BREEDING POTENTIAL OF CASSAVA (*Manihot esculenta* Crantz) IN MOZAMBIQUE

By

Anabela Matangue Zacarias da Silva

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BLOEMFONTEIN

SOUTH AFRICA

Supervisor: Prof. Maryke T. Labuschagne

Co-supervisors: Dr. Elizma Koen

Dr. Edward Eneah Kanju

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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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Anabela Matangue Zacarias da Silva Date

DEDICATION

This work is dedicated to my mother Laurinda Veve Matangue and my late father Zacarias Mambirice, my husband Isidoro Pedro da Silva, my children Junior and Linelle, for the harsh times they have gone through in the course of my studies. My parents brought me up with encouragement to learn. My beloved father passed away while I was preparing to go to the UFS for my MSc studies. May his soul rest in peace.

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CHAPTER 1

GENERAL INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is native to tropical America. It is a major source of energy for more than 500 million people in tropical countries of Africa, Asia and the Americas. Its roots are efficient in carbohydrate production and they constitute the major source of dietary energy (Cock, 1985). It is grown by poor resource farmers, many of them women, as main source for food security and income generation (FAO, 2002). Is adapted to a wide range of environments and is tolerant to drought and acidic soils (Jones, 1959; Kawano *et al.*, 1978), resistant to herbivores and well suited to African farming (Nweke *et al.*, 2002). So far, increases in cassava production in Africa are believed to be reflected by an increased area under cultivation (Hillocks and Thresh, 2001). The high yield potential makes it a viable alternative crop to grains where population pressure has led to tradeoffs between food quality and quantity (Benesi, 2005). Cassava is also classified as classical food security crop with its ability to store the harvestable portion underground until needed (DeVries and Toenniessen, 2001).

All parts of cassava plants are used, but the most common product is the starchy roots. They are prepared in a wide range of forms in different parts of Africa, as fresh or dried chips and pounded (Nweke, 1994; DeVries and Toenniessen, 2001). The leaves are an important vegetable rich in protein, minerals and vitamins (Nweke, 1994; Fregene *et al.*, 2000; IITA, 2001; Benesi, 2005), with excellent nutritional quality for animal and human consumption (Ceballos *et al.,* 2004); and the stem cuttings are commercially used as planting material (Alves, 2002). Traditional farmers have adopted mixed crop systems for generations. It allows the reduction of risk of crop failure and harvesting products at different times during the year (Kizito, 2006).

In Mozambique, cassava is the most important root crop. It is cultivated throughout the country (Zacarias, 1997; FAO/MIC, 2007) and farmers intercrop cassava with other staple food crops. The quantities of cassava produced annually surpass maize in terms of total provision of calories and in market value. The smallholder farmers

contribute 99% of the national production. Presently, cassava accounts for 50% of the agricultural national value of production in the small and medium farm sector and it contributes with 55% of the potential to alleviate income poverty in the smallholder sector (FAO/MIC, 2007).

Cassava breeding began recently compared to other food crops. Breeding results in Africa so far, are reflected in the development of a range of elite clones with resistance to main biotic stresses, such as cassava mosaic disease (CMD) and cassava bacterial blight (CBB) combined with high, stable yield and with other agronomic and consumer quality traits at acceptable levels. Information regarding the inheritance of agronomic traits is scarce (Easwari Amma and Sheela, 1995; Pérez *et al.,* 2005; Cach *et al*., 2005). The knowledge of relative importance of additive and non-additive gene action is limited (Pérez *et al.,* 2005).

Farmers have taken the advantage of cassava vegetative propagation and its hybridisation *in nature* to develop new varieties for thousands of years. As a result, cassava landraces play an important role compared to other crops (Ceballos *et al.,* 2004). Adoption rates of improved technology have been slow, because the end-users, are not ready to accept them as they do not address their preferences and requirements (Nweke, 1994; Benesi, 2005).

Genetic diversity studies using molecular markers (random amplified fragment lenght polymorfism or RAPD), showed that the Mozambican cassava germplasm has wide genetic diversity (Zacarias *et al.,* 2004). The use of morphological and molecular markers combined with diallel analysis and heterotic groups will increase the efficience of development of a strategic breeding programme.

Cassava faces new chalenges in the country. New improved varieties are urgently needed to respond to the demands of food security and emerging and diversified markets. The sucess of a breeding programme relies mainly on the knowledge of the available germplasm, especially genetic diversity (Meredith and Bridge, 1984).

This study, therefore, represents the first comprehensive genetic study based on local cultivars of Mozambique. The genotypes involved represent genebank accessions and progeny obtained in the first batch of crosses conducted by the national programme. The main objectives of this study were:

1. To use diallel crosses to study the importance of combining ability, heterosis, correlations and heritability of the most important cassava traits.
2. To use the AFLP technique to study the genetic distance of 17 different cassava assessions, which represented the first set of parents in our breeding programme.
3. To compare genetic similarities and dendrograms produced from morphological and molecular markers and determine the relatedness between studied varieties.
4. To establish an efficient procedure to screen segregating progeny under cassava brown streak disease pressure.

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CHAPTER 2

LITERATURE REVIEW

2.1 The importance of cassava

Cassava (*Manihot esculenta* Crantz) is native to tropical America. It is a major source of energy for more than 500 million people in tropical countries of Africa, Asia and Latin America (Cock, 1985) and constitutes the most important tropical root crop (Onwuene, 1978; Roa *et al.*, 1997; Mkumbira, 2002). Onwuene (1978) and Cock (1985) reported that cassava roots are efficient in carbohydrate production and they constitute the major source of dietary energy. The tuber root contains nearly the highest starch content among root and tuber crops (Moorthy, 1994).

Cassava is adapted to a wide range of environments and is tolerant to drought and to acidic soils (Jones, 1959; Kawano *et al.*, 1978), to herbivores and well suited to African farming (Nweke *et al.*, 2002). In Sub-Saharan Africa (SSA) it is grown exclusively as food in 39 African countries, stretching through a wide belt from Madagascar in the south-east to Senegal in the north-west (Raji *et al.*, 2001a; Benesi, 2005). An increase in cassava production in Africa has been reported. Hillocks (2002) believe that most of the increase in cassava production has been due to an increase in area under cultivation rather than increases in yield per hectare. Cassava has high yield potential, which makes it a viable alternative crop to grains where population pressure has led to tradeoffs between food quality and quantity (Benesi, 2005), in addition, the ability to store the harvestable portion underground until needed, makes it a classic food security crop (DeVries and Toenniessen, 2001). All parts of the cassava plant are used. The roots are prepared in a wide range of forms in different parts of Africa, as fresh or dried and pounded (Nweke, 1994; DeVries and Toenniessen, 2001). The leaves are an important vegetable, rich in protein, minerals and vitamins (Jones, 1957; Onwuene, 1978; Nweke, 1994; Fregene *et al.*, 2000; IITA, 2001; Benesi *et al.*, 2001, Benesi, 2005), and the stem cuttings as commercial planting material (Alves, 2002).

More recently, cassava has been used increasingly in industry. In Africa, cassava is likely at the beginning stage of major use as raw material in textiles, as binding agent, animal feed and partial substitution for wheat flour in the food industry. With increased demand, it becomes an important source of cash income to a large number of small farmers, consequently saving foreign exchange for nationals (Benesi, 2005). Opportunities for product and market diversification are excellent in several countries, such as Nigeria, Uganda, Malawi, and recently in South Africa (CGIAR Research, 2001; Benesi, 2005).

Economic importance in Mozambique

Mozambique ranks as the fifth largest cassava producer in Africa (FAO, 2006) with average yield estimated at 10.5 ton/ha (Andrade and Naico, 2003; FAO/MIC, 2007). Cassava and maize are the most important staple food crops in the country, while cassava counts as the number one root crop (Zacarias, 1997; Walker *et al*., 2006; FAO/MIC, 2007). It is cultivated throughout the country (Zacarias, 1997; Zacarias and Cuambe, 2004; FAO/MIC, 2007) (Figure 2.1), but cassava production is concentrated in four provinces, namely, Cabo Delgado, Nampula, Zambezia and Inhambane. These provinces contribute about 93% of the national production (FAO/MIC, 2007). In areas prone to drought and floods, cassava is the main crop (IIAM, 2006). The quantities of cassava produced yearly surpass maize in terms of total provision of calories and in market value (FAO/MIC, 2007). Recent studies indicated that cassava accounts for 50% of the agricultural national value of production in the small and medium farm sector and it contributes 55% of the potential to alleviate income poverty in the smallholder sector (Walker *et al*., 2006).

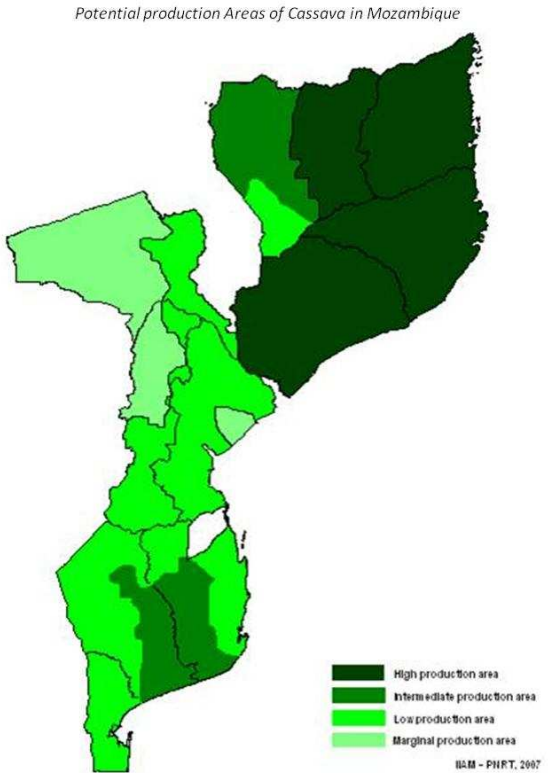

Figure1. Map of Mozambique representing cassava main production areas. Source: IIAM-PNRT, 2007. Adapted from SNAPS-MINAG and INIA-DTA database

2.2 Taxonomy

Cassava (*Manihot esculenta* Crantz), is a member of family Euphorbiaceae (Rogers and Appan, 1973; Onwuene, 1978). This family is characterised by latex production (Hershey, 2005). Rogers and Appan (1973) recognised 98 species that belong to the genus *Manihot,* and cassava is the only species that is widely cultivated for food production (Rogers and Appan, 1973; Onwuene, 1978; Mkumbira, 2002; Nassar, 2005). The cultivated specie may be derived from the wild progenitor *M. flabellifolia* (Fregene *et al.*, 1994; Roa *et al.*, 1997).

