental field of Kenya Agricultural Research Institute (KARI), Kiboko Research Station in Makindu, Eastern Kenya. The site is located at latitude 2º15′ S, longitude 37º 75′ E, and an elevation of 975 m asl. The experimental field is characterized by a Acri-orthic Ferralsol soil. Texturally, the soil belongs to sandy clay loam overlying sandy clay (Table 3.1). This soil has good physical properties; primarily, an excellent structure, which allowed water to enter the soil freely. Despite this, it has reduced weatherable minerals and a low Cation Exchange Capacity (CEC). The low CEC reduces its capacity to retain cation nutrients like potassium, calcium and magnesium. Ferralsol are rich in sesquioxides, especially iron III oxide, which form insoluble precipitates with the orthophosphate ions, leading to fixation of nutrients.

Table 3.1. Physical soil characteristics (%) of Kiboko Research Station, Makindu, Kenya.

Depth (cm)	Sand	Silt	Clay	Depth (cm)	Sand	Silt	Clay
0-19	74	5	21	35-72	54	5	41
19-35	70	5	25	72-110	54	7	39

Source: Kenya Soil Survey, unpublished data

3.2.2. Climate

Kiboko is characterized by a bimodal type of rainfall with the main season occurring from late February to mid May and the minor season from late October to mid December. The total amount of rainfall per annum is about 530 mm, although in the last 10 years, it has been very erratic. Relative humidity and temperature have been fairly constant from 1998 to 2008. Mean maximum and minimum temperatures are 35.1ºC and 14.3ºC, respectively. During the experimental period, the mean annual rainfall was 585 mm with mean temperature of 24.4ºC and mean relative humidity of 79.0% (Figure 3.1).After very high rains in March, the dry season was one month longer than in the long term mean.

3.2.3. Germplasm and field planting

A total of 31 putative drought-tolerant and drought-susceptible cassava germplasm accessions were considered in this study. Accessions consisted of breeding materials from IITA. The materials were obtained from IITA as aseptically cultured *in vitro* micro-propagules. The plantlets were hardened and multiplied (Table 2.1, Chapter 2). They were presumed to be either drought-tolerant, drought-susceptible or disease-resistant, although no detailed supportive data was available. Accessions were classified into five groups according to available information (Table 3.2). The experiment was laid out in a randomized block design with 2 treatments, well-watered and water-stressed, and 4 replicates per treatment. Cassava stakes were hand-planted in single row plots, consisting of 5 plants each, on April 7th 2008. The stakes were planted at a spacing of 1 m between them in each row, and rows were also spaced 1 m apart. An interblock distance of 7 m was planted with cassava guard plants to separate the treatments. No herbicides, fertilizers or plant protection measures were applied. The plantlets were watered twice a week, up to the first week of July (90 days after planting, DAP) to ensure a homogeneous establishment. Then, irrigation was withheld from the water-stressed treatment until plants were harvested in November (210 DAP). Plants in the well-watered blocks were irrigated to field capacity twice a week throughout the growing period.

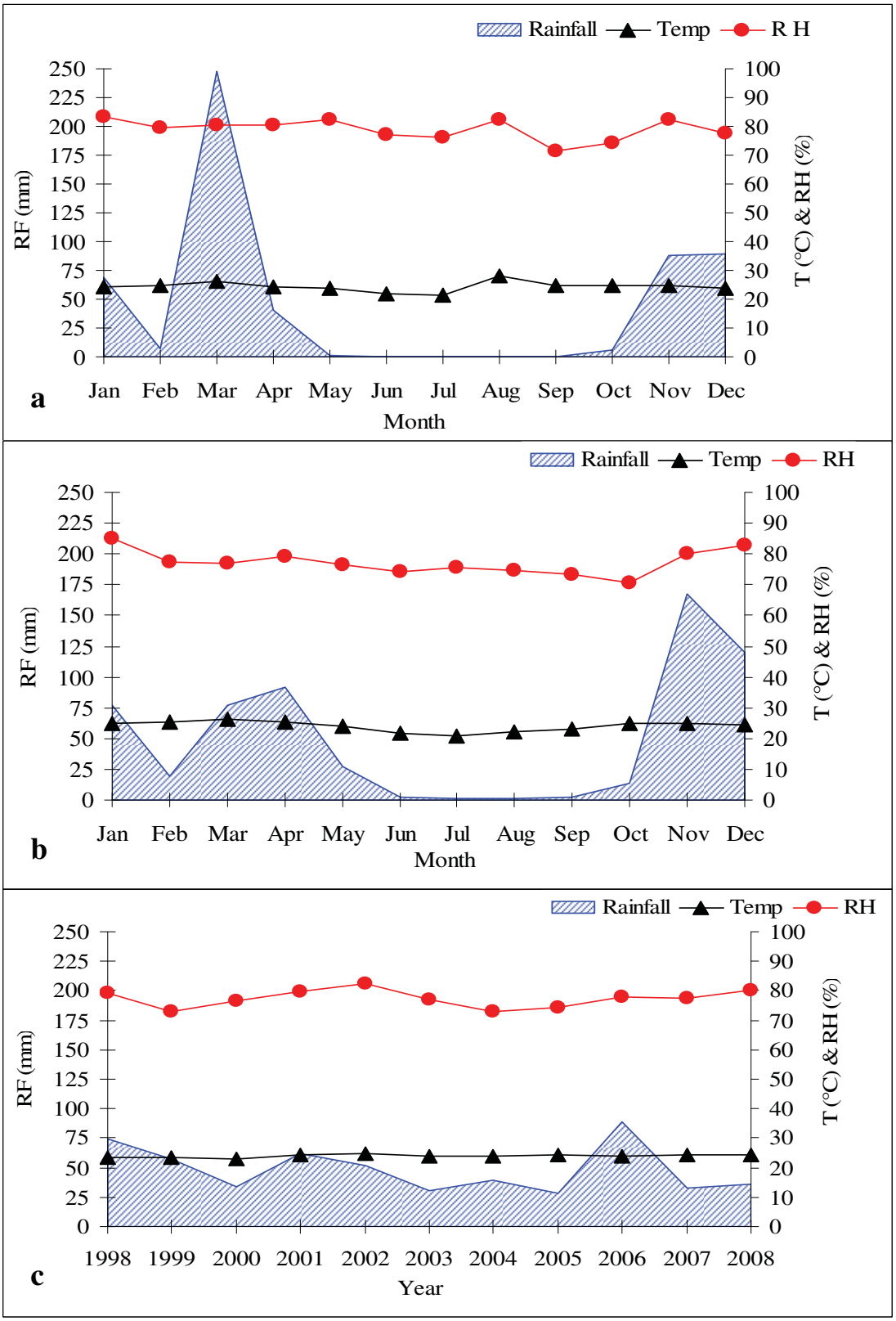

Figure 3.1. Elements of climate at Kiboko Research Station, Makindu, Kenya; (a) mean monthly rainfall, temperature and relative humidity in 2008; (b) mean monthly rainfall, temperature and relative humidity between 1998 and 2008; and (c) mean annual rainfall, temperature and relative humidity between 1998 and 2008. (**Source:** Kiboko Research Station)

Table 3.2. Putative drought-tolerant and drought-susceptible African cassava germplasm accessions utilized for field evaluation at Kiboko Research Station, Makindu, Kenya (CBSD, Cassava Brown Streak Disease resistance).

Accession No	Accession ID	Known Characteristic	Accession No.	Accession ID	Known Characteristic
G1	TME 4	CBSD resistant	G11	97/3200	Stay green
G2	I96/1089A	CBSD resistant	G12	94/0020	Stay green
G6	TME 117	CBSD resistant	G13	96/1087	Stay green
G24	96/1569	CBSD resistant	G14	I91B/00462	Stay green
G10	I92/0057	Mild drought resistant	G16	97/2205	Stay green
G19	TME 7	Mild drought resistant	G18	95/0289	Stay green
G5	92B/00061	Drought resistant	G20	99/0204	Stay green
G26	I92/0326	Drought resistant	G21	01/0090	Stay green
G7	I30572	Drought susceptible	G22	I91/1934	Stay green
G15	I95/0104	Drought susceptible	G25	M98/0068	Stay green
G17	14(2)1425	Drought susceptible	G27	96/0160	Stay green
G23	97/4779	Drought susceptible	G28	96/0596	Stay green
G3	I91/02312	Stay green	G29	96/1708	Stay green
G4	95/0166	Stay green	G30	I91/02327	Stay green
G8	98/0581	Stay green	G31	94/0026	Stay green
G9	01/0014	Stay green	G15	I95/0104	

3.2.4. Traits

A range of ordinal, interval and binomial data was recorded on different morphological and agronomic traits at varying periods of treatment imposition (Table 3.3). The morphological measurements were carried out on the two middle plants per plot for each accession after tagging since the traits were all non-destructive until harvest time. Assessments were carried out at 90, 120, 150, 180 and 210 DAP. Traits were selected based on the IPGRI and Portuguese-translated Embrapa descriptor list (Morag Ferguson, personal communication).

Yield traits were all destructive and were recorded at harvest (210 DAP). Estimation of dry matter content (DM) was based on the principle of a close relationship between specific gravity with DM according to Kawano et al. (1987). To determine the specific gravity, root samples of between 2- 3.5 kg were wiped free of soil and other debris and weighed in air (Wa) using a weighing balance (Scout® Pro-balance SP6000, d = 1 g; Ohaus Corporation, USA). The weight of the same roots fully immersed in water was determined (Ww). A sisal basket with perforations, whose own weight was negligible, was used to determine the two weights. This allowed soil

and debris to fall through. A sturdy wire was used to support the basket firmly to the weighing balance. The specific gravity and DM were computed from the two readings as follows (Kawano et al., 1987).

Specific gravity (**X**) = Wa / (Wa-Ww) (Equation 3.1)

Percentage DM = 158.3**X**-142 (Equation 3.2)

To determine harvest index (HI), the first 3 plants, per plot, per accession were uprooted. The roots and the above-ground biomass (stems, branches and leaves) were weighed separately. HI was computed only on fresh weight basis as described by Kawano (1990).

HI = fresh weight of roots / (fresh weight of roots + fresh weight of above-ground biomass) (Equation 3.3)

3.2.5. Statistical analysis

Analysis of variance (ANOVA) was carried out for all agronomic and morphological traits using PLABSTAT (Utz, 1997). A linear model:

$$Y_{ijkl} = \mu + G_i + T_j + P_l + R\,(GT)_{ijk} + G_iT_j + G_iP_l + T_jP_l + G_iT_jP_l + RGTP_{ijkl} \quad \text{(Equation 3.4)}$$

was used, where, Y_{ijkl} was the observed phenotypic value of the i^{th} genotype, in the k^{th} replication, of the j^{th} treatment and in the l^{th} stress phase; μ was the overall population mean of the trait, G_i is the genotype effect (i=1, 2, 3…31), T_j is the treatment effect (j=1, 2), P_l is the stress phase effect (l=1, 2, 3, 4), $R\,(GT)_{ijk}$ is the replication within the treatment x genotype interaction effect (k=1, 2, 3, 4), G_iT_j is the treatment x genotype effect, G_iP_l is the genotype x stress phase interaction effect, T_jP_l is the effect associated with treatment and stress phase effect, $G_iT_jP_l$ is the genotype by treatment by stress phase interaction effect and $RGTP_{ijkl}$ is the experimental error associated with each observation.

Broad sense heritability (h^2) of traits was estimated based on the analysis of variance. It was computed as:

$$h^2 = \sigma_G^2 / \{\sigma_G^2 + (\sigma_{GT}^2 / t) + [\sigma_E^2 / (rt)]\} \quad \text{(Equation 3.5)}$$

where σ_G^2 was the genotypic variance, σ_{GT}^2 genotypic X treatment variance, σ_E^2 was the residual variance, and r and t the number of replicates and treatment respectively Bernier et al. (2007).

Spearman's rank coefficient of correlation was calculated to determine relationships between traits.

Table 3.3. Agronomic and morphological traits studied when evaluating putative drought-tolerant and drought-susceptible cassava germplasm accessions at Kiboko Research Station, Makindu, Kenya. All agro-morphological traits were assessed five-fold, at 90, 120, 150, 180 and 210 days after planting (DAP).

Trait	Abbreviation	Unit	Remark/state
Agro-morphological traits			
Number of primary stems	NPS	No.	Side branches were not recorded
Number of branching levels	NBL	No.	Side branches were not recorded
Height of primary stem	HPS	cm	Recorded to one decimal place If many, highest was measured
Height of secondary stem	HSS	cm	Recorded to one decimal place If many, one was measured
Leaf retention	LR	%	Recorded as either 100, 75, 50, 25 based on visual estimation
Height of leafless stem	HLS	cm	Measured from ground level to where canopy started. In presence of branches, measured vertically to first leaf
Length of expanded leaf	LL	cm	Central leaf lobe was measured from point of interception to end of lobe
Width of expanded leaf	LW	cm	Widest part of one lobe measured
Harvest traits			
Above-ground biomass	AGB	kg	Determined from 3 plants combined
Storage root fresh weight	SRFW	kg	Determined from 3 plants combined
Harvest index			Relation of SRFW to AGB determined from 3 plants combined
Stem diameter	SD	cm	Measured 10 cm from the ground
Number of storage roots	NSR	No.	Counted from 3 plants at harvest
Dry matter content	DM	%	Determined by root specific gravity

3.3. Results

From the agronomic and morphological evaluation of cassava germplasm accessions conducted at Kiboko, Kenya, there were significant differences between the two water stress treatments for most of the traits assessed. Only number of primary stems and harvest index were not affected by moisture stress (Table 3.4, 3.6).

Table 3.4. Responses to water stress of 31 cassava accessions at harvest (210 days after planting, DAP) evaluated at Kiboko Research Station, Makindu, Kenya (h^2 = heritability).

Trait	Treatments		
	Water-stressed	Well-watered	h^2
Agro-morphological traits	Mean	Mean	
Number of primary stems	1.46	1.44	0.45
Number of branching levels	1.02	1.24	0.58
Height of primary stem	55.69	68.42	0.56
Height of secondary stem	17.22	28.79	0.64
Leaf retention	51.69	65.24	0.62
Height of leafless stem	21.22	22.38	0.32
Length of expanded leaf	9.33	10.31	0.38
Width of expanded leaf	2.90	3.14	0.53
Harvest traits			
Above-ground biomass	1.17	2.64	0.32
Storage root fresh weight	1.07	2.97	0.50
Harvest Index	0.46	0.53	-0.06
Stem diameter	2.88	3.43	0.42
Number of storage roots	4.92	10.33	0.54
Dry matter content	31.20	34.36	0.02

3.3.1. Plant height

Before imposing the stress, mean accession height of primary and secondary stem did not vary in relation to treatments. Significant differences were observed after one month of treatment imposition (Figure 3.2). At harvest, the mean height of plants in the well-watered treatment was 143.5 cm, whereas that of the water-stressed treatment was 98.7 cm (Table 3.5). In relation to accessions, significant differences were also observed after one month of stress imposition and throughout the crop cycle. Among the 31 accessions evaluated in the two treatments, G4, G10, G13, G24 and G28 had

outstanding height of more than 145 cm under water-stressed conditions. The same genotypes attained a mean height of >145 cm under well-watered conditions except G4 with 130 cm. At harvest (210 DAP), heights of accessions under well-watered conditions ranged from 85.4 to 210.8 cm, whereas those under water-stressed conditions were between 10.7 and 160.3 cm (Table 3.5).

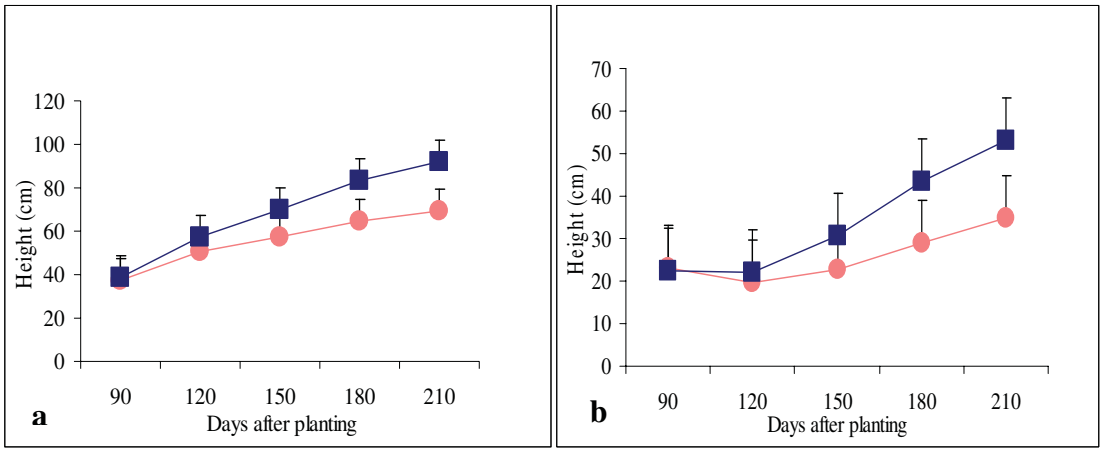

Figure 3.2. Mean plant heights of 31 cassava accessions at different stress phases (early, 90-120; mid season, 120-180; terminal 180-210 days after planting, DAP) evaluated at Kiboko Research Station, Makindu, Kenya; (a) primary (HPS) and (b) secondary (HSS) stems (■ well-watered; ● water-stressed; vertical bars =±s.e).

3.3.2. Leaf retention

The ANOVA of estimated leaf retention revealed significant differences among accession means in well-watered or water-stressed treatments after 1 month of stress imposition (Table 3.4, Figure 3.3). At 150 DAP, when the plants were experiencing mid-season stress, two thirds of the stems of most water-stressed accessions had lost their leaves. At 180 DAP, accessions in both treatments started loosing their leaves at a higher rate (Figure 3.3). Among the 31 accessions evaluated, G11, G13, G20, G22 and G26 had less than 2.5% difference in leaf retention under well-watered or water-stressed conditions (Figure 3.4). Accession G11 had even slightly higher leaf retention in the water-stressed treatment than in the well-watered environment.

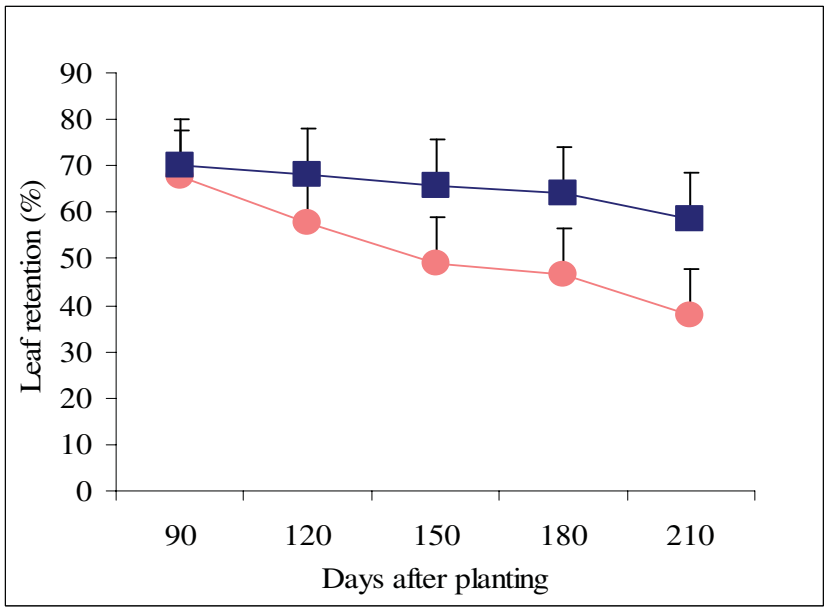

Figure 3.3. Estimated mean percentage leaf retention of 31 cassava accessions at different stress phases (early, 90-120; mid season, 120-180; terminal 180-210 days after planting, DAP) evaluated at Kiboko Research Station, Makindu, Kenya (■ well-watered; ● water-stressed; vertical bars =±s.e.).

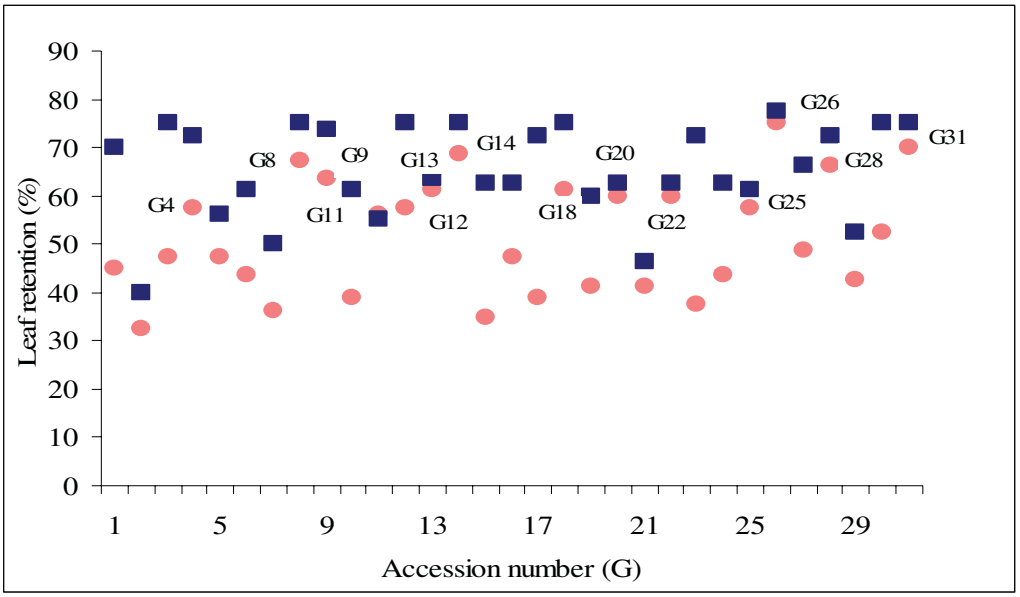

Figure 3.4. Estimated percentage leaf retention of 31 cassava accessions at harvest, 210 days after planting, evaluated at Kiboko Research Station, Makindu, Kenya (■ well-watered; ● water-stressed), only accessions with more than 55% leaf retention in water-stressed treatment were labeled.

Table 3.5. Mean plant height of 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya during different stress phases (WS, water-stressed; WW, well-watered; early stress, 90-120; mid season, 120-180; terminal, 150-210 days after planting, DAP).

Accession	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
No.	90 DAP		120 DAP		150 DAP		180 DAP		210 DAP	
G1	27.0	45.9	47.8	68.8	58.6	92.4	75.1	119.2	79.6	145.2
G2	23.6	31.0	40.1	55.8	43.9	69.7	47.3	77.6	51.3	85.4
G6	38.1	39.3	52.3	69.0	60.9	85.2	80.3	105.7	90.8	122.2
G24	46.1	62.8	89.3	98.0	108.5	121.5	120.6	154.7	150.5	173.6
G10	44.7	49.3	81.0	87.0	108.5	119.6	144.1	155.1	158.5	178.4
G19	37.3	50.0	59.9	76.9	62.8	96.7	73.4	109.9	78.4	125.6
G5	25.9	39.2	43.8	69.8	53.2	86.2	59.6	110.1	66.7	123.3
G26	40.4	37.7	57.6	61.3	73.7	92.0	92.5	116.4	109.7	150.5
G15	39.0	32.8	9.3	82.0	9.7	98.0	10.0	132.4	10.7	147.1
G7	29.3	30.4	42.2	47.8	46.6	61.1	52.9	90.3	58.7	88.3
G17	27.3	30.8	37.3	53.3	49.1	83.8	64.1	116.0	73.3	131.1
G23	29.0	65.7	34.7	114.3	46.3	160.1	60.4	174.5	65.6	197.3
G11	44.4	37.4	61.3	55.8	88.6	80.1	98.2	99.1	102.9	110.7
G12	35.0	54.1	51.8	96.8	68.1	133.0	94.2	168.1	110.2	188.2
G13	47.4	79.5	103.5	109.5	124.4	131.0	149.1	157.7	160.3	170.9
G14	34.0	33.3	48.8	73.3	58.5	87.4	77.8	111.6	85.7	138.7
G16	42.8	30.4	69.0	66.0	74.2	98.0	85.7	122.1	93.4	134.5
G18	34.0	31.6	55.5	68.5	70.9	98.8	81.3	129.8	88.2	139.1
G20	43.7	46.4	62.3	62.0	71.3	85.0	95.2	102.3	116.4	119.0
G21	29.9	33.5	40.4	49.5	46.6	72.7	54.4	87.0	56.5	106.0
G22	39.5	46.1	64.6	68.3	77.9	93.1	93.0	114.5	108.7	130.7
G25	52.1	40.2	73.3	70.8	82.5	92.3	91.2	120.1	99.5	135.5
G27	16.0	36.7	31.3	53.0	33.3	70.1	42.1	86.3	45.2	103.7
G28	53.1	61.3	92.9	86.3	110.8	120.3	131.9	147.8	145.9	163.9
G29	36.8	30.9	49.3	50.0	69.0	84.5	91.4	125.5	97.5	172.0
G30	49.8	37.2	82.0	86.8	85.4	105.0	109.0	151.0	115.5	160.9
G31	54.3	60.7	76.7	131.8	104.7	155.9	133.9	190.3	142.9	210.8
G3	32.1	29.3	42.3	54.6	56.2	70.8	75.6	102.7	85.1	121.2
G4	47.5	34.2	93.0	64.0	104.5	86.5	128.3	117.4	148.8	130.8
G8	47.8	66.9	76.8	86.8	98.7	124.1	120.3	144.7	124.3	170.4
G9	52.2	43.5	71.0	86.3	103.1	113.3	132.5	156.5	140.4	175.4
Mean	38.7	43.5	59.4	74.3	72.6	99.0	89.2	125.7	98.7	143.5
% CV	37.3	45.6	47.0	39.7	46.8	35.5	44.1	31.7	43.9	30.0
SE	2.6	3.6	5.0	5.3	6.1	6.3	7.1	7.1	7.8	7.7

Table 3.6. Analysis of variance of 31 cassava accessions evaluated at Kiboko Research Station, Makindu, Kenya (** significant at P≤0.01; * significant at P≤0.05; T, treatment; A, accession; D, days after planting, TxA, treatment by accession; TxD, treatment by days after planting, AxD, accession by days after planting; ND, not determined).

Trait	Variance components					
	T	A	D	TxA	TxD	AxD
Agro-morphological traits						
Number of primary stems	1.00	21.07**	10.73**	8.40**	4.00	120.00
Number of branching levels	56.07**	11.49**	587.95**	3.45**	4.00	2.36**
Height of primary stem	234.84**	29.11**	325.67**	8.54**	21.89**	120.00
Height of secondary stem	265.94**	19.68**	383.89**	4.65**	17.76**	1.57**
Leaf retention	311.26**	20.34**	83.92**	5.16**	18.18**	120.00
Height of leafless stem	5.31*	9.34**	253.87**	5.03**	5.10**	1.34*
Length of expanded leaf	78.24**	9.64**	217.73**	4.63**	35.08**	1.86**
Width of expanded leaf	40.52**	9.10**	307.32**	3.17**	22.21**	1.47**
Harvest traits						
Above-ground biomass	3477.12**	19.65**	ND	10.43**	ND	ND
Storage root fresh weight	3997.47**	24.97**	ND	8.64**	ND	ND
Harvest Index	0.00	0.00	ND	0.00	ND	ND
Stem diameter	333.63**	19.00**	ND	7.97**	ND	ND
Number of storage roots	1855.69**	23.82**	ND	7.51**	ND	ND
Dry matter content	387.93**	16.87**	ND	16.20**	ND	ND

3.3.3. Number of branching levels

Significant differences between the two treatments were observed after 150 DAP (Table 3.4, 3.6). The well-watered plants had a slightly higher number of branching levels after one month of stress imposition. After three months of stress exposure, accessions in both treatments reached a peak value (Figure 3.5). Results also showed genotypic differences at various stress phases (Table 3.6).

3.3.4. Leaf length and width

The two treatments also caused significant differences for leaf size traits (Table 3.4). It was interesting to note that water-stressed plants had slightly more expanded leaves after one month of stress imposition. After two months of stress exposure, the water-stressed accessions had significantly smaller leaves than the well-watered plants. This showed that mid-season stress had implications on leaf expansion. Results also

showed genotypic differences at different stress phases. It was noted that at 180 DAP, there was a sharp decrease in the leaf length and width in both treatments in all accessions (Figure 3.6).

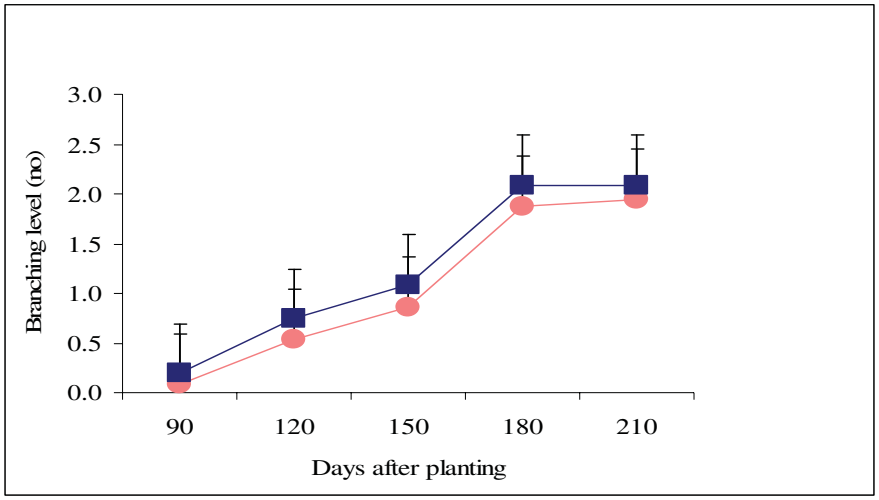

Figure 3.5. Mean number of branching levels of 31 cassava accessions at different stress phases (early, 90-120; mid season, 120-180; terminal 180-210 days after planting DAP) evaluated at Kiboko Research Station, Makindu, Kenya (■ well-watered; ● water-stressed).

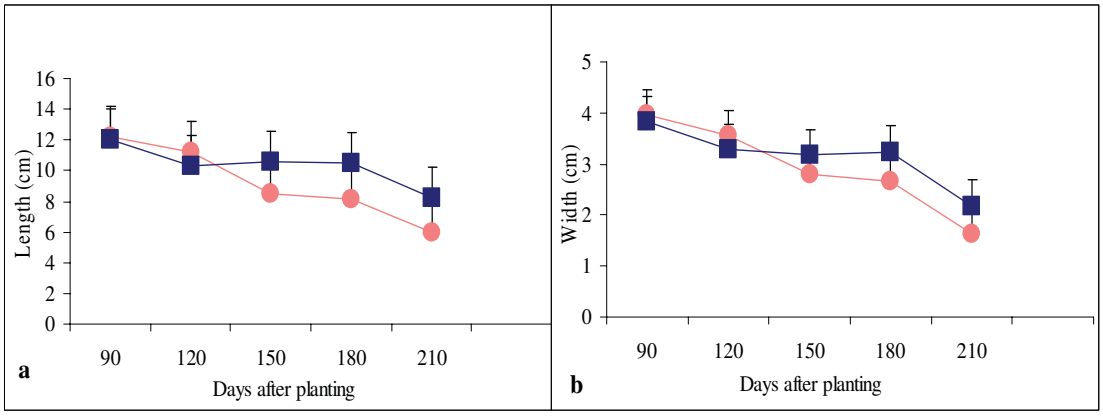

Figure 3.6. Mean leaf size of 31 cassava accessions at different stress phases (early, 90-120; mid season, 120-180; terminal 180-210 days after planting DAP) evaluated at Kiboko Research Station, Makindu, Kenya; (a) leaf length and (b) width; (■ well-watered; ● water-stressed).

