Assessment of genetic diversity, combining ability, stability and farmer preference of cassava germplasm in Ghana

by

Elizabeth Yaa Parkes

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UNIVERSITY OF THE FREE STATE
BLOEMFONTEIN
SOUTH AFRICA

Supervisor: Prof. Maryke T. Labuschagne
Co-supervisor: Dr. Martin A. Fregene

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DECLARATION

"I declare that, the thesis hereby submitted by me, for the degree of Philosophiae Doctor in Agriculture at the University of the Free State, is my own independent work and has not previously, been submitted by me to another University/faculty.

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DEDICATION

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AGDP	Agricultural Gross Domestic Product
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
AP-PCR	Arbitrary Primed Polymerase Chain Reaction
AS-PCR	Allele Specific Polymerase Chain Reaction
AYT	Advanced Yield Trials
BC_1	Backcross F_1
Bp	Base pair
BAS	Bulk Segregant Analysis
CAPs	Cleaved Amplified Polymorphic sequences
CBB	Cassava Bacterial Blight
CBLS	Cassava Brown Leaf Spot
CGM	Cassava Green Mite
CIAT	International Center for Tropical Agriculture
CM	Cassava Mealy bug
cM	centi Morgan
CMD	Cassava Mosaic Disease
COSCA	Collaborative Study on Cassava in Africa
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
CSIR-CRI	Council for Scientific and Industrial Research-Crops Research Institute
DAF	DNA Amplification Fingerprinting
DArTs	Deletion Amplified Regions Tags
DM	Dry Matter
DNA	Deoxyribonucleic acid
dNTPs	deoxy Nitro Triphosphate
DRC	Democratic Republic of Congo
EDTA	Ethylene Diamine Tetraacetic Acid
ESTs	Expressed Sequenced Tags
FAO	Food and Agriculture Organization
GCA	General Combining Ability

GCP	Generation Challenge Programme
GDP	Gross Domestic Product
G x E	Genotype by Environment
GLSS	Ghana Living Standards Survey
HC	Hydrogen Chloride
Gst	Genetic differentiation
HI	Harves Index
HCN	Hydrogen Cyanide
Ht	Heterozygosity
IBPGR	International Board for Plant Genetic Resources
IFPRI	International Food Policy Research Institute's
IITA	International Institute of Tropical Agriculture
ISA	Inter Simple sequence repeats Amplification
LSD	Least Significant Difference
M	Molar
MAP	Months After Planting
MAS	Marker-Assisted Selection
mg	Milligram
Mg	Magnesium
$MgCl_2$	Magnesium Chloride
Mm	Milli mole
MOLCAS	Molecular Diversity Network of Cassava
Mp-PCR	Microsatellite Primed Polymerase Chain Reaction
Mwt	Molecular weight
NaCl	Sodium Chloride
NARP	National Agriculture Research Programme
NCD II	North Carolina design II
NEPAD	New Partnership for African development
ng /µl	Nanogram per micro litre
NRCRI	National Root Crops Research Institue
^oC	Degrees Celsius
PAGE	Polyacrylamide Gel Electrophoresis
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction

pH	Hydrogen ion concentration
PIC	Polymorphism Information Content
PSA	Proportion of Shared Alleles
QMP	Quality Maize Phenotype
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RCBD	Randomised Complete Block Design
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolutions per minute
SCAR	Sequence Characterized Amplified Region
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
SPSS	Statistical Programme for Social Sciences
STS	Sequence-tagged sites
t/ha	ton per hectare
TAE	Tris-acetic-EDTA buffer
TME	Tropical *Manihot esculenta*
TMS	Tropical Manioc Species
UgV	Ugandan variant
UPGMA	Unweighted pair group arithmetic means algorithm
US	United States
UYT	Uniform Yield Trials
µl	micro litre
µM	micro molar

LIST OF TABLES

LIST OF FIGURES

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Cassava is the most important vegetatively propagated food crop and the second most important food staple in terms of calories per capita for more than 500 million people in Africa (Jennings and Hershey 1985; Nweke et al. 2002). It provides 50% of the calorie requirement of over 200 million people in sub-Saharan Africa (Osiru et al. 1996). It is processed into various food forms in many African countries. The main nutritional component of cassava is carbohydrate, which is derived from starch accumulated in the tuberous storage roots. This is processed into various food forms, such as garri (from fresh cassava), dried chips and flour (Nweke et al. 1994). The leaves are reported to have excellent nutritional value for humans and animals (Ceballos et al. 2004). The tender shoots and leaves are eaten as vegetables in many parts of Africa and it provides protein with a high content of lysine, minerals and vitamins (7 g protein per 100 g edible portion) (Hahn 1989; IITA 1990; Nweke et al. 1994; Fregene et al. 2000; Benesi 2005). The cassava woody stem cuttings are commercially used as planting materials (Ekanayake et al. 1997; Alves 2002). The seed is processed for oil and seed cake, used for formulating feed for livestock. The seed is also processed into a medicinal product to cure skin diseases (Popoola and Yangomodou 2006). Most farmers have kept and cultivated ancient cassava varieties for generations (Beeching et al. 1993; Okai 2001; Manu-Aduening et al. 2005).

Cassava is adapted to a wide range of environments. It is a rustic crop tolerant to drought and acidic soils, with good performance on degraded soils where other crops fail (Jones 1959; Kawano et al. 1978; Jaramillo et al. 2005). The Food and Agriculture Organization (FAO 2006) reported that Africa produced half of the world's cassava. However, the recorded increase in production was attributed to increased area under cultivation rather than yield produced per unit area (Hillocks 2002). Cassava is earmarked as one of the alternatives to compete with maize for industrial processes in the tropics (Jaramillo et al. 2005). Its high yield potential makes it a suitable option for other grain staples where population pressure and crop failure are a challenge (Al-Hassan et al. 1993; Nweke 1996; Benesi 2005). The exceptional ability of cassava roots to be stored in the ground and harvested when needed, makes it a food security (DeVries and Toenniessen 2001) and famine reserve crop (Nweke et al. 2002).

Ghana is the third largest producer of cassava in Africa (FAO 2006). Cassava ranks first in the area under cultivation and utilization in Ghana (NARP 1994), and it is grown in all ten regions of Ghana (Okai 2001). Traditional farmers are mostly women, who inter-crop cassava with other staple food crops (Francis 1990; FAO 2001; 2002; Dapaah et al. 2003). In the Ghana Living Standards Survey (GLSS) Dapaah (1996) reported that 83% of the 1.73 million households sampled were engaged in cassava production. Cassava has changed its status from a traditional food crop in the hands of subsistence farmers to an important industrial crop for cash and jobs for the rural communities (Nweke et al. 2002; Dixon and Ssemakula 2008). Al-Hassan and Diao Xinshen (2007) reported the potential of cassava as an important crop to reduce poverty and promote economic growth in northern Ghana, which is among the most poverty stricken areas. Many reports on Ghana's economic growth and development have identified cassava as a single commodity that could generate desired economic growth and fight poverty (Dapaah 1991; 1996; Al-Hassan and Diao, 1993; Nweke 2004). There is a growing importance of cassava in Ghana (Dapaah 1991; Al-Hassan et al. 1993; Manu-Aduening et al. 2006); requiring therefore, that the demands of the growing cassava-based industry in both Ghana and Nigeria be met in order to promote economic growth in Ghana and West Africa (Nweke 2004; Al-Hassan 2007; Dixon and Ssemakula 2008). Cassava still faces challenges, such as poor yields, pest and disease pressure, and post harvest deterioration and new, improved materials are urgently needed to respond to the demands of food security, poverty alleviation, and new emerging threats of some pests and diseases.

Genetic improvement of cassava through breeding began recently and has lagged behind many crops such as the cereals and legumes. Compared with other staple foods, little scientific effort has been made to improve cassava (Cock 1985; Ceballos et al. 2004). Breeding efforts in Africa is reflected in the work of the International Institute for Tropical Agriculture (IITA) in developing elite clones with resistance to pest and diseases (Hahn et al. 1989). The Tropical Manioc Species (TMS) developed by the IITA are improved cassava clones bred for resistance to cassava mosaic disease (CMD), cassava bacterial blight (CBB), cassava green mite (CGM), with high stable yields, agronomic and good consumer quality traits for testing and adoption in Africa. Nweke et al. (2002) attributed the increase in cassava production in Africa to the cultivation of the TMS clones in Africa. There is paucity of information on inheritance of agronomic traits (Easwari Amma et al. 1995; Pérez et al. 2005;

Cach et al. 2006). Data on relative importance of additive and non-additive gene action is limited (Pérez et al. 2005; Jaramillo et al. 2005)

Although cassava is well integrated into the diverse traditional farming systems (Francis 1990; Dapaah et al. 2003; Zacarias 2008) very little genetic improvement has been achieved. Adoption rates for improved technologies have been rather slow, because the end users are not ready to use them as they do not satisfy their unique preferences and requirements (Nweke et al. 1994; Manu-Aduening et al. 2005; Benesi 2005). Farmers have taken advantage of natural hybridization and vegetative propagation of cassava to develop new varieties over thousands of years. This has rendered cassava landraces that offer a rich source of genetic diversity and provide a valuable source of genetic material for crop improvement (Attere 1997; Ceballos et al. 2004; Zacarias 2008). Cassava planting materials are selected and distributed by farmers (Beeching et al. 1993; Kizito et al. 2005; Manu-Aduening et al. 2005). Farmers have selected genotypes that best fit their needs and different ethnic groups have given numerous names according to their uses and many other factors. This nomenclature has led to confusion in the exact numbers and identity of cassava varieties under cultivation (Mignouna and Dixon 1997; Okai 2001; Baafi and Sarfo-Kantanka 2008).

Genetic diversity assessment using morphological and molecular markers Random Amplified Polymorphic DNAs (RAPDs) of some Ghanaian cassava cultivars showed a greater genetic diversity with molecular markers than morphological markers (Okai 2001). Genetic diversity of cassava clones from four districts of the Brong Ahafo region was assessed with four RAPD markers (Asante and Offei 2003). The use of morphological markers is environmentally influenced and highly subjective. Genetic crosses of cassava towards improvement have not been practiced in Ghana. In order to set the platform for the improvement of cassava landraces in Ghana, genetic analysis of a large representative collection of landraces would be ideal. A nation-wide collection of landraces from farmers' fields could be analysed with robust molecular markers available, to offer useful information on the genetic diversity and structure and heterotic groups that may exist and with useful mating schemes like the diallel, it could serve as a guide for planned cassava improvement of landraces or local cultivars for end users. .

The success of a breeding programme depends largely on the genetic diversity that exists in available germplasm (Meredith and Bridge 1984). The study of genetic diversity in cassava

landraces in Ghana using Simple Sequence Repeat (SSR) markers represents the first comprehensive genetic study based on landraces or local cultivars from all regions of Ghana. The genotypes used represent a national collection of landraces or local cultivars from farmers' fields in all ten regions of Ghana and improved clones from the International Center for Tropical Agriculture (CIAT) and the Institue of Tropical Agriculture (IITA). The objectives of this study were:

1. To investigate the genetic diversity and genetic structure among landraces using SSR markers
2. To study combining ability and heterotic groupings in the landraces
3. To use diallel crosses to study the importance of heterosis and heritability of important cassava traits
4. To develop superior hybrids through participatory evaluation
5. To improve landraces with CIAT materials for CMD resistance

All this information will contribute to the structuring of an effective breeding programme for Ghana.

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CHAPTER 2

LITERATURE REVIEW

2.1 Importance of cassava

2.1.1 General importance

Cassava (*Manihot esculenta* Crantz), a perennial crop native to tropical America, was among the first to be domesticated (Okigbo 1980; Allem 2002). It is an important food crop for more than 900 million people in the tropics and sub tropics (FAO 1996; Nassar 2005). Cassava is fourth after rice, sugarcane and maize as a source of calories in the human diet, and contributes about 500 cal/day for more than 500 million people in tropical countries in Africa, Asia and Latin America (Cock 1982; 1985; Iglesias et al. 1997; Henry and Hershey 2002; Hillocks 2002; Onwueme 2002). As a staple food, it is the sixth most important crop in the world (Mann 1997). Cassava is the only non-native crop in Africa that has assumed a major staple food status (Tewe 1992). It constitutes the most important tropical root crop (Onwueme 1978; Dapaah 1991; Roa et al. 1997; Mkumbira 2002; Nweke 2004). Cassava roots are efficient in carbohydrate production (Onwueme 1978; Cock 1985) and contain nearly the highest starch content among all root and tuber crops (Moorthy 1994).

The FAO (2006) reported that cassava produced in Africa accounts for more than half of the total production in the rest of the world. In 2005, the largest producing countries were Nigeria (40%), Democratic Republic of Congo (19%), Ghana (10%), Tanzania (7%) and Mozambique (6%). The increase in cultivation was partially due to declining soil fertility and increased cost of inorganic fertilizers (FAO 1998). The productivity per unit area in Africa (8.2 t/ha) is low compared to the world average of 9.8 t/ha (FAO 1998). Low yields have been attributed to production constraints and abiotic factors (Nweke 1996).

Cassava is adapted to a wide range of environments. It is a rustic crop that grows well in conditions where few other crops survive; it is tolerant to drought and can produce in degraded soils. It is naturally tolerant to acidic soils (Jones 1959; Kawano et al. 1998; Jaramillo et al. 2005) and suitable for farming systems in Africa (Nweke et al. 2002).

There are 39 countries that grow cassava as a staple food crop. The cultivation of cassava stretches over a wide ecological belt from Madagascar in the south-east to Senegal and Cape

Verde in the north-west (Raji et al. 2001; Nweke et al. 2002; Benesi 2005). An increase in cassava production in Africa has been reported (FAO and IFAD 2001). The increase has been attributed to land area cultivated and not the crop yield increase as reported by Hillocks (2002). Cassava has the potential to become one of the alternatives to compete with maize for industrial processes in the tropics (Jaramillo et al. 2005). Its high yield potential makes it a suitable alternative for other grain staples where population pressure and crop failure are a challenge (Al-Hassan et al. 1993; Nweke 1996; Benesi 2005).

The collaborative study on cassava in Africa (COSCA) has revealed and described cassava as a poverty fighter (Nweke 2004) that is playing a leading role in African agriculture, not only as a famine reserve crop and a famine reserve crop but also as a cash crop for urban consumption and jobs for the rural communities (Nweke et al. 2002; Dixon and Ssemakula 2008). The leaves are reported to have excellent nutritional value for humans and animals (Ceballos et al. 2004). Cassava leaves are served as a vegetable in many parts of Africa, providing a rich source of proteins and other trace elements. The seeds have medicinal properties and are also used for animal feed formulation (IITA 1990; Fregene et al. 2000; Benesi 2005). Propagation is mainly done with woody stems, which are sold to generate income (Alves 2002; Popoola and Yangomodou 2006).

There is a growing interest in cassava beyond its traditional role as a rural and urban staple due to its good adaptation to drought and poor soils (Dapaah 1991). The New Partnership for African Development (NEPAD) has proposed a Pan African Cassava Initiative to increase productivity and industrial use of cassava in Africa. The transformation of cassava from a traditionally subsistence crop to an industrial crop has become evident in recent times. Dapaah (1991) reported that root crop production, especially cassava, can improve Africa's economy. Furthermore, there is an emerging market and growing industry for cassava based products in Ghana and Nigeria (Nweke et al. 2002; Nweke 2004). The two countries are at the third stage of cassava transformation, hence the proliferation of cassava based industries. The cassava based industry is growing, expanding its activities to cover the production of starch and its derivatives, ethanol, glucose syrup, composite flour, "gari" and feed for livestock (Al-Hassan 1989; 1993; Nweke et al. 2002).

Cassava plays a major role in reducing food crisis (Egesi et al. 2007), and there is an increased demand for cassava, the large acreages of mechanized farms and farmer groups in

cooperatives, the supply of raw cassava tuberous roots to the growing cassava based industry, is becoming important (Nweke et al. 2002; Nweke 2004; Manu-Aduening et al. 2006). There are excellent opportunities for product and market diversification in several African countries (Dapaah 1996; Benesi 2005; Al-Hassan and Diao Xinshen 2007; Dixon and Ssemakula 2008).

2.1.2 Importance of cassava in Ghana

Ghana is among the first African countries where cassava was introduced. It was planted around coastal trading castles. The Ghanaian name for cassava "bankye" may be a contraction of the *akan* phrase "aban kye" meaning 'gift from the castle or government'. Cassava became widely grown in the coastal plains by the second half of the 18th century (Adams 1957). The adoption of cassava as a major food crop within Ghana was slow. Most people in the forest zone preferred plantains and cocoyam and the northern part used sorghum and millet (Ministry of Agriculture 1990). In 1983, there was a national drought in Ghana, which resulted in crop failure for major food crops except cassava. Thereafter, cassava cultivation and utilization spread throughout the country (Korang-Amoako et al. 1987; Manu-Aduening et al. 2005).

Ghana is the third largest producer of cassava in Africa (FAO 2006). Cassava ranks first in both the area under cultivation and utilization in Ghana (NARP 1994). Cassava is grown in all the agro-ecological zones (Figure 2.1) across the ten regions of Ghana (Okai 2001; Manu-Aduening et al. 2005; Amenorpe et al. 2006). Dapaah (1996) reported that the roots and tuber crops contributed 59% of Ghana's Agricultural Gross Domestic Product (AGDP) excluding forestry. Cassava contributed 19% (AGDP) compared to 4% for maize, 2% for rice and 13% for cocoa. In the Ghana Living Standards Survey (GLSS) Dapaah (1996) reported that 83% of the 1.73 million households sampled were engaged in cassava production. Cassava has undergone a transformation from being a traditional food crop in the hands of subsistence farmers to an important industrial crop for cash and jobs for the rural communities (Nweke et al. 2002; Dixon and Ssemakula 2008). Al-Hassan and Diao Xinshen (2007) reported the potential of cassava as an important crop to reduce poverty and promote economic growth in northern Ghana, which is among the most poverty stricken areas. Many reports on Ghana's economic growth and development have identified cassava as a single commodity that could generate desired economic growth and fight poverty (Dapaah 1991; 1996; Al-Hassan et al. 1996; Nweke 2004; Al-Hassan and Diao Xinshen 2007).

There is a growing importance of cassava in Ghana (Dapaah 1991; Al-Hassan et al. 1996; Manu-Aduening et al. 2006). There is a need to meet the demands of the growing cassava based industry in Ghana and Nigeria, to promote economic growth in Ghana and West Africa (Nweke 2004; Al-Hassan and Diao Xinshen 2007; Dixon and Ssemakula 2008). It is reported (Al-Hassan 1993; Nweke et al. 2004; Dixon and Ssemakula 2008) that cassava has tremendous potential in Ghana and Africa's economy for food, feed, and industry and to provide cash and jobs for the rural communities. Cassava is processed into varied food forms based on its tuberous root attributes (Amenorpe et al. 2006; Baafi and Sarfo-Kantanka 2008). Cassava starch and its derivatives are used in the textile, pharmaceutical, paper and ply wood industry. Glucose syrup is also used in the food and confectionery industry. Cassava flour is a composite part of wheat flour for bakery and confectionaries in the local markets and instant 'fufu'. Cassava is used for ethanol production, and as animal feed (Nassar 2006).

The production, processing and utilization of cassava offer jobs to large communities, especially women in the tropical countries (Francis 1990; Haleegoah and Okai 1992; Thro et al. 1995). In a survey conducted, Collinson et al. (2001) reported the significant potential of cassava to contribute to rural incomes and to meet urban food needs in processing instant 'fufu'. They indicated an estimated annual potential in the range of 1000 to 17000 mt which translates to 2000 to 34200 mt of fresh cassava roots. There is an increasing importance of cassava as food and cash crop in Ghana (Manu-Aduening et al. 2006; Baafi and Sarfo-Kantanka 2008). In northern Ghana (comprising the upper west, upper east and northern region) where poverty is the highest, Al-Hassan and Diao Xinshen (2007) reported on staple crops whose production growth exerts the largest effect on poverty reduction as groundnut, cassava and cowpea. Cassava was identified as the most important of the three crops. Additionally, agriculture-led economic growth had a larger poverty reducing effect than non-agriculture-led growth. The growth in staple crop production reduced poverty more than export of crops (Al-Hassan and Diao Xinshen 2007).

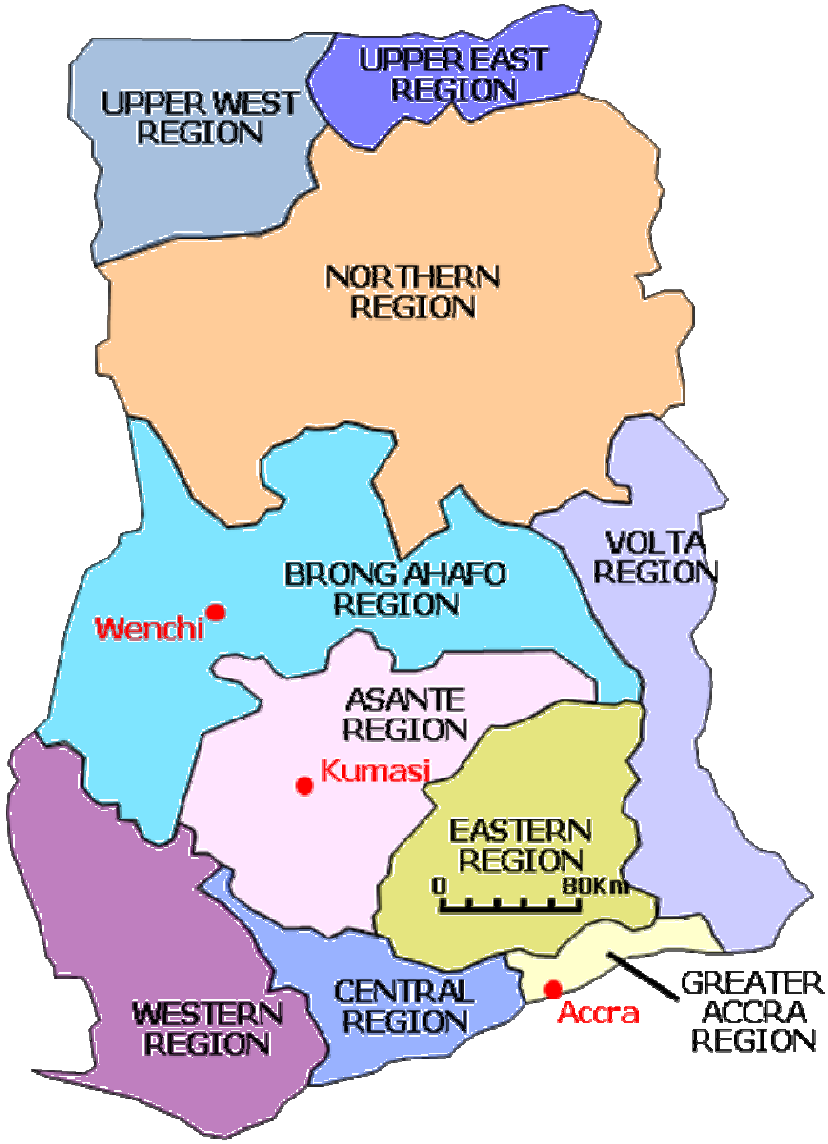

Figure 2.1 Map of Ghana showing 10 regions where cassava is grown

Cassava has a long growth cycle; low seed set and is allogamous in nature, with a complex genetic structure. Cassava breeding is therefore considerably slowed down by the biology of the crop (Kawano et al. 1998; Fregene et al. 2001). However cassava still faces challenges, such as poor yields, pest and disease pressure, and post harvest deterioration. New improved materials are urgently needed to respond to the demands of food security, poverty alleviation, and new emerging trends of some pests and diseases. There is the need for cassava improvement programmes to take advantage of modern tools to meet population growth and pressure on the environment (Bertrand et al. 2008).

2.2 Cassava in Africa

Fregene et al. (1994) reported that all species of the genus *Manihot* are native to the New World tropics and occur naturally only in the western hemisphere between southern USA (33^0N) and Argentina (33^0S). Reports indicate that cultivated and wild species were introduced in the old world tropics in the 16th century (Jones 1959) by Portuguese explorers when they established forts, trading stations and settlements on African coastal and nearby islands. The first cultivation of cassava in Africa dates from 1558 (Carter et al. 1992). The crop spread throughout Africa by various mechanisms of which the initial contact with the Portuguese-Brazilian culture appears to be the most remarkable. Cassava got to West Africa via the Bight of Benin and the Congo River at the end of the 16th century. The crop reached the east coast of Africa via the islands of Reunion, Madagascar and Zanzibar at the end of the 18th century (Jennings 1976).

The spread of cassava became possible by river and overland trade by merchandise, and by mass migration (Jones 1959; Carter et al. 1992). In the 19th and 20th centuries, the colonial administrators promoted its spread and increased cultivation. Cassava possesses botanical characteristics that enables it to compete well with weeds and therefore can survive under various conditions of neglect. The ability to regenerate from stem cuttings may have enhanced the spread of the crop to many new locations (Carter et al. 1992). The diffusion of cassava is seen from historical documents and travelogues in Africa. Gulick et al. (1983) defined the primary, secondary and tertiary levels of diversity for *M. esculenta* in modern times. An important source of secondary diversity lies in Africa, outside the crop's centre of origin (Lefevre and Charrier 1993; Fregene et al. 2000; 2003; Hurtado et al. 2008). Cassava is adapted to a wide range of ecologies. The growing zone lies within latitudes 30^o north and south of the equator at elevation of up to 2000 meters above sea level in temperatures ranging from 18^{o}C to 25^{o}C, to rainfall of 50-5000 mm/year and to poor soils with pH from 4 to 9. This stretches through a wide ecological belt from Madagascar in the south-east to Senegal and Cape Verde in the Northwest (Raji et al. 2001; Nweke 2002; Nassar 2007). There are 39 countries that grow cassava as a staple food crop. An increase in cassava production in Africa has been reported (FAO and IFAD 2001).

Cassava has been cultivated around Accra since 1785. Since its introduction, initial attempts at improving cassava in Ghana were made by the Crops Research Institute at Kwadaso,

Kumasi where introduced cultivars, particularly from East Africa were tested, screened and elite clones such as K357, K102, K680 were allowed to cross with the local cultivars to generate high yielding and disease resistant clones (Doku 1969). In collaboration with the International Institute of Tropical Agriculture (IITA), a number of introductions, cassava genotypes TMS have been tested and screened to identify genotypes of high and stable yields and resistance to pests and diseases across different agro-ecological zones in Ghana. Since 1992 several varieties have been released to farmers. However, there has been low adoption of cassava technologies since farmers did not find preferred attributes that were suitable for their food needs (Nweke et al. 2002; Benesi 2005; Manu-Aduening et al. 2005). Some varieties bred for CMD lost their resistance to the disease over the years. This is due to high CMD virus builds up with the use of vegetative material for propagation and new emerging strains of the virus (Nweke et al. 2002; Pujol et al. 2002; Kizito et al. 2005; Peroni et al. 2007). There is a need for national agriculture programmes in Africa to improve cassava to solve emerging trends and meet demands of the growing cassava based industry (Okogbenin et al. 2006).

2.3 Morphological characteristics of cassava

Cassava (*Manihot esculenta* Crantz, synonymous with *Manihot ultissima* Pohl) (Onwueme 1978) is a dicotyledonous crop. It belongs to the family Euphorbiaceae and genus *Manihot.* There are 98 species in the genus that are useful as gene resources in cassava improvement (Rogers and Appan 1970; Fregene et al. 1994). The Euphorbiaceae family has several commercial plants as members (Hershey 2005). It is characterised by lactiferous vessels composed of secretory cells for latex production. Some of these are rubber trees (*Hevea brasiliensis*), castor oil plants (*Ricinus comunis*), root crops (*Manihot spp*) and ornamentals (Euphorbia *spp*) (Ekanayake et al. 1997; Fregene et al. 2006). Cassava is the only species from the genus that is widely cultivated for food production (Rogers and Appan 1973; Onwueme 1978; Mkumbira 2002; Nassar 2006). *Manihot glaziovii* and *Manihot eaerulescene* have minor uses as alternative sources of latex for rubber production (Franche et al. 1991; Fregene 1996).

The *Manihot* species have 2n=36 chromosomes and are regarded as polyploids with n=18 (Jennings 1976; Hershey 1993). It is reported that *Manihot* species behave meiotically as diploids (Nassar 2003). Studies carried out on the pachytene of *M. glaziovii* and in

comparison with karyology of cassava, suggests that the species is probably a segmental allotetraploid (Magoon et al. 1969; Jennings 1976) derived from a combination of two diploid taxa whose haploid complement has six common and three different chromosomes (Jennings 1976). Inheritance of several isozymes supports this evidence and showed disomic heredity, thus confirming diploid behavior (Jennings and Hershey 1985; Hussein et al. 1987; Lefevre and Charrier 1993). Cassava, though a major global crop with enormous potential, has little research focus (Olsen 2004; Gbadegesin et al. 2008). Indeed, it is genetically the least understood among the major staple crops (such as rice, maize, wheat and potato) for food production (Gomez et al. 1996; Fregene et al. 1997; 2001). However, research towards the development of a molecular linkage map is likely to provide a better structural definition of the cassava genome (Fregene et al. 2001).

Cassava is an outcrossed crop (Allard 1960) and pollination is mainly done by bees and wasps. Spontaneous hybrids between cassava and other *Manihot* species have been reported to occur naturally in Africa and Brazil (Nassar 1994; 2007). Earlier hybridization studies in the late 1930s suggested that tree cassava is a natural hybrid between cassava and *M. glaziovii* Allem (Fregene 1996). This was later confirmed by other workers who observed normal pairing at meiosis in the first filial generations of two sets of crosses, between cassava and *M. glaziovii* and cassava and arborescent cassava (Magoon 1967; Bai 1982; IITA 1988). Farmers make cuttings from the spontaneous or volunteer seedlings in their fields for subsequent planting (Harlan 1992; Okai 2001; Pujol et al. 2002; 2007; Kizito et al. 2005; Manu-Aduening et al. 2005; Peroni et al. 2007).

Cassava is a perennial woody shrub, cultivated mainly for its starchy tuberous roots. It is mainly propagated from stem cuttings (IITA 1990; Hallack 2001) however, spontaneous or volunteer seedlings may be used by farmers (Lefevre and Charrier 1993; Okai 2001; Kizito et al. 2005; Manu-Aduening et al. 2005). The average height of a mature plant ranges from one to two meters; some cultivars can reach four meters (Onwueme 1978; Ekanayake et al. 1997; Hershey 2005). Most farmers harvest within the first two years, however abandoned stands may continue to grow for several years (Onwueme 1978). Cassava propagation from true seed occurs under natural conditions and is widely used in breeding programmes (Iglesias et al. 1994). Plants generated from true seeds take a longer time to become established and are smaller and less vigorous than plants from woody stem cuttings (Ekanayake et al. 1997;

Alves 2002). Such seedlings are genetically segregated due to outcrossing in cassava (Osiru et al. 1996).

2.3.1 Flowering in cassava

Cassava is monoecious and predominantly out-crossing (Fregene et al. 1997). Flowering may begin as early as the sixth week after planting (Jennings and Iglesias 2002). There are frequent and regular flowering cultivars, while in others it is rare or non-existent (Onwueme 1978; IITA 1990). The availability of flowers is influenced by plant habit and is generally formed in the intersection point of the reproductive branching (Jennings and Iglesias 2002; Hershey 2005). Male flowers occur near the tip, while female flowers occur close to the base of the inflorescence. The female flowers normally open 10 to 14 days before the males on the same branch, encouraging cross-pollination. However, self-fertilisation can occur on different plants or branches of the same genotype that open simultaneously (Onwueme 1978; Osiru et al. 1996; Jennings and Iglesias 2002). Generally the pollinating agents are insects, particularly bees and wasps (Onwueme 1978; IITA 1990; Mkumbira 2002).

The stigma of the female flowers remains receptive six hours after flower opening, between the hours of 11:00 to 12:00. The pollen from the male flowers lose viability after two days of flower opening (Kawano 1978).

2.3.2 Fruit and seeds

Cassava produces mature fruits between 75 to 90 days after pollination and fertilization (Ghosh et al. 1988). There is variability in the fertility among cultivars; an average of one seed is obtained per fruit instead of a maximum of three from the tri-locular ovary (Jennings and Iglesias 2002). The mature fruit is a globular capsule, which naturally splits explosively to release the seeds, a process called autochory. Myrmecochory (seed dispersal by ants) help move seeds over short distances, leading to the constitution of a cassava seed bank in the soil. The seeds are buried in the soil, protected from predators during prolonged dormancy and lethal temperatures during land preparation (Elias et al. 2000; Pujol et al. 2002). When the field is cleared and burnt, cassava seeds that have persisted during the fallow period since the previous crop, germinate in large numbers as volunteer seedlings. Farmers select from healthy and vigourous plants and add them to their varieties (Onwueme 1978; IITA 1990; Osiru et al. 1996; Pujol et al. 2002; 2007).

Newly harvested cassava seeds remain dormant and require 3-6 months storage at ambient temperature before germination. Cassava seeds germinate within 16 days after planting. Seed germination can be hastened by temperature treatment. Ellis et al. (1982) found that the best germination rates were obtained when temperatures for part of the day exceeded 36^{o}C and the mean temperature was at least 33^{o}C. A treatment of 14 days at 60^{o}C is appropriate for freshly harvested seeds. It is unclear whether germination of cassava seeds is triggered by fluctuation between high and low temperatures or simply by high temperatures (Pujol et al. 2002). Cassava seeds stored at ambient temperatures lose viability within a year (Kawano 1978).

2.3.3 Leaves

The number of cassava leaves produced, leaf longevity and whole plant products are determined by genotype and environmental conditions. Cassava leaves are arranged alternately in a spiral order on the stem. The pyhllotaxis or arrangement of the leaves on the stem is 2/5 spiral. Cassava leaves are simple with lobed lamina and petiole. Each leaf is subtended by three to five stipules, each about 1 cm long. The number of lamina lobes varies between three and nine, usually odd numbers. Most cassava varieties grown in Africa have lobes that are elliptical or lanceolated (Onwueme 1978; Okai 2001). When cassava is propagated, the development of the first true leaf marks the start of active photosynthesis. Food and nutrients are transported to all parts of the plant including the roots (Simwambana 1988). Leaf area approaches its maximum size between 4-5 months depending on the type (Ekanayake et al. 1997).

2.3.4 Root system

The cassava root system is made up of feeder roots and storage roots. Cassava propagated from hard woody stem cuttings, develop adventitious roots at the base of the cutting during the first two to three weeks (Ekanayake et al. 1997). These adventitious roots subsequently develop into a fibrous root system, which absorbs water and nutrients from the soil. The fibrous root system may reach 200 cm or more in length (IITA 1990). Storage roots formation begins about eight weeks after planting. A few of the fibrous roots (between three and 10) start to bulk and become storage roots (Alves 2002). Most of the other fibrous roots remain thin and continue to function for water and nutrient absorption. The fibrous roots that become storage roots, lose their ability to absorb water and nutrients considerably. Cassava plants propagated by true botanical seeds develop a typical tap root system, similar to dicot species within 30-60 days. Some roots increase in diameter and become tuberous storage

roots. As tuberisation proceeds, the storage tuberous roots are developed from the activity of cambium and starch accumulation (Alves 2002). Cassava roots have the shortest post-harvest shelf life compared to any of the major root crops (Ghosh et al. 1988). Roots are highly perishable and deteriorate within 24-72 hours after harvest due to a rapid physiological deterioration process (Wheatley et al. 1985; Wheatley and Chuzel 1993).

2.4 Cropping systems

Cassava is intercropped with long or short season staple crops (Cock 1985; Alves 2002). It is produced under a low input and low output system particularly when grown as a food crop (Leihner 2002). In Africa and the Americas cassava is intercropped with maize and legumes (Mutsaers et al. 1993; Alves 2002). An estimated one third of cassava grown in the world is reported (Cock 1985) to be intercropped, to minimise the risk of crop failure (Kizito et al. 2007). Traditionally farmers in Ghana have adopted mixed cropping. Cassava is intercropped with other food crop staples to ensure food security and also offer farmers the opportunity to harvest different crops from the same piece of land (Francis 1990; Okai 2001; Dapaah et al. 2003; Manu-Aduening et al. 2006; Baafi and Sarfo-Kantanka 2008). Production and processing are mostly done by women, and they produce it as food and also process it into “gari” and other products (Al-Hassan 1989; Nweke et al. 1994)

2.5 Genetic diversity

Genetic diversity has been defined by Frankel et al. (1995) as the product of interplay of biotic factors, physical environment, artificial and plant characters such as size, mating system, mutation, migration and dispersal. Genetic variability and genetic diversity of a taxon are of great importance to plant geneticists, breeders and taxonomists (Prince et al. 1995). In populations, the genetic composition and genetic diversity are derived from wild parents. This has been influenced by evolutionary processes such as mutation, recombination, genetic drift, migration, natural selection (Hartl and Clark 1997) and adaptation to a range of environments.

A clear knowledge and understanding of the distribution of genetic diversity and relationship among individuals, populations and gene pools is important for efficient management of germplasm collections and breeding programmes (Geleta 2003; Shiying Yang et al. 2006)

and the potential performance would be useful for all phases of crop improvement. The concept of genetic relationships among lines, populations or species has been a vital tool for effective management of genetic diversity in a given gene pool (Manjarrez-Sandoval et al. 1997).

Lee (1995) reported on variability and inter-relationships of growth and storage root yield and characteristics in cassava. The evaluation of genetic diversity among adapted or elite germplasm provided estimates of genetic variation among segregating progenies from pure line development (Manjarrez-Sandoval et al. 1997) and the degree of heterosis in the progenies of parental combinations (Barbosa-Neto et al. 1997; Cox and Murphy 1990; Geleta 2003).

An understanding of the genetic diversity is the first step to harness the genetic variability in the germplasm (Hurtado et al. 2008). The success of a breeding programme depends greatly on the genetic diversity that exists in available germplasm (Meredith and Bridge 1984). The study of genetic diversity has been of great importance to plant breeders and germplasm curators. It is a process where variation among individuals or groups of individuals is analysed (Mohammadi and Prasanna 2003). It has been found useful in plant species in the study of the evolution of germplasm as possible sources of genes that can improve the performance of cultivars (Yang et al. 1996; Geleta 2003). Studies based on DNA sequence and SSR marker data revealed that genetic variation found in cassava is a sub-set of that found in its putative progenitor (Olsen and Schaal 2001).

2.5.1 Genetic distance

The genetic distance measures the extent of the gene differences between cultivars, as measured by allele frequencies at sample loci (Nei and Li 1979) while the genetic relationship among individuals and the populations can be measured by similarity of any number of quantitative characters (Souza and Sorrels 1991). Genetic distance measurements are indicators of relatedness among populations or species and are useful for reconstructing the history and phylogenetic relationships among such groups.

There are two basic approaches for measuring genetic distance, these are the cluster analysis and the parsimony analysis and they represent the genetic and phylogenetic relationship, respectively. The data input for this analysis involve numerical or a combination of different

variables provided by a range of markers that can be used to measure genetic distance. This includes pedigree data, morphological traits, isozymes and recently DNA-based markers such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SCARs) and several others. The molecular markers are recognized as significant tools to enhance plant resource conservation management. This provides a means to accurately estimate the genetic diversity and structure for species of interest (Hamrick and Godt 1997).

2.5.2 Genetic diversity of cassava

Knowledge of the genetic variability in any population forms the fundamental basis for cassava improvement (Hurtado et al. 2008). The genetic distances within the population affords a better comprehension of germplasm organisation and efficient parental selection during genotypic sampling (Meredith and Bridge 1984). It also has implications on the choice of parents for crosses and gene introgression from exotic germplasm. The cassava gene pool ranges from a great variety of wild species to numerous domesticated species with very specific characteristics. The methods employed to investigate the origin and variability of cassava is the concept of the taxonomy of the species, the biological species, biosystematics and quantitative molecular genetics. Genetic diversity can be assessed by a number of methods including pedigree data, morphological data, agronomic performance, biochemical data and more recently DNA-based data (Mohammadi and Prasanna 2003). The DNA-based molecular markers reveal polymorphisms at the DNA level and are extensively used in various fields of plant breeding and germplasm management. These markers can identify many genetic loci simultaneously with excellent coverage of an entire genome. They are phenotypically neutral and can be applied at any developmental stage (Jones et al. 1997).

The molecular markers are not subject to environmental change, making them especially informative and superior to any traditional methods of genotyping (Tanksley et al. 1989; Messmer et al. 1993). The use of DNA-based markers has contributed to cassava breeding and genetics in the understanding of the phylogenetic relationships in the genus (Fregene et al. 1994; Roa et al. 2000; Olsen and Schaal 2001) and assessing the genetic diversity (Beeching et al. 1993, Second et al. 1997; Okai 2001; Elias et al. 2001; Mkumbira et al. 2003; Kizito et al. 2007). It has also helped with the development of genetic maps and identification of quantitative loci for traits of importance (Fregene et al. 1997; Jorge et al. 2000; 2001; Mba et

al. 2001; Okogbenin and Fregene 2002; 2003; Lokko et al. 2005; Ojulong 2006; Okogbenin et al. 2006). Other molecular tools used in cassava breeding include single nucleotide polymorphisms (SNPs) identified from whole genome scans, Deletion Amplified Regions Tags (DArTs) and Expressed Sequence Tags (ESTs) (Hurtado et al. 2008; Kawuki et al. 2009). Molecular markers have been successfully used to recommend cultivars for a given region (Vieira et al. 2007). It also helps breeders to concentrate their breeding efforts on the most promising combinations (Ceballos et al. 2004; Brennan and Martin 2007; Betrand et al. 2008). Crop plants are distinguished by classical morphological descriptors which are highly subject to environmental influences. These show low numbers of polymorphisms leading to a low accuracy of quantitative genetic parameters (Vieira et al. 2007), however these methods remain effective and play a complementary role especially for QTL (quantitative triat loci) studies (Okogbenin et al. 2006; 2007).

2.6 Marker techniques

2.6.1 Morphological markers

Classical breeding uses recorded morphological traits of plants growing in the field as bases for identification. It has been effectively used as a powerful tool in the classification of cultivars and the study of their taxonomic status (Rogers and Appan 1973). The certification of new cultivars or varieties is usually based on the genetic purity of a particular crop. However, traditionally these assessments depend on botanical traits (Stegemann 1984) for cultivar identification. Breeders and geneticists have used morphological characteristics such as leaf and flower attributes to follow segregation of genes and hybrids, but most agronomic traits are not associated with easily observable phenotypic markers (Kochert 1994).

Most of the descriptors are ambiguous and have limited use (Stegemann 1984). Such traits are controlled by multiple genes and are subject to varying degrees of environmental modifications and interactions. Morphological traits have higher heritability than the agronomic ones and they are the basic descriptors recommended for gene bank characterization. Mathura et al. (1986) reported that phenotypic variance in cassava was higher than genotypic variance for traits of agronomic importance, such as weight of tuberous roots. Many of these traits are also difficult to analyse because they do not have a simple genetic control assumed by many populations in genetic models (Liu and Furnier 1993) hence they have limited use (Tanksley et al. 1989). Morphological characterisation has been

used to identify duplicates, study genetic variation patterns and correlation with characteristics of agronomic importance. The use of morphological traits involve a lengthy survey of plant growth over time that is costly, labour intensive (Cock 1982) and vulnerable to environmental conditions. They must also be assessed during the fixed vegetative phase of crop development.