The *Manihot* species have 2n=36 chromosomes (Jennings, 1976). Nassar (2002) reported that *Manihot* species behave meiotically as diploids. Studies conducted on the pachytene on *M.glaziovii* and comparison with karyology of cassava, suggested that the species is probably a segmental allotetraploid (Magoon *et al.*, 1969;

Krishnam *et al.*, 1970) derived from a combination of two diploid taxa whose haploid complement has six common and three different chromosomes (Jennings, 1976; Magoo *et al.*, 1969). Inheritance of several isoenzymes supports this evidence and indicated disomic heredity confirming diploid behaviour (Jennings and Hershey, 1985; Hussein *et al.,* 1987; Lefevre and Charrier, 1993). On-going research towards the development of a molecular linkage map is likely to provide better structural definition of the cassava genome (Fregene *et al.,* 1997).

2.3 Morphology and growth habit

Cassava is a perennial woody shrub that generally grows from one to three meters in height (Onwene, 1978; Hershey, 2005). Although in agriculture, farmers usually harvest it during the first or second year (Onwene, 1978).

The plant

Cassava has two types of growth habit, erect with or without branches on the top, and the spreading type (Alves, 2002). Plants with branches higher than 1 m are preferred by farmers. The plant is propagated either vegetatively or by sexual seeds. Commercial plantings are often by stem cuttings, while the seeds are important for the breeding programmes in the first cycle (Onwuene, 1978; IITA, 1990; Nassar, 2005; Benesi, 2005). Cassava seeds have a dormancy period that can be shortened by filing the micropylar end until the white embryo is just visible, or a wet treatment. Both methods have been reported to improve seed germination (Onwuene, 1978). Propagation of cassava through true seeds (sexual seeds) is a promising technique (Rajendran *et al.*, 2005), but the seedlings genetically segregate into different types (Osiru *et al.*, 1996) which constitutes a major drawback in sexual propagation. Plants originating from seeds are likely to be weak. They are homozygous for recessive and prejudicial genes, conferring a distinct competitive disadvantage to plants originating from cuttings that have a genetic heterozygous structure (Hershey, 2005; Nassar, 2005). When cuttings are planted in moist soils under favourable conditions, they produce sprouts and roots within weeks (Osiru *et al.*, 1996). Upon sprouting, one or more axilar buds on the stem piece develop and form, in sequence, nodal units consisting of the node, bud, palmate leaf blade subtended by a long petiole, and

internodes whose length and mass depend on the genotype, age of the plant and environment (Hershey, 2005). The shoot lengthens and the roots extend downwards and spread. The shoot shows marked apical dominance and new leaves are produced in sequence of the main stem.

Flowering

Flowering may begin as early as six weeks after planting, although the exact time of flowering depends on the cultivar and the environment (Jennings and Iglesias, 2002; Hershey, 2005). Cassava flowers are monoecious and predominantly out crossing (Fregene *et al.*, 1997). The flowering is controlled by complex interaction of a range of genetic and environmental factors. In some areas, cassava will flower abundantly all year round, while in other locations, flowering is seasonal (Alves, 2002) or shy. Flowers are regular in some varieties and rare to non-existing in others (Onwene, 1978; IITA, 1990). Flower availability is influenced by plant habit and is generally formed in the insertion point of the reproductive branching (Jennings and Iglesias, 2002; Hershey, 2005).

Leaves and roots

When the first leaf appears, photosynthesis starts, contributing positively to all plant parts, including storage roots (Cours, 1951; Simwambana, 1998). The maximum size of the leaves is observed at four to five months, depending on the planting time (Osiru *et al.*, 1996). It starts with the initiation of secondary roots, a process observed three weeks after planting (Veltkamp, 1986; IITA, 1990; Hershey, 2005). Six weeks after planting, the roots are differentiated and some start thickening rapidly. Starch deposition in the roots begins when the supply of photosynthesis exceeds the requirement of growth of stems and leaves (Cock *et al.*, 1979; Tan and Cock, 1979). The tuberous roots continue to increase in size by swelling due to the deposition of large amounts of starch within the tuberous root tissues. The root harvesting must be delayed until an appreciable amount of starch has accumulated (Onwuene, 1978). The exact time in terms of months after planting, when it is best to harvest cassava, depends on the cultivar. It varies from seven months after planting (MAP) to 18 MAP (Onwuene, 1978). As a result, the starch content of cassava tuberous roots depends on many factors such as variety, soil type and climate, in addition to the age of the plant (Corbishley and Miller, 1984).

2.4 Growth conditions and cropping system

Cassava is often grown in a wide range of ecologies. It is produced on a range of edafic and climatic conditions, between 30^{o}N and 30^{o}S latitude and in regions from sea level to 230 m altitude. It is produced under low input and output production, particularly when grown as food crop. It is also tolerant to low fertility, and pests. Most areas under cassava production are considered marginal for other crops (Alves, 2002). All these attributes place cassava in an important position in traditional tropical cropping systems, particularly to the small scale and subsistence farmers. In this system, cassava is usually found intercropped with a variety of other crops, with long or short cycles and food or cash crops (Ramannujan *et al.*, 1984; Cock, 1985; Alves, 2002). In Africa and the Americas, cassava is commonly intercropped with grains and legumes (Mutsaers *et al.,* 1993; Alves, 2002). Cock (1985) estimated that at least one third of cassava grown worldwide is intercropped by minimizing the risk of crop failure. In Mozambique, in general, farmers intercrop cassava with other staple food crops (INIA/IITA, 2003; Zacarias and Cuambe, 2004). Although, within the main cassava production region, such as the Zambezia province, it is estimated that 41.1% of farmers cultivate cassava as sole crop (Zacarias and Cuambe, 2004). Traditional farmers have adopted mixed crops for generations, where this production system allows reducing the risk of crop failure and harvesting products at different times during the year (Kizito, 2006). This also gives the opportunity to use available land and labour resources and provides the household with a balanced food diet.

2.5 Genetic diversity

Genetic variability and genetic diversity of a taxon is of great importance for plant geneticists, breeders and taxonomists (Prince *et al.*, 1995). In populations, the genetic composition and genetic diversity are derived from wild progenitors and it has been influenced by evolutionary processes such as mutation, recombination, genetic drift, migration, natural selection (Hartl and Clark, 1997) and adaptation to different environments. Frankel *et al.* (1995) defined genetic diversity as the product

of interplay of biotic factors, physical environment, artificial and plant characters such as size, mating system, mutation, migration and dispersal.

In general, the knowledge of genetic diversity and relationship among sets of germplasm as well as the potential merit would be beneficial to all phases of crop improvement (Lee, 1995; Geleta, 2003). Evaluation of genetic diversity among adapted or elite germplasm provides the estimation of genetic variation among segregating progeny for pure line development (Manjarrez-Sandoval *et al.*, 1997) and the degree of heterosis in the progeny of certain parental combinations (Barbosa-Neto *et al.*, 1997; Cox and Murphy, 1990; Geleta, 2003). The information about genetic diversity in available germplasm is important for the optimal design of a breeding programme (Geleta, 2003) and the notion of genetic relationships among lines, population or species has been an important tool for effective management of genetic diversity in a given gene pool (Manjarrez-Sandoval *et al.*, 1997). The study of genetic diversity has been of interest to plant breeders and germplasm curators. It is a process where variation among individuals or groups of individuals is analyzed using specific methods of combination (Mohammadi and Prasana, 2003). In plant species, it can assist in the evolution of germplasm as possible sources of genes that can improve the performance of cultivars (Yang *et al.*, 1996; Geleta, 2003). More recently, breeding efforts started to also contribute to the generation of genetic variability (H. Ceballos – personal communication).

2.5.1 Genetic distance

Genetic distance is the extent of the gene differences between cultivars, as measured by allele frequencies at a sample of loci (Nei, 1987) while the genetic relationship among individuals and populations can be measured by similarity of any number of quantitative characters (Souza and Sorells, 1991). Genetic distance measures are indicators of relatedness among populations or species and are useful for reconstructing the historic and phylogenetic relationships among such groups.

Genetic distance has been measured using two approaches, the parsimony analysis and the cluster analysis, and they represent the phylogenetic and genetic relationship, respectively. The data used in this analysis involve numerical or a

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

2.5.2 Genetic diversity of cassava

The cassava gene pool ranges from a great variety of wild species to numerous domesticated species with very specific characteristics. The methods used to investigate the origin and variability of cassava comprises the taxonomic species concept, the biological species concept, biosystematics and quantitative molecular genetics. Genetic diversity can be revealed by a number of methods including pedigree data, morphological data, agronomic performance, biochemical data and recently molecular (DNA-based) data (Mohammadi and Prasanna, 2003). The DNA-based molecular markers reveal polymorphisms at a 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, 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) and give rise to a higher number of polymorphisms (Karp *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). DNA markers have been successfully used in cassava and contributed to cassava breeding and genetics in understanding the phylogenetic relationship in the genus (Fregene *et al.*, 1994; Roa *et al.*, 1997; Olsen and Schaal, 1999), assessing the genetic diversity (Beeching *et al.*, 1993; Second *et al.*, 1997; Mkumbira *et al.*, 2003; Elias *et al.*, 2000; 2001; Kizito *et al.*, 2006), helping with the development of genetic maps and identification of quantitative loci (QTL) for some traits of importance

(Fregene *et al.*, 1997; Jorge *et al.*, 2001; Okogbenin and Fregene, 2002; Lokko *et al.*, 2005).