3.3.5. Harvest traits

In general, there were highly significant differences between the treatments for all yield components except harvest index (HI). All observed yield parameters were higher in well-watered than in the water-stressed treatment (Table 3.4). The largest differences between the two treatments were found in storage roots fresh weight (Figure 3.7). Differences among accessions were apparent. Accessions G26, G11, G8 and G31 produced the highest storage root FW of 36.7, 33.3, 23.3 and 21.7 kg/m^2, respectively, under water-stressed conditions. These accessions had outstanding above-ground biomass and number of roots (Table 3.7, Figure 3.7). It was interesting to note that two of the best genotypes for yield under stress (G11 and G26) were small dwarfed above-ground. They had good yields due to their ability to retain a high HI under stress. This indicates that above-ground appearance alone cannot be used as a guide to the best genotypes. G15 which had been classified as drought-susceptible produced the lowest FW root yield.

3.3.6. Relationship between traits

The degree of relationship between traits assessed by Spearman's rank correlation coefficient varied considerably. The highly significant correlations between leaf retention and all yield traits except harvest index showed that leaf retention was a good character to be assessed. The number of storage roots was highly correlated with above-ground biomass FW and storage roots FW (r=0.71** and 0.61** respectively). Dry matter content was highly correlated with number of storage roots and harvest index (r=0.61** and 0.44*, respectively). Also, storage roots FW was correlated with HI at r=0.63** (Table 3.8). In figure 3.7a, there appears to be some genotype by environment effects for HI in that some genotypes hold their HI high even under stress, while in others it gets drastically diminished. Accessions that showed the stay green characteristic were also tall as reflected in high correlation coefficients of the height of primary and secondary stems with leaf retention (r=0.54** and 0.48**, respectively) (Table 3.8). It was interesting to note that correlation coefficient for individual treatments varied in their level of significance. For example, the correlation coefficient for storage roots FW was significant at $P < 0.05$ in the water-stress treatment whereas in the well-watered conditions, the differences were not significant (Table 3.9).

3.3.7. Heritability

The ANOVA results of this study revealed intermediate broad sense heritability estimates for most agro-morphological and harvest traits evaluated, however, dry matter content and harvest index showed very weak genotypic effect (Table 3.4).

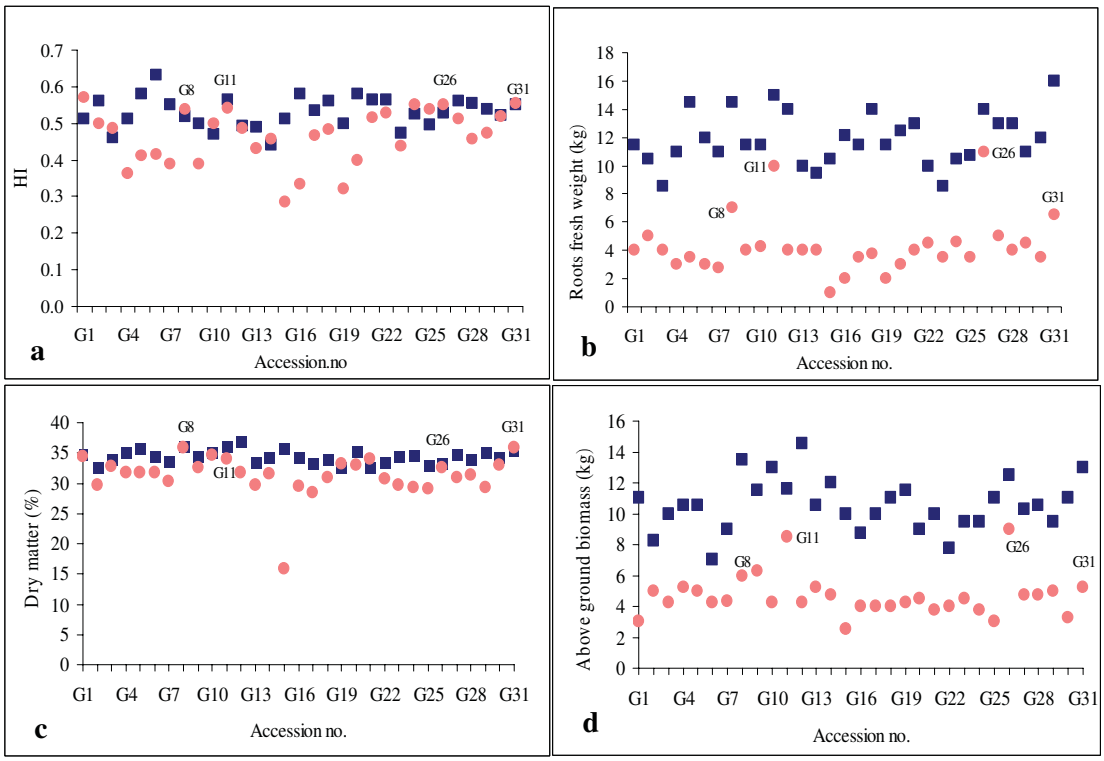

Figure 3.7. Mean yield parameters of 31 cassava accessions evaluated at Kiboko Research Station, Makindu, Kenya; (a) harvest index; (b) storage roots fresh weight; (c) percentage dry matter content; and (d) above-ground fresh biomass at harvest (■ well-watered; ● water-stressed); only accessions outstanding in water stressed environment for all four traits were labeled.

Table 3.7. Means of yield traits at harvest of 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya under well-watered (WW) and water-stressed (WS) treatments.

	Storage root FW (kg/m^2)		Above-ground biomass FW (kg/m^2)		Harvest Index		Dry matter content (%)		Number of storage roots	
Accession	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
G1	13.3	38.3	10.0	36.7	0.57	0.51	34.4	34.9	5.0	10.3
G2	16.7	35.0	16.7	27.5	0.50	0.56	29.7	32.5	5.0	9.3
G6	10.0	40.0	14.2	23.3	0.41	0.63	31.7	34.5	3.5	8.8
G24	15.3	35.0	12.5	31.7	0.55	0.53	29.3	34.7	4.3	8.8
G10	14.2	38.3	14.2	43.3	0.50	0.47	34.6	35.0	4.8	9.8
G19	6.7	38.3	14.2	38.3	0.32	0.50	33.2	32.6	2.5	12.8
G5	11.7	48.3	16.7	35.0	0.41	0.58	31.7	35.7	4.8	10.8
G26	36.7	46.7	30.0	41.7	0.55	0.53	32.7	33.2	9.8	13.5
G15	3.3	35.0	8.3	33.3	0.29	0.51	16.0	35.7	0.0	6.5
G7	9.2	36.7	14.5	30.0	0.39	0.55	30.4	33.6	2.5	9.0
G17	11.7	38.3	13.3	33.3	0.47	0.53	28.4	33.1	4.3	9.8
G23	11.7	28.3	15.0	31.7	0.44	0.47	29.6	34.5	5.3	12.8
G11	33.3	50.0	28.3	38.7	0.54	0.56	33.9	36.0	8.3	8.5
G12	13.3	46.7	14.2	48.3	0.48	0.49	31.8	36.8	5.3	13.8
G13	13.3	33.3	17.5	35.0	0.43	0.49	29.6	33.4	4.8	10.3
G14	13.3	31.7	15.8	40.0	0.46	0.44	31.5	34.2	5.8	11.5
G16	6.7	40.3	13.3	29.2	0.33	0.58	29.5	34.3	2.8	7.0
G18	12.5	46.7	13.3	36.7	0.48	0.56	30.8	34.1	4.8	11.3
G20	10.0	41.7	15.0	30.0	0.40	0.58	32.9	35.2	4.5	10.5
G21	13.3	43.3	12.5	33.3	0.52	0.57	33.9	32.6	5.0	8.8
G22	15.0	33.3	13.3	25.8	0.53	0.56	30.6	33.5	5.5	10.3
G25	11.7	35.8	10.0	36.7	0.54	0.49	29.1	33.0	3.0	9.3
G27	16.7	43.3	15.8	34.2	0.51	0.56	31.0	34.8	4.5	10.3
G28	13.3	43.3	15.8	35.0	0.46	0.55	31.3	34.1	5.3	14.0
G29	15.0	36.7	16.7	31.7	0.47	0.54	29.4	35.0	5.8	9.0
G30	11.7	40.0	10.8	36.7	0.52	0.52	33.0	34.3	3.5	7.5
G31	21.7	53.3	17.5	43.3	0.55	0.55	35.9	35.4	9.0	13.3
G3	13.3	28.3	14.2	33.3	0.48	0.46	32.9	34.1	5.8	11.8
G4	10.0	36.7	17.5	35.0	0.36	0.51	31.7	35.0	4.5	9.0
G8	23.3	48.3	20.0	45.0	0.54	0.52	35.9	36.1	8.0	10.5
G9	13.3	38.3	20.8	38.3	0.39	0.50	32.5	34.5	5.3	12.3
Mean	14.2	39.7	15.5	35.2	0.46	0.53	31.3	34.4	4.9	10.3
% CV	46.8	15.4	28.8	15.6	17.20	11.30	10.9	3.1	38.8	18.8
SE	1.20	1.10	0.80	0.99	0.01	0.01	0.61	0.19	0.3	0.3

Table 3.8. Spearman's rank correlation coefficient between traits (abbreviations in Table 3.3) assessed for 31 cassava accessions evaluated at Kiboko Research Station, Makindu, Kenya.

Trait	NPS	NBL	HPS	HSS	LR	HLS	LL	LW	AGB	SRFW	HI	SD	NSR
NBL	-0.28												
HPS	-0.54**	0.63**											
HSS	-0.42*	0.87**	0.79**										
LR	-0.21	0.41*	0.54**	0.48**									
HLS	-0.47**	0.38*	0.73**	0.47**	0.15								
LL	-0.33	0.26	0.58**	0.31	0.48**	0.25							
LW	-0.38*	0.42*	0.51**	0.48**	0.67**	0.15	0.53**						
AGB	-0.41*	0.16	0.41*	0.29	0.57**	0.25	0.26	0.59**					
SRFW	-0.12	0.12	0.15	0.16	0.39*	0.07	-0.14	0.39*	0.75**				
HI	0.32	0.21	-0.11	0.11	0.08	-0.14	-0.48**	0.01	0.12	0.63**			
SD	-0.61**	0.56**	0.68**	0.69**	0.54**	0.33	0.51**	0.64**	0.53**	0.17	-0.12		
NSR	-0.12	0.32	0.35	0.29	0.63**	0.26	0.21	0.59**	0.71**	0.61**	0.33	0.54**	
DM	-0.11	0.28	0.29	0.28	0.38*	0.16	0.14	0.49**	0.54**	0.56**	0.44*	0.35	0.61**

Table 3.9. Spearman's rank correlation coefficient for individual treatments (abbreviations in Table 3.3) assessed for 31 cassava accessions evaluated at Kiboko Research Station, Makindu, Kenya (WW=well-watered; WS=water stressed).

Trait	WW_NPS	WW_NBL	WW_HPS	WW_HSS	WW_LR	WW_HLS	WW_LL	WW_LW	WW_AGB	WW_SRFW	WW_HI	WW_SD	WW_NSR	WW_DM
WS_NPS	1.00	-0.02	-0.16	-0.13	-0.15	-0.10	-0.02	-0.19	-0.19	-0.02	0.25	-0.35	-0.03	-0.09
WS_NBL	-0.07	1.00	0.52	0.67	0.19	0.23	0.15	0.27	0.22	0.02	-0.08	0.37	0.27	-0.01
WS_HPS	-0.22	0.60	1.00	0.81	0.35	0.62	0.10	0.23	0.40	0.02	-0.31	0.56	0.38	0.25
WS_HSS	-0.15	0.70	0.81	1.00	0.28	0.46	0.16	0.28	0.42	0.05	-0.26	0.52	0.32	0.16
WS_LR	0.03	0.21	0.26	0.29	1.00	-0.11	0.05	0.27	0.29	0.02	-0.26	0.33	0.16	0.07
WS_HLS	-0.29	0.32	0.58	0.51	0.09	1.00	-0.02	0.10	0.22	-0.04	-0.20	0.25	0.34	0.05
WS_LL	-0.07	0.08	0.11	0.14	0.09	0.03	1.00	0.45	0.12	0.10	-0.07	0.11	0.03	0.08
WS_LW	-0.03	0.17	0.11	0.12	-0.03	0.05	0.10	1.00	0.28	0.21	-0.16	0.25	0.25	0.14
WS_AGB	-0.06	0.09	0.11	0.09	0.28	0.22	0.12	0.10	1.00	0.29	-0.34	0.51	0.42	0.07
WS_SRFW	0.07	0.08	0.06	0.04	0.22	0.05	-0.01	0.17	0.64	1.00	0.30	0.04	0.23	0.17
WS_HI	0.22	0.19	0.07	0.21	0.14	-0.01	0.02	0.12	0.16	0.56	1.00	-0.46	-0.18	0.00
WS_SD	-0.22	0.21	0.45	0.48	0.26	0.39	0.23	0.30	0.19	0.17	0.19	1.00	0.44	0.08
WS_NSR	0.09	0.19	0.15	0.15	0.20	0.18	0.01	0.22	0.54	0.80	0.49	0.23	1.00	-0.15
WS_DM	0.04	0.21	0.15	0.24	0.13	0.23	0.09	0.27	0.20	0.27	0.37	0.21	0.30	1.00

Significance at P< 0.1
Significance at P< 0.05
Significance at P< 0.01

3.4. Discussion

During the trial period, temperature and relative humidity at Kiboko were within the optimum requirements for cassava and were relatively uniform (Figure 3.1). This indicates that the performance of accessions was little affected by variation in these two parameters. There was hardly any rainfall during treatment imposition, so that the two treatments differed in the amount of artificial water supplied. Water at field capacity probably contributed to higher plant heights in the well-watered treatment. Onwueme (1978) and IITA (1990) showned that cassava height is affected by environmental conditions. Results also indicate that genotypes responded differently to different stress conditions (e.g., Figures 3.4 and 3.7). This may be due to genotype by environment interaction in addition to genetic variation.

Aina et al. (2007) demonstrated that germplasm introduction provides a unique source of variability to broaden the genetic base for drought tolerance in cassava. Selecting drought-tolerant cassava plants that have the ability to grow tall is advantageous since cassava is a vegetatively propagated crop. Cassava multiplication in farmers' fields is commonly through stem cuttings. The number of nodes per stake is of prime importance since these are regions for shoot development. Selection should be geared towards drought-tolerant tall plants with close inter-node spacing since more cuttings can be obtained and, hence, a higher multiplication rate.

The International Plant Genetic Resources Institute (IBPGR, 1982, cited in Gulick et al., 1983), while defining traits useful for cassava characterization, identified the branching habit as a stable morphological trait. This trait has been shown to be of adaptive, agronomic and market importance (Gulick et al., 1983). Cassava forms one or more axillary buds on the stem upon sprouting. These buds develop and sequentially form nodal units consisting of a node, a bud, a palmate leaf blade subtended by a long petiole, and an inter-node whose length and mass depend on genotype, age of the plant and environment (El-Sharkawy, 2003). The shoot shows apical dominance and indeterminate growth habit. This leads to formation of new leaves sequentially, in a spiral manner on the main stem depending on genotype and environmental conditions. Once apical dominance ceases and the apex becomes reproductive, 1-6 axillary buds develop and produce a branching characteristic in

cassava. In this study, most accessions in the water-stressed treatment had significantly reduced number of branching levels (Table 3.4, 3.6). Well-watered plants had higher branching levels, which resulted from increased numbers of axillary buds. Genotypic variation observed agrees with the findings by CIAT (1979) and Cock (1987), who found differences in timing and number of branching levels in cassava accessions.

Leaf retention/stay green trait has been identified as one of the most desirable characteristics in achieving high yields in crops (Borrell et al., 2000; Lenis et al., 2006). This is a drought tolerance mechanism that confers forbearance to plants under severe soil moisture stress. Cereals like maize, sorghum, millet, wheat and rice have vegetative and reproductive stages (phasic) of crop development, which are separated in time. The vegetative phase takes about 70 to 75% of the growth cycle during when the leaves, stems and inflorescences develop. This is followed by a shorter reproductive stage of between 25 to 30% of the growth cycle during which grain filling with carbohydrate occurs (El-Sharkawy and Cock, 1987a). In this pattern of crop growth and development, no competition exists for partitioning the photosynthetic assimilates between the source (leaves) and sink (grain) development. Unlike these cereal crops, cassava experiences simultaneous growth and development of the economic plant part (roots) and the photosynthetic sites (leaves). Lenis et al. (2006) reported that cassava accessions with greater leaf longevity can produce more total fresh biomass and a 33% higher root DM compared to drought-susceptible cultivars. Accessions with this characteristic are potentially drought-tolerant, which is an important trait in the complex sub-Saharan drought-hit regions.

In this study, the leaf retention in some accessions was almost equal in both the well-watered and water-stressed treatment (Figure 3.4). This suggests that this condition may be an inherent physiological characteristic of individual accessions and not only a response to stress. The genotypic ability for leaf retention was positively correlated with storage root FW and above-ground biomass (Table 3.8). Thus it may be advantageous to breed and select for longer leaf life and, hence, better leaf retention when developing varieties adapted to dry areas.

After 180 DAP, however, a significant decrease in leaf retention was observed, irrespective of the water regime. These results concur with the findings by El-

Sharkawy and Cadavid (2002) who observed a decrease in leaf formation after 6 months of cassava growth. This is mainly due to the dynamics of cassava development that control and favor partitioning of photosynthetic assimilates after 6 months towards the most important economic plant part (roots) as compared to leaf formation. In addition after 6 months, lower canopy leaves senescence and abscise due to aging and are, therefore, shed at accelerating rates (Pellet and El-Sharkawy, 2001). Despite our observations on leaf retention, there is need to refine and standardize the technique of quantifying leaf retention since the method used in this study was based on visual observation, which can be biased.

Water deficit is one of the most important environmental factors affecting leaf area development in cassava. Although the crop experiences simultaneous growth and development of the source and sink (El-Sharkawy and Cock, 1987a), El-Sharkawy (2003) has reported that the formation of leaves in cassava has preference for available assimilates over storage roots in the first 3 months of growth, after which more competition exists for partitioning the photosynthetic assimilates between the source and sink. In this study, results revealed a decline in leaf length and width between 90 to 120 DAP in both water regimes may-be due to the increased competition among different plant tissues.

Connor and Cock (1981) observed that in regions with high temperatures, cassava leaves are fully expanded in two weeks and the size increases with plant age up to about four months and then declines. During the mid season stress, the water-stressed plants had significant reduction in leaf length and width. This is in agreement with Porto (1983) who found that leaves produced under prolonged water stress are small, maybe to conserve carbohydrate reserves (El-Sharkawy and Cadavid, 2002). The observation that leaf length and width in well-watered plants reached a peak value at 180 DAP after which there was a sharp decrease in all accessions, agrees with findings by Pellet and El-Sharkawy (2001) on fertilized and unfertilized cassava, which is mainly due to the intrinsic dynamics of crop development.

Research has shown that cassava can be highly productive under favorable environments. In the absence of production constraints, it compares well with major staple food crops in the tropics, and it has been ranked as the second greatest energy producer after sugarcane (El-Sharkawy, 1993). Despite this, yield stability, which is

more important from the farmer's point of view, requires genotypes that also produce well under prolonged stress conditions. This is even more important as water continues to become a rare commodity especially in the semi- arid tropics.

Cock et al. (1979), using a computer-based simulation model, reported that an ideal cassava plant should produce about 90 kg/m^2 of fresh roots under optimal growth conditions. In this study, the maximum root yield observed under well-watered conditions was 53.3 kg/m^2, which was high considering that Kiboko is characterized by an Acri-orthic Ferralsol soil. Studies by El-Sharkawy (1993) showed that cassava yields of 8–16 t ha^{-1} of fresh roots are normally attained with local, traditional varieties on marginal soils without application of agrochemicals. Of the 31 accessions evaluated in this study, 4 showed outstanding performance under water-stressed conditions in that they attained fresh root yields of >21 kg/m^2, suggesting that they maybe drought-tolerant (Table 3.7). Also Bakayoko et al. (2009) observed outstanding performance in one of these 4 accessions (G11).

Harvest index, which is the ability to convert biomass to yield in crops, is a valuable trait in cassava breeding in that, selections based on this trait are stable across evaluation stages. El-Sharkawy and Cadavid (2002) observed that under prolonged water stress, cassava produces less total biomass but an increased harvest index, implying that nutrient use efficiency for root production is greater in stressful environments than in favorable ones. Although there were no significant differences observed between the two water regimes for this parameter, HI was higher for most genotypes under well-watered conditions. There were some exceptional genotypes that had high HI under stress (Figure 3.7a). Studies by Okogbenin et al. (2003), on the adaptation responses of cassava to drought stress in Nigeria, found considerable variation for HI amongst varieties and no significant differences in the mean HI amongst the water table sections. This indicates that the primary effect of the HI differences amongst the varieties may-be attributed to genetic effects and that, perhaps, it is an important trait to phenotype under limiting water conditions.

Dry matter content is a major component of cassava yield. Cassava roots have mean DM of about 35 percent, which is high compared to most roots and tubers. Starch and sugar comprise about 90 percent of this DM. Westby (2002) has shown that DM in cassava can vary from 20 to 45 percent depending on variety, growing conditions

(especially temperature and soil moisture), and health of the plant. In this study, soil moisture seems to have significantly influenced root DM in that well-watered plants had a slightly higher percentage than those in water stressed conditions (Figure 3.7c). This might also be due to foliage growth and photosynthate partitioning.

Determination of phenotypic variation resulting from genetic effects provides useful information to plant breeders to formulate effective breeding strategies. Low to high broad sense heritabilities for most cassava traits have been reported, e.g. 80-92% for DM; 55% for leaf retention (Kawano et al., 1987; Lenis et al., 2006), 91% for plant height, 83% for branching levels, 80% for leaf length, 90% for leaf width and 27% for length of stems. 87% for harvest index, 71% for fresh shoot weight, 50% for fresh root yield and 36%for number of storage roots (Okogbenin and Fregene, 2003). Although intermediate heritability values were observed in this experiment for most traits, their use is for comparison of traits in this study and not between our data and others given that this was a single year, single location study. In addition, there is need to do a multi-location study so as to determine the interaction between the accessions and environment.

3.5. Conclusion

In general, there were differences between treatments and accessions for several of the traits assessed. The relationship between most traits was strong. The results from this study suggest that the leaf retention trait combined with drought tolerance mechanisms commonly found in cassava, is advantageous in terms of total biomass and yield production under prolonged drought conditions. Length and width of leaves, branching level, leaf retention and harvest parameters could be important traits to phenotype African cassava germplasm under favorable and water stress conditions.

Four accessions G26, G11, G8 and G31 were more tolerant than the rest of the genotypes evaluated, calling for further research and their involvement in agricultural experimentation under drought-prone conditions. This information on phenotypic plasticity although, it is environment-dependent, will be important in breeding for climatic uncertainty and extreme environments.

4. Metabolites Analysis in African Cassava Germplasm Accessions Evaluated at Kiboko Research Station, Makindu, Kenya

Abstract

Thirty one African cassava germplasm accessions, consisting of diverse breeding materials from the International Institute of Tropical Agriculture (IITA) were analyzed for 7 metabolic traits under well-watered and water-stressed conditions, each at 3 time points (120, 150 and 180 days after planting), and in different tissues. The objective of the study was to identify secondary traits that could be used for phenotyping breeding materials for drought tolerance and to determine the concentrations of metabolites in different tissues. The ANOVA results showed that for all the traits, except protein and amylose contents, the population had genotypic differences as indicated by the highly significant probabilities. Variation was also observed between treatments. However, no significant differences were observed at different stress phases. The abscisic acid (ABA), sucrose and glucose contents decreased under water-stress, which, on the other hand, did not lead to a marked change in fructose concentration. The decrease in ABA was not consistent with reports on other cassava accessions and other species, probably, because of differences in stress intensity. Starch content per g dry weight was significantly higher in water-stressed accessions due to increased synthesis. The relationship between traits varied considerably. From the results of this study, the relative contribution of the traits to drought-tolerance cannot be determined. Further work will be required to identify and quantify the concentrations of the traits in relation to water-use efficiency of these varieties under limited available soil water.

Keywords: accession; cassava; drought; evaluation; genetic diversity; Kenya; *Manihot esculenta*; metabolic

4.1. Introduction

Cassava *(Manihot esculenta* Crantz) is a major staple food for nearly a billion people in 105 developing countries, where the roots provide a third of their daily calories (Onwueme, 2002; FAO, 2008). Since 1970, world cassava production has risen by 2.2% per annum (FAO, 1997). In Africa, the increase has been by 2.9%, which is roughly the same as the population growth rate (Westby, 2002). This growth, much of which occurred after the severe drought of 1982-83, has been attributed to the expansion in area under cultivation rather than to rising productivity (Hillocks et al., 2002; Nweke et al., 2002). Seventy percent of the global production (which is estimated to be over 128 million metric tons of dry roots annually) is used for human consumption either directly after cooking or in processed forms; the remaining 30% is used for animal feed and other industrial products (El-Sharkawy, 2003; FAO, 2008). Cassava is the cheapest known source of starch, and is used in more than 300 industrial products including ethanol as a possible source for biofuel (FAO, 2008).

Cassava is usually grown in monoculture; although, mixed cropping with tree crops, annual legumes and cereals is also common (Leihner, 1983; El-Sharkawy and Cock, 1987a). It is widely grown in tropical Africa, Asia and Latin America, mainly by resource-limited small-scale farmers over a range of environments. This is because of its remarkable tolerance to abiotic stresses and adverse environments, as compared to the capital-intensive and input-demanding Green Revolution cereal crops such as wheat, rice and maize (El-Sharkawy, 2003). For instance, unlike most other staple crops, cassava almost never fails due to drought (Burrell, 2003; Ceballos et al., 2004). This is perhaps due to its indeterminate growth habit, which may give it the ability to resume growth after an extended drought, or continue to develop a deeper fine root system to access water out of reach by other crops (seed/cereal), which are determinate. In addition, it is replacing yam in the humid zone, maize in the non-humid environment and other food crops in the sub-humid zone (IITA, 1997). Because of its undemanding nature in terms of soil fertility and inputs, together with its versatility in production and processing systems, it is an appropriate target for meeting goals of food security, equity, poverty alleviation, and environmental protection in the escalating African population.

Starch is the key storage reserve of carbohydrates in plants. Cassava has a remarkable sink capacity to store food reserves. It ranks very high among crops that convert the greatest amount of solar energy into soluble carbohydrates per unit ground area (Raheem, 2006; Adeniyi et al., 2007). Amongst the starchy staples, cassava amasses carbohydrate, which is about 40% higher than rice and 25% more than maize (Nyerhovwo, 2004; Lacerda et al., 2008). This makes cassava the cheapest source of calories for both human nutrition and as animal feed.

Drought is the most common environmental factor limiting crop productivity, growth and survival in the agricultural rain-fed areas (Bohnert and Jensen, 1996; Saxena et al., 2002). It affects more than 43% of the world population engaged in agriculture since it poses a threat to food security and sustainability of production systems to the people living in drought-prone areas (Saxena et al., 2002). It will continue to be a serious problem in agriculture because water is becoming scarcer due to increased use by the escalating population, declining and erratic precipitation, and less potable water availability. In response to drought, plants have developed various physiological, biochemical and genetic systems to tolerate, avoid or escape drought stress. These coping systems determine the survivability and persistence of plants in water-limited environments (Wu et al., 2006). Cassava is tolerant to drought and many other stresses.

It is often referred to as a “scavenger crop” because of its ability to efficiently absorb nutrients from low-nutrient soils (Howeler, 2002). In addition, it grows well on soils that are acidic and in drought conditions. Traits that contribute to cassava’s productivity in unfavorable environments include a response of the plant hormone abscisic acid (ABA) and accumulation and utilization of non-structural carbohydrates. The phytohormone is involved in root to shoot signaling, particularly, through regulation of stomata behavior, leaf growth and senescence, seed development, germination, defense against pathogens, and synthesis of storage proteins and lipids (Davies, 2004; Schwartz and Zeevaart, 2004; Wu et al., 2006). Sugars, the products of photosynthesis, are known to play a role in controlling a number of vital processes, including development, photosynthesis, germination and growth (Humby and Durnford, 2006). ABA is involved in plant response to drought stress by serving as a signal molecule and a key mediator for regulating specific pathways (Wu et al., 2006).

Cassava responds to water deficit with a stress avoidance syndrome, although, there is a wide variation within the cassava germplasm for response to prolonged drought (El-Sharkawy and Cock, 1987a; Setter and Fregene, 2007). This involves the highly sensitive stomatal closure, leaf drooping, leaf loss and halt of leaf growth, all of which influence the amount and concentration of the phytohormone ABA and sugars (Setter and Fregene, 2007). On the other hand, studies on such changes of the phytohormone and sugars, for detecting differences among African cassava germplasm accessions have not yet been done.

The present study was conducted within the framework of the project "Identifying the physiological and genetic traits that make cassava one of the most drought-tolerant crops" implemented since 2005 by the Brazilian Agricultural Research Corporation, (Embrapa); the International Center for Tropical Agriculture, (CIAT); the International Institute of Tropical Agriculture (IITA); Cornell University, USA, and the University of Goettingen, Germany.

Main objectives of the present study was to identify secondary traits that could be used for phenotyping breeding materials for drought tolerance and to determine the concentrations of metabolites in different tissues.

4.2. Materials and methods

4.2.1. Plant materials and sample preparation

Thirty one putative drought-tolerant and drought-susceptible African cassava germplasm accessions were considered in this study. These accessions, consisting of breeding materials from IITA were presumed to be either drought-tolerant or drought-susceptible. They represent a range of tolerances, including intermediate behavior, although, there is no firm knowledge available of the tolerance for each genotype (Table 3.2). The plants were grown under well-watered and water-stressed conditions at the experimental field of Kenya Agricultural Research Institute (KARI), Kiboko Research Station in Makindu, Eastern Kenya (Chapter 3, 3.2.1). For sugars, starch, ABA and protein contents, leaf disks, petioles and stem discs were sampled at 120, 150 and 180 days after planting (DAP) and immersed in 1 ml ice-cold 80% methanol. Three leaf disks, 0.3 cm in diameter each, were sampled from the mid fully expanded leaves using a leaf punch. For the petioles, approximately three 0.3 cm thick slices were sampled using a scalpel blade. One 0.3 cm disc per accession was sampled from the stem using a cork borer. Samples were transported in cooler boxes to IITA-Biosciences for eastern and central Africa (BecA) laboratories in Nairobi (Kenya), a journey that took about 3 hr. They were stored at -20ºC for 3 days to exodiffuse sugars and ABA. They were then dried at 45ºC for 1 week and transported to Cornell University, New York, USA in 96-well plates for various analyses. For both well-watered and water-stressed treatments, one root was sampled from each of the 3 plants at harvest. Following washing, approximately 10 mg slice was taken from the middle section of the tuber and transported to IITA-BecA for starch extraction. The starch was packed in C/7 envelopes and transported to University of the Free State, Bloemfontein, South Africa for analysis.

4.2.2. Chromatography separation, abscisic acid extraction and quantification

Abscisic acid was extracted from the leaf disks, petioles and stem discs in 600 µl of 80% (v/v) methanol. Two hundred microlitres of the supernatant were pipetted and dried overnight at 45°C using a non-vacuum drying incubator fitted with a turbulent fan. Dried samples were re-suspended in 100 µl of 30% (v/v) methanol and 20 µl of 0.04% bromecresol green (tracer) and homogenized for 15 min. using a shaker to re-

dissolve. ABA was separated with C_{18} chromatography on Supelco columns (DSC-18 SPE-96, J.T. Baker Chemicals, Phillipsburg, New Jersey) built on a 96-well vacuum manifold apparatus and packed with 25 mg of 40 µm diameter C_{18} silica material using the procedure described by Setter et al. (2001). Bromecresol green indicated more than 90% recovery of ABA. ABA fractions were dried at 45°C overnight using a non-vacuum incubator.