Dixon and Nukenine (2000) reported that the variation in traits observed do not reflect only the genetic constitution of the cultivar, but also the interaction of the genotype with the environment (G X E) within which it is expressed. In cassava breeding programmes emphasis has been on the collection and conservation of gene pools for characterization. The International Board for Plant Genetic Resources (IBPGR) descriptors have been used to characterize cultivars. A set of relatively stable morphological traits useful for characterization of cassava cultivars has been identified by IBPGR. The descriptors include qualitative and quantitative traits for cassava roots and shoot characters.

Morphological characters have been mainly used to classify the *Manihot* species (Hershey and Ocampo 1989; Elias et al. 2001). Due to the influence of different ecological environments on cassava morphology, morphological classification based on variable traits is complex. These descriptors measure traits of the shoot and root which include: colours of unexpanded apical leaves, mature leaf colour, tip shoot colour, height at first branching, length of petiole, stem colour, petiole colour, leaf shape, root shape, rind and pulp colour and many more (Gulick et al. 1983; Zacarias 1997; Okai 2001; Alves 2002; Benesi 2002; Nassar 2006; Amenorpe et al. 2006). There is extensive diversity for most characters assessed. They are either monogenic or polygenic traits (constant or variable respectively). The variable traits are associated with GXE interaction. Wanyera (1993) and Efisue (1993) have used morphological descriptors in cassava to access diversity among the *Manihot* species and within populations.

Results from studies using phenotypic markers have been useful to show the single gene control for leaf lobe width, root surface colour, albinism, stem collenchyma, stem growth habit, root flesh pigmentation and male sterility (Hershey and Ocampo 1989). It is reported that the phenotypic variance in cassava is higher than genotypic variance for traits of agronomic importance like tuberous root weight (Mathura et al. 1989). Studies on the phylogeny of *Manihot* carried out by Bertram (1993) showed a high degree of homoplasy in

many of the morphological characters. Reports from several studies have suggested the use of markers that are not environmentally influenced. It is therefore appropriate to use better and robust approaches to elucidate genetic diversity and differentiation in cassava.

Phenotypic markers still play an important role in classical plant breeding and complement the use of molecular tools. It is useful in the identification of specific markers linked to traits and QTL (Fregene et al. 2000; Akano et al. 2002; Mkumbira et al. 2002; Kizito et al. 2007; Okogbenin et al. 2002; 2003; 2007).

2.6.2 Molecular characterization

Advances in molecular biology have introduced DNA based procedures for cultivar identification and genetic improvement of food crops. Several DNA based markers that reveal polymorphism at DNA level (Kumar et al. 2000) have been developed for measuring similarity in agricultural crops. They have been proven to be powerful in the assessment of genetic variation within and between populations and the elucidation of genetic relationships among adapted cultivars (Lee 1995; Karp et al. 1996). DNA sequences show greater variation than amino acid changes in isozymes. The composition of DNA is also consistent between tissues and this is not affected by environmental changes (Beeching et al. 1993). Target genes in a segregating population can be identified with the help of DNA markers so as to shorten traditional breeding time (Thottappilly et al. 2000).

There are two major applications of DNA markers. The first is the extensive use of these molecular markers for the development of detailed genetic and physical chromosome maps in a variety of organisms among animal and human systems and among plant systems. Molecular markers in plant systems have been found very useful in conventional breeding by carrying out indirect selection through molecular markers linked to traits of interest. The second is the use of molecular markers for simple loci and QTL because the environment does not influence these markers and they can be used at all stages of plant growth. In addition to these two major applications, DNA markers can also be used in plant systems for germplasm characterization, genetic diagnosis, characterization of transformants, whole genome study and organization and phylogenetic analysis (Rafalski and Tingey 1993). Although each marker system has some merits and demerits, the choice of any marker system is dictated to a large extent by the intended application, convenience, cost and time

consideration, number of samples, how quickly the data is needed and the techniques that will best yield the maximum data (Ribaut and Hoisington 1998; Thottappilly et al. 2000).

Molecular markers can be broadly grouped into two: Firstly hybridization based DNA markers such as RFLP and oligonucleotide fingerprinting. Secondly, polymerase chain reaction (PCR) based DNA markers such as RAPDs, which can also be converted into SCARs, simple sequence repeats or microsatellites (SSRs), sequence-tagged sites (STS), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats amplification (ISA), cleaved amplified polymorphic sequences (CAPs) and amplicon length polymorphism (ALPs). Thirdly DNA chip and sequencing based DNA markers such as single nucleotide polymorphisms (SNPs). In addition to the above three groups of markers, there are derivatives. Microsatellite primed polymerase chain reaction (MP-PCR), arbitrarily primed PCR (AP-PCR), allele specific PCR (AS-PCR) and DNA amplification fingerprinting (DAF) have also proven useful in the detection of polymorphism (Thottappilly et al. 2000)

2.6.3 The PCR technique

PCR (Saiki et al. 1988) has been the basis of a growing range of new techniques for genome analysis based on the selective amplification of genomic DNA fragments. Williams et al. (1990) reported the use of PCR with short oligonucleotide primers of arbitrary (random) sequence to generate markers, the basis of RAPD. Welsh and McClelland (1990) also reported on arbitrary primed polymerase chain reaction (AP-PCR) while Caetano-Anolles et al. (1991) reported on DAF. The introduction of the PCR technique has revolutionalised standard molecular techniques and has allowed for the proliferation of new tools for detecting DNA polymorphism (Hu and Quiros 1991). The electrophoresis pattern of fragments generated by each primer for one isolate can be used as DNA fingerprints for assaying diversity (Tommerup et al. 1995). Polymorphism between two individuals is generally scored as a presence or absence (non amplification) of a particular DNA fragment. The absence may result from deletion of a priming site or insertion rendering the site too distant for successful amplification. Insertion can change the size of a DNA fragment without preventing its amplification (Williams et al. 1990). PCR is simple, fast, specific, sensitive and relatively low cost.

Advantages linked to molecular markers have made an immense contribution to cassava breeding and genetics. Areas covered include the development of genetic maps (Fregene et al.

1997; Mba et al. 2001; Okogbenin et al. 2006), the assessment of genetic diversity (Beeching et al. 1993; Lefevre and Charrier 1993; Bonierbale et al. 1997; Mignouna and Dixon 1997; Fregene et al. 2000), taxonomy studies (Second et al. 1997), understanding the phylogenetic relationships in the genus (Calvalho et al. 1993; Roa et al. 1997; 2000; Olsen and Schaal 2001), confirmation of ploidy (Lefevre and Charrier 1993; Fregene et al. 1994) and cultivar identification (Ocampo et al. 1992; Wanyera 1993; Laminski et al. 1997). When planning a molecular experiment, one of the most important decisions is the marker system and technique to be used. Molecular markers, which include biochemical (isozymes and storage proteins) and DNA markers, exist in every genotype and can be exploited to improve breeding programmes.

2.6.4 Isozyme markers

Isozymes are protein markers based on the use of naturally occurring enzymes that share a common substrate but differ in electrophoretic mobility. They are revealed when tissue extracts are subjected to electrophoresis in enzyme specific gels. The number and relative mobilities of various enzyme products with appropriate genetic analysis become transformed into single or multi-locus genotypes for each analysed individual. Isozymes were among the earliest markers used for plant analysis (Brewbaker et al. 1968; Mäkinen and Brewbaker, 1976). Wanyera (1993) demonstrated the usefulness of isozymes in confirming true hybrids in a cross between *M. glaziovii* and *M. esculenta*. Lefevre and Charrier (1993) detected genetic diversity among several cassava clones using isozyme markers. Based on the inheritance of the markers the study confirmed that cassava is a true diploid. Ocampo et al. (1992) used the esterase isozyme to fingerprint the cassava germplasm collection held at CIAT. Fregene et al. (1997) placed three isozymes markers on the cassava genetic linkage map developed at CIAT. Isozyme markers were used to develop a procedure for identifying cassava varieties (Ramirez et al. 1987). The main limitation of isozyme markers is that only a few gene products can be revealed. They are difficult to work with due to a limited amount of polymorphism, low levels of reproducibility (since they are influenced by tissue type and developmental stage of the plant(Zacarias 1997) and are unevenly distributed throughout the genome (Neilsen and Scandalios 1974). Nevertheless, isozymes have been successfully applied in cassava.

2.6.5 DNA based markers

A DNA marker is basically a small region of DNA showing sequence polymorphism in different individuals within and between species (Liu, 1998). DNA fingerprinting is a technique, which has been widely adopted to differentiate among organisms at the species and subspecies levels (McClean et al. 1994). The techniques used for the cultivar identification are designed to detect the presence of specific DNA sequences or combination of sequences that uniquely identify the plant. Cultivar identification can be achieved more accurately using DNA fingerprinting data, especially materials characterized by high genetic variation between cultivars. The most closely related cultivars are usually distinguished with the DNA fingerprinting methods (Beckman and Soller 1986). Another advantage of DNA fingerprinting over morphological markers is the dominance and the absence of environmental effects. The application of DNA fingerprinting could be very valuable in the identification of cultivars and species, and could help to create more efficient breeding programmes through the detection of genetic linkages between DNA fingerprinting described in humans, animals and plants allowing the identification of different individuals, genotypes and species (Lin et al. 1993).

These markers are based on the enormous variation or polymorphism in DNA sequences of organisms. DNA markers eliminate the limitations in genome investigation using morphological and isozyme markers, such as gene expression and environmental interaction, heritability, and low map resolution (Vogel et al. 1996). In addition to identifying and discriminating closely related cultivars, DNA markers can be applied in assessing taxonomic and phylogenetic relationships, pedigree analysis and linkage mapping. DNA-based marker systems can be used for indirect selection of tagged loci affecting qualitative or quantitative traits and to monitor loci during introgression or selection programmes, thus reducing the number of backcross generations (Baird et al. 1996). PCR based fingerprinting involves the *in vitro* amplification of particular DNA sequences using specific or arbitrary primers and a thermostable DNA polymerase.

DNA markers vary in level of detected polymorphism and the amount of information generated. The most informative DNA markers are characterised by high polymorphism information content (PIC), indicating relatively large numbers of alleles with similar frequencies in each locus (Botstein et al. 1980). Polymorphism may be a result of single site alterations due to mutations, which abolish or create a restriction or primer binding site,

and/or insertions, deletions, or inversions between two restriction and primer binding sites. As a result the level of polymorphism may be low, or it may be due to a variable number of tandem repeats resulting in a marker system with high levels of polymorphism that can detect variation between closely related relatives. Markers that detect single site alterations include RAPDs, AFLPs and SSRs.

Restriction Fragment Length Polymorphism (RFLP)

The first DNA markers to be used were fragments produced by restriction enzyme digestion. Restriction fragments from a given chromosome locus often vary in size in different individuals. The differences are what are referred to as RFLP markers (Botstein et al. 1980; Wyman and White 1980). The development of RFLP technology represented an important contribution to breeding programmes (Burr et al. 1983; Young et al. 1988). RFLP was developed in the 1980s to overcome problems encountered with isozymes and phenotypic markers (Botstein et al.1980; Helentjaris et al. 1986). Since RFLPs represent the entire genome and are both co-dominant and multi-allelic (Brettschneider 1998), RFLPs have been and are still used in cassava. The RFLP technique generates relatively high levels of detectable loci and alleles, it is not sensitive to environmental factors, and can be used at any developmental stage of the organism (Kelley 1995). This has allowed the extensive use of RFLP analysis in genetic studies (Tanksley et al. 1989), in the exploration of evolutionary relationships among different species (Song et al. 1990) and populations (Bonierbale et al. 1997; Miller and Tanksley 1990), for identification of genotypes (Smith et al. 1990; Melchinger et al.1991; Livini et al. 1992), and for mapping genes that control quantitative as well as qualitative traits (Beavis and Grant 1991). The most important advantage of RFLP markers is that they are co-dominantly inherited, being able to distinguish between homozygous and heterozygous loci (Rafalski and Tingey 1993). RFLP markers have contributed to DNA marker technology in cassava. Angel et al. (1993) initiated work on a detailed genetic map of cassava for tagging agronomically important traits and to clone cassava genes.

Fregene et al. (1997) constructed a linkage map using 132 RFLP, 30 RAPD, three microsatellite and three isozyme markers from a heterozygous female parent of an inter-specific cross. The map consisted of 20 linkage groups spanning 931.6 cM. A second map was constructed from the segregation of 107 RFLP, 50 RAPD, one microsatellite and one isozyme marker from the male parent. RFLP has been used to assess the genetic diversity

within cassava and between *Manihot* species. Beeching et al. (1993) assessed the genetic diversity within a collection of cassava germplasm using RFLPs and recommended the use of RFLPs in the genetic diversity analysis within collections of cassava. Beeching et al. (1994) compared RFLPs and RAPDs in assessing genetic diversity within cassava and between *Manihot* species and found that RFLPs and RAPDs were comparable in revealing genetic diversity but that at least 30 probes or primers should be used to achieve these relationships. RFLPs have been applied in studies of analysis for phylogenetic relationships of species within the genus *Manihot* (Haysom et al. 1994). The disadvantages of RFLP analysis are that it is time consuming, costly, labour intensive (Marsan et al. 1993), and requires specific probes (Tommerup et al. 1995). The complexity in performing RFLP analysis, coupled with the widespread use of short lived radio-isotopes, has led to its limitation for routine application in large scale crop improvement programmes (Yamamoto et al. 1994). In addition, the RFLP technique requires a substantial amount of DNA and involves special manipulations to come up with pure DNA which requires high levels of expertise and skill (Beeching et al. 1994).

Random Amplified Polymorphic DNA (RAPD)

RAPD primers usually have 10 bases that are used to amplify unknown and arbitrary regions of a genome. The short sequences of the primers allow a multitude of possible primer binding sites throughout the genome. Efficient amplification of DNA fragments may occur when two primer-binding sites occur in close proximity. RAPD markers are dominant since the polymorphism is detected as a failure of one allele to amplify due to mutations in the primer-binding site (Williams et al. 1990; Welsh et al. 1991) or due to size differences of the amplified fragments due to insertions or deletions. RAPD markers have been the most extensively used markers in cassava biotechnology, especially in determination of phylogenetic relationships and genetic diversity in *Manihot* species (Marmey et al. 1994; Laminski et al. 1997; Schaal et al. 1997). The RAPD methodology is simple, rapid and requires only small amounts of DNA. Michelmore et al. (1991) were the first to report three RAPD markers linked to major disease resistance genes using contrasting DNA bulks composed of F_2 individuals of known genotype.

Marmey et al. (1994) demonstrated genetic diversity among African cassava accessions using RAPD markers. Mignouna and Dixon (1997) demonstrated genetic differences among several African landraces with varying levels of resistance to CMD using RAPD analysis.

Zacarias et al. (2004) assessed genetic diversity of cassava germplasm from Mozambique using RAPDs. Results showed that the cassava germplasm had wide genetic diversity, and accessions did not group according to geographical distribution. Raji et al. (2001) assessed the diversity of 500 African landraces of cassava using RAPD and AFLP analysis. Results showed that both markers provided similar genetic relationship of the population. However, the AFLP technique detected a much higher level of polymorphism giving a better diversity structure than RAPD analysis. However, reproducibility of RAPDs between runs and/or laboratories is a problem (Weeden et al. 1992). The homozygous presence of a fragment is not distinguishable from its heterozygote, since polymorphisms detected by RAPDs are inherited in a dominant fashion (Williams et al. 1990; Welsh et al. 1991). Buso et al. (1994) suggested that the RAPD technique had limitations for use in cassava, due to cassava's high level of heterozygosity.

Microsatellite or simple sequence repeats (SSR)

Simple sequence repeats or microsatellites are usually tandem repeats of short (2 - 6) sequences such as (GT)n or (TA,CA,GTG,TAA,GATA). Hamada et al. (1982) demonstrated the large number and widespread occurrence of short tandem repeats in eukaryotic genomes. This finding was verified by Tautz and Renz (1984). The first report of SSR in plants was by Condit and Hubbel (1991), who suggested their abundance in the plant system. Later Akkaya et al. (1992) reported on the length of sequences and polymorphisms of SSR in soya bean. Simple or short sequence repeats, also known as short tandem repeats or microsatellites, can be used to generate polymorphisms because of the frequent variation in the length of the repeat regions. The fragment polymorphisms are due to variation in the total sequence length as determined by the number of repeat units. Such differences are detected on polyacrylamide or agarose gels, where repeat lengths migrate different distances according to size (Robinson and Harris 1999). SSR primers are developed in three ways, firstly from closely related species (Provan et al. 1996; White and Powel 1997), secondly by a search from sequenced databases (Bell and Eker 1994) and thirdly, by screening cDNA or small insert library with tandemly repeated oligonucleotides and sequencing candidate clones (Powell et al. 1996). Although the procedure for obtaining microsatellites is laborious and expensive, their conversion to PCR markers allows screening of large numbers of alleles at defined loci.

SSRs have a high level of allelic diversity as a result of the variable number of repeat units within their structure, making them valuable as genetic markers (Hamada et al. 1982;

Morgante and Olivieri 1993). SSRs are often multi-allelic and can be multiplexed and automated for high-throughput genotyping. SSR analysis is easy and convenient to exchange between laboratories (Powell et al. 1996; Chen et al. 1997). SSR markers are co-dominant and heterozygotes can be identified. This makes SSRs a suitable option for mapping and molecular characterisation of cassava, given the fact that cassava is highly heterozygous (Agyare-Tabbi et al. 1997; Chavarriaga-Aguirre et al. 1998). Microsatellite marker loci were developed for various crops, such as maize (Taramino and Tingley 1996), wheat (Devos et al.1995), barley (Russell et al. 1997) and potato (McGregor et al. 2000). CIAT identified 186 SSR markers for cassava (Chavarriaga-Aguirre et al. 1998; Mba et al. 2001).

The SSR technique has been used extensively in various studies in cassava (Chavarriaga-Aguirre et al. 1998). The authors isolated and characterised 14 highly heterozygous G A rich microsatellite DNA regions in cassava. A total of 521 accessions from the cassava core collection at CIAT were successfully screened for genetic diversity with SSR primers (Chavarriaga-Aguirre et al. 1999). The study revealed between-country allele number and frequency variation, which agreed with between-country allele size variations at the same loci. Unique alleles were present in countries such as Brazil, Colombia and Guatemala. Cassava microsatellites were used to assess genetic diversity among cassava accessions and between cassava and its wild relatives (Roa et al. 2000). Fregene et al. (1997) placed 77 SSR markers on the cassava linkage map. Mba et al. (2001), developed and characterised 172 new SSR markers and placed 36 of these on the cassava linkage map. An SSR marker linked to CMD resistance was identified with the aid of Bulk Segregant Analysis (BSA) (Akano et al. 2002). Fregene et al. (2001) assessed the SSR diversity at 67 unlinked loci in 303 accessions of cassava land races from Tanzania, Nigeria, Brazil, Colombia, Peru, Venezuela, Guatemala, Mexico and Argentina. Results revealed that more than 90% of the loci were polymorphic in all samples and estimates of genetic diversity and differentiation ranged widely from locus to locus. It was observed that factors that contributed to differences in allele frequency at SSR loci in this predominantly vegetatively propagated crop were spontaneous recombination and selection (Fregene et al. 2001). An SSR cassava genetic map from F_2 cassava progeny has been developed (Okogbenin et al. 2007).

The genetic map constructed from F_1 crosses of non-inbred parents was used with SSR, RFLP, AFLP and EST markers to map genes for cassava bacterial blight resistance. Furthermore nine QTL located on linkage groups were identified to explain the pathotypic

response of cassava to *Xanthomonas*. Mkumbira et al. (2001) and Peroni et al. (2007) used SSR markers to study the traditional way farmers classified cassava varieties as sweet/cool (safe for direct consumption) and bitter (toxic and needs to be processed before consumption). Results showed that farmer's classification into bitter and sweet cultivars corresponded to genetic subdivision with differences in cyanogenic glucoside levels. The necessity to differentiate between bitter and cool based on cyanogenic glucoside levels seem to have influenced the genetic structure of cassava in the areas studied in Malawi (Mkumbira et al. 2001).

Hurtado et al. (2008) assessed the superiority of SSR in cassava genetic diversity study to DArTs. The authors found SSR loci markers more polymorphic than the DArTs. This observation could be due to the fact that the DArTs were designed from cassava and its wild relatives, hence its low sensitivity. Kawuki et al. (2009) reported on the efficiency of SSRs in a diversity study, where SNP markers did not discriminate the accession as much as the SSR, and more SNPs would be required for efficiency. In general, SSRs have been used to assess the genetic diversity in cassava (Beeching et al. 1993; Mkumbira et al. 2003; Elias et al. 2000; 2001; Benesi, 2002; Fregene et al. 2003; Zambrano et al. 2007; Hurtado et al. 2008; Kawuki et al. 2009). Studies on the genetic diversity of some cassava cultivars in Ghana has been done based on phenotypic and molecular markers (Okai 2001; Asante and Offei 2003), SSR markers are robust and polymorphic and have been used for genetic diversity studies of cassava. Molecular analysis matrices are very informative tools for estimation of genetic distances (Vieira et al. 2007)

Amplified fragment length polymorphism (AFLP)

AFLP is based on PCR amplification that uses selective restriction fragments generated by specific restriction enzymes (Vos et al. 1995). AFLP involves digestion of genomic DNA two restriction endonucleases, followed by ligation terminal of adapter sequences to generate template DNA for PCR amplification. Separate adapters are needed for each of the different restriction enzymes. A large number of restriction fragments are generated which are selectively reduced by PCR primers modified by adding two to three selective nucleotides. PCR products are separated on polyacrylamide gels (Vos et al.1995; McGregor et al. 2000).

The AFLP technique can be used for DNA of any origin and complexity. It does not require any prior sequence knowledge before using a set of primers. The number of fragments

generated in a single reaction can be regulated or tuned by selective nucleotides. AFLP analysis is robust and reliable because stringent reaction conditions are used for primer annealing. Fingerprint profiles can be used to distinguish between closely related organisms, including near isogenic lines and allows scoring for very large numbers of markers in a given population (Vos et al. 1995; Winter and Kahl 1995; Powell et al. 1996; Blears et al. 1998).

AFLP is highly sensitive for DNA fingerprinting. It has been used in genome mapping for constructing high density genetic maps of genomes or genome fragments for bridging the gap between genetic and physical maps. Many mapping studies have applied AFLP markers, in sorghum (Xu et al.1994), Oryza (Liakat Ali 1999), tef (Bai et al. 1999), Solanum (Bradshaw et al. 1998) and Zea (Xu et al. 1999). AFLP is described as an efficient way to generate a large number of markers that are linked to target genes (Xu et al. 1999). Lin et al. (1996) found the AFLP technique to be the most efficient in detecting polymorphism in soybean in comparison with three different DNA mapping techniques, RFLP, RAPD and AFLP. It is highly reproducible, rapid in generating high frequency of identifiable polymorphisms. It is also used for identifying polymorphisms and for determining linkages in segregating populations (Vos et al. 1995; Winter and Kahl 1995; Powell et al. 1996; Blears et al. 1998).

The AFLP technique has been applied in various cassava studies. Bonierbale et al. (1997) assessed the genetic diversity of 105 genotypes using AFLP analysis to estimate genetic similarities among taxa and evaluated intra- and inter-specific variability. Results showed individuals grouped according to prior taxonomic classification. The germplasm presented a narrower range of variation than most wild species. Some wild species showed specific bands which could be useful for identification and classification of germplasm and introgression studies (Bonierbale et al. 1997).

Second et al. (1997) assessed the numerical taxonomy and genetic structure of 358 clones representing the geographic and ecological range of distribution of *Manihot* species along with classical botany and ecology. AFLP analysis was used to characterise the genetic structure of cassava in relation to its wild relatives and to elucidate the domestication process of cassava. Genetic diversity of cassava itself was high, but the diversity was narrow in a single Amazonian field. Although domestication appeared to have evolved primarily from *M. esculenta* ssp. *flabellifolia* and *peruviana,* it seemed that some other species also contributed.

Results suggest the importance of genetic recombination at the origin of the diversity of cassava, which was postulated as being a favourable perspective for various strategies of genetic mapping and gene tagging since cassava is vegetatively propagated.

Mapped AFLP and SSR markers were used as evidence of introgression (Morillo et al. 2001). Results showed that AFLP and SSR bands that appeared in some varieties of cassava and not in *M. esculenta* ssp. *flabellifolia*, the presumed ancestor of cassava, were considered as introgressed bands. This study showed evidence of introgression from *M. glaziovii* in some genotypes. Narváez-Trujillo et al. (2001) used AFLP and SSR markers to study the traditional cassava varieties from various Amerindian communities from French Guiana and Ecuador. They found that recently bred varieties tended to be hybrids derived between sweet and bitter varieties.

AFLPs, just like RAPDs are usually scored as dominant markers. The technique is more reliable than RAPDs (Vos et al.1995), yet more laborious and time consuming (McGregor et al. 2000; Powell et al. 1996).

2.7 Cassava breeding

2.7.1 Classical breeding for yield

Efforts to improve cassava yield and other traits of interest are generally not geared towards the highest possible yield under favourable conditions, but rather towards obtaining stable yields and durable traits in marginal conditions where cassava is grown at present and is likely to expand in future (Cock 1984; El-Sharkawy 2003). High yield is achieved firstly by selecting plants that have both a genetic and a plant structure which maximises performance, and secondly by adding resistance or tolerance to factors which limit yield (Ellis et al. 1982). Hybrid vigour through heterozygosity is the main requirement for the genetic structure of new varieties and a major objective of breeding programmes (Nassar et al. 2004). Conventional cassava breeding and selection starts from crosses, or open pollinated seeds, to the nursery, to clonal evaluation trials to preliminary yield trails then to advanced yield trial, uniform yield trials to multi-locational testing and on farm testing towards release (Jennings and Iglesias 2002; Kawano 2003). This process takes between eight to ten years before a variety is released. Over the years efforts have been made to reduce the number of years required for breeding cassava varieties. Modern molecular tools have been used and participatory breeding is introduced at an earlier stage of the breeding cycle. Some of the

stages such as the UYT, AYT, multi-locational trial and on farm testing could be done concurrently (Dixon et al. 2007).

2.7.2 Modern cassava breeding

Classical breeding methods have produced large advances in root yields of cassava. Jennings and Hershey (1985); Hershey (2005) reported improvements of over 200% during the period from 1976 - 1990 at CIAT, and remarkable advances at IITA (Jennings and Iglesias 2002). However, the rate of improvement in average national cassava yields in the most important producing countries has not paralleled progress at experimental level, except for some Asian countries (Kawano 1978).

Some progress in understanding the inheritance of agronomic traits in cassava has been achieved and articles regarding the inheritance of quantitative traits have been published (Easwari Amma et al. 1995; Easwari Amma and Sheela 1998; Calle et al. 2005; Jaramillo et al. 2005; Cach et al. 2006). To this end, cassava is unique because a molecular map has already been developed (Fregene et al. 1997; Mba et al. 2001; Okogbenin et al. 2006) and yet it is complemented with limited knowledge regarding traditional genetics. CIAT cassava breeding programmes are engaged in projects to provide some relevant information on yield related attributes across different agro ecological zones (Ceballos et al. 2004).

Progress in cassava improvement

The potential to increase cassava yields through genetic improvement has been demonstrated with considerable progress and success (Hahn et al. 1980; IITA 1982; 1993; Ceballos et al. 2006). However, despite the proven record in cassava improvement, many challenges remain. Lawson (1988) noted that cassava genotypes find optimum physiological expression of their genetic potential within narrow ranges of biophysical conditions. Cock (1987) and El-Sharkawy (2003) found that few cassava cultivars were stable over a wide range of ecological conditions. There exists growing consensus that stable productivity in cassava depends on a number of factors acting synergistically: abiotic factors (soils, temperature, photoperiod and latitude), biotic factors (diseases, pests and nematodes) and management practices (Allem and Hahn 1991). Genetic control mechanisms and environmental influences on important characteristics of cassava are largely unknown. Carter et al. (1992) reported that 19% of cassava in Africa is found in mid-altitudes where trends in socio-economic and physical environment favour increased cassava production. This has stimulated considerable interest

in increasing cassava production within this ecology since earlier research focused on the lower altitudes of the tropics where cassava finds its most suitable growth environments (IITA 1993; FAO 1996). Cooper and Hammer (1996) suggested that the analysis of variation in plant adaptation is linked with understanding environmental factors that influence the differential yield performance of genotypes. Understanding the nature of the influence of the environment is therefore a critical component of improving efficiency of plant breeding programmes.

2.7.3 Marker Assisted Selection/Breeding (MAS/MAB)

Marker assisted selection (MAS) is the use of DNA and other markers for selection of desired individuals within a population, by linking the molecular marker to a desired trait. The closer the marker is linked to a desired trait, which often is a QTL, the more useful it becomes. Molecular markers are now well integrated into many breeding programmes. These new technologies can accelerate the development of new varieties, the introduction of new traits, or the inexpensive screening of large populations (Ribaut and Hoisington 1998; Brennan and Martin 2007). These advances in molecular breeding has offered great progress in crop improvement aided by new biotechnology tools such as genetic linkage map construction (Bertrand et al. 2008). Genetic linkage mapping is used for localizing and isolating both simple and complex traits. Molecular markers placed on genetic maps allow the development and efficient use of indirect selection schemes for germplasm improvement, thereby increasing precision in the manipulation of both qualitative and quantitative traits (Stuber and Edwards 1986; Baird et al. 1996; Bertrand et al. 2008). This is the basis for MAS, where markers closely associated with traits of interest can be used to introgress a specific gene(s) of interest into a desired background (Taylor et al. 2004). Genetic linkage maps can provide a more direct method for selecting desirable genes via their linkage to easily detectable molecular markers (Tanksley et al. 1989). Once a trait is identified and mapped, MAS could be used to introduce the trait into a wide variety of populations. It has been used to reduce large population sizes, several continuous recurrent testing and the time required to develop a superior line. In cassava the application of MAS has been developed more recently compared to other staple crops.

The use of MAS in cassava has become possible with the construction of genetic linkage maps using RLFP, isozymes and SSR markers (Fregene et al. 1997; Mba et al. 2001; Okogbenin et al. 2007). Cassava genetics has used information from the cassava genetic map,

even with its low saturation of loci and randomly distributed marker loci over linkage groups. Cassava mosaic disease (CMD) is the main biotic constraint in cassava production in sub-Saharan Africa. Over six CMD viruses are reported from Africa (Legg et al. 1999; Thottappilly et al. 2006).Viruses are particularly important in vegetatively propagated crops like cassava since they become virus-infected during year to year propagation. Eighteen different viruses have been reported in cassava (Calvert and Thresh 2002). MAS has been so useful and continues to be so with the improvement of the cassava genetic map. Genes for resistance to CMD have been mapped, including a major one (CMD2) (Akano et al. 2002). MAS for breeding CMD resistance has been successfully applied for introducing resistance into elite gene pools at CIAT (CIAT 2003; Fregene and Mba 2004) and introgression of resistance to cassava green mite (CGM) and CMD in local Tanzanian varieties (Kullaya et al. 2004) and Ghanaian landraces. New sources of CMD resistant genes are being looked for and others are ready for validation (Thottappilly et al. 2006; Okogbenin personal communication). The potential use will be to pyramid the useful genes into elite cassava clones. Fregene et al. (2007) reported on effective use of MAS in common beans and in cassava. MAS would be a routine activity for classical breeders with time.

2.7.4 Participatory plant breeding

Participatory plant breeding is the set of approaches that apply in situations where clients need different varietal traits, and are involved in the development process. It is poorly understood and difficult to diagnose with conventional market research methods. Indeed farmers have been doing informal breeding for a long time and their selections are the progenitors of most crops planted in modern times (Harlan 1992). Plant breeding exploits existing variability, generation manipulation and combination of new variability into plant forms most useful to humans. This has been done by farmers for thousands of years as food production is the oldest profession of humanity. Although early ‘plant breeding’ was developed essentially as an art, the scientific basis became well established with the discovery of laws of genetics and the applications of principles of genetics to crop improvement. Although farmers essentially were unschooled, they intuitively looked for, skillfully recovered and successfully propagated genetic recombinants that exhibited desirable traits (Jauhar 2006).

Cassava is an out crossed crop (Allard 1960) and vegetatively propagated. Spontaneous crosses do occur during its growth phase. Cassava seeds are produced in the fields before

cultivated plants are harvested. The buildup of seeds in the field seed bank, eventually germinate and produce volunteer seedlings from which farmers select and add to their cultivated genotypes (Elias et al. 2001; Kizito et al. 2007; Manu-Aduening et al. 2005; Mkumbira et al. 2003; Nassar 2006; Peroni et al. 2007; Pujol et al. 2002; 2007).

In a study on the patterns of genetic diversity, results suggested that incorporation of volunteer seedlings leads to increase in intravarietal genetic diversity (Elias et al. 2000; Pujol et al. 2007). Volunteer seedlings originating from seeds may confer important agronomic advantage. Systemic pathogens are usually not transmitted by seed, seedlings often are relatively free of viral and other pathogens that tend to accumulate in vegetatively progagated plants, and seedlings may supply healthier cuttings (Pujol et al. 2002; Rocha et al. 2008). The seed bank in the soil increases diversity at a local scale, the exchange of locally adapted materials and acts as a buffer to respond to catastrophic crop failure, where a fresh stock of clones is regenerated. The seed bank increases genetic diversity (Pujol et al. 2007).

This informal breeding over many years have made farmers skillful in selecting varieties with preferred attributes. Most scientists have gone to farmers for indigenous knowledge on the cultivation of many crops (Francis 1990; Dapaah et al. 2003; Manu Aduening et al. 2006). It has been observed that technologies that have been developed with little farmer participation have low adoption (Nweke 2004; Manu-Aduening et al. 2005; Zacarias et al. 2004). Cassava breeding with farmers in a participatory approach has been very successful. Farmers' perception has been different from that of breeders and their priorities too (Manu-Aduening et al. 2005). Kizito et al. (2007) indicated that farmers used some stable morphological traits like height at first apical branching, petiole colour and culinary attributes such as taste to differentiate and name varieties. Also, farmers adopted improved varieties when there was a nearby market, high CMD incidence and good extension service. Farmer participatory breeding to enhance technology adoption is vital for cassava improvement (DeVries and Toenniessen 2001; Ssemakula et al. 2001; Dixon 2003). Farmer participation at an early stage has been proposed to 'fast track' or accelerate the cassava improvement cycle (Dixon et al. 2007).

2.8 Statistical designs and calculations

2.8.1 Diallel designs

The diallel design is an important tool in plant breeding aimed to improve yield and other parameters. Diallel crosses are commonly used to study the genetic properties of inbred lines in plant and animals breeding experiments. The concept of diallel design was first introduced by Schmidt in animal breeding in 1919 (Pirchner 1983). Later, Sprague and Tatum (1942) introduced it in the field of plant breeding by making all possible matings among a set of maize inbred lines. It has gained more attention and has been subjected to more theoretical and practical application than any other mating design (Wright 1985). The concept was later redefined by Sughrue and Hallauer (1997), as making all possible crosses among a group of genotypes.

Diallel is the most popular method used by breeders to obtain information on the value of the varieties as parents, to assess the gene action involved in the various characters and thereby develop appropriate selection procedures and understand heterotic patterns of progenies at an early stage of hybridisation programmes (Dikerson 1969; Le Gouis et al. 2002; Egesel et al. 2003). Diallel mating designs permit the estimation of the magnitude of additive and non additive components of the heritable variance (Griffing 1956; Mather and Jinks 1977). Data obtained from such cross combination is useful and can be analysed in several ways, but the most commonly used are as proposed by Hayman (1954) and Griffing (1956). Based on this, a test of validity is calculated on the additive and dominance components of heritability from the mean squares of these mating designs (Hayman 1954; Mather and Jinks 1977). The diallel mating design has been specifically designed to investigate the combining ability of parents and to identify superior parents for use in hybrid and cultivar development.

2.8.2 North Carolina design II

Another mating design that is similar to the diallel is the North Carolina design II (NCD II). Both designs provide genetic interpretations including combining abilities and on the inheritance of quantitative traits (Kang 1994). The NCD II mating scheme is a cross-classification design that was first proposed by Comstock and Robinson (1948). It differs from the diallel in that different sets of parents are used as males and females. It accommodates more parents in determining combining abilities than a diallel and provides the same type of genetic information (Hallauer and Miranda 1988). Main effects of males and females are equivalent to general combining ability (GCA) and the female x male interaction

is equivalent to specific combining ability (SCA) (Calle et al. 2005; Jaramillo et al. 2005; Cach et al. 2006). Both the diallel and NCD II mating designs have been used to obtain genetic information on morphological and agronomical traits of importance in cassava (Hahn et al. 1989; Rajendran 1989; Amma et al. 1995; Calle et al. 2005; Jaramillo et al. 2005; Cach et al. 2006). Rajendran (1989) reported additive gene action for storage root yield and non additive gene action for yield components (HI, storage root number and storage root weight). Amma et al. (1995) reported that root quality traits, namely starch, dry matter (DM) and hydrogen cyanide (HCN) content are predominately non-additive. It has been extensively used in rice improvement (Gravois 1994; Zhou et al. 2003).Rajendran (1989) suggested that selection of parents based on their *per se* performance is not reliable in breeding for root yield in cassava, it is necessary to estimate the combining abilities of parents before formulating specific breeding programmes. Specifically, diallel crosses were devised to investigate GCA of parents and to identify superior parents for use in hybrid and cultivar development (Ortiz et al. 2001; Yan and Hunt 2002).

2.8.3 Combining ability

Combining ability is defined as the performance of hybrid combinations (Kambal and Webster 1965). It plays an important role in the selection of superior parents for hybrid combinations and studying the nature of genetic variation (Duvick 1999). Griffing (1956) proposed a method to analyse combining ability by using the genetic estimates of the parents and the hybrid components of diallel analysis, represented by the GCA and SCA. Sprague and Tatum (1942) introduced the concepts of GCA and SCA. GCA describes the average contribution of the lines in the hybrid combination and consists of additive epistatic variances (Matzinger 1963). Parents with good combining ability for specific characters may be useful in a hybridization programme for improvement of that trait (Woldegiorgis 2003). SCA is where certain hybrid combinations do relatively better or worse than would be expected on the basis of the average of the performance of the lines involved. It is the deviation to a greater or lesser extent from the sum of GCA of the parents. SCA consist of dominance and all types of epistatic variances and is regarded as estimates of effects of non-additive gene actions (Falconer and Mackay 1996).

The relative amount of improvement to be obtained from GCA and SCA will be proportional to their variances. It estimates the type of gene action which controls a particular trait. The ratio has been studied as an indicator of the nature of genetic variability in diallel analysis

(Sayed 1978; Quick 1978). Thus the relative sizes of mean squares (GCA:SCA ratios) have been used to assess the relative importance of GCA and SCA. High value of the ratio indicates the performance of additive genes in determining a particular trait. The closer the ratio is to the unit (1), the greater is the magnitude of additive genetic effects.

There has been very little progress on understanding the inheritance of traits with agronomic relevance in cassava (Easwari Amma et al. 1995; Calle et al. 2005) and few papers regarding the inheritance of quantitative characteristics have been published (Easwari Amma et al. 1995; Jaramillo 2005; Calle et al. 2005; Cach et al. 2006) despite the molecular map that has already been developed (Fregene et al. 1997; Mba et al. 2001). Lokko et al. (2005) concluded that GCA was more important in controlling CMD resistance among the crosses made. Cach et al. (2006) concluded that dominance plays an important role in the case of harvest index and fresh root yield but had relatively little importance in some traits such as dry matter content and height at first branching.

2.8.4 Heterosis or hybrid vigour

Agronomist and animal breeders have long known that crossbreeding or hybridisation of two lines often gave positive fitness-related effects in the F1 progeny (Darwin 1859; 1876; Turnton 1981; Sprague 1983). The phenomenon has been known for more than a century (Shull 1908) and has been exploited in animal and plant breeding over the years. There are several definitions of heterosis. An F_1 performance that exceeds the average parental performance is generally referred to as hybrid vigour or heterosis (Shull 1952). Heterosis is the increased vigour, size, fruitiness, speed to develop, resistance to disease and pest or to climatic vigour of any kind. Hartl and Clark (1997) defined it as a phenomenon of enhanced hybrid performance. However, there are two predominant theories of heterosis called dominance and over-dominance hypothesis (Crow 1952). Heterosis under the dominance hypothesis is produced by masking of deleterious recessive alleles in one strain by dominant or partially dominant alleles in the second strain, whereas, under the over-dominance hypothesis it is due to the heterozygous superiority and therefore, increased vigour is proportional to the amount of heterozygosity (Lamkey and Edwards 1999; Lamkey et al. 1995). Wricke and Weber (1986) noted that there are several hypotheses to genetically explain this phenomenon: a) Partial dominance of the large number of loci, b) over dominance of several loci, c) several types of epitasis. The authors indicated that for hybrid breeding a substantial number of the loci should show dominance.

Heterosis results from combined action and interaction of allelic and non allelic factors and is usually closely and positively correlated with heterozygosity (Burton 1968). Heterosis is brought about by bringing together in the F1 the dispersed genes of dominant alleles showing directional dominance and non-allelic interactions, but not by heterozygote superiority or complementary epitasis. Coors et al. (1999) showed that interaction within loci (dominance) and among loci (epitasis) are the major genetic factors in the exploitation of heterosis. If the population crossed does not differ in gene frequency there will be no heterosis (Kawano 2003).

Heterosis can be expressed as mid-parent, better-parent and standard heterosis. Mid-parent heterosis or hybrid vigour is defined as the difference between the hybrid and the mean of the two parents (Falconer and Mackay 1996). Lamkey and Edwards (1999) noted that the mid-parent heterosis or performance of F1 hybrids over the better-parent is preferred in some circumstances, particularly in self pollinated crops, for which the goal is to find better hybrids than either of the parents. It has been observed that out crossing does not always increase fitness. The heterosis observed in the F_1 population could have much of it lost in the F_2 generation due to recombination loss (Dikerson 1969). In some cases the F_2 progenies are less fit than the members of the parental lines. A cross between different species or distantly related populations can lead to complete loss of viability or fecundity (Barton and Hewitt 1981).The gene interaction requires an optimum degree of out crossing to take advantage of hybrid vigour or heterosis (Lynch and Walsh 1998; Ceballos et al. 1998).

Heterosis in plant breeding has been exploited extensively over the years (Duvick 2001). Duvick (1999) and Jauhar (2006) reported a steady increase in grain yield for major cereal crops such as wheat, maize, sorghum, pearl millet by reason of heterosis. Vasal et al. (2006) indicated that exploitation of heterosis continues to be the most appropriate means to increase grain yield relatively rapidly. Hybrid rice (*Oryza sativa* L.) is grown extensively in China and India (Brar and Khush 2006). Heterosis is used for large increases in production per unit area, thus making available large acreages of land for other uses. It is remarkable that in 2001 more than 70 hybrids were under cultivation on 6 million hectares of the total of the 10 million hectares of pearl millet area in India (Jauhar 2006). Few studies on the inheritance of quantitative and qualitative traits of cassava have been reported (Easwari et al. 1995; Easwari and Sheela 1998; Pérez et al. 2005; Chavez et al. 2005). Unikrishnan et al. (2004) assessed hybrid vigour for root yield over better-parent values and root yield performance was

associated with heterosis for yield components. Further studies by the same authors on the nature and magnitude of heterosis in cassava, showed that the hybrids displayed substantial differences in their heterotic response. Heterosis over mid-parents and better–parents values were observed in all traits studied (Unikrishnan et al. 2004).