2.6 Marker techniques

2.6 1 Morphological characterisation

Traditional identification of plants is based on morphological traits recorded in the field during plant growth. It has been used as a powerful tool in the classification of genotypes and to study taxonomic status. Certification of new varieties is based on the genetic purity of a particular crop. However, these assessments depend on botanical traits (Stegemann, 1984). Most of them are controlled by multiple genes and are subject to varying degrees of environmental modifications and interactions, hence are ambiguous and have limited use for cultivar identification. The morphological traits have higher heritabilities than the agronomic ones and they are the basic descriptors recommended for gene bank characterization. Mathura *et al.* (1986) observed that phenotypic variance in cassava was higher than genotypic variance for traits of agronomic importance, like tuberous root weight. Many of these traits are difficult to analyze because they do not have the simple genetic control assumed by genetic models (Liu and Furnier, 1993) and are of very little use (Tanksley *et al.*, 1989).

Morphological characterisation has been used for various purposes including identification of duplicates, studies of genetic variation patterns, and correlation with characteristics of agronomic importance. These evaluations of genetic relationships among germplasm using morphological traits are lengthy and costly (Cock, 1992; Patterson and Weatherup, 1984) and vulnerable to environmental conditions (CIAT, 1993). They must also be assessed during the fixed vegetative phase of the crop development.

The *Manihot* species have traditionally been classified using morphological characters (Hershey and Ocampo, 1989; Elias *et al.*, 2001; Zacarias and Cuambe, 2004). Due to the influence of different ecological environments on cassava morphology, morphological classification based on variable traits is difficult. These

traits include: hairiness of unexpanded apical leaves, colours of unexpanded apical leaves, mature leaf colour, leaf vein colour, flowers and seeds, leaf shape and size, mature stem colour, tip shoot colour, petioles length and colour, phyllotaxi, flowering, leaf lobe shape, number of leaf lobes, petiole colour, plant and first branch height, and root peduncle lengths, root shape, root surface, inner root skin, root pulp and root position (Gulick *et al.*; 1983; Zacarias, 1997; Benesi, 2002; Alves, 2002; Nassar, 2005). Extensive diversity exists for most cassava traits examined so far. They are grouped as either variable (polygenic) or constant (monogenic). The variable characters are associated with large genotype by environment interaction. Wanyera *et al.* (1992), Efisue (1993) and Simwanbana *et al.* (1996), have used morphological descriptors in cassava to access diversity among the *Manihot* species and within populations. They suggested that the characterisation of cassava accessions should be based on descriptors that are less influenced by the environment.

Studies using phenotypic markers have been useful to demonstrate 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). On the other hand, phenotypic variance in cassava is higher than genotypic variance for traits of agronomic importance like tuberous root weight (Mathura *et al.*, 1986). The picking of cassava leaves for use as vegetable causes morphological changes of the cassava plant (Onwuene, 1978; Simwambana *et al.*, 1996). On the other hand, studies on the phylogeny of *Manihot* conducted by Bertram (1993) observed a high degree of homoplasy in many morphological characters. Therefore, based on several reports it is imperative to employ a better approach to resolve the issues of duplication and genetic diversity.

Phenotypic markers are still playing an important role in conventional plant breeding as well as in identification of specific markers and quantitative trait loci (QTLs) using molecular markers (Fregene *et al.*, 2000; Akano *et al.*, 2002; Mkumbira, 2002).

2.6.2 Isozymes

Isozymes are protein markers based on use of naturally occurring enzymes that share a common substrate but differ in electrophoretic mobility. Isozymes were among the earliest markers used for plant analysis (Brewbaker *et al.*, 1968; Mäkinen and Brewbaker, 1976). Isozymes have been useful tools for genetic fingerprinting and studies of genetic diversity in cassava (Hussein *et al.*, 1987; Ramirez *et al.*, 1987; Ocampo *et al.*, 1992; Lefevre and Charrier, 1993). Isozymes have been used to complement morphological descriptors for the identification of duplicates in the collection at CIAT (Ocampo *et al.*, 1995). However, isozymes are difficult to work with due to a limited amount of polymorphism and 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). However, isozymes have been successfully applied in cassava breeding and genetics (Ojulong, 2007).

2.6.3 DNA markers

DNA markers have been widely adopted for genetic improvement of food crops. Several DNA based markers that reveal polymorphism at DNA level (Kumar *et al.*, 2000) have been developed for measuring genetic similarity in agricultural crops. It has 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). The DNA markers are distinguished in two types (Karp *et al.*, 1996), firstly, those that rely on hybridisation between probe and homologous DNA segments within the genome, RFLP, and secondly, those that are polymerase chain reaction (PCR) based (Mulis *et al.*, 1986). PCR based molecular markers are most commonly used (Taylor, 1991). They lead to an introduction of several new techniques for genome analysis based on selective amplification of DNA fragments. The potential application of molecular markers in plant breeding have been in fingerprinting of genotypes for plant variety identification and protection and in the assessing of genetic similarity among parents for prediction of quantitative-genetic parameters such as heterosis or progeny variance (Bohn *et al.*, 1999). PCR based methods include: random amplified polymorphism (RAPD), mplified fragment

length polymorphism (AFLP), simple sequence repeat (SSR), expressed sequence tags (EST) and their derivatives.

2.6.3.1 Restriction fragment length polymorphism (RLFP)

RFLP was developed in the 1980's to overcome the problems encountered with isozymes and phenotypic markers (Botstein *et al.*, 1980; Helentjaris *et al.*, 1986). 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. RFLPs were superior to isozymes and phenotypic markers since they 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 more detectable loci and alleles, 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.*, 1988; 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).

RFLP has been used particularly in mapping species that display a high level of interspecific variation. Several maps have been reported in different crops such as, maize (Burr *et al.*, 1983; Helentjaris *et al.*, 1986; Gardiner *et al.*, 1993), barley (Garmer *et al.*, 1993), sorghum (Xu *et al.*, 1994), sunflower (Berry *et al.*, 1995), rice (McCouch *et al.*, 1988) and wheat (Chao *et al.*, 1989). A preliminary linkage map of cassava was drawn from F1 segregation data of a single dose of polymorphisms of RFLP and random amplified polymorphic DNA (RAPD) markers. The map comprised of 200 loci corresponding to genomic clones selected from PstI, HindIII and EcoRI random genetic libraries (RFLP markers; Fregene *et al.*, 1994; 1995; 1997). RFLP has also 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 at least 30 probes or primers should be used to achieve these relationships. RFLPs have been applied in studies of phylogenetic relationships of species within the genus *Manihot* (Haysom *et al.*, 1994).

2.6.3.2 Random amplified polymorphic DNA (RAPD)

Random amplified polymorphism (RAPD) analysis is done by the use of single short oligonucleotide primers that can frequently recognises similar sequences that are opposed to each other at distances close enough for the intervening sequence to be amplified by PCR. Single short random primers are allowed to anneal at a relatively low temperature, priming amplification of DNA fragments distributed at random in the genome (Williams *et al.*, 1990). Amplification products are visualised by separation on an agarose gel and stained with ethidium bromide (Williams *et al.*, 1990; Whitkus *et al.*, 1992).

RAPD analysis has been used for identification purposes in many crops including maize (Stojsin *et al.*, 1996), potato (Hosaka *et al.*, 1994; Demeke *et al.*, 1996; Sosinski and Douches, 1996; Milbourne *et al.*, 1997; McGregor *et al.*, 2000), soybean (Maughan *et al.*, 1996), Brassica species (Lanner-Herrera *et al.*, 1997; Lazaro and Aquinagalde, 1998; Geraci *et al.*, 2001), and red pines (De Verno and Mosseler, 1997).

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 interspecific cross. The map consisted of 20 linkage groups spanning 931.6 cM. A second map was constructed from the segregation of 50 RAPD, 107 RFLP, one microsatellite and one isozyme marker from the male parent. RAPD has been used to explore genetic diversity in cassava collections. Raji *et al.* (2001b) assessed the diversity of 500 African landraces of cassava using RAPD and AFLP. Results showed that both markers provided similar genetic relationships of the population, however, the AFLP technique detected a much higher level of polymorphism giving a

better diversity structure than RAPD. Zacarias and Cuambe (2004) assessed genetic diversity of cassava germplasm from Mozambique using RAPDs. Results showed that the cassava germplasm had wide genetic diversity, and the accessions did not group according to geographical distribution.

2.6.3.3 Expressed sequence tags

Boventius and Weller (1994) suggested using ESTs as candidate loci of quantitative traits to increase the accuracy of mapping complex traits. ESTs are generated by sequencing random cDNA clones from libraries obtained from different tissues at various stages of an organism's development (Suárez *et al.*, 2000). A method is needed for selecting and mapping suitable ESTs. The application of the AFLP technique to cDNA libraries proved to be a highly effective tool for displaying genes that are differentially expressed during the life cycle of an organism (Bachem *et al.*, 1996). Constructing cDNA libraries from different tissues and developmental stages are important to studying certain traits, and combined with AFLP analysis, it yields highly informative transcript-derived fragments (TDF) for mapping the trait in question. Sequencing of differentially expressed TDFs converts them into ESTs (Suárez *et al.*, 2000).