The ABA fractions from C_{18} chromatography were re-dissolved in 150 µl azide water (0.02% w/v, NaN_3). They were then assayed for ABA by indirect enzyme-linked immunosorbant assay (ELISA) using the method described by Setter et al. (1991) with the following minor modifications. Round-bottom 96-well microtiter plates (Costar High Binding #3366, Corning Inc., Corning, New York) were coated overnight at 5°C with 1.4 µg of ABA-bovine serum albumin (BSA) conjugate in 200 µl of 50 mM $NaHCO_3$, pH 9.6 and 0.02% NaN_3 as an antimicrobial agent. Plates were washed 4 times with Tris-buffered saline-Tween detergent (TBST) solution, which contained Tris-buffered saline (TBS; 10 mM Tris-hydroxymethyl amino methane, pH 7.5, 1 mM $MgCl_2$, 100 mM NaCl and 0.02% NaN_3) to which 0.1% Tween-20 (P-7949, Sigma Chemical Co., St. Louis) was added.

Samples were then incubated with primary antibody with the following in each well: 60 µl 3-N-morpholino propane-sulfonic acid (MOPS) solution, which contained MOPS-buffered saline MBS (MBSA; 50 mM MOPS, 1 mM $MgCl_2$, 100 mM NaCl, 0.02% NaN_3, pH 7.5, with 0.1% BSA) (A-8022, Sigma Chemical Co., St. Louis), 40 µl of C_{18} eluate and 100 µl of MBS containing 1 µg of anti-ABA monoclonal antibody (clone 15-I-C15, FEBS Lett 160:269, 1983). On each plate, a set of positive ABA standards (Sigma Chemical Co., St. Louis) containing a 1:2 dilution series of 12 values from 2 to 0.01 pmol per well served as a calibration curve. The antibody was added last to all wells on the plates using a 12-channel pipette with rapid, turbulent outflow so that solutions could swirl together and mix immediately. Plates were sealed fully using a cling film to prevent evaporative loss and incubated at 5°C overnight. On the following day, plates were washed 4 times with TBST solution and 200 µl of secondary antibody solution containing 20 µl of anti-mouse IgG-alkaline phosphatase conjugate (A-3562, Sigma Chemical Co., St. Louis) in MBSA was added into each well. After incubating overnight at 5°C, plates were washed 4 times with

TBST and 200 µl *para*-nitrophenyl phosphate (PNPP) reagent mixed with diethanolamine (DEA) buffer (0.9 M DEA, 3 mM $MgCl_2$, pH 9.8, 1 mg/ml PNPP) was added into each well. Samples were incubated at room temperature for 2 hr. and the absorbance was read at 405 nm with a plate reader spectrophotometer (model 750, Cambridge Technology, Watertown, MA). ABA content in samples was determined by calculations based on positive ABA calibration standards and a fit logit-transformation of data.

4.2.3. Determination of sugar content

Sugar analysis was performed on aliquots from the same leaf, petiole and stem extracts (80% methanol) used for ABA analysis. Glucose concentration was determined before and after the enzymatic hydrolysis of sucrose and fructose was assessed subsequent to the determination of glucose. The concentrations of glucose were determined using an assay based on enzyme-coupled reaction of peroxidase/glucose oxidase (PGO) (Trinder, 1969), where D-glucose reacts with O_2, catalyzed by glucose oxidase, to transfer electrons from glucose to O_2 and form gluconic acid and H_2O_2. The H_2O_2 immediately reacts in a coupled reaction catalyzed by peroxidase to accept electrons from *para*-hydroxybenzoic acid, a colorless electron donor, to create a pink quinone-imine dye complex with 4-amino-antipyrine. The reaction is highly specific for α-D-glucose (Lott and Turner, 1975). One hundred and fifty µl of PGO (100 mM KH_2PO_4, pH 7.0, 9 mg/ml *para*-hydroxybenzoic acid, 0.3 mg/ml 4-aminoanti pyrene, 0.1% BSA, 0.01% NaN_3, 0.33 µl/ml glucose oxidase and 2 µl/ml peroxidase) was added to each of the supernatants (100 µl leaves, 75 µl petioles and 40 µl stems). Concurrently, a duplicate set of glucose standards containing a series from 3 to 32 µg per well were added to each plate to serve as a calibration curve. Plates were incubated at room temperature for 2 hr. and the absorbance was read at 490 nm with a plate reader spectrophotometer (model 750, Cambridge Technology, Watertown, MA). For the sucrose assay, enzymatic inversion technique was used where; 50 µl of invertase solution (β-fructosidase) (250 mM acetate buffer, pH 4.5, 2 µl/ml invertase and 0.1% NaN_3) was added to the sucrose standards, glucose calibration standards and samples on the plate. Plates were incubated at room temperature for 4 hr. after which 200 µl of PGO reagent was added. After about 1 hr., plates were read at 490 in a plate reader spectrophotometer.

The sucrose concentration was calculated from the difference of the glucose concentration before and after enzymatic inversion. All enzymes were from Sigma Chemical Co., St. Louis.

4.2.4. Determination of protein content

The stems, leaves and petioles were ground using the Genogrinder (Bridgewater, NJ, USA). The to and fro high speed of the Genogrinder, which was run for 10 min. allowed the metallic balls (2 balls per sample, per well) to hit the wall of the well in between and during which the samples crushed. The resulting powder was centrifuged at 1000 rpm for 5 min. Two hundred and fifty µl of 0.01% NaN_3 was added to the samples. Forty µl of the supernatant was aliquoted for protein assay. Protein concentration in this supernatant was estimated by the method of Bradford (1976) using BSA as a standard and Coomassie Plus Protein Assay Reagent (Rockford, Illinois, USA). Absorbance was read at 590 nm after 30 min. incubation at room temperature.

4.2.5. Determination of starch content

Starch analysis was performed on the insoluble debris from the same leaf, petiole and stem used for protein analysis. Starch was gelatinized by heating at 80°C for 2 hr. in an oven. After cooling, starch was completely hydrolyzed to glucose with 200, 400 and 600 µl of amyloglucosidase solution (250 mM acetate buffer, pH 4.5, 0.15 mg/ml amyloglucosidase, 0.15 mg/ml α-amylase, 0.1% NaN_3 and 0.1% BSA) in leaves, petioles and stems, respectively. Samples were incubated at 40°C for 36 hr. with agitation. The amount of glucose released from starch hydrolysis was analyzed using the same procedure described above for sugar in extracts.

4.2.6. Determination of amylose content in roots

Native cassava starch was extracted using the method described by Benesi et al. (2004) with a few modifications. Approximately 2 g of fresh tuberous roots were washed, peeled, washed again and chopped to about 0.5 cm^3 cubes. After adding 250 ml of water, the chopped tuberous roots were pulverized in a blender (Phillips domestic blender, Model: HR1720/50) for 5 min. The pulp was suspended in 10x its

volume water, stirred for 2 min. and filtered using a double cheese cloth (muslin). The filtrate was allowed to stand for 2 hr. to facilitate starch sedimentation and the top liquid was decanted and discarded. The sediment was broken, water added as in the first step, and the whole process was repeated. The sediment was washed and then air-dried for three days. Amylose content was determined by first removing amylopectin as an aggregate with concanavalin A (Con A) by precipitation followed by amylose assay as above using an amylose/amylopectin assay kit (Megazyme International Ireland Ltd., Bray, Ireland) according to the manufacturer's protocol

4.2.7. Generation of derived variables

For total non-structural carbohydrates (TNC), total sugars and starch values were added. The sucrose to starch ratio was also calculated.

4.2.8. Statistical analysis

Abscisic acid, sugars, protein and starch in different tissues were expressed on an estimated tissue area basis. Analysis of variance (ANOVA) was carried out for the metabolic traits using PLABSTAT (Utz, 1997). A linear model:

$$Y_{ijl} = \mu + G_i + T_j + P_l + G_iT_j + G_iP_l + T_jP_l + GTP_{ijl} \qquad \text{(Equation 4.1)}$$

was used, where, Y_{ijl} was the observed phenotypic value of the i^{th} genotype, of the j^{th} treatment and in the l^{th} stress phase; μ was the overall population mean of the trait, G_i is the genotype effect (i=1, 2, 3…31), T_j is the treatment effect (j=1, 2), P_l is the stress phase effect (l=1, 2, 3,), G_iT_j is the treatment x genotype effect, G_iP_l is the genotype x stress phase interaction effect, T_jP_l is the effect associated with treatment and stress phase effect, $G_iT_jP_l$ is the genotype by treatment by stress phase interaction effect.

To determine the differences between treatments, accessions and tissues (leaf, petiole or stem), the linear model:

$$Y_{ijl} = \mu + G_i + T_j + R_l + G_iT_j + G_iR_l + T_jR_l + GTR_{ijl} \qquad \text{(Equation 4.2)}$$

was used, where, Y_{ijl} was the observed phenotypic value of the i^{th} genotype, of the j^{th} treatment and in the l^{th} tissue; R_l was the tissue effect (l=1, 2, 3,), and the other symbols being as defined in equation 4.1.

Spearman's rank correlation coefficients were calculated to determine the relationships between variables.

4.3. Results

Metabolic evaluation of African cassava germplasm accessions was carried out in three tissues (leaf, petiole, and stem) for both well-watered and water-stressed treatments. Amylose content was determined only in the roots. In general, there were significant differences between the treatments for the traits evaluated except protein and amylose content (Table 4.1). Due to genotypic differences of the cassava germplasm evaluated and the interaction between accessions and environments, the performance in individuals was variable. Insignificant differences were observed between the different stress phases.

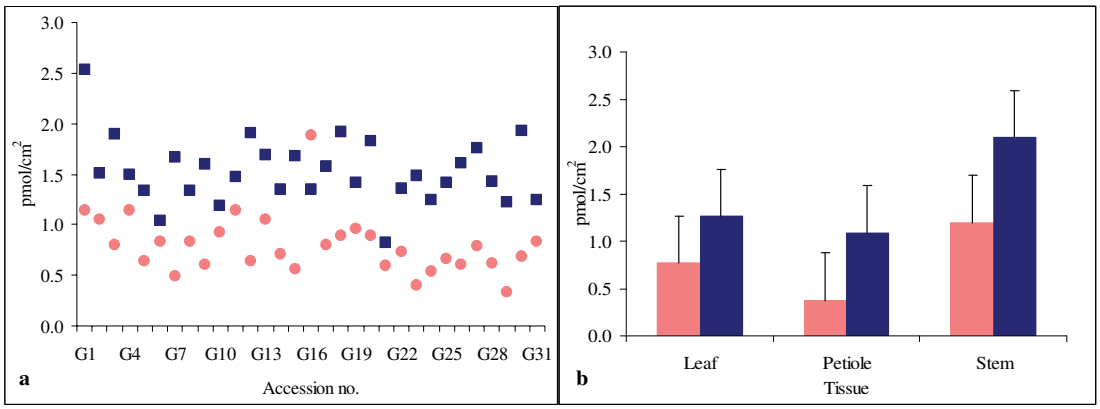

Figure 4.1. Abscisic acid concentration in 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya; (a) mean ABA concentration in different accessions across 3 samplings (120, 150, 180 days after planting) and across 3 tissues; (b) mean ABA in different cassava tissues (leaf, petiole, stem) (■ well-watered; ● water-stressed).

4.3.1. Abscisic acid in cassava tissues

The ANOVA of the ABA content revealed significant differences between the two treatments and among the accessions evaluated (Table 4.1). It was surprising to note that, the well-watered accessions accumulated more ABA than their water-stressed counterparts except in G16 (Figure 4.1a). However, there was not a consistent ranking of genotypes according to ABA concentration in the leaves, petioles and stems. Relative to the corresponding well-watered treatment, water-stress reduced ABA concentration by ca. 2-fold in most genotypes (Figure 4.1a). In general, ABA content

decreased from stem to leaf, and the petioles had the least amounts in both treatments (Figure 4.1b).

Table 4.1. Analysis of variance of 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya (TxA, accession by treatment interaction; **, *, significant at P<0.01 and 0.05 respectively).

	Variance components		
	Treatment (T)	Accession (A)	TxA
Trait			
Abscisic acid	109.47**	5.34**	30.00
Total sugars	19.44**	3.13**	2.21**
Glucose	18.56**	4.50**	2.38**
Sucrose	13.34**	2.44**	1.71*
Protein	1.00	30.00	30.00
Starch	7.74**	20.02**	30.00
Amylose	1.00	30.00	30.00

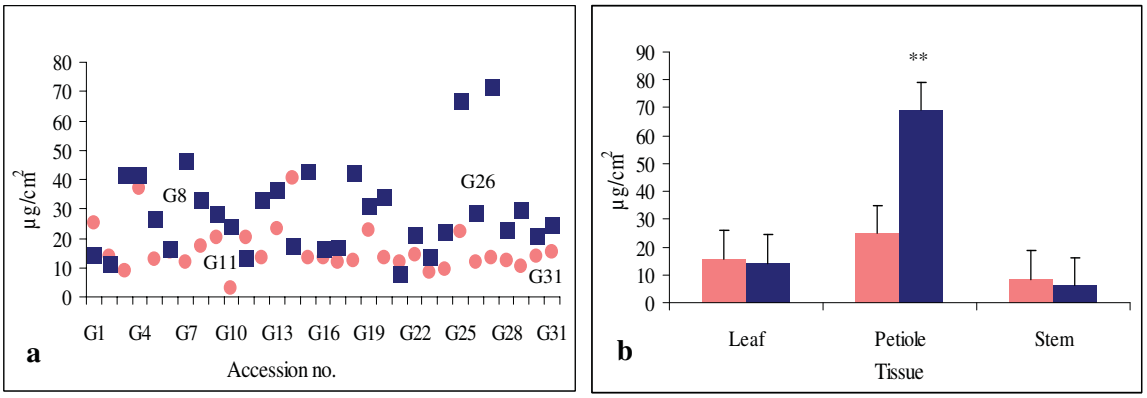

Figure 4.2. Glucose concentration in 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya (a) mean glucose concentration in different accessions across 3 samplings (120, 150, 180 days after planting) and across 3 tissues; (b) mean glucose in different cassava tissues (leaf, petiole, stem) (■ well-watered; ● water-stressed). ** The differences between well-watered and water-stressed treatments are significant at 1% level of probability (only the 4 accessions that had higher yields in water-stressed condition were labeled).

4.3.2. Assimilates content in cassava tissue

Drought stress decreased all total sugars in the cassava accessions evaluated (Appendix 4.1). Concentrations of total sugars, glucose and sucrose were significantly higher in the petioles of the well-watered accessions. On the other hand, the sugars

remained somewhat similar in the stems and the leaves (Figure 4.2, 4.3). The interaction of cassava accessions and water treatment (TxA) was significant only for sugars, indicating that water stress influenced these parameters, but differently according to the accessions (Table 4.1).

Protein content of the genotypes was not only meager in quantity in the accessions evaluated, but also showed no significant variation between the two treatments (Table 4.1). Among the tissues, the stems had an insignificant low amount of protein.

The water-stressed accessions accumulated more starch than their well-watered counterparts (Figure 4.4a). Leaf and petiole had markedly lower amounts of starch in both treatments (Figure 4.4b). Total non-structural carbohydrate (total sugars + starch) was higher in the water-stressed treatment (Figure 4.5 a). Relative to the well-watered accessions, the sucrose to starch ratio decreased under drought stress (Figure 4.5 b). Imposition of water stress did not significantly affect the amounts of amylose (Table 4.1).

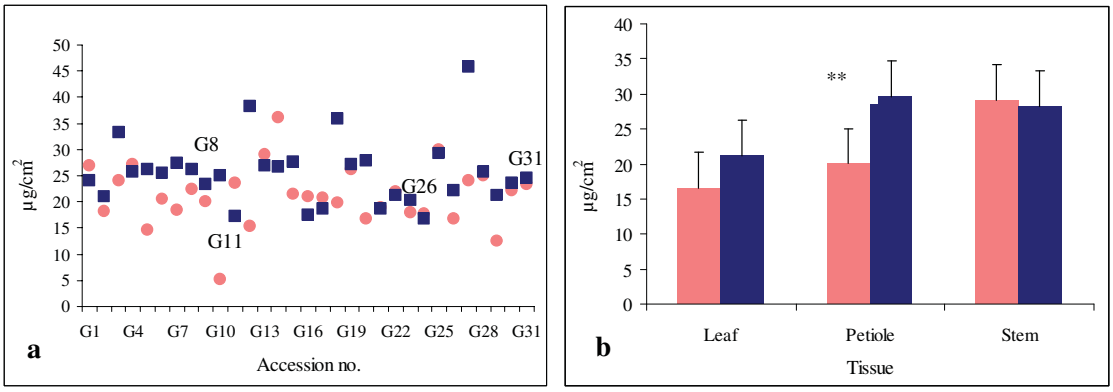

Figure 4.3. Sucrose concentration in 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya (a) mean sucrose concentration in different accessions across 3 samplings (120, 150, 180 days after planting) and across 3 tissues; (b) mean sucrose in different cassava tissues (leaf, petiole, stem) (■ well-watered; ● water-stressed). ** The differences between well-watered and water-stressed treatments are significant at 1% level of probability (only the 4 accessions that had higher yields in water-stressed condition were labeled).

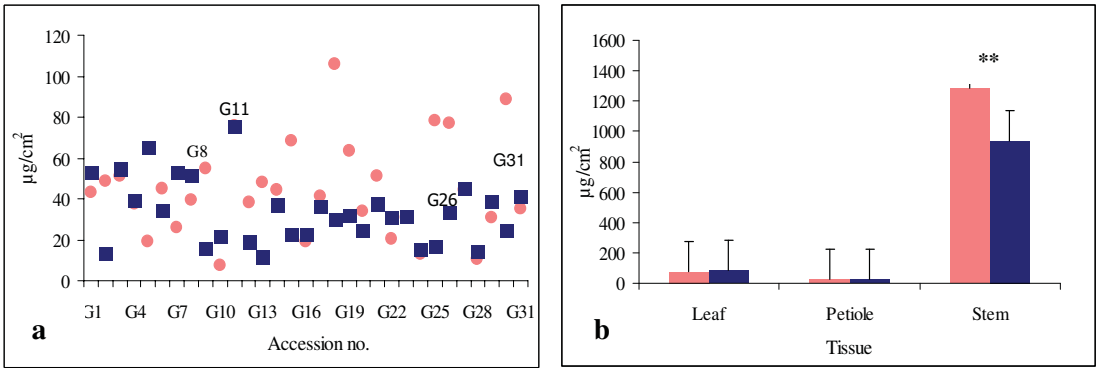

Figure 4.4. Starch concentration in 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya (a) mean starch concentration in different accessions across 3 samplings (120, 150, 180 days after planting) and across 3 tissues; (b) mean starch in different cassava tissues (leaf, petiole, stem) (■ well-watered; ● water-stressed). ** The differences between well-watered and water-stressed treatments are significant at 1% level of probability (only the 4 accessions that had higher yields in water-stressed condition were labeled).

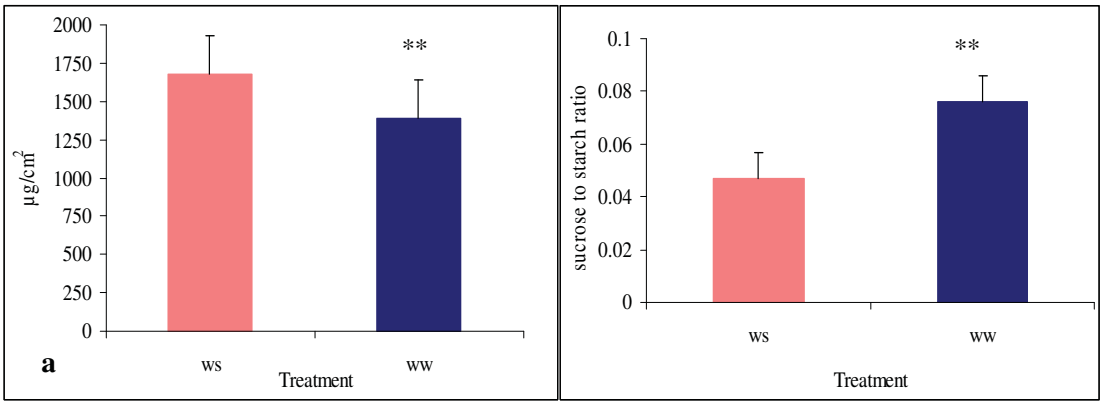

Figure 4.5. The effect of drought stress imposed on 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya; on (a) total non-structural carbohydrate (TNC) (total sugars + starch); and (b) sucrose to starch ratio (■ well-watered; ● water-stressed). ** The differences between well-watered and water-stressed treatments are significant at 1% level of probability.

4.3.3. Relationship between traits

Spearman's rank correlation analysis was used to investigate the inter-relationships amongst the various traits. In general, relationships between traits varied considerably (Table 4.2). Abscisic acid was positively correlated with sucrose and starch content ($P < 0.01$), while it was inversely correlated with total sugar ($P < 0.05$) and glucose ($P < 0.01$). Sugars were also significantly correlated among each other (Table 4.2). For example, total sugar was positively correlated with glucose ($P < 0.01$) and sucrose ($P < 0.05$).

Table 4.2. Spearman's rank correlation coefficient for various metabolic traits evaluated for 31 cassava accessions across 3 samplings (120, 150, 180 days after planting) and 3 tissues (leaf, petiole and stem) at Kiboko Research Station, Makindu, Kenya.

Trait	Abscisic acid	Total sugar	Glucose	Sucrose	Protein	Starch
Total sugar	-0.424*					
Glucose	-0.603**	0.965**				
Sucrose	0.510**	0.398*	0.145			
Protein	-0.176	-0.14	-0.02	-0.456**		
Starch	0.857**	-0.379*	-0.574**	0.581**	-0.414*	
Amylose	-0.164	-0.09	-0.036	-0.212	0.013	-0.025

4.4. Discussion

Although the role of the phytohormone ABA in stress physiology has received much attention, efforts to correlate ABA production and drought tolerance in plants have yielded conflicting results (Chen et al., 1997). In this study, the well-watered accessions accumulated more ABA than their water-stressed counterparts. This drought-induced decrease in ABA concentration of water-stressed plants is inconsistent with previous reports on the accumulation of the plant hormone in young expanding leaves of water-stressed cassava plants under controlled green-house conditions after 6 days of water-stress (Alves and Setter, 2000; 2004). Our finding also disagreed with the observations on castorbean (*Ricinus communis* L.) (Zeevaart, 1977) and moleplant (*Euphorbia lathyris* L.) (Sivakumaran and Hall, 1978), which, like cassava, are members of the family *Euphorbiaceae*. Despite these observations, studies by Hsiao (1973) have shown that only mild to moderate stress is able to induce an ABA increase. Reports by Hiron and Wright (1973) have indicated that ABA accumulates most readily in wheat leaves if the loss in fresh weight does not exceed 9%; and in sugar cane leaves, before wilting appears (Most, 1971). Mizrani (1970) has shown that 1 day of wilting in *Nicotiana* species increased ABA content in the leaves. In this study, since sampling for phytohormone analysis was done from 120 DAP onwards, the stress might have been too intense and the period quite long to cause a significant increase in ABA, or to stimulate its biosynthesis. In addition, the stressed plants might have had a lower capacity to catabolize ABA. Regardless of these probable reasons, our finding needs substantiation, particularly with more data on the phase and degree of water stress.

In this study, accessions differed in ABA concentration during water-stress and well-watered conditions (Figure 4.1a). This may be because, since these accessions consisted of breeding materials from IITA, they varied in their “known characteristics” (Table 3.2, chapter 3), and their regions of origin have distinctly different climatic ecosystems that vary in relation to drought pressure. Another possibility is that when the tissues were sampled, the stressed tissues were senescing and so had lower levels of ABA due to their half-dead condition. In addition, maybe the tissues from stressed plants represented a less advanced development stage (due to stress arresting development) such that they had low ABA concentrations due to their "younger" stage of development.

ABA content varied in the 3 tissues evaluated. This is a common phenomenon and has been reported in numerous studies and for various crops such as maize (Pekic and Quarrie, 1987; Ribaut and Pilet, 1991), soybean (Liu et al., 2003) and chick pea (Nayyar et al., 2005). Variation in ABA might have occurred due to a lower capacity to metabolize (break down) and translocate ABA to different plant parts leading to higher net ABA accumulation (Jaschke et al., 1997).

Soluble sugars (glucose, sucrose and fructose) not only supply a significant source of calories in the diets of many people but also they make food more palatable. Sucrose is the major sugar used by most plants to translocate photoassimilates from the leaves (source tissue) to non-photosynthetic tissues (sink tissues), possibly because of its high solubility, low reactivity and energy storage capacity (Sawkins et al., 2006). Data from this experiment demonstrated that there was a significant reduction in the concentration of sucrose during drought-stress, and that this was coupled with a decrease in glucose (Figure 4.2 and 4.3). This is in accordance with previous reports on the accumulation of sugars in cassava plants subjected to 6 days of water-stress (Alves and Setter, 2004). Zinselmeier et al. (1999) also showed that photosynthetic activity is severely reduced under water-stress conditions, which affect the availability of sucrose. In addition, sucrose might have been diverted to the vacuole and further hydrolysed into fructose (Epron and Dreyer, 1996). David et al. (1998) also found a lower glucose concentration in *Lupinus albus* under controlled drought conditions. The decline in sugar concentration under water-stressed conditions may be attributed to a decrease in carbon assimilation (Gebeyehu, 2006). Although the changes in sugar concentration may have a role in the drought tolerance of these accessions, the relative contribution of sugars to drought stress cannot be determined from the available data. Further work will be required to identify and quantify sugar concentrations in relation to osmotic adjustment and, hence, their exact contribution to water stress.

There was no marked difference in soluble protein concentration between well-watered and water-stressed plants. Unfortunately, because of logistical limitations, we did not determine the accumulation of drought responsive proteins in this study, which are thought to confer osmoprotective function during water stress Thus, within

the scope of this study, the effect of water-stress on protein accumulation cannot be determined.

Starch is one of the major end products of carbon assimilation, and a principal storage carbohydrate in most plants. It is found in stems, seeds and underground storage organs such as roots and tubers. In this study, it was surprising to note that the water-stressed accessions accumulated more starch than their well-watered counterparts. This increase in starch concentration due to water-stress is inconsistent with findings for cassava by Duque and Setter, (unpublished) and grapevines (Patakas and Noitsakis, 2001) under controlled conditions. It might be that in this scenario, starch synthesis was highly promoted in water-stressed accessions after restriction of sucrose synthesis, since starch serves as a transient sink to accommodate excess photosynthate that cannot be converted to sucrose (Paul and Foyer, 2001; Gebeyehu, 2006). Stitt and Quick (1989) showed that a decreased demand for sucrose leads to either an increase in starch synthesis or to a restricted rate of photosynthesis. In addition, during the timeframe of our study we could have expected a decline in starch if the water-stressed plants were utilizing stem and petiole starch (via remobilization to various plant organs) to sustain a small amount of growth and respiration during a time of zero net photosynthetic carbon assimilation, but in this case they did not make use of it. The stems had markedly large amounts of starch, most probably for sustaining tissue metabolism under stress conditions. In addition, starch accumulates early during stem elongation and maturation than in other plant parts. Our data shows almost no starch in the petioles. This corroborates findings by Duque and Setter (unpublished) that petiole starch is usually depleted first than in the stem. There were no significant differences observed between the relative concentrations of the two starch polymers, amylose and amylopectin.

The increased total non-structural carbohydrate (TNC) in water-stressed plants was due to a significantly high amount of starch in the water-stressed accessions. Increased ratio of sucrose to starch has been implicated as one of the adaptive features to different types of stresses including drought (Silva and Arrabaça, 2004). In this study, we observed a reduced sucrose to starch ratio in favor of starch, which might be probably due to down-regulation of the enzymes sucrose synthase and sucrose phosphate synthase (Geigenberger et al., 1999). In addition, it may be due to reduced

starch hydrolysis in water-stressed accessions (Jones et al., 1980) and/or a changes in the partitioning between starch and sucrose synthesis (Vassey and Sharkey, 1989).

The effect of water-stress is composite in its mode of action and highly erratic in response as a result of interacting factors (Ramirez-Vallejo and Kelly, 1998). Results from this study did not differentiate the well-watered and water-stressed treatments well. In addition, ABA was reverse the expected published effects. Maybe the well-watered plants were experiencing an incipient stress due to low humidity, warming from direct sun, or due to slight soil moisture depletion which were not ascertained in this study. Despite these probable reasons, interpretation of ABA data can be tricky to the extent that treatment effects and genotypic differences can depend on delicate timing of tissue sampling, with respect to oscillations in cycles of induction and attenuation of ABA accumulation. This calls for further research in both controlled and field conditions to determine the time course of ABA accumulation as a cassava plant goes from its young stage to aging. This information on contrasting water-limited conditions would be helpful so as to know the stage at which ABA data are most informative. In addition, more research is required on these breeding materials to identify secondary traits that could be used for phenotyping for drought tolerance.

Appendix 4.1 Means of metabolites of 31 cassava germplasm accessions evaluated at Kiboko Research Station, Makindu, Kenya under well-watered (ww) and water-stressed (ws) treatments

	Abscisic acid		Total sugar		Glucose		Sucrose		Protein		Starch		Amylose	
Accession	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
G1	1.31	2.53	21.50	12.77	27.40	14.20	27.00	24.10	18.20	36.37	49.02	52.79	20.54	30.46
G2	1.05	1.51	10.63	10.80	13.90	11.50	18.10	21.00	24.24	22.37	48.90	13.32	28.79	25.92
G6	0.84	1.04	10.80	13.90	11.90	16.20	20.60	25.50	18.00	12.72	93.88	34.67	26.27	32.39
G24	0.74	1.24	12.90	12.93	13.40	22.00	25.40	16.80	31.47	18.98	29.26	15.09	27.47	33.43
G10	0.51	1.18	7.77	16.43	7.80	24.20	15.50	25.10	14.77	17.93	22.88	21.82	39.09	51.32
G19	0.74	1.42	12.43	19.37	13.00	31.30	24.10	27.10	37.55	15.45	62.39	32.27	28.83	18.81
G5	0.83	1.34	10.30	17.53	8.10	26.50	22.80	26.20	8.52	14.99	55.37	65.21	34.86	36.78
G26	0.78	1.61	25.03	16.83	41.00	28.50	34.10	22.10	16.32	21.52	38.39	32.97	26.09	26.83
G15	0.69	1.68	10.47	23.43	10.20	42.90	21.10	27.60	25.24	16.62	69.93	22.54	32.60	32.23
G7	0.47	1.67	6.97	24.57	9.70	46.30	11.20	27.40	6.13	9.74	33.84	53.15	37.99	32.86
G17	0.60	1.58	12.63	11.83	19.60	17.00	18.50	18.70	9.94	49.73	41.38	36.32	32.86	36.12
G23	0.88	1.48	17.33	11.30	23.40	13.80	28.40	20.20	36.99	17.60	34.97	31.21	40.41	30.46
G11	1.15	1.47	14.57	10.17	20.20	13.40	23.70	17.20	42.90	24.36	75.79	75.40	31.68	33.47
G12	0.55	1.90	9.40	23.87	10.60	33.30	17.50	38.30	10.59	26.00	43.74	19.02	27.57	26.38
G13	0.91	1.68	13.57	21.10	16.80	36.30	24.20	26.90	58.78	10.16	36.56	11.78	31.32	32.40
G14	1.77	1.35	12.80	14.73	15.10	17.40	23.20	26.70	22.74	13.40	38.78	36.80	32.79	31.05
G16	0.78	1.35	16.70	11.23	20.50	16.30	29.40	17.50	17.31	12.77	66.21	22.71	37.82	36.19
G18	0.72	1.92	9.90	26.13	14.50	42.40	15.20	35.90	8.51	15.14	44.63	30.17	31.93	35.43
G20	0.76	1.83	10.57	20.60	14.80	33.90	17.00	27.80	9.88	10.45	29.92	24.43	29.12	24.69
G21	0.63	0.83	12.60	8.90	13.70	8.10	24.10	18.60	20.23	47.04	26.93	37.69	36.94	33.43
G22	0.91	1.35	18.53	14.10	27.30	21.10	28.40	21.30	11.21	10.32	25.39	30.64	27.97	18.54
G25	0.53	1.42	9.60	31.90	11.30	66.70	17.30	29.30	10.63	19.48	74.99	16.69	31.59	23.43
G27	0.92	1.76	11.47	39.13	12.30	71.80	22.10	45.80	15.29	28.82	59.02	44.62	24.98	24.14
G28	0.80	1.43	9.57	16.00	11.80	22.60	17.40	25.70	11.08	35.96	32.22	14.43	42.69	34.12
G29	0.73	1.23	15.83	16.93	22.30	29.50	25.10	21.20	29.45	32.07	42.13	38.62	26.09	40.72
G30	0.53	1.92	10.73	14.67	13.50	20.50	18.90	23.60	15.35	12.40	70.25	24.51	28.82	26.83

Appendix 4.1. continued.