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CHAPTER 3

GENETIC DIVERSITY ANALYSIS OF GHANAIAN CASSAVA GENOTYPES USING SSR MARKERS

Abstract

A total of 320 cassava landraces assembled from all 10 regions of Ghana were analysed with 33 SSR markers to assess the genetic diversity in the germplasm. The genetic distance, based on the proportion of shared alleles (PSA), was obtained from the raw allele size data. Distances between the accessions were subjected to principal component analysis (PCA) to obtain a structure of relationship between the landraces. The genetic diversity and differentiation were calculated from allelic data. The average number of alleles for each locus was close to five and ranged from three to seven. The probability that two randomly selected alleles in a given accession are different, also known as average gene diversity, was 0.5245±0.0045. Average gene diversity was comparable across all regions with the exception of the central region in the coastal savannah zone. Genetic diversity parameters, including total heterozygosity (Ht) and genetic differentiation (Gst) ranged widely across the 33 markers. Genetic differentiation, as estimated by F_{ST} (theta), was very low for samples between regions, an overall value of 0.04 was recorded. The genetic diversity and differentiation revealed clustering that may represent heterotic groups. Sixty three duplicates or related genotypes were identified. The overall heterozygosity was high, with a low level of differentiation. A loose structure was observed in the collection, but there was a unique sub-structure in the landraces from the upper regions of Ghana, the arid and semi arid climatic zones. The heterotic groupings were distinct for the regions in the savannah ecological zones and the forest ecological zone regions. Parents could be selected from the groupings for crosses to test for heterotic patterns and combining ability.

3.1 Introduction

An understanding of genetic diversity is the first step to harness the genetic variability in germplasm (Hurtado et al. 2008). The success of a breeding programme depends greatly on the genetic diversity that exists in available germplasm (Meredith and Bridge 1984). The knowledge of genetic distances of gene pools in a breeding programme is useful. It generates

a better understanding of germplasm organization and efficient parental selection during genotypic sampling. It also has implications on the results of choice of parents for crosses and gene introgression from exotic germplasm. Genetic diversity can be assessed by a number of methods including morphological data and DNA based data (Mohammadi and Prasanna 2003). The DNA based molecular markers reveal plymorphisms at the DNA level and is extensively used in various fields of plant breeding and germplasm management. These markers can identify many genetic loci simultaneously with excellent coverage of the entire genome. They are phenotypically neutral and can be applied at any developmental stage (Jones et al. 1997).

Simple sequence repeats (SSRs) or microsatellites are usually two to six base pair repeat motif (example TA, CA, GTG, TAA and GATA) repeats. The first report of SSR in plants was by Condit and Hubbel (1991), who suggested their abundance in the plant system. This was subsequently confirmed by Akkaya et al. (1992). The repeats generate polymorphisms due to the frequent variation in the length of the repeat regions. Such differences are detected on polyacrylamide or agarose gels, where repeat lengths migrate different distances according to size (Robinson and Harris 1999). SSRs are co-dominant markers with a high level of allelic diversity. SSR markers are a suitable option for genetic studies for a highly heterozygous crop such as cassava (Agyare-Tabbi et al. 1997; Chavarriaga-Auguirre et al. 1998). Molecular analysis matrices are very informative tools for estimation of genetic distances (Vieira et al. 2007). Microsatellite marker loci were developed for various crops, such as maize (Taramino and Tingley 1996), soybean (Devos et al. 1995), barley (Russell et al. 1997) and potato (McGregor et al. 2000). CIAT has identified 186 SSR markers for cassava (Chavarriaga-Aguirre et al. 1998; Mba et al. 2001).

Local cassava germplasm in Africa represents decades or centuries of selection by farmers to meet local culinary and agronomic needs. The various local names given suggest that farmers associate a number of useful traits with the varieties. Names given connote: early bulking ‘Bosomnsia’ (six months), source of income ‘Tuaka’ (pays off debt) or ‘Cedi Bankye’ (cassava that brings money or cedis, Ghana’s currency), sweet or cool and tasty ‘Bankye Santum’ (potato like cassava), high yielding ‘Ahyengyanka’ (career of orphans) or ‘Bokentenma’ (produces a basket full), and ‘Debor’ (traditionally old or ancient variety that yields for all generations). The selection of genotypes by farmers is influenced by their uses and culinary attributes, farming system and the growing industry. In northern Ghana some

farming communities chew fresh uncooked cassava on the fields and would describe those varieties as sweet and soft to chew. These are the 3-6 months maturing varieties. There is also the use of cassava for 'konkonte' and dry chips for a special meal called 'Tuo Zaafi' (TZ) which is a major food form unique to the north as 'fufu' is also unique to the southern part of Ghana. In regions where cassava is prepared into food forms that require high dry matter genotypes such as for the preparation of 'fufu', 'gari' and cassava dough, the selection criteria for farmers would differ and this would influence the varieties found in a particular region (Okai 2001; Amenorpe et al. 2006; Baafi and Sarfo-Kantanka 2008).

High heterosis for yield components, starch and number of roots has been observed in cassava and hence hybrid breeding is considered a promising method of genetic improvement (Easwari Amma and Sheela 1998; Pèrez et al. 2005). Heterotic groups identified in maize in the early 20th century (Schull et al. 1952) have been the basis of a very successful hybrid seed industry. DNA-based markers, especially SSR have been successfully used in the study of cassava genetic diversity (Beeching et al. 1993; Chavarriaga-Aguirre et al. 1999; Elias et al. 2000; 2001; Fregene et al. 2000; Benesi 2002; Fregene et al. 2003; Mkumbira et al. 2003; Zambrano et al. 2007; Hurtado et al. 2008; Kawuki et al. 2009). This study is the first comprehensive molecular characterization of Ghanaian landraces from all cassava growing areas across all regions and ecological zones in Ghana. The objective of this study was to use SSR marker analysis to assess genetic diversity and differentiate cassava genotypes in Ghana.

3.2 Material and methods

3.2.1 Collection of germplasm

Plant materials

A total of 320 cassava genotypes were collected from farmers' fields during the 2002 season. Cassava landraces were collected from 45 villages visited during the Collaborative Study on Cassava in Africa (COSCA) and an additional 28 villages where cassava production is important. The villages were located in the forest, guinea savannah, coastal savannah, arid and semi arid agro ecological zones across the ten regions of Ghana. Some regions share more than one of the ecological zones. Information on the passport data for each accession was collected, which included details on accession code, name of cultivar or landrace, meaning of the name of the cultivar, sample status, name of farmer, district, region ethnic group, village, names of individuals in the collection team, collection date, maturity period,

uses, preferred characteristics, period over which the landrace had been kept by the farmer, source of the cultivar and other crops grown with cassava or farming system practiced. The list of genotypes, passport data and characteristics can be found at the home page of the Molecular Diversity Network of Cassava (MOLCAS) (http://www.ciat.cgiar.org/molcas).

The 320 genotypes collected were mostly local varieties but also included some improved clones from IITA. Stem cuttings of 20-30 cm of the accessions were first planted in pots at the University of Ghana after each collection trip. The varieties were established in single rows at ten plants per row at 1 m x 1 m spacing in the experimental field of the Crops Research Institute (CRI), Pokuase station, Accra, Ghana. A copy of the collection was also sent as stem cuttings to the IITA, Ibadan Nigeria for field conservation.

3.2.2 DNA extraction

Fresh young leaf samples were collected from the field at IITA and stored on ice and then transferred to the laboratory for DNA extraction. About 0.1 g of fresh young leaves was ground in liquid nitrogen and DNA extracted using the Qiagen (Palo Alto, CA, USA) kit. DNA was stored in absolute ethanol. SSR marker analysis was done at CIAT (Colombia). The DNA was precipitated, dried and re-suspended in Tris-EDTA and quantified using a fluorometer. Genomic DNA was diluted to a final concentration of 10 ng/ul and used for PCR reactions using SSR marker primer pairs (Table 3.1). The DNA was checked on 1% agarose gels.

3.2.3 SSR marker analysis

A set of 36 SSR markers, selected earlier to cover the entire cassava genome, two from each of the 18 haploid chromosomes or linkage groups of the cassava genome, and clear reproducible PCR amplification patterns was employed for molecular marker analysis. PCR amplification and polyacrylamide gel analysis, and data scoring were as described by Fregene et al. (2003). PCR assays of SSR markers used 30 ng of genomic DNA in 15 ul reactions containing 0.1 mM dNTP, 1X PCR buffer, 2.5 mM MgCl2, 0.2 uM of each primer and 1U *Taq*DNA polymerase. PCR amplification was performed using the following thermal profile: 2 min at 95˚C, 30 cycles of 30 s at 94˚C, 55/45˚C at 1 min and 60 s at 72˚C and a final step of 5 min at 72˚C. PCR products were denatured and electrophoresis was done on 6% polyacrylamide gels and visualized by silver staining. The raw SSR data were scored as allele sizes and allele numbers used for statistical analysis.

Table 3.1 Cassava SSR markers used for studying genetic diversity

Locus	Type of repeat	Primer	size (bp)
SSRY4-R	$(GA)_{16}$	ATAGAGCAGAAGTGCAGGCG	287
SSRY4-F		CTAACGCACACGACTACGGA	
SSRY9-R	$(GT)_{15}$	ACAATTCATCATGAGTCATCAACT	278
SSRY9-F		CTAACGCACACGACTACGGA	
SSRY12-R	$(CA)_{19}$	ACTGTCAAACCATTCTACTTGC	266
SSRY12-F		GCCAGCAAGGTTTGCTACAT	
SSRY19-R	$(CT)_8(CA)_{18}$	TGTAAGGCATTCCAAGAATTATCA	214
SSRY19-F		TCTCCTGTGAAAAGTGCATGA	
SSRY20-R	$(GT)_{14}$	CATTGGACTTCCTACAAATATGAAT	143
SSRY20-F		TGATGGAAAGTGGTTATGTCCTT	
SSRY24-R	$(GA)_{26}$	CCTGCCACAATATTGAAATGG	192
SSRY24-F		CAACAATTGGACTAAGCAGCA	
SSRY34-R	$(GGC)_5GGTGGC\ (GGT)_2$	TTCCAGACCTGTTCCACCAT	279
SSRY34-F		ATTGCAGGGATTATTGCTCG	
SSRY38-R	$(CA)_{17}$	GGCTGTTCGTGATCCTTATTAAC	122
SSRY38-F		GTAGTTGAGAAAACTTTGCATGAG	
SSRY51-R	$(CT)_{11}CG(CT)_{11}(CA)_{18}$	AGGTTGGATGCTTGAAGGAA	298
SSRY51-F		GGATGCAGGAGTGCTCAACT	
SSRY52-R	$(GT)_{19}$	GCCAGCAAGGTTTGCTACAT	266
SSRY52-F		AACTGTCAAACCATTCTACTTGC	
SSRY59-R	$(CA)_{20}$	GCAATGCAGTGAACCATCTTT	158
SSRY59-F		CGTTTGTCCTTTCTGATGTTC	
SSRY63-R	$(GA)_{16}$	TCAGAATCATCTACCTTGGCA	290
SSRY63-F		AAGACAATCATTTTGTGCTCCA	
SSRY64-R	$(CT)_{13}CG(CT)_6$	CGACAAGTCGTATATGTAGTATTCACG	194
SSRY64-F		GCAGAGGTGGCTAACGAGAC	
SSRY69-R	$(CT)_{18}ATT(AT)_2(N)_7$	CGATCTCAGTCGATACCCAAG	239
SSRY69-F		CACTCCGTTGCAGGCTTA	
SSRY82-R	$(GA)_{24}$	TGTGACAATTTTCAGATAGCTTCA	211
SSRY82-F		CACCATCGGCATTAAACTTTG	
SSRY100-R	$(CT)_{17}TT(CT)_7$	ATCCTTGCCTGACATTTTGC	210
SSRY100-F		TTCGCAGAGTCCAATTGTTG	
SSRY102-R	$(GT)_{11}$	TTGGCTGCTTTCACTAATGC	179
SSRY102-F		TTGAACACGTTGAACAACCA	
SSRY103-R	$(GA)_{22}$	TGAGAAGGAAACTGCTTGCAC	272
SSRY103-F		CAGCAAGACCATCACCAGTTT	
SSRY105-R	$(GT)_6GC(GT)_2(GA)_{16}$	CAAACATCTGCACTTTTGGC	225
SSRY105-F		TCGAGTGGCTTCTGGTCTTC	
SSRY106-R	$(CT)_{24}$	GGAAACTGCTTGCACAAAGA	270
SSRY106-F		CAGCAAGACCATCACCAGTTT	
SSRY108-R	$(CT)_{24}CCT$	ACGCTATGATGTCCAAAGGC	203
SSRY108-F		CATGCCACATAGTTCGTGCT	
SSRY110-R	$(GT)_{12}$	TTGAGTGGTGAATGCGAAAG	247
SSRY110-F		AGTGCCACCTTGAAAGAGCA	
SSRY135-R	$(CT)_{16}$	CCAGAAACTGAAATGCATCG	253
SSRY135-F		AACATGTGCGACAGTGATTG	

SSRY47-R		GGAGCACCTTTGCTGAGTT	113
SSRY47-F		TTGGAACAAAGCAGCATCAC	
SSRY148-R		GGCTTCATCATGGAAAAACC	114
SSRY148-F		CAATGCTTTACGGAAGAGCC	
SSRY151-R		AGTGGAAATAAGCCATGTGATG	182
SSRY151-F		CCCATAATTGATGCCAGGTT	
SSRY155-R		CGTTGATAAAGTGGAAAGAGCA	158
SSRY155-F		ACTCCACTCCCGATGCTCGC	
SSRY161-R	$(CT)_{11}TT(CT)_{21}(CA)_{19}$	AAGGAACACCTCTCCTAGAATCA	220
SSRY161-F		CCAGCTGTATGTTGAGTGAGC	
SSRY164-R	$(GA)_{29}$	TCAAACAAGAATTAGCAGAACTGG	187
SSRY164-F		TGAGATTTCGTAATATTCATTTCACTT	
SSRY169-R	$(GA)_{19}(A)_3(GAA)_2$	ACAGCTCTAAAAACTGCAGCC	100
SSRY169-F		AACGTAGGCCCTAACTAACCC	
SSRY171-R	$(TA)_5CATA(GATA)_8$	ACTGTGCCAAAATAGCCAAATAGT	291
SSRY171-F		TCATGAGTGTGGGATGTTTTTATG	
SSRY177-R	$(CCT)_6CT(N)_{65}(CT)4AT(CT)_{18}$	ACCACAAACATAGGCACGAG	268
SSRY177-F		CACCCAATTCACCAATTACCA	
SSRY179-R	$(GA)_{28}$	CAGGCTCAGGTGAAGTAAAGG	226
SSRY179-F		GCGAAAGTAAGTCTACAATTTTCTAA	
SSRY180-R	$(GA)_{16}(G)_4(GA)_5$	CCTTGGCAGAGATGAATTAGAG	163
SSRY180-F		GGGGCATTCTACATGATCAATAA	
SSRY181-R	$(GA)_{22}(G)_3C(GA)_3GGAA(GA)$	GGTAGATCTGGATCGAGGAGG	199
SSRY181-F		CAATCGAAACCGACGATACA	
SSRY182-R	$(CA)_{17}(N)_{23}GAGG(GA)_8$	GGAATTCTTTGCTTAGATGCC	253
SSRY182-F		TTCCTTTACAATTCTGGACGC	

3.2.4 Data analysis

Gene diversity and genetic differentiation analyses were carried out with data from 33 SSR markers. Genetic distance, based upon the PSA, was obtained from the raw allele size data using the computer programme Micro Sat (Minch, 1993) available at http://.hpgl.stanford.edu/projects/microsat/microsat.html. Distances between the accessions were subjected to PCA using JMP (SAS Institute 1995) to obtain a structure of relationship between the landraces. Parameters of genetic diversity and differentiation were calculated from allelic data using the computer packages GENSURVEY (Vekemans and Lefebvre 1997) and FSTAT (Goudet 1995).

3.3 Results and discussion

Data from a total of 33 out of 36 SSR loci was used to derive estimates of genetic diversity and differentiate genetic distances between individual genotypes. Three markers gave poor quality data (Figure 3.1).

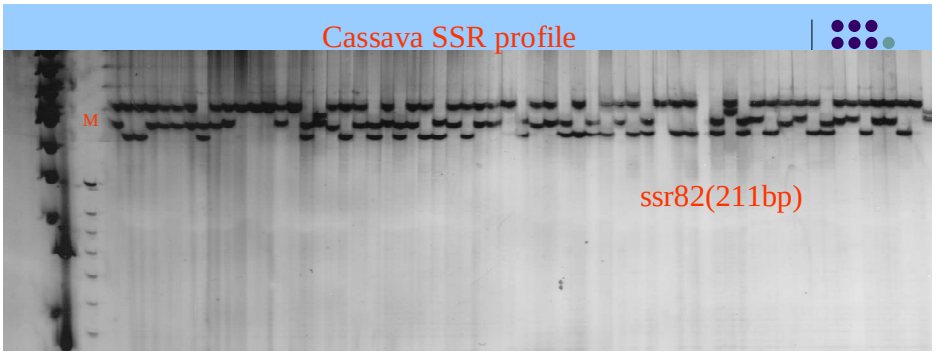

Figure 3.1 Silver stained polyacrylamide gel electrophoresis of PCR amplification product with primers from SSR marker 82 for local cassava varieties from Ghana

The cassava accessions used for the study had the three regions in the north (Northern, Upper East and Upper West regions) contributing 162 acessions and the remaining seven regions 125 accessions. The average number of alleles for each locus was close to five and ranged from three to seven (Figure 3.1 and Table 3.2) and was similar to that found for a study of landraces from Nigeria, Tanzania and seven neo-tropical countries (Fregene et al. 2003; Hurtado et al. 2008). The probability that two randomly selected alleles in a given accession are different, also known as average gene diversity, was 0.5245±0.0045 and it is lower than that found in a study carried out in CIAT (Fregene et al. 2003) under the MOLCAS project (Table 3.2). Average gene diversity was comparable across all regions with the exception of the central region of the coastal savannah zone. Genetic diversity parameters, including total heterozygosity (Ht) and genetic differentiation (Gst) ranged widely across markers. Genetic differentiation, as estimated by F_{ST} (theta), was very low for samples between regions. An overall value of 0.04 was recorded, with the exception of some accessions from northern Ghana that showed moderate to high genetic differentiation (Table 3.3 and Figure 3.2). The results found here support previous findings that agricultural practices and the allogamous nature of cassava produces a large pool of volunteer seedlings that natural and human

selection acts upon to maintain a high level of diversity and low differentiation (Doyle and Luckow 2003; Fregene et al. 2003).

Table 3.2 Intra-population and inter-population estimates of genetic diversity parameters of cassava landraces from different agro-ecologies represented by regions of Ghana

Population	n	#loc	#loc_P	PLP	K	K_P	HO_p	HE_p	HEc_p
Ashanti	11	33	30	90.9	3.9	4.1	0.5285	0.5017	0.5262
Brong Ahafo	37	33	31	93.9	5.4	5.6	0.5012	0.5267	0.5339
Central	8	33	29	87.9	3.3	3.7	0.5082	0.4701	0.4999
Eastern	27	33	30	90.9	5.4	4.9	0.5322	0.5123	0.5223
Western	4	33	27	81.8	2.9	3.4	0.5404	0.4670	0.5405
Greater Accra	10	33	29	87.9	3.7	4	0.5016	0.5065	0.5336
Volta	28	33	31	93.9	5.3	5.5	0.5133	0.5735	0.5839
Northern	109	33	31	93.9	6.9	7.2	0.5369	0.5779	0.5806
Northern I (upper east & upper west)	53	33	31	93.9	6.3	6.5	0.5479	0.5851	0.5908
Mean				90.57	4.69	4.98	0.5234	0.5245	0.5457
Std deviation				4.13	0.36	1.31	0.0176	0.045	0.0317

PLP: Percentage of polymorphic loci at the 5% level within accessions
K: Mean number of alleles per locus within accessions
K_P: Mean number of polymorphic alleles per locus within accessions
HO_p: Observed heterozygosity
HE-p: Average gene diversity
HEc_p: Average gene heterozygosity corrected for small sample sizes

Table 3.3 Parameters of genetic diversity, Ho, Hs, Ht, Dst, Gst and Gst' (correction for differences in sample size) by SSR locus

Loc Name	Ho	Hs	Ht	Dst	Dst'	Ht'	Gst	Gst'
SSRY4	0	0.346	0.439	0.093	0.104	0.45	0.212	0.23
SSRY5	0.499	0.474	0.481	0.006	0.007	0.481	0.014	0.015
SSRY9	0.463	0.582	0.587	0.005	0.006	0.587	0.009	0.01
SSRY12	0.704	0.597	0.598	0.001	0.001	0.598	0.001	0.001
SSRY19	0.811	0.738	0.764	0.026	0.029	0.766	0.034	0.037
SSRY20	0.79	0.73	0.76	0.03	0.033	0.764	0.039	0.043
SSRY21	0.596	0.487	0.514	0.027	0.03	0.517	0.053	0.058
SSRY34	0.479	0.428	0.425	-0.004	-0.004	0.424	-0.009	-0.01
SSRY38	0.043	0.08	0.082	0.001	0.002	0.082	0.018	0.02
SSRY47	0.429	0.668	0.739	0.071	0.079	0.747	0.096	0.106
SSRY51	0.79	0.694	0.751	0.057	0.063	0.757	0.076	0.084
SSRY52	0.752	0.603	0.616	0.014	0.015	0.618	0.022	0.025
SSRY59	0.152	0.639	0.701	0.062	0.069	0.708	0.089	0.098
SSRY63	0.445	0.484	0.519	0.036	0.04	0.523	0.069	0.076
SSRY64	0.725	0.67	0.689	0.02	0.022	0.691	0.028	0.031
SSRY69	0.557	0.552	0.568	0.016	0.018	0.57	0.028	0.031
SSRY82	0.846	0.84	0.858	0.018	0.02	0.86	0.021	0.024
SSRY100	0.725	0.779	0.798	0.019	0.021	0.8	0.024	0.027
SSRY102	0.007	0.01	0.009	0	0	0.009	-0.037	-0.041
SSRY103	0.804	0.76	0.764	0.004	0.004	0.764	0.005	0.005
SSRY106	0.829	0.761	0.768	0.008	0.009	0.769	0.01	0.011
SSRY108	0.422	0.361	0.373	0.012	0.014	0.375	0.033	0.036
SSRY110	0.279	0.272	0.274	0.002	0.002	0.274	0.008	0.009
SSRY120	0.814	0.629	0.653	0.024	0.026	0.656	0.036	0.04
SSRY148	0.08	0.083	0.086	0.002	0.003	0.086	0.029	0.032
SSRY151	0.689	0.79	0.806	0.015	0.017	0.807	0.019	0.021
SSRY155	0.072	0.595	0.632	0.037	0.041	0.636	0.059	0.065
SSRY164	0.528	0.658	0.668	0.01	0.011	0.669	0.014	0.016
SSRY169	0.258	0.316	0.321	0.004	0.005	0.321	0.013	0.015
SSRY171	0.544	0.555	0.591	0.037	0.041	0.596	0.062	0.068
SSRY179	0.845	0.726	0.776	0.05	0.056	0.781	0.065	0.071
SSRY181	0.672	0.532	0.535	0.003	0.004	0.536	0.006	0.007
SSRY182	0.605	0.705	0.761	0.055	0.062	0.767	0.073	0.08
Overall	0.523	0.55	0.573	0.023	0.026	0.575	0.04	0.045

Ho Average observed heterozygosity within country
Ht Total heterozygosity in the entire data set
Hs Gene diversity within country averaged over the entire data set
Dst Average gene diversity between populations
Gst Coefficient of gene differentiation

3.3.1 Estimates of genetic distance from cluster analysis

Genetic distances between all pairs of individual accessions were calculated by the proportion of shared alleles (1-PSA) and are presented graphically by a PCA (Figure 3.2). The PC1 and PC2 accounted for 26% and 16% of the total variance respectively. The PCA showed loose clustering of the landraces by region but of note is the sub-structure of some landraces from northern Ghana (Figure 3.2). A similar sub-structure as observed in the accessions from Northern Ghana, was observed in accessions from Nigeria and Tanzania in earlier studies (Fregene et al. 2003).

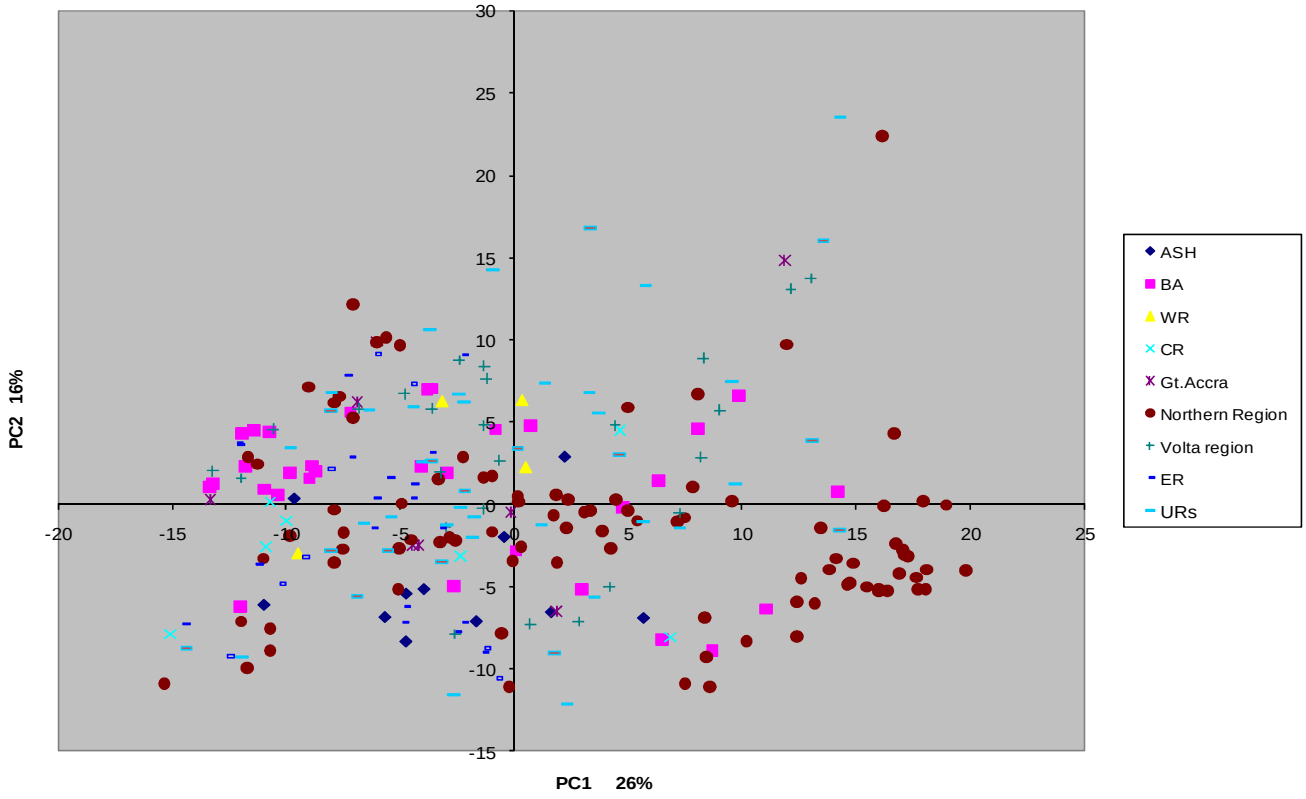

Figure 3.2 Principal Component Analysis (PCA) of genetic distance (proportion of shared alelles-PSA) of cassava landraces from Ghana (PC1 = 26%, PC2 = 16%)

Key ASH- Ashanti region, BA- Brong Ahafo region, WR- Western region, CR-Central region, GA- Greater Accra region, ER- Eastern region, URs- Upper regions (Upper East and Upper West)

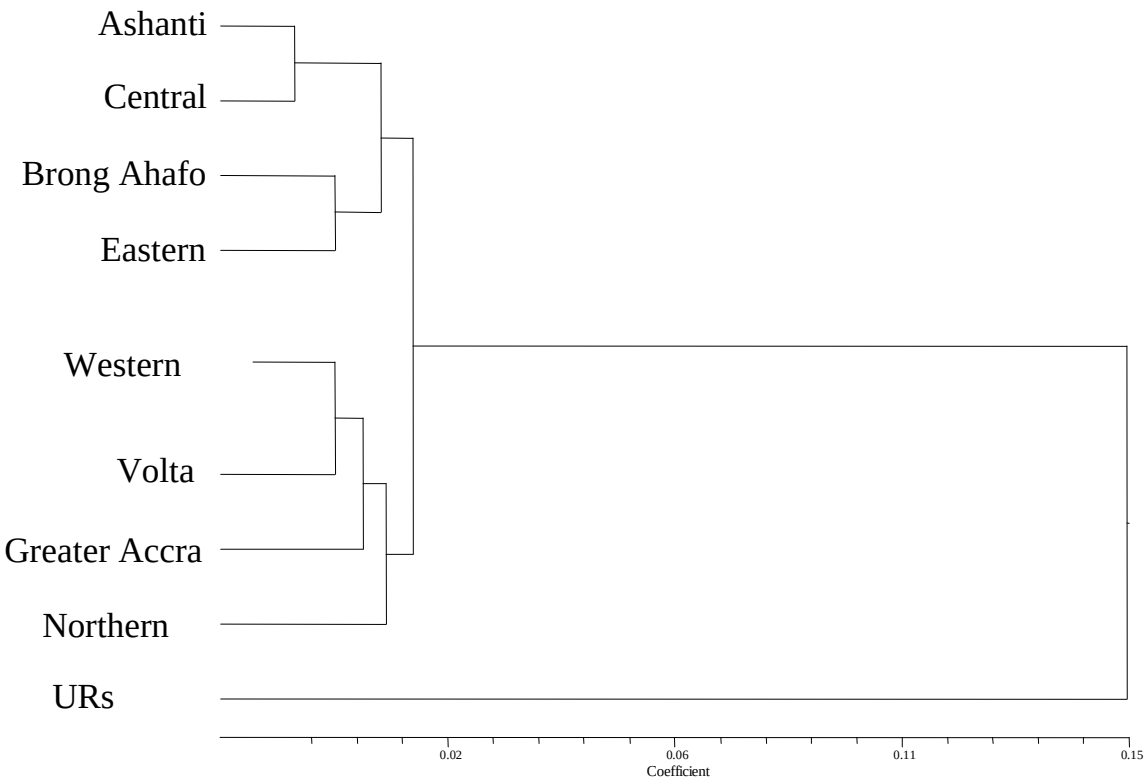

Figure 3.3 UPGMA tree of pair-wise F_{ST} data calculated between samples from 10 different regions of Ghana

(URs comprise Upper East and Upper West regions)

The presence of a defined sub-structure in the genetic relationship of cassava landraces from Africa appears to be a common feature of cassava germplasm in a number of countries but the underlying factors for the groupings are yet to be understood (Fregene et al. 2000; Hurtado et al.2008).

UPGMA cluster analysis of F_{ST} estimate of genetic differentiation amongst landraces was able to group the landraces into loose clusters according to regions also representing agro-ecologies, with a group of genotypes from the upper regions in the guinea savannah, the arid and semi arid ecological zone sub-structure being the most differentiated (Figure 3.3). A total of 63 duplicates or closely related accessions were identified in the collection. The cassava accessions used for the study had the Nothern region, Upper East and Upper West regions contributing 162 acessions and the remain seven regions contributed 125 accessions. This

may be a possible reason for the sub-structure observed in the accessions from northern Ghana. The regions with well established cassava-based industry (gari, chips, ethanol, starch and starch derivatives,) in the coastal and forest ecological zones of Ghana (Al-Hassan et al. 1996; Henry and Gottret 1996; Nweke 2004) will, influence the selection of varieties grown in these areas. Farmers who grow cassava as raw materials to the industry will grow only varieties that will be acceptable for processing. The quite high average gene heterozygosity of 0.573-0.589 for the Volta and northern regions could be the effect of introductions of unique germplasm from Burkina Faso and the Togo borders with the active trade of cassava products (Al-Hassan et al. 1996). The sub group structure (Figure 3.3) observed among the local genotypes from northern Ghana could be due to many factors; of which the influence of exchange of cassava materials from the bordering country, Burkina Faso, culinary attributes suitable for particular food forms for northern Ghana, the arid and semi arid climatic conditions with a monomodal rainfall pattern and historically the late movement of cassava from the coast to northern Ghana in the 1980s and population growth (Plucknett et al. 1998; Ministry of Agriculture 1990) could be important. The selection and distribution of cassava by famers is based on shared or common preferred attributes suitable to preferred food forms.These are reason for high number of duplicates or closely related accessions in the collection.The vegetative nature of cassava, allows the addition of useful volunteer cassava to farmers' varieties which may be closely related to farmers varieties (Okai 2001**;** Elias et al 2001). The uses, climatic factors and agronomic importance have also influenced the landraces grown in Ghana (Amenorpe et al. 2006; Baafi and Sarfo-Kantanka 2008). This also explains the low adoption of IITA technology for cassava in Ghana (Nweke et al. 1994).

The forest and coastal savannah regions have cassava based-industries that will, with time, influence the diversity and differentiation and also affect the genetic base of cassava in Ghana, if farmers grow few improved varieties to meet industrial needs for income generation. An understanding of the genetic diversity present in the Ghanaian genotypes is the first step in harnessing the phenotypic variability for crop improvement (Meredith and Bridge 1984). This understanding also lead to the establishment of a core or reference samples that represent allelic richness in the entire collection that can be evaluated for traits of agronomic interest and selected for use as parental lines in crop breeding (Hurtado et al. 2008). Furthermore, releasing improved cassava varieties takes eight to ten years and the choice of parental materials with good combining ability is important (Ceballos et al. 2004). Assessment of genetic diversity can help delineate heterotic pools for a more systematic improvement of

combining ability via recurrent reciprocal selection. The SSR marker loci detected 63 duplicates or closely related genotypes. Previous studies to assess genetic diversity of some cassava genotypes in Ghana, using RAPD and morphological markers also detected some duplicates or closely related genotypes that had different names (Okai 2001; Asante and Offei 2003).

Based upon clusters obtained, genotypes representative of the clusters from the northern regions and the regions in the forest zones like the western areas could be selected as parents for a diallel experiment to search for heterotic patterns. The genetic diversity would form a basis for planned cassava improvement towards the development of new varieties necessary to meet new trends of biotic and abiotic stress tolerance and the growing cassava-based industry in Ghana, Nigeria and West Africa in a participatory approach to enhance adoption of technology.

3.4 Conclusions

The assessment of genetic diversity and differentiation in a collection of local cassava varieties in Ghana using SSR markers revealed clustering that may represent heterotic groups. SSR marker loci detected 63 duplicates or closely related genotypes. From the study it was concluded that SSR is a powerful robust molecular tool for assessing the genetic diversity within the Ghanaian landraces for utilization in the breeding programme. A total of 33 SSR loci were assessed and 63 duplicates or related genotypes were identified. The overall heterozygosity was high, with a low level of differentiation. There was a loose structure but a unique sub-structure in the landraces from the upper regions of Ghana, and the arid and semi arid climatic zones. The heterotic groupings were distinct for the regions in the savannah and the forest ecological zone regions. Parents could be selected from the groupings for crosses to test for heterotic patterns and combining ability.

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CHAPTER 4

THE COMBINING ABILITY AND HETEROSIS OF CASSAVA GENOTYPES FOR CASSAVA MOSAIC DISEASE AND CASSAVA BACTERIAL BLIGHT, YIELD AND ITS RELATED COMPONENTS IN TWO ECOLOGICAL ZONES IN GHANA

Abstract

In 2007 and 2008, seven cassava genotypes were crossed in a half-diallel cross design (Griffing's method 2). The seven progenitors and their 21 F1 progenies were planted in a Randomised Complete Block Design with three replications in two different locations for two seasons in the forest ecology. A second set of five parents were also crossed in the same diallel fashion for one season in 2008. The five progenitors and their 10 F1 progenies were planted in a RCBD with three replications and tested in two locations in the coastal savannah ecological zone of Ghana. Both experiments were evaluated for CMD and CBB resistance, fresh root yield, dry root yield, root number, harvest index, dry matter content, plant height at maturity and height at first branching, levels of branching and plant vigour. Results of the combined analysis of variance (ANOVA) revealed that the environment effect was significant for all the traits. General Combining Ability (GCA) and Specific Combining Ability (SCA) effects were significant for most of the traits. Narrow sense heritability was significant for plant vigour, root number, CMD and CBB in both the zones. CMD and root number also had a predictability ratio of close to one, indicating the importance of additive gene effects. In assessing best-parent heterosis, mid-parent heterosis, GCA and SCA of progenitors and F1 progenies, Dabodabo x Tuaka, Dabodabo x Debor, Debor x Lagos and Debor x Tuakab were the four best combinations for CMD resistance, Lagos x Kwasea and Tuaka x Afebankye (of the forest and savannah trials respectively) also proved to be the best crosses to increase fresh root yield.

4.1 Introduction

Cassava is the most important vegetatively propagated food crop and the second most important food staple in terms of calories per capita in Africa (Nweke et al. 2002). Over 50% of the world production of cassava is in Africa (about 121 million metric ton) with Nigeria as the leading producer (FAO 2009).

CMD is caused by a Gemini virus that causes an estimated total crop yield loss of about US$440 million per annum on the continent (Thresh et al. 1997). During epidemics root yield losses of up to 100% have been recorded (Jennings 1994; Thresh et al. 1997). Yield losses from 20% to 90% are common in farmer's fields (Muimba-Kankolongo and Phuti. 1987; Moses 2008). Breeding for resistant genotypes is the best strategy to offset the destructive effect of the virus in cassava (Thresh et al. 1997). The initial breeding effort started in 1937 at the Amani Research station in Tanzania, Ghana obtained resistant genotypes to start breeding for resistant genotypes (Hillocks and Jennings 2003).

The landraces are known to have a higher frequency of genes for traits of interest, hence analysis for genetic diversity was carried out (Chapter 3) which was used to select parents from the various clusters and a diallel analysis was employed to test for the combining ability for disease resistance and yield characteristics and to test the predictability of heterosis in the population in two sets of diallels in the forest and savannah ecological sites in Ghana over different seasons.

4.2 Material and methods

The diallel crosses of the cassava landraces done for this study constitute one of the major attempts to carry out the first comprehensive genetic crosses for cassava in Ghana. Prior to this study the Crops Reseach Institute collaborated with IITA to develop genotypes for testing. Evaluation and screening of IITA improved materials had been the routine breeding approach over the years to date.

Two sets of diallel parents were selected for the forest and the savannah ecological zones. A five-parent diallel cross for the savannah ecology was tested at the Pokuase and Ohawu research stations of the CSIR-Crops Research Institute for one season.

A seven-parent diallel for the forest ecology was carried out in two locations, Fumesua and Ejura research stations, over two seasons. The genotypes used for both experiments were selected from the landraces used for the genetic diversity study (Chapter 3) based on their agronomic performance, flowering ability, clusters obtained from the diversity results and farmer's and end-user preference.

4.2.1 Development of progeny

The five parents for the savannah zone trials were planted in the crossing block at Pokuase station of the CRI in the 2004 and 2005 cropping seasons. The seven parents for the forest zone trials were planted at Fumesua and Wenchi research stations in Ghana in 2004 and 2005. Controlled pollinations were carried out following the standard procedures described by Kawano (1980). The five parents were crossed in a diallel mating design (Griffing 1956) to produce 10 F1 families or crosses without reciprocals. The seven parent diallel produced 21 F1 families with no reciprocals. Mature seeds were generated from each cross. The seed viability was tested by the floatation method (CIAT 2003). The dry seeds were mixed with water and floated. Non viable seeds were discarded. The crosses were carried out in two seasons to produce between 77-120 viable seeds per cross. A total of 70 seeds from each cross were germinated in seed trays in a greenhouse in Accra and at the screen house at the Fumesua station in 2006. Seedlings were transplanted on ridges at 10 weeks after planting in the seedling nursery. The savannah experiment seedlings were transplanted in the experimental field at the Pokuase station.

The F1 seedlings for the forest and savannah experiments were transplanted in the seedling nursery field at the Fumesua station in the minor season in September 2006. The seedlings were established in single row plantings at 50 cm by 1 m spacing within and between rows, respectively to produce adequate stem cuttings for the study. On 9 May 2007, selected F1s were harvested at 8 MAP. Stakes (25-30 cm long) were planted in the major season on 15 May 2007 to produce sufficient woody stems for the experiment. At 12 MAP, the seedlings were harvested and 15 stakes (30 cm) were made for each family for each location. The five progenitors for the savannah zone and the seven for the forest zone were also planted in the field together with the progeny. The plants were established under natural conditions without irrigation or fertilization. Harvesting was done at 12 MAP. In the forest nursery, each F1 family and parents produced 30 genotypes on average and each genotype was vegetatively propagated into 15 stakes. Similarly in the savannah zone, 15 stakes were obtained from each of the 25 genotypes from each F1 family and parents in May 2008. The selection of progeny was done on the basis of their ability to produce enough good quality vegetative cuttings required for the next stage of evaluation. F1 seedlings were planted in the minor season and 8 MAP, they were harvested and five woody cuttings of 20-25 cm were made from the first planting for a second planting done on the 19th of May 2007 in the major season. This was

harvested at 12 MAP in May 2008. Sufficient woody stems of 30 cm long cuttings were obtained for each cross for clonal evaluation at each location.

4.2.2 Field trials

Field arrangement

A RCBD with three replications was used to plant 15 entries consisting of 10 F1, and five parents at two locations, Pokuase and Ohawu stations in June 2008 in the coastal savannah ecological zone for one season. A second season trial was not possible due to land issues and insufficient resources.

The same design was used to plant 28 entries of 21 F1, and seven parents at two locations in the forest ecological zone, at the Fumesua and Ejura stations. The forest experiment was planted in two consecutive years, in May 2008 and May/June 2009. The planting was done during the raining season and all the locations had a bimodal rainfall pattern. Each of the three replicates contained all 15 and 28 entries. Spacing was 1 m between rows and within rows, hence a population of 10 000 plants per hectare. Routine field maintenance practices were followed to keep the fields clean of weeds and no irrigation or fertilizers was applied during the growth. Data from three inner plants of each entry was averaged before analysis was done.

Weather data for minimum and maximum temperature, monthly total rainfall and altitudes of experimental sites were recorded as well as soil series and properties (Table 4.1a-c). Detailed information is given in the appendix.

4.2.3 Agronomic and morphological characteristics measured

During the growth period, data was collected on incidence and severity of CMD and CBB. At harvest, data on yield and its related characteristics were measured. The incidence and severity of CMD was scored using a scale of 1-5 where 1 represented no symptoms and 5 severe damage (IITA 1990). The scoring was done at 1, 3 and 6 MAP and an average score for analysis was determined. Plant vigour was also measured. Prior to harvesting at 12 MAP, the plant height was measured at the highest branch. At harvesting, the height at first branching and the levels of branching were recorded. Plants were hand-harvested and averaged results for each F1 cross were determined for each replication at each location. The marketable roots were counted and weighed separately. Harvest index was measured as a

ratio of root weight to total biomass. Dry matter was determined by measuring the weight of the storage roots in water and air and the values calculated according to Kawano et al. (1998).