Studies in cassava on the development of ESTs from TDFs indicated that the cDNA-AFLP technique using EcoRI-MseI restriction enzymes, for generating TDFs between parents of a mapping cross, is a quick, reliable, and a potentially powerful way to identify candidate loci that control agronomic traits that differ in the parents (Bachem *et al.*, 1996). Suárez *et al.* (2000) recommended the application of the cDNA-AFLP technique in the generation of ESTs as differentially expressed sequences in time and between different varieties as a way of developing ESTs around specific traits for a candidate locus approach to mapping complex traits.

2.6.3.4 Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP), a PCR based assay for plant DNA fingerprinting, combines the specificity of restriction analysis with PCR amplification (Zabeau, 1992; Zabeau and Vos, 1993; Vos *et al.*, 1995). AFLP involves digestion of

genomic DNA with restriction endonucleases followed by ligation of terminal adapter sequences to generate template DNA for amplification. Selective PCR primers are modified by adding two or three selective nucleotides (Vos *et al.*, 1995; McGregor *et al.*, 2000).

The AFLP technique can be used for DNA of any origin or complexity. Fingerprints are produced without prior sequence knowledge using a set of generic primers. The number of fragments detected in a single reaction can be tuned by selection of specific primer sets, and in variation of the number of selective nucleotides. Fingerprints can be used to distinguish between closely related organisms, including near isogenic lines (NILs) and allows scoring very large numbers of markers in a given population. AFLP analysis is robust and reliable because stringent reaction conditions are used for primer annealing (Vos *et al.*, 1995; Winter and Kahl, 1995; Powell *et al.*, 1996; Blears *et al.*, 1998).

AFLP is a highly sensitive method for DNA fingerprinting (Vos *et al.*, 1995; Blears *et al.*, 1998). Vos *et al.* (1995) were primarily interested in genome mapping using AFLP markers, i.e. construction of high density genetic maps of either genomes or genome fragments for bridging the gap between genetic and physical maps. Since then many studies have applied this technique to mapping studies, e.g. Oryza (Zhou *et al.*, 1998), Zea (Xu *et al.*, 1999) and Solanum (Bradshaw *et al.*, 1998). Xu *et al.* (1999) suggested that AFLP is the most efficient way to generate a large number of markers that are linked to target genes. Thomas *et al.* (1995) reported the use of AFLP technology in the identification of tightly linked markers flanking (within 15.5 kb) the Cf-9 resistance gene of tomato, concluding that AFLP technology can be exploited for gene isolation by positional cloning (Thomas *et al.*, 1995).

Restrepo *et al.* (1998) characterised Colombian Xanthomonas isolates for genetic diversity using AFLP analysis. Results obtained were consistent with those obtained with RFLP analysis, using plasmid DNA as a probe. Some primer combinations differentiated Xanthomonas strains that were not distinguished by RFLP analysis. It was concluded that AFLP fingerprinting allowed a better definition of genetic relationships among Xanthomonas strains.

The AFLP technique has been applied in cassava in various studies. For example, Bonierbale *et al.* (1997) assessed the genetic diversity of 105 genotypes using AFLP analysis to estimate genetic similarities among taxa and evaluate intra- and inter-specific variability. Results showed individuals grouped according to prior taxonomic classification. *M. aesculifolia*, *M. brachyloba* and *M. carthaginensis* were the most distant taxa to cassava (*M. esculenta*). These results agreed with the proposal that the subspecific taxa of *M. esculenta* is most related to cassava and supported the hypothesis that ancestors of cassava can be found in this group. The crop 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.

Second *et al.* (1997) assessed the numerical taxonomy and genetic structure of 358 plants representing the geographic and ecological range of distribution of *Manihot* species along with classical botany and ecology using AFLP analysis 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 *peruvian*, it seemed that some other species also contributed. Results suggested 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 this crop is multiplied vegetatively.

Morillo *et al.* (2001) used mapped AFLP and SSR markers as evidence of introgression in a set of 60 plants. Results indicated 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) and Elias *et al.* (2000; 2001) have used AFLP and SSR markers to study the traditional cassava varieties from various Amerindian communities.

As in the case of RAPDs, AFLPs are dominant markers but technical refinements to distinguish homozygous and heterozygous genotypes have recently been achieved

(Vos and Kuiper, 1998). The technique is more reliable than RAPD (Vos *et al.*, 1995), but more laborious and time consuming (McGregor *et al.*, 2000; Powell *et al.*, 1996).

2.6.3.5 Simple sequence repeats

DNA sequence with repeated motifs (2-6 bp) are called simple sequence repeats (SSR) or microsatellites (Hamada *et al.*, 1982; Litt and Lutt, 1989; Epplen *et al.*, 1991; Todocoro *et al.*, 1995). 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).

SSR markers have been used in studies and have generally been developed by three routes: (1) transfer from closely related species (Provan *et al.*, 1996; White and Powel, 1997); (2) search sequence database (Sanwell *et al.*, 2001; Bell and Eker, 1994) and (3) screening cDNA or small insert library with tandemly repeated oligonucleotides and sequencing candidate clones (Powell *et al.*, 1996).

Some studies indicated that SSR primers may amplify the same SSR region in closely related taxa. For example, White and Powell (1997) amplified DNA from seven of the 11 microsatellite loci in other Swietenia species, six loci in other genera of the same tribe, and four to six loci in species of the same family. Wang *et al.* (2005) evaluated 210 SSR markers developed from maize, sorghum, wheat and rice (major cereals) for transferability to minor grass species like finger millet (*Eleusine coracana*), seashore paspalum *(Paspalum vagnatum*) and Bermudagrass (*Cynodon dactylon*). Results indicated that 412 cross-species polymorphic amplifications were identified.

Microsatellite markers were developed for various crops, including maize (Taramino and Tingley, 1996), soybean (Devos *et al.*, 1995), barley (Russell *et al.*, 1997) and potato (McGregor *et al.*, 2000). CIAT identified 186 SSR makers for cassava (Chavarriaga-Aguirre *et al.*, 1998; Mba *et al.*, 2001).

In cassava, the SSR technique has been applied in various studies: a SSR marker linked to CMD resistance was identified with the aid of bulk segregant analysis (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 appeared to be spontaneous recombination.

Mkumbira *et al.* (2001) used SSR markers to study the traditional way farmers in Malawi classify cassava varieties. Restrepo *et al.* (2001) used the recently constructed molecular genetic map from F_1 crosses of non-inbred parents using SSR, RFLP, AFLP and EST markers to map genes of resistance for CBB. Nine QTLs located in linkage groups B, D, L, N, and X were found to explain the crops' pathotypic variance response to *Xanthomonas* in the green house, while linkage group D was found to be involved in field resistance.

Apart from the prerequisite of knowledge of sequence information of the organism being analysed, another disadvantage of microsatellites is that it only surveys one locus at a time while AFLP surveys the whole genome at once (Robinson and Harris, 1999). Maughan *et al.* (1996) found that AFLPs produced more polymorphic loci than SSRs.

2.7 Diallel analysis

The diallel design is an important tool in plant breeding programmes aimed to improve yield and other parameters. Diallel crosses are commonly used to study the genetic properties of inbred lines in plants and animal breeding experiments. The concept of diallel design was firstly introduced by Schmidt in animal breeding in 1919 (Pirchner, 1979). 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 attracted more attention and has been subject to more theoretical and practical

application that 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 (Egesel *et al.*, 2003; Le Gouis *et al*., 2002; Saghrouse and Hallauer, 1997). Diallel mating designs permit the estimation of magnitude of additive and non-additive components of heritable variance (Griffing, 1956; Mather and Jinks, 1977). Data obtained from such cross combination can be analysed in several ways, but the commonly used are proposed by Hayman (1954) and Griffing (1956). On the basis of this premises, a test of validity of the additive and dominance components of heritability components of variation from the mean squares of these mating designs (Hayman, 1954; Mather and Jinks, 1977) is calculated. Thus, the diallel mating design has been specifically designed to investigate the combining ability of the parents and to identify superior parents for use in hybrid and cultivar development (Yan and Hunt, 2002).

2.7.1 Combining ability

Combining ability is defined as the performance of hybrid combinations (Kambal and Webster, 1965). It plays an important role in selecting superior parents for hybrid combination 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 parent and hybrid components of diallel analysis, represented by general combining ability and specific combining ability. Sprague and Tatum (1942) introduced the concepts of general combining ability and specific combining ability. General combining ability (GCA) designates the average contribution of the lines in the hybrid combination. GCA consists of additive and additive epistatic variances (Matzinger, 1963). Parents with good combining ability for specific characters may be helpful in a hybridisation programme for improvement of that character (Woldegiorgis, 2003). Specific combining ability (SCA) is where certain hybrid

combinations do relatively better or worse than would be expected on the bases of the average of 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 come from GCA and SCA will be proportional to their variances. It estimates the type of gene action which controls a particular character. The ratio has been studied as 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 (Kanju, 2000). High value of the ratio indicates the performance of the additive genes in determining a particular character. The closer the ratio to the unit, the greater is the magnitude of additive genetic effects.

Owolade *et al.* (2006) reported that both additive and non-additive gene effects were present as conclusion of a study conducted to determine the relative importance of GCA and SCA of anthracnose in cassava. The crosses between disease resistance and susceptible lines showed intermediate disease reaction, suggesting a polygenic system of resistance to the disease. Cach *et al.* (2006), in a study conducted on cassava on the inheritance of agronomic traits in cassava, such as reaction to trips, fresh root and foliage yields, harvest index, dry matter content and root dry matter yield, suggested that dominance plays an important role in complex traits such as root yield.

Very little progress on understanding the inheritance of traits with agronomic relevance on cassava has been achieved (Easwari Amma *et al.*, 1995; Calle *et al.*, 2005) and few articles 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.* (2004) concluded that GCA was more important in controlling CMD resistance among the crosses made. A study conducted by 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 the reaction of thrips, dry matter content and first branching.