	Abscisic acid		Total sugar		Glucose		Sucrose		Protein		Starch		Amylose	
Accession	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
G31	0.84	1.24	12.80	16.40	15.20	24.80	23.30	24.50	10.86	25.69	35.30	41.19	34.19	28.45
G3	0.76	1.89	11.77	24.80	14.90	41.50	20.40	33.20	11.93	14.32	27.33	54.73	25.17	22.42
G4	0.66	1.49	11.70	22.53	14.20	41.70	20.90	25.70	12.84	28.30	25.13	39.46	35.68	27.42
G8	0.90	1.34	3.37	19.67	4.10	33.00	6.30	26.20	22.53	12.66	31.48	52.00	32.94	23.42
G9	0.47	1.60	8.70	17.20	9.50	28.20	16.60	23.30	21.24	19.31	23.03	15.71	26.34	24.45
Mean	0.80	1.52	12.35	18.12	15.55	28.93	21.22	25.50	19.70	21.05	44.81	33.61	31.34	30.15
% CV	32.99	21.17	34.36	36.94	46.23	51.55	26.43	24.68	60.03	49.54	41.33	47.19	16.51	22.26
SE	0.05	0.06	0.76	1.20	1.29	2.68	1.01	1.13	2.12	1.87	3.33	2.85	0.93	1.21

5. Genetic Mapping in Cassava (*Manihot esculenta* Crantz) using SSR's and EST-derived SSR's

Abstract

Cassava is an important crop in sub-Saharan Africa, due to its efficient production of food energy, flexible harvest date and tolerance to abiotic stresses. In a first step to identifying quantitative trait loci (QTL) associated with drought tolerance in cassava, a genetic linkage map was constructed from an F_1 population of 228 individuals derived from a cross between COL 1734 (drought-tolerant) and BRA 1149 (drought-susceptible) at the International Center for Tropical Agriculture (CIAT) in Colombia. A set of 307 simple sequence repeat (SSR) primers and 70 expressed sequence tag (EST) derived SSRs (ESSR's) were screened for polymorphism between the two parents. The segregating progenies were used to generate two genetic linkage maps using 110 polymorphic markers. The female map (COL 1734) has 56 markers spanning 519.2 cM, assembled over 14 linkage groups, whereas the male map (BRA 1149) spans 468.3 cM distributed on 13 linkage groups. The mean distance between markers is 9.3 cM in the female map and 8.2 cM in the male map. Homology between the two maps was established between seven linkage groups using 27 allelic bridges. Although the two maps are not saturated, they will form the basis for identifying QTLs associated with drought tolerance. In addition they provide map locations for 46 new and previously unmapped SSR's and ESSR',s which can be incorporated into other cassava genetic linkage maps to build a consensus map for use in genetic analysis of Manihot esculenta.

Keywords: Cassava; ESSR; linkage map; *Manihot esculenta*; marker; SSR;

5.1. Introduction

Tropical root and tuber crops such as cassava (*Manihot esculenta* Crantz), cocoyams (*Colocasia* and *Xanthosoma* spp.), potato (*Solanum tuberosum* L.), sweet potato [*Ipomoea batatas* (L.) Lam] and yams (*Dioscorea* spp.) play an important role in the world's food supply. They are consumed by a third of the world's population, mainly comprising the lower socio-economic groups (Chandra, 1994). In the face of rapid population growth and climate change, African countries have continued to heavily depend on these crops. They act as food security crops at both household and national levels by providing a cheap source of carbohydrates and, hence, are often referred to as 'insurance crops' (Onwueme and Charles, 1994). Among these, cassava is the dominant root crop (Dapaah, 1994).

Cassava is one of the leading staples in sub-Saharan Africa, owing to its efficient production of food energy, year round availability and tolerance to extreme abiotic stresses. It is a valuable crop in regions where annual rainfall is low, seasonal, and often highly variable. The crop can withstand prolonged periods of drought in which most other food crops fail. An estimated 500 million people obtain more than 60% of their daily calorie intake from cassava roots, which constitutes one of the world's largest staple crops for starch (Wenham, 1995).

Between 1991 and 1999, more than 1000 Brazilian accessions were evaluated for drought tolerance in four representative ecosystems of Brazil's semi-arid northeast, which have homologous counterparts in sub-Saharan Africa (Figure 5.1). Results from this project revealed extraordinary ability of certain accessions to withstand prolonged drought. The selection criteria for these drought-tolerant accessions was based on sprouting percentage, tolerance to moisture stress, resistance to mites (the main pests of the region), dry matter content, root yield and cyanogenic potential. These accessions were officially released to farmers in the Semi-arid region of North East Brazil (Fukuda and Saad, 2001), although the genetic traits that make these genotypes more drought-tolerant have not been documented.

Although agriculture has realized exponential gains in productivity in the recent past, cassava has traditionally received less attention from researchers working on temperate crops, leaving fundamental questions about its genetics unanswered (Cock,

1985; Okogbenin et al., 2008). This may, in part, be due to the biological characteristics of cassava, making it a difficult crop to breed by conventional methods. It is traditionally a vegetatively propagated crop through stem cuttings, and seed production is low. This is a serious limitation to genetic improvement, which relies on recombination during crossing for progress. In addition, the crop's phenology is highly influenced by the environment, affecting time to flowering. It has a long growth cycle, and breeding of a new variety can take between 8-12 years with no guarantee for the release and adoption of an improved variety. Cassava has a heterozygous genetic background and quickly suffers from inbreeding depression. Although this heterozygosity makes it difficult to consolidate genetic gain in the breeding process due to inherent instability of the heterozygous status, it is advantageous in that it creates variation within the crop and facilitates a directional selection of additive genes towards desirable traits (Hahn et al., 1990). For these reasons, it is extremely difficult, time-consuming and expensive to combine an array of preferred characteristics both agronomic and organoleptic. In addition, since precise measurements are required for the expression of traits, field environments might not offer an ideal condition for selection of complex traits, which is a major objective in many plant breeding programs today. Biotechnology tools, which enable trait selection with cost effectiveness, can play a major role in increasing the accuracy and efficiency of cassava genetic improvement through molecular marker technology (Kizito, 2006).

The use of biochemical and DNA markers for genetic analysis and manipulation of important agronomic traits has become an increasingly important tool in plant genetic improvement. Molecular markers have enhanced the operation of genetic improvement programs through a number of ways. These include fingerprinting of genetic stocks; assessment of genetic relationships; confirmation of ploidy levels; gene cloning; whole genome scanning; increasing the efficiency of selection for difficult traits; and making environment-neutral selection possible (Ejeta et al., 2000; Fregene and Puonti-Kaerlas, 2002; Fregene et al., 2003; Zhang et al., 2004). The greatest potential of these markers appear to be in the construction of genetic maps, which is the first step towards locating genes or quantitative trait loci (QTL) that condition economically important traits (Zhang et al., 2004; Semagn et al., 2006).

Most DNA-based marker systems employ either the Polymerase Chain Reaction (PCR) technique (Mullis, 1990) or the DNA-DNA hybridization gel technique (Botstein et al., 1980). Both techniques are able to detect single and/or multiple locus differences in addition to being inherited in either a dominant or co-dominant fashion. These markers include amplified fragment length polymorphisms (AFLP), cleaved amplified polymorphic sequence (CAPS), diversity arrays technology (DArT), expressed sequence tags (EST's), inter simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLPs), single nucleotide polymorphisms (SNP's), and simple sequence repeats (SSR's). Among these, SSR's or microsatellites remain a standard for linkage mapping. This is because they provide high information content, have a co-dominant mode of inheritance, are reproducible, locus-specific, highly transferable across laboratories, and have ease for automation for high-throughput capillary electrophoresis (CE) (Semagn et al., 2006).

Most *Manihot* species studied have 36 somatic chromosomes. Cassava is generally considered a diploid, with a haploid number of n=18; although an allopolyploid with basic chromosome number x=9 and segmental allotetraploidy have been postulated (Hahn et al., 1990). It has a DNA content of 1.67 pg per cell nucleus (Awoleye et al., 1994). This value corresponds to 772 mega base pairs in the haploid genome and puts cassava's genome size at the lower end of the range of higher plants (Bennett and Smith, 1991). The relatively small size of the cassava genome favors the development of a saturated genetic map that would contribute to an understanding of the inheritance of important agronomic traits despite the crop's heterozygous nature (Fregene et al., 1997).

The first genetic linkage map for cassava, constructed predominantly with RFLP markers, was drawn from an F_1 progeny segregating for early root bulking, disease resistance and root quality (Fregene et al., 1997). Although an SSR-based map is also available for these traits (Okogbenin et al., 2006), the cassava genetic map needs to be saturated with SSR molecular markers especially those derived from EST's, henceforth referred to as expressed simple sequence repeats (ESSR's). This is because, if an ESSR marker is found to be genetically associated with a trait of interest, it is possible that the mapped gene directly affects the trait. These markers are

also very useful in comparative mapping across different species in that they have a high degree of sequence conservation and are more likely to be transportable across a pedigree and species than the non-ESSR markers. ESSR's can be used as a basis for genetic mapping in other species if their DNA sequence information is lacking (Semagn et al., 2006). Thus, linkage mapping in a crop like cassava using ESSR markers would enable a more rapid transfer of genetic information between species (Cato et al., 2001). A densely populated cassava map will make genetic improvement more effective and fast in that it will provide molecular breeding approaches with more variety in the quality and type of markers and additional probability of polymorphic markers in an important chromosome interval (Somers et al., 2004).

The present study was conducted within the framework of the project "Identifying the physiological and genetic traits that make cassava one of the most drought tolerant crops" implemented since 2005 by the Brazilian Agricultural Research Corporation, (Embrapa); the International Center for Tropical Agriculture, (CIAT); the International Institute of Tropical Agriculture (IITA); Cornell University, USA, and the University of Goettingen.

Main objectives of the present study were:

- to screen parents from two mapping populations for marker polymorphism and genotype the mapping populations using genomic SSR's and ESSR markers;
- to perform linkage analysis so as to place markers on a molecular genetic framework based on their segregation in the mapping populations.

5.2. Materials and methods

5.2.1. Mapping populations

5.2.1.1. Selection of contrasting parental accessions

Twenty eight drought-tolerant and 12 drought-susceptible cassava germplasm accessions with contrasting characteristics for drought tolerance were selected by Embrapa and CIAT. The selection of these accessions was based on sprouting percentage, tolerance to moisture stress, resistance to mites, dry matter content, root yield and cyanogenic potential (Appendix 5.1). The accessions were multiplied and evaluated in 4 representative ecosystems of Brazil's semi-arid Northeast in the field and in the greenhouse under stressed and irrigated conditions (Figure 5.1). Based on this evaluation, four best divergent parental combinations were selected to form a base population for developing a mapping population. These were accessions BRA 255 and COL 1734 and BRA 1149 and COL 1468 (Appendix 5.1).

5.2.1.2. Generation of crosses between contrasting parents

Cassava stakes from the four contrasting parents were planted at CIAT headquarters in Colombia. During flowering, which started after 6 weeks, crosses were made by hand between the male and the female parents (Table 5.1) (IITA, 1990). Mature pollen grains (when anthers changed from green to yellow) were collected in the morning, and mature unopened female flowers were bagged with a white paper to prevent honey bees or other insects from pollinating opened female flowers. Pollination was performed in the afternoon by rubbing the male flower on the stigma of the female flowers. After pollination, the pollinated flowers were bagged to prevent unwanted pollen grains landing on the stigma. The mature unopened female flowers were also pollinated through emasculation by removing the perianth. The plants were uncovered 5-6 days after pollination, and mature seed were obtained from 70 to 90 days.

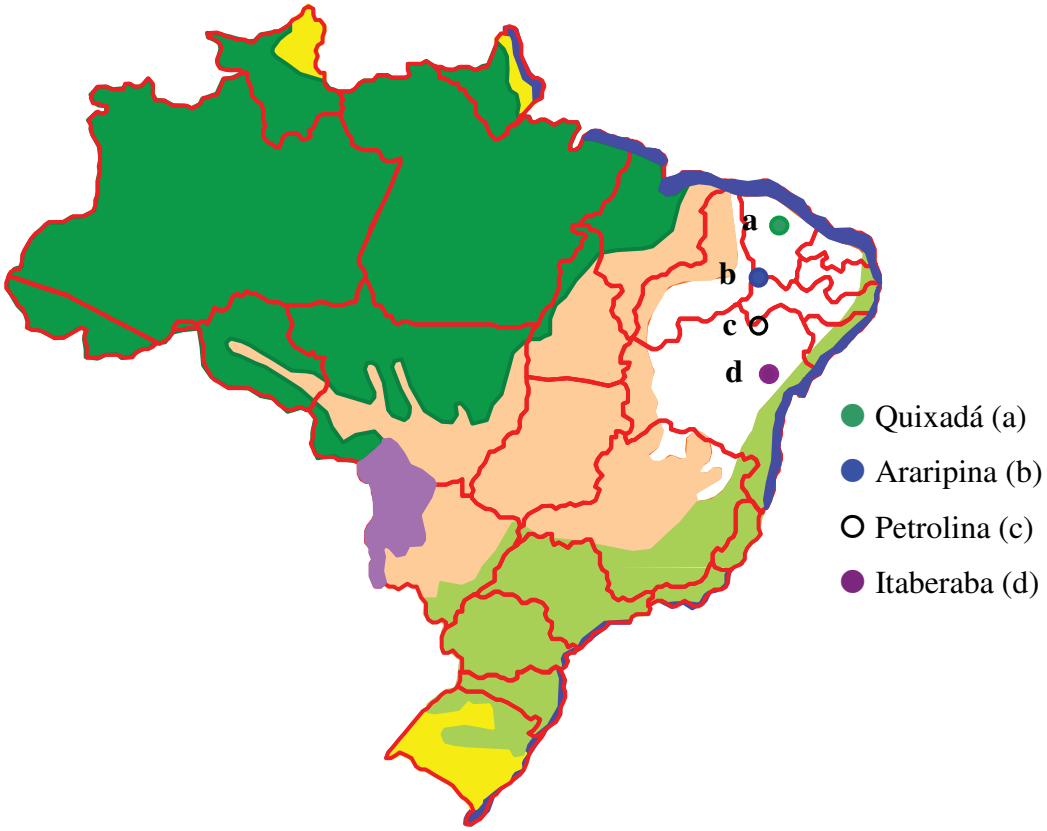

Figure 5.1. Location of four representative locations of Brazil's semi-arid Northeast (indicated in white on the map) where field evaluations of 28 drought-tolerant and 12 drought-susceptible cassava germplasm accessions under stressed and irrigated conditions were performed (a, Quixadá; b, Araripina; c, Petrolina; and d, Itaberaba).
Source: (CIAT and Embrapa, 1996)

Table 5.1. Population name, cross name and status of cassava germplasm accessions used in the generation of segregating populations (accessions marked with * were considered drought-tolerant).

Population name	Cross name	Female	Male	Number of individuals
A	CTS1A	COL 1734*	BRA 1149	228
B	CTS2A	MCOL 1468	BRA 255*	23
B	CTS2B	BRA 255*	MCOL 1468	33

5.2.1.3. Segregating populations

At CIAT in Colombia, mature seeds were treated with concentrated sulphuric acid for 50 min. (CIAT, 2003). They were thoroughly washed with water and soaked for 30 min. The seeds were surface-sterilized by immersion in 70% alcohol for 5 min. They were then immersed in 5% sodium hypochlorite and Tween-20 for 20 min., before they were rinsed three times with sterile water. Under aseptic conditions, they were split along the longitudinal axis and the embryos were removed using a sterile forcep and scapel. Excised embryos were placed in 17N medium (growth medium) with their radicles down. The embryo cultures were incubated in darkness for three days to promote radicle growth and then transferred to growth chambers with a 12 hr photoperiod. Plantlets remained in the growth chamber for 6 weeks before being shipped to Kenya for molecular analysis. In Kenya, the accessions were acclimatized as described in section 2.2.2 (Chapter 2) for furnishing young leaves for DNA extraction.

5.2.2. Marker analysis

Molecular work was carried out at the International Institute of Tropical Agriculture (IITA), Biosciences for eastern and central Africa (BecA) laboratory, Nairobi-Kenya Campus.

5.2.2.1. DNA isolation

The progenies used for map construction consisted of 228 individuals produced from population A (Table 5.1). Population B was eliminated from further analysis due to inadequate number of individuals. From each full-sib F_1 progeny, approximately 0.5 g of young leaf tissue from green-house acclimatized plants was collected in a 1.5 ml 96-well round bottomed extraction plate containing one 4 mm stainless steel bead. The plates were sealed with mats and immediately frozen in dry ice. In the BecA laboratory, the samples were stored at -80°C. Genomic DNA was extracted from the frozen leaf samples of each individual of the F_1 population and from the parents after grinding the samples at 1500 strokes for 10 min. using a Geno/Grinder (Grinder Spex CertiPrep™, USA). A modified protocol by Dellaporta et al. (1983) was followed. The DNA was purified two times using chloroform: isoamylalcohol (24:1v/v) mixture.

Following precipitation of the DNA with cold isopropanol, samples were washed twice with 70% ethanol, air-dried before re-suspending in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Samples were incubated at 65°C in a shaking water bath for 1 hr. to ensure good re-suspension. DNA concentrations were measured using a NanoDrop™ ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA). DNA quality was assessed on 0.8% agarose gels prepared with TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0). The gels were run for 1 hr. at 100 volts. Samples were finally diluted to a standard concentration of 50 ng/µl with TE buffer.

5.2.2.2. Optimization of primers and labeling of ESSR's

A set of 307 locus-specific SSR primers from the cassava genomic library (unpublished data, M. Fregene et al., 2002) and 70 ESSR's were employed in this study (Appendix 5.2).

Primer pairs for each marker were synthesized by Eurofins MWG Operon, Ebersberg, Germany. They were reconstituted with TE buffer (1 mM EDTA, 10 mM Tris–HCl, pH 8.0) to make a stock solution of 100 pmols/µl. Primer aliquots of 1 pmols/µl for each forward (F) and reverse (R) marker were prepared. Amplification reactions for annealing temperature optimization were carried out using 0.4 pmols/µl F and R primers and 1 DNA sample. The 10 µl reaction mixture contained 9 µl of amplification mix [1 x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP's, 0.375 *Taq* DNA-polymerase (New England Biolabs)] and 1 µl of the diluted DNA as template. The PCR amplification was performed using a Techne TC-512 Thermal Cycler (Global Medical Instrumentation, GMI, USA). The thermo cycler was set through a gradient program from 52°C to 62°C. The temperature/time profile of the cycles was a hot start at 95°C/120 sec. for denaturing the DNA, and then 30 cycles of 95°C/30 sec. denaturing, 52-62°C/60 sec. annealing, and 72°C/30 sec. extension. A final step of 30 min. extension and incubation was carried out at 72°C. The PCR products were separated on 2% agarose gels stained with ethidium bromide. The optimal annealing temperature was determined by visual inspection as the sharpest amplification of the marker. For the optimization of primer, $MgCl_2$ and dNTP's, the protocol developed by IITA, BecA laboratory was used (Table 5.2). The amount and concentration of DNA and *Taq* DNA-polymerase were kept constant.

Fragments for analysis in capillary electrophoresis (CE) need to be fluorescently labeled with a suitable dye for detection on the Applied Biosystems (ABI) 3730 analysis platform. The SSR's used in this study were synthesized with an added 5' labeled tail on the F primer by Eurofins MWG Operon, Ebersberg, Germany. The ESSR's were labeled with either FAM (Blue), VIC (Green), NED (Yellow) or PET (Red) (Applied Biosystems, dye set G5) fluorescent dyes. A universal unlabelled 'tail' ($^{5'}$ GCTACAGAGCATCTGGCTCACTGG $^{3'}$) that had been raised against an octopus was added to the 5' end of the F primer and a complementary labelled oligo (Table 5.3), which was incorporated into the product during amplification was added to the PCR mix. The decision on which dye to add to which ESSR marker was such that loci with overlapping or close allele ranges were differently labeled, so that up to 4 marker loci could be co-loaded on the ABI. The amplification reactions were carried out using the optimized conditions for each marker and 0.175 pmoles/µl of the tail.

Table 5.2. Polymerase Chain Reaction (PCR) optimization conditions developed by the International Institute of Tropical Agriculture (IITA), at Biosciences for eastern and central Africa (BecA) Laboratory for cassava genotyping (IITA, unpublished). The primers, magnesium chloride ($MgCl_2$) and deoxynucleotide triphosphates (dNTPs) varied for conditions A, B and C.

Component	Condition A	Condition B	Condition C
Primer F and R	0.4 pmols/µl	0.8 pmols/µl	1.2 pmols/µl
Mg (mM)	1.5 mM	2 mM	2.5 mM
dNTP (mM)	0.15 mM	0.2 mM	0.25 mM

5.2.2.3. Polymorphism screening and high throughput genotyping

Amplification reactions were set using the optimized conditions for each marker and the diluted parental DNA samples in 96 PCR plates to identify the polymorphic markers. One µl each of fluorescence-labeled PCR products (i.e. 4 µl total product for 4 PCR products) were combined in one new plate. The products were briefly vortexed and centrifuged at 3500 rpm for 30 sec. Nine µl formamide-standard mix (0.11 µl GS500 LIZ and 8.89 µl Hi-Di Formamide, Applied Biosystems) was added into each well of a new, empty plate. The standard, which allows the alignment of peaks for

analysis, is labeled with an orange dye (LIZ) (dye set "G5", Applied Biosystems). One µl of the PCR product mixture was added to 9 µl formamide-standard mix. The pooled plate was vortexed and centrifuged at 3500 rpm for 60 sec. It was denatured at 95°C for 5 min., placed on ice for 5 min., centrifuged, and then loaded into the ABI 3730. Capillary electrophoresis separates DNA fragments based on their size-dependant mobility when passing through a sieving matrix. Following separation, DNA fragments were analyzed for fluorescent signal as well as fragment size to check for polymorphisms. The SSR and ESSR genotyping in the F_1 progeny of the cassava mapping populations was performed using the polymorphic markers. An auto-Lid Dual 384-Well GeneAmp® PCR System 9700 (Applied Biosystems) was used. Marker panels comprised of SSR's and ESSR's with non-overlapping allele sizes.

Table 5.3. Properties of dyes used for fluorescent labeling of ESSR markers

Dye	Color	Sequence
FAM	blue	TTTCCCAGTCACGACGTTG
VIC	green	GCGGATAACAATTTCACACAGG
NED	yellow	TAAAACGACGGCCAGTGC
PET	red	GCTTACAGAGCTGGCTCACTGG

5.2.2.4. Data scoring and linkage analysis

Polymorphic alleles were scored using the GeneMapper software (version 3.7, Applied Biosystems). Peaks common to both parents were discarded from the analysis (Figure 5.2). Polymorphic markers with non-specific amplifications and/or which fell below the range of ABI-automated allele sizing of 1000 relative fluorescent units (rfu) were eliminated from the final population assay. Ambiguous genotypes were treated as missing data for map construction. Linkage analysis was carried out using the two-way pseudo-testcross method as described by Grattapaglia and Sederoff (1994) for markers segregating in the 1:1 ratio. Markers that segregated in the 3:1 and 1:1:1:1 ratios were treated according to Maliepaard et al. (1997). Two data sets, one segregating in the gametes of the female parent (COL 1734) and the other for the male parent (BRA 1149) were obtained. Linkage maps were constructed using the JoinMap® 3.0 software package, which permits linkage analysis in outbred progenies involving markers with different segregation types (Stam and Van Ooijen, 1995; Van Ooijen and Voorrips, 2001). The cross-pollinated (CP) population type was used. Markers were considered linked at a logarithm of odds (LOD) value of ≥3.0. A LOD

threshold of 2.0 was used to classify the linkage phase of each locus. The jump threshold value in the goodness-of-fit was set at 3.0. This represented the difference in goodness of fit chi-square value before and after adding a locus to the map, and was used to make the decision, whether or not a locus should remain on the map during the first and second rounds in the process of map construction. Reasonable values for the jump threshold are usually in the range of 3.0 to 5.0, while a higher jump represents a poor fit of the added marker and may justify its elimination from the map (Van Ooijen and Voorrips, 2001; Cavalcanti and Wilkinson, 2007).

Recombination frequency (REC) threshold was set at 0.45. REC were converted to map distances (cM) using the Kosambi mapping function (Kosambi, 1944). Adding a locus may influence the best possible map order and, to avert it from becoming ensnared in a local optimum of the goodness-of-fit, a ripple 2 function was performed so as to define the best map order. During this step, all permutations of three neighboring markers were considered for every map order, corresponding goodness-of-fit calculated, and the best order was chosen. Tests of similarity among loci and individuals were performed using a threshold value of 0.95 and a suspect linkage test was performed for each linkage group in each map using a REC threshold of 0.6 (Van Ooijen and Voorrips, 2001).

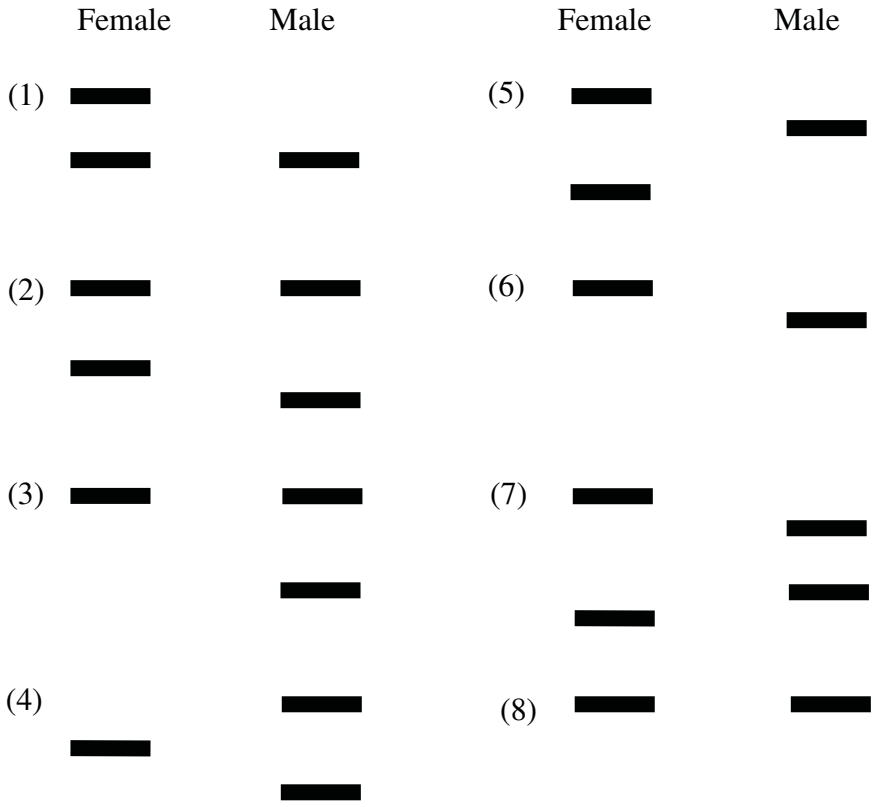

Figure 5.2. Category of polymorphic markers observed during the parental screen and used to identify unique alleles in cassava germplasm accessions for the construction of male and female linkage maps, and to determine the segregation ratio of each locus in the mapping population. Markers class (6) and (8) were not used to screen the segregating population.

5.3. Results

5.3.1. Labeling of ESSR's, polymorphism screening and marker segregation

In this study, the ESSR allele sizes were overlapping (between 165-218 bp). These markers had a maximum of 53 bp differences, which could not allow good co-separation (Appendix 5.2). Therefore, there were no ESSR markers with the same dye that were multiplexed.

Out of the 377 SSR and ESSR markers screened, 144 SSR and 11 ESSR loci revealed a unique allele in at least one of the parents and were used to screen the mapping population A. Thirty five percent of the markers showed a unique allele for both parents, whereas 29 markers had monomorphic double bands. The markers showed the same level of heterozygosity for both female and male parents in that the number of alleles observed ranged from 2 to 4. The size of the amplified fragments ranged from 80 to 391 bp.

The markers showed different segregation types, the ones considered in this study being **(a)** the lm x ll, where most fragments segregated according to Mendelian expectation 1:1 as a result of heterozygosity in the female parent and homozygosity in the male (maternally informative); **(b)** nn x np, where segregation ratio was 1:1 as a result of heterozygosity in the male and homozygosity in the female (paternally informative); **(c)** ef x eg, in which fragments were present in both parents as a result of heterozygosity on both sides segregating 3:1 in the progeny; and **(d)** ab x cd, in which four alleles segregated at one locus resulting in a 1:1:1:1 segregation (fully informative) (Table 5.4). Seventy one percent of the markers evaluated segregated according to 1:1, 1:1:1:1 and 3:1 ratios. Thirty seven markers (24%) showed significant deviation from Mendelian segregation patterns and were eliminated from the mapping assay. These markers were found in 12 linkage groups (LG's) and the number varied from 1-3 per LG. The extreme example of segregation distortion was found with marker SSRY99 where 2 out of 228 plants were "lm" heterozygotes. Eight markers (5%) had ambiguous, failed or weak amplification.

Table 5.4. Segregation types of markers mapped in the progeny of accessions COL 1734 x BRA 1149 and the number of polymorphic markers of fluorescent-labeled SSR's and ESSR's observed in the parents of the segregating population (a to g and l to p stand for different alleles).

Segregation type	**(a)** lm x ll	**(b)** nn x np	**(c)** ef x eg	**(d)** ab x cd	**Total**
Number of markers	57	43	42	13	155

5.3.2. Map construction

Two maps, one for each parent, were generated according to the inheritance patterns of the markers and ordering of loci within a linkage group. The number of linkage groups in the two maps did not correspond to the haploid number of chromosomes of cassava (n=18). One hundred and ten markers were employed in the linkage analysis. Among these, 74 and 65 % were used for the female and male map, respectively. Of the 81 markers used for female map construction, 56 of them could be assigned to 14 linkage groups (LG1 – LG 14). The length of the linkage groups ranged from 0.5 cM (LG14) to 65.6 cM (LG1), and the number of markers varied from 2 to 8 per group (Figure 5.3). The male framework map consisted of 57 markers, which could be assigned to 13 linkage groups (LG1 – LG 13), also with 2 to 8 markers, and a linkage group length varying from 1.4 cM (LG13) to 83.4 cM (LG1) (Figure 5.4). Twenty two markers remained unlinked in both the female and male map. Both the tests of similarity among loci and individuals detected 13 loci showing strong similarity (0.99). Suspect linkage was only identified in the male map in which one linkage (SSRY8 and SSRY53) was observed exhibiting recombination frequencies of 0.75. Inclusion of these markers in the map caused discrepancy in the resultant map and so these markers were eliminated.