Table 4.1a The soil series for the four locations used for the experiment

Research site	Soil series	Soil type
Fumesua	Bomso-Asuansi series (ferric acrisol)	Sandy loam top soil over sandy clay
Ejura	Amantin series	Sandy loam top soil
Ohawu	Toje-Alajo	Loamy top soil over sandy loam
Pokuase	Adams series	Sandy loam

Table 4.1b Temperature range and rainfall distribution for experimental sites

Site	Eco-zone	Rainfall (mm)	Months of rainfall	Temperature (^{o}C)
Fumesua	Forest zone	1460-1750	Bimodal April-July Sep-Nov	20-34
Ejura	Forest-transition zone	1300-1400	Bimodal April-July Sep-Nov	21-34
Ohawu	Coastal Savannah zone	1000-1500	Bimodal March-July Sep-Nov	24-34
Pokuase	Coastal Savannah zone	800-1500	Bimodal May-Aug Sep-Nov	21-30

Table 4.1c The coordinates and altitude for the locations used for the experiments

Site	Longitude	Latitude	Altitude
Fumesua	1°30’0’’W	6°42’0’’N	277
Ejura	1°22’0’’W	7°23’0’’N	240
Ohawu	0°54’0’’E	6°8’0’’N	24
Pokuase	0°16’36’’E	5°41’0’’N	65

Altitude = meter above sea level. Source: CSIR- Soil Research Institute and Ministry of Food and Agriculture

Pokuase and Ohawu have a bimodal rainfall pattern with the major rains in May/June to August and late September to November (Table 4.1b). The locations used for the trials experience bimodal rainfall with rains starting between April and May. The effective rain fall is 7-8 months with varied distribution. The 2008 season had fairly regular rainfall distribution within the period the trials were carried out. For all the seasons, the peak of rainfall was

observed in June/July. The average monthly and maximum temperatures ranged between 21.9 to 23.5°C for the 2008 and the 2009 seasons.

4.2.4 Data analysis

Analysis of variance and diallel analysis (Griffing, method 2) were done for each year using a fixed model (SAS 2004).

Combining ability

Analysis of GCA and SCA for the forest and savannah experiments was performed, and mean squares of GCA and SCA were used to determine GCA:SCA ratios (Beil and Atkins 1967; Haussmann et al.1999).

Phenotypic correlation

Phenotypic correlation (rp) between the traits measured was calculated using the plot means as follows:

$$rp = \frac{Covxy}{d}$$

Where Cov xy = phenotypic covariance between characteristic X and Y; and d = phenotypic variance of characteristics Y.

Genetic parameters

The contribution component of genetic variance was determined to obtain estimates of GCA variance (δ^2gca) and SCA variance (δ^2sca) for each trait assessed. Additive (Va) and dominance (Vd) variance were estimated as Va = 2(δ^2gca) and Vd = (δ^2sca). Phenotypic (Vp) and genotypic variance (g) were also estimated as Vg = Va +Vd where Vp = Vg +Ve.

Broad sense (h^2b) and narrow sense (h^2n) heritability was calculated from the estimated variances as

h^2b = Vg/Vp and h^2n = Va/Vp, respectively.

The relative size of variances due to GCA and SCA on progeny performance was estimated following Baker's prediction ration (Baker 1978) as PR= $2\delta^2$gca/($2\delta^2$gca + δ^2 sca)

The average degree of dominance was estimated as $\sqrt{}$H/D= $\sqrt{}$(δ^2gca/δ^2sca) (Singh and Chaudhary 1979).

Estimates of predicted heterosis

$$Ht\ (\%) = \frac{F^1 - BP}{MP} X\ 100$$

$$Hbt\ (\%) = \frac{F^1 - BP}{BP} X\ 100$$

Where
Ht= Heterosis
Hbt= Heterobeltiosis
MP= Mid Parent Value
BP= Better Parent Value

The 't' test was done to determine whether F1 hybrid means were statistically different from mid parent and better parent means as follows (Wynne et al. 1970):

$$tij = \Gamma_1 ij \quad \frac{MP}{\sqrt{\frac{3}{8} * EMS}}$$

$$tij = F_1 ij - \frac{BP}{\sqrt{\frac{1}{2} * EMS}}$$

Where
F_1 ij = The mean of the ijth F1 cross
MP ij = The mid parent for the ijth cross
BP ij = The better parent values for ijth cross
EMS = Error mean square

4.3 Results and discussion

4.3.1 Phenotypic correlation

Eleven agronomic and morphological characters were combined for this analysis (Tables 4.2-4.3). Results from the study indicated that fresh yield and dry yield per plant were almost 100% correlated. This agrees with results by Ojulong (2006). Selection of any two traits that are negatively correlated will result in compromising one trait for the other. A significant positive correlation between plant height and first branch height and root number was found for both eco zones (forest and savannah). A significant correlation between plant height and root number has also been reported by Ntawuruhunga and Dixon (2010). This is very important because root number correlated positively with fresh yield per plant and dry yield (Tables 4.2-4.3). A negative correlation between CBB and fresh yield has also been reported by Ssemakula and Dixon (2007), although, in this study the correlation was not significant. The significantly negative correlation between CMD and all the yield related traits studied; fresh yield, root number and dry yield in the savannah zone, confirms the potential yield losses that can be caused by CMD. These correlations were also negative, but not significant in the forest zone. In the forest zone CMD significantly negatively affected the plant vigour and plant height.

The study showed a significantly positive correlation between root number produced per plant, plant height and height at first branching for all the trials. There was a significant positive correlation between CMD and CBB in the forest zone and a positive, but not significant correlation in the savannah zone. This could be useful for screening genotypes for resistance. Hence selection for CMD resistance would imply selection for CBB resistance. It is imperative that breeding efforts are geared towards searching for new sources of resistance and pyramiding of resistance genes into some major varieties. In the same vein, biotechnological tools like MAS would be a choice to strengthen traditional breeding towards making faster progress in the improvement of a long season crop such as cassava.

Table 4.2 Phenotypic correlation among some traits of cassava clones for the forest zone (combined for seasons and locations)

	CBB	CMD	PLTHT	FBRHT	BRLEV	ROOTNO	FYLD/PLT	HI	DM	DRYLD
CMD	0.51**									
PLTHT	-0.28	-0.57**								
FBRHT	0.32	0.03	0.18							
BRLEV	-0.19	-0.30	0.09	-0.36						
ROOTNO	0.30	-0.06	0.51**	0.60***	-0.24					
FYLD/PLT	-0.07	0.23	-0.02	0.36	-0.74***	0.12				
HI	0.15	-0.11	-0.03	0.42*	-0.08	0.20	0.11			
DM	0.18	-0.01	0.43*	0.12	0.37*	0.57**	-0.30	-0.01	1	
DRYLD	-0.03	0.24	0.04	0.39*	-0.70***	0.21	0.99***	0.11	-0.16	
VIGOUR	-0.05	-0.44*	0.25	0.09	0.48**	0.31	-0.62***	0.33	0.45*	-0.60***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

CBB=Cassava Bacterial Blight, CMD=cassava mosaic disease, PLTHT= plant height, FBRHT=first branch height, BRLEV=branching level, ROOTNO=root number, FYLD/PLT=fresh yield per plant, HI=harvest index, DM=dry matter content, DRYLD=dry yield

Table 4.3 Phenotypic correlation among some traits of cassava genotype for the savannah zone (combined for locations)

	CBB	CMD	PLTHT	FBRHT	BRLEV	ROOTNO	FYLD/PLT	HI	DM	DRYLD
CMD	0.30									
PLTHT	0.50	-0.04								
FBRHT	0.31	-0.32	0.39							
LEVELS	-0.63*	-0.07	-0.02	-0.39						
ROOTNO	0.26	-0.61*	0.56*	0.65**	-0.09					
FYLD/PLT	-0.46	-0.79***	-0.20	0.35	0.02	0.45				
HI	-0.24	-0.04	-0.29	0.24	-0.19	-0.12	0.49			
DM	0.10	0.02	0.33	0.54*	0.16	0.34	0.21	0.48		
DRYLD	-0.40	-0.73**	-0.09	0.44	0.02	0.48	0.98***	0.58	0.18	
VIGOUR	-0.62	0.28	-0.58*	-0.25	0.38	0.01	0.39	0.05	-0.26	0.28

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

CBB=Cassava Bacterial Blight, CMD=cassava mosaic disease, PLTHT= plant height, FBRHT=first brach height, BRLEV=branching level, ROOTNO=root number, FYLD/PLT=fresh yield per plant, HI=harvest index, DM=dry matter content, DRYLD=dry yield

4.3.2 Estimation of combining ability variances

The evaluation of combining ability to determine additive and non-additive gene action within any breeding population, is essential for the determination of the type of breeding methods that will successfully improve the performance of the traits of interest (Dudley and Moll 1969)

Mean squares for general and specific combining ability

GCA was significant for CMD, first branch height and plant height in all the trials. Dry yield GCA was significant in the forest zone in the first season. GCA for vigour, root number, fresh root yield, dry matter content and CBB were significant in three of the four tables (Tables 4.4-4.7).

SCA was significant for plant vigour, plant height, first branch height, dry yield per plant and CBB in all trials. The ratio between GCA and SCA mean squares was calculated. Where the ratio was closer or higher than a unit it indicates the predominance of additive gene action. For root number, plant height, CMD and CBB in the forest zone for both seasons, additive gene effects were predominant, indicating that they are highly heritable and should react positively on selection. The problem with the disease scores is that high ombining ability scores are not desirable as this would indicate high disease scores. In the savannah zone trials the GCA:SCA ratio was more than one for eight of the 11 tested characteristics. In the Pokuase trial only five characteristics had a value of more than one.

Table 4.4 Mean squares for GCA and SCA for CMD, CBB, yield and yield components of cassava genotypes for the two locations of the forest zone combined for 2008/2009

Source	Df	Vig	Rootno	Pltht	HI	Fryld	Fbrht	Dyld	Dm	CMD	CBB	Brlev
GCA	6	0.74**	22.53**	3773.74**	0.02	2.02	1050.18*	0.21	6.77*	0.87**	0.47	1.52*
SCA	21	1.96**	9.48**	1170.80**	0.02*	4.31**	1461.58*	0.32**	4.30**	0.50**	0.45*	1.66**
Error	108	0.16	2.52	171.96	0.012	0.22	225.93	0.023	0.973	0.099	0.115	0.109
GCA:SCA		0.38	**2.38**	**3.22**	1.00	0.47	0.719	0.66	**1.57**	**1.74**	**1.04**	0.92

Df= Degree of freedom, Vig=plant vigour, Rootno=average root number per plant, Pltht= plant height, HI=harvest index, Fryld=fresh root yield, Fbrht=first branch height,Dyld=Dry yield per plant, Dm=Dry matter content, CMD= cassava mosaic disease,CBB= cassava bacterial disease, Brlev= branching levels GCA=general combining ability, SCA=specific combining ability

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.5 Mean squares for GCA and SCA for CMD, CBB, yield and yield components of cassava genotypes for two locations of the forest eco-zone combined for 2009/2010

Source	Df	Vig	Rootno	Pltht	HI	Fryld	Fbrht	Dyld	Dm	CMD	CBB	Brlev
GCA	6	0.18ns	30.29**	1698.76*	0.03	3.08*	2211.15**	0.25*	2.85*	0.64**	0.89**	0.36
SCA	21	1.44**	11.97**	1285.55*	0.06	7.47**	1550.18**	0.65**	4.36**	0.54**	0.35*	0.40
Error	108	0.08	2.72	290.83	0.05	0.27	336.12	0.02	0.89	0.11	0.16	0.19
GCA:SCA		0.13	**2.53**	**1.32**	0.5	0.41	**1.43**	0.38	0.65	**1.19**	**2.54**	0.9

Df= Degree of freedom, Vig=plant vigour, Rootno=average root number per plant, Pltht= plant height, HI=harvest index, Fryld=fresh root yield, Fbrht=first branch height, Dyld=Dry yield per plant, Dm=Dry matter content, CMD= cassava mosaic disease, CBB= cassava bacterial disease, Brlev= branching levels GCA=general combining ability, SCA=specific combining ability

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.6 Mean squares for GCA and SCA for CMD, CBB, yield and yield components of cassava clones at Pokuase (savannah zone)

Source	DF	Vig	Rootno	Plth	HI	Fryld	Fbrht	Dryld	Dm	CMD	CBB	Brlev
GCA	4	0.69*	13.86*	1126.03*	99.72	19.68*	1130.79*	1.38ns	7.63*	0.52*	0.31*	0.59*
SCA	10	0.51*	7.66*	1718.45**	42.50	26.54*	1382.10**	1.96*	4.33*	0.24	0.33*	0.18
Error	30	0.17	2.929	392.02	59.05	6.7	201.56	0.55	2.21	0.14	0.11	0.15
GCA:SCA		**1.35**	**1.81**	0.66	**2.35**	0.74	0.82	0.70	**1.76**	**2.17**	0.94	**3.28**

Df= Degree of freedom, Vig=plant vigour, Rootno=average root number per plant, Pltht= plant height, HI=harvest index, Fryld=fresh root yield, Fbrht=first branch height, Dyld=Dry yield per plant, Dm=Dry matter content, CMD= cassava mosaic disease, CBB= cassava bacterial disease, Brlev= branching levels GCA=general combining ability, SCA=specific combining ability

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.7 Mean squares for GCA and SCA for CMD, CBB, yield and yield components of cassava clones at Ohawu (savannah zone)

Source	DF	Vig	Rootno	Plth	HI	Fryld	Fbrht	Dryld	Dm	CMD	CBB	Brlev
GCA	4	0.67*	11.35	2301.86**	0.012*	5.90**	1088.51**	0.62**	2.67	1.27*	0.39*	0.35
SCA	10	0.12*	38.16	2819.67**	0.01*	4.99**	940.16**	0.46**	5.97	0.52	0.35*	0.22
Error	30	0.1982	3.793	392.07	0.004	0.79	263.44	0.09	2.86	0.29	0.08	0.14
GCA:SCA		**5.530**	0.298	0.816	**1.200**	**1.182**	**1.158**	**1.348**	0.447	**2.442**	**1.114**	**1.591**

Df= Degree of freedom, Vig=plant vigour, Rootno=average root number per plant, Pltht= plant height, HI=harvest index, Fryld=fresh root yield, Fbrht=first branch height, Dyld=Dry yield per plant, Dm=Dry matter content, CMD= cassava mosaic disease, CBB= cassava bacterial disease, Brlev= branching levels GCA=general combining ability, SCA=specific combining ability

* $P \leq 0.05$, ** $P \leq 0.01$

4.3.3 General and specific combining ability effects

Parents Debor and Agric had significant negative GCA effects for root number in the first season (Table 4.8) and Debor had a significant negative value in the second season as well (Table 4.10). Dabodabo had a significant positive GCA effect for dry matter content in the first season in the forest zone. Kwasea and Tuaka had significant negative CMD GCA for the first season, which is very good, as it will not contribute to high values for CMD. Debor and Agric had significant positive GCA for CMD, which means that they will contribute to high values for this characteristic.

In the first season in the forest zone, there was a negative SCA effect for fresh yield for Dabodabo x Agric (Table 4.9). For root number Dabodabo x Debor had a negative SCA and Dabodaba x Afebakye a significant positive value. For plant vigour only combination Agric x Tuaka showed significant SCA (positive). For harvest index only combination Afebankye x Kwasea had a significant value (positive). For the second season in the forest zone (Table 4.11) there were also very few positive SCA values. Dabodabo x Lagos had a positive SCA for harvest index and CMD. For CMD there were three desirable negative values.

The differences observed between years evaluated might be due to differences in environmental conditions, especially rainfall which differed in amount and distribution pattern during the two years. During 2008 rainfall was better distributed compared to 2009. Significant GCA x environmental interaction effects for fresh roots yield and dry matter content were reported by Jaramillo et al. (2005).

For the savannah zone (Table 4.12) parent Afisiafi had positive significant GCA for plant vigour. Two parens, Afisiafi and Sisipe had desirable negative GCA effects for CBB. For the SCA effects there were very few significant values (Table 4.13). There was a significant negative value for CMD in the Afisiafi x Santumbankye combination.

Table 4.8 Estimates of GCA effects for some traits of cassava at the forest zone (both locations) of Ghana in the 2008/2009 growing season

Parents	Fyld	Rootno	Vig	HI	DM	CMD	Dryld
Dabodabo	-0.11	0.27	0.06	-0.03	**0.51***	-0.12	-0.02
Debor	-0.06	**-0.60***	0.11	0.01	-0.34	**0.16***	-0.04
Agric	-0.26	**-0.92****	0.01	-0.02	-0.10	**0.18****	-0.09
Lagos	-0.07	-0.20	-0.07	-0.03	-0.07	-0.01	-0.09
Tuaka	0.35	0.52	0.03	0.02	-0.40	**-0.15***	0.08
Afebankye	-0.07	0.29	-0.10	0.02	0.20	-0.03	0.004
Kwasea	-0.16	0.63	-0.04	0.04	0.20	**-0.36***	0.07

Fyld=fresh root yield, Rootno=average root number per plant, Vig=plant vigour, HI=harvest index, DM=Dry matter content, CMD=cassava mosaic disease, Dryld=Dry yield per plant, GCA=general combining ability

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.9 Estimates of SCA effects for some traits of cassava for F1 progenies evaluated in the forest zone (both locations) of Ghana in 2008/2009

Crosses/F1 progenies	Fryld	Rootno	Vig	HI	DM	CMD	Dryld
Dabodabo x Debor	0.33	**-1.73****	0.16	0.07	-0.58	-0.11	0.06
Dabodabo x Agric	**-0.80***	-0.26	-0.14	-0.06	-0.46	0.08	-2.26
Dabodabo x Lagos	-0.21	-0.06	0.01	-0.19	0.28	-0.06	-0.04
Dabodabo x Tuaka	0.47	-0.02	0.11	0.06	-0.63	-0.12	0.07
Dabodabo x Afebankye	-0.22	**1.38***	-0.10	-0.07	0.46	-0.07	-0.04
Dabodabo x Kwasea	0.43	0.68	-0.05	0.01	**0.93***	0.26	0.16
Debor x Agric	0.28	-0.06	-0.11	0.01	0.09	-0.11	0.07
Debor x Lagos	0.11	0.04	0.07	0.01	0.09	-0.05	0.04
Debor x Tuaka	-0.49	1.08	-0.19	-0.04	**1.08***	0.04	-0.08
Debor x Afebankye	-0.17	-0.31	-0.06	-0.05	-0.29	0.26	-0.06
Debor x Kwasea	-0.06	0.97	0.08	-0.02	-0.32	-0.04	-0.04
Agric x Lagos	0.39	1.03	0.09	0.04	0.52	0.09	0.14
Agric x Tuaka	0.32	0.04	**0.28***	0.05	-0.18	-0.09	0.10
Agric x Afebankye	-0.19	-0.48	0.07	-0.01	-0.77	0.15	-0.04
Agric x Kwase	-0.001	-0.27	-0.19	-0.03	-0.19	-0.13	-0.04
Lagos x Tuaka	-0.23	-0.95	-0.19	0.01	-0.77	0.14	-0.10
Lagos x Afebankye	0.37	0.12	0.03	0.03	-0.03	-0.04	0.08
Lagos x Kwasea	-0.44	-0.18	-0.02	-0.07	-0.09	-0.09	-0.12
Tuaka x Afebankye	0.04	0.17	-0.10	-0.04	0.31	-0.14	0.008
Tuaka x Kwasea	-0.11	-0.33	0.08	-0.04	-0.11	0.15	-0.009
Afebankye x Kwasea	0.18	-0.88	0.10	**0.14*****	-0.26	0.16	0.05

Fyld=fresh root yield, Rootno=average root number per plant, Vig=plant vigour HI=harvestindex, DM=Dry matter content, CMD=cassava mosaic disease, Dryld=Dry yield per plant, GCA=general combining ability

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.10 Estimates of GCA effects for some traits of cassava progenitors evaluated in the forest zone (both locations) of Ghana for the 2009/2010 growing season

Progenitors	Fryld	Rootno	Vig	HI	DM	CMD	Dryld
Dabodabo	**-0.35***	**-0.66***	0.03	0.03	0.05	0.07	**-0.10***
Debor	**-0.51****	**-0.67***	0.06	0.007	-0.24	0.07	**-0.16****
Agric	-0.27	-0.42	-0.06	-0.04	**0.33***	-0.01	-0.05
Lagos	0.07	-0.12	-0.05	0.02	0.15	0.06	0.02
Tuaka	0.27	0.58	0.06	0.03	0.09	-0.09	0.08
Afebankye	**0.42***	0.78	-0.03	-0.04	-0.18	-0.10	**0.11***
Kwasea	**0.36***	0.51	0.03	0.00	-0.21	-0.004	0.09

Fryld=fresh root yield, Rootno=average root number per plant, Vig=plant vigour HI=harvest index, DM=Dry matter content, CMD=cassava mosaic disease, Dryld=Dry yield per plant, GCA=general combining ability

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.11 Estimates of SCA effects for some traits of cassava progenies evaluated in the forest zone (both locations) of Ghana for the 2009/2010 growing season

Crosses/F1 progenies	Fryld	Rootno	Vig	HI	DM	CMD	Dryld
Dabodabo x Debor	0.45	0.32	0.14	-0.07	0.13	0.07	0.15
Dabodabo x Agric	0.56	0.76	-0.02	-0.03	-0.54	-0.06	0.09
Dabodabo x Lagos	-0.61	**-1.71***	-0.07	**0.29*****	0.17	**0.29***	-0.18
Dabodabo x Tuaka	-0.40	-1.08	-0.04	-0.13	-0.16	-0.01	-0.09
Dabodabo x Afebankye	-0.17	1.06	-0.10	-0.04	0.47	0.11	0.02
Dabodabo x Kwasea	0.19	0.66	0.08	-0.03	-0.07	**-0.40****	0.05
Debor x Agric	0.40	-0.74	-0.04	0.06	0.20	-0.05	0.14
Debor x Lagos	0.19	0.79	0.04	-0.07	**-0.67***	**-0.53*****	-0.002
Debor x Tuaka	-0.19	0.42	-0.08	0.132	0.24	-0.06	-0.04
Debor x Afebankye	-0.08	0.22	0.001	0.01	-0.20	0.04	-0.04
Debor x Kwasea	**-0.77***	-1.01	-0.06	-0.06	0.31	**0.52*****	**-0.21***
Agric x Lagos	-0.24	0.22	0.04	-0.04	-0.20	**0.28***	0.08
Agric x Tuaka	-0.32	0.02	0.04	-0.03	-0.03	-0.02	-0.08
Agric x Afebankye	-0.07	-0.01	-0.03	0.06	0.36	-0.19	0.02
Agric x Kwase	-0.33	-0.24	0.008	-0.03	0.22	0.03	-0.10
Lagos x Tuaka	0.09	0.39	-0.12	-0.12	-0.08	0.15	-0.03
Lagos x Afebankye	0.11	-0.81	0.09	-0.02	0.30	-0.11	0.06
Lagos x Kwasea	0.60	1.12	0.01	-0.05	0.48	**-0.30***	**0.23***
Tuaka x Afebankye	0.46	0.16	0.14	-0.20	0.02	-0.14	0.10
Tuaka x Kwasea	0.55	0.09	0.06	**-0.17***	0.01	0.08	0.14
Afebankye x Kwasea	-0.24	-0.61	-0.11	0.004	**-0.95****	0.07	-0.11

Fryld=fresh root yield, Rootno=average root number per plant, Vig=plant vigour, HI=harvestindex, DM=Dry matter content, CMD=cassava mosaic disease, Dryld=Dry yield per plant, GCA=general combining ability

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.12 Estimates of GCA effects for some traits of cassava at the savannah zone (both locations) of Ghana in the 2008/2009 season

	Vigour	Rootno	Pltht	HI	Fyld	Dryld	CMD	CBB	Brlev	DM	Fbrht
Afisiafi	**0.277***	0.292	5.052	0.001	-0.146	-0.044	-0.067	**-0.143***	0.137	-0.310	-5.227
Bosomia	0.086	0.620	3.628	-0.004	0.238	0.072	0.033	0.096	-0.024	0.062	-4.549
Sisipe	-0.076	-0.808	-7.637	-0.010	-0.346	-0.117	0.183	**-0.149***	-0.008	-0.371	-0.699
Bokentenma	**-0,220***	0.140	-0.498	-0.004	0.154	0.050	-0.167	0.046	-0.080	0.496	2.957
Santumbankye	-0.037	-0.247	-0.576	0.018	0.099	0.039	0.017	**0.151***	-0.024	0.123	7.520

Vigour=plant vigour, Rootno=average root number per plant, Pltht=plant height, HI=harvest index, Fyld=fresh root yield, Dryld=Dry yield per plant, DM=Dry matter content CMD=cassava mosaic disease, CBB= cassava bacterial blight, Brlev= number of levels of branching, DM=dry matter content, Fbrht=height at first branching

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.13 Estimates of SCA effects for some traits of cassava at the savannah zone (both locations) of Ghana in the 2008/2009 season

	Vigour	Rootno	Pltht	HI	Fyld	Dyld	CMD	CBB	Brlev	DM	Fbrht
Afisiafi x Bosomia	0.111	**-1.931***	**14.667***	0.033	-0.406	-0.153	**0.350***	-0.114	-0.089	-0.314	-6.469
Afisiafi x Sisipe	0.106	0.231	-5.589	0.006	-0.106	-0.014	0.200	0.014	**0.228***	0.719	**-11.069***
Afisiafi x Bokentenma	-0.150	0.681	12.306	-0.033	0.294	0.119	-0.167	-0.014	-0.050	-0.264	**14.225****
Afisiafi x Santumbankye	-0.067	1.019	7.950	-0.006	0.217	0.047	**-0.383***	0.114	-0.089	-0.142	3.314
Bosomia x Sisipe	-0.083	0.936	13.456	-0.006	0.244	0.086	-0.283	0.142	-0.211	-0.519	**17.986*****
Bosomia x Bokentenma	0.011	0.936	7.933	-0.011	0.094	0.003	-0.100	0.014	0.094	0.081	**-10.719***
Bosomia x Santumbankye	-0.039	0.058	-6.722	-0.017	0.067	0.064	0.033	-0.042	0.206	0.753	-0.797
Sisipe x Bokentenma	0.006	-0.853	-13.439	0.011	-0.122	-0.042	0.000	-0.042	0.028	0.297	-3.953
Sisipe x Santumbankye	-0.028	-0.319	-6.800	-0.011	-0.017	-0.031	0.083	-0.114	-0.044	-0.497	-2.964
Bokentenma x Santumbankye	0.133	-0.764	5.052	0.033	-0.267	-0.081	0.267	0.042	-0.072	-0.114	0.447

Vigour=plant vigour, Rootno=average root number per plant, Pltht=plant height, HI=harvest index, Fyld=fresh root yield, Dryld=Dry yield per plant, DM=Dry matter content CMD=cassava mosaic disease, CBB= cassava bacterial blight, Brlev= number of levels of branching, DM=dry matter content, Fbrht=height at first branching

* P≤0.05, ** P≤0.01

4.3.4 Estimates of genetic parameters

Broad sense heritability ranged from 0.12 to 0.67 and narrow sense heritability across environments ranged from 0.30 to 0.64 for the savannah zone (Table 4.14). The GCA variances were low in general compared to SCA variances for the two seasons in the forest zone. Broad sense heritability ranged from 0.05 to 0.68 and narrow sense heritability across environments and seasons ranged from 0.25 to 0.68 for the forest zone (Table 4.15). Heritability estimates (h^2) were classified as high (>0.50), medium (0.30-0.50) and low (<0.30) according to Bhateria et al. (2006). All the traits except fresh yield and dry yield had higher values of δ^2_{sca} than δ^2_{gca} in the savannah zone while plant vigour, root number, harvest index, fresh yield, dry yield, dry matter, CMD and CBB had higher values of δ^2_{sca} in both seasons than δ^2_{gca} in the forest zone, indicating contribution of non additive genetic effects. Selection for these traits will be problematic using direct selection in the respective zones; hence, heterosis and use of hybrid vigour could be applied for improving them.

All the traits except first branch height and plant height had high broad sense heritability for the two zones. High broad sense heritability indicated that the traits had high genetic variance, both additive and non-additive. In this study, almost all the traits had high broad sense heritability. Narrow sense heritability is important for breeding programmes as it estimates the relative importance of the additive portion of the genetic variance that can be transmitted to the next generation. In the savannah zone a number of characteristics had high narrow sense heritability > 0.57 (plant vigour, root number, fresh yield, first branch height, plant height, dry yield, dry matter content, CMD, CBB and branching level) (Table 4.14). In the forest zone a number of characteristics also had high narrow sense heritability > 0.5 (plant vigour, root number, harvest index, CMD and CBB) (Table 4.15).

The magnitude of heritability of a given trait is affected by the type of genetic material involved (Ceccarelli 1994). Cassava is a vegetatively propagated crop with the advantage that in every new hybrid the genes are fixed, the new commercial variety is produced by simply multiplying the stem cuttings. For this reason Kawano et al. (1998) suggested that

heritability of cassava after hybridization is mainly broad-sense in nature, lower narrow sense heritability was caused by low additive effects and high dominant gene action.

The average degree of dominance for almost all the traits were less than one except fresh yield, dry yield and harvest index in the savannah zone (Table 4.14); and root number, first branch and branch levels in the forest zone (Table 4.15), suggesting partial dominance. The greater degree of dominance for the three traits above suggests the presence of over dominance for those characters.

Baker (1978) indicated that when SCA means are not important, the hypothesis is that performance of single cross progeny can be adequately predicted on the basis of GCA. Moreover, if the SCA mean squares are significant, the relative importance of GCA and SCA should be determined by estimating the components of variance to predict the progeny performance. The closer the ratio of 2GCA/(2GCA+SCA) is to 1, the more important the additive gene effects. The predictability ratio in this study varied from the lowest 0.58 to 0.84 (Table 4.14) and 0.43 to 0.93 (Table 4.15). CMD and root number had a ratio closer to one for the savannah and forest zones indicating the importance of GCA and additive gene action.

Table 4.14 Estimates of genetic parameters for various traits evaluated across two locations in the savannah eco-zone for season 2008/2009

	Genetic parameter								
Source	δ^2_{gca}	δ^2_{sca}	δ^2_{gcaEnv}	δ^2_{scaEnv}	δ^2_{e}	$h^2b(\%)$	$h^2n(\%)$	PR	$\sqrt{(H/D)}$
Vig	1.481	1.563	0.533	1.762	0.019	0.663	0.569	0.73	0.973
Rootno	1.17	1.199	0.371	5.954	3.499	0.601	0.577	0.58	0. 987
Pltht	0.855	6.778	0.58	1.708	341.6	0.121	0.639	0.61	0.355
HI	0.989	0.982	2.352	0.725	0.013	0.316	0.303	0.82	1.003
Fyld	2.937	0.608	0.131	5.538	3.54	0.628	0.601	0.79	2.197
Fbrht	1.803	1.853	0.137	3.454	235.6	0.115	0.642	0.74	0.986
Dryld	2.829	0.685	0.118	4.59	0.314	0.663	0.613	0.79	2.032
DM	1.207	3.436	0.586	0.947	2.451	0.637	0.614	0.69	0.592
CMD	2.075	3.959	1.418	0.721	0.215	0.664	0.565	**0.84**	0.723
CBB	0.782	20.07	3.918	0.306	0.107	0.666	0.607	0.64	0.197
Brlev	0.686	1.789	1.899	0.717	0.137	0.662	0.614	0.68	0.987

Vig=plant vigour, Rootno=average root number per plant, Pltht=plant height, HI=harvest index, Fyld=fresh root yield, Fbrht=first branching height, Dryld=Dry yield per plant, DM=Dry matter content, CMD=cassava mosaic disease, CBB= cassava bacterial blight Brlev=number of levels of branching, h^2b=broad sense heritability, h^2n=narrow sense heritability, PR=performance ratio, H/D=Degree of dominance

Table 4.15 Estimates of genetic parameters for various traits evaluated across two locations in the forest eco-zones for two seasons 2008/2009 and 2009/2010

		Genetic parameter								
Traits	Years	δ^2_{gca}	δ^2_{sca}	δ^2_{gcaEnv}	δ^2_{scaEnv}	δ^2_{e}	$h^2b(\%)$	$h^2n(\%)$	PR	√(H/D)
Vig	2008/09	0.387	5.799	1.227	0.205	0.165	0.666	0.659	0.430	0.081
Vig	2009/10	0.241	5.683	1.411	2.863	0.089	0.665	0.593	0.200	0.205
Rootno	2008/09	2.266	3.403	0.604	1.102	2.529	0.644	0.612	0.826	0.816
Rootno	2009/10	2.298	2.497	0.686	1.757	2.728	0.639	0.586	0.835	0.959
Pltht	2008/09	2. 725	1.977	0.728	3.442	171.97	0.186	0.562	0.865	1.173
Pltht	2009/10	0.447	2.095	6.317	2.109	290.84	0.056	0.265	0.725	0.462
HI	2008/09	1.496	1.621	0.223	1.18	0.012	0.666	0.623	0.684	0.960
HI	2009/10	0.694	1.804	1.949	0.707	0.047	0.665	0.501	0.546	0.620
Fyld	2008/09	0.665	1.558	1.041	12.079	0.229	0.661	0.499	0.483	0.653
Fyld	2009/10	0.645	2.962	0.477	9.14	0.276	0.662	0.617	0.452	0.467
Fbrht	2008/09	1.872	0.922	3.150	7.015	225.93	0.104	0.246	0.934	1.425
Fbrht	2009/10	0.567	2.025	4.833	2.276	336.12	0.052	0.303	0.740	0.529
Dyld	2008/09	0.728	1.476	1.208	9.486	0.024	0.667	0.473	0.564	0.702
Dyld	2009/10	0.613	3.301	0.434	7.54	0.084	0.666	0.625	0.439	0.431
DM	2008/09	0.608	2.487	5.596	1.776	0.974	0.648	0.314	0.759	0.495
DM	2009/10	0.925	3.270	0.121	1.487	0.897	0.654	0.655	0.566	0.531
CMD	2008/09	1.194	2.097	1.796	2.406	0.099	0.665	0.467	0.777	0.754
CMD	2009/10	1.053	2.292	1.214	2.003	0.119	0.678	0.527	0.701	0.678
CBB	2008/09	1.226	3.405	0.342	1.154	0.115	0.664	0.678	0.678	0.599
CBB	2009/10	2.151	3.158	0.976	0.707	0.161	0.665	0.577	0.833	0.825
Brlev	2008/09	0.57	2.058	2.998	7.364	0.109	0.664	0.385	0.647	0.526
Brlev	2009/10	1.371	0.64	0.507	3.209	0.197	0.662	0.4774	0.642	1.464.

Vig=plant vigour, Rootno=average root number per plant, Pltht= plant height, HI=harvest index, Fyld=fresh root yield, Fbrht=first branching height, Dyld=Dry yield per plant, DM=Dry matter content, Brlev=number of levels of branching, h^2b=broad sense heritability, h^2n=narrow sense heritability, PR=performance ratio, H/D=Degree of dominance

4.3.5 Estimates of mid and better parent heterosis

The estimates of heterosis over respective mid and better parent were significantly different between the traits studied for the forest zone (Tables 4.16a-4.17a). For plant vigour, values for all the combinations were significant but negative for better parent heterosis in both seasons. Most of the hybrids showed negative heterosis for root number in both seasons (Tables 4.16a and 4.17a). Dabodabo x Afebankye and Dabodabo x Kwasea showed positive heterosis for root number over better parent for the 2008 planting season, the mid-parent heterosis was positively significant (Table 4.16a). In 2009, both combinations showed negative significant mid and better parent heterosis for root number (Table 4.17a). Debor x Kwasea (2008) was the best combination for first height at branching, showing positive significant mid and better parent heterosis (27.60% and 28.90%, Table 4.16b).

Many hybrid combinations showed positive significant heterosis over both mid and better parent values for CMD especially in the 2008/2009 season with great susceptibility even up to 94.69% in Dabodabo x Agric. Debor x Tuaka showed negative resistance to CMD at 7.35% more than the better parent in 2008 (Table 4.16c) and 26.09 in 2009 (Table 4.17c). Debor x Lagos also showed 37.89% higher resistance to CMD than both mid and better parent values in the 2009/2010 season. Dabodabo had a negative significant GCA for CMD in 2008. Kwasea had significant negative values for CMD in both seasons of planting but other hybrids with Kwasea as parent, were susceptible to CMD. Some of the hybrids derived from Dabodabo showed resistance and can be used in breeding, especially Dabodabo x Tuaka.

CMD is one of the most important biotic constraints in Ghana and West Africa. Negative values are therefore desirable and significant heterosis values were observed for this trait. The best four combinations observed were Debor x Tuaka, Dabodabo x Debor, Dabodabo x Tuaka and Debor x Lagos. In 2008, only Dabodabo x Agric showed non- significant negative heterosis for fresh yield (Table 4.16b), the rest were positive although some were not significant.

Table 4.16a Mean performance and percentage of better parent and mid-parent heterosis and for plant vigour, root number and plant height in the 2008/2009 season at the forest ecological zone

Geno	Vig	BPH	MPH	Rootno	BPH	MPH	Pltht	BPH	MPH
P1	4.33			12			293.7		
P2	4			13.66			275.7		
P3	4.66			14.33			313.4		
P4	3.33			10.33			259.3		
P5	4.33			17.67			290.5		
P6	4			14.33			303.8		
P7	4			12.33			333.2		
1x2	3.35	-22.63**	-19.57**	10.83	-20.72*	-15.59**	262	-10.79**	-7.97**
1x3	2.95	-36.70**	-34.37**	11.98	-16.40*	-9.00**	277.9	-11.33**	-8.45**
1x4	3.01	-30.48**	-21.41	12.9	7.50	15.54	298.7	1.69	8.02
1x5	3.21	-25.87**	-25.87	13.66	-22.69**	-7.91	301	2.49	3.05
1x6	2.88	-33.49**	-30.85	14.83	3.49	**12.63***	306.9	1.00	**2.71***
1x7	2.98	-31.18**	-28.45	14.46	17.27	**18.85***	296.2	-11.11**	-5.51*
2x3	3.03	-34.98**	-30.02	11.31	-21.07*	-19.19	251	-19.92**	-14.80
2x4	3.13	-21.75**	-14.60*	12.13	-11.20	1.13	286.8	4.01	**7.21****
2x5	2.96	-31.64**	-28.93	13.9	-21.34**	-11.26	288.4	-0.54	1.88
2x6	3.01	-24.75**	-24.75	12.28	-14.31	-12.26*	279.2	-8.05*	-3.65*
2x7	3.16	-21.00**	-21.00	13.9	1.76	**6.95***	296.5	-11.00**	-2.61*
3x4	3.05	-34.55**	-23.65	12.8	-10.68	3.81	280.8	-10.41**	-1.95
3x5	3.33	-28.54**	-25.92	12.53	-29.09**	-21.68**	286.3	-1.46	-5.20
3x6	3	-35.62**	-30.72	11.78	-17.79*	-17.80*	279.3	-10.89**	-9.50*
3x7	2.8	-39.91**	-35.33	12.33	-13.96	-7.51*	285.4	-14.35**	-11.73*
4x5	2.78	-35.80**	-27.42*	12.27	-30.58**	-12.37	284.4	-2.10	**3.46****
4x6	2.88	-28.00**	-21.42*	13.1	-8.58	6.23	292.7	-3.67	**3.95****
4x7	2.88	-28.00**	-21.42	13.13	6.52	**15.90***	306.4	-8.04**	**3.43***
5x6	2.85	-34.18**	-6.40*	13.88	-21.43**	-2.54	307.1	1.08	**1.05***
5x7	3.08	-28.87**	-0.48*	13.72	-22.37**	-2.44*	299.5	-10.11**	0.31
6x7	2.98	-25.50**	-28.45**	12.93	-9.75	2.11	296.4	-11.04**	-3.17

Parents: 1=Dabodabo, 2=Debor, 3=Agric, 4=Lagos, 5=Tuaka, 6=Afebankye, 7=Kwasea, BPH=better parent heterosis, MPH=mid parent heterosis, vig=plant vigour, rootno= root number, Pltht=plant height,
* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.16b Mean performance and percentage of mid-parent heterosis and best parent heterosis for harvest index, fresh yield and first branch height in the 2008/2009 season at the forest ecological zone

Genotype	HI	BPH	MPH	Fryld	BPH	MPH	Fbrht	BPH	MPH
P1	0.5			3.35			109.5		
P2	0.53			2.62			132.8		
P3	0.52			3.65			116.8		
P4	0.53			2.82			113.7		
P5	0.6			4.08			212.6		
P6	0.57			4.05			125.7		
P7	0.53			2.8			136		
1x2	0.53	0.63	**3.23****	4.97	**48.26****	**66.48****	129.2	-2.77	**6.59****
1x3	0.37	-29.49	-27.87**	3.63	-0.46	**3.81****	106.6	-8.78	-5.83**
1x4	0.4	-24.53	-22.58	4.42	**31.84****	43.24	138.1	**21.51***	23.77
1x5	0.53	-11.11	-3.03	5.52	**35.21****	48.43	181.3	-14.71**	**12.59****
1x6	0.4	-29.82*	-25.00	4.47	**10.29**	**20.72***	115.9	-7.74	-1.50
1x7	0.5	-5.66	-3.23	5.3	**58.21****	**72.36***	153.1	12.56	**24.71****
2x3	0.48	-8.81	-7.94	4.77	**30.59****	52.13	121.1	-8.86	-3.02
2x4	0.48	-8.81	-9.38	4.78	**69.62****	76.07	144.5	8.80	17.26
2x5	0.48	-19.44	-14.71	4.6	12.75	37.31	151.7	-28.63**	-12.15**
2x6	0.47	-18.13	-15.15	4.57	12.76	**37.00***	118.4	-10.88	-8.43
2x7	0.52	-2.52	-3.12	4.85	**73.21****	**79.08***	173.3	**27.40****	**28.90****
3x4	0.47	-11.95	-11.11	4.87	**33.33****	50.52	115.8	-0.91	0.46
3x5	0.53	-11.11	-4.48	5.22	**27.86****	**34.91****	153.8	-27.66**	-6.63**
3x6	0.47	-18.13	-13.85	4.35	7.41	**12.99***	109.8	-12.67	-9.47
3x7	0.47	-11.95	-11.11	4.72	**68.45****	**46.25***	139.1	2.25	**10.01****
4x5	0.48	-19.44	-14.71	4.85	**18.87***	40.58	152	-28.49**	-6.80**
4x6	0.5	-12.28	-9.09	5.1	**25.93****	48.54	139.1	10.59	16.17
4x7	0.42	-21.38	-21.87	4.47	**58.39****	**59.05***	121.1	-11.32	-2.96**
5x6	0.48	-19.44	3.57	5.18	**27.04****	**3.84****	132.3	-37.75**	-10.94**
5x7	0.5	-16.67	-3.23	5.22	**27.86****	-3.54**	152.1	-28.45**	-9.03**
6x7	0.68	19.88	30.16	5.15	**27.16****	**52.03****	138	1.43	**14.78****

Parents: 1=Dabodabo, 2=Debor, 3=Agric, 4=Lagos, 5=Tuaka, 6=Afebankye, 7=Kwasea, BPH=better parent heterosis, MPH=mid-parent heterosis, HI=Harvest index, Fryld = fresh tuber yield, Fbrht= height at first branching

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.16c Mean performance and percentage of better parent and mid-parent heterosis for dry yield, dry matter content and CMD in the 2008/2009 season at the forest ecological zone