2.7.2 Heritability

Meredith (1984) has defined heritability as the ratio of the variance due to hereditary difference and genotypic variance due to total phenotypic variance. The higher the ratio the more heritable the trait would be. If the ratio is smaller, the influence of environment on the phenotypic expression of the trait is bigger. Thus, it expresses the proportion of the total variance that is attributed to the average effects of genes. Falconer and Mackay (1996) found that the phenotypic variance allows the breeder to estimate the relative importance of the various determinants of the phenotype, in particular the role of heredity versus environment.

Heritability can be defined in two types, the broad sense and the narrow sense heritability:

Broad sense heritability was defined by Dudley and Moll (1969) as the ratio of the total genes variance to phenotypic variance. It includes total genetic variance (Meredith, 1984).

$h^2 = V_G/V_{P;}$
Where: h^2 = Heritability, V_G= Additive value; V_P = Phenotypic value

Narrow sense heritability is the ratio of additive genetic variances to the genetic variance (Dudley and Moll, 1996) and expresses the extent to which the phenotype is determined by genes transmitted from parents. It is the breeding value of the parents, which determines the genetic prosperity of the progeny. Narrow sense heritability is used for determining the selection progress estimates and selection indexes and determines the degree of resemblance between parents and offspring (Chaudhary, 1991; Meredith, 1984). Narrow sense heritability measures the extent of the correspondence between breeding values and phenotypic variances in the population, which is mainly responsible for changing composition of the population via selection (Falconer, 1989). It gives a basis to predict accuracy with which

selection for genotypes could be made on the phenotypic measurements of individuals or groups of individuals (Falconer, 1989; Dabholkar, 1992).

$h^2 = V_A/V_P$;
Where: h^2 = Heritability, V_A= Genetic value; V_P = Phenotypic value
Also $h^2 = V_A/(V_P = V_A + V_D + V_I + V_E)$
Where: V_A = Additive variance; V_D = Dominance variance; V_I = Interaction variance; V_E = Environmental variance (Falconer, 1989).

Populations which are genetically more uniform are expected to show lower heritability than those genetically more diverse. Since the environment variance forms part of the phenotypic variance, it affects the magnitude of heritability (Falconer, 1989). The knowledge of the relative heritability of various traits and their genotypic and phenotypic correlation can aid in the design of an efficient breeding programme system where many traits need to be improved simultaneously (Jones, 1986), by providing an indication of the expected response to the selection in the segregating population (Burton and De Vane, 1953). Selection is effective when genetic variation in relation to environmental variation is high. The net gain from selection depends on the combination of the heritability, the amount of genetic variation present, and the intensity (Poehlman, 1987).

Heritability in the narrow sense can be useful in making selection progress estimates. Characters with high narrow sense heritability values can be improved more rapidly with less intensive evaluation than those with low values and are useful to make selection progress estimates. Hershey (2005) indicated that estimates of heritability depend on the method used to estimate them, the population from which the estimates are derived and environmental conditions encountered during the test. Heritability estimates in cassava have been suggested as broad sense in nature (Kawano *et al.*, 1998). High heritability estimates for dry matter in cassava has been reported (IITA, 1990; Kawano, 1978; Cach *et al.*, 2006).

2.7.3 Heterosis

Heterosis has been recognised as a phenomenon for almost a century (Shull, 1908). It has been exploited in animal and plant breeding over the years. There are several definitions of heterosis. Schull (1952) defined heterosis as the increased vigour, size, fruitiness, speed of development, resistance to disease and pests, or to climatic vigour of any kind. Hart 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 the heterozygosity superiority and, therefore increased vigour is proportional to the amount of heterozygosity (Lamkey and Edward, 1999). Wricke and Weber (1986) noted that there are several hypotheses to genetically explain this phenomenon: (1) partial dominance of the large number of loci, (2) overdominance of several loci, (3) several types of epistasis. The authors indicated that for hybrid breeding a substantial number of 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 F_1 the disperse genes of dominant alleles showing directional dominance and non-allelic interactions, but not by heterozygote superiority or complementary epistasis. Coors *et al.* (1999) indicated that dominance and epistasis are the principal genetic factors in the exploitation of heterosis. If the population crossed does not differ in gene frequency there will be no heterosis.

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 Edward (1999) noted that the mid-parent heterosis is difficult to interpret from a quantitative genetics point of view. They indicated that high-parent heterosis or performance of F_1 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.

Heterosis in plant breeding has been exploited extensively over the years (Duvick, 2001). Duvick (1999) reported that field crops such as maize (*Zea* mays L.) sorghum (*Sorghum* bicolour (L.) Moench) and sunflower (*Helianthus annus* L.) are produced as hybrids in the industrialised world. Hybrid rice (*Oryza sativa* L.) is grown extensively in China and increasingly in India. Heterosis is used for large increases in production per unit area, thus alleviating large amounts of land for other uses. Few studies on the inheritance of quantitative and qualitative traits of cassava have been reported (Easwari Amman *et al.*, 1995; Easwari and Sheela, 1998; Peréz *et al.*, 2005). Unnikrishnan *et al.* (2001) have assessed hybrid vigour for root yield over better-parent values, and that for root yield was generally associated with heterosis for yield components. Studies undertaken by Unnikrishnan *et al.* (2001), on the nature and magnitude of heterosis in cassava, found that the hybrids displayed substantial differences in their heterotic response, heterosis over mid-parents and better-parents values were observed in all the characters studied.

Heterosis has been reported at both phenotypic and gene level. Comings and MacMurray (2000) after revising the phenotypic data in many species on more than a dozen of genes, concluded that molecular heterosis is common and they occur up to 50% of all the genes association and that many of most of them are gene-, phenotype-, and organ-specific.

2.8 Correlations

The coefficient of correlation is the measure of association between two characteristics. Correlations can be either positive or negative. The positive values are observed when an increase in one variable is accompanied by an increase in another one and negative when an increase in one character is accompanied by a decrease in another (Falconer, 1989). They are of interest for three reasons namely (1) genetic causes of correlation through the pleiotropic action of genes, (2) in connection to changes brought about by selection and (3) in connection with natural selection (Falconer and Mackay, 1996).

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CHAPTER 3

A DIALLEL ANALYSIS OF CASSAVA BROWN STREAK DISEASE, YIELD AND YIELD RELATED CHARACTERISTICS

3.1 Introduction

Cassava is a staple food for more than 50% of the population in the main growing areas of Mozambique. The roots and leaves are used daily as a main source of carbohydrates and vitamins. The outbreak of cassava brown streak disease (CBSD) reported from 1999, now constitutes the main production constraint in the country. CBSD may be found in all plant parts, affecting food security and availability and quality of planting material. The root symptoms are a yellow-brown, corky necrosis in the starch-bearing tissue, making the most severely affected roots unfit for consumption. Losses in root yield may reach 60 to 70% due to CBSD in susceptible cultivars (Hillocks and Thresh, 2001; Cuambe *et al.*, 2007), resulting in food security problems. Survey reports conducted in Northern Mozambique, have shown that farmers have identified local varieties that are tolerant or resistant to CBSD (Hillocks *et al.*, 2002; Zacarias and Cuambe, 2004). Some of these varieties are Chigoma mafia, Nikwaha, Mucudo muevia, Mulaleia, Likonde and Mocuba, reported in this study.

CBSD is caused by Cassava Brown Streak Virus (CBSV) of the genus *Ipomovirus*, family *Potyviridae* (Monger *et al.*, 2001). CBSD was first reported by Storey (1936) in the then called Tanganyika. The disease is endemic in East African coastal cassava-growing areas from southern Kenya, through Tanzania to the Zambezi River in Mozambique, and also occurs in some inland areas of Malawi, Uganda and the Democratic Republic Congo (DRC) (Hillocks *et al.*, 2002; Legg and Raya, 1998; Benesi, 2005).

The first attempt to select for resistance to CBSD dated from 1937 at Amani Research Station (Hillocks and Jennings, 2003), the area where the disease was confined at the time. Crosses were made between wild *Manihot* species, collected in

Surinam and Brazil. However, only the hybrids from *M. glaziovii* and *M. melanobasis* were promising and contained a combination of other required traits in cassava (Hillocks and Jennings, 2003). Seventy years have passed and little is still known about the disease. The disease has since spread to more areas in different countries. More breeding for resistance to CBSD is necessary. It is known that development of resistant varieties could potentially form the basis of a sustainable management strategy for cassava diseases (Asiedu *et al.*, 1998; Mahungu *et al.*, 1994; DeVries and Toenniessen, 2001). Selection of resistant varieties and continuous breeding appears to be the most efficient way to control CBSD.

Studies on improvement for resistance to CBSD are scarce. All breeding programmes in the affected areas should pool information that will help to formulate an efficient strategy for incorporating the resistance genes into high yielding and adapted lines. It is known that the development of new varieties depends greatly on the screening of parental lines to be used for hybridisation programmes. The overall objective of this study was to evaluate the relative importance of general and specific combining ability, inheritance of relevant traits and to identify superior parents for use in further improvement of cassava genotypes for yield and CBSD resistance.

3.2 Material and methods

3.2.1 Parental material

Diallel cross progeny constituted one of the first breeding trials to be implemented in the cassava programme in Mozambique. Eighteen entries maintained at the national cassava gene bank were investigated for their performance based on their agronomic traits, adaptation to the environment and farmer preference (Zacarias *et al.*, 2003). In the end, the choice of parents for the current experiment was based on the ability to produce a reasonable amount of seed for each of the required F_1 crosses in a complete and balanced diallel design. Additionally the relative importance of CBSD for the region played a role in the selection. The selected parents had different degrees of resistance to CBSD (resistant, moderately resistant, and susceptible) (Zacarias and Cuambe, 2004). A five-parent diallel cross was done

at Posto Agronomico de Nampula (PAN)-Mozambique, using two CBSD-resistant parents (Chigoma mafia and Mulaleia), one CBSD-moderately resistant parent (Macia 1) and two CBSD-susceptible parents (MZ 89186 and IMM 30025). The varieties Chigoma mafia, Mulaleia and Macia 1 are landraces collected in Cabo Delgado and Zambezia, while IMM 30025 and MZ 89186 are improved clones selected from true seed received from the International Institute of Tropical Agriculture (IITA), batches of 1986 and 1989 respectively (Table 3.1).