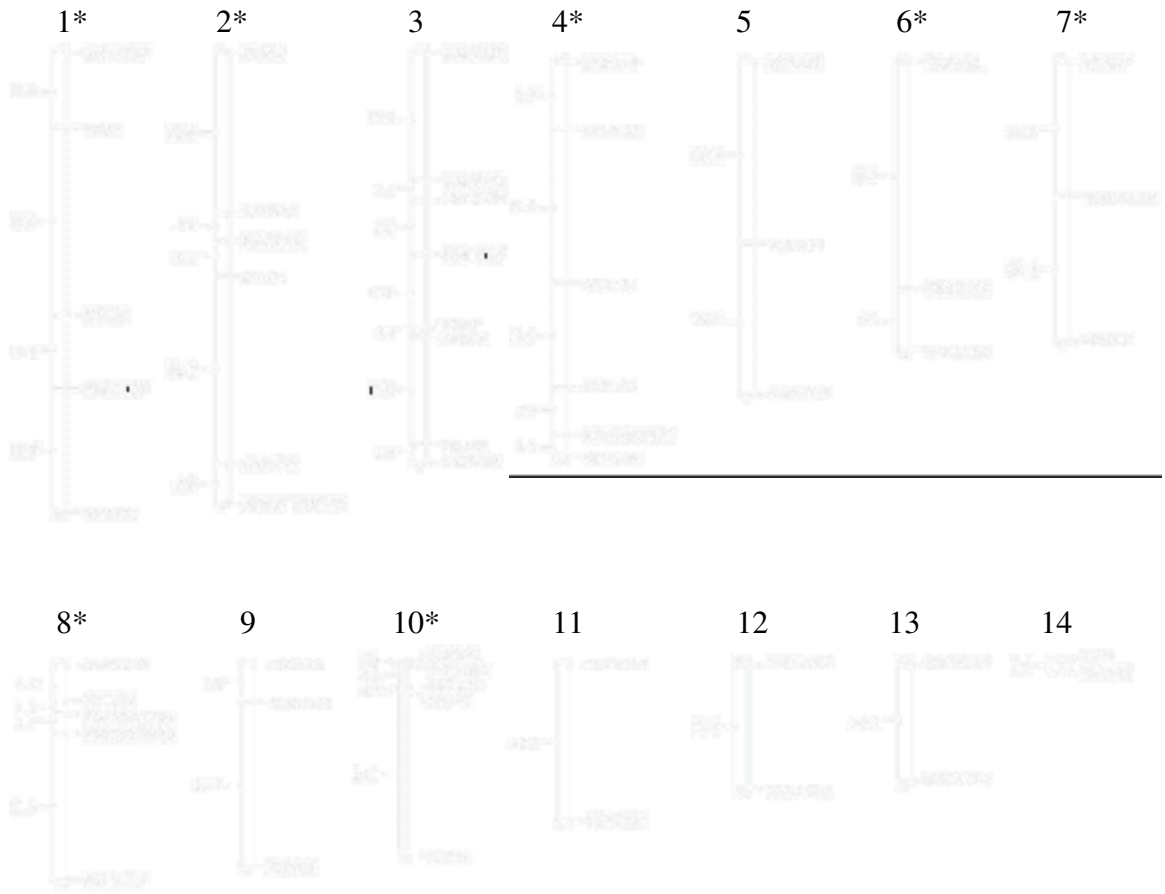

Figure 5.3. Female genetic map of cassava derived from a cross between a drought-tolerant (COL 1734) and a drought-susceptible (BRA 1149) accession. The map shows linear order and interval distance of markers in cM. Linkage groups are numbered sequentially from the longest to the shortest (*Homologous linkage groups).

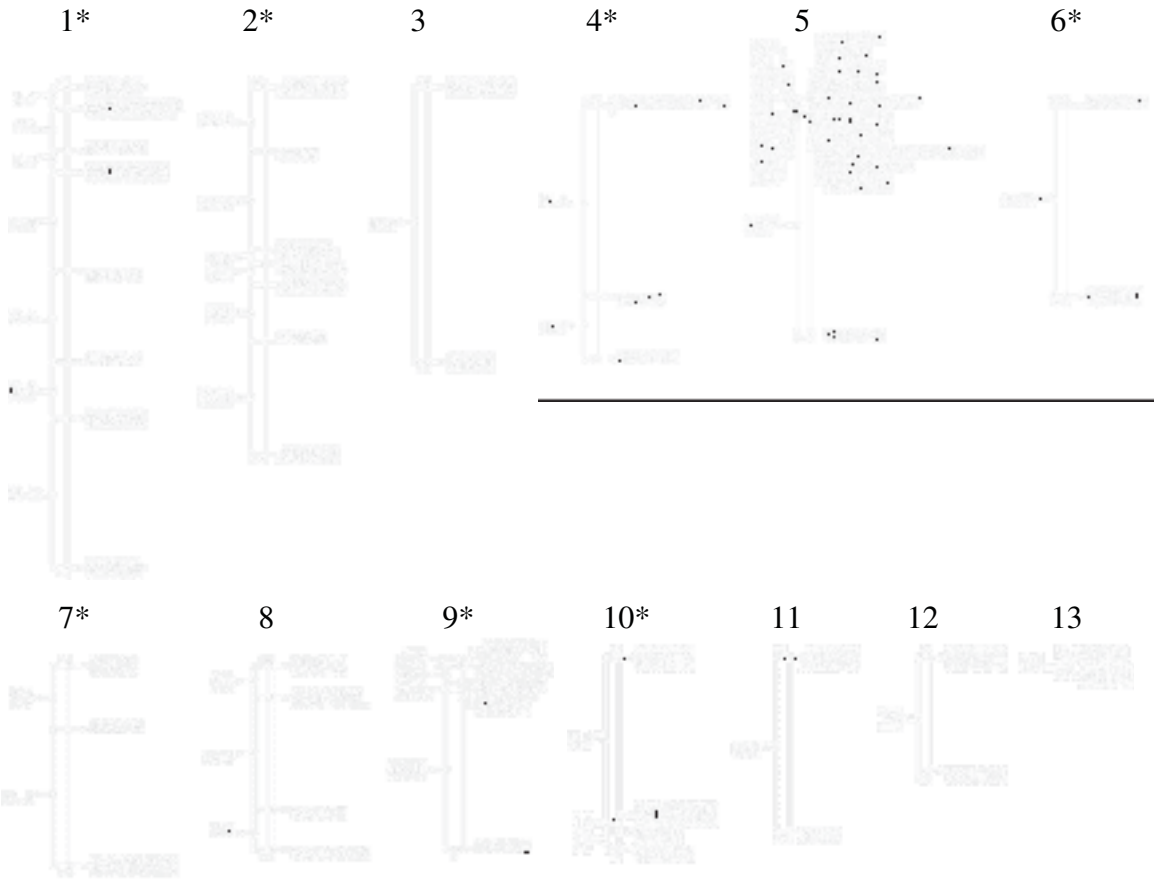

Figure 5.4. Male genetic map of cassava derived from a cross between a drought-tolerant (COL 1734) and a drought-susceptible (BRA 1149) accession. The map shows linear order and interval distance of markers in cM. Linkage groups are numbered sequentially from the longest to the shortest (*Homologous linkage groups).

5.3.3. Female and male map comparison

The distance between markers in both maps varied greatly across the different linkage groups. In total, the female map spanned 519.2 cM with a mean distance between adjacent markers of 9.3 cM. The total length of the male map was 468.3 cM with a mean distance of 8.2 cM between markers (Table 5.6). The intervals between loci were 0.3-32.5 cM and 0.1-47.8 cM in the female and male map, respectively. In general, there were 27 common markers present in both female and male map in the population, which allowed identification of homologous linkage groups. Homologies were identified between 7 linkage groups in the female and male map. Homology between linkage group 4 in the female and 1 in the male map showed the highest

number of allelic bridges (markers that are heterozygous in the gametes of both the male and female parents and share a common allele). In all except one incident, intervals between the male and the female homologous loci were larger in the male than in the female map. In some instances, there were differences in the estimated marker order in the common markers between the individual parental maps (Figures 5.3 and 5.4).

Table 5.5. Details of the cassava female (COL 1734) and male (BRA 1149) genetic maps.

Detail	Map	
	Female	Male
Number of linkage groups	14	13
Number of markers	56	57
Total map size (cM)	519.2	468.3
Mean distance between markers (cM)	9.3	8.2
Number of unlinked markers	22	22
Range of marker number per group	2-8	2-12
Interval between loci (cM)	0.3-32.5	0.1-47.8

Table 5.6. Number of allelic bridges identified between the male and female genetic maps of cassava derived from a cross between a drought-tolerant (COL 1734) and a drought-susceptible (BRA 1149) accession.

Female map	Male map	Number of allelic bridges
LG 1	LG 2	5
LG 2	LG 4	4
LG 4	LG 1	6
LG 6	LG 6	2
LG 7	LG 7	2
LG 8	LG 10	3
LG 10	LG 9	5
Total		27

5.4. Discussion

5.4.1. Labeling of ESSR's, polymorphism screening and marker segregation

Traditional plant breeding methods with or without using biotechnology tools are the two options for improving crops in water-limiting environments. Although both ways aim at identifying traits that confer relative advantages under drought conditions, their approaches are different. Traditional plant breeders use multi-locational testing techniques to identify lines with economic traits. Physiologists and biotechnologists hypothesize field traits that might be of importance, look for variation and then try to link them to genes at molecular level. In a long-cycle crop like cassava, biotechnology tools like marker-assisted selection can complement traditional plant breeding methods through speeding up genetic gain by effectively increasing heritability and also reducing the population sizes. Although the probable value of genetic markers and linkage maps in plant breeding has been known for over 8 decades, it is only in recent times that progress in automated technology has presented the accuracy, expediency, rapidity, and level of throughput that can finally offer relevance to modern plant breeding programs (Crouch and Serraj, 2002). For instance, Mansfield et al. (1995) reported that fluorescence-based SSR detection and allele sizing through laser excitation on an automated DNA fragment analyzer is one of the fastest and most accurate methods for genotyping. Hayden et al. (2008) showed that the use of dyes that fluoresce at certain wavelengths and intensities enables PCR multiplexing and, hence, markers can be separated simultaneously in a single capillary or gel lane as long as the fragment sizes do not overlap.

In this study, the ESSR allele sizes were overlapping (between 165-218 bp). Co-separation was achieved by labeling the ESSR's with spectrally resolvable fluorescent dyes that had different emission wavelengths. This permitted the analysis of multiple loci in the same capillary injection, on the basis of color and size, and also prevented analysis complication caused by spectral overlap. The use of LIZ-labeled size standard in the loading buffer allowed the alignment of peaks. Fluorescence-labeling methods are advantageous in that the fluorophores have a longer shelf life, are safe and their disposal issues are not demanding. Scoring of alleles is also automated and more accurate than autoradiography and silver-staining techniques.

Linkage maps are usually constructed using different types and sizes of mapping populations (Ferreira et al., 2006). Mapping populations are advantageous in that they allow dis-association of many characteristics that normally occur together in cultivars, thus allowing a clearer evaluation of the value of individual loci (Lafitte et al., 2002). Although specific studies relating to the ideal number of individuals in a segregating population required to construct accurate linkage maps have been inconclusive, simulation studies have shown that 200 individuals are required to construct a reasonable and accurate genetic map (Semagn et al., 2006). Studies by Ferreira et al. (2006) showed that, using between 50-1000 individuals, the low number of individuals provided several fragmented linkage groups, inaccurate locus order and imprecise maps. It was on this basis that population B was eliminated from this study since the total number of individuals was 23 only and that of the reciprocal cross 33. The use of large mapping populations is a critical factor in mapping as it facilitates the analysis of quantitative traits such as drought tolerance.

SSR and ESSR markers are powerful tools for genetic analysis because they are co-dominant, multi-allelic, easily assayed, and have wide transportability across different mapping populations (Gupta et al., 1999). They have become the marker class of choice for linkage mapping in many crop species (Roa et al., 2000; Okogbenin et al., 2006). They provide a much more efficient marker system than the dominant type of markers for mapping diploid cassava as well as other polyploids. SSR's, especially EST-derived SSR's are attractive for molecular mapping, since EST's represent the coding regions of the genome. This means that, if ESSR's are found to be associated with a trait of interest, it might be possible that the mapped gene directly affects the drought trait.

The high level of polymorphism (41%) of SSR and ESSR markers that we observed in the reference population is comparable to the results of other crop species and the same as the 40% polymorphism detected with RFLP's in cassava (Okogbenin et al., 2006). Eight percent of the markers revealed monomorphic double bands indicating the possibility of duplicated loci for such genomic regions.

Marker segregation type provides information about the unordered genotypes of the parents in a cross. They also determine the phenotypes that may occur in the offspring (Maliepaard et al., 1997). In this study, apart from segregation types ab x cd and ef x

eg, the other segregation types gave rise to less than four possible offspring genotypes. Segregation types lm x ll and nn x np had only two offspring genotypes and were completely un-informative with regard to the meiosis of the second and the first parent respectively.

Segregation deviations of molecular markers from Mendelian ratios have been reported in many studies. A high percentage of markers showing distorted segregation is frequent in out-crossing species (Gan et al., 2006; Okogbenin et al., 2006). In this study, we observed a 24% segregation distortion, which is within the range of distortions found in other studies. For example, a deviation of 31% has been reported in soybean (Prabhu and Gresshoff, 1994) and 27% in cassava (Okogbenin et al., 2006). Segregation distortion may be due to various processes amongst which can be the partial lethal factors, i.e. elimination of gametes or zygotes controlled by a partial lethal factor located in the region neighbouring the marker (Cheng et al., 1998), an evolutionary force of an organism, as explained by Lyttle (1991). Xu et al. (1997) suggested that segregation distortion may be ascribed to either contamination of the genomic DNA with chloroplast DNA or some degree of preferential pairing or linked deleterious mutations. Chromosome loss, genetic isolating mechanisms, genetic load (Bradshaw and Stettler, 1994), genetic drive, chromosomal re-arrangements between the parents, locus duplication, and technical problems like genotyping and scoring errors may also cause segregation distortions (Cavalcanti and Wilkinson, 2007).

Cassava is an out-crossing species with high genetic load and suffers from severe inbreeding depression (Okogbenin et al., 2006). In this study, a strict criterion was applied for the selection of markers to be included in the mapping analysis on the basis of scoring, peak height, ambiguity, and on segregation ratios approximating to Mendelian expectations, and that is why the 5% markers that had ambiguous or weak amplification were eliminated from the mapping assay. Therefore, deviations from Mendelian segregation in this study may rather be related to the highly heterozygous structure of the crop.

5.4.2. Map construction

Genetic mapping in a full-sib family derived from heterozygous parents involves linkage analysis of markers segregating independently in the female and male gametes, and this leads to two autonomous maps (Maliepaard et al., 1997). This permits the estimation of recombination frequencies for each parent separately, which is not possible in a classical F_2-mapping population. This means that mating types are re-defined at a locus level rather than at all loci in parents. Homologous groups can then be identified amongst the female- and male-derived linkage groups through allelic bridges.

In this study, male and female PCR marker-based genetic linkage maps of cassava were constructed with SSR and ESSR markers. The use of LOD 3.0 was the most appropriate with this data and resolved 14 linkage groups in the female and 13 in the male map. This represents a close approximation to the expected number of 18 linkage groups for a comprehensive linkage map of cassava (2n=36) although, additional markers need to be incorporated into these maps to saturate them. The differences in the number of linkage groups and map length observed may be due to variation in the number of recombination events and mapped loci. Results by Wu et al. (2002) showed that different map distances can also be caused by differences in DNA sequence, DNA content and chromosomal re-arrangements. In addition to low map saturation, marker distribution along the linkage groups was random as evident by the mixture of tightly linked loci and regions with low density in the constructed map. This is an indication that either recombination events or mapped loci were not evenly distributed throughout the genome. The low density of markers in some of the linkage groups could also correspond to regions that are highly homozygous and, hence, show higher recombination frequency events (Castiglioni et al., 1999; Okogbenin et al., 2006).

The mean map size per linkage group was 37.1 cM for COL 1734 and 36.0 cM for BRA 1149 (Table 5.5), which is considerably smaller than the 100-150 cM commonly found in agricultural crops (Maliepaard et al., 1997). This is because, as indicated, some linkage groups were missing, and there was a limited number of mapped markers in the maps. The mean map distance between markers is one of the important components in linkage mapping and for the detection of quantitative loci associated

with traits of interest. In this study, the mean separation between markers was 9.3 cM in the female compared with 8.2 cM in the male map. Although these maps were incomplete in terms of map saturation and the number of linkage groups, when compared with other studies, these means represent good marker coverage. For example, researchers have found means of 6.0 and 10.7 cM in European pears (Yamamoto et al., 2002); 6.7 and 10.8 cM in eucalyptus (Myburg et al., 2003); 8.7 and 9.0 cM in European chestnut (Casasoli et al., 2001); and 7.8 and 8.0 cM in willow (Hanley et al., 2002). A few clusters of markers were evident in both linkage maps. This is a common occurrence and has been reported in many linkage maps irrespective of the organism or technique used to assay DNA polymorphisms. This may be due to suppressed genetic recombination as described by Tanksley et al. (1992).

5.4.3. Comparison with other maps

The F_1 cassava genetic maps by Fregene et al. (1997) and Mba et al. (2001) differed from our maps with respect to marker number, type and density; genome coverage; and number of linkage groups. The former, also the first genetic linkage map for cassava was constructed with predominantly RFLP markers and only 3 SSR markers. The map consisted of 168 markers distributed in 20 linkage groups, spanning 931.6 cM and a mean marker density of 1 per 7.9 cM. In the latter, 36 SSR markers were placed on the former RFLP framework map of cassava to saturate it, and this resulted in a reduction of 2 linkage groups. The F_1 female parent-derived map generated from this study spans 519.2 cM with 56 markers compared to the male map with 468.3 cM and 57 SSR markers. The mean marker density is 1 per 8.7 cM. Of the SSR's mapped in this study, 23 markers are common to both our F_1 female map and the one generated by Fregene et al. (2001), whereas in the male map, 22 allelic bridges where identified. However, some variations were observed. Differences in the order were evident for some markers in LG G (SSRY 135, NS 928, NS 97, SSRY 226 and SSRY 38) and LG 3 (SSRY 226, SSRY 153, SSRY 135, SSRY 165, NS 97, NS 928, NS 189, and SSRY 38) in the map generated from this study. In addition, NS 189 had been mapped in LG G of the male map, whereas in this study, it was mapped in LG 3 in the female map. These may probably be due to the different statistical software and the parameters used. The variation in the mean and lengths of the linkage groups

observed may-be due to the disparity in the number of markers utilized. Twenty five common markers showed colinearity between the maps generated from this study and the F_2 map by Okogbenin et al. (2006) indicating the reliability of both maps. In addition, the map distance of 46 microsatellite markers was determined, which had not been mapped before.

5.4.4. Female and male map comparison

The female and male maps were compared to identify analogous linkage groups based on common markers segregating in both parents. Homologies/locus bridges were identified between 27 markers based on 7 linkage groups. This is advantageous in that, these markers can act as anchor points for more mapping work, and also identification of QTLs after further saturation since they have known sequences. In addition, these markers are easily transferable between laboratories.

In this study, the female genomic map length was higher than the male. This is a common phenomenon and has been well documented. For instance, Graner et al. (1991) reported that, in flowering plants, the females appear to have a higher genomic map length than the males due to variation in the rate of meiotic recombination, whereas in gymnosperms, greater meiotic recombinations occur in male than in female gametes (Groover et al., 1995). A few analogous markers were not uniformly distributed over the maps. In some instances, there were minor differences in the estimated marker order between the two parental maps due to differences in recombination frequencies or the presence of chromosomal re-arrangements of one parental genotype relative to the other in the group. Despite this observation, with a number of common co-dominant markers of the same order present in chromosomes of both parents, with more work, it will be possible to combine the information of markers from different individuals and the available map so as to assemble a comprehensive cassava consensus map. Ideally, a linkage map should contain at least a backbone of co-dominant markers, such as SSR's or ESSR's, which are reproducible and can be transported to another progeny to saturate the more interesting regions of the genome (Maliepaard et al., 1998). Although the present maps fulfills these requirements, they are not ideal to form a core map for cassava drought research due to the limited number of co-dominant markers, but they form an important platform for QTL's associated with drought tolerance in cassava.

Therefore, there is need to develop a saturated cassava consensus map that incorporate SSR's and ESSR markers since it will provide researchers with a greater arsenal of tools for identifying genes associated with economically important traits.

Appendix 5.1. Contrasting cassava germplasm accessions evaluated in Northeast Brazil for the identification of mapping population parentals with wide diversity for drought tolerance traits (Embrapa, Brazilian Agricultural Research Corporation; CIAT, International Center for Tropical Agriculture; N/a, Not available; * diverse accessions that were selected for development of mapping populations).

CIAT Code	Accession identifier 1	Accession identifier 2	Name	Country of origin	Selected by	Remark
BRA 116	BGM 0195	BRA-009849	São João I	Brazil	Embrapa	Tolerant
BRA 134	BGM 0260	BRA-007277	Rosa	Brazil	Embrapa	Tolerant
BRA 165	BGM 0001	BRA-005509	Aipim Bravo	Brazil	Embrapa	Tolerant
BRA 179	BGM 0082	BRA-007161	Branca de Santa Catarina	Brazil	Embrapa	Tolerant
BRA 200	BGM 0537	BRA-001171	Do Céu	Brazil	Embrapa and CIAT	Tolerant
BRA 209	BGM 0478	BRA-001601	Manca	Brazil	Embrapa	Tolerant
BRA 216	BGM 0384	BRA-002062	Sacai	Brazil	Embrapa and CIAT	Tolerant
BRA 255*	BGM 0080	BRA-005819	Engana Ladrão	Brazil	Embrapa and CIAT	Tolerant
BRA 264	BGM 0215	BRA-007293	Saracura	Brazil	Embrapa	Tolerant
BRA 293	BGM 0549	BRA-012611	Amansa Burro	Brazil	Embrapa and CIAT	Tolerant
BRA 534	BGM 0860	BRA-061221	Pornuncia	Brazil	Embrapa	Tolerant
BRA 974	BGM 0211	BRA-008648	Mantiqueira	Brazil	Embrapa	Tolerant
BRA 1142	BGM 0814	BRA-057410	Guiana	Brazil	Embrapa	Tolerant
BRA 1204	BGM 0282	BRA-009083	Manteiga I	Brazil	Embrapa	Tolerant
BRA 1342	BGM 0538	BRA-011304	Macaxeira Preta	Brazil	Embrapa and CIAT	Tolerant
BRA 1346	BGM 0249	BRA-011606	Maragogipe I	Brazil	Embrapa	Tolerant
BRA 1394	BGM 1269	BRA-080560	Engana Ladrão	Brazil	Embrapa	Tolerant
BRA 1400	BGM 0116	BRA-000361	Cigana Preta	Brazil	Embrapa	Tolerant
CM 3306-9	CIAT Hybrid	CIAT Hybrid	CIAT Hybrid	Colombia	CIAT	Tolerant
COL 948D	COR-363	N/a	Algodon	Colombia	CIAT	Tolerant
COL 1719	GUA-316	N/a	Blanca Mona	Colombia	CIAT	Tolerant
COL 1725	GUA-322	N/a	Desconocida III	Colombia	CIAT	Tolerant
COL 1734*	GUA-331	N/a	Negra	Colombia	CIAT	Tolerant
COL 2215	N/a	N/a	Venezolana 1	Colombia	CIAT	Tolerant

Appendix 5.1. continued

CIAT Code	Accession identifier 1	Accession identifier 2	Name	Country of origin	Selected by	Remark
COL 949	COR-364	N/a	Monablanca	Colombia	CIAT	Tolerant
SM 1438-2	CIAT Hybrid	CIAT Hybrid	CIAT Hybrid	Thailand	CIAT	Tolerant
TAI 8	CMR 246343	RYG 60	N/a	Thailand	CIAT	Tolerant
TAI 16	MKUC 28-77-3	N/a	N/a	Thailand	CIAT	Tolerant
BRA 114	BGM 0120	BRA-006939	Paulo Rosa	Brazil	Embrapa	Susceptible
BRA 201	BGM 0382	BRA-001228	Fio de Ouro	Brazil	Embrapa	Susceptible
BRA 253	BGM 0021	BRA-005789	Cachimbo	Brazil	Embrapa	Susceptible
BRA 346	BGM 0070	BRA-006611	Jaboti	Brazil	Embrapa	Susceptible
BRA 835	BGM 0876	BRA-062634	Pretinha V	Brazil	Embrapa	Susceptible
BRA 846	BGM 0063	BRA-007226	Cacau	Brazil	Embrapa	Susceptible
BRA 997	BGM 0600	BRA-012327	Paraguaia	Brazil	Embrapa	Susceptible
BRA 1149*	VEN 77	BRA-014109	MVEN 77	Venezuela	CIAT	Susceptible
BRA 1356	BGM 0867	BRA-066575	Mocotó	Brazil	Embrapa	Susceptible
COL 1468*	CMC 40	BRA-000965	Mantiqueira	Brazil	CIAT	Susceptible
COL 1522	CMC 92	N/a	Algodonera Amarilla	Colombia	CIAT	Susceptible
COL 2066	N/a	N/a	Chiroza Gallinaza	Colombia	CIAT	Susceptible

Appendix 5.2. Features of fluorescent labelled cassava loci screened for parental survey; their primer pairs, type of repeat and their fragment sizes in bp in the two parents (COL 1734 and BRA 1149); n/a, not available; NA, no amplification observed.

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
ESSR1	TCTGCTCAGCTGCCCAGCCA	TCGCAGCAAACCTCTCCCCA	(AG)11	186	186
ESSR2	TGGAAATGCTGAAAGTGAACGCTTGA	AAAACACCAGCAAAATTGCACAGGAC	(AC)12	186	186
ESSR3	GCAACAGGTGCCCGATGTGTAGC	CAGCGGCTGCTCCCATTCCT	(CT)11	223	223
ESSR4	TCTCTCACAGGTCGCCCAACACA	GGTCACGTCAAGTACCTGTCAAGGCA	(CT)8	181, 187	181, 187
ESSR5	TGCCACAACGCCTGTGTAGAATCG	ACCCAATGGAGCCGTAACAAATTCA	(TA)8	188, 206	206
ESSR6	TCCACCATTTCATTCATCAAGGCCA	GGAACGATTTTCTCAACCAAAATGCGA	(CT)8	NA	NA
ESSR7	AGCACTCTAATCATGCAACTCCTTCGG	GCTCAATCAGGTGCCACAGCG	(CT)11	181, 183	181
ESSR8	TTCTGCCGAGCACGAATATTACCCC	TCGACTTGTTTTCAAGTGCATCCCA	(CT)13	165, 175	175
ESSR9	CTCTAGCCTGGAGCTCGTGACGACATT	TCCCAATGTAACCAGCACCACCG	(TC)9	176	176
ESSRI0	AGCCACCACACACACCAAACGC	TCCAGACGCTGCATTTGCCA	(CT)11	NA	NA
ESSR11	GAGGAGGTTTGGGACCCTCCCTG	GGAGGGTGGCTGTGAATCCCG	(CT)8	187	187
ESSR12	TGTCAATACACTGTCAGACACGTTCGC	GCATCGTGACTTTTCTTGATAGGCCAG	(TA)8	NA	NA
ESSR13	TCAGAATTCGAGCTGAGAGTGTTGAGG	CCCTTCTCTGAGGCCAGTCCCA	(TC)8	178, 184	178, 184
ESSR14	ATGGGGTTCTCACAGTGACGGTTCC	TGCTCAGAGAATCCCAAAGGCACA	(AT)12	199	199
ESSR15	GTCAGCCGTCATCCGGCCAT	GCTTTCTCTTCAAGCCAAAAGCGTCC	(TC)8	189, 205	189
ESSR16	TTGCCAGCATTGATACTGCACAAGC	GGCACCTGGGGACCTGTAATCAGTC	(AGC)8	NA	NA
ESSR17	CTATTGGATGTGGGCTGGCGCT	CCACTCGCATGCTCCTCAAGCA	(TAT)6	180, 182	180, 182
ESSR18	CACCGGATCCCACGTGCAAGA	CAAGGGTGACGTCCACTAAATCGACA	(ATT)5	194	194
ESSR19	ACGGTAGTGCCCTTGAGGTTGGG	CAAATGGACAACATCAACGATCACAGG	(TTA)8	190	184
ESSR20	TGTCAATTTGGGTCCAATTGCAACAGT	AGCAAGCACATGCCATTCTTTTCTTTC	(ATT)5	197	197
ESSR21	TACAGGATTGACGTTGCTGTTGCATGT	CACAAATGGTGAAGACACAGAAAACGC	(AAT)6	179, 183	183
ESSR22	TTGGAATGCACTGAAACTCATTTGGGA	TGCATAATGAGGTCAAATGTTTGGGG	(TTAT)5	203	203
ESSR23	GGTTGATGGGAATGTTGTTTGGCTC	TGGAGGGGAAGGAGAGATTTTTCAGA	(AT)6	214	214
ESSR24	GGGACGCGTGAATTCTTGCTTTTG	GGTTTCTGAGAGAAAGCATGCGCAGA	(TA)6	176	174
ESSR25	TCCTCGTCTTCAAAACCCACAAGGC	GGTCAGGCAAAGCAATTGGGC	(ATA)6	180, 184	180

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
ESSR26	GCGTGGAAGCAAGGCAATACTGAAT	TTTCTGCTGCTTTCGAAGCTCTCTGTT	(ATA)10	193	190
ESSR27	AGTTGCTGGGTCCTGCGTTTAAGG	TTCTGGACGTCCTCTTCAGAGCCA	(AT)7	213	196
ESSR28	TTCCTATTGACCGACATCCCTCTCCC	GAGCGAGCGAGACCGAGCGA	(CT)6	170	170
ESSR29	TTCGCGTCTTCAATCCGTAGCCA	GCCGGTGTGAGTCGCGAGAA	(TC)6	203	203
ESSR30	CGTGTTGTGCATCTGGGCCG	CCTTCGAAGTACAACCAAAGCCATGA	(TG)6	193	189, 193
ESSR31	CGGCCGCTGCATCAGAGCTT	TGCCTCTTGGCGGGGGTCTT	(TC)6	NA	NA
ESSR32	GGGGAAATCACAAACTCCAAGCCA	TGGATCATCGGAGACCCCTCG	(AG)6	202	202
ESSR33	CCGCAAGCAACGGCCAAGA	GGAATATCAACGGTGATGCCGGA	(CT)7	214	214, 216
ESSR34	TCACAGGCTGGAGTTTATGAAGGCG	ACTGCAGCCGCTCCTCCCAA	(TA)7	206, 208	206, 208
ESSR35	GGGTCCTGAGCCACCTGCATC	TGCTCCCGGTAACCAGTGGTGG	(TA)7	208	208, 210
ESSR36	ACGATGTTTGTCTTTGAGGATTGGTGG	TGAGACAACACAGGTGGATTGCAGC	(CT)7	173	173
ESSR37	TGGAGGCAGGGCCTTCTTTGC	TGCATCCCAAGCAAGAGAGGAGAA	(AT)6	181	183
ESSR38	TGATCATAAAGCTGGAGCAGAGGCTGA	AAACTCATGCCCCTCGTGAAAACAA	(AT)6	194, 196	194, 196
ESSR39	GTTGCAGCAAAGCTTGCTATCCAATCA	GGAGGCTCCACTCCCCACTGA	(AG)6	188	186, 188
ESSR40	GGGAGTACCTCGAGTACAACGAAGCAA	ATCGCATGCCTCTGCGTGGA	(TC)6	178	176
ESSR41	TCCGCGAAAACAATTTGGCACA	TCCAATTCCATTTTCATCACCAGCA	(GA)6	200	200
ESSR42	TCATTCTTTCCCTGTTTTGCCTTCG	GCCTTCGTCAGGCAAGGAGCA	(TA)7	193	193
ESSR43	TTTGCTCACCAGCACCAGCGA	TCACGAGCTGACACGTTGCCG	(CT)6	189	189
ESSR44	TTCCTCGTTAACGCTGGCCTTGTG	GAGAAAACGCAATTCCGAGCCAA	(AT)6	204	204
ESSR45	GTCTCAGTCCCTGCCAGACCCG	TCGCCTTCCTCTTCTTTCTGTGTCCA	(TC)6	177	177
ESSR46	TCCGTCAACTCTCTCACTCTGCGTTG	GCCTTGGTTCTAAGAGGGTGGGC	(AG)7	197	197
ESSR47	TTCGCTTCTTGACATCTTCCGCC	TGCAGAGTCCATGGTTTGGCGA	(GA)6	181	181
ESSR48	TCTCCGCCCTTCCCCCATCT	CACGGAAAGCTTGGTGTTTTTGGC	(TC)6	175	175
ESSR49	CTGGCACAAAGTGCAGTTGGAGTTG	GCTGTGAAAATGAACTGCATGCCAC	(AG)7	NA	NA
ESSR50	ACGCCAACTAGCCTCTGATTTCTCACA	GGCCAAAATCTTTGCAACGTGGT	(AG)6	200	200