Genotype	Dryld	BPH	MPH	DM	BPH	MPH	CMD	BPH	MPH
P1	0.98			29.88			1.5		
P2	0.77			29.98			2.53		
P3	1.02			28.1			1.13		
P4	0.75			26.8			1.67		
P5	1.25			30.32			2		
P6	1.22			29.88			2.1		
P7	0.85			29.98			1.4		
1x2	1.38		**58.10****	28	-6.60**	-6.46	1.98	**32.22***	-1.65**
1x3	1.05	2.94	**5.00****	28.37	-5.06*	-2.16	2.2	**94.69****	**67.09****
1x4	1.32	**34.35****	51.92	29.13	-2.50	2.79	1.87	24.44	**17.89***
1x5	1.52	21.33*	35.82	27.9	-6.63**	-7.31	1.67	11.11	-4.76*
1x6	1.33	9.29	**21.21***	29.58	-2.43	-1	1.83	22.22	1.85
1x7	1.6	**63.27****	**74.55***	30.05	0.57	0.39	2.17	**54.76****	49.43
2x3	1.32	**29.08***	**47.66***	28.07	-6.38*	-3.36	2.28	**102.06****	24.55
2x4	1.37	**77.49****	80.22	28.1	-6.27*	-1.03	2.15	**28.74***	**2.38****
2x5	1.33	6.67	32.23	28.68	-5.40*	-4.86	2.1	5.00	-7.35*
2x6	1.28	5.19	**29.41***	27.98	-6.66**	-6.51	2.43	15.87	5.04
2x7	1.37	**60.78****	**69.07***	27.95	-6.77**	-6.78	2.15	**53.57****	9.32
3x4	1.42	**38.89****	60.38	28.77	2.37	4.8	2.32	**76.84****	**65.48****
3x5	1.47	17.33	**29.41****	28.1	-7.32**	-3.79	2	**76.99****	27.66
3x6	1.25	2.46	**11.94***	28.33	-5.18*	-2.27	2.35	**107.96****	45.36
3x7	1.32	**29.08***	**41.07***	28.37	-5.38*	-2.32	2.08	**84.37****	64.47
4x5	1.35	8.00	35.00	27.18	-10.35**	-4.81	2.03	21.76	10.91
4x6	1.45	**18.85***	47.46	28.52	-4.56	0.62	1.97	17.76	**4.42****
4x7	1.32	**54.90****	**64.58***	28.45	-5.10*	0.21	1.93	**38.10***	26.09
5x6	1.47	17.33	**2.92****	28.53	-5.58*	-0.72	1.73	-13.33	-0.95
5x7	1.52	**21.33***	-2.67**	28.1	-7.02**	-3.02	2.03	**45.24****	6.09
6x7	1.5	**22.95***	**48.76***	28.55	-4.77	-4.54	1.83	30.95	12.82

Parents: 1=Dabodabo, 2=Debor, 3=Agric, 4=Lagos, 5=Tuaka, 6=Afebankye, 7=Kwasea, BPH=better parent heterosis, MPH=mid-parent heterosis, Dryld= dry yield, DM= dry matter content, CMD= Cassava Mosaic Disease

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.16d Mean performance and percentage of better parent and mid-parent heterosis for CBB and branching level in the 2008/2009 season at the forest ecological zone

Genotype	CBB	BPH	MPH	Brlev	BPH	MPH
P1	1.33			4.5		
P2	2.5			2.83		
P3	1.33			2.5		
P4	2.17			3.67		
P5	2.33			3		
P6	2.3			3		
P7	1.5			4		
1x2	1.8	35.34	-6.09*****	2.57	-42.96**	-30.00**
1x3	1.73	30.33	**30.00****	2.85	-36.67**	-18.57**
1x4	1.7	27.82	-2.86	2.7	-40.00**	-33.88**
1x5	1.97	**47.87***	**7.27***	1.9	-57.78**	-49.33**
1x6	1.93	45.36	6.42	2.57	-42.96**	-31.56**
1x7	2.07	**55.39****	45.88	2.63	-41.48**	-38.04**
2x3	1.85	**39.10***	-3.48	2.47	-12.84	-7.50
2x4	1.7	-21.66	-27.14**	2.67	-27.34**	-17.95**
2x5	1.93	-17.02	-20.00*	2.58	-13.89	-11.43
2x6	1.55	-32.61**	-35.42	2.88	-3.89	-1.14
2x7	1.9	26.67	-5.00	2.47	-38.33**	-27.80**
3x4	1.65	24.06	-5.71**	2.72	-25.98**	-11.89**
3x5	1.95	**46.62***	6.36	2.35	-21.67*	-14.55*
3x6	2.13	**60.40****	17.43	2.92	-2.78	6.06
3x7	1.93	**45.36***	36.47	2.73	-31.67**	-15.90**
4x5	1.8	-17.05	-20.00*	2.4	-34.60**	-28.00**
4x6	1.92	-11.67	-14.18**	2.45	-33.24**	-26.50**
4x7	1.98	32.22	8.18	2.92	-27.08**	-23.91**
5x6	1.72	-25.36*	-11.97	2.57	-14.44	14.93
5x7	1.78	18.89	-11.57	2.33	-41.67**	**2.94****
6x7	1.65	10.00	2.06	2.58	-35.42**	-35.42**

Parent: Parent: 1=Dabodabo, 2=Debor, 3=Agric, 4=Lagos, 5=Tuaka, 6=Afebankye, 7=Kwasea, BPH=better parent heterosis, MPH=mid-parent heterosis, CBB=cassava bacteria blight, Brlev= branching levels

* P≤0.05, ** P≤0.01

Table 4.17a Mean performance and percentage of better parent and mid-parent heterosis for plant vigour, root number and plant height in the 2009/2010 season at the forest ecological zone

Genotype	Vigour	BPH	MPH	Rootno	BPH	MPH	Pltht	BPH	MPH
P1	3.83			12.17			269.3		
P2	3.83			14			318.5		
P3	4.17			14.67			302.5		
P4	3.83			11.17			279.3		
P5	4.17			18.33			292.7		
P6	3.67			15			296.3		
P7	3.67			14			302.5		
1x2	3.17	-17.39**	-17.39**	11.33	-19.05*	-13.38	266.4	-16.37**	-9.38*
1x3	2.88	-30.80**	-27.92**	12	-18.20*	-10.56	303.4	0.31	6.13
1x4	2.85	-25.65**	-25.65**	9.83	-19.20	-15.71	256	-8.35	-6.68
1x5	2.98	-28.46**	-25.42**	11.17	-39.08**	-26.78**	278.8	-4.74	-0.78
1x6	2.83	-26.02**	-24.44**	13.5	-10.00	-0.61	296.6	0.09	4.87
1x7	3.02	-21.24**	-19.56**	12.83	-8.33	-1.91	291.9	-3.50	2.09
2x3	2.9	-30.46**	-27.50**	10.5	-28.43**	-26.74**	259.2	-18.61**	-16.52**
2x4	2.98	-22.11**	-22.17**	12.33	-11.90	-1.99	290.5	-8.78*	-2.80
2x5	2.97	-28.86**	-25.83**	12.67	-30.90**	-21.65**	293.9	-7.72	-3.82
2x6	2.97	-22.54**	-20.89**	12.67	-15.56	-12.64	289.4	-9.14*	-5.87
2x7	2.9	-24.28**	-22.67**	11.17	-20.24*	-20.24*	272.9	-14.31**	-12.10**
3x4	2.87	-31.25**	-28.33**	12	-18.20*	-7.10	290.9	-3.84	-0.01
3x5	2.97	-28.86**	-28.80**	12.5	-31.81**	-24.24**	281.2	-7.05	-5.51
3x6	2.82	-32.45**	-28.09**	12.67	-15.56	-14.61*	301.6	-0.31	0.71
3x7	2.85	-31.65**	-27.23**	12.17	-17.06*	-15.12*	282.7	-6.55	-6.55
4x5	2.82	-32.45**	-29.58**	13.17	-28.17**	-10.73	285.2	-2.56	-0.29
4x6	2.95	-22.98**	-21.33**	12.17	-18.89*	-7.01	289.8	-2.21	0.68
4x7	2.87	-25.15**	-23.56**	13.83	-1.19	9.93	297.9	-1.52	2.41
5x6	3.1	-25.66**	-20.85**	13.83	-24.53**	-17.00*	315	6.30	6.96
5x7	3.02	-27.66**	-22.98**	13.5	-26.35**	-16.49*	303.1	0.19	1.85
6x7	2.77	-24.61**	-5.41	13	-13.33	-1.27	295.2	-2.43	0.31

Parents: 1=Dabodabo, 2=Debor, 3=Agric, 4=Lagos, 5=Tuaka, 6=Afebankye, 7=Kwasea, BPH=better parent heterosis, MPH=mid-parent heterosis, vig=plant vigour, rootno= root number, Pltht=plant height

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.17b Mean performance and percentage of better parent and mid-parent heterosis for harvest index, fresh yield and first branch height in the 2009/2010 season at the forest ecological zone

Genotype	HI	BPH	MPH	Fryld	BPH	MPH	Fbrht	BPH	MPH
P1	0.5			2.92			125.33		
P2	0.5			2.98			120.33		
P3	0.4			3.4			137.67		
P4	0.53			2.83			119.52		
P5	0.6			3.32			187.9		
P6	0.57			3.25			143.8		
P7	0.53			3.4			156.17		
1x2	0.5	0.00	0.00	4.77	**59.96****	**61.58****	131.05	4.56	6.69
1x3	0.5	0.00	11.11	5.1	**50.00****	**61.48****	155.68	13.08	**18.39***
1x4	0.88	**66.67***	**70.97***	4.3	**47.26****	**49.57****	133.48	6.51	9.03
1x5	0.47	-22.22	-15.15	4.7	**41.57****	**50.80****	143.22	-23.78**	-8.56
1x6	0.48	-15.20	-9.38	5.08	**56.41****	**64.86****	128.32	-10.77	-4.64
1x7	0.53	0.63	3.23	5.38	**58.33****	**70.45****	161.03	3.11	14.41
2x3	0.57	13.33	25.93	4.8	**41.18****	**50.39****	138.5	0.60	7.36
2x4	0.5	-5.66	-3.23	4.93	**65.55****	**69.63****	164.42	**36.64****	**37.10****
2x5	0.7	16.67	27.27	4.75	**43.07****	**50.79****	140.17	-25.40**	-9.05
2x6	0.52	-9.36	-3.12	5.02	**54.36****	**60.96****	166.52	**15.80***	**26.09****
2x7	0.48	-8.81	-6.45	4.27	**25.49***	**33.68****	130.92	-16.17**	-5.30
3x4	0.48	-8.81	3.57	4.78	**40.69****	**53.48****	143.95	4.56	11.94
3x5	0.5	-16.67	0.00	4.87	**43.14****	**44.91****	142.32	-24.26**	-12.57
3x6	0.52	-9.36	6.90	5.27	**54.90****	**58.40****	160	11.27	13.69
3x7	0.47	-11.95	0.00	4.95	**45.59****	**45.59****	118.62	-24.05**	-19.26*
4x5	0.47	-22.22	-17.65	5.43	**63.65****	**76.69****	131.93	-29.79**	-14.17
4x6	0.5	-12.28	-9.09	5.78	**77.95****	**90.14****	142.78	-0.71	8.45
4x7	0.52	-2.52	-3.12	6.22	**82.84****	**99.47****	146.85	-5.97	6.54
5x6	0.5	-16.67	-14.29	6.33	**90.76****	**92.89****	165.93	-11.69*	0.05
5x7	0.73	22.22	29.41	6.37	**87.25****	**89.58****	145.53	-6.81	-15.40*
6x7	0.5	-12.28	-1.64	5.73	**68.63****	9.55	163.67	4.80	13.13

Parent: 1=Dabodabo, 2=Debor, 3=Agric, 4=Lagos, 5=Tuaka, 6=Afebankye, 7=Kwasea, BPH=better parent heterosis, MPH=mid parent heterosis, HI=Harvest index, Fryld= fresh root yield, Fbrht = height at first branching, * P≤0.01

* P≤0.05, ** P≤0.01

Table 4.17c Mean performance and percentage of better parent and mid-parent heterosis for dry yield, dry matter content and CMD in the 2009/2010 season at the forest ecological zone

Genotype	Dryld	BPH	MPH	DM	BPH	MPH	CMD	BPH	MPH
P1	0.85			29.23			1.8		
P2	0.92			30.53			2.7		
P3	0.9			26.7			1.8		
P4	0.8			28.38			2.7		
P5	1			29.65			2.7		
P6	0.98			30.48			2.1		
P7	0.98			28.33			1.8		
1x2	1.42	**53.99****	**60.38****	28.25	-7.47*	-5.47**	2.3	24.77	0.74
1x3	1.47	**62.96****	**67.62****	28.15	-3.69	0.66	2.1	14.81	13.76
1x4	1.27	**49.02****	**53.54****	28.68	-1.87	-0.43	2.5	**35.70***	10.37
1x5	1.42	**41.67****	**53.15****	28.28	-4.61	-3.93	2	11.11	-9.63
1x6	1.52	**54.76****	**65.45****	28.65	-6.00*	-4.05*	2.2	17.49	10.26
1x7	1.57	**59.86****	**70.91****	28.08	-3.92	-2.43	1.7	-2.07	-3.70
2x3	1.45	**57.61****	**59.63****	28.6	-6.32	-0.06	2.1	15.74	-7.41
2x4	1.38	**50.36****	**61.17****	27.55	-9.76**	-6.48**	1.7	-37.58**	-37.89**
2x5	1.4	**40.00****	**46.09****	28.4	-6.98**	-5.62**	2	-25.72*	-26.09**
2x6	1.43	**46.26****	**50.88****	27.68	-9.32**	-9.26**	2.1	0.64	-12.59
2x7	1.25	**27.55***	**31.58****	28.17	-7.74*	-4.30	2.7	49.72**	18.66
3x4	1.42	**57.41****	**66.67****	28.6	0.78	3.84	2.4	**33.33***	7.46
3x5	1.47	**46.67****	**54.39****	28.7	-3.20	1.86	2	8.33	-12.69
3x6	1.6	**63.27****	**69.91****	28.82	-5.46	0.79	1.8	-1.85	-8.62
3x7	1.47	**49.66****	**55.75****	28.65	1.13	4.12	2.1	17.70	16.82
4x5	1.6	**60.00****	**77.78****	28.47	-3.99	-1.90	2.2	-18.23	-18.13*
4x6	1.72	**75.17****	**92.52****	28.58	-6.22	-2.89	2.1	3.06	-9.86
4x7	1.87	**90.48****	**109.35****	28.73	1.25	1.32	1.8	2.64	-18.05
5x6	1.82	**81.67****	**83.19****	28.23	-7.37**	-6.10**	1.7	-16.26	-26.76**
5x7	1.83	**83.33****	**84.87****	28.2	-4.89	-2.73	2.1	15.82	-7.52
6x7	1.62	**64.97****	4.86	26.97	-11.53*	-4.94*	2	14.88	4.72

Parents: 1=Dabodabo, 2=Debor, 3=Agric, 4=Lagos, 5=Tuaka, 6=Afebankye, 7=Kwasea, BPH=better parent heterosis, MPH=mid-parent heterosis, Dryld= dry yield, DM= dry matter content, CMD= Cassava Mosaic Disease

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.17d Mean performance and percentage of better parent and mid-parent heterosis for CBB and branching level in the 2009/2010 season at the forest ecological zone

Genotype	CBB	BPH	MPH	Brlev	BPH	MPH
P1	1.33			3		
P2	2.5			3		
P3	1.33			2.17		
P4	2.33			2.67		
P5	2.5			2.83		
P6	2.17			2.67		
P7	1.5			2.33		
1x2	1.75	31.58	-8.70	2.5	-16.67	-16.67
1x3	1.73	30.33	30.00	2.67	-11.11	3.23
1x4	1.85	39.10	0.91	2.67	-11.11	-5.88
1x5	1.87	40.35	-2.61	2.83	-5.56	-2.86
1x6	1.77	32.83	0.95	2.83	-5.56	0.00
1x7	1.63	22.81	15.29	2.33	-22.22*	-12.50
2x3	1.73	30.33	-9.57	2.67	-11.11	3.23
2x4	1.77	-24.18	-26.90*	2.5	-16.67	-11.76
2x5	1.78	-28.67*	-28.67**	2.5	-16.67	-14.29
2x6	1.95	-10.14	-16.43	2.33	-22.22*	-17.65
2x7	2.15	**43.33***	7.50	3	0.00	12.50
3x4	2.02	**51.63***	10.00	2.67	-0.12	10.34
3x5	1.92	44.11	0.00	2.33	-17.55	-6.67
3x6	1.93	45.36	10.48	2.33	-12.61	-3.45
3x7	2.1	**57.89***	**48.24***	3	**28.76***	**33.33***
4x5	2.02	-13.45	-16.55	2.83	0.12	3.03
4x6	1.9	-12.44	-15.56	2.67	-0.12	0.00
4x7	1.82	21.11	-5.22	2.33	-12.61	-6.67
5x6	1.78	-17.82	-23.57	2.5	-11.66	-9.09
5x7	1.75	16.67	-12.50	2.83	0.12	9.68
6x7	1.95	30.00	14.71	2.17	-18.85	-16.13

Parents: 1=Dabodabo, 2=Debor, 3=Agric, 4=Lagos, 5=Tuaka, 6=Afebankye, 7=Kwasea, BPH=better parent heterosis, MPH=mid-parent heterosis, CBB= Cassava Bacteria Blight
Brlev= branching levels
* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.18a Mean performance and percentage of better parent and mid-parent heterosis for plant vigour, root number and plant height in the 2008/2009 season at the savannah ecological zone

Geno type	Vigour	BPH	MPH	Rootno	BPH	MPH	Pltht	BPH	MPH
P1	3.33			7.83			240.67		
P2	2.67			4			238.33		
P3	3			8.67			230		
P4	3.17			11			262		
P5	3.67			8.92			186.33		
1x2	3.3	-1.00	**10.00****	9.18	17.74	**55.21***	261.2	8.53	9.06
1x3	3.13	-6.00	-1.05	9.92	13.98	20.20	258.98	7.61	10.05
1x4	2.73	-18.00**	-15.90**	11.32	2.88	20.18	284.02	8.40	**13.00***
1x5	3	-18.18**	-14.29**	11.27	29.50	**34.53***	279.58	**36.61****	**30.95****
2x3	2.78	-7.22*	-1.76	10.95	25.86	**72.89****	276.63	**16.07***	**18.14****
2x4	2.73	-13.68**	-6.29	11.9	8.18	**58.67****	278.25	**16.75***	**11.23***
2x5	2.87	-21.82**	-9.47**	10.63	19.48	**64.65****	263.52	10.57	**24.11****
3x4	2.57	-18.95**	-16.76**	8.68	-21.06	-11.69	245.58	-6.27	-0.17
3x5	2.72	-25.91**	-18.50**	8.83	-0.75	0.47	264.52	**15.01***	**27.07****
4x5	2.73	-25.45**	-20.00**	9.33	-15.15	-6.28	259.28	-1.04	**15.67***

Parents: 1=Afisiafi, 2=Bosomnsia, 3=Sisipe, 4=Bokentenma, 5=Santumbankye, BPH=better parent heterosis, MPH=mid-parent heterosis, vig=plant vigour, rootno= root number, Pltht=plant height,

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.18b Mean performance and percentage of better parent and mid-parent heterosis for 2008/2009 season for harvest index, fresh yield and first branch height at the savannah ecological zone

	HI	BPH	MPH	Fryld	BPH	MPH	Fbrht	BPH	MPH
P1	0.52			4.38			96.33		
P2	0.5			2.3			61		
P3	0.02			7.98			120.2		
P4	0.5			8.85			98.33		
P5	0.57			6.67			94.33		
1x2	0.5	-3.22	-1.64	4.47	1.90	33.67	94.57	-1.83	20.21
1x3	0.47	-8.50	**75.00***	4.18	-47.60*	-32.35	93.82	**14.18****	-13.33
1x4	0.43	-16.13	-14.75	5.08	-42.56*	-23.17	122.8	**24.85***	**26.13***
1x5	0.48	-14.70	-10.77s	4.95	-25.75	-10.41	116.4	20.85	**22.12***
2x3	0.45	-10.00	**74.19***	4.92	-38.41*	-4.38	123.6	2.82	**36.39****
2x4	0.45	-10.00	-10.00	5.27	-40.49*	-5.53	98.5	0.17	23.64
2x5	0.47	-17.64	-12.50	5.18	-22.25	15.61	113	19.77	**45.47****
3x4	0.47	-17.64	**80.65***	4.47	-49.41**	-46.93**	109.1	-9.20	-0.12
3x5	0.47	-6.67	**60.00***	4.52	-43.42*	-38.34*	114.7	-4.58	6.92
4x5	0.52	-8.81	-3.12	4.77	-46.14*	-38.56*	121.7	23.80	**26.37***

Parents: 1=Afisiafi, 2=Bosomia, 3=Sisipe, 4=Bokentenma, 5=Santumbankye, BPH=best parent heterosis, MPH=mid-parent heterosis, HI=Harvest index, Fryld= fresh tuber yield, Fbrht=first branch height

* P≤0.05, ** P≤0.01

Table 4.18c Mean performance and percentage of better parent and mid-parent heterosis for dry yield, dry matter content and CMD in the 2008/2009 season at the savannah ecological zone

Genotype	DRYLD	BPH	MPH	DM	BPH	MPH	CMD	BPH	MPH
P1	1.27			29.2			2.6		
P2	0.6			26.2			3.1		
P3	2.42			30.4			2.5		
P4	2.42			27.7			1.6		
P5	1.63			26			2.1		
1x2	1.23	-2.58**	32.14	27.9	-4.45	0.81	2.9	11.54	1.75
1x3	1.18	-51.03	-35.75	28.5	-6.04	-4.25	2.9	**16.00****	13.73
1x4	1.48	-38.62*	-19.46	28.4	-2.79	-0.18	2.2	**37.50***	**4.76***
1x5	1.4	-14.28*	-3.45	28.2	-3.65	2.08	2.1	0.00	**-10.64***
2x3	1.4	-42.07	-7.18	27.7	-8.89*	-2.15	2.5	0.00	**-10.71****
2x4	1.48	-38.62*	-1.66	29.1	5.17	**8.13***	2.3	43.75	-2.13
2x5	1.53	-6.10*	37.31	29.4	**12.41***	**12.92***	2.7	28.57	**3.85****
3x4	1.25	-48.28*	-48.28**	28.9	-4.77	-0.40	2.6	62.50	26.83
3x5	1.25	-48.28*	-38.27*	27.8	-8.61*	-1.45	2.9	38.10	26.09
4x5	1.37	-43.45*	-32.51	29	4.69	**8.11***	2.7	68.75	**45.95***

Parents: 1=Afisiafi, 2=Bosomia, 3=Sisipe, 4=Bokentenma, 5=Santumbankye, BPH=best parent heterosis, MPH=mid parent heterosis, Dryld= dry yield, DM= dry matter content, CMD= Cassava Mosaic Disease

* $P \leq 0.05$, ** $P \leq 0.01$

Table 4.18d Mean performance and percentage of better parent and mid-parent heterosis for CBB and number of branching levels in the 2008/2009 season at the savannah ecological zone

Geno	CBB	BHP	MHP	Brlev	BHP	MHP
P1	1			3.33		
P2	1.66			2.67		
P3	1.33			2.5		
P4	1			3		
P5	1.33			2.5		
1x2	1.61	**61.00***	21.05	2.63	-12.2222*	-20.9209
1x3	1.5	50.00	28.76	2.97	1.772442	-10.9109
1x4	1.66	**66.00***	**66.00****	2.62	-17.3249*	-21.4214
1x5	1.9	**90.00****	**63.09****	2.63	-9.66266*	-20.9209
2x3	1.86	**39.85***	24.41	2.37	-8.44616	-11.3608
2x4	1.93	**93.00****	**45.11***	2.6	-8.28924	-13.3333
2x5	1.98	**48.87***	**32.44***	2.77	7.027724	3.620475
3x4	1.63	**63.00***	**39.91***	2.55	-7.27273	-15
3x5	1.66	**66.00***	24.81	2.53	1.333333	1.333333
4x5	2.01	**51.13***	**72.53****	2.43	-11.5152	-18.8889

Parents: 1=Afisiafi, 2=Bosomia, 3=Sisipe,, 4=Bokentenma, 5=Santumbankye , BPH=best parent heterosis, MPH=mid parent heterosis, , CBB= Cassava Bacteria Blight, Brlev=number of branching levels
* P≤0.05, ** P≤0.01

All the combinations showed positive significant heterosis for mean root number above both mid and better parent values except Afebankye x Kwasea that showed no significant mid-parent heterosis in 2009. Tuaka x Afebankye showed very high mid and better parent heterosis values of 90.76% and 92.89% respectively. All the combinations were good for fresh yield but the best was Lagos x Kwasea that showed 99.47% heterosis over the better parent. Debor x Lagos and Debor x Tuaka showed resistance to CBB in both seasons (Tables 4.16d and 4.17d).

For the savannah zone (Table 4.18 a-d), the entire hybrid combinations showed negative plant vigour heterosis except Afisiafi x Bosomnsia that showed positive significant mid-parent heterosis (Table 4.18a). Most of the combinations showed negative significant

heterosis for root number with, 5x7, 2x3, 2x5, 3x5, 1x5 with highly significant negative heterosis for both better and mid-parent and heterosis with combination1x5 being the highest of (-39.08** and -26.78**) (Table 4.18b). None of the combinations showed positive significant heterosis for fresh yield.

Hybrid combinations that showed positive heterosis for root number were 1x5, 2x3, 2x4 and 2x5 of 34.53, 58.67, 64.65, 72.89 mid-parent heterosis respectively (Table 4-18a). The hybrid 3x4 showed the highest significant harvest index mid-parent heterosis, and negative highest significance for fresh and dry tuber yield of -46.93 and -48.28 respectively. The hetoritic gain in dry matter content was observed in 2x4, 2x5 and 4x5 with significant mid-parent heterosis of 12.41, 12.92 and 8.11. The combinations that showed resistance to CMD were 1x5 and 2x3 of -10.64 and -10.17 mid-parent heterosis. The same hybrid 1x5 also showed the highest positive significan heterosis foe CBB, which inidicates susceptibility (Tables 4 18a-d)

The results of this study showed that the heterosis of an accession is dependent on both the environment and the genetic background within which it is being tested (Lokko et al. 2006). Selection of parents in breeding for resistance to CMD cannot be tested based on the performance of the accession alone. Progeny testing in different environments is essential to enhance selection of the best combinations because some of the hybrids responded to CMD differently in the different environments.

It is an established fact that the amounts of heterosis obtained by hybrids depend largely on the genetic divergence of the populations from which the parental lines have been selected (Moll et al. 1962). Dabodabo was identified as the best parent for CMD resistance breeding, although its progeny needs to be tested in different environments to ascertain the stability for the trait across locations in the F1 progeny. Lagos x Kwasea can be tested in different environments for fresh yield. The positive heterotic influence experienced in certain crosses such as Lagos x Kwasea, has been also been reported by Unikrishnam et al. (2004) who confirmed hybrid vigour for root yield. He also reported heterosis over mid-parents and better-parents values in all the traits studied.

4.4 Conclusions

The present study generated relevant information for planning a more efficient cassava breeding programme for Ghana and the cassava community. The analysis of variance and the GCA:SCA ratio indicated that the GCA was larger than SCA for average root number, CBB and branch levels, indicating the presence of additive gene effects and a possibility for improvement of the characters by selection. GCA:SCA ratio indicated that the SCA was larger than GCA for harvest index and fresh root yield indicating environmental effects on the trait and confirms the non-additive effects mainly determining expression of root yield.

The parental genotypes were selected from a particular region where CMD is the main economic constraint. Debor had the best GCA effect for cassava mosaic severity reaction; while the clone Tuaka had the best GCA effect for fresh root weight. However, the combination of Afebankye x Kwasea will reduce yield. Dabodabo x Agric showed negative significant heterosis for fresh yield and the SCA was also higher than GCA, hence will reduce yield. Debor x Kwasea was the best progeny for CMD resistance development for the forest ecology study whereas Afisiafi x Santumbankye and Bosomnsia x Bokentenma were the best for the savannah ecology. Debor and Kwasea were the best parents for resistance breeding to CMD. These two parents are important for different traits and should be considered as parents in the next hybridisation scheme.

CMD had high broad and narrow sense heritability with a predictability ratio close to unit, confirming that this character is highly heritable and largely under additive genetic action.

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CHAPTER 5

PARTICIPATORY EVALUATION OF ELITE CIAT GENOTYPES IN GHANA

Abstract

A major staple in Ghana and contributing 22% of Agricultural Gross Domestic Product (AGDP), cassava has assumed industrial and cash crop status in the country. However, this good and reliable cheap source of carbohydrates is susceptible to cassava mosaic virus (CMD) and other devastating diseases and pests, as well as post harvest physiological deterioration. Cassava breeding in Ghana, with the support of the International Institute of Tropical Agriculture (IITA), has yielded several varieties. Adoption challenges and susceptibility of some varieties to diseases and pests have necessitated this study. New improved varieties are needed to feed the growing cassava-based industry. Marker assisted selected genotypes resistant to pests and diseases developed by the International Center of Tropical Agriculture (CIAT) and evaluated by the Crops Research Institute (CRI) together with farmers and end-users, identified outstanding clones CR52A-31 and CR52A-25 with starch levels between 26-30% and yields of 50 to 62 t/ha. Genotype CR42-4 recorded the highest yield of 101.2 t/ha at Ejura but the second highest yield across all locations. Farmers preferred two other genotypes CR52A-4 and AR14-10.

5.1 Introduction

In Africa cassava is the most important vegetatively propagated food crop and the second most important food staple in terms of calories per capita, consumed regularly by more than 200 million people (Nweke et al. 2002). It is also one of very few crops with the potential to produce starch competitively for industries (Robertson and Ruhode 2001). Cassava is the only non-native crop in Africa that has assumed a major staple food status (Tewe 1992), and constitutes the most important tropical root crop (Dapaah 1991; Roa et al. 1997; Mkumbira 2002; Nweke 2004). Cassava roots are efficient in carbohydrate

production (Onwueme 2002; Cock 1985) and contain the highest starch content among all root and tuber crops (Moorthy 1994).

The reliability and productivity of cassava has made it the most important locally produced food in a third of the world's low-income, food-deficient countries. Cassava is the main staple in sub-Saharan Africa, providing food for over 300 million people, many of them among the poorest in the world. In 15 sub-Saharan countries, 30 million people get up to 60% of their daily energy intake from cassava (Cock 1985; CIAT 2003). In spite of its economic importance, resource-poor farmers lose 48 million ton of fresh storage roots, which accounts for 30% of the total world production, valued at US$1.4 billion every year to pests, diseases and post harvest physiological deterioration (FAO 2003).

Cassava is the least researched crop among the major crops of the world. Breeding efforts to improve cassava began only recently compared with other major crops. Hence many fundamental questions regarding its genetics still remain unresolved. Cassava is mostly grown as an intercrop. It offers the advantage of a flexible harvesting date, allowing farmers to keep the roots in the ground until needed. Its tolerance of low field labour inputs and variability in planting and harvesting dates makes it much less tightly constrained by seasonality compared with other staple crops. The majority of small-scale cassava farmers worldwide are self-reliant and self-providing for their planting materials with a few having access to planting material from breeding programmes (Manu-Aduening et al. 2005). In managing their crops to meet their needs for food or cash the small-scale farmers are involved in various activities which include choosing which variety to grow, the size of the cultivated population allotted to a given variety, acquisition of the planting material and the percentage to buy or exchange from various sources. Planting material acquisition strategies include both saving from previous harvests and replacing it with cuttings of cassava stems obtained from other farmers. These strategies can lead to high turnover of varieties and renaming (Doku 1969). During germplasm collection for the genetic diversity studies, it was found that many farmers got their planting materials from ancestors as inheritance from one generation to another;

from distant relatives and some sourced planting material from fallowed forest fields with volunteer cassava seedlings.

Total world cassava use is expected to increase from 172.7 million ton to 275 million ton in the period 1993-2020 using the International Food Policy Research Institute's (IFPRI) base line data. A higher prediction of demand and products puts the 2020 production at 291 million ton (Scott et al. 2000). In both projections, cassava use in Africa is equivalent to 62% of total world production.

In Africa, cassava is used almost exclusively as human food (either directly or sold for consumption). The total cassava consumption more than doubled from 24 million ton per year in the early 1960's to 58 million ton per year in the early 2000s (FAO 2006)

Cassava has been grown as a subsistence and famine reserve crop in Africa for centuries, and in the past years its importance as a reliable crop giving acceptable yields with limited inputs has grown. Cassava as the major food staple and as a cheap source of calories is important in ensuring food security in Africa and all other countries that depend on it for livelihood. The small-scale and subsistence farmers in Africa have little opportunity to invest in commercially produced inputs, e.g. fertilizers or pesticides needed to increase their yields, and thus have little possibility of meeting the increasing demand for food. Crops that can be produced with limited inputs offer a potential of increasing food security in these countries. Cassava takes 10-12 months to mature and this long growth cycle poses a challenge in breeding programmes. Breeding of cassava may take 10-12 years to complete a normal cycle. This imposes limitations on the frequency of generating elite genotypes for production. In the light of this, there is a need to generate new elite material in a relatively short period and with farmers' readiness to adopt them in order to achieve the desired food security in communities that depend on cassava as their source of livelihood.

The objectives of this study were to:

1. Evaluate elite genotypes of cassava introduced from CIAT through the National Root and Tuber Crop Research Institute (NRCRI) into Ghana
2. To employ participatory evaluation to enhance effective adaptation and adoption.

5.2 Material and methods

Ten elite inter-specific hybrid cassava genotypes introduced from CIAT through NRCRI, Umudike under the Generation Challenge Programme (GCP) were tested for yield and disease resistance with two checks, one IITA released variety in Ghana, TMS 30572, called Afisiafi and a land race called Sisipe. Two of the CIAT genotypes were discarded due to susceptibility to scale insects, CMD and mites and only eight were evaluated with two local checks in a study for three years from 2006 to 2009. The first year was used to multiply the elite planting materials on station in single row plantings at 1 x 1 m and 10 plants per row. The cuttings of 20 cm of selected stems were planted in a RCBD with three replications for clonal evaluation in 2007. The experiment was conducted in four locations representing three of the five agro-ecological zones namely Ejura (forest transition), Pokuase (coastal savannah), Ohawu (coastal savannah) and Fumesua (forest zone) (data on locations given in Table 4.1). The plot size was 4 x 10 m and a spacing of 1 x 1 m was used.

5.2.1 On station evaluation

The eight introduced elite cassava genotypes and the two checks were planted in the field and at 1MAP data on vigour, percent establishment and CMD severity and incidence were taken per plot in the 2007 and 2008 planting seasons. Data on disease and pest reaction at 3, 6 and 9 MAP were also measured. At 12 MAP the plant height and yield related data were taken. The number and weight of roots per plot were recorded. The above ground and the below ground weight were taken for the determination of the harvest index in two of the locations.

5.2.2 Participatory evaluation

Field evaluation of plant and root characteristics

Each location had 30 farmers drawn from districts within the region to assess the cassava genotypes on station during the time of harvesting at 12 MAP, therefore 120 farmers were involved in the on station evaluation.

The genotypes were evaluated with farmers on station in 2008 and 2009. After the 2008 evaluation, during the harvesting stage, farmers were randomly selected across the districts in the regions to assist with the assessment of the yield and cooking quality performance of the elite CIAT materials and the two checks on station during a farmer field day. In 2008, on farm testing was done concurrently with an on station trial to evaluate the genotypes for another season under farmer supervision and managed by researchers. The majority of the respondents (73.3%) had farm sizes between 0.2 to 2.5 acres. A collaborator, who mechanises cassava production on a large scale of more than 100 000 ha, has a scheme of growers who feed the cassava company. On farm testing was done with ten of these growers from his district. These growers work closely with the mechanised farmer who buys their cassava roots to feed his starch and high quality processing factory. A few farmers who cluster around processing companies had farm sizes of 0.5 ha to 2 ha.

Each farmer prepared up to 0.25 acre of land for planting the eight CIAT genotypes and the two checks. The plot size was 4 x 10 m with four rows at 1 x 1 m spacing. Each plot contained 40 plants planted under the supervision of researchers to avoid errors. As a criterion for cassava variety release, the candidate varieties are concurrently evaluated in the agro-ecological zones in Ghana, on station and on farmers' fields. If this is not done side by side it takes close to five years before the final inspection by the varietal release committee at the inspection plots. Recently, the on-station testing and on farm evaluation has been done concurrently after enough planting materials have been generated, to save time and fast track the release of new cassava varieties. Routine agronomic practices were followed. There was no application of fertilizers or irrigation, the experiments were rain fed. Data on the plant vigour, percentage establishment, CMD incidence and severity

(on a scale of 1-5 with 1 being clean and 5 the worst infected), CBB, yield data on the number of roots harvested, the yield of harvested roots and plant height), harvest index and food quality were assessed. A total of 40 farmers out of the 120 invited for the 2008 harvesting were used for a farmer managed yield evaluation on their farms. Structured questionnaires were administered to assess the desired plant characteristic above ground and below ground and information on farmer preferences on the trials planted at the station and managed by the scientists. Samples of the tubers were boiled and sensory evaluation was done to assess the poundabilty and mealiness and taste. Data was analysed with the Statistical Programme for Social Sciences (SPSS 2010) and the field data analysed with Genstat.

Sensory evaluation

Cassava roots were peeled, cut, washed and boiled for 30-45 minutes. Three samples were prepared simultaneously for each test and assessed by a panel of 30 people. The panel was selected and trained to evaluate taste, colour and mealiness. Participants were seated and arranged to do independent assessment in a well lit open shed. The mealiness was measured by taking a small portion of the boiled sample and pressing it between the thumb and the index finger. When it is soft and can form a sticky paste then it is considered mealy. On the contrary the hard and difficult to press root will not form a sticky paste and is considered non mealy and suitable for 'ampesi' (that is boiled and eaten) or for 'fufu'. However, non mealy genotypes can be used for cassava dough 'agbelima', or dried for 'konkonte', cassava chips or processed into gari. Components include: mealiness on a scale of 0-3 (0 = non mealy 1 = mealy, 2 = very mealy and 3 = excellent), fresh root colour on a scale of 1-3 (1 = yellow, 2 = green, 3 = white) and taste on a scale of 1-3 (1 = bitter, 2 = bland and 3 = sweet) (Raji et al. 2007). After evaluation, farmers were asked to rank the different genotypes depending on their preference.

The data was analysed using SPSS Version 16 and the analysis was mainly descriptive statistics that employed means, ranges, percentages and farmers' preferences

5.2.3 Determination of starch content

Percentage starch content was determined by the specific gravity method. A sample of 5 kg of each genotype was weighed and weighed again submerged in water held in a receptacle on a balance with gradation for the starch values. The starch amount was estimated by reading the value when the scale was balanced.

5.3 Results

5.3.1 On station clonal evaluation

The results obtained showed that genotypes CR52A-25 and CR52A-31 had fresh tuber yields of 48.12 and 60.68 t/ha (Table 5.1).

Table 5.1 On station evaluation of fresh root yield (t/ha) of CIAT elite genotypes in four locations in 2007/2008 season

Genotype	Fumesua	Ejura	Pokuase	Ohawu	Mean
Afisiafi	45.7	42.7	33.9	40.2	40.62
AR14-10	43.3	58.4	23.4	25.7	37.7
AR15-5	47.2	68.9	37.3	41.4	48.70
CR41-10	48.5	21.3	23.4	24.7	29.5
CR42-4	50.1	101.4	27.5	37.8	54.2
CR52A-25	45.1	64.4	39.8	43.2	48.12
CR52A-31	55.2	75.8	54.3	57.4	60.68
CR52A- 4	55.5	50.2	40.6	42,7	47.25
CR59-4	29.6	52.2	25.0	29.4	34.05
Sisipe	50.0	31.9	33.0	45.6	40.12
LSD	8.9	9.5	9.2	10,6	9
Mean	47.0	55.6	33.4	36.67	43.17
SED	13.8	29.5	15.39	16.7	23.29

LSD=least significant difference SED= standard error of deviation

Genotype CR 42-4 recorded the highest yield of 101.2 t/ha at Ejura but the second highest yield across all locations. The first season results were significantly different among the locations used. Pokuase was identified as the location with best plant establishment, but the lowest yield. However performace of all the genotypes over a period of two years across three locations showed that Sisipe, a landrace, produced the highest yield of 63.34 t/ha followed by CR52A-25 (57.48), CR52A-31 (56.44 t/ha) CR52A-4 (55.39),and Afisiafi (53.68 t/ha). The higest amount of starch of (22.52%) was produced by CR52A-4. The genotype Sisipe was the most suitable for its mealiness (Table 5.2).

5.3.2 On station participatory evaluation

At each of the three locations and during harvesting at 12 MAP farmers, processors, scientists and consumers were brought in to evaluate the cassava genotypes. Preliminary results showed that farmers identified the best genotypes based on their preferred attributes or characteristics (Table 5.3). They had their own perception on healthy and diseased genotypes and causes for the plant diseases (Figure 5.1).

Figure 5. 1 On station participatory evaluation of cassava genotypes

Table 5.2 On station performance of CIAT genotypes for measured traits across three locations for two seasons 2007-2009

Genotypes	Root yield(t/ha)	CMD score	HI	DM (%)	Starch (%)	Architecture	CBB	Vigour	Mealiness	Plant stand (plot)
Afisiafi	53.68	2.26	0.502	28.20	18.26	2.89	2.556	3.67	2.330	12.110
AR14-10	35.70	1.52	0.383	30.09	17.79	2.89	3.111	3.11	2.560	12.110
AR15-5	43.42	1.17	0.554	29.41	16.18	3.00	3.000	3.78	2.780	14.330
CR41-10	36.96	1.26	0.368	29.29	20.57	2.63	3.000	3.89	3.000	9.780
CR42-4	41.11	1.44	0.459	31.08	19.92	2.67	3.667	3.78	3.780	11.000
CR52A-25	57.48	1.69	0.528	29.93	21.42	2.78	3.111	3.44	3.330	12.000
CR52A-31	56.44	1.76	0.491	29.58	22.17	3.11	2.889	3.78	2.110	14.560
CR52A-4	55.39	1.83	0.556	29.81	22.52	3.11	3.000	4.11	2.330	11.000
CR59-4	27.20	1.17	0.325	29.13	20.08	3.33	3.000	3.56	3.220	10.780
Sisipe	63.34	2.24	0.535	30.96	19.00	3.22	3.000	4.22	4.220	9.110
LSD (0.05)	10.31	0.20	0.055	2.44	2.60	0.435	0.600	0.444	0.933	3.440

LSD= least signicant difference, CMD=cassava mosaic disease, HI= Harvest index, DM= dry matter content, CBB= cassava bacteria blight

Table 5.3 Characteristics named by farmers as reasons for rejection of cassava cultivars or genotypes

Reason given	Coastal	Forest	Transition	Total
Diseased	26	47	-	73
Root rot	-	56	-	56
Total				129
Indirect measures of resistance to pests, diseases or weeds				
Low yield	24	54	30	108
Poor soil storage	-	7	-	7
Total				115
Criteria not related to resistance to pests, diseases or weeds				
Not poundable into fufu	21	53	16	90
Low price	17	49	19	85
Not good for gari	21	32	-	53
Late maturing	-	18	14	
Watery tubers	-	21	-	21
Poisonous tubers	-	-	-	-
Bad taste	-	-	-	-
Irregular tuber shape	-	-	8	8

Reasons for choosing or rejecting a variety

Farmers used several criteria to choose or reject cassava genotype for cultivation.In the forest ecological zones farmers rejected cassava genotypes with root rot and other diseases. Low yields was a major reason for rejection where the ability for in –ground storage was a minor concern for the forest ecology, whereas the savanna and the transition were not concered due to the processing of cassava in these areas (Table 5.3) %3 and 49 farmers indicated in the forest zone that non-poundability is a major reason for rejection, low price and latematurity and cassava with low dry matter content. The major concern for all the locations was low yield which the savanna, forest and transition had (24, 54 and 30) respondents indicating would rejection genotype. Indeed it could be inferred that disease resistant, high yielding and high dry matter content genotypes will be choosen by the farmers.