3.2.2 Development of progeny

The five parents were planted in the breeding nurseries at Posto Agronomico de Nampula (PAN) in 15 Octobre 2002 season. Controlled pollinations were performed following the standard procedures described by Kawano (1980). The parents were crossed in a full diallel mating design (Griffing, 1956) to produce 20 F_1 crosses, including reciprocals. Twenty seeds from each cross were harvested. The seeds were germinated and grown in a greenhouse in plastic bags at PAN. They were watered twice a day to ensure good germination and development. Forty five days after planting (DAP) the plants were transported and transplanted (10 December 2003) to the experimental field in Mogincual Substation, during the rainy season as a seedling nursery. Established seedlings were planted in a single row, 50 cm and 1 m spacing within and between rows, respectively, to produce enough woody cuttings for the study. Mature stems (25 cm long) of the five parents were also planted in the field. No irrigation and fertiliser was applied at this stage. At harvest time, 12 months after planting (MAP), five vegetative cuttings for each of the 20 genotypes of each F_1 family and parents were obtained. They were selected based on the capacity of the plant to produce five good quality vegetative cuttings to proceed with evaluation.

3.3 Field experiments

Field arrangement

A randomised complete block design with five replicates was used to plant a total of 25 entries consisting of 10 F_1, 10 reciprocals and five parents (Table 3.1). The experiment was planted in two consecutive years, 2004 and 2005. The planting date was 15/12/2004

and 18/12/2005 at Mogincual Experimental Station. The planting period started at the beginning of the raining season, as this area is characterised by short rain periods, from December to March. Each replication contained the 25 entries (parents and crosses), planted together in the respective plots of each replication. The plant spacing was 1 m between and within rows, giving a plant population of 10 000 plants per hectare. The field was kept weed free manually and no supplement of irrigation or fertiliser was applied during the growth period. Data from three plants (inner plants), of each entry was averaged before analysis.

Trial site

Trials were planted at Mogincual, a district of the Nampula province. Weather data for minimum and maximum temperatures, monthly total rainfall and altitudes of trial sites were recorded. The soils are predominantly sandy, brown-grey, deep with good drainage, however they were moderately acidic with low organic matter (INIA, 1995). Data of temperature, rainfall and relative humidity are presented in Figure 3.1.

Mogincual is located at 15 34' latitude south and 040 45' longitude east, 35 m above sea level (INIA, 1995). It is situated along the coast with an average incidence of CBSD of 90% annually (Zacarias and Cuambe*,* 2004). In this area, Tomo is the predominant local variety grown by farmers. It is classified as highly susceptible to CBSD and presents severe symptoms in the leaves and in the roots with necrosis severity of 5 (scale 1 to 5 where 1 = no symptoms and 5 = very severe symptoms). The roots have a bitter taste. The plant produces two to three stems which can reach a height of 2.5 to 3 m at 11 MAP and doesn’t flower.

Table 3. 1 List of parents and F1 progeny used in the diallel trial

	Entry codes	Pedigree	Remarks
Parents	1	Chigoma mafia	Landrace
	2	Mulaleia	Landrace
	3	MZ 89186[a]	Improved clone
	4	MZ IMM 30025 [a]	Improved clone
	5	Macia 1	Landrace
F1	1x2	Chigoma mafia x Mulaleia	F1
	1x3	Chigoma mafia x MZ 89186	F1
	1x4	Chigoma mafia x IMM 30025	F1
	1x5	Chigoma mafia x Macia 1	F1
	2x1	Mulaleia x Chigoma mafia	Reciprocal
	2x3	Mulaleia x MZ 89186	F1
	2x4	Mulaleia x IMM 30025	F1
	2x5	Mulaleia x Macia 1	F1
	3x1	MZ 89186 x Chigoma mafia	Reciprocal
	3x2	MZ 89186 x Mulaleia	Reciprocal
	3x4	MZ 89186 x IMM 30025	F1
	3x5	MZ 89186 x Macia 1	F1
	4x1	IMM 30025 x Chigoma mafia	Reciprocal
	4x2	IMM 30025 x Mulaleia	Reciprocal
	4x3	IMM 30025 x MZ 89186	Reciprocal
	4x5	IMM 30025 x Macia 1	F1
	5x1	Macia 1 x Chigoma mafia	Reciprocal
	5x2	Macia 1 x Mulaleia	Reciprocal
	5x3	Macia 1 x MZ 89186	Reciprocal
	5x4	Macia 1 x IMM 30025	Reciprocal

[a] Selection from IITA seeds received in batch 1986 to 89

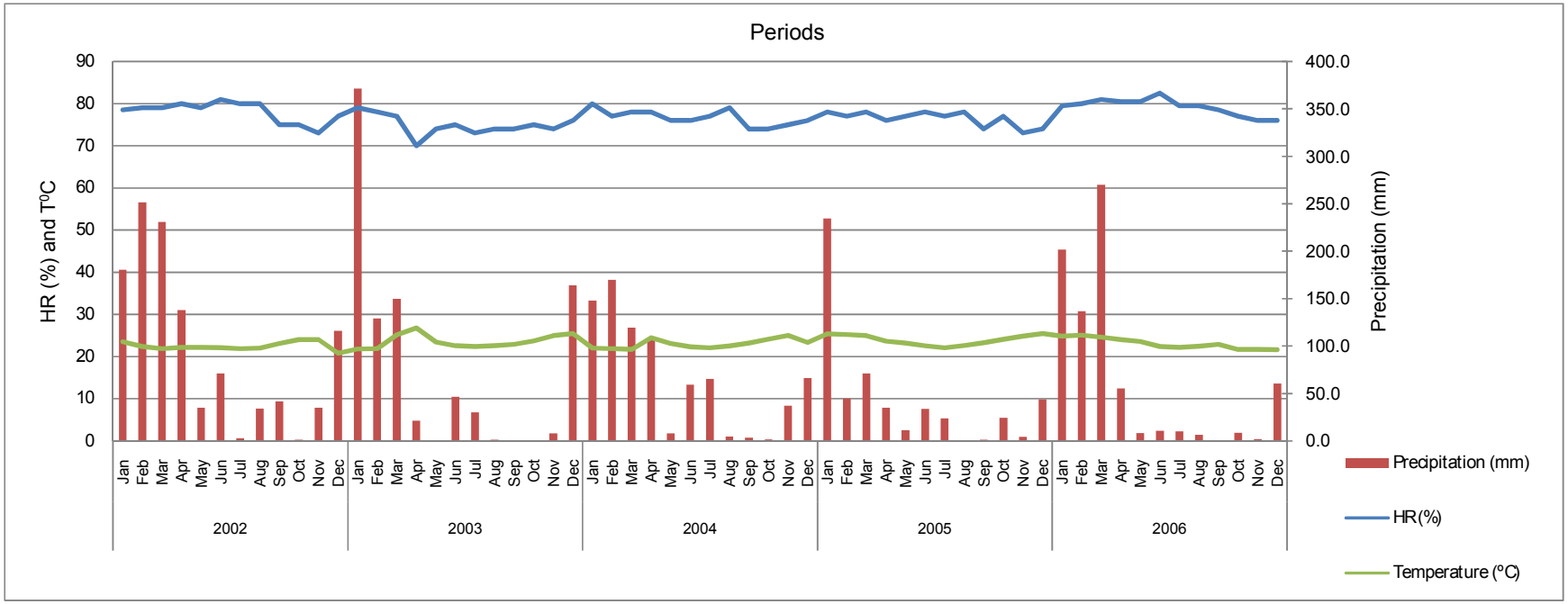

Figure 3.1 Temperature, rainfall and relative humidity of Mogincual data collected from January 2002 to December 2006 (Source: IIAM, 2006)

3.4 Agronomic and morphological characters measured

During the growth period, data was collected on morphological traits and cassava mealybug (CM) infestation. At harvest, data of eight agronomic characters were recorded (Table 3.2). Cassava mealybug (*Phenacoccus manihotis* Mat.-Ferr) severity damage was scored on the top apical part of the plant in the field with a scale of 1 to 5 where 1=no symptoms and 5=severe damage (IITA, 1990). Prior to harvest, 12 MAP, the plant height was measured at the highest branch. Plants were hand-harvested individually and results averaged across plants from each F_1 cross. The roots were counted and weighed separately. Every root was sliced to score for necrosis 1= no symptoms, 5= totally damaged (Hillocks *et al.,* 1996). Harvest index was measured as a ratio of root weight to total biomass. Root taste was scored using scale 1=bitter and 2=sweet, using adapted sensorial methodology described by Padonou *et al.* (2005). Root pulp hardiness was measured using a subjective scale of 1 to 5, where 1 = very hard, 2 = hard, 3 = moderate, 4 = soft and watery, 5 = soft and very watery. Farmers frequently use the chewing technique to get an indication of the dry matter content. Roots with a lower score, 1 to 3, are the best, while the ones with a high score, 4 to 5, will be rejected because such roots will be too soft.

Fresh root and harvest index are important traits related to yield (Byrne, 1984). Root pulp hardness is of the same importance as dry matter content in cassava. Cassava brown streak root necrosis (CBSDr) is the most important economical constraint in the region (Hillocks and Jennings, 2003; Zacarias and Cuambe, 2004). Root taste and plant height, are important traits for farmers (Zacarias and Cuambe, 2004).

3.5 Data analysis

Analysis of variance and diallel analysis (Griffing, Method 1) were done for each year using Agrobase (2000) using a fixed model.

3.5.1 Combining ability

Analysis of general (GCA) and specific (SCA) combining ability for individual 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).