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
ESSR51	AGATGGAGAGGCAATGCTGGGC	CACTTGTTCCTGTGCTTAACCCACCTT	(AT)6	196	196
ESSR52	ATGGGTGTCCTTGTGCCTACTGGA	CCCCAATTGCAGCAAGGCGT	(AG)6	NA	NA
ESSR53	TCCCACTTCCCAGTCAACGCC	TCGCCTATGCCGACGGAGGA	(TC)7	218	202
ESSR54	TGATCATAAAGCTGGAGCAAAGGCTG	TGGTAAAACTCATGCCCCTCGTGA	(AT)6	176	176
ESSR55	AGGGTTGGAGGCTGAGCTGGC	TGGTGTAAGCGGCTCACCATTCTC	(AGC)6	NA	NA
ESSR56	TTGGGTGACGATGACGCCGA	GGAGGTACCGGCTTGAAGGGGA	(GCC)5	183	183
ESSR57	TCCATGAGCAGTGAAGGAGCTTCAAGT	GGCACATCATCTTCTCCAATCATAGCC	(AAC)5	212	212
ESSR58	TGGATCTGATGAGGAAGGGGATCA	GGATGATCACCATCTTGCAAGCCTAA	(TGA)5	188	188
ESSR59	GGAATGGTTGAAACGGGAAAGCC	CCAGTGATGATTGGGCTTCATGGTC	(TCT)7	175	175
ESSR60	CGTTTCCCTTGCGCTCTCCG	CCCACTAAGCTGATTGGTTGCTGCT	(AGC)5	175	175
ESSR61	CAGCAGCAGCAACAACATCCGC	CAAGCAGCCCTGCAATCCTCTTTC	(GCA)5	219	219
ESSR62	AGCATGAGCGCATGTCTGTGAGC	TGGGTCGACACCAAATCTACCATTCA	(AGA)6	198	198
ESSR63	CCACCAACATCCTCATCATGGAAGAC	AAGGTCATGATGAACGACTGGAGCA	(CAT)5	NA	NA
ESSR64	GCTACGGGGGATTACACGACCTTTG	TGCACCACTCGCTCGTTCACC	(TGC)5	NA	NA
ESSR65	GATGGAGCCGCTGACCTCCG	TGATCGCCGCTTCGACGACTT	(CTC)5	215	215
ESSR66	CGTCTCTCCGGTGACGTTGTCG	GACGGAGCAAATTATCATCATCGAACC	(TCT)10	182, 184	182, 184
ESSR67	TATGATCCAGCGCCCAGCGG	GGTGCATCTGGCGGAATGTCAA	(AGA)5	NA	NA
ESSR68	CGCCGCCCTCGCTTAGCC	GCTGGAGGGTATGCTGCAGTGG	(CTT)7	188	188
ESSR69	TGGAGGCTGTAATGGCTTGCTGG	TGGGGACAAGAGGACCAAATCCC	(TGA)5	NA	NA
ESSR70	TTGACAGGCCCGCAGCTGGT	TGGTCTTCAGTCAGGGGAACAGGA	(CCA)7	195	195
ESTSSRY5	AATGCAATTTGCTGCTTCCT	CCAAGGGAAGTCCAGAAGAA	ATGG(5)	208, 210	206, 210
ESTSSRY11	AAGAGCTTTAGGCGGTCACA	TTGGGGTTCTCCCTTAATCC	GAA(5)	243	243
ESTSSRY12	AAAAATGATCCAACATCTAGCAA	TTGGGGCCCGATAATAAGAT	TTC(5)	181	181
ESTSSRY14	TCGATGCCCTTATTGGTAGG	GGCAGAGCTGGATTCATCA	AAG(5)	199, 203	199, 203
ESTSSRY15	GAGCAATTTCCACCACCATC	CGGAATGGTCAATACCCTTG	GAT(5)	283	283
ESTSSRY16	TCGAAGCATTCTGTGTGTCC	TCATCCATAAGATCATCTCTGTTACTG	AGA(5)	264	264

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
ESTSSRY17	GGCCATGCTCGTTTTAGAAG	CACCCCATTGTTCTTTGACA	CCT(5)	203	205, 203
ESTSSRY18	AGTAGGTTTTCGCGGTCTCC	ACCTCCAGAAGGTCGTCGT	TCA(5)	200	200
ESTSSRY20	CGTCAGCCCATGAAATCTCT	GCAAAGACAAAGAGGCATCC	TCC(5)	205	205
ESTSSRY21	CAAAGCATAACCGCGAATTT	AGTGCCCGATTATTGGAGTG	AAC(5)	200	200, 212
ESTSSRY22	CCAACCCACAACCTTCTGAT	AATCCAATCCCCTTGGACTC	CAT(5)	212	200
ESTSSRY45	GCCGGTAAACGGTAAAATCA	AATCAAAGAGACGGCGAAGA	TTA(7)	NA	NA
ESTSSRY47	GTGTCGCTGCTAGATCTTGACT	TCATCAGATCACCACCATCAA	TCT(7)	249	247, 249
ESTSSRY48	GCCCCCATAAAAATCACCAT	GAGAGCAAACAACCAAAGTTGA	TCC(8)	179, 183	179, 185
ESTSSRY49	GACCACGTCTCGTCGTTTTA	TGCCACGACATCTTCTTCTG	TC(8)	200, 202	208
ESTSSRY54	GCAAATTGGGGGAATGTTTT	AAGACACGAAGACGGTTGCT	GA(8)	NA	NA
ESTSSRY55	TGCCCTCAAATTTTCTCCAT	TTGGAAGATTCCTTTTCCATAGAC	TG(8)	NA	NA
ESTSSRY61	TTCAATGATGGCTGAGCAAG	TCGCAGCACTAAACATCTCG	TGA(8)	146	146, 150
ESTSSRY62	CCATGGCACAATAACATTGG	TGGGCGTAGGACAGTAAGAGA	TG(8)	219	215, 221
ESTSSRY63	CCCTTCCCGAGCAAGAAC	CCTGTATCCAAGCTGCCATAA	TC(8)	159	159
ESTSSRY65	GCACCAGTCAACATTCCTGA	CATCATTCACAACCCCATGA	ATG(9)	158	158
ESTSSRY66	CGCTTACAACACCACCTTCA	TGACGTCCTTAGCCATCCTC	AG(9)	NA	NA
ESTSSRY70	GCCAATTTTGCTGGGTTTAC	GCTGATGAACCCTTCACGTT	CT(9)	NA	NA
ESTSSRY73	AGGAGTCCACCGAGGAAGTT	GTCAGAGCGGACACAACAAC	CT(9)	NA	NA
ESTSSRY115	AGCACAGGTTTCATGCTAAT	TGGACTTGAGGAAGAGACAT	GA(5)	251, 253	253, 257
ESTSSRY117	CAGAGAGAGAGCCTGAAAGA	CAGAGCTCCGTAGCTTATGT	AG(5)	261	261
ESTSSRY264	TCCACTTCAAATCTTCTGCT	CTCTTTGGTTCTGGAAAATG	TTC(6)	NA	NA
ESTSSRY266	CACGATCATCTAAACCAACC	GTCATCAGAATCCTCCTCTG	GAA(6)	245, 251	245, 255
ESTSSRY272	CACTCCTTTTCCACAAAACA	TGTATACCGAGCCTTTGACT	CT(7)	266, 272	278
ESTSSRY273	GGCAATACAGAAGAGGACAC	CACAAAACCGAACACCATA	CT(7)	212, 220	214
ESTSSRY274	CTGAGCTTCTCCTCCTCTTT	AGTACGACAACCCACATCTC	TC(7)	238	238
ESTSSRY280	GGTGAATTTTGGGGTTAGAT	AGAAGCGGATTGTAAGATCA	TC(7)	NA	NA

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
ESTSSRY281	ATGTGGTGGTGATGGTTACT	AAGAACCTGTGGTTCCTTTT	GT(7)	NA	NA
ESTSSRY284	GAGAAGTTTTGCCACCATAG	CTTTCCAAGCAAACTGGTAG	GA(7)	246	246
ESTSSRY287	GAGAACTTGGCAACACACTT	AGAAACCCCTAGAGTGAAGG	TG(8)	255	221, 227
NS6	TTCCATCCAAGCTCCTTCAG	GCTCGCATATTCCCATCAAT	n/a	268	266
NS10	GTCATTACGGCGAATCTGCT	CGATTTTGCGAATTACCACC	n/a	202	202
NS22	CAGCCAAATCAACATCCCTT	CAAGCCCCATCATCATTTTC	n/a	289	295
NS40	GCTGTTACGGCCAGAGTAGA	GATGTCTGAAATCCCTCTCTTT	n/a	155, 173	173
NS53	CCAACGTATGGAATGTGCTG	CCTAAGTTGTTAGCCAGTGATTAGA	n/a	202, 210	200, 202
NS57	ACCAAAATCTCCACACCCTG	CAACAATTGGACTAAGCAGCA	n/a	250	256, 262
NS59	CATGAGCATATCCCTCCTCA	CTCCTGCCAAAATTGACCAC	n/a	113	119
NS74	TCGCTGTATGCAATACTTCGTT	TAGTGTTGGGGACTCTTTCG	n/a	NA	NA
NS78	AGCAATGCCTTGATCTTGAG	AAGATGGCAATTCAAGCAAG	n/a	380, 384	368, 384
NS92	ACTTCATTGGTGCTGGTGCT	AGCGTTTAGCACGTCAGAGC	n/a	262	240, 262
NS97	TTAAAAGCACCTGTGGGTCC	GATACCCACAAGCCCAAAGA	n/a	242, 248	242
NS109	AACTGCAAACAAAGCCGAAG	TCCTTGGCATAGCCAAAATC	n/a	162	162
NS119	GGGAAGTGAGCAGAGACTGG	GATGGTGGTGATGATGATGC	n/a	186, 188	186, 198
NS149	TCTTGCTCAAGGGCTCAAAT	TTTGATTCCACGAAATCTAGAGAA	n/a	300	300
NS160	CAATAAGAGTATAACCATTACCTGTG	ATGCATCTTCCTGGTTTTGT	n/a	123, 133	123
NS166	ACAAAAGCAATCAGGCAAGC	TTGTGCACCATGAAACCATT	n/a	303	273, 303
NS169	GTGCGAAATGGAAATCAATG	GCCTTCTCAGCATATGGAGC	n/a	306, 314	324, 334
NS178	TTACAGGTGCCCGATGTGTA	CGTTCGAGTTGCATTCATTC	n/a	168	168
NS185	AGTTAAGGGCCAATTCCTGC	CCTTTCTGATGTTCTCTCTGCAT	n/a	155, 165	155, 169
NS189	TGGGCTGTTCGTGATCCTTA	CATGAGTTTAAAAATTATCACATCCG	n/a	91, 106	91
NS190	CCAAGCAACCATCATTCAGA	TTCATGTGTGGGTTTCCTCA	n/a	NA	NA
NS193	TTGGGGGCTTTAAGTTGTTG	AAAGCCCATCCCCTCTATGT	n/a	255	249
NS197	TGTAAAATTTAAGACTTTCCATCTTCA	ATGGCATTTCTGCAGCTCTT	n/a	170	170
NS210	AAAGGGAGATTTGCAGAGCA	TGGCTTTGGTTGTATGTGGA	n/a	244, 252	238, 252

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
NS217	TCAGCTGTATGTTGAGTGAGCA	AGGGAAGGAACACCTCTCCTA	n/a	224, 232	224, 232
NS235	CCAAAACATAGGGAGCGAAA	AATTATGGGCAGGAGAAGCC	n/a	189	193
NS260	TCAGCTGTATGTTGAGTGAGCA	AGGGAAGGAACACCTCTCCTA	n/a	185	225, 235
NS267	ACACGCAACAAATCAACCAA	CCGCTCCAGGTGCTTTTAC	n/a	171	151
NS272	TGTAAAATTTAAGACTTTCCATCTTCA	ATGGCATTTCTGCAGCTCTT	n/a	168	168
NS306	AAGACCCACCAGAAAGCTGA	CAATTTCCAATGTGGTCTCAAA	n/a	302	270
NS308	GGAAATTGGTTATGTCCTTTCC	CGCATTGGACTTCCTACAAA	n/a	123, 141	137, 143
NS319	TTCTAAAGGTTGATTAAAGCTCTGTG	AGGGAAGGAACACCTCTCCTA	n/a	165, 167	165, 167
NS340	GCCAGCAAGGTTTGCTACAT	TTGCTAAAAATCCCTGGACC	n/a	NA	NA
NS346	CTGCAAAGTCGATGCCTACA	TCTCCATCAGCAGTGCAAAC	n/a	289	289
NS347	AAAGGGAGATTTGCAGAGCA	TGGCTTTGGTTGTATGTGGA	n/a	242, 250	236, 250
NS349	CACTGCGTACAAGCAACACC	GCAAAAGTGAAAAGGACGGA	n/a	262	262, 264
NS350	TCTGGAGCATCAAACTGCTG	GGTGTTGCTTGTACGCAGTG	n/a	216	216
NS356	CAATAGTATTACATGTCCTGCATACG	CGCATTTTGCTTGCAGATTA	n/a	240	240, 250
NS376	TCAAGACCCTTGCTTTGGTT	GGACTATCAAGGCGCAAAAG	n/a	204, 208	208
NS391	TCTCAACCTCAGAATGTTCCAA	ATTTCCTGCACCCGGATAA	n/a	132	132
NS576	ATGAGTGAGAAATCTGCCGC	GAGAGGAAGGAAGTTAGAAATCCA	n/a	145	145
NS602	AGTGGATGTATTTGTGTTTTG	GCCTTTGTCCAGTCCATAGG	n/a	112, 120	120
NS622	TAAGTCGCGCAAATCCTTCT	AGCCCACAACAACTGTGTGA	n/a	146, 150	138, 140
NS656	AAGAACCCAACGCATTTGTC	TGCCTCTAAGAAGATTGGAAGC	n/a	180, 190	188, 192
NS658	CATGATGGCCCGAAGATAGT	TCGTTGGAGCCATTACATTTC	n/a	166, 196	160, 166
NS664	GGGTGCCAAACTCTCATTGT	GGTGAGAGCCTAACCTGTGC	n/a	276, 296	288, 296
NS701	TCTCTTGTTCATTTGTTGCGTT	TCCATTTGAGCCAAAATTTTATT	n/a	343	295
NS717	GCCAAATCGCCAAGGTAATA	GGTGAGTGATAAGGTTACGGC	n/a	196, 212	224
NS720	CCATTACTTACACATTGGACTTCCT	GGAAATTGGTTATGTCCTTTCC	n/a	134, 150	146, 152
NS774	AACCCGCAGAGAATCATGG	TCTCTTGCTTCTGTCACAACG	n/a	127	127
NS847	CAAACTTAAACTCCGTCCGC	TTGGCCTGTAAGGTTCCATC	n/a	280, 286	272, 280

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
NS890	TAAATTGGGGGTTCTTGCTC	TGCTTACTCTTTGATTCCACG	n/a	321	321
NS905	CAAACTTAAACTCCGTCCGC	TTGGCCTGTAAGGTTCCATC	n/a	269, 277	269, 277
NS909	CAAACTTAAACTCCGTCCGC	TTGGCCTGTAAGGTTCCATC	n/a	334	334
NS911	TGTTGTTCAGACGATGTCCAA	TTGAAGCAGTTATGAACCGT	n/a	113, 123	113, 123
NS928	GATACCCACAAGCCCAAAGA	GACCCACCCATCCACTAGAA	n/a	272, 278	272
NS945	GCAAGGCTCCATTAAAAGTCC	TGTTTGAAATAGTGTTGCTTCTTGA	n/a	381, 391	391
NS963	TTTTTGTCTGCTGCATATGTTT	GAAGAAACCACCCAAGTGGA	n/a	80	80, 120
NS964	AAGGGACACGACTTGGTCAC	TGGTTAAAATTTCTTTTGTGAACTG	n/a	107, 113	113
NS977	TTCCTCGCATGGCAGAAG	GACCCTTTGCCGTTACTCAT	n/a	302	302
NS978	TGTTGGCCATATTTCCCATT	TTGAACACACTTGGCCAGAA	n/a	239	239
NS980	TTTTGCCTTCCTCTTAGCCA	TGATCCCAAGGATCTTCCAG	n/a	232	232
NS983	GCTTCAAACATCAAACCCTAAC	TCTGCAGATGCAACAAATCC	n/a	NA	NA
NS995	CATGAGTTTAAAAATTATCACATCCG	GGGCTGTTCGTGATCCATA	n/a	91	91
NS1003	TGCAATTGTAAGGGC CAAAT	AATTTGGAGCTCAAGCGATG	(GA)5(AAGAGA)3 (GA)3	267	267
NS1008	AAATGGCCTAGAAATCCATGA	AACCCACTCAAGTGTCTCCG	(TC)8N2(CT)2N(CT)5	259	259, 269
NS1019	CTGGAGAAGACCACCCAGAA	AAGCAGTGGCTCAGTTTGGT	(TTG)2N8(TAA)5	215, 225	215, 223
NS1020	TCCAAGCCTCAAAACTTGCT	GAGAAAGACTCAGGTTCGGC	(CGG)2	255	255
NS1021	TCACAATGAAGCCCAGTGAA	TTGTATCTGAGCCTTGCGTG	(CT)2N3(CA)2N2(CTT)9	318, 330	318, 330
NS1035	TTCCTTGCTTCTCAAGGCAT	TGCTCCTGCTGTACTGGTTG	(CAA)2(AG)2N2(GCT)6N3(GCT)3	215, 221	215
NS1039	TATTCAAGAACCGGGCAGAC	CGGTCGCAAATAACGAAAAC	(ATTT)2N2(TA)2N6(ATT)4	249	249
NS1043	CTCACCATGGCTCATTCTCA	AAAGCCTGCAAAGAAAACCA	(GA)5(GGGA)2	285	285
NS1047	TATGCACATTGCCTCCAAAA	AACTCAACCCCTCCCATTTC	(GA)2N2(GA)5	308	308
NS1052	GAGTGTTGTCCGGCAGTTTC	CCATTCCATGGGTTTTGTTT	(CA)8(TA)5(TGTA)3(TA)2	360, 368	360, 366
NS1054	CTGGTGATGGTGGGAAAAAT	ACCCAACCATGAGAAGCAAC	(TA)7	176, 180	176
NS1056	GCTTAAACTCATTGGGCTGC	TTCCTTTGGCTCTCCCTGTA	(CT)2N(CT)5	351	353
NS1063	TGCATACAAAACTGCCCTCA	AAGTTTGGGAATGCAAACTG	(TA)10N4(ATCCAA)2	198	196
NS1077	TGCCTTTGAGTTAACTTCTTATTCG	CCTTGGCTACTTTTCTGTCACC	(CA)2N5(TGG)4	NA	NA

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
NS1099	GAGTTCGAGAATGTGCGTGA	ATTTCTTTCTGCGCAAGCAT	(CCA)3N7(CT)7N(TA) 2N4(AAT)2N3(TAA)2	216	216
NS1109	TGTGCATGGTACGAGGGTTA	CACACGCAATTCAATCCATC	(GA)3N2(GA)12	NA	NA
NS1113	ACCATTCCATTCTGGGCATA	GGTGGGGACTGCTATTCTGA	(CT)5	324	324
NS1116	AAACATGCATTCTACCCCCA	TGGCCAGCCACTATAAAAGG	(GA)2(GAC)2	322	322
NS1121	TTTTGCTCCCTCACATGAAA	TGCTGTTGAGGAGCTGTTGT	(TA)6	398	398
NS1141	CTGATGATTGCAAGGTGTGG	TTGGTGCATAAAGGGGAGAG	(GA)2N3(GAGGA)2N8 (GGA)2N3(GA)2N7(GA)2	348	342, 348
SSRY1	GCAGCTGCCGCTAATAGTTT	CCAAGAGATTGCACTAGCGA	GCC(6)	190	190
SSRY2	CGCCTACCACTGCCATAAAC	TGATGAAATTCAAAGCACCA	CT(18)CC CT(16)	106	106, 128
SSRY4	ATAGAGCAGAAGTGCAGGCG	CTAACGCACACGACTACGGA	GA(16)TA GA(3)	266, 270	284
SSRY5	TGATGAAATTCAAAGCACCA	CGCCTACCACTGCCATAAAC	GA(38)	107	107, 129
SSRY6	TTTGTTGCGTTTAGAAAGGTGA	AACAAATCATTACGATCCATTTGA	CA(7)N(51)CA(17) N(47)CA(15)	298	298
SSRY7	TGCCTAAGGAAAATTCATTCAT	TGCTAAGCTGGTCATGCACT	CT(26)	217	217
SSRY9	ACAATTCATCATGAGTCATCAACT	CCGTTATTGTTCCTGGTCCT	GT(15)	248, 262	274
SSRY11	TGTAACAAGGCAAATGGCAG	TTCTTGTGTCGTGCAACCAT	GA(19)	243, 261	249
SSRY12	AACTTCAAACCATTCTACTTC	CCACAATTTCTACAT	CA(19)	255, 265	265
SSRY13	GCAAGAATTCCACCAGGAAG	CAATGATGGTAAGATGGTGCAG	CT(29)	218, 220	220, 232
SSRY14	TTTGCATCGATTCCATCATC	TTGACCTTAGCACATTTAAGGATTC	CA(6)	300	300
SSRY15	TGAAAGCCTGCATTCAAACA	TGATGCAGGTAGCAAGGATG	GA(24)	214	214
SSRY16	GCACTGCAAAAATATCATCTTGA	CTGGAAAGATGGGACGTGTT	GAAGAGG GA(13) CA GA(3)TGAATA GA(4)	222, 232	222, 226
SSRY19	TGTAAGGCATTCCAAGAATTATCA	TCTCCTGTGAAAAGTGCATGA	CT(8)CA(18)	205, 213	199, 213
SSRY20	CATTGGACTTCCTACAAATATGAAT	TGATGGAAAGTGGTTATGTCCTT	GT(14)	128, 144	140, 146
SSRY21	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA	GA(26)	163, 185	163, 185
SSRY22	GGCGTGGACTAACCTGTTCT	CTTGCCACTAGAACAGCCAC	GT(13)	281	275, 281
SSRY25	TGGCTACATGATAGCAACATCAA	CGCATGGTTTGTCTCGTTTA	GA(27)	292	292

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
SSRY26	GCAGCTTTTTAGCATAACAATCAA	TGCTAATTGCAGGAAATAGGAT	GA(18)	124	120
SSRY27	CCATGATTGTTTAAGTGGCG	CCATTGGAGAACTTGGCAAC	CA(14)	268, 266	262, 266
SSRY28	TTGACATGAGTGATATTTTCTTGAG	GCTGCGTGCAAAACTAAAAT	CT(26)AT(3) AC AT(2)	159, 165	159, 175
SSRY29	TGGTAGCTTTTGAATATCTGATGG	TGCCAACCAAACCATTATAGAC	CT(18)	264, 296	264
SSRY31	CTTCATCACGTGTTAATACCAATC	ATTGTTGTGGTTGCAGGACA	GA(21)	186, 198	168, 198
SSRY32	TCCACAAAGTCGTCCATTACA	CAAATTTGCAACAATAGAGAACA	CA(11)	306	286, 302
SSRY33	AACTCTTTTGACTGAAGATGCTGA	CATGATTACCGCCAAGGCT	CT(18)	NA	NA
SSRY36	CAACTGTTTCAACCAACAGACA	ATTCTCGTGAACTGCTTGGC	CT(15)	129 , 133	129
SSRY37	ATGGCAAAAGATCGAGCAAC	GGCCAGTAATTCCTCAAGGC	CT(33)	NA	NA
SSRY38	GGCTGTTCGTGATCCTTATTAAC	GTAGTTGAGAAAACTTTGCATGAG	CA(17)	107, 121	107
SSRY39	TCAATGCATAGGATTTTGAAAGTA	AATGAAATGTCAGCTCATGCT	CT(24)AT CT(3)AT(3)	292, 302	292, 302
SSRY41	TATCACAATCGAAACCGACG	TTTTCCAACAATCTGATACTCGT	CT(4)TC CT(3)CGCC CT(20)	268, 274	264, 274
SSRY43	TCAGACGTTGATACCTCACTTCA	CCAGAGCATGGTCTTTCTGA	CT(25)	237	243, 253
SSRY44	GGTTCAAGCATTCACCTTGC	GACTATTTGTGATGAAGGCTTGC	GA(28)	105	105, 111
SSRY45	TGAAACTGTTTGCAAATTACGA	TCCAGTTCACATGTAGTTGGCT	CT(27)	195, 205	195, 223
SSRY46	TCAGGAACAATACTCCATCGAA	CGCTAAAGAAGCTGTCGAGC	CT(19)	259, 265	265
SSRY47A	GGAGCACCTTTTGCTGAGTT	TTGGAACAAAGCAGCATCAC	CA(17)	248	240, 266
SSRY48	AGCTGCCATGTCAATTGTTG	TCATAAAGCTCGTGATTTCCA	CA(11)	286	286, 292
SSRY50	CCGCTTAACTCCTTGCTGTC	CAAGTGGATGAGCTACGCAA	CA(6)N(6)GA(31)	254, 268	254
SSRY51	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT	CT(11)CG CT(11) CA(18)	280, 298	260, 280
SSRY52	GCCAGCAAGGTTTGCTACAT	AACTGTCAAACCATTCTACTTGC	GT(19)	257, 267	267
SSRY53	CCATGCAGTAGTGCCATCTTT	ATTTTCACCAACCGCAACTC	CT(8)CC CT(8)	139, 141	141
SSRY54	GCGACTTTCTGGATGGATTC	TGCAAATGACAAATAACCATCTC	GT(18)GA(18)	127, 143	127, 137
SSRY55	GCAATTTGCAAAGACATACCA	TGTGGAGCTTGATTTTGCAG	GA(16)	129, 145	135, 145
SSRY56	AACTCTTAATGGCTAAAATTATTGATG	TTTTAGTTTAGTTTAGTTAGTTGCGCT	GA(21)G GA(5)	123	123
SSRY58	GAAGGACAAGCAAAGAAGCAA	TGGAATCCAATATTGATGACTAAGA	GA(6)AA GA(31)	182, 206	182, 218
SSRY59	GCAATGCAGTGAACCATCTTT	CGTTTGTCCTTTCTGATGTTC	CA(20)	148	148

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
SSRY60	CGGCCACCAACTCAAATAAC	TTGCAATGATATCAACGGCT	CT(20)	113, 127	117, 131
SSRY63	TCAGAATCATCTACCTTGGCA	AAGACAATCATTTTGTGCTCCA	GA(16)	284, 296	284, 296
SSRY64	CGACAAGTCGTATATGTAGTATTCACG	GCAGAGGTGGCTAACGAGAC	CT(13)CG CT(6)	191, 197	199
SSRY68	GCTGCAGAATTTGAAAGATGG	CAGCTGGAGGACCAAAAATG	CT(12)CC CT(17)	246, 254	246
SSRY69	CGATCTCAGTCGATACCCAAG	CACTCCGTTGCAGGCATTA	CT(18)ATT AT(2) CTTT CTT CTTT(2)CCTTCT	234	216, 234
SSRY70	CGCTATTAGAATTGCCAGCAC	CGCTTGTTGTATCCATTGGC	GT(18)	243, 247	235, 243
SSRY71	TGATGCAGGTAGCAAGGATG	TGAAAGCCTGCATTCAAACA	CTT(5)N(10)CT(9)TT CT(15)CCT	219, 223	219
SSRY72	AAGCATCAGTGGCTATCAACA	TTTTGCTGTGCTATTTCTGAGC	CA(10)	127, 135	141, 145
SSRY74	TTGCTCGAATTCCACACAAT	GGTCAGGTGAGTAATAAAGAACAGTG	CT(21)	85	85, 113
SSRY75	TCTGGTAAACCTACTAGTGCTCCA	TTCATGCACGTCCTGATACA	GA(23)	256, 284	284
SSRY76	AAAGGAAGCAACCTTCAGCA	CATGATTTGGATTTTGGAATGA	GT(17)GA(21)	264	264
SSRY77	CAGGAGGTGGCAGATTTTGT	GCATGTTCCACCTGCATAAG	GT(20)CT GT(2)	276	276
SSRY79	CAAACCAATGGTCATGCTGT	CAGCATCAGAAAGACAAAAACAA	CT(19)T CT(7)	197, 207	197
SSRY81	GGCGATTTCATGTCATGCTT	TGATTTTCTGCGTGATGAGC	GA(22)	196, 204	204
SSRY82	TGTGACAATTTTCAGATAGCTTCA	CACCATCGGCATTAAACTTTG	GA(24)	192, 200	184, 210
SSRY83	TGGCTAGATGGTGATTATTGCTT	TGCTTACTCTTTGATTCCACG	GTCGTG GT(22)CT GT(2)	NA	NA
SSRY84	TTCCTTTCATTCATCCTGGC	AGAACTTCATGCACACAAGTTAAT	GA(24)	175	175, 201
SSRY85	AAGGTGGCAGCACTTTTCTG	AAGAATACTATACGGACTACATGCCA	CT(27)	266, 288	294
SSRY87	CTCATCTCATGAAGAACTTGTGC	AGAGCACGCATTGTGCATTT	GA(18)	91, 95	91, 99
SSRY90	AGGTTATGGCGGTGGCAG	GCGATTTTGCGAATTACCAC	GGA(5)TAT GGA(2)	195	195
SSRY91	GTCTGCATGGCTCGATGAT	TGCCTGCTTCATATGTTTTTG	GA(16)	295, 305	295, 303
SSRY92	CCAATGCTCAGTTTGACAACTC	TCGGCTTAAGGTATGAACGC	GT(14)	159, 161	159
SSRY93	TTTGTTGCTCACATGAAAACG	CAGATTTCTTGTGGTGCGTG	CT(25)	264	266, 282
SSRY94	AGGATGGACTTGGAGATGGA	GGTGGAAGTAAGGCTGTTAGTG	CA(23)TC CA(3)CT(3) TCCTTCCAT CCT(2)CT(2)	254	242, 274
SSRY96	CTTTACCTGCATGCCATTGA	CTCCATGTTATCCAAGGTTGC	GT(12)	147, 155	149, 151

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
SSRY98	ACCAATCCAAGCTGCAAATC	GTGATTGGTAGTGGTGGCCT	GT(11)	219, 221	209, 221
SSRY99	ATCAAGGCGCAAAAGTCAAT	CTTGCTTTGGTTCCAATTATTTA	GT(15)GA(13)AA GA(3)	193, 199	197
SSRY100	TTCGCAGAGTCCAATTGTTG	ATCCTTGCCTGACATTTTGC	CT(17)TT CT(7) CCCT	206, 240	200, 240
SSRY101	AAATACCACCACAA	ACACACAATCACCATTTC	CT(13)	211, 213	205
SSRY104	AGGCCATGGCAATTACTGAA	TTCTTGATATGCGCAACAGC	CA(4)GA CA(2)C CA(4)TAG A CA(16)GA(2)GATA(3)GA(21)	255, 267	255, 257
SSRY106	AAACTCTTCACAAAA	CACAAACCATCACCATTT	CT(24)	255, 259	269
SSRY107	CCATTTTCTCTTCTTCTTCA	TTTTAATCCTATAAAATCCTT	CT(23)	116	90, 116
SSRY110	TTATTAATCAAA	ATCCACCTTAAAACA	T(12)	248	248
SSRY112	CGCAAGGTAAATCGGAGCTA	ACAATCAAAGGAGTCGTGTAATC	CT(15)C CT(3)	NA	NA
SSRY113	TTTCTACCTCCACAATA	TCAACAATTACTAACAC	A(19)	171, 193	171, 193
SSRY115	CAACCGCTTTCGATGGTATT	TGCCATCACAATTTTGCCTA	GA(8)	NA	NA
SSRY117	TAAAGTTTGGCATGCCTGTG	GCAAATGTGTTTTCAATATAAGGC	GA(12)GTCA GA(3)N(32)GA (5)GT(2)ATGTGC GT(3)N(123)GA(5)	143	159, 143
SSRY119	AACATACATTAAATTTCA	CAAATTTTTTCAATATAAC	A(8)(3)A(3)N(4)A (3)N(32)A(5)T(2)ATTC T(3)	157	157, 171
SSRY122	AACCAATTTTTATTC	TCTTTTTATCCTT	A(12)T A(3)T A(3)T A(6)N(41)A(14)	275	275
SSRY123	AGCAGATCCAAATCACTGAAA	TTCAACAATAAAGCTCAGAAAGAG	CT(14)	135, 143	131
SSRY124	CTGCTGGACGGAGGATTCTA	TGGCATCAATTTTTGCTTCA	GA(9)A GA(2)AGA	139	139, 141
SSRY125	CAGGACATGACGCAATTCTG	GCATGTTAGAAGTTTTTGCAATTT	GT(2)T(8)GT(6)	248	248
SSRY126	AATGGATCATGTTCAATGTCTTC	TTGAAATACGGCTCAAGCTC	GT(2)T GT(5)GC(4)	250	250
SSRY129	CTTTTTGCCAGTCTTCCTGC	AATGGATCATGTTCAATGTCTTC	GC(3)G CA(6)A CA(3)	202	202
SSRY130	GGTCCCTGATAGTTGATAATGGAT	CTTTTTGCCAGTCTTCCTGC	GT(2)T GT(5)GC(3)	227	227
SSRY132	CTTTTTGCCAGTCTTCCTGC	TGTCCAATGTCTTCCTTTCCTT	CA(6)ACA(2)	194	194
SSRY134	TCCACAAAGATAAGCTAAGCG	GCAAGTTCAAAAGGAGCAGC	CAGA CA(4)GA(4)	211	211
SSRY135	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	CT(16)	250, 256	248, 250