A whole range of genotypes with different local names were grown by the farmers with a few farmers (12%) cultivating improved varieties. The main reasons given for growing genotypes were for the ability to be pounded or mealy and therefore they can be used for fufu, also high yielding and mature early (Table 5.4).

Table 5.4 Farmers' reasons for variety preference

Preferred variety	Most important reason	Frequency	Percentage
One	Good for fufu (poundable/mealy	14	23.3
	High yielding	9	15.0
Two	Good for fufu, ampesi or gari (poundable/mealy)	9	15.0
	High yielding	8	13.3
Three	Good root tuber shape (maturity)	7	11.7
	Architecture		6.7

Assessment of individual genotypes

The individual genotypes were assessed by farmers for its utilization. Colour was also an important consideration and most of the clones were either white or cream except one clone which was yellow. In terms of mealiness, 50% of the clones assessed had average mealiness of 2 (Table 5.5).

Ranking assessment of genotypes in order of importance

Farmers were asked to select the clones that they preferred and would cultivate on their individual fields, and the main criteria used by them were that of poundability or mealiness, gari processing quality, dry matter content and yield. Farmers' best varieties in order of importance were genotypes CR52A-31, CR52A-25 CR52A-4 and AR14-10 in addition to Afisiafi and the landrace Sisipe.

Table 5.5 Participatory evaluation of CIAT elite genotypes on station

Clones	Food use	%	Colour	%	Taste	%	Mealiness	%
Afisiafi	Gari	63.3	White	56.7	Bland	55.0	Non-mealy	60.0
AR14-10	Gari	70.7	White	45.0	Sweet	23.3	Mealy	28.3
AR15-5	Fufu	68.7	Cream	41.7	Sweet	68.3	Excellent	38.3
CR41-10	Fufu	40.0	Cream	45.0	Sweet	53.3	Very mealy	55.0
CR52A-25	Gari & Agbelima	63.4	White	61.7	Bland	46.7	Very mealy	36.7
CR52A-31	Gari	75.3	White	36.7	Bland	56.7	Non- mealy	66.7
CR52A-4	Fufu	63.3	Yellow	46.7	Bland	43.3	Non-mealy	38.3
CR59-4	Gari	60.0	Cream	41.7	Bland	45.0	Non- mealy	50.0
Sisipe	Gari	73.6	White	45.0	Bland	51.7	Non- mealy	83.3

Table 5.6 Ranking assessment of CIAT elite genotypes in order of importance

Genotype	Reason	Percentage
CR52A-25	Fufu/Gari/Agbelima	23.3
CR52A-31	Fufu/Gari	26.7
AR14-10	Gari/Fufu	21.7

Table 5.7 Ranking assessment of genotypes for specific culinary characteristics

	Characteristics assessed							
	Food use	%	Colour	%	Taste	%	Mealiness	%
Afisiafi								
Fumesua	Gari	60.0	White	60.0	Bland	60.0	Non – mealy	90.0
Ejura	Gari	90.0	White	60.0	Bland	55.0	Non – mealy	60.0
Pokuase	Gari/Fufu	70.0	White	100.0	Bland	50.0	Mealy	45.0
Ohawu	Gari	70.0	White	100.0	Bland	50.0	Mealy	45.0
AR14-10								
Fumesua	Gari	70.0	White	90	Bland	55.0	Non–Mealy	60.0
Ejura	Gari	75.0	White	45.0	Bitter	55.0	Non–Mealy	35.0
Pokuase	Gari	50.0	White	90.0	Sweet	50.0	Non–Mealy	50.0
Ohawu	Gari	50.0	White	90.0	Sweet	50.0	Non–Mealy	50.0
AR15-5								
Fumesua	Fufu	45.0	White	60.0	Sweet	55.0	Excellent	70.0
Ejura	Fufu	65.0	Cream	55.0	Sweet	85.0	Very – mealy	50.0
Pokuase	Fufu/Gari	90.0	Cream	90.0	Sweet	65.0	Mealy	45.0
Ohawu	Fufu/Gari	50.0	White	90.0	Sweet	50.0	Mealy	50.0
CR41-10								
Fumesua	Fufu	30.0	White	40.0	Bland	50.0	Mealy	55.0
Ejura	Fufu	95.0	Cream	65.0	Sweet	75.0	Mealy	80.0
Pokuase	Gari	85.0	Cream	95.0	Bland	40.0	Non – mealy	70.0
Ohawu	Gair	50.0	White	90.0	Sweet	50.0	Non–mealy	50.0
CR42-4								
Fumesua	Agbelima	30.0	White	55.0	Bland	85.0	Non – mealy	45.0
Ejura	Fufu	60.0	White	45.0	Bland	45.0	Mealy	65.0
Pokuase	Fufu	90.0	Cream	75.0	Sweet	35.0	Mealy	80.0
Ohawu	Fufu/Agbelima	50.0	White	65.0	Bland	40.0	Mealy	35.0
CR52A-25								
Fumesua								
Ejura	Gari	45.0	White	40.0	Bland	80.0	Non– mealy	65.0
Pokuase	Fufu	50.0	White	80.0	Sweet	80.0	Mealy	60.0
Ohawu	Fufu	50.0	White	65.0	Bland	40.0	Mealy	35.0
	Agbelima	50.0	White	65.0	Bland	40.0	Non– mealy	35.0
CR52-31								
Fumesua	Gari	45.0	White	40.0	Bland	80.0	Non – mealy	65.0
Ejura	Fufu	50.0	White	80.0	Sweet	80.0	Mealy	60.0
Pokuase	Fufu/Agbelima	50.0	Cream	65.0	Bland	40.0	Mealy	45.0
Ohawu	Agbelima	50.0	White	65.0	Bland	40.0	Non=mealy	30.0
CR52-4								
Fumesua	Gari	45.0	White	40.0	Bland	80.0	Non – mealy	65.0
Ejura	Gari	50.0	White	80.0	Sweet	80.0	Non – mealy	60.0
Pokuase	Agbelima	50.0	White	65.0	Bland	40.0	Non – mealy	35.0
Ohawu	Agbelima	50.0	White	65.0	Bland	40.0	Non– mealy	35.0
CR59-4								
Fumesua	Gari	45.0	White	40.0	Bland	80.0	Non– mealy	60.0
Ejura	Gari	50.0	White	80.0	Bland	80.0	Non–mealy	65.0
Pokuase	Agbelima	50.0	White	65.0	Bland	40.0	Non – mealy	30.0
Ohawu	Agbelima	50.0	White	65.0	Bland	40.0	Non– mealy	40.0
Sisipe								
Fumesua								
Ejura	Fufu/Gari	45.0	Yellow	40.0	Sweet	80.0	Mealy	65.0
Pokuase	Fufu/Gari	50.0	Yellow	80.0	Sweet	80.0	Mealy	67.0
Ohawu	Agbelima	50.0	Cream	65.0	Bland	40.0	Non– mealy	35.0
	Gari/Agbelima	50.0	Cream	65.0	Bland	40.0	Non– mealy	35.0

The farmer preferences for genotypes are given in Table 5.8, and farmers indicated that they would like to plant these clones on their farms. There were similarities in terms of reasons given for selecting these clones. Clones were chosen because of their culinary attributes, no disease (clean), high yield and good architecture (closes canopy hence smoulders weeds and reduces weeds infestation). Farmers could generate good income from these new genotypes. (Table 5.7) In terms of the preferences by ecological zone (Table 5.9) AR14-10 and CR52A-25 were preferred in all the zones.

Table 5.8 On station best three preferred genotypes with reasons for selection, by location

Fumesua	AR14-10	CR52A-25	CR52A-31	Good for fufu (poundable) , high yielding, ampesi and clean
Ejura	AR14-10	CR52A-4	CR52A-25	Good for fufu, and for ampesi
Pokuase	CR52A-25	AR 14-10	CR52A-4	Good for gari and ampesi, high yielding and good architecture
Ohawu	CR52A-4	CR 52A-25	AR14-10	Good for gari and ampesi, high yielding and good architecture

Table 5.9 On-farm best three preferred genotypes by ecological zone

Forest zone Ashanti	AR14-10	CR52A-25	CR52A-31	Good for fufu (poundable) , high yielding, ampesi and clean
Forest transition Ejura/BA	AR14-10	CR52A-4	CR52A-25	Good for fufu, and for ampesi
coastal savannah GA region	CR52A-25	AR14-10	CR52A-4	Good for gari and ampesi, high yielding and good architecture
Coastal savannah and Volta region	CR52A-4	CR52A-25	AR14-10	Good for gari and ampesi, high yielding and good architecture

5.4 Discussion

Of the 10 interspecific hybrids developed with CMD and green mite resistance selected with MAS in CIAT, eight had resistance and two were heavily susceptible. Two of the genotypes had CMD incidence of 100% and CMD severity of score 5 and were discarded after the first year (2006 season). The remaining eight tested with the two checks showed yields better than the checks. However, the root form and shape, texture and rind colour were not so appealing to farmers. The on farm testing done on the genotypes and farmers checks showed that farmers consider utilization in selection of varieties. The attributes the farmers used were similar across all agro-ecological zones. In the coastal savannah, Pokuase research station and community where cassava fresh root tubers are processed into cassava dough “abgelima” and “gari”, genotypes such as CR52A-25 and CR52A-4 were preferred due to its high yield, dry matter and root form. Most of the genotypes had an average score on suitability for fufu as 2. The genotypes would be suitable for processing.

The released IITA variety, TMS 30572 called Afisiafi in Ghana (meaning everywhere) and resistant to CMD, has been used by farmers as a variety useful for processing, yields are stable and higher than in the land races. The challenge with TMS 30572 in recent times is its susceptibility to CMD (Moses 2008). The buildup of the virus inoculums after several years of cultivation has resulted in reduced yields. Studies conducted with cassava farmers in Uganda showed that farmers had attributes which they used to select or abandon genotypes. A similar observation was made when farmers described the various cassava diseases and indicated the symptoms and stage of infection (Manu-Aduening et al. 2005). At the on station participatory evaluation, farmers selected and took with them stakes or stems of genotypes they preferred. This was a clear indication of the interest of farmers for new genotypes. The materials selected by farmers had high starch and dry matter content and high yields. The introduction of farmers to participate in the evaluation of on station evaluation and the scheme of running the on farm and on station evaluation concurrently helps to cut down the number of years for developing a cassava variety. As selected materials are at an advanced stage towards varietal release, this marks the usefulness of participatory breeding.

5.5 Conclusions

Farmers made their choices based on their experience. Genotypes were selected because they gave high yields, had good architecture, beautiful or healthy plants, high dry matter content or starch and superior cooking characteristics and taste. Farmers made informed decisions that guided their choice or rejection of genotypes. The most preferred elite CIAT varieties across all the agro-ecological zones tested were CR52A-25, AR14-10, CR52A-4 and CR52A-31. A community where "gari" is processed close to the Pokuase Research station has adopted the materials they preferred from the on farm and on station evaluation in which they participated. The genotypes have become popular in the community, even though they have not been officially released.

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CHAPTER 6

STABILITY ANALYSIS OF CIAT ELITE GENOTYPES AND SELECTED F1 DIALLEL PROGENIES

Abstract

Stability of eight CIAT elite cassava genotypes bred for CMD and green mite resistance was evaluated in a RCBD with three replications over three agro-ecological zones of Ghana for two seasons. Likewise 14 selected F1 progenies from the diallel experiments described in Chapter 4 were evaluated for stability for one season over four locations in an RCBD with three replications. The genotype CR59-4 showed remarkable resistance to CMD but had low yields. Afisiafi, CR52A-4, CR52A-31 and Sisipe recorded the highest yields. CR52A-25 was found to be the highest yielding F1 clone, but very unstable. The relative magnitude of the main effects and their interactions for the traits measured as proportion of the total sum of squares showed that genotype main effect impacted more on root weight, CMD and dry matter than the other effects, indicating that the traits can be improved by direct selection. High variability existed among the cassava clones for the traits studied. The AMMI biplots presented excellent graphical presentation of the data in terms of mean yields and stability.

6.1 Introduction

A major aim for cassava breeding is the genetic enhancement that aims at increasing cassava productivity through the use of broad-based improved germplasm that combines multiple disease resistance ability with high yield and other desirable traits (Aina et al. 2009). The conservation and maintenance of valuable genetic resources such as landraces could help achieve this. To broaden the genetic base, germplasm with unique traits can be introduced and introgressed into existing germplasm. Cassava contributes 16-22% of the AGDP of Ghana. Most of the cassava produced is consumed in the form of “fufu” and “gari” but there are many small-scale enterprises currently in Ghana that process cassava into diverse foods and starch for industrial uses (Manu-Aduening et al.

2006). Cassava also has tremendous potential as feed for animal production and other industrial uses such as starch and ethanol and in food processing, pharmaceutical, and paper and textile industries (Al-Hassan 1993).

Locally adapted cassava landraces constitute an integral part of the traditional diet in Sub-Saharan Africa, Asia and Latin America (FAO 2002). Nevertheless, a number of African cassava landraces possess certain agronomic and food quality characteristics that could potentially be utilized for further quality and productivity improvement (Raji et al. 2007). Despite this, the use of African genetic resources for cassava improvement has been limited compared to the resources from Latin America and Asia. One of the factors impeding the development of African landraces has been their shy flowering habit. Other major constraints include lack of information on tuber quality and agronomic traits (Ceballos et al. 2004; Chavez et al. 2005*)*. Landraces therefore constitute important starting material from where desirable traits can be tapped for improvement purposes.

Cassava is a rich source of carbohydrate cultivated under different edaphic and climatic conditions throughout the world, because of its efficient carbohydrate production (Kawano 2003). As a consequence of its diverse cropping conditions, cassava shows a strong and significant G x E interaction effect (Fukuda 1996; Kvitschal et al. 2009), which makes selection difficult. Selection for superior cassava genotypes should be performed taking G x E interaction into consideration. A detailed assessment of G x E interaction, magnitude and significance is important to ensure greater precision in the release of high yielding and stable genotypes (Kvitschal et al. 2009).

Stable yield plays a major role in developing countries, where small-scale farmers, particularly those living in marginal areas, are working towards risk-minimization (Adugna and Labuschagne 2002). Farmers prefer a constantly superior performance of genotypes on their farms, adapted to other conditions and needs, and which have a high degree of stability over time (Ceccarelli 1994). Dixon et al. (1994) defines this G x E interaction as the change in a cultivar's relative performance over environments from differential response of the cultivar to various edaphic, climatic and biotic factors.

Cassava germplasm enhancement programmes should seek to widen and improve the genetic base of the cassava crop in Africa and maintain its adaptability through population improvement targeted to specific agro-ecological zones. It is therefore necessary to develop new cassava varieties and to select breeding material for target environments. Each ecological zone has specific challenges and objectives because of the diversity of the environment, utilization and local consumer preferences.

Cassava genotype improvement should be done using landraces and introduction of elite genotypes to broaden the genetic base and to meet the demand for new varieties that satisfy the requirements of end-users. This includes higher starch yield, mealiness and stable fresh root yield. The objectives of this study were to evaluate the performance of cassava breeding clones and CIAT elite materials in three agro-ecological zones in Ghana to assess their yield stability. Specifically:

1 To evaluate the influence of genotype (G), environment (E) and G x E interaction on fresh root yield and dry matter percentage and CMD resistance.

2. To test variability in root yield of cassava clones as influenced by environment

3. To determine the stability of clones with respect to CMD resistance and root yield, starch and dry matter content.

6. 2 Material and methods

Three locations representing different agro-ecological zones were used for this study. The locations were Pokuase (coastal savannah), Fumesua (forest zone) and Ejura (forest savannah transitional zone) (data on locations given in Table 4.1 in Chapter 4).

6.2.1 Background of genotypes

A total of 320 landraces were collected from all 10 regions of Ghana and the diversity studied (Chapter 3). Heterotic pools identified were used to select parents for crosses. At the end of the study, heterotic pools that were identified were used to select parents to do crosses so that heterosis can be tapped (Chapter 4). Out of the diallel crosses 12 F1 genotypes and two checks were selected for clonal evaluation in four locations for the

2010 season (Table 6.1). The F1 progenies were selected based on their yield performance and CMD resistance. The eight CIAT genotypes evaluated in Chapter 5 were also analysed in this study, together with two checks, Afisiafi and Sisipe (Table 6.2).

Table 6.1 F1 progenies used for clonal evaluation

Code	Pedigree Male progenitor	Pedigree Female progenitor	Remarks	Source
Debor	-	-	Progenitor	Landrace
Afisiafi	-	-	Progenitor	IITA TMS30572
Dad07	Dabodabo	Debor	F1 progeny	Forest diallel
Dad06	Dabodabo	Debor	F1 progeny	Forest diallel
Dad02	Dabodabo	Debor	F1 progeny	Forest diallel
Dabu02	Dabodabo	Tuaka	F1 progeny	Forest diallel
Dafe01	Dabodabo	Afebankye	F1 progeny	Forest diallel
Daba01	Dabodabo	Agric	F1 progeny	Forest diallel
Delag05	Debor	Lagos	F1 progeny	Forest diallel
Delag06	Debor	Lagos	F1 progeny	Forest diallel
Delag07	Debor	Lagos	F1 progeny	Forest diallel
Alag07	Agric	Lagos	F1 progeny	Forest diallel
Alag26	Agric	Lagos	F1 progeny	Forest diallel
Bnaf01	Bosomnsia	Afisiafi	F1 progeny	Savannah diallel
Bnaf03	Bosomnsia	Afisiafi	F1 progeny	Savannah diallel
Bnaf06	Bosomnsia	Afisiafi	F1 progeny	Savannah diallel

Table 6.2 CIAT cassava genotypes used for the clonal experiment

Genotypes	Source
Afisiafi	IITA material released in Ghana
AR14-10	CIAT
AR15-5	CIAT
CR41-10	CIAT
CR42-4	CIAT
CR52A-25	CIAT
CR52A-31	CIAT
CR52A-4	CIAT
CR59-4	CIAT
Sisipe	Local check

6.2.2 Field studies

Field studies were carried out on-station and off-station of the CRI in the forest, coastal and transitional zones of Ghana. The F1 clones were planted across the various locations with two checks, Debor and Asifiasi. Meteorological and soil data were sourced from the Meteorological Services Department Offices at the various locations where the study was conducted and also from the Soil Research Institute (Chapter 4, Table 4.1).

The field establishment of the trials was done at three different dates for the two locations. Cassava stem cuttings of 25 cm were planted in the field at Fumesua on 12 June 2009, Pokuase on 22 June 2009 and Ohawu on 29 June 2009. The plots were four rows at 1 m x 1 m spacing and 10 plants per row, 40 plants per plot. The experimental design was a RCBD with three replications.

The second experiment was the evaluation of CIAT elite cassava clones in four locations in the three agro-ecological zones for two seasons. The trials were planted from 2007-2009. The experiment was planted as a RCBD with three replications. Replacement of

cuttings which did not sprout was done two weeks after planting. The experiment was planted under rain-fed conditions. Four hand weedings were done at one, four, seven and 10 MAP.

Harvesting was done at 12 MAP. The pest and disease scores were recorded during the growth cycle. At harvest, yield data was collected.

6.2.3 Disease assessment

Disease assessment was done at 1, 3 and 6 MAP based on the scale described by a Reference Manual (IITA 1990). Symptom severity was determined by inspection of leaves or stem depending on disease type. Ten plants representing the plot were sampled.

Cassava Mosaic Disease (CMD)

Scoring for CMD was done at 1, 3 and 6 MAP using the following scoring system: No symptoms – 1

Mild chlorotic pattern on entire leaflets – 2

Strong mosaic pattern on entire leaf – 3

Severe mosaic and distortion of two-thirds of leaflets- 4

Severe mosaic and distortion of four-fifths of leaflets - 5

6.2.4 Yield and yield components of cassava roots

Fresh weight of storage roots/plot

The weight of the storage roots of 10 plants at each harvesting was measured using a hanging scale.

Harvest Index = Root weight/Total biomass

Where total biomass = tuber weight + top weight

Dry matter percentage

Dry matter percentage was determined from a random bulk sample of two to four plants from the 10 plants harvested per plot. Duplicate samples of 100 g of fresh roots were dried at 105˚C for 48 h in an air-drying oven to estimate the dry weights.

$$\text{Hence DM (\%)} = \frac{\text{Fresh weight}}{\text{Dry weight}} \times 100\%$$

Determination of starch content

Percentage starch content was determined by the specific gravity method. A sample of 5 kg of each genotype was weighed and weighed again submerged in water held in a receptacle on a balance with gradation for the starch values. The starch amount was estimated by reading the value when the scale was balanced.

6.2.5 Statistical analysis

The additive main effects and multiplicative interaction (AMMI) statistical model (MATMODEL 2.0, Gauch 1993) was used to analyse the yield data to obtain AMMI analysis of variance and mean estimates. Agronomic and biotic constraints data were analysed using GENSTAT and Least Significant Difference (LSD) was used to separate the means where significant differences existed.

6. 3 Results

6.3.1 Yield performance and stability of F1 genotypes over four locations

Combined analysis of variance using the AMMI Matmodel indicated that genotype main effect was highly significant ($P<0.001$) for all five traits. Location was highly significant ($P<0.001$) for CMD and for harvest index and significant ($P<0.05$) for root weight (Table 6. 3) but was not significant for dry matter content (Table 6.4). Interaction (G x E) effect was highly significant ($P<0.001$) for harvest index, root weight and CMD, starch ($P<0.01$) and dry matter ($P<0.05$).

The relative magnitude of the main effects and their interactions (Tables 6.3 and 6.4) for the five traits measured as proportion of the total sum of squares showed that genotype main effect impacted more on root weight, CMD and dry matter than the other effects and interactions. Interaction effect impacted more on harvest index , CMD and starch than the main effects.

In the AMMI analysis of stability and adaptability for the traits, two principal component axes (IPCA's) were necessary to explain the whole G x E interaction. The first IPCA was significant ($P<0.001$) for harvest index, root weight, CMD and ($P<0.01$) for dry matter and starch and explained 76.6%, 66.2%, 89.4%, 83.9% and 76.5% of variation respectively. On the other hand, the second IPCA was significant ($P<0.05$) for only harvest index and root weight ($P<0.01$). For these two figures, IPC1 was plotted against IPC2 and not against the main effect as for the other characteristics.

Table 6.3 AMMI analysis of CMD score, root weight and harvest index of F1 genotypes

Source	Df	SS	MS	% Total SS	% Interaction SS
CMD score					
Total	167	93.70	0.561		
Treatments	41	76.65	1.869**		
Genotypes	13	52.29	1.022**	55.8	
Environments	2	1.70	0.850**	1.8	
Block	9	0.84	0.093		
Interaction	26	22.66	0.871**	24.2	
IPCA 1	14	20.25	1.446**		89.4
IPCA 2	12	2.41	0.201		10.6
Error	117	16.22	0.139		
Root weight					
Total	167	31671	189.6		
Treatments	41	20390	497.3**		
Genotypes	13	11891	914.7**		
Environments	2	1491	745.6*		
Block	9	1220	135.5**		
Interaction	26	7008	269.5**		
IPCA 1	14	4642	331.6**		
IPCA 2	12	2365	197.1**	66.2	
Error	117	10062	86		
Harvest index					
Total	167	1.2333	0.00739		
Treatments	41	0.7718	0.01882**		
Genotypes	13	0.3504	0.02695**	28.4	
Environments	2	0.0642	0.03211**	5.2	
Block	9	0.0439	0.00488		
Interaction	26	0.3572	0.01374**	29	
IPCA 1	14	0.2736	0.01954**		76.6
IPCA 2	12	0.0836	0.00697*		23.4
Error	117	0.00357	0.00357		

** P<0.001 * P<0.05

Table 6.4 AMMI analysis of dry matter content and starch content of F1 genotypes

Source	Df	SS	MS	% Total SS	% Interaction SS
Dry matter content					
Total	167	1668.2	9.99		
Treatments	41	1121.0	27.34**		
Genotypes	13	941.3	72.41**	56.4	
Environments	2	9.6	4.78	0.6	
Block	9	87.3	9.70*		
Interaction	26	170.1	6.54*	10.2	
IPCA 1	14	142.7	10.19**		83.9
IPCA 2	12	27.4	2.28		16.1
Error	117	459.9	3.93		
Starch content					
Total	167	2263.4	13.55		
Treatments	41	1070.1	26.10**		
Genotypes	13	487.4	37.49**	21.5	
Environments	2	84.6	42.31*	3.7	
Block	9	98.1	10.90		
Interaction	26	498.1	19.16**	22	
IPCA 1	14	381.0	27.22**		76.5
IPCA 2	12	117.1	9.76		23.5
Error	117	1095.2	9.36		

** P<0.001 * P<0.05

The AMMI biplot graphically represents the relationships between the means of the main effects and the first principal component axis (IPCA) scores of the G x E interaction of both genotypes and environment for root weight, dry matter, harvest index, CMD and starch (Fig 6.1-6.5). The treatment sum of squares was partitioned into two components; 88.4%, 97.5%, 89.2%, 96.8% and 89.0% due to pattern (main effects and IPCA) and 11.6%, 2.5%, 10.8%, 3.2% and 11% as residue for root weight, dry matter, harvest index, CMD and starch respectively.

Dad02, an F1 progeny of the forest diallel and Debor, a landrace and a progenitor were identified as the highest yielding genotypes for root weight, with Debor being the most stable genotype. Alag26 was adapted to Env1 and Env2 (Fumesua and Ohawu) fig 6.1. Two of the F1 genotypes Alag07 and Delag06 good root weight and were moderately

stable (Figure 6.1). Most of the genotypes showed stability and an appreciable content of dry matter but Delag07 was identified the most stable for the trait (Figure 6.2). Two environments Env 2 and 3 (Ohawu and Pokuase both in the coastal savannah ecology were similar and showed no difference (Figure 6.2). Daba01, Alag07, and Delag07 were more adapted to Env1 (Fumesua) Figure 6.3. Alag26 was considered as the most stable genotype for harvest index, with Dabu01 and Dad02 showing high and fairly stable performance (Figure 6.3). Afisiafi had the highest CMD score. Delag05 and Delag06 were the most stable genotypes for CMD but Dad02 had the lowest score for the disease (Figure 6.4). The most stable genotypes for starch content were Delag07, Bnaf03 and Dabu01 but they recorded low starch values. Daba01 had the highest starch content with moderate stability (Figure 6.5).

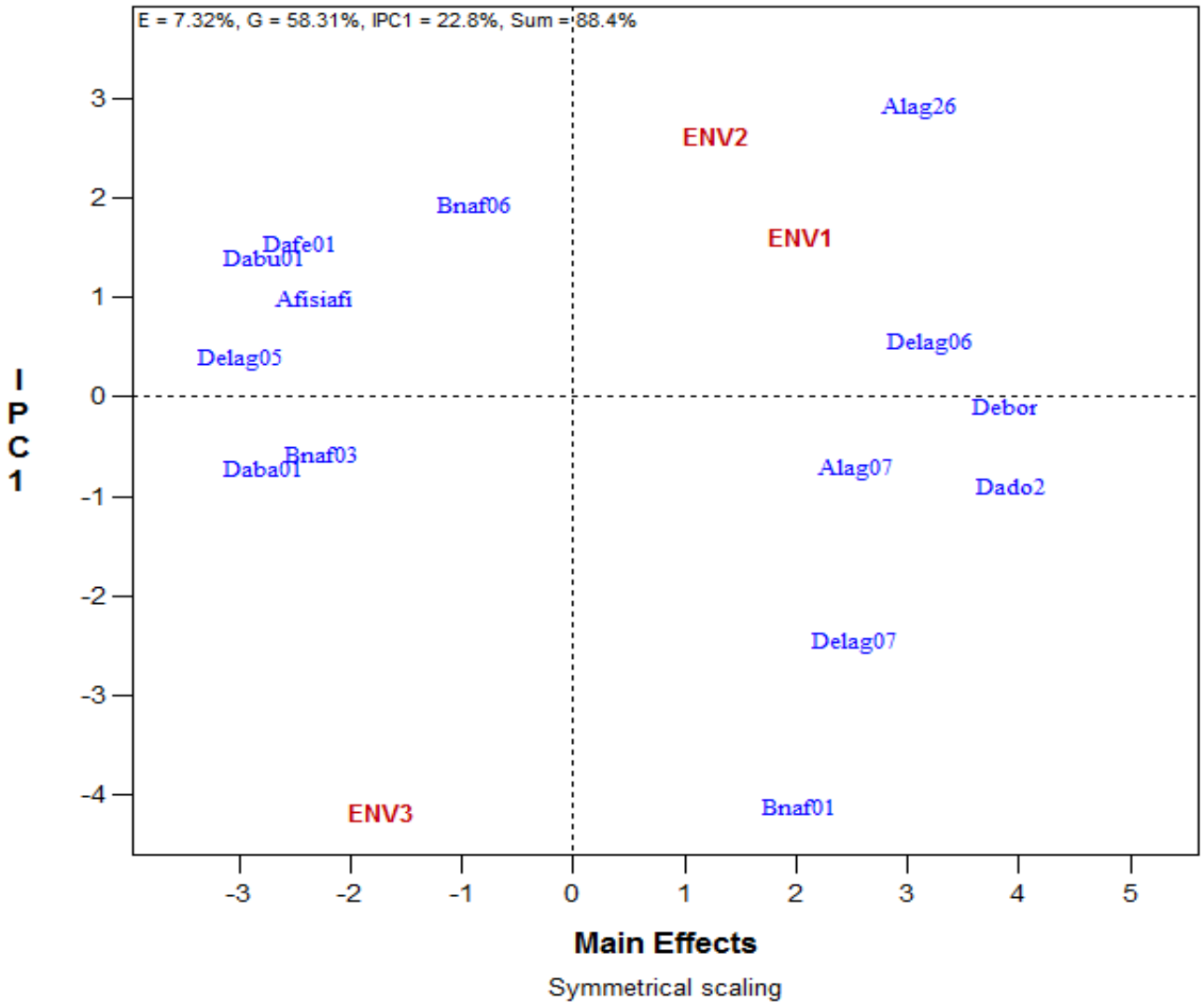

Figure 6.1 Biplot of the first AMMI interaction (IPCA) scores (Y-axis) plotted against main effects (X-axis) of root weight for 14 cassava genotypes in three environments

ENV1 = Fumesua, ENV 2 = Ohawu, ENV 3 = Pokuase

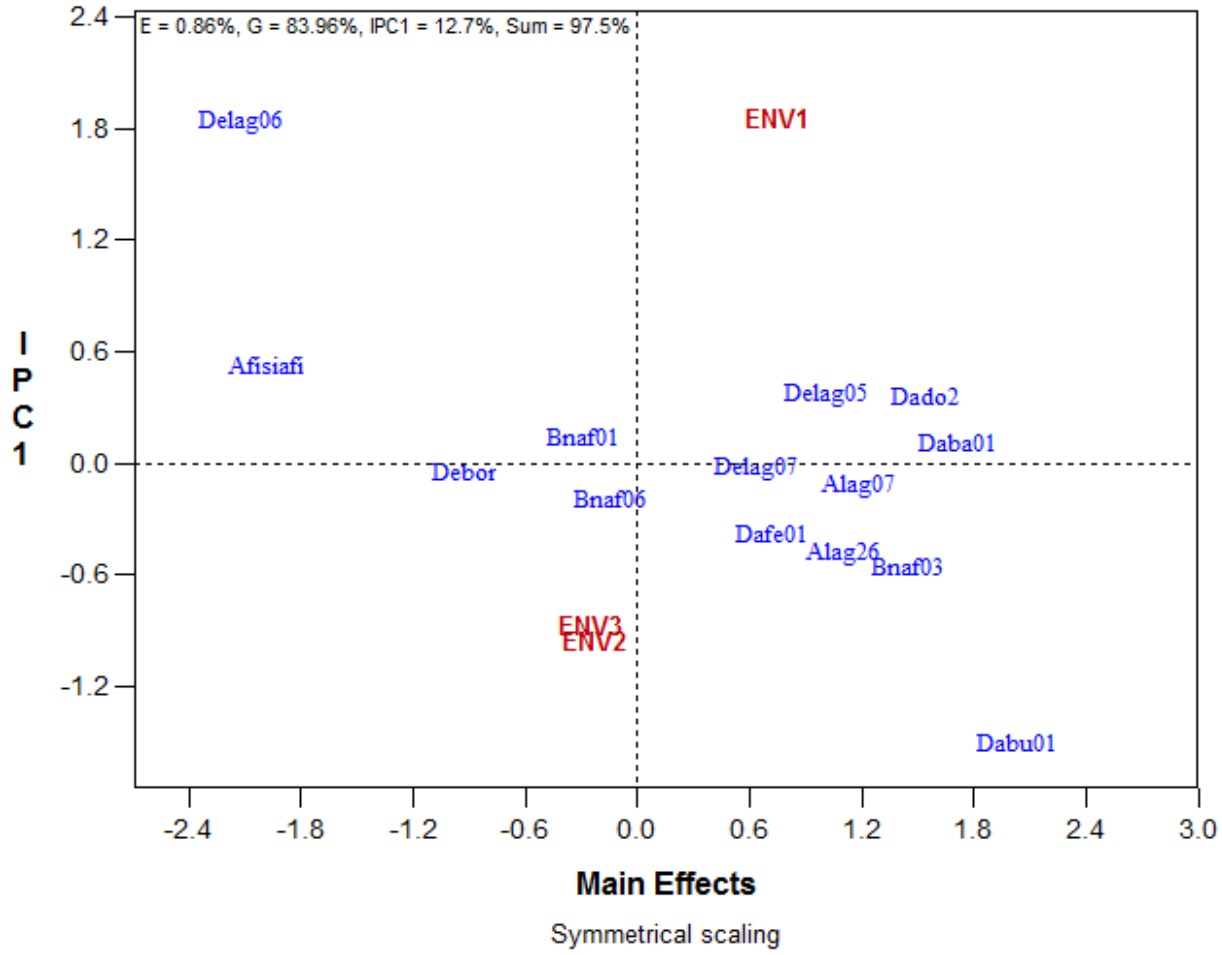

Figure 6.2 Biplot of the first AMMI interaction (IPCA) scores (Y-axis) plotted against main effects (X-axis) of dry matter for 14 cassava genotypes in three environments

ENV1 = Fumesua, ENV 2 = Ohawu, ENV 3 = Pokuase

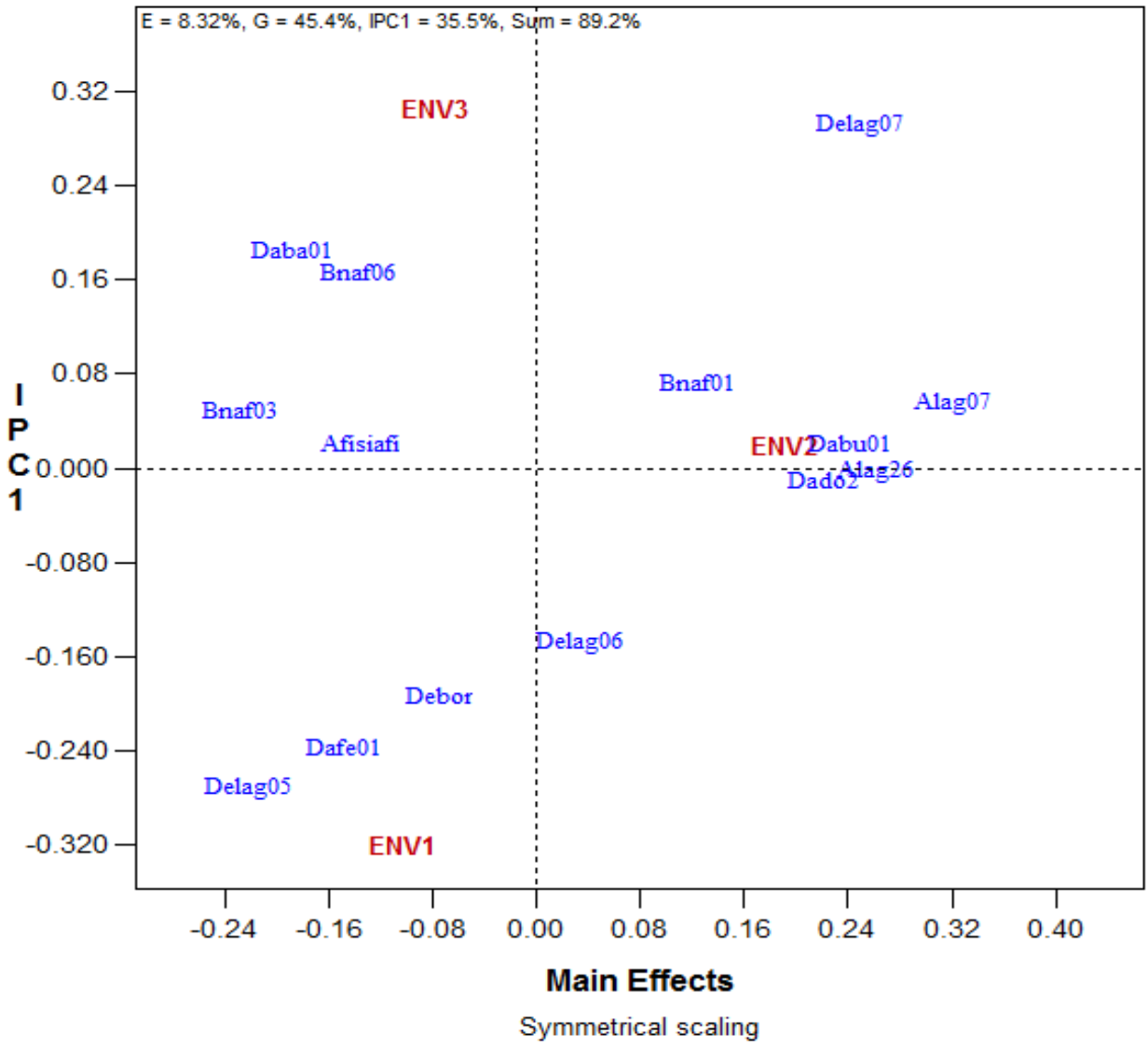

Figure 6.3 Biplot of the first AMMI interaction (IPCA) scores (Y-axis) plotted against main effects (X-axis) of harvest index for 14 cassava genotypes in three environments

ENV1 = Fumesua, ENV 2 = Ohawu, ENV 3 = Pokuase

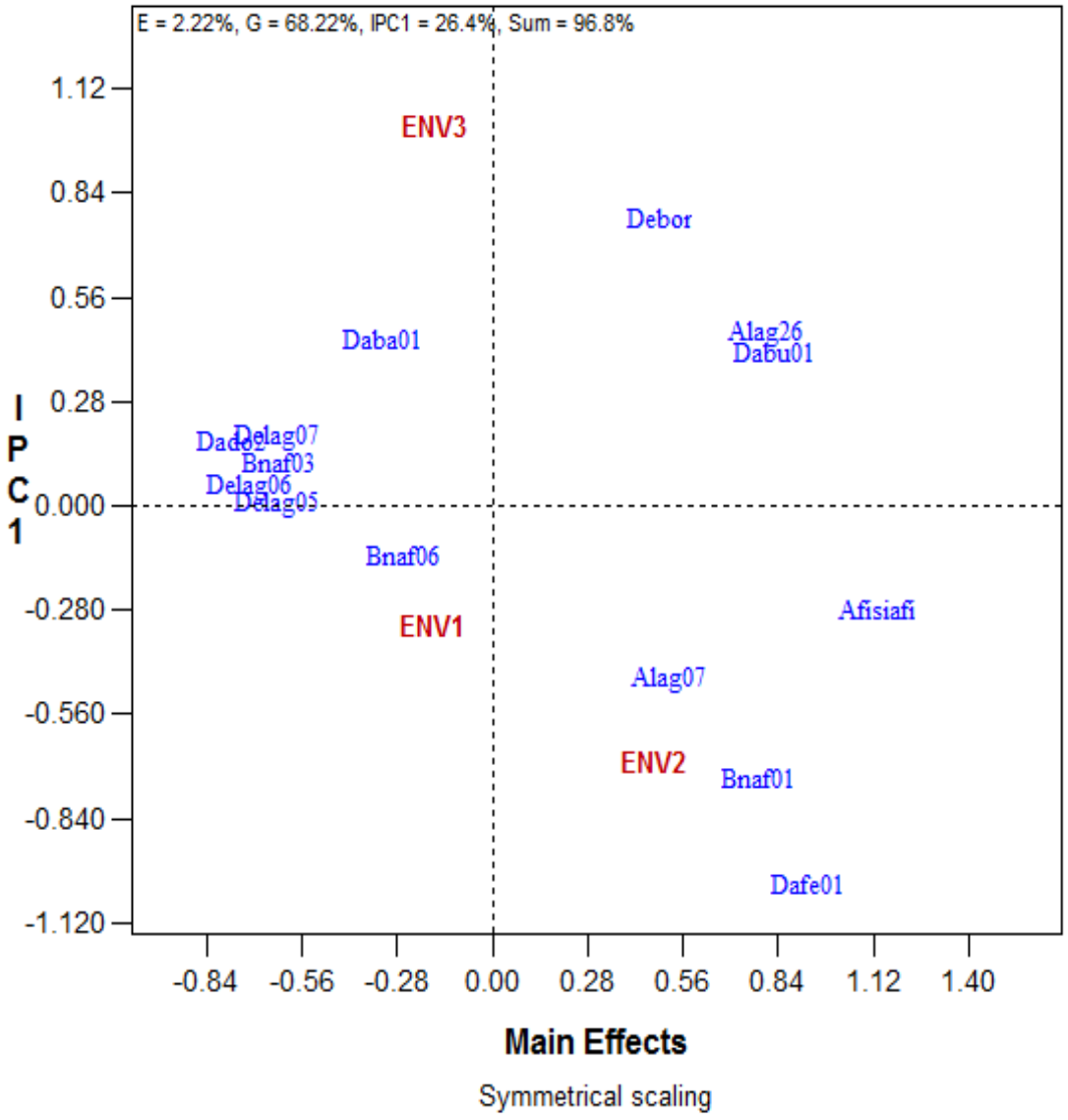

Figure 6.4 Biplot of the first AMMI interaction (IPCA) scores (Y-axis) plotted against main effects (X-axis) of CMD score for 14 cassava genotypes in three environments

ENV1 = Fumesua, ENV 2 = Ohawu, ENV 3 = Pokuase

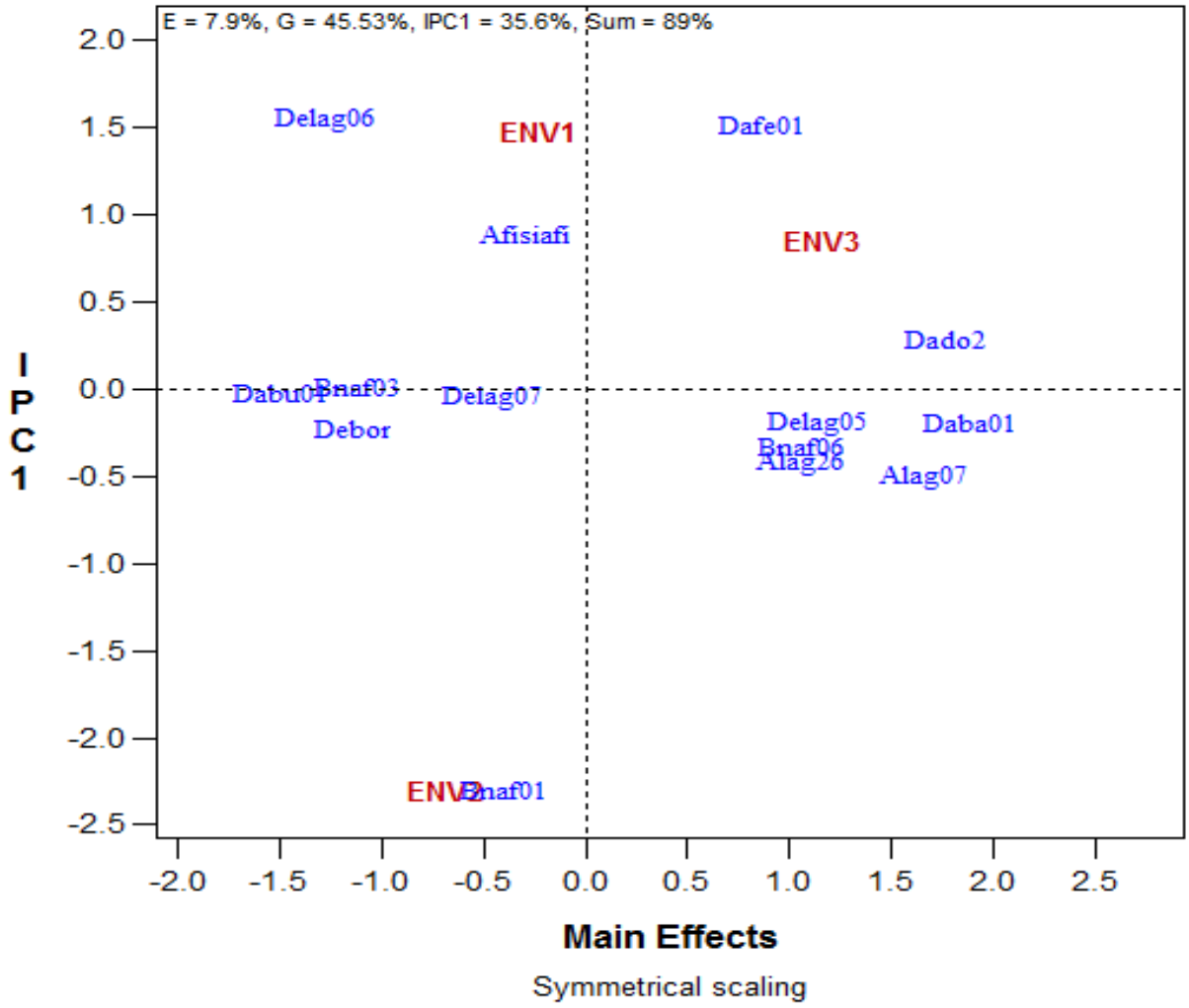

Figure 6.5 Biplot of the first AMMI interaction (IPCA) scores (Y-axis) plotted against main effects (X-axis) of starch content for 14 cassava genotypes in three environments

ENV1 = Fumesua, ENV 2 = Ohawu, ENV 3 = Pokuase

The results showed that one check and one of the F1 progenies (Bnaf01) and Afisiafi recorded high scores for CMD and Dad02 was identified as the most stable genotype for the five traits studied.