3.5.2 Phenotypic correlation

Phenotypic correlation (r_P) between agronomic and morphological characters was calculated using the plot means as follows:

$$r_P = Cov_{XY}/(\delta^2_x \delta^2_Y)$$

where Cov_{XY} = phenotypic covariance between characteristic X and Y; δ^2_X = phenotypic variance of characteristics x, and δ^2_Y = phenotypic variance of characteristics Y.

3.5.3 Genetic parameters

The relative contribution of genetic components was determined to obtain estimates of GCA variance (δ^2_{gca}) and SCA variance (δ^2_{sca}) for each character studied. Additive (V_a) and dominance (V_d) variance were estimated as $V_a = 2(\delta^2_{gca})$ and $V_d = (\delta^2_{sca})$. Phenotypic (V_p) and genotypic variance (V_g) were also estimated as $V_g = V_a + V_d$, where $V_p = V_g + V_e$.

Broad (h^2b) and narrow (h^2n) sense heritability was calculated from the estimated components of variance as:
$h^2_b = V_g / V_p$ and $h^2_n = V_a / V_p$, respectively.

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

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

3.5.4 Estimates of heterosis

Heterosis was calculated as:
Mid-parent heterosis (MPH) (%) = $(F_1\text{-}MP)/MP \times 100$

where, F_1 = F_1 hybrid performance, MP = $(P1+P2)/2$ in which *P1* and P2, are the performance of inbred parents, respectively.

Statistical significance of mid-parents and high-parent heterosis values was tested by comparing these values with the LSD values.

3.6 Results and discussion

Analysis of variance of the characters evaluated during 2004 and 2005 in Mogincual, showed that the mean squares of genotypes were significant for all characteristics presented in this study (Table 3.3).

3.6.1 Weather and climate at trial site

Temperature, rainfall and relative humidity data of the trial site are given in Figure 3.1. Mogincual has four months of effective rain per season. Annual rainfall showed a reducing tendency from 2001 to 2005. The total rainfall was 1137 mm for 2001, 921.6 mm for 2002, 790.9 mm for 2003, 524 mm for 2004 and 769.7 mm for 2005 (Figure 3.1). At most parts of the country, trial sites had a monomodal type of rainfall, with effective rain starting between December and January. The effective rain period is only for four months with varied rainfall distribution. The season 2004/05 was the most irregular with January receiving 234 mm and other months less than 100 mm. For all seasons the peak of rainfall was observed between February to March. The average monthly and maximum temperatures ranged between 21.9 to 23.5^{o}C for 2001, 20 to 29.8^{o}C for 2002, 21.4 to 25.4^{o}C for 2003, 22.05 to 24.4^{o}C for 2004 and 21.6 to 25.1^{o}C for 2005 season.

Table 3. 2 Agronomical and morphological characters recorded for the diallel study in Mogincual, 2004 and 2005

Characters	Code	Description
Agronomic	PH	Plant height
	ARN	Average root number
	FRW	Average fresh root yield per plant
	CBSDr	Root necrosis due to CBSD
	RTST	Root taste
	CM	Cassava mealybug
	RPH	Root pulp hardness
	HI	Harvest index in percentage
Morphological	PBs	Leaf pubescence
	NL	Number of lobes per leaf
	SHL	Shape of the central lobe
	ULC	Unexpanded leaf colour
	MLC	Mature leaf colour
	CNC	Leaf vein colour
	PLC	Petiole colour
	PTP	Petiole position
	PTL	Petiole length
	MSC	Mature stem colour
	ASC	Apical stem colour
	StH	Stem habit

3.6.2 Estimation of combining ability variances

Evaluation of combining ability of additive and non-additive gene performance within breeding populations is important to determine the type of breeding methods that will successfully improve the performance of the studied characters (Dudley and Moll, 1969).

General and specific combining ability effects

General combining ability (GCA) mean squares were highly significant for plant height, CBSDr, root taste and root pulp hardness. GCA was not significant for average root nember, fresh root weight, cassava mealybug and harvest index. Specific combining hability (SCA) was significant for average root number, CBSDr, root taste, root pulp hardness and harvest index (Table 3.3). Reciprocal effects were significant only for harvest index.

The GCA:SCA ratios indicated that GCA was higher than SCA for average root number, root taste, root pulp hardness and harvest index, for two years of evaluation, where ratio closer or higher than a unit indicates the predominance of additive gene action. However, the GCA was lower than SCA for fresh root yield and cassava mealybug for the second year of evaluation, showing the influence of the environment on these traits. The SCA was higher than GCA for plant height and CBSDr, demonstrating that these characters are largely under the influence of non-additive gene action.

The parent Chigoma mafia had positive GCA effects for plant height, average root number per plant, average fresh root weight, and root taste, but negative GCA for dry matter content and CBSDr for both years, but positive in one year and negative in the other year for harvest index (Table 3.4). However, Chigoma mafia had the best combining ability for CBSDr followed by Mulaleia. Mulaleia had poor GCA effects for average root number, fresh root yield, root taste and root pulp hardness. The parent MZ 89186, had negative GCA effects for plant height, average root number, fresh root yield, root taste and mealy bug. MZ 89186 had the second best GCA score for root pulp hardness and harvest index. The clone IMM 30025 had a positive GCA effect for average root number, the highest GCA effect for fresh root yield and root pulp hardness, but the worst combining ability for CBSDr and cassava mealybug. The parent Macia1 had the best GCA effect for plant height, but a negative GCA effect for average root number, fresh root yield, root taste, harvest index. Its GCA performance for root pulp hardness was among the best observed but it also had the poorest GCA for mealy bug.

The GCA effects contributed 21.4% and 25.1%, during year 1 and 2 respectively while SCA contributed 78.6% and 74.9%, to the sum of squares for CBSDr, indicating the importance of non-additive gene action in the expression of CBSDr. Negative GCA effects for CBSDr indicated better performance based on the scales used.

Table 3. 3 Mean squares for GCA and SCA and GCA:SCA ratios for different cassava characters studied in a diallel trial during 2004 and 2005

Source	Df	PH (cm)		ARN (kg/pl)		FRW (kg/pl)		CBSDr (1-5)	
		2004	2005	2004	2005	2004	2005	2004	2005
Genotypes	24	2889**	1342*	214*	1694*	0.14**	34.5**	1.03**	2.1*
GCA	4	1158**	6.6*	8.15	402	0.03	2.88	0.2**	0.48**
SCA	10	5144	455	3.73*	168*	0.02	14.9	0.32**	0.57**
Reciprocal	10	4092	186	3.7	485	0.04	0.48	0.09	0.23
Error	96	142	223	3	227	0.03	2.5	0.07	0.26
GCA:SCA		0.23	0.01	2.19	2.39	1.87	0.19	0.63	0.84

PH=plant height, ARN=average root number, FRW=average fresh root weight per plant, CBSDr= cassava brown streak necrosis, RTST= root taste, RPH=dry matter content; CM=cassava mealybug, HI=harvest index, Y1=2004, Y2= 2005, Df= degrees of freedom, GCA=general combining ability, SCA=specific combining ability, **P<0.05,**P<0.01*

Table 3.3 Cont...

Source	Df	RTST (1-2)		RPH (1-5)		CM (1-5)		HI (%)	
		2004	2005	2004	2005	2004	2005	2004	2005
Genotypes	24	0.26*	0.24**	2.04**	3.2*	0.41*	0.41*	0.01*	0.02**
GCA	4	0.14*	0.13*	0.87**	1.1*	0.17	0.03	0.04	0.01
SCA	10	0.06**	0.05**	0.45**	0.88**	0.13	0.08	0.03*	0.01*
Reciprocal	10	0.01	0.01	0.18	0.22	0.04	0.11	0.01**	0.02**
Error	96	0.02	0.2	0.1	0.17	0.01	0.01	0.03	0.02
GCA:SCA		2.33	2.6	1.93	1.25	1.31	0.38	1.33	1.2

PH=plant height, ARN=average root number, FRW=average fresh root weight per plant, CBSDr= cassava brown streak necrosis, RTST= root taste, RPH=root pulp hardness; CM=cassava mealybug, HI=harvest index, Y1=2004, Y2= 2005, Df= degrees of freedom, GCA=general combining ability, SCA=specific combining ability, **P<0.05,**P<0.01*

Table 3. 4 Estimates of general combining ability (GCA) effects for various characters evaluated during 2004 and 2005

Parent	PH (cm)			ARN (kg/pl)			FRW (kg/pl)			CBSDr (1-5)		
	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean
Chigoma mafia	9.28*	0.5	141	1.18**	9.73**	6.98	0.03	0.07	3.22	-0.03*	-0.35**	1.55
Mulaleia	-0.32	0.6	177	-0.5	-0.26	5.46	-0.05	0.55*	2.07	-0.18*	-0.03	1.34
MZ 89186	-1.4	-0.74	146	-1.1	0.74	5.32	-0.05	-0.49	3.32	0.01	0.08	2.59
IMM 30025	-6.7*	1.11*	169	0.65	0.67	7.55	0.07	0.53	2.96	0.22**	0.24**	2.32
Macia 1	11.7**	-0.47	184	-0.3	-0.81	7.84	-0.13	0.57*	2.82	-0.01*	0.06	2.92
SD (G_i)	9.55	4.22	163	0.49	4.25	6.63	0.05	0.44	2.88	0.07	0.14	2.14
SD (G_i-G_j)	5.34	8.7	22.4	0.77	6.7	2.63	0.11	0.71	0.99	0.19	0.23	0.67

PH=plant height, ARN=average root number, FRW=average fresh root weight, CBSDr= cassava brown streak necrosis, RTST= root taste, RPH=root pulp hardness, CM=cassava mealybug, HI=harvest index, SD (G_i)= standard error for any GCA effect; SE(G_i -G_j) = standard error of the difference between any two effects, **P<0.05,**P<0.01*

Table 3.4 Cont...