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
SSRY136	CGACTGCATCAGAACAATGC	AGCATGTCATTGCACCAAAC	CAA CA(2)TA CA(4) CAAA(2)CAA	294	294
SSRY137	TAGTTAGCTCGGTTCGTCCG	TTTTGATAGATCAAGAGAGTTTTTGAA	ATT(3)ATTT ATT(2) T(9)N(9)AT AAT(4)ATAAAT	159	159
SSRY138	AGAATGTCTCTTTATTCTTGACAATTT	TTCAGGAAACATGCACAAACA	TA TTA(8)CT(5)	NA	NA
SSRY140	CAGTGAGCAGAAACTAAAAACATTG	GGCACTTTGGAAAGGAAGAG	ATT(4)CATTATTTA TTATTT ATTTT(3)ATTT	NA	NA
SSRY143	GCTCATGAACTGAGCCTTCA	AGCAGATCCAAATCACTGAAA	GA(12)	156, 164	152, 156
SSRY144	TAATGTCATCGTCGGCTTCG	GCTGATAGCACAGAACACAG	n/a	115, 125	115
SSRY145	GATTCCTCTAGCAGTTAAGC	CGATGATGCTCTTCGGAGGG	n/a	136, 146	136
SSRY148	GGCTTCATCATGGAAAAACC	CAATGCTTTACGGAAGAGCC	n/a	NA	NA
SSRY149	AGCAGAGCATTTACAGCAAGG	TGTGGAGTTAAAGGTGTGAATG	n/a	178	160
SSRY150	CAATGCAGGTGAAGTGAATACC	AGGGTGCTCTTCAGAGAAAGG	n/a	162	162
SSRY151	AGTGGAAATAAGCCATGTGATG	CCCATAATTGATGCCAGGTT	n/a	NA	NA
SSRY153	TTCCAGAAAGACTTCCGTTCA	CTCAACTACTGCACTGCACTC	n/a	101, 115	95, 115
SSRY156	TTCAAGGAAGCCTTCAGCTC	GAGCCACATCTACTCGACACC	n/a	NA	NA
SSRY158	CCTTACTTGTGTTTCTTACTGACAAG	CCAAGTCCTCACCTCCAAAG	CT(23)ATGTAT GT(7)	162	162
SSRY159	CTTATCCTGTCCCCTCCACC	GACAATTGCATAGGAAGCACA	CT(4)TT CT(16)TCT	154, 162	154, 162
SSRY160	CTGGCTCTTCCAGACACCTT	GGCAAGAGAAGCCATAAAGC	CT(30)	122, 154	126, 154
SSRY161	AAGGAACACCTCTCCTAGAATCA	CCAGCTGTATGTTGAGTGAGC	CT(11)TT CT(21)CA(19)	177	221
SSRY162	TTTAGTTAGTTGCGCTAGCTTCC	AACTCTTAATGGCTAAAATTATTGATG	CT(28)	NA	NA
SSRY163	TCATGATGCTATTCCAAGTGTG	AGGCCTCCAACAATTAGCCT	CTTCTCC CT(21)CA(19)	NA	NA
SSRY164	TCAAACAAGAATTAGCAGAACTGG	TGAGATTTCGTAATATTCATTTCACTT	GA(29)	175, 185	185
SSRY165	AAATGAGTTGCAAAGGCCAA	GGTAAACAAATGATGTGGTGTTC	GT(17)GA(18)	234, 240	230, 246
SSRY166	AATAACAACAAGAGTTGTGGAAAAA	TATCCATGACTGTGATGCGG	GA(16)GGGA(2)GA(2)	235	235, 245
SSRY167	AAAATTGGATGGGACCGTTT	AAGGAAAGGGAGAAATCAAAGA	CT(27)	157, 185	157
SSRY169	ACAGCTCTAAAAACTGCAGCC	AACGTAGGCCCTAACTAACCC	GA(19)A(3)GAA(2)	101	101

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
SSRY171	ACTGTGCCAAAATAGCCAAATAGT	TCATGAGTGTGGGATGTTTTTATG	TA(5)CATA GATA(8) GC GA(23)GTGA(2)	292	292
SSRY172	TCCAACTGGCTTAACTTGAGG	TTTAGTTTTTGAAACAATGATGAAA	CT(17)	194	202
SSRY173	TGTAAATATGCAAAGAAGCACGA	TACCTTTGGTGGAGTTTGCC	GT(3)GC GT(2) GA(20)GG GA(2))	NA	NA
SSRY174	AACAAAACCATTTTCATGTTGA	TTGCATACTCATCTCCATCTTCA	GA(16)	288	288
SSRY175	TGACTAGCAGACACGGTTTCA	GCTAACAGTCCAATAACGATAAGG	GA(38)	90, 94	90, 96
SSRY176	TGGCTAAATTATTGATGTTTTAGTGT	TTTTTCAAAATAGAGGGACCAA	GA(19)	NA	NA
SSRY178	GGCCCGTAAGGTTTACAGAG	CTGCAAAAACACGATCCCTT	GA(20)N(123)GA(6)	106, 110	96, 106
SSRY179	CAGGCTCAGGTGAAGTAAAGG	GCGAAAGTAAGTCTACAACTTTTCTAA	GA(28)	203	203
SSRY180	CCTTGGCAGAGATGAATTAGAG	GGGGCATTCTACATGATCAATAA	GA(16)G(4) GA(5)	NA	NA
SSRY181	GGTAGATCTGGATCGAGGAGG	CAATCGAAACCGACGATACA	GA(22)G(3)C GA(3) GGAA GA(4)	191, 195	187, 195
SSRY182	GGAATTCTTTGCTTATGATGCC	TTCCTTTACAATTCTGGACGC	CA(17)N(31)GAGG GA(8)	226, 230	226
SSRY183	TGCTGTGATTAAGGAACCAACTT	TTAACTTTTTCCAGTTCTACCCA	GAGC GA(8)	213	213
SSRY184	TCATCCCAAAAATACCTCTAACA	CTCCGACAAGCATGTGAATG	ATT(4)T ATT(3)T(7)	161	161
SSRY185	GAAGAAGACGGTTAAAGCAAGTT	ATGCCAGTTTGCTATCCAGG	GC(3)AC GC(2)A CA(2)N(3)CA(10) GA(8)	241, 247	241, 247
SSRY186	GCTTTGTGTAAACAACCTCGC	AATGACCATGCCAACACAAG	CA(13)	97	97
SSRY219	GCTCTCTTGGGGAGGTGTCT	CGAGAACAACAGGGTTCTACA	(CA)6(GA)6TA(GA)10	195, 201	195
SSRY 220	GTTGCATCAATGCCTTCAGT	TGCTCTCTGATCTTGCACTAGC	(TA)6(N)8(GTTGT)3GT	193, 195	193, 195
SSRY 226	AACGTTTCGCCGATTACAAG	AACTGGAGTAGAGAAACTGGAGGA	(GA)8(N)7(GA)5	221, 231	217, 231
SSRY 229	TGATCAGCGAAATCGTGGTA	AGTCCAGCCTCTTCCTTCGT	(CT)9TT(CT)5	182, 188	182, 198
SSRY 233	TGTGGCCATCACACTCATTT	CGAAACGATCGAAGTTCCAC	(CT)9	207, 209	209, 211
SSRY 235	CAGCAAAATGACATGAGTGTATCTC	CAGCTTTGCCATCCAATTTT	(CA)8(N)13CAGA(CA)6(GA)2	218, 222	218, 222
SSRY 236	CCGTATCCTATGGCAACACC	TGAAAAACGTTCCTTCCCTTT	(CT)2T(CT)13	185, 189	185
SSRY 238	GCTTCTGCGAATTCGTTTCT	GGCCTTAAGCCACCATTCTA	(CA)4CC(CA)7	217	217
SSRY 240	AGCTAGGAGCAACGCAGTTC	TCGGCTTTTAACATCCTTCG	(GA)22	165, 181	159, 165

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
SSRY 242	TAATGCCTGGAGGGTAATGG	TGGGTTCGAAAACAGCAAAC	(CT)5AT(CT)16	297, 301	309
SSRY 248	CCTCGTACAGAAAACTCAAGCA	TGTTTGCTGAGTGCAGTCCT	(GA)12	240	236, 240
SSRY 250	AATTGGAAGGGAAAGCCAAA	GATCGGATGTCTGAGGAGGA	(CT)18(ATCT)2CTAT(CT)2	175	175, 185
SSRY 252	CCCACCTCCGAAATCACTAA	GACGCGAGAACTGACAAGTTT	(GA)5AA(GA)10	206, 218	206, 216
SSRY 262	CTCGACATTCCCTTCACTTTG	TTCAGTTTCAGGAGACAGAAAGG	(GA)11	126	130
SSRY 269	TCAATCACAAGCCAGACACA	AATAGTTTCAGGCAAGGGTGA	(GA)55(N)22(GA)36 (N)26(GA)11(N)30(GA)20	158, 172	158, 172
SSRY 274	TCAGCGACATCTCTCTTCCA	TTCTTCCCCGTTCACGAAT	(CT)17A(CT)2	227	253
SSRY 282	TCCAACACTACGCTGTGTTTCT	CCATCCCCAACAAACAAGAT	(CT)10TT(CT)5	193	193
SSRY 284	GGAACATTTTAGCGGTCAGG	TCCTTCCAAAGCCAGACTTG	(CT)12	201	191
SSRY 295	AGACAAGCACCCACCAGAAT	GCTTAGCTTATCCTTGGACCTTG	CTC(CT)17	204, 206	196, 204
SSRY 300	ATAGAAGGGCCCGAGACTGT	CCATTTCCAGGCATTTCATC	(GAA)5GA	294	294
SSRY 305	AAAACACAATCAAATCCCTCA	AAGCTTGGCTAAGGTTCTGC	(CT)22(CCT)7	209	209
SSRY 306	CGGAGTGCTCCTCTCCATTA	GGACAGCCTCGTCATTTCAC	(CT)3C(CT)17CG(CT)2	286, 298	286, 316
SSRY 309	GCCACAGGCTAAGGAAACAA	TCCTCCTCCCTCTTCAGATTC	(CT)11CCT	224, 230	220, 234
SSRY 312	ATGGAAGGCTTGAAGTGGTG	TGATTGCAGCAGAAAGCAAG	(CT)13	204	204
SSRY 314	GCTCTCTCAAGCGCAGATTT	CAAACATTGGCAAAGCTTCA	(CAT)7	189, 193	189, 193
SSRY 316	GAGCTTTACGGTCTCTGTCTCTG	ACATCGTGAGGTCCAAGAGG	(GT)12	195	195
SSRY 331	TGCTTTGCTTCTCAAGTCCA	TCAGATCCCTCGGTTCTCAG	(CT)11	290, 292	280
SSRY210	TTGCCGGCTTTTCTGATTAC	GACCAGTTGCTGTTCGTCAA	(CA)4(TA)4	218, 220	218, 220
SSRY227	CCCATCAATGGAAACCTCAC	CCAAGGTTGTTGGGTAAGGA	(GCA)4(N)3GCA	200	200
SSRY228	TTTCTTATCCTCCGCTATCCA	TGGCAGAGATTTTGAGACGA	(CT)21	NA	NA
SSRY231	GGGCGCCTATTACTGTGAAA	CCACAGAAGAACACCAAACTG	(GA)7(G)2(GA)4	287	291
SSRY246	TGTAGGGCTGAGCAAGTTCC	TCCCCTCAGATGCTCAAGAC	(TA)6(TTA)2(TA)3	198	198
SSRY253	GCTGATGGAGGAGTAGCAGTG	TCCAGAGGGAGAGATCTGACA	(CT)11	NA	NA
SSRY254	TGTCAGATCTCTCCCTCTGGA	GACGCATCTCCTGCACAATA	(GT)6	225	225
SSRY255	ACCAGCACTGTTGTGTCCAG	GAAGAGATGCGACGATGGTT	(GAA)6GA	183	183

Appendix 5.2. continued

Marker	Right Primer	Left Primer	Repeat motif	COL 1734	BRA 1149
SSRY256	GGTGGTGGAGGTCCTGATTA	AGAACAAAGGGCTCCATTCA	(GAT)7	206	202
SSRY265B	CTGCTCCACGCTGTTTATCA	GCTGCTGGTCAAAAGAGTCC	(TA)8(CA)9	207	209, 211
SSRY268	GCAATATCTTCTTGGAGTTCAATTCT	CCAATATAAGCGGCGTCATT	(CT)18	NA	NA
SSRY277	GGTTTGGGTCCGGATTCTAT	CTGAGTGGTGATTCCGCTAA	(GAA)4GA	188	188
SSRY279	ACGCGTGGGTTTTATGCTT	GGTCCTGCCTTGCACTGTAT	(CT)5TT(CT)2	NA	NA
SSRY280	TGTGCATGGAGAGATTGACAG	AAGTCGTTTATTGCCGATGC	(CT)3CC(CT)2CC(CT)2CC(CT)7	NA	NA
SSRY283	AAAAGCGTGGGCTTTTGA	TGGAACTCAGTGAAGCGATG	(GA)11	NA	NA
SSRY286	ATCCACAATCCTCCCTACCC	TGGGCCCAGAGATAGAGTGT	(CT)5(N)3(CT)5	203	203
SSRY288	TGGTTTAAGGTTTGGGCGTA	TCAGGCATCCCTAATATTTCTTTC	(TA)3TTAAATA	178	178
SSRY289	AACACCCAACAGCAACATCA	ATCTTCTTGGTGGGTCATGG	(CCA)3ACA(CCA)6	188	188
SSRY297	CCCAGGCACAGGTACTCTCA	TTGTGCTCCAATGAAAATGG	(GA)19	190, 198	190, 204
SSRY299	TCTTTTTCACAGAACCAACTGAA	ATGCGAAACAGAGAGGAGGA	(CT)10	208, 210	208
SSRY317	CCTCTGCTACTGGCTATTTTCA	CGAAGAAGCCTCATTCCTGA	(CT)12	223, 227	223, 225
SSRY325	AGCCAAAAACCATACCCACA	GCTATTGCTGTGTGGTCCAG	(GT)2AT(GT)7	225, 231	231
SSRY330	CCACCATCATCATCGTCATC	TTCTTCTTCTTCCCCATTGC	(CT)2TCTC(CT)8	NA	NA
SSRY332	CAGGCTCGAGGTCTTCTTTG	CCACCCCATCTTCAACATTT	(GA)11	225	225
SSRY333	TACTTTGGGCCTTCCTTTCA	GGTTGGAGGAGCCATAGGTT	(CT)11	NA	NA
SSRY336	CAGTCTCTAACGATCCCCTCA	CATTTTCAGCAGCCTTTTCC	(CT)17	NA	NA
SSRY337	TCGCTCACAAAAACAATCCA	AGATCTTTGCACGTTCACCA	(CT)11	208	212
SSRY342	TCTGCTTTTGGCTGGAATTT	GGTTCGAAGCATTCTATGGTTA	(CT)18TTCT	187, 193	193
SSRY343	CACTCTTGTGCAGAACTTTGCT	CTCAATCCGCTCCATCTCTC	(CT)16	NA	NA

6. Summary

Drought is one of the most important factors limiting crop production in sub-Saharan Africa. This has detrimental effects to the people living in this region, and whose population is increasing more rapidly than their domestic food production. Noticeably, pressure on agricultural land has continued to intensify. Cassava is one of the staple crops with remarkable tolerance to drought. It is adapted to diverse and poor soil conditions, in addition to its flexibility in planting and harvesting times. Understanding its physiological and molecular basis of drought tolerance may help to target the key traits that limit crop yield under drought conditions. To improve our understanding on drought tolerance mechanisms in cassava, the project "Identifying the physiological and genetic traits that make cassava one of the most drought-tolerant crops" was initiated in 2005 by the Brazilian Agricultural Research Corporation (Embrapa) in collaboration with the International Center for Tropical Agriculture (CIAT); the International Institute of Tropical Agriculture (IITA); Cornell University and University of Goettingen. The ultimate goal of the project was to identify morphological, physiological and molecular traits related to drought tolerance mechanisms in cassava for further progress, and for their application in cassava and other crop breeding programs.

The present study was conducted within the framework of this project with 31 African cassava germplasm accessions from IITA and a mapping population developed at CIAT. The objectives of this study were,

1) To develop a protocol for hardening and rapid micro-propagation of cassava plantlets under local, low-cost conditions;
2) To identify agro-morphological attributes that are related to drought tolerance in cassava;
3) To identify drought-tolerant and drought-susceptible cassava germplasm from a selection of African accessions;
4) To identify secondary traits that could be used for phenotyping breeding materials for drought tolerance;
5) To screen the CIAT mapping population with simple sequence repeats (SSR) and expressed simple sequence repeat (ESSR) markers for linkage analysis.

Thirty one putative drought-tolerant and drought-susceptible African cassava germplasm accessions from IITA were micro-propagated using direct and in-direct techniques, at Kenya Agricultural Research Institute (KARI), Nairobi, Kenya. In direct micro-propagation, plantlets were hardened using vermiculite and multiplied through nodal cuttings. In in-direct micro-propagation, plantlets were first multiplied through sub-culturing and later hardened. The direct micro-propagation method had a higher multiplication rate. The number of plantlets obtained in 7 months using the direct method were 1173 as compared to 722 attained using the in-direct micro-propagation. Rapid micro-propagation through nodal cuttings was cheaper in terms of consumables and an effective alternative to enhance rates of multiplication, over the in-direct method and the more conventional technique like the use of stem cuttings.

Agronomic and morphological evaluation of contrasting African cassava germplasm accessions was carried out in water-stressed and well-watered environments at 5 time points. The trial was conducted at the experimental field of KARI, Kiboko Research Station in Makindu, Eastern Kenya, a site characterized by Acri-orthic Ferralsol soil. Analysis of variance was performed using the agronomic and morphological data, and broad sense heritability was estimated.

In general, significant differences were observed among the accessions, suggesting a strong genetic basis for the phenotypic variation observed. Variation was also notable in water-stressed and well-watered environments for a majority of traits evaluated. This was due to the artificial water applied since, during the trial period, there was hardly any rainfall.

At harvest, leaf length and width of certain accessions at the water-stressed site approached that of the well-watered treatment. On average, the estimated mean percentage leaf retention was high in the well-watered treatment. However, leaf retention in some of the accessions assessed was almost the same in both treatments. These accessions tended to produce higher yields. Thus, it may be desirable to select for higher leaf retention when developing varieties adapted to dry areas. The range of yields under stress was from 3.3 to 36.7 kg/m^2, whereas, under the well-watered treatment, it was smaller: 28.3 to 53.3 kg/m^2. Differences among accessions in yield and overall above-ground fresh biomass showed that these are important primary traits to phenotype germplasm under favorable and water-stress conditions. In

addition, 4 accessions G26, G11, G8 and G31 were more tolerant than the rest of the genotypes evaluated, calling for further research and their involvement in agricultural experimentation under drought-prone conditions.

Considering relationships between traits, genotype ability for both accumulation of above-ground fresh biomass and to partition carbon into roots (harvest index) were among the traits most correlated with root yield. However, accessions, environment and the interaction of both influenced the traits strongly. Thus, it is important that agro-morphological field trials be conducted in several locations for several seasons for effective evaluation of their influences on traits that might be relevant for phenotypically assessing drought tolerance.

Unlike for agro-morphological traits, where a drought-tolerant accession could be identified from the yield, which is of primary concern, selection of an outstanding accession in a water-limited environment using metabolic traits was not achieved in this study. Maybe the well-watered plants experienced an incipient stress due to low humidity, symptomless diseases or nutrient deficiency, which was not ascertained in this study. Despite this, significant differences were observed between the water-stressed and well-watered treatments for the traits evaluated, except protein and amylose content. Performance in individuals was variable, although insignificant differences were observed between the different stress phases.

Changes in sugar concentration have a role in the drought-tolerance of the accessions evaluated, although, their relative contribution to drought stress could not be determined from the available data. Further work is imperative to identify and quantify sugar concentrations in relation to osmotic adjustment in these accessions. In addition, further research to determine the time course of ABA accumulation, as a cassava plant goes from its young stage to aging, is required so as to know the stage at which ABA data are most informative.

Two genetic linkage maps were constructed using a South American mapping population of 228 individuals derived from a cross between a drought-tolerant and a drought-susceptible parent. A set of 377 simple sequence repeats (SSR) and expressed simple sequence repeats (ESSR's) were utilized for the initial polymorphism screening. Differences in map size, interval, number and mean distance between

markers were apparent between the two maps. The female map had 14 linkage groups as compared to 13 in the male map. Twenty seven allelic bridges were noticeable between the two maps. In addition, 25 markers showed collineality with other available cassava maps. Forty six markers, whose map distances had not been determined previously, were mapped in this study. These maps form an important platform upon which to characterize the genetic basis of drought tolerance in cassava. Continued addition of more markers in these maps will refine the utility of the resource for future cassava breeding efforts.

In conclusion, four African cassava accessions apparently have the ability to withstand severe drought. However, a majority of the accessions evaluated gave poor response in adaptability to water-limited conditions. This suggests that further agro-ecologically based research is required on these materials, since they represent diverse improved accessions from IITA breeding activities. This, coupled with marker-assisted genetic analysis, would be an appropriate approach for the identification of drought-tolerant accessions.

References

Acevedo, E. Fereres, E. Hsiao, T. and Henderson, D. (1979). Diurnal growth trends, water potential, and osmotic adjustment of maize and sorghum leaves in the field. Plant Physiology 64: 476-480.

Adeniyi, O. Kovo, A. Abdulkareem, A. and Chukwudozie, C. (2007). Ethanol fuel production from cassava as a substitute for gasoline. Journal of Dispersion Science and Technology 28: 501-504.

Aerni, P. (2006). Mobilizing science and technology for development: The case of the Cassava Biotechnology Network (CBN). The Journal of Agrobiotechnology Managemnet and Economis 9: 1-14.

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

Akkaya, M. Shoemaker, R. Specht, J. Bhagwat, A. and Cregan, P. (1995). Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Science 35: 1439-1445.

Almazan, A. (1990). Effect of cassava flour variety and concentration on bread loaf quality. Cereal Chemistry 67: 97-99.

Alves, A. (2002). Cassava botany and physiology. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: Biology, Production and Utilization. CABI Publishing, Wallingford, NY, pp 67-89.

Alves, A. and Setter, T. (2000). Response of Cassava to water deficit. Leaf area growth and Abscisic acid. Crop Science. 40: 131-137.

Alves, A. and Setter, T. (2004). Abscisic acid accumulation and osmotic adjustment in cassava under water deficit. Environmental and Experimental Botany 51: 259-271.

Awoleye, F. Duren, M. Dolezel, J. and Novak, F. (1994). Nuclear DNA content and *in vitro* induced somatic polyploidization cassava (*Manihot esculenta* Crantz) breeding. Euphytica 76: 195-202.

Awoyinka, A. Abegunde, V. and Adewusi, S. (1995). Nutrient content of young cassava leaves and assessment of their acceptance as a green vegetable in Nigeria. Plant Foods for Human Nutrition 47: 21-28.

Babu, R. Pathan, M. Blum, A. and Nguyen, H. (1999). Comparison of measurement methods of osmotic adjustment in rice cultivars. Crop Science 39: 150-158.

Bakayoko, S. Tschannen, A. Nindjin, C. Dao, D. Girardin, O. and Assa, A. (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: 21-27.

Balagopalan, C. (2002). Cassava utilization in food, feed and industry. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: Biology, Production and Utilization. CABI Publishing, Wallingford, NY, pp 301–318.

Barany, M. Hammett, A. Sene, A. and Amichev, B. (2001). Nontimber forest benefits and HIV/AIDS in sub-Saharan Africa. Journal of Forestry 99: 36-41.

Benesi, I. Labuschagne, M. Dixon, A. and Mahungu, N. (2004). Stability of native starch quality parameters, starch extraction and root dry matter of cassava genotypes in different environments. Journal of the Science of Food and Agriculture 84: 1381-1388.

Bennett, M. and Smith, J. (1991). Nuclear DNA amounts in angiosperms. Philosophical Transactions: Biological Sciences 334: 309-345.

Bernier, J. Kumar, A. Ramaiah, V. Spaner, D. and Atlin, G. (2007). A Large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Science 47:507–518

Blum, A. (1998). Improving wheat grain filling under stress by stem reserve mobilisation. Euphytica 100: 77-83.

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

Bohnert, H. and Jensen, R. (1996). Strategies for engineering water-stress tolerance in plants. Trends in Biotechnology 14: 89-97.

Borrell, A. Hammer, G. and Henzell, R. (2000). Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Science 40: 1037-1048.

Botstein, D. White, R. Skolnick, M. and Davis, R. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32: 314-331.

Bottema, J. and Henry, G. (1990). History, current status and potential of cassava use in Asia. In: Howeler, R.H. (ed.). Proceedings of the regional workshop on cassava breeding, agronomy and utilization research in Asia. International Centre for Tropical Agriculture (CIAT). Cali, Colombia, pp 3-26

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72: 248-254.

Bradshaw, H. and Stettler, R. (1994). Molecular genetics of growth and development in Populus. II. Segregation distortion due to genetic load. Theoretical and Applied Genetics 89: 551-558.

Bryson, J. (1981). Women and agriculture in sub-Saharan Africa: Implications for development (an exploratory study). Journal of Development Studies 17: 29-46.

Burrell, M. (2003). Starch: the need for improved quality or quantity-an overview. Journal of Experimental Botany 54: 451-456.

Carter, S. Fresco, L. Jones, P. and Fairbairn, J. (1992). An Atlas of Cassava in Africa: Historical, Agroecological and Demographic Aspects of Crop Distribution. International Centre for Tropical Agriculture (CIAT). Cali, Colombia, pp 90

Carter, S. Fresco, L. Jones, P. and Fairbairn, J. (1993). Introduction and diffusion of cassava in Africa. International Institute of Tropical Agriculture. (IITA), Ibadan, Nigeria, pp 34

Casasoli, M. Mattioni, C. Cherubini, M. and Villani, F. (2001). A genetic linkage map of European chestnut (*Castanea sativa* Mill.) based on RAPD, ISSR and isozyme markers. Theoretical and Applied Genetics 102: 1190-1199.

Castiglioni, P. Ajmone-Marsan, P. Van Wijk, R. and Motto, M. (1999). AFLP markers in a molecular linkage map of maize: codominant scoring and linkage group ditsribution. Theoretical and Applied Genetics 99: 425-431.

Cato, S. Gardner, R. Kent, J. and Richardson, T. (2001). A rapid PCR-based method for genetically mapping ESTs. Theoretical and Applied Genetics 102: 296-306.

Cavalcanti, J. and Wilkinson, M. (2007). The first genetic maps of cashew (*Anacardium occidentale* L.). Euphytica 157: 131-143.

Ceballos, H. Iglesias, C. Pérez, J. and Dixon, A. (2004). Cassava breeding: opportunities and challenges. Plant Molecular Biology 56: 503-516.

Chandra, S. (1994). Contributions of tropical root crops to socio-economic improvement of the developing world. In: Ofori, F. and Hahn, K. (eds.) Symposium on tropical root crops in a developing economy. ISHI Acta Horticulturae 380: 31-36.

Chen, S. Wang, S. Altman, A. and Huttermann, A. (1997). Genotypic variation in drought tolerance of poplar in relation to abscisic acid. Tree Physiology 17: 797-803.

Cheng, R. Kleinhofs, A. and Ukai, Y. (1998). Method for mapping a partial lethal-factor locus on a molecular-marker linkage map of a backcross and doubled-haploid population. Theoretical and Applied Genetics 97: 293-298.

Chiwona-Karltun, L. (2001). A reason to be bitter. Cassava classification from the farmer's perspective. PhD thesis, Karolinska Institute, Uppsala.

CIAT (1979). Annual Report. International Centre for Tropical Agriculture (CIAT). Cali, Colombia.

CIAT (2003). Annual Report. International Centre for Tropical Agriculture (CIAT). Cali, Colombia.

CIAT and EMBRAPA (1996). Development of cassava germplasm for the drier tropics and subtropical Agroecosystems of Africa, Asia and Latin America. International Centre for Tropical Agriculture (CIAT). Cali, Colombia, pp 30.

CIAT and EMBRAPA (1999). Programme for the enhancement of income-generation potential through the development and transfer of improved cassava genetic material and adoption of post-harvest technologies. International Centre for Tropical Agriculture (CIAT). Cali, Colombia, pp 40.

Close, T. Fenton, R. and Moonan, F. (1993). A view of plant dehydrins using antibodies specific to the carboxy terminal peptide. Plant molecular biology 23: 279-286.

Cock, J. (1985). Cassava: new potencial for a neglected crop. Westview Press Boulder, pp 240.

Cock, J. (1987). Stability of performance of cassava genotypes. In: Hershey, C. (ed.) Cassava breeding: a multidisciplinary review: proceedings of a workshop held in the Philippines. International Centre for Tropical Agriculture (CIAT), pp 312.

Cock, J. Franklin, D. Sandoval, G. and Juri, P. (1979). The ideal cassava plant for maximum yield. Crop Science 19: 271-279.

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

Cortés, M. García-Escudero, V. Hughes, M. and Izquierdo, M. (2002). Cyanide bystander effect of the linamarase/linamarin killer-suicide gene therapy system. Journal of Gene Medicine 4: 407-414.

Crouch, J. and Serraj, R. (2002). DNA marker technology as a tool for genetic enhancement of drought. tolerance at ICRISAT. In: Saxena, N and O'Toole, J (eds.) Field screening for drought tolerance in crop plants with emphasis on Rice. Proceedings of an international workshop on field screening for drought tolerance in Rice, International Crops Research Institute for the Semi Arid Tropics (ICRISAT), India, pp 155-170.

Dapaah, S.K. (1994). Contributions of root and tuber crops to socioeconomic changes in the developing world: the case of Africa, with special emphasis on Ghana. In: Ofori, F. and Hahn, K. (eds.) Symposium on tropical root crops in a developing economy. ISHI Acta Horticulturae 380: 380:18-25.

David, M. Coelho, D. Barrote, I. and Correia, M. (1998). Leaf age effects on photosynthetic activity and sugar accumulation in droughted and rewatered *Lupinus albus* plants. Australian Journal of Plant Physiology 25: 299-306.

Davies, P. (2004). Plant hormones: biosynthesis, signal transduction, action! Kluwer Academic Publishers, The Nertherlands, pp 750.

De Tafur, S. El-Sharkawy, M. and Calle, F. (1997). Photosynthesis and yield performance of cassava in seasonally dry and semiarid environments. Photosynthetica 33: 249-257.

Dellaporta, S. Wood, J. and Hicks, J. (1983). A plant DNA minipreparation: version II. Plant Molecular Biology Reporter 1: 19-21.