6.3.2 Yield performance and stability of elite CIAT genotypes across three locations for two seasons

The clones showed mild symptoms in response to CMD across the three locations. All the elite genotypes showed milder symptoms to the disease than the checks (Table 6.5). There were significant differences among the clones in the combined analysis. All the elite clones had average root yield of 35 t/ha or more except CR59-4 (27.20 t/ha) but this genotype recorded the least response to CMD. All the genotypes from the CR52A family recorded the highest starch content and root weight (Table 6.5).

Table 6.5 Mean root weight, harvest index, starch, CMD and dry matter of 10 CIAT elite cassava clones across three locations for two growing seasons

Genotypes	Root yield t/ha	CMD score	HI	DM	Starch
Afisiafi	53.68	2.26	0.502	28.20	18.26
AR14-10	35.70	1.52	0.383	30.09	17.79
AR15-5	43.42	1.17	0.554	29.41	16.18
CR41-10	36.96	1.26	0.368	29.29	20.57
CR42-4	41.11	1.44	0.459	31.08	19.92
CR52A-25	57.48	1.69	0.528	29.93	21.42
CR52A-31	56.44	1.76	0.491	29.58	22.17
CR52A-4	55.39	1.83	0.556	29.81	22.52
CR59-4	27.20	1.17	0.325	29.13	20.08
Sisipe	63.34	2.24	0.535	30.96	19.00
LSD (0.05)	**10.31**	**0.20**	**0.055**	**2.44**	**2.60**

The AMMI biplot (Figure 6.6) showed that environment explained 40.62%, genotype 32.86% and the IPCA1 19.8% reflecting 93.3% of the root weight variation. The biplot showed Sisipe, Afisiafi, Cr52a-4 and Cr52a-31and CR 52A-31 as high yielding genotypes. Genotypes Cr42-4 and Cr59-4 were the most stable but their yields were below the average yield. Cr52a-25 also had a high yield but was the most unstable

genotype. Afisiafi was identified as the most productive variety in terms of stability and root weight. It supports the fact that it is a released variety and has been tested across locations in Ghana, therefore can be used in a breeding programme to develop populations. AR15-5 was more adapted to Env4 (Fumesua: Season 2) and CR52A-25 was adapted to Env1 (Fumesua: Season 1), Env 2 (Ohawu: Season 1) and Env 3 (Pokuase: Season 1).

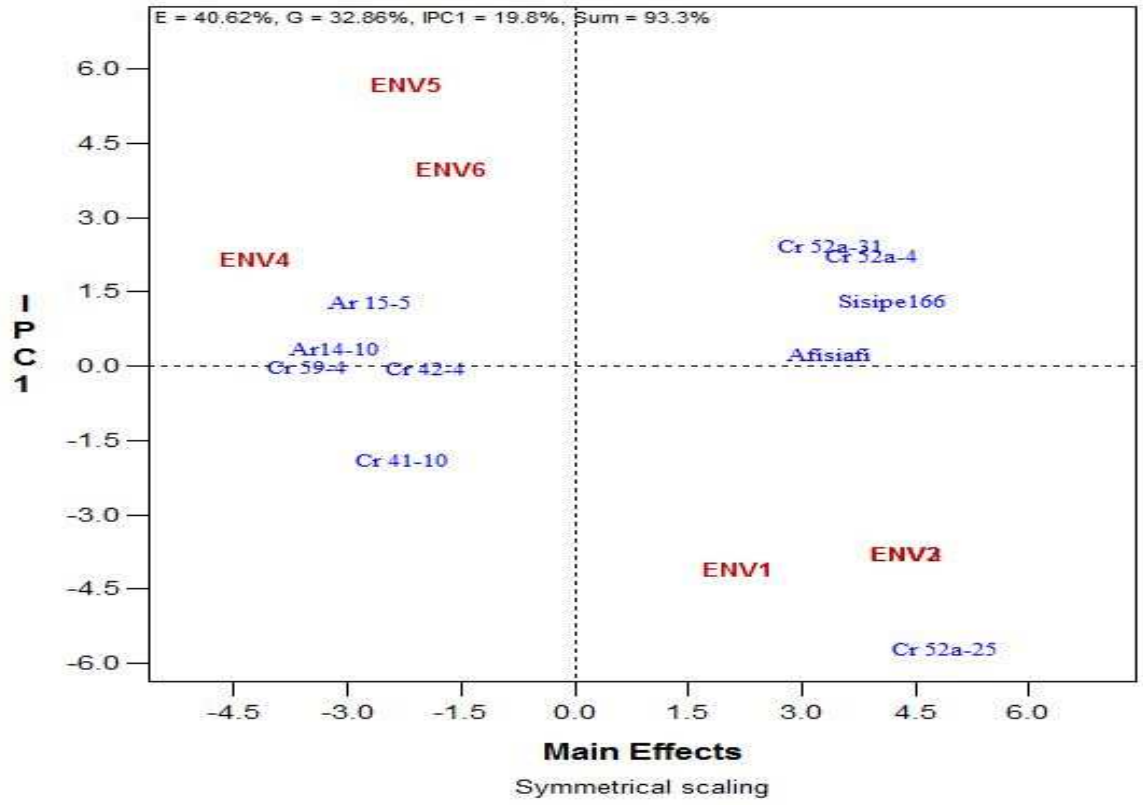

Figure 6.6 Biplot of the first AMMI interaction (IPCA) scores (Y-axis) plotted against main effects (X-axis) of root weight for CIAT cassava genotypes in three environments and two seasons

ENV1 = Fumesua year 1, ENV 2 = Ohawu year 1, ENV 3 = Pokuase year 1, ENV4 = Fumesua, year 2, ENV 5 = Ohawu year 2, ENV 6 = Pokuase year 2

The AMMI biplot for the mean root yield and stability of the genotypes studied (Figure 6.7) is based on the distance of the genotypes from the double arrow line, Cr52a-4,

Afisiafi and Sisipe166 had the highest mean root weight. Afisiafi, Cr59-4 and Cr42-4 which were close to the single arrow line were identified as being stable. Afisiafi was selected as the most productive genotype because of high yield and stability as Cr52a-25 was identified as the most unstable genotype. This supports the result of Figure 6.6 above.

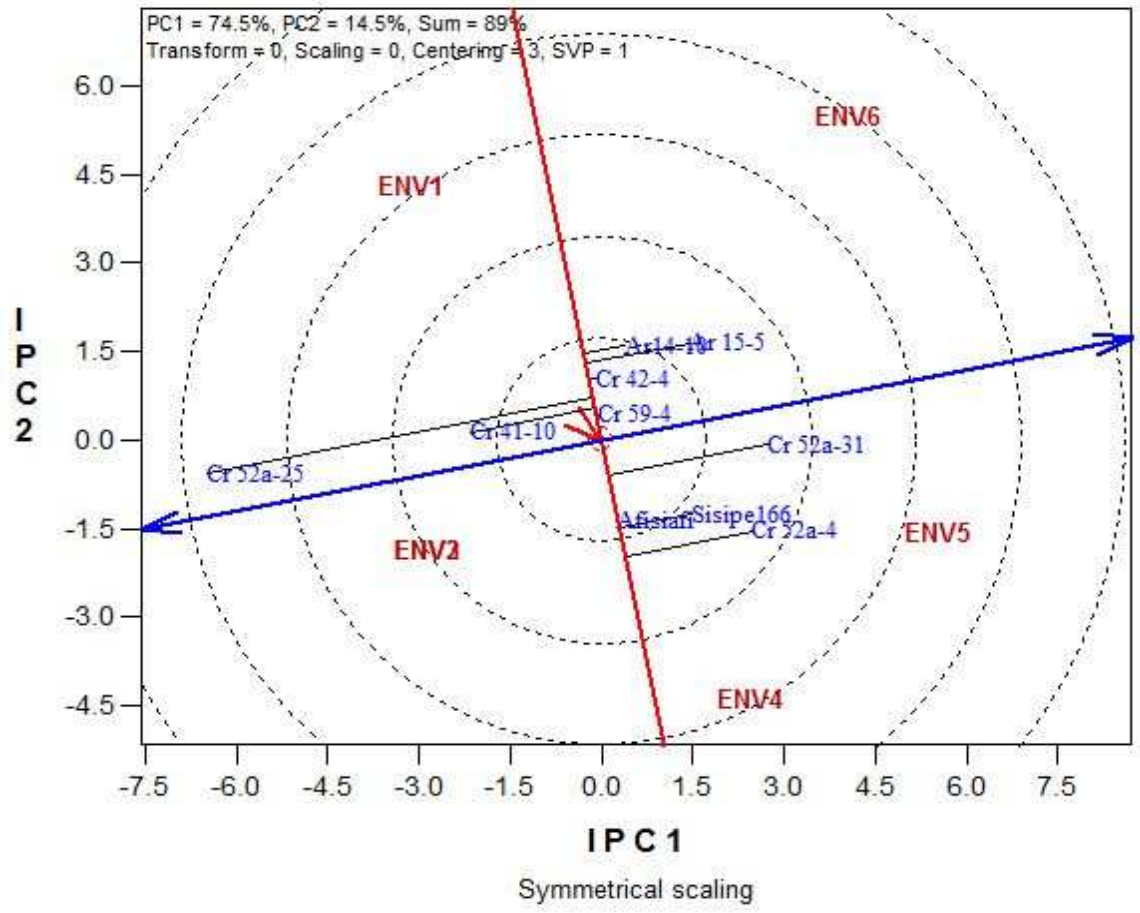

Figure 6.7 Biplot of the first AMMI interaction (IPC1) scores (X-axis) plotted against the second interaction (IPC2) scores (Y-axis) for root yield for CIAT genotypes for three locations and two seasons

ENV1 = Fumesua year 1, ENV 2 = Ohawu year 1, ENV 3 = Pokuase year 1, ENV4 = Fumesua year 2, ENV 5 = Ohawu year 2, ENV 6 = Pokuase year 2

Dry matter was highest in genotype Cr42-4 as shown on Figure 6.8. Cr41-10 was the most stable but with average dry matter. Cr52a-25 yielded 57 t/ha and was a stable genotype for root yield, thus the most productive.

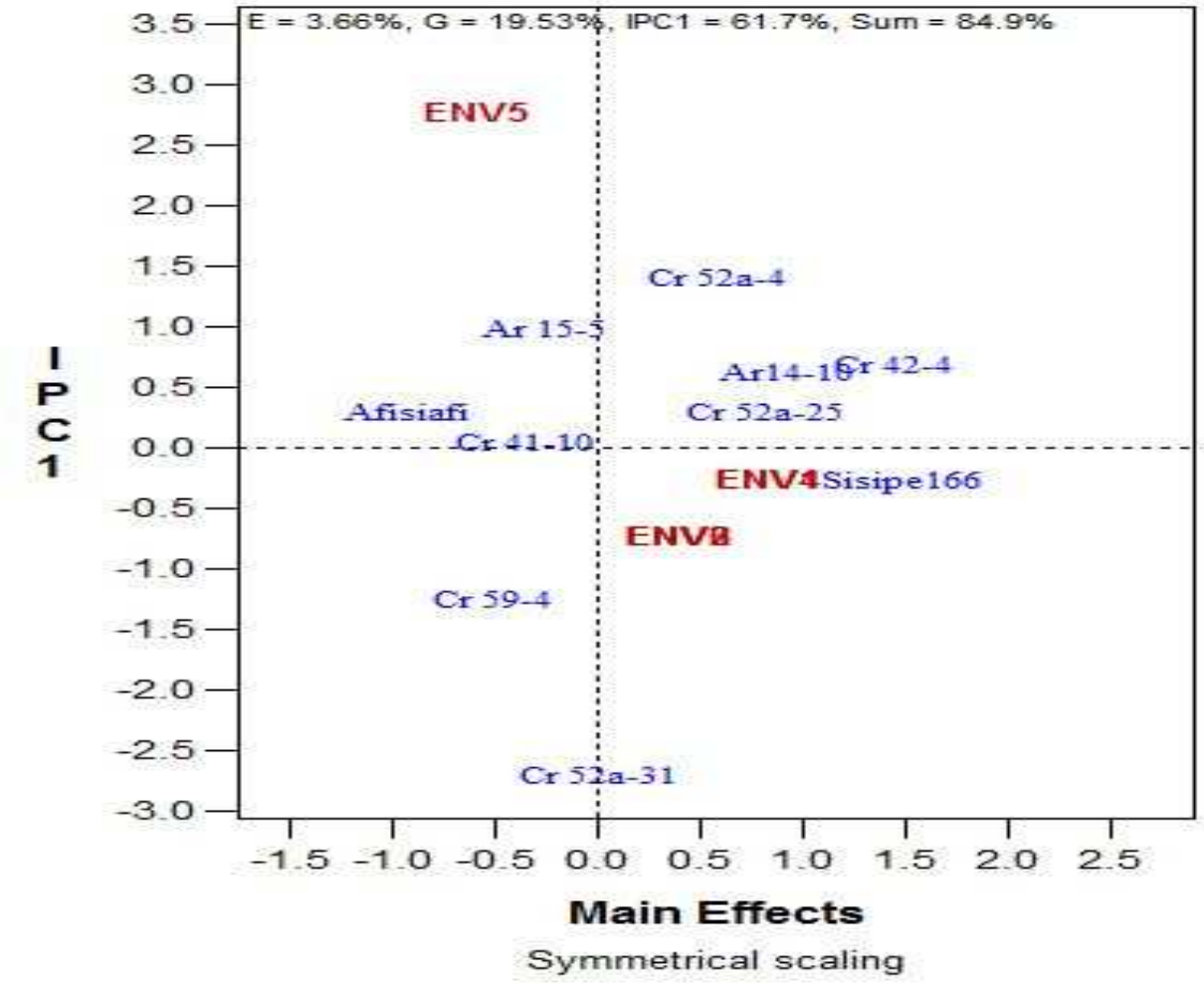

Figure 6.8 Biplot of the first AMMI interaction (IPCA) scores (Y-axis) plotted against main effects (X-axis) of dry matter content for CIAT cassava genotypes in three environments and two seasons

ENV1 = Fumesua year 1, ENV 2 = Ohawu year 1, ENV 3 = Pokuase year 1, ENV4 = Fumesua year 2 , ENV 5 = Ohawu year 2, ENV 6 = Pokuase year 2

Cr42-4 and Cr59-4 were identified as the most stable for harvest index but in terms of productivity and stability, Cr42-4 and Cr52a-4 were selected because of their moderate yield and stability (Figure 6.8).

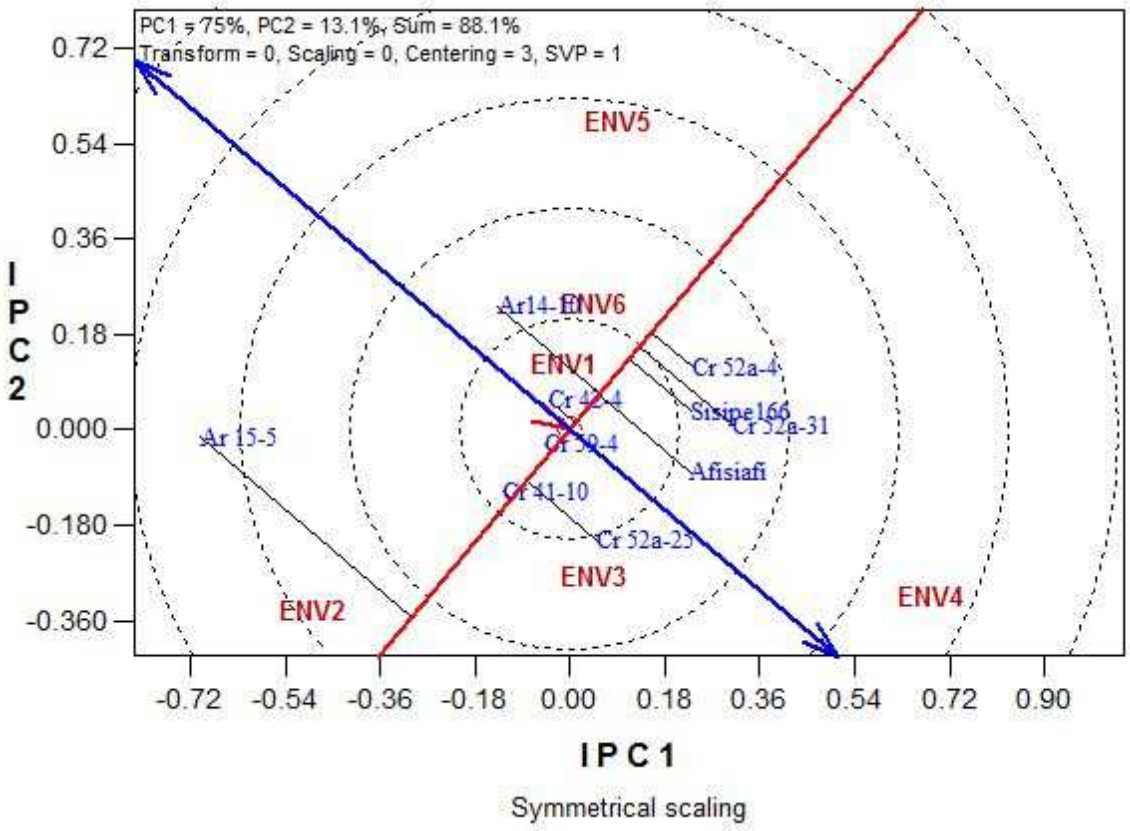

Figure 6.9 Biplot of the first AMMI interaction (IPC1) scores (Y-axis) plotted against the second interaction (IPC2) scores (X-axis) for harvest index for CIAT genotypes for three locations and two seasons

ENV1 = Fumesua year 1, ENV 2 = Ohawu year 1, ENV 3 = Pokuase year 1 ENV4 = Fumesua year 2 = ENV 5 = Ohawu year 2 ENV 6 = Pokuase year 2

Afisiafi and Cr52a-31 were considered to be the most affected genotypes by CMD based on their projection to the X-axis (Figure 6.10). This indicates high susceptibility to the disease. The genotypes demonstrated variable CMD score stability across environments, indicating environmental specificity for the incidence of the disease. Genotype Cr14-10 had the lowest CMD score. Cr42-4 and Cr52a-25 were the most stable compared to the

other genotypes, but Cr42-4 was selected because of its low CMD score. This can be used in a cassava breeding programme.

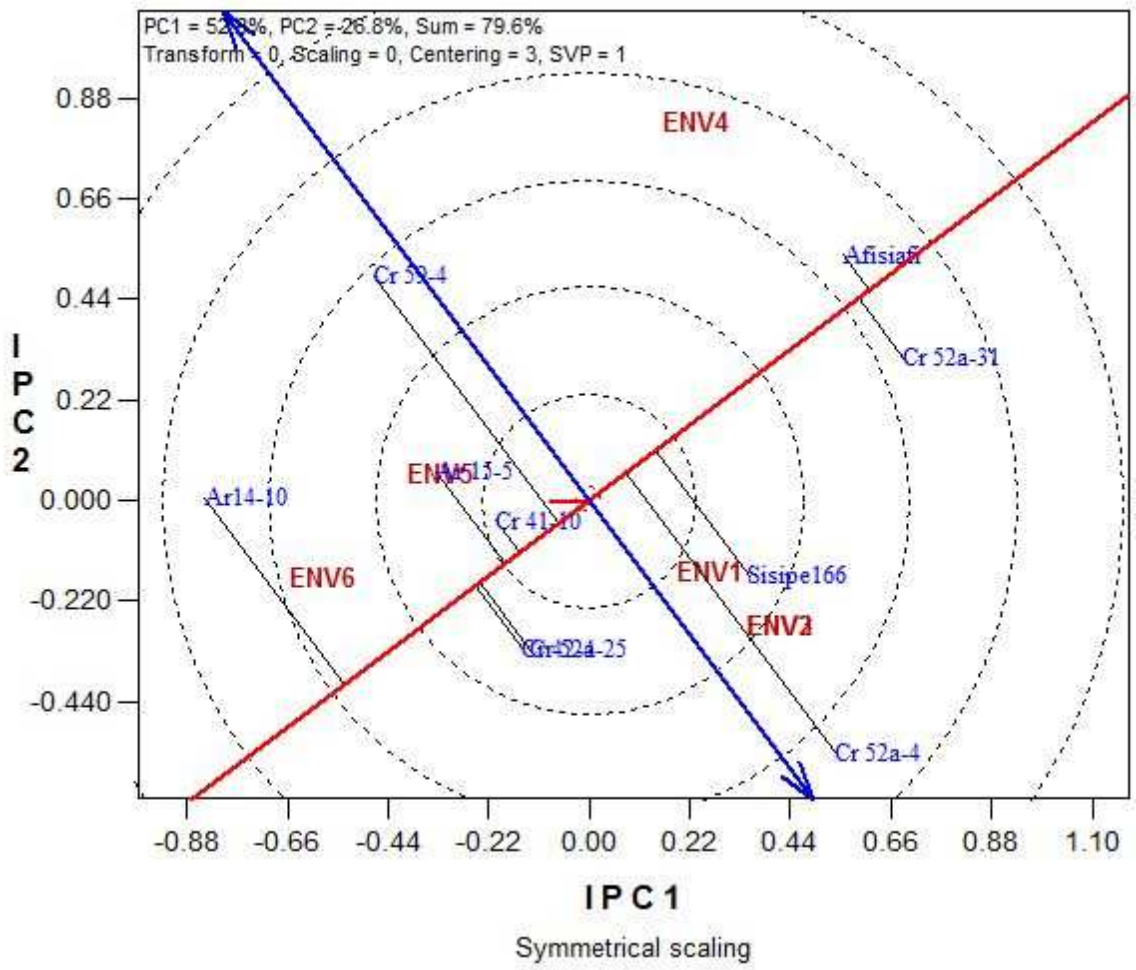

Figure 6.10 Biplot of the first AMMI interaction (IPC1) scores (Y-axis) plotted against the second interaction (IPC2) scores (X-axis) for CMD score for CIAT genotypes for three locations and two seasons

ENV1 = Fumesua year 1, ENV 2 = Ohawu year 1, ENV 3 = Pokuase year 1, ENV4 = Fumesua year 2, ENV 5 = Ohawu year 2, ENV 6 = Pokuase year 2

Combined analysis of variance indicated that all the sources of variance were highly significant for harvest index (Table 6.6). Genotype main effect and Genotype x Year (G xY) were highly significant (P<0.001) for all five traits studied except dry matter.

Location main effect was not significant for root weight and dry matter. Genotype x Location x Year (G x L x Y) was highly significant (P<0.001) for harvest index, (P<0.05) for starch and CMD (Table 6.6), (P<0.01) for harvest index, (P<0.05) for root weight (Table 6.6). The relative magnitude of the main effects and their interaction (Tables 6.6) for the five traits measured as proportion of the total sum of squares showed that genotype main effect impacted more on root yield, CMD and harvest index than the other effects and interaction. G x L and G x Y effects impacted more on dry matter and starch respectively than the main effects.

Table 6.6 Mean squares for five traits in 10 elite cassava genotypes evaluated in three locations for two seasons

Source	DF	Root yield	Starch	DM	HI	CMD
Genotype (G)	9	2506.47***	72.39***	13.16	0.125***	2.88***
Location (L)	2	178.33	321.31***	7.05	0.101***	1.59***
Rep	6	890.32	5.05	10.65	0.041***	0.08
G*L	18	464.04**	25.93*	11.76	0.023*	0.18*
Year (Y)	1	10178.66***	168.03**	3.04	0.031***	17.36***
L*Y	2	3290.02***	114.56**	3.04	0.271***	0.26
G*Y	9	1458.50***	87.20***	8.56	0.068***	0.56***
G*L*Y	18	384.25	29.68*	8.56	0.019***	0.19*

*** P<0.001, ** P<0.01, * P<0.05, DM = dry matter content, HI = harvest index, CMD = cassava mosaic disease

Table 6.7 Sum of squares for 10 elite cassava genotypes evaluated in three locations for two seasons

Source	DF	SS	% Total SS
Root yield			
Genotype (G)	9	22558.24	22.24
Location (L)	2	356.65	0.35
Rep(L)	6	5341.89	5.27
G*L	18	8352.79	8.23
Year (Y)	1	10178.66	10.03
L*Y	2	6580.05	6.49
G*Y	9	13126.55	12.94
G*L*Y	18	6916.59	6.82
Total	179	101442.87	
CMD			
Genotype (G)	9	25.89108	37.18
Location (L)	2	3.176774	4.56
Rep(L)	6	0.50733	0.73
G*L	18	3.18329	4.57
Year (Y)	1	17.36404	24.94
L*Y	2	0.524926	0.75
G*Y	9	5.005611	7.19
G*L*Y	18	3.387365	4.86
Total	179	69.633	
HI			
Genotype (G)	9	1.12348	26.09
Location (L)	2	0.202288	4.69
Rep(L)	6	0.247188	5.74
G*L	18	0.415943	9.66
Year (Y)	1	0.030961	0.72
L*Y	2	0.541291	12.57
G*Y	9	0.612776	14.24
G*L*Y	18	0.348719	8.11
Total	179	4.305	
DM			
Genotype (G)	9	118.4048	5.37
Location (L)	2	14.09761	0.64
Rep(L)	6	63.9243	2.9
G*L	18	211.6911	9.59
Year (Y)	1	3.043548	0.14
L*Y	2	6.083599	0.28
G*Y	9	77.07032	3.49
G*L*Y	18	154.0748	6.98
Total	179	2206.89	
Starch			
Genotype (G)	9	651.4828	12.35
Location (L)	2	642.6268	12.17
Rep(L)	6	30.30377	0.57
G*L	18	466.7646	8.84
Year (Y)	1	168.034	3.18
L*Y	2	229.1212	4.34
G*Y	9	784.7854	14.87
G*L*Y	18	534.297	10.12
Total	179	5279.204	

6.4 Discussion

For the F1 progenies, the high genotype but small environment effects and relatively low G x E interaction for dry matter content may indicate evaluation over fewer environments to distinguish genotypes with high and stable performance.

The high impact of genotype on root weight indicates that evaluation and selection can be done in fewer environments to distinguish genotypes with high and stable performance but this is contrary to work done by Ssemakula et al. (2007) when working on stability of total carotenoid concentration and fresh yield of selected yellow-fleshed cassava genotypes. The genotype Debor was identified as stable. This is an ancient variety that farmers have kept for years and it is grown in most parts of the country, hence its stability. The low starch levels observed may be due to physiological changes that starch undergoes during the growth cycle, a comprehensive study would have to factor in time of harvesting, climatic changes and it may require testing in diverse and multiple environments to identify genotypes with broad and specific adaptation due to the high impact of location and interaction. The F1 progeny Dad02 with its performance can be tested on farmers' field for release, it can also be used in a breeding programme to pyramid other sources of CMD resistance to add on to resistance to CMD and higher root yield.

Cassava genotypes with high dry matter, root weight and resistance to CMD can be identified easily and selection for the traits in a single environment can be done. This multivariate analysis is useful especially where the location impacted very little on the performance of the genotypes. However, G x E interaction on three traits (root weight, dry matter and CMD) indicates that some genotypes may not respond positively.

In the CIAT trial, Afisiafi was identified as the most productive variety in terms of stability and fresh root yield. It supports the fact that it is an IITA improved variety (TMS 30572) which had been tested extensively before its release as a variety. The name given means 'every where' though Afisiafi has CMD as a challenge it can be improved for this

trait. The mechanised cassava farmers use this variety because of its stable yield (personal communication Chris Quarshie, CALTECH, Ghana). Afisiafi has been tested across locations in Ghana. The study also identified genotype Cr59-4 as a CMD resistant genotype. Although the fresh root yield was poor, it can be used in cassava breeding programmes. Among the CIAT elite materials genotype Cr52A-31 and Cr52A-5 were high yielding genotypes.

6.5 Conclusions

High variability existed among all the cassava clones (CIAT material and F1's) for the traits studied. The AMMI biplots presented excellent graphical presentation of the data in terms of mean yields and stability. The genotype CR59-4 showed remarkable resistance to CMD but had low yields. Afisiafi, Cr52a-4, Cr5sA-31 and Sisipe recorded the highest yields. Cr52A-25 was found to be the highest yielding F1 clone, but it was very unstable. The relative magnitude of the main effects and their interactions for the five traits measured as a proportion of the total sum of squares showed that genotype main effect impacted more on root yield, CMD and dry matter than the other effects, indicating that the traits can be improved by direct selection.

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CHAPTER 7

IMPROVING GHANAIAN CASSAVA LANDRACES FOR CMD RESISTANCE USING CIAT ELITE GENOTYPES AND MARKER ASSISTED SELECTION

Abstract

Cassava, *Manihot esculenta* Crantz, is a major staple and food security crop in Africa. It produces large amounts of energy per unit land area under uncertain rainfall and low fertility conditions compared to other crops. However, it suffers from several pests and diseases that reduce yield by nearly 48 million ton yearly in Africa, about 50% of its current production, valued at US$1.4 billion. The CMD2 resistance gene is being deployed in Africa for breeding in national programmes. A total of eight CMD resistant genotypes from CIAT were selected and used for crosses with three cassava landraces and one IITA breeding genotype. CMD evaluation was done on the 12 parental genotypes and 525 F_1 progenies comprising individuals pre-selected for CMD resistance based on average severity scores of 1 and 2. Multiple marker analysis was used to screen for the CMD2 gene in this material. Of the tested genotypes 83% showed at least a marker allele for the CMD2 gene. Two CIAT genotypes (CR52A-31 and AR14-10) had all four marker alleles associated with CMD2 indicating that it is the probable source of CMD resistance in these genotypes. Only two of the local progenitors (Dabodabo and TME11) were phenotypically identified as resistant. TME11 had three alleles (RME-1, 158, 169) associated with CMD2 while Dabodabo had one marker allele (SSRY28) associated with the gene. This suggests that Dababo may have an alternative gene or genes than CMD2 for CMD resistance, but this needs to be confirned. The F_1 families showed segregation for the CMD2 gene. A total of 88 individuals (17%) had the four marker alleles associated with the CMD2 gene. A total of 179 individuals had between one to three marker alleles each associated with CMD2. A total of 91 individuals had no marker allele associated with the CMD2 gene.

7.1 Introduction

Despite the low average yields of 9 t/ha, over 50% of the world's production of cassava is in Africa. Africa produced nearly 121 million metric ton of the total world production of 242 million metric ton in 2008, with Nigeria as the leading producer and Ghana the third (FAO 2009). Cassava production is seasonless and hence is used as a food reserve against famine and plays a major role in reducing food shortages (Egesi et al. 2007). Cassava is used in different parts of the world. However, in developing countries, especially in sub Saharan Africa, cassava serves as a major food crop or staple. Its importance to many in Africa is epitomised in the Ewe (a language spoken in Ghana, Togo and Benin) name for the plant, ''Agbele" meaning 'there is life' (Manu-Aduening 2005). Cassava accounts for a daily calorie intake of 30% in Ghana and it is cultivated by nearly every farming family or household (FAO 2006). Over 60% of cassava production in Ghana depends on landraces, though a number of improved varieties have been released since 1993 (Nweke et al. 1999; Manu-Aduening 2005). The landraces are low yielding and stressed by biotic factors. Yield losses due to CMD range between 20-95% in susceptible genotypes (Muimba-Kankolongo and Phuti 1987; Moses 2008). In Ghana, for example, genotypes susceptible to CMD yield below 10 t/ha in most farming communities compared to yields of 30 t/ha or more that can be obtained from improved genotypes resistant to CMD (Moses 2008). In a survey by the collaborative study of cassava in Africa (COSCA), covering 80% of the cassava cultivated in Africa, it was found that farmers would continually abandon some old cassava cultivars and introduce new ones (Nweke et al. 1994) indicating a need for better varieties.

A number of diseases affect production of cassava in Ghana, but the most common is CMD. The disease is caused by viruses of the genus Begomivirus in the family Geminiviridae and transmitted by *Bemisia tabaci,* the whitefly, and disseminated in the stem cuttings used routinely for propagation (Pita et al. 2001; Thottappily et al. 2006). It is the most important disease of cassava and is associated with national or regional epidemics flaring up every few decades. Epidemics are particularly ravaging, with root losses as high as 100% (Jennings 1994; Thresh et al. 1997). Even in the absence of a

serious outbreak, yield losses of 20% to 90% are common in farmers' fields (Muimba-Kankolongo and Phuti 1987; Moses 2008). Estimated total crop yield losses due to CMD on the continent amounts to about US $440 million annually (Thresh et al. 1997).

High disease incidence (disease occurrence in > 50% of the plants) in various parts of Africa, have been attributed to high inoculums from infected fields, population outburst of the vector and cultural practices of the farmers (Fauquet and Fargette 1990). However, it is unclear whether the low incidence (disease incidence < 50%) of disease recorded in some areas is due to the inherent resistance of varieties or to the lack of inoculums (Thresh and Mbwana 1998). The virus can infect all cultivars although disease susceptibility varies greatly (Fauquet and Fargette 1990).

The most appropriate and only strategy to overcome the virus in cassava, is by breeding for resistance to CMD (Thresh et al. 1997; Lokko et al. 2005). CMD resistance should be introgressed into farmer preferred genotypes. Landraces have been considered as a valuable source for useful genes in breeding programmes, including resistance to CMD, because they contain co-adapted gene complexes with tolerance and adaptation to disease and specific ecological conditions (Harlan 1975; Zeven 1998; Akano et al. 2002; Okogbenin et al. 2007). New sources of CMD resistance genes would be useful for pyramiding into farmer preferred genotypes.

The conventional breeder has the challenge of screening for large numbers of progenies and difficulty in screening at the seedling stage where most genotypes look vigorous and healthy until a later stage. There are several cycles of selection (preliminary, advanced and uniform yield trials) before the multi-location yield trials, a process that takes not less than eight years. The number of years required for the evaluation of promising genotypes, approximately 10 years, is prohibitive and is a bottleneck to increased productivity. Hence an effective means to speed up identification of genotypes with high performance is clearly required for effective breeding. Biotechnology offers this opportunity with tools such as Marker Assisted Selection (MAS).

MAS is the use of DNA markers for selection of individuals with desired traits within a population, by linking molecular markers to a desired trait. To be of utmost benefit, the markers should be closely linked to one or more of the target loci, which may often be QTLs (Goff and Salmeron 2004). In the past decade many research institutes and breeding companies have started applying MAS to increase the effectiveness of selection in breeding to shorten the time required developing varieties (Ribaut and Hoisington 1998). This has been applied effectively in maize improvement, thus reducing the number of generations needed for recovery of the recurrent genome in a shorter time (from eight to three generations, Frisch et al. 1999). In maize breeding it has been used to transfer quality protein maize (QPM) genes, controlled by a mutant allele of a gene called opaque2 from one elite maize inbred line to another (CIMMYT 1999). In rice, MAS has centered on pyramiding disease resistance genes, particularly for blight and blast (Koebner 2003). In cassava, the application of MAS has been developed more recently compared to other major crops, with the construction of genetic linkage maps. Despite the low saturation of loci in the genetic maps of cassava, the marker loci are randomly distributed over linkage groups and the information from these maps has been used in cassava genetics. The genes for resistance to CMD have been mapped, including a major one (CMD2) (Akano et al. 2002). MAS for breeding CMD resistance has successfully been applied for introducing resistance into elite gene pools at CIAT (CIAT 2003; Fregene and Mba 2004;Fregene et al. 2007) and also to introgress resistance to cassava green mite (CGM) and CMD in local Tanzanian varieties (Kullaya et al. 2004). Precise identification of genotypes without the confounding effect of the environment is made possible (Meuwissen et al. 2001). The environmental effect or interaction and subjective nature of characterising using morphological markers alone are reduced.

The potential use of the identified genes in cassava will be in pyramiding the disease resistance genes together with those for agronomic traits into one genotype. Several other factors will also influence the efficiency of the use of MAS in the future: new developments and improvements in marker technology such as the use of SNPs (Collard et al. 2005), high density maps and the integration of functional genomics with QTL mapping such as microarray analysis and ESTs (Morgante and Olivieri 1993; Anderson

et al. 2004). To enhance the efficiency of MAS, knowledge of DNA sequences of the genes allows the development of the 'perfect marker' which is actually located within the interested gene sequence (Collard et al. 2005). The application of knowledge of gene sequences for useful traits, especially in cassava, is still a new area.

The objectives of this study were:

1. To introgress CMD resistance into farmer preferred genotypes
2. To use MAS to improve selection of cassava introgressed with CMD from CIAT genotypes
3. To search for potential new sources of CMD resistance from landraces

7.2 Material and methods

The CIAT genotypes were introduced to Ghana to broaden the narrow genetic base of the germplasm and were first evaluated on station and later on farmers' fields (Chapter 5). A total of eight CMD resistant genotypes from CIAT were selected and used for crosses with three cassava landraces and one IITA breeding genotype at the CSIR-CRI, Fumesua, near Kumasi. The number of crosses carried out for each family and seeds generated are shown in Table 7.1.

The station is located in the forest zone and it is a CMD high pressure zone or hot-spot. The progenitors were planted in single rows in completely randomised plots. The CIAT materials were used as male parents and the three landraces and one IITA breeding genotype as females. Controlled genetic crosses were carried out between 6-8 MAP. Fertilized flowers were covered with mesh pollination bags to prevent pollen contamination. The F1 seeds of the crosses were harvested after eight weeks, and then dried and tested for viability by floating in water (seeds that floated were considered not viable) and planted as F1 progenies. In 2009 F1 seedlings were planted for evaluation at two locations, Ohawu and Fumesua in high disease pressure zones or hot-spots for CMD.

Table 7.1 Crosses of progenitors or parental genotypes and number of F1 progenies

Female parent	Male parent	Number of progenies
Afeb	CR52A-4	46
	CR52A-25	41
	CR41-10	1
TMEII	CR52A-31	47
	AR15-5	32
	CR52A-25	47
	CR41-10	37
	CR12-7	9
	AR12-50	14
	AR14-12	2
Dabodabo	CR52A-4	40
	CR52A-31	25
	CR52A-25	48
	CR41-10	12
	A514-10	17
	AR15-5	17
Tuaka	CR52A-4	45
	CR52A-31	34
	AR15-5	32
	CR52A-25	28
	CR41-10	5

Figure 7.1a CMD resistant cassava plant

Figure7.1b CMD infected cassava plant

Figure 7.1c Cassava white fly and mealy bug

The best progenies were selected for multiplication and clonal evaluation in 2010 (data not given). Climatic conditions for the locations are given in Table 4.1 in Chapter 4.

7.2.1 Plant materials

The evaluation was done on 12 parental genotypes and 525 F_1 progenies comprising individuals pre-selected for CMD resistance based on average severity score of 1 and 2 (where 1 is no symptoms on the leaves, and 2 is when leaves show slight mosaic pattern symptoms) (Figures 7.1a-b). The parental lines included three landraces, one IITA breeding genotype and eight CIAT developed genotypes developed for improved CMD resistance using the CMD2 gene.

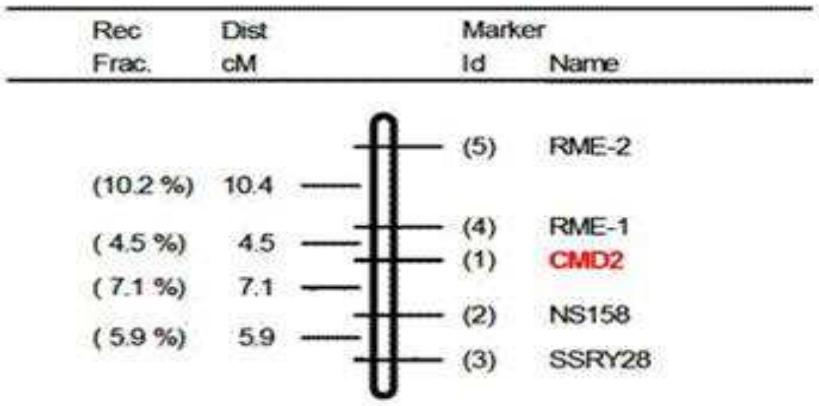

Figure 7.2. Markers and their distances from the CMD2 gene (Fregene et al. 2001)

7.2.2 Phenotyping

A total of 525 F1 individuals and their parents were evaluated for CMD in one season. Two locations (Fumesa and Ohawu) were used for the screening of the CMD reaction of the genotypes. At Fumesa, 399 genotypes were evaluated while the remaining 126 genotypes were evaluated for the disease at Ohawu. The splitting was done for each family to have representation in both locations. The purpose for splitting was to assess the disease reaction in the two major cassava producing regions so that assessing the F1 with

end-users and farmers in the location would be less expensive and cost effective. The effect of the two locations on the performance of the F1s selected for the clonal evaluation in the following season has not been reported in this study. The virus symptom severity scores were assessed on a scale of 1-5, where 1 indicates no symptoms and 5 indicates severe mosaic with distortion of entire leaf (IITA 1990).