Duque, L. O. and. Setter, T. L. (2005). Interdrought II, The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress. Rome, pp. L 5.09.

Dure III, L. (1993). Structural motifs in Lea proteins. Plant responses to cellular dehydration during environmental stress. Current Topics in Plant Physiology 10: 91–103.

Ejeta, P. B. Goldsbrough, M. R. Tuinstra, E. M. Grote, A. Menkir, Y. Ibrahim, N. Cisse, Y. Weerasuriya, A. and Melake-Berhan, C.A. Shaner (2000). Molecular marker applications in sorghum In: Haussmann, B. Geiger, H. Hess, D. Hash, C. and Bramel-Cox, P. (eds.) Application of molecular markers in plant breeding. Training manual for a seminar held at IITA. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Andhra Pradesh, pp 81-89.

Ekanayake, I. (1998). Screening for abiotic stress resistance in root and tuber crops. IITA Research Guide 68, International Institute of Tropical Agriculture, Ibadan, Nigeria, pp 46.

Eke-Okoro, O. N. Njoku, D. N. and Ikeorgu, J. G. (2008). Evaluation of the productivity of low cyanide cassava varieties in cassava/soybean intercrop. Scientific Research and Essays 3: 495-499.

El-Sharkawy, M. (1993). Drought-tolerant cassava for Africa, Asia, and Latin America. BioScience 43: 441-451.

El-Sharkawy, M. (2003). Cassava biology and physiology. Plant molecular biology 53: 621-641.

El-Sharkawy, 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 of Plant Physiology 19: 257-286.

El-Sharkawy, M. and Cadavid, L. (2002). Response of cassava to prolonged water stress imposed at different stages of growth. Experimental Agriculture 38: 333-350.

El-Sharkawy, M. Cock, J. and Hernandez, A. (1985). Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. Photosynthesis Research 7: 137-149.

El-Sharkawy, M. and Cock, J. (1987a). Response of cassava to water stress. Plant and Soil 100: 345-360.

El-Sharkawy, M. and Cock, J. (1987b). C_3-C_4 intermediate photosynthetic characteristics of cassava (*Manihot esculenta* Crantz). Photosynthesis Research 12: 219-235.

Epron, D. and Dreyer, E. (1996). Starch and soluble carbohydrates in leaves of water-stressed oak saplings. Annals of Forest Science 53: 263-268.

Escobar, R. Hern Andez, C. Larrahondo, N. Ospina, G. Restrepo, J. Mu Noz, L. Tohme, J. and Roca, W. (2006). Tissue culture for farmers: Participatory adaptation of low-input cassava propagation in Colombia. Experimental Agriculture 42: 103-120.

FAO (1997). Production Yearbook. Food for Agriculture Organization (FAO), Rome,

FAO (2003). The state of food insecurity in the world . Counting the hungry: Latest estimates. Food and Agriculture Organization of the United Nations, Rome.

FAO (2004). Twenty seventh FAO regional conference for the near East. Forest and tree contribution to environment, water and food security, Doha, Qatar.

FAO (2008). Investing in cassava research and development could boost yields and industrial uses. Food and Agriculture Organization, [Cited on 25/07/08].

Farooq, S. and Azam, F. (2002). Molecular markers in plant breeding: Concepts and characterization. Pakistan Journal of Biological Sciences 5: 1135-1140.

Ferreira, A. Silva, M. Silva, L. and Cruz, C. (2006). Estimating the effects of population size and type on the accuracy of genetic maps. Genetics and Molecular Biology 29: 187-192.

Fischer, R. and Maurer, R. (1978). Drought resistance in spring wheat cultivars. Grain yield responses. Australian Journal of Agricultural Research 29: 897-912.

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

Fregene, M. Okogbenin, E. Mba, C. Angel, F. Suarez, M. Janneth, G. Chavarriaga, P. Roca, W. Bonierbale, M. and Tohme, J. (2001). Genome mapping in cassava improvement: Challenges, achievements and opportunities. Euphytica 120: 159-165.

Fregene, M. and Puonti-Kaerlas, J. (2002). Cassava biotechnology. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: Biology, production and utilization. CABI Publishing, Wallingford, NY, pp 179-207.

Fregene, M. Suarez, M. Mkumbira, J. Kulembeka, H. Ndedya, E. Kulaya, A. Mitchel, S. Gullberg, U. Rosling, H. and Dixon, A. (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.

Fukuda, W. M. G. and Saad, N. (2001). Participatory research in cassava breeding with farmers in Northeast Brazil. Embrapa/CNPMF, Brazil, pp 42.

Gan, S. Li, F. and Li, M. (2006). Genome mapping in tropical tree species. Molecular Biology of Tropical Plants 2: 75-103.

Gebeyehu, S. (2006). Physiological response to drought stress of common bean (*Phaseolus Vulgaris* L.) Genotypes differing in drought resistance. PhD thesis, Justus Liebig University, Germany, pp 116.

Geigenberger, P. Reimholz, R. Deiting, U. Sonnewald, U. and Stitt, M. (1999). Decreased expression of sucrose phosphate synthase strongly inhibits the water stress-induced synthesis of sucrose in growing potato tubers. The Plant Journal 19: 119-129.

Ghaouti, L. (2007). Comparison of pure line cultivars with synthetic cultivars in local breeding of faba bean (*Vicia faba* L.) for organic farming. PhD thesis, Goettingen University, Germany, pp 147

Gleick, P. (2003). Global freshwater resources: soft-path solutions for the 21st century. Science 302: 1524-1528.

González, A. Schöpke, C. Taylor, N. Beachy, R. and Fauquet, C. (1998). Regeneration of transgenic cassava plants (*Manihot esculenta* Crantz) through *Agrobacterium*-mediated transformation of embryogenic suspension cultures. Plant Cell Reports 17: 827-831.

Graner, A. Jahoor, A. Schondelmaier, J. Siedler, H. Pillen, K. Fischbeck, G. Wenzel, G. and Herrmann, R. G. (1991). Construction of an RFLP map of barley. Theoretical and Applied Genetics 83: 250-256.

Grattapaglia, D. and Sederoff, R. (1994). Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137: 1121-1137.

Groover, A. Williams, C. Devey, E. Lee, J. and Neale, D. (1995). Sex-related oiffiej-ences in meiotic recombinaftion frequency in *Pinus taeda*. Journal of Heredity 86: 157-158.

Gulick, P. Hershey, C. and Esquinas Alcazar, J. (1983). Genetic resources of cassava and wild relatives. International Board for Plant Genetic Resources (IBPGR). Rome, pp 60.

Gupta, P. Varshney, R. Sharma, P. and Ramesh, B. (1999). Molecular markers and their applications in wheat breeding. Plant Breeding 118: 369-390.

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

Hall, A. (1993). Is dehydration tolerance relevant to genotypic difference in leaf senescence and crop adaption to dry environments? In: Close, T. J. and Bray E.A. (eds.) Plant response to cellular dehydration during environmental stress. The American Society of Plant Physiologists, Rockville, Maryland, pp 1-10

Halsey, M. Olsen, K. Taylor, N. and Chavarriaga-Aguirre, P. (2008). Reproductive Biology of Cassava (*Manihot esculenta* Crantz) and Isolation of Experimental Field Trials. Crop Science 48: 49-58.

Han, Y. Gómez-Vásquez, R. Reilly, K. Li, H. Tohme, J. Cooper, R. and Beeching, J. (2001). Hydroxyproline-rich glycoproteins expressed during stress responses in cassava. Euphytica 120: 59-70.

Hanley, S. Barker, J. Van Ooijen, J. Aldam, C. Harris, S. Åhman, I. Larsson, S. and Karp, A. (2002). A genetic linkage map of willow (*Salix viminalis*) based on AFLP and microsatellite markers. Theoretical and Applied Genetics 105: 1087-1096.

Hayden, M. Nguyen, T. Waterman, A. McMichael, G. and Chalmers, K. (2008). Application of multiplex-ready PCR for fluorescence-based SSR genotyping in barley and wheat. Molecular Breeding 21: 271-281.

Hershey, C. (1987). Cassava breeding: a multidisciplinary review: proceedings of a workshop held in the Philippines. International Centre for Tropical Agriculture (CIAT), pp 312.

Hillocks, R. J. (2002). Cassava in Africa. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: Biology, production and utilization. CABI Publishing, Wallingford, NY, pp 41-54.

Hillocks, R. J. Thresh, J. M. and Bellotti, A. (2002). Cassava: Biology, production and utilization. CABI Publishing, Wallingford, NY, pp 352.

Hiron, R. and Wright, S. (1973). The role of endogenous abscisic acid in the response of plants to stress. Journal of Experimental Botany 24: 769-780.

Howeler, R. H. (1978). The mineral nutrition and fertilization of cassava. International Centre for Tropical Agriculture (CIAT) Cali, Colombia, 247-292.

Howeler, R. H. (2002). Cassava mineral nutrition and fertilization. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: biology, production and utilization. CABI Publishing, Wallingford, NY, pp 115-147.

Hsiao, T. (1973). Plant responses to water stress. Annual Review of Plant Physiology 24: 519-570.

Hsiao, T. Acevedo, E. Fereres, E. and Henderson, D. (1976). Water stress, growth, and osmotic adjustment. Philosophical Transactions of the Royal Society of London 273: 479-500.

Humby P. L. and Durnford D. G. (2006). Photoacclimation: Physiological and molecular responses to changes in light environments. In: Huang, B. (ed.) Plant environment interactions. CRS Press, Boca Raton, pp 69-101.

Iglesias, C. Hershey, C. Calle, F. and Bolaños, A. (2008). Propagating cassava (*Manihot esculenta*) by sexual seed. Experimental Agriculture 30: 283-290.

IITA (1990). Cassava in Tropical Africa. A Reference Manual. Ibadan, Nigeria, pp 108.

IITA (1997). Cassava in Africa: past, present and future. Report prepared for IITA by Dunstan Spencer and associates. International Institute of Tropical agriculture (IITA), Ibadan, Nigeria.

IAEA (2004). Low cost options for tissue culture technology in developing countries. IAEA-TECDOC-1384. International Atomic Energy Agency, Austria

Jaschke, W. Peuke, A. Pate, J. and Hartung, W. (1997). Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communis* L.) under phosphate deficiency and moderate salinity. Journal of Experimental Botany 48: 1737-1747.

Jenks, M. Hasegawa, P. and Jain, S. (2007). Advances in molecular breeding toward drought and salt tolerant crops. Springer, The Netherlands, pp 817.

Jennings, D. and Iglesias, C. (2002). Breeding for crop improvement. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: Biology, Production and Utilization. CABI Publishing, Wallingford, NY, pp 149-166.

Jones, J. and Zur, B. (1984). Simulation of possible adaptive mechanisms in crops subjected to water stress. Irrigation Science 5: 251-264.

Jones, M. M. Osmond, C. B. and Turner, N. C. (1980). Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. Australian Journal of Plant Physiology 7: 193-205.

Jorge, V. Fregene, M. Duque, M. Bonierbale, M. Tohme, J. and Verdier, V. (2000). Genetic mapping of resistance to bacterial blight disease in cassava (*Manihot esculenta* Crantz). Theoretical and Applied Genetics 101: 865-872.

Kassianof, E. (1992). Biomass partitioning characteristics, tissue culture and transformation for the genetic improvement of cassava (*Manihot esculenta* Crantz), PhD thesis, University of Zimbabwe, Zimbabwe, pp 312.

Kaushik, A. D. (2008). Disasters update, Issue No. 1072. From: http://nidm.gov.in/News%20in%20PDF/2008/May/30-05-08.pdf [Cited on 30/10/2009].

Kawano, K. Fukuda, W. and Cenpukdee, U. (1987). Genetic and environmental effects on dry matter content of cassava root. Crop Science 27: 69-74.

Kawano, K. (1990). Harvest index and evoluation of major food crop cultivars in the tropics. Euphytica 46: 195-202.

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

Khush, G. (1999). Green revolution: preparing for the 21st century. Genome 42: 646-655.

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, pp 127.

Kosambi, D. (1944). The estimation of map distances from recombination values. Ann Eugen 12: 172-175.

Kramer, P. and Boyer, J. (1995). Water relations of plants and soils. Academic Press, San Diego, pp 481.

Kumar, P. Gupta, V. Misra, A. Modi, D. and Pandey, B. (2009). Potential of molecular markers in plant biotechnology. Plant Omics Journal 2: 141-162.

Lacerda, L. Azevedo, J. Carvalho Filho, M. Demiate, I. Schnitzler, E. Vandenberghe, L. and Soccol, C. (2008). Thermal characterization of partially hydrolyzed cassava (*Manihot esculenta*) starch granules. Brazilian Archives of Biology and Technology 51: 1209-1215.

Lafitte, H. R, Courtois, B. Atlin, G. N. (2002). The International Rice Research Institute's Experience in Field Screening for Drought Tolerance and Implications for Breeding. In: Saxena, N. P and O'Toole, J. C (eds) Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice. The Rockefeller Foundation, New York, pp 25-40

LaRosa, P. Hasegawa, P. Rhodes, D. Clithero, J. Watad, A. and Bressan, R. (1987). Abscisic Acid Stimulated Osmotic Adjustment and Its Involvement in Adaptation of Tobacco Cells to NaCl. Plant Physiology 85: 174-181.

Lecoeur, J. Wery, J. Turc, O. and Tardieu, F. (1995). Expansion of pea leaves subjected to short water deficit: cell number and cell size are sensitive to stress at different periods of leaf development. Journal of Experimental Botany 46: 1093-1101.

Leihner, D. (1983). Management and evaluation of intercropping systems with cassava. International Institute of Tropical Agriculture (CIAT). Cali, Colombia, pp 70.

Lelley, T. Stachel, M. Grausgruber, H. and Vollmann, J. (2000). Analysis of relationships between *Aegilops tauschii* and the D genome of wheat utilizing microsatellites. Genome 43: 661-668.

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

Liu, F. Andersen, M. and Jensen, C. (2003). Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structures in soybean. Functional plant biology 30: 271-280.

Lopez, C. Piegu, B. Cooke, R. Delseny, M. Tohme, J. and Verdier, V. (2005). Using cDNA and genomic sequences as tools to develop SNP strategies in cassava (*Manihot esculenta* Crantz). Theoretical and Applied Genetics 110: 425-431.

Lott, J. and Turner, K. (1975). Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine. Clinical Chemistry 21: 1754-1760.

Ludlow, M. and Muchow, R. (1990). A critical evaluation of traits for improving crop yields in water-limited environments. Advances in Agronomy 43: 107-153.

Ludlow, M. (1992). Physiological mechanisms of drought resistance. In: Mabry, T. Nguyen, H. Dixon, R. and Bonnes, M. (eds.) Biotechnology of arid plants. The University of Texas, Dallas, pp 11-34.

Lyttle, T. (1991). Segregation distorters. Annual Review of Genetics 25: 511-581.

Mahungu, N. and Kanju, E. (1997). Cassava breeding manual. Reference document of Southern Africa Root Crops Research Network (SARRNET) Regional workshop on cassava. Kibaha, Tanzania, pp 53.

Maliepaard, C. Jansen, J. and Van Ooijen, J. (1997). Linkage analysis in a full-sib family of an outbreeding plant species: overview and consequences for applications. Genetics Research 70: 237-250.

Maliepaard, C. Alston, F. Van Arkel, G. Brown, L. Chevreau, E. Dunemann, F. Evans, K. Gardiner, S. Guilford, P. and Van Heusden, A. (1998). Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theoretical and Applied Genetics 97: 60-73.

Mansfield, D. Brown, A. Green, D. Carothers, A. Morris, S. Evans, H. and Wright, A. (1995). Automation of genetic linkage analysis using fluorescent microsatellite markers. Genomics 24: 225-233.

Manyong, V. Bamire, A. Sanusi, I and Awotide, D. (2004). African association of agricultural economists. Shaping the future of African agriculture for development: The role of social scientists. Proceedings of the inaugural symposium. Nairobi, pp 8.

Martin, C. and Smith, A. (1995). Starch biosynthesis. Plant Cell. 7: 971-985.

Mathur, M. Sampath, S. and Ghosh, S. (1969). Studies on tapioca: effect of 50 and 100 percent replacement of oats by tapioca in the concentrate mixture of dairy cows. Indian Journal Dairy Science 22: 193–199.

Mba, R.E.C. Stephenson, P. Edwards, K. Melzer, S. Nkumbira, J. Gullberg, U. Apel, K. Gale, M. Tohme, J. and Fregene, M. (2001). Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: Towards a SSR-based molecular genetic map of cassava. Theoretical and Applied Genetics 102: 21–31.

Mizrahi, Y. Blumenfeld, A. and Richmond, A. (1970). Abscisic acid and transpiration in leaves in relation to osmotic root stress. Plant Physiology 46: 169-171.

Mkumbira, J. Chiwona-Karltun, L. Lagercrantz, U. Mahungu, N. Saka, J. Mhone, A. Bokanga, M. Brimer, L. Gullberg, U. and Rosling, H. (2003). Classification of cassava into 'bitter'and 'cool' in Malawi: From farmers' perception to characterisation by molecular markers. Euphytica 132: 7-22.

Most, B. (1971). Abscisic acid in immature apical tissue of sugar cane and in leaves of plants subjected to drought. Planta 101: 67-75.

Moustafa, M. El-Kady, M. and El-Shibini, F. (2002). A strategy to combat desertification in the Nile basin International conference on drought mitigation and prevention of land desertification in the Nile basin. Bled, Slovenia.

Mullis, K. (1990). The unusual origin of the polymerase chain reaction. Scientific American 262: 56-65.

Murashige, T. and Skoog, F. F.(1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia plant 15: 473-497.

Myburg, A. Griffin, A. Sederoff, R. and Whetten, R. (2003). Comparative genetic linkage maps of *Eucalyptus grandis*, *Eucalyptus globulus* and their F_1 hybrid based on a double pseudo-backcross mapping approach. Theoretical and Applied Genetics 107: 1028-1042.

Nayyar, H. Bains, T. and Kumar, S. (2005). Low temperature induced floral abortion in chickpea: relationship to abscisic acid and cryoprotectants in reproductive organs. Environmental and Experimental Botany 53: 39-47.

Ng, N. and Ng, S. (2002). Genetic resources and conservation. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: Biology, Production and Utilization. CABI Publishing, Wallingford, NY, pp 167-178.

Nhassico, D. Muquingue, H. Cliff, J. Cumbana, A. and Bradbury, J. (2008). Rising African cassava production, diseases due to high cyanide intake and control measures. Journal of the Science of Food and Agriculture 88: 2043-2049.

Nweke, F. (2004). New challenges in the cassava transformation in Nigeria and Ghana. International Food Policy Research Institute (IFPRI), Washington, pp 103.

Nweke, F. Spencer, D. and Lynam, J. (2002). The cassava transformation: Africa's best-kept secret. Michigan State University Press, East Lansing, pp 273.

Nyerhovwo, J. (2004). Cassava and the future of starch. Electronic Journal of Biotechnology 7: 5-8.

Ojulong, H. Labuschangne, M. Herselman, L. and Fregene, M. (2008). Introgression of genes for dry matter content from wild cassava species. Euphytica 164: 163-172.

Okogbenin, E. Ekanayake I. J. and Porto M. C. M. (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. and Fregene, M. (2003). Genetic mapping of QTLs affecting productivity and plant architecture in a full-sib cross from non-inbred parents in Cassava (*Manihot esculenta* Crantz). Theoretical and Applied Genetics 107: 1452-1462.

Okogbenin, E. Marin, J. and Fregene, M. (2006). An SSR-based molecular genetic map of cassava. Euphytica 147: 433-440.

Okogbenin, E. Marin, J. and Fregene, M. (2008). QTL analysis for early yield in a pseudo F 2 population of cassava. African Journal of Biotechnology 7: 131-138.

Olsen, K. and Schaal, B. (2001). Microsatellite variation in cassava (*Manihot esculenta*, Euphorbiaceae) and its wild relatives: further evidence for a southern Amazonian origin of domestication. American Journal of Botany 88: 131-142.

Omole, T. and Eshiet, R. (1992). The use of cassava for feeding rabbits. In: Hahn, S. K. Reynolds, L. and Egbunike, G.N (eds.) Cassava as Livestock Feed in Africa. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, pp 159.

Onwueme, I. (1978). The tropical tuber crops. John Wiley & Sons Ltd, NY, pp 79.

Onwueme, I. (2002). Cassava in Asia and the Pacific. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: Biology, Production and Utilization. CABI Publishing, Wallingford, NY, pp 55-65.

Onwueme, I. C. and Charles, W. B. (1994). Tropical root and tuber crops: production, perspectives and future prospects. FAO Plant Production and Protection Paper 126, Rome, pp 228.

Osiru, D. S. Porto, M. C. and Ekanayake, I. J. (1996). Morphology of cassava plant. Interntional Institute of Tropical Agriculture (IITA). Ibadan, Nigeria.

Passioura, J. (1983). Roots and drought resistance. Agricultural Water Management 7: 265-280.

Patakas, A. and Noitsakis, B. (2001). Leaf age effects on solute accumulation in water-stressed grapevines. Journal of Plant Physiology 158: 63-69.

Paul, M. and Foyer, C. (2001). Sink regulation of photosynthesis. Journal of Experimental Botany 52: 1383-1400.

Pekic, S. and Quarrie, S. (1987). Abscisic acid accumulation in lines of maize differing in drought resistance: a comparison of intact and detached leaves. Journal of Plant Physiology 127: 203-217.

Pellet, D. and El-Sharkawy, M. (2001). Cassava varietal response to fertilization: Growth dynamics and implications for cropping sustainability. Experimental Agriculture 33: 353-365.

Porto, M. (1983). Physiological mechanisms of drought tolerance in cassava (*Manihot esculenta* Crantz). PhD thesis, University of Arizona, Tucson, pp 125.

Prabhu, R. and Gresshoff, P. (1994). Inheritance of polymorphic markers generated by DNA amplification fingerprinting and their use as genetic markers in soybean. Plant Molecular Biology 26: 105-116.

Prasad, P. V. V. and Stagggenborg, S. A. (2008). Impacts of drought and/or heat stress on physiological, development, growth and yield processes of crop plants. In: Ahuja, L. Reddy, V. Saseendran, S. and Qiang, Y. (eds.) Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes. ASA-CSSA-SSSA, USA, pp 301-356.

Raheem, B. (2006). Developments and microbiological applications in African foods: Emphasis on Nigerian wara cheese. PhD thesis, University of Helsinki, Finland, pp 53.

Ramirez-Vallejo, P. and Kelly, J. D. (1998). Traits related to drought resistance in common bean. Euphytica 99: 127–136.

Ribaut, J. and Pilet, P. (1991). Effects of water stress on growth, osmotic potential and abscisic acid content of maize roots. Physiologia Plantarum 81: 156-162.

Roa, A. Chavarriaga-Aguirre, P. Duque, M. Maya, M. Bonierbale, M. Iglesias, C. and Tohme, J. (2000). Cross-species amplification of cassava (*Manihot esculenta*) (Euphorbiaceae) microsatellites: allelic polymorphism and degree of relationship. American journal of botany 87: 1647-1655.

Rogers, D. and Appan, S. (1973). *Manihot* and *Manihotoides* (Euphorbiaceae), a computer-assisted study. Flora Neotropica, Monograph n 13. Hafner Press, NY, pp 272.

Rosielle, A. and Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non-stress environment. Crop Science 21: 943-946.

Rout, G. Das, P. Goel, S. and Raina, S. (1998). Determination of genetic stability of micropropagated plants of ginger using random amplified polymorphic DNA (RAPD) markers. Botanical Bulletin of Academia Sinica 39: 23-27.

Saini, H. and Westgate, M. (1999). Reproductive development in grain crops during drought. Advances in Agronomy 68: 59-96.

Santiveri, F. Royo, C. and Romagosa, I. (2004). Growth and yield responses of spring and winter triticale cultivated under Mediterranean conditions. European Journal of Agronomy 20: 281-292.

Sawkins, M. DeMeyer, J. and Ribaut, J. (2006). Drought adaptation in maize. In: Ribaut, J. M. (ed.) Drought adaptation in cereals. Food Products Press, NY, pp 259–299.

Saxena, N and O'Toole, J. (2002). Field screening for drought tolerance in crop plants with emphasis on Rice. Proceedings of an international workshop on field screening for drought tolerance in Rice. International Crops Research Institute for the Semi Arid Tropics (ICRISAT), India, pp 414.

Schwartz, S. H. and Zeevaart, J. A. D. (2004). Abscisic acid biosynthesis and metabolism In: Davies, P. (ed.) Plant hormones: biosynthesis, signal transduction, action! Kluwer Academic Publishers, The Netherlands, pp 137-155.

Semagn, K. Bjornstad, A. and Ndjiondjop, M. (2006). An overview of molecular marker methods for plants. African Journal of Biotechnology 5: 2540-2568.

Senior, M. and Heun, M. (1993). Mapping maize microsatellites and Polymerase Chain Reaction confirmation of the targeted repeats using a CT primer. Genome 36: 884-889.

Setter, T. L. Flannigan, B. A. and Melkonian, J. (2001). Loss of kernel set due to water deficit and shade in maize. Carbohydrate supplies, abscisic acid, and cytokinins. Crop Science 41: 1530-1540.

Setter, T. and Fregene, M. (2007). Recent advances in molecular breeding of cassava for improved drought stress tolerance. In: Jenks, M. Hasegawa, P. and Jain, S. (eds.) Advances in molecular breeding toward drought and salt tolerant crops. Springer, The Netherlands, pp 701-711.

Sharp, R. Wu, Y. Voetberg, G. Saab, I. and LeNoble, M. (1994). Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. Journal of Experimental Botany 45: 1743–1751.

Silva, J. and Arrabaça, M. (2004). Contributions of soluble carbohydrates to the osmotic adjustment in the C_4 grass *Setaria sphacelata*: A comparison between rapidly and slowly imposed water stress. Journal of Plant Physiology 161: 551-555.

Sivakumaran, S. and Hall, M. (1978). Effects of age and water stress on endogenous levels of plant growth regulators in *Euphorbia lathyrus* L. Journal of Experimental Botany 29: 195-205.

Smith, M. Biggs, B. and Scott, K. (1986). *In vitro* propagation of cassava (*Manihot esculenta* Crantz). Plant Cell, Tissue and Organ Culture 6: 221-228.

Somers, D. Isaac, P. and Edwards, K. (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 109: 1105-1114.

Ssemakula, G., and Dixon, A. (2007). Genotype x environment interaction, stability and agronomic performance of carotenoid-rich cassava clones. Scientific Research and Essays 2: 390-399.

Stam, P. and Van Ooijen, J. (1995). JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen.

Stitt, M. and Quick, W. (1989). Photosynthetic carbon partitioning: its regulation and possibilities for manipulation. Physiologia Plantarum 77: 633-641.

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

Tanksley, S. Ganal, M. Prince, J. De-Vicente, M. Bonierbale, M. Broun, P. Fulton, T. Giovannoni, J. Grandillo, S. and Martin, G. (1992). High density molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141-1160.

Taylor, N. and Fauquet, C. (1997). Transfer of rice and cassava gene biotechnologies to developing countries. Biotechnology International. 1: 239–246.

Taylor, N. Chavarriaga, P. Raemakers, K. Siritunga, D. and Zhang, P. (2004). Development and application of transgenic technologies in cassava. Plant Molecular Biology 56: 671-688.

Théberge, R. (1985). Common African pests and diseases of cassava, yam, sweet potato and cocoyam. International Institute of Tropical Agriculture. Ibadan, Nigeria, pp 116.

Thomas, D. and Twyman, C. (2005). Equity and justice in climate change adaptation amongst natural-resource-dependent societies. Global Environmental Change 15: 115-124.

Trinder, P. (1969). Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. Journal of Clinical Pathology 22: 158-161.

Tuinstra, M. Grote, E. Goldsbrough, P. and Ejeta, G. (1997). Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Molecular Breeding 3: 439-448.

UNAIDS (2006). Report on the global AIDS epidemic. UNAIDS, Geneva.

UNFPA (United Nations Population Fund), (1999). State of the World population. From: http://www.unfpa.org/swp/1999/chapter2e.htm [Cited on 15/12/2009]

Utz, H. (1997). PLABSTAT A computer program for the statistical analysis of plant breeding experiments. Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany.

Van Ooijen, J. and Voorrips, R. (2001). JoinMap® 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands.

Vassey, T. and Sharkey, T. (1989). Mild water stress of *Phaseolus vulgaris* plants leads to reduced starch synthesis and extractable sucrose phosphate synthase activity. Plant Physiology 89: 1066-1070.

Vavilov, N. (1951). Phytogeographic basis of plant breeding. The origin, variation, immunity and breeding of cultivated plants. Chronica Bot 13: 1-366.

Vries, C. Ferweda, J. and Flach, M. (1967). Choice of food crops in relation to actual and potential production in the tropics. Netherlands Journal of Agricultural Science 15: 241-248.

Wang, Y. Ying, J. Kuzma, M. Chalifoux, M. Sample, A. McArthur, C. Uchacz, T. Sarvas, C. Wan, J. and Dennis, D. (2005). Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant Journal 43: 413-424.

Weising, K. Nybom, H. Wolff, K. and Kahl, G. (2005). DNA fingerprinting in plants: principles, methods, and applications. Taylor and Francis, Boca Raton, pp 444.

Wenham, J. (1995). Post-harvest deterioration of cassava: A biotechnology perspective. Food & Agriculture Organisation of the United Nations, Rome, pp 85.

Westby, A. (2002). Cassava utilization, storage and small-scale processing. In: Hillocks, R. J. Thresh, J. M. and Bellotti, A. (eds.) Cassava: Biology, Production and Utilization. CABI Publishing, Wallingford, NY, pp 281–300.

Wheatley, C. and Chuzel, G. (1995). Cassava: the nature of the tuber and use as a raw material. In: Macrae, R. Robinson, R. and Sadler, M. (eds.) Encyclopaedia of Food Science, Food Technology and Nutrition. Academic Press, San Diego, pp 734-743.

Whyte, J. (1987). Breeding cassava for adaptation to environmental stress. In: Hershey, C. (eds.) Cassava breeding: a multidisciplinary review: proceedings of a workshop held in the Philippines. International Centre fo Tropical Agriculture (CIAT), Cali, Colombia, pp 312.

Wu, R. Ma, C. Wu, S. and Zeng, Z. (2002). Linkage mapping of sex-specific differences. Genetics Research 79: 85-96.

Wu, Y. Ballif, J. and Huang, B. (2006). Molecular mechanisms in hormonal regulation of drought tolerance: In: Huang, B. (ed.) Plant environment interactions. Taylor and Francis, NY, pp 101-114.

Xia, L. Peng, K. Yang, S. Wenzl, P. Carmen de Vicente, M. Fregene, M. and Kilian, A. (2005). DArT for high-throughput genotyping of cassava (*Manihot esculenta)* and its wild relatives. Theoretical and Applied Genetics 110: 1092-1098.

Xu, Y. Zhu, L. Xiao, J. Huang, N. and McCouch, S. (1997). Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Molecular and General Genetics 253: 535-545.

Yamamoto, T. Kimura, T. Shoda, M. Imai, T. Saito, T. Sawamura, Y. Kotobuki, K. Hayashi, T. and Matsuta, N. (2002). Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. Theoretical and Applied Genetics 106: 9-18.

Zeevaart, J. (1977). Sites of Abscisic Acid Synthesis and Metabolism in *Ricinus communis* L. Plant Physiology 59: 788-791.

Zeevaart, J.A.D. and Creelman, R. A. (1988). Metabolism and physiology of abscisic acid. Annual Review of Plant Physiology and Plant Molecular Biology 39: 439-473.

Zhang, M. Zheng, X. Yu, A. Xu, J. and Zhou, H. (2004). Molecular marker application in sugarcane. Sugarcane Biotechnology 6: 251-259.

Zhu, Y. Strassmann, J. and Queller, D. (2001). Insertions, substitutions, and the origin of microsatellites. Genetics Research 76: 227-236.

Zinselmeier, C. Jeong, B. and Boyer, J. (1999). Starch and the control of kernel number in maize at low water potentials. Plant Physiology 121: 25-36.

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