7.2.3 Marker Assisted Selection

DNA isolation

Fresh young tender leaf samples of the 12 progenitors and all the F1 progenies were isolated using the Qaigen kit. Methodology is as given in Chapter 3 for the genetic diversity study.

Marker analysis

Four DNA based markers associated with the CMD2 gene which confers CMD resistance were used for molecular analysis. They included one SCAR marker (RME1) and three SSR markers (SSRY28, 158 and 169). The genetic distance of the markers from the CMD2 gene and their sizes is given in Figure 7.2. The primer sequence for the four markers associated with the CMD2 gene is shown in Table 7.2. Two genotypes were also included as controls for the marker analysis. These are TME3 which is the source of the CMD2 gene which was used as the positive control for presence of the CMD2 genes while Nga2 was used as the negative control and absence for the band (marker allele) for the CMD2 gene.

SSR PCR reaction profile and cocktail mixtures

The PCR amplification reactions were performed in 10 ul volumes for the three markers The reaction mixture composition was 50 ng template DNA, 1X PCR buffer, 1.5 Mm $MgCl_2$, 0.2 mM dNTP, 250 nM each of forward and reverse primers and 0.25U Taq polymerase, 0.5 ul was used for the 10 ul PCR reaction. PCR amplification was carried out in an M&J Thermal cycler. The three SSR marker (158, 169 and 22RY28) profiles programmed temperature involved an initial denaturation step of 95^{o}C for 2 minutes followed by 29 cycles at 94^{o}C for 30 seconds, annealing at 55^{o}C for 1 minute and

extension at 72°C for 1 minute; a final elongation cycle of 72°C for 5 minutes was included. Amplification products were left at 4°C prior to electrophoresis. The SCAR marker profile had an initial denaturation of 95°C for 2 minutes followed by 34 cycles at 94°C for 30 seconds, annealing at 50°C for 1 minute and extension at 72°C for 1 minute, a final elongation of 72°C for 5 minutes. DNA loading dye was added to the PCR amplification products and separated by electrophoresis on 1.5% agarose and 6% polyacrylamide gels to compile marker profiles for the SSR reaction.

Table 7.2 Primer sequences for DNA analysis

Name Position	Type of repeat	Left primer	Right primer	Product size	Ann. Temp(°C)	MgCI (mM)
SSRY28 13.0cM	SSR CT(26)AT(3) AC AT(2)	TTGACATGAG TGATATTTTC TTGAG	GCTGCGTGCAA AACTAAAAT	180	55	1.5
158 7.1cM	SSR	GTGCGAAAT GGAAATCAA TG	TGAAATAGTGA TACATGCAAAA GGA	166	55	2.5
169 70cM	SSR	GTGCGAAAT GGAAATCAA TG	GCCTTCTCAGC ATATGGAGC	319	55	2.5
RMEI 4.5cM	SCAR	ATGTTAATGT AATGAAAGA GC	AGAAGAGGGTA GGAGTTATGT	700	50	2.5

Table 7.3 Marker profiles for progenitors or parental genotypes used for crosses

					Marker		
Genotype	Parent	Source	AvScore	SCAR	SSRY 28	158	169
1	TME11	IITA	2	1	0	1	1
2	DABO	LOCAL	2	0	1	0	0
3	TUAKA	LOCAL	3	1	0	0	0
4	AFEB	LOCAL	3	0	0	0	1
5	AR15-5	CIAT	1	1	0	1	1
6	CR52A-4	CIAT	2	1	0	0	1
7	CR52A- 25	CIAT	2	0	0	0	1
8	CR42-4	CIAT	3	1	0	0	0
9	CR59-4	CIAT	2	1	0	0	1
10	AR14-10	CIAT	1	1	1	1	1
11	CR41-10	CIAT	1	1	1	0	0
12	CR52A-31	CIAT	1	1	1	1	1

7.3 Results

7.3.1 CMD reaction for progenitors

With the exception of CR42-4, the other seven CIAT elite lines were CMD resistant. The elite lines were developed at CIAT having CMD2 donor parents in their pedigree and resistance is based on CMD2. The landraces from Ghana used in these crosses had a different reaction to CMD, with landraces Dabodabo and IITA genotype TME11 being resistant with an average score of 2 while landraces Afeb and Tuaka were moderately tolerant to the disease with an average score of 3 (Table 7.3).

7.3.2 SSR marker analysis

Multiple marker analysis was used to screen for the CMD2 gene in the progenitors (landraces, IITA genotype and CIAT elite genotypes) and progenies.

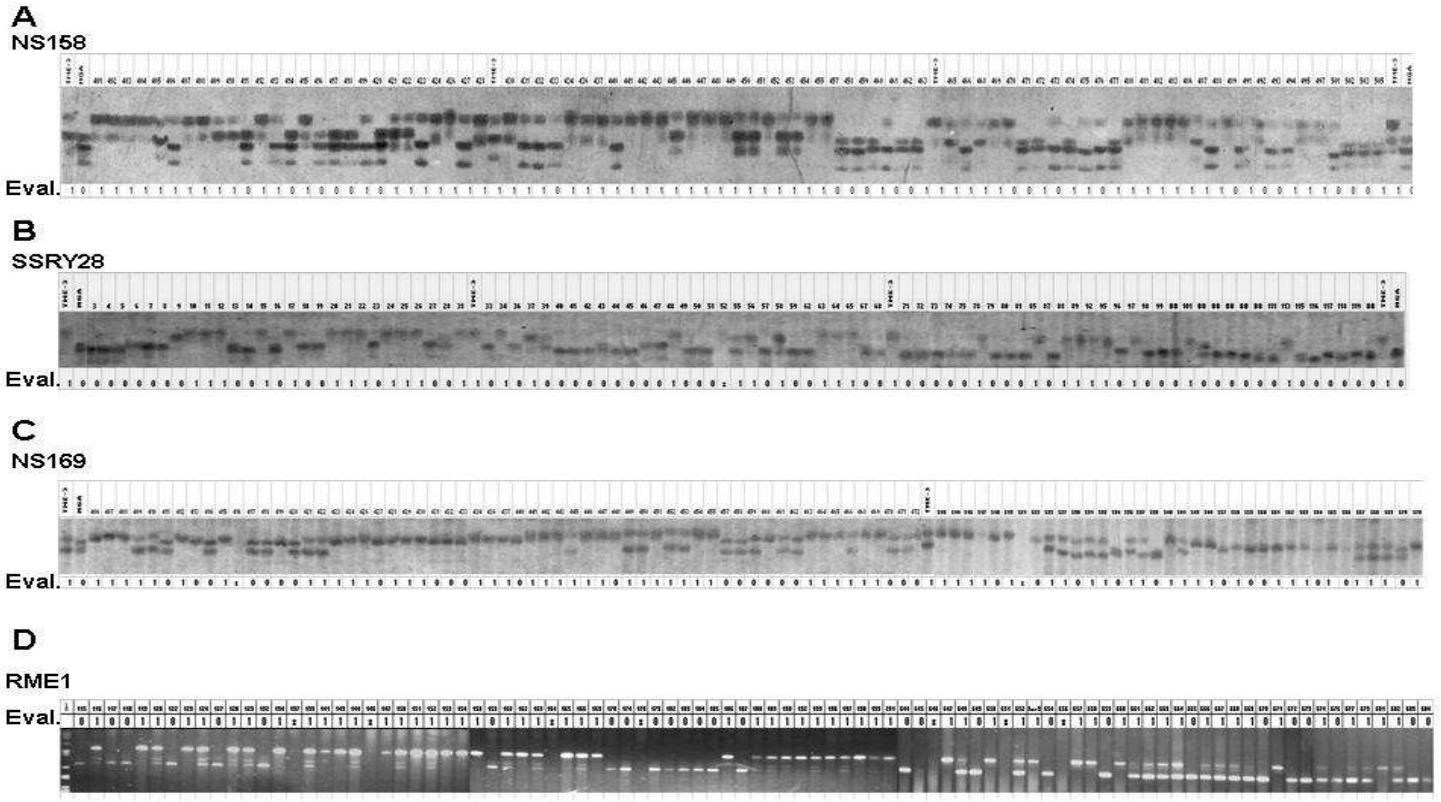

Figure 7.3 Silver stained PAGE (1A, B and C) and agarose stained gel (1D) of PCR amplificatio of Ghana cassava genotypes

Table 7.4 Evaluation of progenies showing values of 1 and 2 in CMD average score using four markers associated with CMD2 and resistance to CMD

Genotype	Father	Mother	Site	AV.SCO	Marker				Genotype	Father	Mother	Site	AV.SCO	Marker			
					SCAR	SSRY 28	NS 158	NS 163						SCAR	SSRY 28	NS 158	NS 163
95	AFEB	CR 52A-25	FUMESUA	1	1	1	1	1	143	DABO	CR 25A-25	FUMESUA	2	1	1	1	1
96	AFEB	CR 52A-25	FUMESUA	1	1	0	1	1	144	DABO	CR 52A-25	FUMESUA	2	1	0	1	0
97	AFEB	CR 52A-25	FUMESUA	2	1	1	1	1	146	DABO	CR 25A-25	FUMESUA	2	0	x	1	0
98	AFEB	CR 52A-25	FUMESUA	1	1	0	1	x	147	DABO	CR 52A-25	FUMESUA	2	1	0	1	0
99	AFEB	CR 52A-25	FUMESUA	1	1	0	1	x	150	DABO	CR 41-10	FUMESUA	2	1	0	1	0
100	AFEB	CR 52A-25	FUMESUA	1	1	0	1	1	151	DABO	CR 41-10	FUMESUA	2	1	x	1	0
101	AFEB	CR 52A-25	FUMESUA	2	0	1	0	1	152	DABO	CR 41-10	FUMESUA	1	1	0	1	0
104	AFEB	CR 52A-25	FUMESUA	1	0	0	0	0	153	DABO	CR 41-10	FUMESUA	1	1	0	1	0
105	AFEB	CR 52A-25	FUMESUA	1	0	0	1	1	154	DABO	CR 41-10	FUMESUA	1	1	0	1	0
106	AFEB	CR 52A-25	FUMESUA	2	1	0	1	1	155	DABO	CR 41-10	FUMESUA	1	0	0	1	0
107	TUAKA	CR 52A-25	FUMESUA	2	0	0	0	0	156	DABO	CR 41-10	FUMESUA	1	1	0	1	1
108	TUAKA	CR 52A-25	FUMESUA	2	0	0	0	0	157	DABO	CR 41-10	FUMESUA	2	0	x	1	1
111	TUAKA	CR 52A-25	FUMESUA	2	1	0	1	1	158	DABO	CR 41-10	FUMESUA	2	1	0	1	1
113	TUAKA	CR 52A-25	FUMESUA	2	0	1	0	0	159	DABO	CR 41-10	FUMESUA	2	0	0	1	1
115	TUAKA	CR 52A-25	FUMESUA	2	0	0	1	1	160	TME11	CR 41-10	FUMESUA	2	1	0	1	1
116	TUAKA	CR 52A-25	FUMESUA	1	1	0	1	1	162	TME11	CR 41-10	FUMESUA	1	1	0	1	1
117	TUAKA	CR 52A-25	FUMESUA	1	0	0	1	1	163	TME11	CR 41-10	FUMESUA	2	1	0	1	1
118	TUAKA	CR 52A-25	FUMESUA	2	0	0	1	1	164	TME11	CR 41-10	FUMESUA	2	0	0	1	1
119	TUAKA	CR 52A-25	FUMESUA	1	1	0	1	1	165	TME11	CR 41-10	FUMESUA	2	1	1	1	1
120	DABO	CR 52A-31	FUMESUA	2	1	0	1	x	166	TME11	CR 41-10	FUMESUA	2	1	0	1	0
122	DABO	CR 52A-31	FUMESUA	1	0	0	0	0	169	TME11	CR 41-10	FUMESUA	2	1	0	0	1
123	DABO	CR 52A-31	FUMESUA	2	1	0	1	1	170	TME11	CR 41-10	FUMESUA	2	0	1	1	1
124	DABO	CR 52A-31	FUMESUA	2	1	0	1	1	174	TME11	CR 41-10	FUMESUA	2	0	1	1	0
127	DABO	CR 52A-31	FUMESUA	1	0	0	1	1	178	TME11	CR 41-10	FUMESUA	2	0	0	0	0
128	DABO	CR 52A-31	FUMESUA	1	1	0	1	0	179	TME11	CR 41-10	FUMESUA	2	0	0	1	0
129	DABO	CR 52A-31	FUMESUA	1	1	1	1	0	182	TME11	CR 41-10	FUMESUA	2	0	0	1	0
132	DABO	CR 52A-31	FUMESUA	2	0	1	1	0	183	TME11	CR 41-10	FUMESUA	2	0	1	1	0
134	DABO	CR 52A-25	FUMESUA	1	1	1	1	1	184	TME11	CR 41-10	FUMESUA	1	0	0	1	1
137	DABO	CR 52A-25	FUMESUA	2	0	0	1	1	185	TME11	AR 14-10	FUMESUA	2	0	0	1	0
139	DABO	CR 25A-25	FUMESUA	2	1	1	1	1	186	TME11	AR 14-10	FUMESUA	1	1	0	0	0
141	DABO	CR 52A-25	FUMESUA	2	1	0	1	1	187	AFEB	CR 41-10	FUMESUA	2	0	1	1	0

Genotype	Father	Mother	Site	AV.SCO		Marker			Genotype	Father	Mother	Site	AV.SCO		Marker		
					SCAR	SSRY 28	NS 158	NS 169						SCAR	SSRY 28	NS 158	NS 169
188	DABO	AR 14-10	FUMESUA	1	1	0	1	0	243	DABO	CR 52A-25	FUMESUA	1	x	1	1	0
189	DABO	AR 14-10	FUMESUA	1	1	1	1	1	244	DABO	CR 52A-25	FUMESUA	1	0	0	1	0
190	DABO	AR 14-10	FUMESUA	1	1	1	1	1	247	DABO	CR 52A-25	FUMESUA	1	0	1	1	0
192	DABO	AR 14-10	FUMESUA	1	1	0	1	1	248	DABO	CR 52A-25	FUMESUA	2	x	0	1	0
193	DABO	AR 14-10	FUMESUA	1	1	0	1	1	250	DABO	CR 52A-25	FUMESUA	2	0	0	1	1
196	DABO	AR 14-10	FUMESUA	1	1	1	1	1	251	DABO	CR 52A-25	FUMESUA	2	0	0	1	0
197	DABO	AR 14-10	FUMESUA	2	1	0	1	0	254	DABO	CR 52A-25	FUMESUA	1	x	1	1	0
198	DABO	AR 14-10	FUMESUA	1	1	1	1	1	255	DABO	CR 52A-25	FUMESUA	1	0	0	1	1
199	DABO	AR 14-10	FUMESUA	1	1	1	1	1	256	DABO	CR 52A-25	FUMESUA	1	x	1	1	1
201	DABO	AR 14-10	FUMESUA	1	1	0	1	1	257	DABO	CR 52A-25	FUMESUA	2	0	0	1	0
202	DABO	AR 14-10	FUMESUA	1	0	0	1	0	258	DABO	CR 52A-25	FUMESUA	2	1	1	1	0
203	DABO	AR 14-10	FUMESUA	1	0	0	1	1	259	DABO	CR 52A-25	FUMESUA	2	1	0	1	0
206	TME11	CR 15-7	FUMESUA	1	0	0	0	0	260	DABO	CR 52A-25	FUMESUA	1	0	1	1	1
207	TME11	CR 15-7	FUMESUA	2	0	0	0	0	261	DABO	CR 52A-25	FUMESUA	1	0	0	1	0
208	TME11	CR 15-7	FUMESUA	1	0	0	1	0	263	TME11	CR 52A-25	FUMESUA	2	1	1	1	0
215	TME11	AR 12-50	FUMESUA	2	0	0	0	0	264	TME11	CR 52A-25	FUMESUA	1	x	1	1	0
216	TME11	AR 12-50	FUMESUA	2	1	0	1	1	265	TME11	CR 52A-25	FUMESUA	2	1	1	1	0
217	TME11	AR 12-50	FUMESUA	2	1	0	1	1	266	TME11	CR 52A-25	FUMESUA	1	0	1	1	1
220	TME11	AR 12-50	FUMESUA	1	0	0	0	0	267	TME11	CR 52A-25	FUMESUA	2	0	1	1	1
222	TME11	AR 12-50	FUMESUA	2	1	0	0	1	268	TME11	CR 52A-25	FUMESUA	1	1	1	1	1
223	TME11	AR 12-50	FUMESUA	1	0	0	1	0	269	TME11	CR 52A-25	FUMESUA	2	0	1	1	1
224	TME11	AR 12-50	FUMESUA	1	0	0	1	0	270	TME11	CR 52A-25	FUMESUA	1	0	1	1	1
225	TUAKA	CR 52A-25	FUMESUA	2	0	0	1	1	271	TME11	CR 52A-25	FUMESUA	2	1	1	1	1
227	TUAKA	CR 52A-25	FUMESUA	1	1	0	1	0	273	TME11	CR 52A-25	FUMESUA	2	1	0	1	1
235	DABO	CR 52A-25	FUMESUA	1	0	0	1	1	274	TME11	CR 52A-25	FUMESUA	2	1	0	1	1
236	DABO	CR 52A-25	FUMESUA	1	x	1	1	1	275	TME11	CR 52A-25	FUMESUA	1	1	1	1	1
237	DABO	CR 52A-25	FUMESUA	1	1	0	1	1	277	TME11	CR 52A-25	FUMESUA	1	0	1	1	1
238	DABO	CR 52A-25	FUMESUA	2	0	0	1	1	278	TME11	CR 52A-25	FUMESUA	2	1	0	1	1
239	DABO	CR 52A-25	FUMESUA	1	0	1	1	1	279	TME11	CR 52A-25	FUMESUA	2	0	1	1	0
240	DABO	CR 52A-25	FUMESUA	2	1	0	1	1	280	TME11	CR 52A-25	FUMESUA	1	1	0	1	0
241	DABO	CR 52A-25	FUMESUA	1	x	0	1	0	282	TME11	CR 52A-25	FUMESUA	1	1	1	1	0

The presence of the CMD2 gene in the CMD resistant parental genotypes requires the presence of an allele associated with CMD2 and the co-segregation with CMD resistance in the progeny. Four markers were used to analyze for the CMD2 gene. The presence of alleles associated with CMD2 in the four markers in a genotype is an indication that the most probable source of CMD resistance is CMD2. When less than four markers (i.e. one to three) show presence of alleles associated with the CMD2 gene, then CMD2 gene resistance cannot be determined with certainty in such genotypes due to recombination. The gel profile of all the markers is given in Figure 7.3.

7.3.3 Marker screening for the CMD2 gene in parental genotypes

Susceptible landraces Afeb and Tuaka

In each of these landraces only one of the four markers had alleles associated with the CMD2 gene. Therefore, CMD2 gene resistance cannot be confirmed in these landraces based on the genotypic data. However, the phenotypic data available suggest that these two landraces are susceptible to the CMD disease and therefore cannot have the dominant CMD2 gene.

Resistant parental genotypes Dabodabo and TME11

In the genotype TME11, three of the four markers revealed alleles associated with the CMD2 gene; however, only one marker had an allele associated with the CMD2 gene in Dabodabo. Since not all alleles of the four markers were found in these two genotypes, (landrace and IITA material), CMD2 resistance cannot be confirmed with certainty in these genotypes. Nonetheless, both landraces were phenotypically classified as resistant. Further study will be needed to confirm the source of resistance to CMD in this parent. Further crosses involving these landraces with a susceptible parent will be required to verify this. If results are consistent with the banding patterns as observed, then CMD2 gene resistance will be inferred. If results are not consistent, then both genotypes may represent a new source of CMD resistance in these genotypes.

CIAT progenitors or parental genotypes

Two CIAT genotypes (CR52A-31 and AR14-10) had all four marker alleles associated with CMD2 indicating that it is the probable source of CMD resistance in these genotypes. Phenotypic data showed no symptoms for the disease in these two genotypes. Each of the other six CIAT lines had one to three marker alleles associated with CMD2 (three marker alleles in AR15-5; two marker alleles in CR52A-4, CR 41-10, CR59-4; and one marker allele in CR52A-25 and AR42-4 (Table 7.3). With the exception of AR42-4 which is susceptible, the other five genotypes were phenotypically resistant to the disease. The five genotypes were developed in CIAT for CMD resistance using the CMD2 gene through MAS and therefore, CMD2 is the probable source of resistance in these genotypes. The phenotypic results confirmed CMD resistance in these five lines. Generally, CIAT has been deploying CMD2 donor parents as the source of resistance in Latin American cassava germplasm and this has remained the main source of CMD resistance in the AR and CR series of cassava genotypes as used in this study Although AR42-4 was developed using the CMD2 donor parent in its pedigree, its susceptibility to CMD indicates that the RME-1 marker allele for the CMD2 gene as found in this genotype from marker analysis was a false positive which could occur through recombination.

7.3.4 F1 progeny disease reaction

The F_1 families showed segregation for the CMD2 gene. Out of the total of 684 genotypes initially planted, phenotypic data indicated that 525 individuals were resistant with 159 individuals being susceptible. The progenies scored with CMD severity of 1 and 2 thus showing resistance were used in the molecular analysis.

Results indicated that 83% of the genotypes showed at least a marker allele for the CMD2 gene (Table 7.4). If CMD2 was responsible for all the resistance observed in the F_1 families, it would imply that the markers associated with the gene was 83% successful in identifying resistant genotypes in the phenotypically selected resistant individuals analyzed with molecular markers. A total of 88 individuals (17%) had the four marker alleles associated with the CMD2 gene. A total of 179 individuals had between one to

three marker alleles each associated with CMD2. A total of 91 individuals had no marker allele associated with the CMD2 gene.

7.4 Discussion

The CIAT genotypes, in addition to other important traits which they possess, such as high dry matter content and yield, also served as donor parent for the CMD2 gene in these crosses. The F_1 families were therefore expected to show segregation for the CMD2 gene. Although 684 individuals were initially planted, phenotypic data indicated that 525 individuals were resistant with 159 individuals being susceptible. The progenies used in the molecular analysis were those selected for CMD resistance based on phenotypic scoring and should be expected to express marker alleles or corresponding bands for the CMD2 gene.

Results indicated that 83% of the genotypes showed at least a marker allele for the CMD2 gene. If CMD2 was responsible for all the resistance observed in the F_1 families, it would imply that the markers associated with the gene was 83% successful in identifying resistant lines in the phenotypically selected resistant individuals analyzed with molecular markers. Generally, CMD2 markers have been found to be 68% effective in MAS, but this is in populations that were not pre-selected phenotypically.

A total of 88 individuals (17%) had the four marker alleles associated with the CMD2 gene. In these individuals, the most probable source of CMD resistance is CMD2. A total of 179 individuals had between one to three marker alleles each associated with CMD2. The results in Table 7.3 showed that the progenitors Dabodabo and TME11 were phenotypically resistant. TME11 appeared consistent with the CMD2 gene resistance (showing bands present for alleles of this gene in three markers). However, Dabodabo was less consistent, showing presence of bands for the corresponding allele for CMD2 gene resistance in only one of the four markers and may represent a new possible source of CMD resistance gene(s). The further use of Dabodabo in crosses with susceptible

CMD parent(s) to test for new CMD resistance gene in this landrace would confirm the observation.

The 179 genotypes with between one and three marker alleles for the CMD2 gene were phenotypically shown to be resistant to the disease. Since they resulted mainly from crosses between CMD2 donor parents (from seven CIAT lines) and landraces (with TME11 and Dabodabo being CMD resistant), resistance in these genotypes may result from the CMD2 source alone or from both the CMD2 source and any other new source of CMD resistance if we assume that the CMD resistant landrace progenitors (Dabodabo and TME11) represent a new source of CMD resistance genes.

A total of 91 individuals had no marker alleles associated with the CMD2 gene. This would imply that either these individuals have resistance different from the CMD2 gene or that they are false resistant genotypes (Table 7.4). The former could apply to individuals which are progenies of Dabodabo and TME11 if CMD resistance in the two genotypes is different from CMD2 resistance. Further study is still required to confirm resistance in these two genotypes. The latter possibility (false resistant genotypes) would be most probable in individuals with no marker allele for CMD2 derived from crosses between CIAT genotypes and the susceptible landraces (Afeb and Tuaka). The expected source of CMD resistance for such individuals should be the CMD2 gene from the CIAT donor parents and since the individuals do not have any allele of the four markers used for CMD2 selection, then CMD2 resistance is most probably not present in these individuals and they may therefore be false resistant genotypes. MAS has been used successfully in common bean and cassava (Fregene et al. 2007) and in cassava for studying CMD resistance (Okogbenin et al. 2007). It should be noted that these materials were phenotypically evaluated in one season. Under high CMD infestation, disease evaluation in one season (one year) is very efficient. Previous studies (Okogbenin et al. 2007) have indicated that under high disease pressure, there is a highly significant correlation in disease expression between one season data and two-season data. However, at locations where disease pressure is not very high, some susceptible individuals may escape and could be misclassified, although this is relatively unusual even in moderate

CMD pressure zones. Disease pressure levels for Fumesua and Ohawu are extremely high as compared to areas like Damongo in the derived savannah zone with very low CMD pressure (Cudjoe pers comm.). Hence, the choice of these two locations was appropriate for classification of CMD reaction. The greatest impact from MAS will be realized when breeding systems use high throughput techniques for large populations for genotyping for multiple target traits. The advantage would be to achieve the same breeding progress in a much shorter time than through conventional breeding alone and from pyramiding genes of several traits that could not be readily combined through other means.

7.5 Conclusions

Only two of the local and IITA progenitors (Dabodabo and TME11) were phenotypically identified as resistant. TME11 had three alleles (RME-1, 158, 169) associated with the CMD2 gene while Dabodabo had one marker allele (SSRY28) associated with the gene. The IITA genotype TME11 has been known to be CMD resistant and has been tested as a CMD donor parent in a back cross in a research study at the Crops CRI. The genotype Dabodabo, a landrace, would need to be evaluated further in new crosses with CMD susceptible parents to determine if there is consistency with CMD2 resistance. Where results indicate that there is no consistency then a new source of CMD resistance genes may be implied in these genotypes and Bulk Segregant Analysis may be used to identify the new gene(s). Selected progenies with resistance and high yields have been identified for yield trials in three agro ecological zones and participatory evaluation with farmers and end-users.

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CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

This study constitutes the first detailed breeding research on cassava landraces in Ghana. It has generated useful information that would serve as relevant guide to plan cassava and other root and tuber crop improvement programmes in Ghana. The experiences, skills developed and knowlegde acquired will be shared among the communities involved in cassava improvement and production where knowledge sharing is of great importance.

The genetic diversity study revealed high overall heterozygosity. The 33 SSR markers used for the analysis detected 63 duplicates or closely related genotypes. A loose genetic structure was observed overall but a unique sub-structure was seen in the landraces from the upper regions of Ghana, the arid and semi-arid areas, thus suggesting possible heterotic pools in the population. The heterotic groupings were distinct for the savannah and the forest ecological zones. The selection of the progenitors was guided by the clusters and agronomic performance of the genotypes.

The phenotypic correlation among diseases and yield and yield components for cassava showed that there was a strong correlation between root number per plant, plant height and height at first branching. There was a strong positive correlation between CMD and CBB in the forest zone, hence CMD evaluation in cassava breeding must be done with molecular tools that allows better precision on detection.

To assess the combining ability for the progenitors selected from the genetic pools and to predict heterosis, two sets of cassava progenitors selected from the landraces were included in a diallel experiment. Selection of the progenitors was based on location or source of parent in the collection, agronomic performance, preference by farmers and the cluster it fell into from the molecular characterization. The progenitors were crossed in a diallel experiment in the forest and the savannah ecological zones of Ghana. The

progenitors and progenies were assessed for disease and yield components for two seasons. The analysis of variance and the GCA:SCA ratios indicated that the GCA was larger than SCA for average root number, CBB and branch levels, indicating the presence of additive gene effects and a possibility for improvement of the characters through selection. The GCA:SCA ratio indicated that the SCA was larger than GCA for harvest index and fresh root yield indicating environmental effects on the traits and it confirms the non additive effects mainly in inheritance of root yield.

The amount of heterosis expressed in some crosses between parents selected from the genetic diversity study showed some good potential. Among the progenitors and progenies for the forest diallel experiment, all crosses showed positive significant heterosis, both MPH and BPH, for fresh yield in the forest second season experiment. Four crosses in the forest ecology study showed resistance to CMD. Dabodabo was identified as a progenitor that combined very well for CMD resistance. Kwasea showed the highest GCA for dry matter. Traits with high GCA:SCA ratio indicate high heritability and additive gene effects, indicating that they will show good response to selection.

Clonal evaluation of selected F1 progenies from the diallel for the forest zone was carried out in three ecological zones and four locations in the 2010 season. Yield performance, disease resistance, and stability were likewise assessed for two seasons for introduced CIAT genotypes on station. Farmers and end-users preferred genotypes for culinary suitability, high yields and high dry matter. At the end of the study CR25-31A, CR25-5 and CR25-4 from the CIAT genotypes were selected as the most preferred genotypes. Each ecological zone preferred particular attributes that guided their choice. CMD has been identified as a major constraint to cassava production in all cassava growing areas. Debor had the best GCA effect for cassava mosaic severity reaction; while the genotype Tuaka had the best GCA effect for fresh root weight. However, the combination of Afebankye x Kwasea had reduced yield. Debor x Kwasea was the best F1 cross for CMD resistance development. Highly heritable traits such as disease resistance can be selected for in routine breeding activities. CIAT elite clones evaluated

on station and with farmers showed that farmers are interested in genotypes that are high yielding and suitable for different types of food.

Participatory breeding at an early stage of the breeding cycle reduces the several years of multilocation evaluation. The clonal evaluation and stability studies for the CIAT genotypes and the F1s revealed that Debor, an ancient landrace, was stable in most of the environments. Afisiafi an IITA improved genotype (TMS 30572) was found to be extremely stable and this is an important finding. This confirms its name in Ghana meaning “everywhere”. It was tested extensively in this study and was found to be extremely stable. The recommendation is to use Afisiafi in crosses to improve its CMD resistance. The stability in the yield performance explains why farmers would treasure varieties such as Debor (meaning “it still yields or ancient treasure”).

MAS has become a routine technique to speed up cassava breeding. CIAT introduced genotypes were used to introgress the CMD2 gene into local material and look for new sources of resistance genes for CMD. F1 progenies developed from crosses between CIAT elite materials and three land races and an IITA genotype were assessed for CMD resistance using four markers linked to the CMD2 gene (SCAR and SSR markers). The landrace Dabodabo was seen as a putative source with CMD resistance not conferred by the CMD2 gene. This putative new source of CMD resistance needs to be confirmed by crossing Dabodabo to CMD susceptible genotypes. Further studies could be carried out to tag the gene and together with the other known sources, the genes could be pyramided into a unique genotype.

From this study progenitors that combine very well for certain traits could be used in crosses to develop mapping populations to look for genes controlling the traits. A systematic national programme of germplasm collection every 10-15 years will help broaden the genetic base.

CHAPTER 9

SUMMARY

Key words: Cassava, diversity, combining ability, heterosis, heritability, clonal, hybridization, crosses, participatory breeding, Ghana

The aim of this study was to assess genetic diversity, combining ability, heterosis, heritability of traits, stability and farmer preference of cassava germplasm in Ghana in order to structure an effective breeding programme. The assessment of genetic diversity in a collection of local cassava genotypes using 33 SSR markers revealed a high overall heterozygosity. The SSR markers identified 63 duplicates in the collection. The heterotic pools were distinct for the savannah and the forest ecological zones and this was the basis for the selection of progenitors for the rest of the study. Seven and five cassava progenitors were crossed in a diallel design without reciprocals in the forest and savannah ecologies respectively. The progenitors and progenies were evaluated for CMD, CBB, yield and its components in two seasons in the forest and one season in the savannah zone. The GCA:SCA ratio indicated that the GCA was larger than SCA for average root number, indicating additive gene effects and a possibility for improvement by selection. SCA was larger than GCA for harvest index and fresh root yield, indicating environmental effects on the trait and confirms the non-additive effects mainly determining expression of root yield. The parental genotypes were selected from a particular region where CMD is the main economic constraint. Debor had the best GCA effect for cassava mosaic severity reaction; while Tuaka had the best GCA effect for fresh root weight. Debor x Kwasea was the best combination for CMD resistance. Debor and Kwasea were the best parents for resistance breeding to CMD. The predictability ratio varied between 0.58 and 0.84. CMD and root number had a ratio close to one for the savannah and forest zones, showing that these traits were highly heritable and influenced by additive gene action. Marker assisted selection was used to search for new sources of CMD resistance in the progenies and progenitors. Crosses among CIAT elite genotypes and landraces revealed progenitor Dabodabo as a putative source of new genes to CMD resistance.

OPSOMMING

Sleutelwoorde: Cassava, diversiteit, kombineervermoë, heterose, oorerflikheid, klone, hibridisasie, kruisings, deelnemende teling, Ghana

Die doel van hierdie studie was om genetiese diversiteit, kombineervermoë, heterose, oorerflikheid van eienskappe, stabiliteit en die boere se voorkeur vir cassava kiemplasma in Ghana te evalueer sodat ‘n effektiewe teelprogram gestruktureer kan word. Die evaluasie vir genetiese diversiteit met 33 SSR merkers in ‘n versameling van plaaslike cassava genotipes het baie heterosigose getoon. Die SSR merkers het 63 duplikate in die versameling uitgewys. Die heterotiese groepe was duidelik verskillend vir die savannah en die woud ekologiese sones en dit was die basis van seleksie van ouers in hierdie studie. Sewe en vyf cassava ouers onderskeidelik is gekruis in ‘n dialleel ontwerp sonder resiproke, in die woud en savannah ekologiese sones. Die ouers en die nageslag is geëvalueer vir CMD, CBB, opbrengs en die opbrengs komponente in twee seisoene in die woud en een seisoen in die savannah sone. Die GCA:SCA verhouding het gewys dat die GCA groter was as SCA vir die aantal wortels, wat additiewe geenaksie wys, dus is verbetering moontlik met seleksie. SCA waardes was hoër as GCA vir oesindeks en vir vars wortel opbrengs wat wys dat daar groot omgewingseffekte op opbrengs is en dat nie-additiewe geeneffekte ‘n groot rol speel. Die ouers is geselekteer uit ‘n spesifieke gebied waar CMD die grootste ekonomiese beperking is. Debor het die beste GCA effekte vir CMD reaksie gehad; terwyl Tuaka die beste GCA effekte vir vars wortel opbrengs gehad het. Debor x Kwasea was die beste kombinasie vir CMD weerstand. Debor en Kwasea was die beste ouers vir CMD weerstandsteling. Die voorspelbaarheidsverhouding het gevarieer tussen 0.58 en 0.84. CMD en aantal wortels het ‘n verhouding van na aan een gehad vir die woud en savannah sones, wat getoon het dat hierdie eienskappe hoogs oorerflik is, en deur additiewe geenaksie bepaal word. Merker ondersteunde teling en soeke na nuwe bronne van CMD weerstand in die ouers en nageslag is gedoen in kruisings tussen CIAT elite genotipes en landrasse. Die ouer Dabodabo is as moontlike bron van nuwe CMD weerstandsgene uitgewys.

APPENDIX Climatic data for experimental sites

Climatic data for Fumesua and Ejura in the forest zone													
Fumesua	rainfall												
Total	Jan	Feb	Mar	April	May	June	July	Aug	sep	Oct	Nov	Dec	Year
1,159.80	111.1	98.4	112.8	66.9	187.3	145.4	66.7	65.2	111.4	158.4	32.5	3.7	2006
1,794.40	0.2	16.4	56.2	310.9	164.2	176	192.9	117.7	534.5	153.9	51.7	19.8	2007
1,452.00	0	53.7	97.4	132	239.6	286.7	131.1	192.6	170.7	75.1	18.3	54.8	2008
1,530.70	TR	131.4	110.6	139.8	164.6	376.7	273.5	17.6	99.3	138.6	45.2	33.4	2009
1,392.40	4.2	56.7	41.3	129.4	132.6	203.3	166.8	134.9	201.8	163.3	111.1	47	2010
Ejura	rainfall												
1470.7	32.7	23.9	66.4	54.8	383.7	167.9	79.4	8.7	251.9	366.8	34.5	0	2006
1487.5	3.9	0	91.7	209.8	188	155.9	165	23.1	311.8	237.7	47.6	53	2007
1287.8	0	2.4	136.2	138.1	201.5	248.1	159.2	77.9	205.9	94.4	13.2	10.9	2008
1319	0	48.6	102.4	162.7	102.6	289.4	235.6	60	78.7	128.9	97.2	12.9	2009
1572	0	38.2	61.5	127.6	222.5	166.1	161.3	248.3	198.2	221.3	127	0	2010
Max and min temp for Fumesua													
Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Year	
Min 22.6	23.1	22.5	23.3	22.9	22.5	21.8	21.3	31	21.5	21.8	20.7	2006	
19.9	22.5	23.5	21.7	22.2	22.6	22.2	22	22.1	21.9	22.2	22.2	2007	
19.2	21.9	22.6	22.9	22.8	22.5	22.3	22	22.3	22.6	23.4	23	2008	
21.8	23.2	23	22.9	22.9	22.1	21.5	21.9	22.1	22.1	22.2	23.1	2009	
22.7	23.3	23.4	23.4	23.4	22.8	21.5	21.8	21.9	22	22.5	22.1	2010	
32.1	32.6	32.5	33.3	31.5	31	29.5	29	29.8	31.4	32.6	33	2006	
33.8	34.5	35	32.9	32.1	30.7	29.4	28.9	29.7	31.1	31.8	32.1	2007	
32.2	35.1	34.2	32.6	32.1	30.6	29.3	29	29.8	31.5	32.8	30.5	2008	
33.4	33.6	33.4	32.6	32.3	30.7	29.1	28	29.8	30.9	32	32.7	2009	
33.4	35.5	34.5	32.4	33.1	31.2	29.7	29	29.7	31	32.5	32.4	2010	
20.9	23.5	22.3	23.3	22.9	22.5	22	21.9	20.8	23.3	23.5	24	2006	
19.5	23	23.9	23.1	23.5	22.7	22.3	22.3	22.1	22.2	21.2	22.6	2007	
17.2	22.4	23.8	23.1	23	22.7	22.5	22.4	22.7	22.6	23.1	22.7	2008	
20.7	23.7	23.9	23.6	23.8	22.6	22.1	22.9	22.8	23	22	22.8	2009	
22.7	24.3	24.9	24.5	24.4	23.8	22.5	22.6	23.1	24.5	23.4	22.4	2010	
Max 34.8	36.5	36	35.6	33.5	32.6	31.9	30	30.6	32	33.3	34.8	2006	
34.6	36	36.5	34.5	33.9	32.3	31.8	30.8	31.4	32.3	32.7	33.6	2007	
33.2	36	36	34.6	33.9	32.4	30.8	30.9	31.4	32.4	33.7	34.4	2008	
35	36.1	35.4	35.1	34.4	32.8	31.1	30.3	31.6	32.4	32.8	33.7	2009	
35.6	36.7	36	35.7	33.9	32.1	30.8	30.4	30.5	32	33.1	34	2010	

Climatic data for Pokuase								
Pokuase								
Month	2007	2008	2009	2010				
Jan	27.4	0	0	39.2				
Feb	45.6	0	5	14.7				
March	50.1	48	91.6	105.9				
April	81.3	105.5	127.7	67.5				
May	189.1	54.6	256.9	90.9				
June	81.6	103	122.2	204.7				
July	45.5	101.5	88.3	67.5				
August	9.9	97.2	115.7	44.3				
Sep	126.2	58.6	20	12.6				
Oct	89	160.2	44.9	40.3				
Nov	85.9	47.8	52.2	87.8				
Dec	61	17	42.1	26.7				
Total	892.6	793.4	966.6	802.1				
Max	and	min	Temp.	Pokuase				
	2007		2008		2009		2010	
Month	Max	min	Max	Min	Max	Min	Max	Min
Jan	33.1	23.4	33.9	19.2	34.4	17.6	33.5	21.5
Feb	34.1	23.3	34	23.7	34.8	22.7	33.8	23.6
March	33.8	23.1	34.2	23.7	34.3	23.2	34.2	23.7
April	34.3	23.7	33.7	23.8	33.8	23.1	33.2	23.6
May	32.5	23.1	32.9	23.6	33	22.9	33	23.3
June	32.3	23	31.8	23.6	32.8	23.3	30.6	23.5
July	31.5	23.4	29.7	23	32.3	23.1	28.7	23
August	29.6	23.1	31	23.1	32.1	22.8	28.8	23.2
Sep	30.4	23.3	32	23.2	31.5	23.5	30.6	23.6
Oct	32	23.5	32.5	22.8	32.8	23.3	32.6	23.7
Nov	33.3	23.3	32.7	22.6	32.7	22.9	32.9	22.9
Dec	33.5	22.7	33.7	22.7	33.2	23.2	33.6	23.1

Climatic data for Ohawu				
Ohawu				
Month	2007	2008	2009	2010
Jan	0	14.3	0	37.3
Feb	2.8	2.4	31.6	35.1
March	50	64.8	28.6	109.3
April	189.7	58.7	131.7	118.3
May	101.7	191.6	144.6	56.5
June	178.3	161.9	305.8	130
July	165.4	36.7	35.9	15.2
August	81.7	56.4	34.6	71.1
Sep	0	114.4	19.4	98
Oct	0	137.9	71.4	158.3
Nov	80	54.9	7.3	78.7
Dec	65.9	14.4	4.8	10.8
Total	915.5	908.4	815.7	918.6

Max	and	min	Temp.	Ohawu				
	2007		2008		2009		2010	
Month	Max	min	Max	Min	Max	Min	Max	Min
Jan	33.7	23.4	33.9	19.8	34.7	19.6	33.9	22.7
Feb	34.9	23.3	34.5	23.9	34.8	21.7	34.8	22.9
March	33.0	23.1	34.6	23.7	34.5	23.4	34.5	23.9
April	34.7	23.7	34.7	23.8	33.8	22.4	33.9	23.8
May	32.9	23.1	33.9	23.7	33.5	22.7	34	23.7
June	32.6	23	33.8	23.8	32.9	23.5	31.6	23.5
July	30.5	23.4	31.7	23.3	32.4	22.3	29.7	23.1
August	29.6	23.1	32.8	23.1	32.2	22.8	29.8	22.9
Sep	31.4	23.3	33.4	23.5	33.5	23.5	31.9	23.5
Oct	32.5	23.5	33.5	21.8	34.8	23.3	33.7	23.8
Nov	33.7	23.3	34.7	22.7	33.7	22.9	32.9	21.9
Dec	33.5	22.7	33.8	22.9	33.8	23.2	34.7	23.3