Parent	RTST (1-2)			RPH (%)			CM (1-5)			HI (%)		
	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean
Chigoma mafia	0.2**	0.19**	1.56	-0.3	-0.4**	2.27	0.11**	-0.01	1.1	0.05**	-0.02	0.5
Mulaleia	-0.12	0.11**	1.13	-0.3	-0.27**	3.04	-0.1**	0.03	1.02	0.05**	0.01	0.55
MZ 89186	-0.03	0.01	1.12	0.13	0.31**	3.32	-0.09**	-0.09**	1.1	0.03**	0.02**	0.63
IMM 30025	-0.01	0.02	1.3	0.39**	0.31**	4.06	-0.1**	0.02	1.11	-0.01	0.03**	0.47
Macia 1	-0.04	0.04	1.04	0.07	0.05	2.62	0.18**	0.05*	1.32	0	-0.03	0.39
SD (G_i)	0.03	0.04	1.23	0.08	0.11	3.1	0.02	0.02	1.07	0.01	0.01	0.51
SD (G_i-G_j)	0.06	0.06	0.23	0.14	0.18	0.6	0.04	0.04	0.22	0.01	0.01	0.1

PH=plant height, ARN=average root number, FRW=average fresh root weight, CBSDr= cassava brown streak necrosis, RTST= root taste, RPH=root pulp hardness, CM=cassava mealybug, HI=harvest index, SD (G_i)= standard error for any GCA effect; SE(G_i -G_j) = standard error of the difference between any two effects, *$P<0.05$,**$P<0.01$*

The differences observed between years of evaluation might be due to differences in environmental conditions, especially rainfall, which differed in amount and pattern during the two years of the trial (Fig. 3.1). During 2004, the rainfall was better distributed compared to 2005, where half of the total amount (524.4 mm) fell in only one month of January. Significant GCA x environment interaction effects for fresh root yield and dry matter content were reported by Jaramillo *et al.* (2005).

For overall performance for GCA and SCA, Macia 1 and Chigoma mafia, were among the three parents that were the best general combiners for average root number, fresh root yield and root pulp hardness (Table 3.4). The best average performance for root number was observed with crosses MZ 89186 x IMM 30025 (8.50) and MZ 89186 x Macia 1 (7.57), while the highest SCA effect was in the cross Chigoma mafia x IMM30325 (0.90) (Table 3.5). The fresh root weight Chigoma mafia x MZ 89186 (0.90) had the best performance and the best SCA effect was observed with crosses Macia 1 x Chigoma mafia (0.26) followed by IMM 30325 x MZ 89186 (0.14). For root necrosis, the best performance was observed with combination Chigoma mafia x Mulaleia (1.63) followed by Macia 1 x Chigoma mafia (1.70), but the best SCA effects where observed with reciprocal Macia 1 x Chigoma mafia (-0.51).

The best average performance and SCA effect for root taste was observed in the cross Chigoma mafia x Mulaleia, 1.2 and –0.20, respectively. Root pulp harness has been used to classify the root quality. In this study, it was classified using a scale of 1 to 5, where the lower classes (1 – 3) were the best, and the higher (4 – 5) the poorest and rejected by farmers. The best performance was found in the cross Chigoma mafia x Mulaleia, and the best SCA effect between Macia 1 x Chigoma mafia (-0.49) in 2004. For dry matter content, these results are similar to findings reported by Jaramillo *et al.* (2005) and this was a confirmation of the importance of non-additive effects for root pulp hardness in cassava.

The GCA effect was lower than SCA for CBSDr. Thus the GCA:SCA ratio was lower than unit, suggesting the presence of non-additive gene action (Baker, 1978). A negative GCA effect in the parent line for disease, in this case CBSDr, is

an indication of the contribution towards the resistance to the disease (Owolade *et al.*, 2006), while a positive value represents the susceptibility due to the scale used. Mulaleia ranked as the best for GCA effects for CBSDr, followed by Chigoma mafia. During the field trips and surveys conducted from 1999 to 2004 in the affected region (Hillocks, 2003; Zacarias and Cuambe, 2004), farmers ranked these cultivars as resistant to CBSDr. However, in this study, the best average performance was observed in the cross Chigoma mafia x Mulaleia (1.63), but the best SCA effects with the combinations with the lowest values were those between MZ 89186 x IMM 30325 (-0.59), susceptible x resistant parents, and the cross Macia 1 x Chigoma mafia (-0.51), moderate x resistant parents, according to the available information so far collected in the affected areas. The magnitude of GCA and SCA for a given trait depends on the environment and genotypes involved (Ntawuruhunga, 2000; Ojulong, 2006).

Table 3. 5 Specific combing ability effects and combined mean performance of different characters evaluated during 2004 and 2005

Crosses and reciprocals	PH (cm)			ARN (kg/pl)			FRW(kg/pl)			CBSDr (1-5)		
	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005
1x2	141.30	0.96	0.63	6.35	0.45*	0.55	2.06	0.01	-0.53*	1.63	0.20*	-0.09
1x3	132.50	-11.51**	-13.16**	6.11	0.33*	0.59	3.54	0.20**	-0.42*	2.13	0.35**	0.10*
1x4	147.80	-2.62	-2.34	6.94	0.90*	-0.03	2.69	0.13**	-1.59**	2.32	0.01	0.24**
1x5	132.60	-3.66**	-13.97**	5.59	-0.33	-1.71**	1.59	-0.04	-2.15**	1.57	-0.11*	0.12*
2x3	142.20	-14.20**	-4.49	6.97	0.13	0.89*	2.07	-0.02	-0.66*	2.02	0.16*	0.60**
2x4	135.40	-4.54*	-13.53**	5.21	0.79*	-0.75*	2.09	-0.04	-1.23*	1.93	-0.03	0.31**
2x5	154.60	-5.75*	-7.32**	6.43	1.10*	0.14	2.99	0.07	-2.16**	2.08	-0.10*	0.19*
3x4	140.90	-6.24*	-9.97**	8.50	0.21	1.33**	2.26	-0.08	-1.55**	1.75	-0.27*	-0.59**
3x5	142.80	-7.13*	1.54	7.73	0.43*	-0.62**	2.63	-0.09	-0.03	1.76	-0.47**	-0.90**
4x5	141.10	-12.25**	1.03	5.22	-0.21	-2.23**	2.09	-0.04	-1.99**	1.87	-0.36**	-0.02
2x1	152.35	-18.30**	-0.72	1.07	0.04	-1.13	2.70	-0.15**	-0.27*	2.01	-0.11	-0.20*
3x1	128.63	-4.26*	2.44	7.57	0.23**	-1.61**	2.58	-0.10	-0.55	1.74	-0.05	0.10
3x2	135.00	27.78**	10.54*	7.26	0.01	-0.27	2.31	0.09	-0.22	2.41	0.27*	-0.52**
4x1	135.00	3.02	-3.02	6.94	0.34**	-0.92	2.53	0.09	-0.44	1.83	-0.03	0.20
4x2	169.40	-7.36*	-16.84**	6.60	-0.13	-1.40	3.26	0.03	-0.58	2.68	-0.14	-0.40**
4x3	150.40	3.58*	-0.02	7.36	-0.11	1.80	2.70	0.14**	0.41	2.06	-0.02	-0.60**
5x1	166.70	-18.97**	-15.50**	5.47	-0.26	-0.18	2.72	0.26**	-0.78	1.70	-0.51**	-0.10
5x2	153.00	-4.33*	6.95*	4.89	-1.12	0.52	2.14	0.09	0.41*	2.10	0.03	-0.50**
5x3	158.50	-2.87	-8.02*	4.12	1.10**	2.02	1.09	0.21**	0.69*	1.70	0.07	0.10
5x4	160.80	-21.41**	-10.31*	4.43	-0.23	0.72	3.13	0.02	-0.22	2.70	-0.19*	-0.05
SE (S_{ij})	146.05[1]	3.38	4.22	6.04[1]	0.18	0.61	2.46[1]	0.21	0.22	1.99[1]	0.10	0.14
SE (S_{ij}-S_{kl})	22.34[2]	9.25	11.56	2.63[2]	1.34	1.17	0.99[2]	0.13	1.33	0.67[2]	0.31	0.39
SE (R_{ij})		8.44	10.55		1.22	10.64		0.12	1.12		0.19	0.36

Parent: 1=Chigoma mafia, 2=Mulaleia, 3=MZ 89186, 4=IMM 30025, 5=Macia 1, PH=plant height, ARN=average root number, FRW=average fresh root weight, CBSDr= cassava brown streak necrosis, RTST= root taste, RPH=root pulp hardness, CM=cassava mealybug Y= year, HI=harvest index, SE=Standard error, [1]=average, [2]=LSD (5%), **P<0.05,**P<0.01*

Table 3.5. Continuation ....

Crosses and reciprocals	RTST (1-2)			RPH (%)			CM (1-5)			HI (%)		
	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005
1x2	1.20	-0.20**	-0.19**	2.77	-0.08*	0.03	1.00	-0.10**	-0.16**	0.4	0.02*	-0.02**
1x3	1.58	0.05*	0.04*	3.26	0.33**	0.05	1.00	-0.12**	0.01	0.39	0.03**	0.03**
1x4	1.44	0.03*	0.02	3.90	0.27**	-0.01	1.00	-0.11**	-0.10**	0.47	0.01*	0.01*
1x5	1.29	-0.20**	-0.20**	3.34	0.32**	0.10*	1.88	0.64**	0.29**	0.34	0.02*	0.02**
2x3	1.29	0.19**	0.18**	3.77	0.54**	-0.13*	1.00	0.09**	0.43**	0.41	-0.07**	-0.07**
2x4	1.29	-0.04*	-0.04*	3.79	0.12*	-0.20**	1.00	0.09**	-0.18**	0.53	0.02*	0.02**
2x5	1.42	0.10	0.10*	3.68	0.42**	-0.10	1.00	-0.18**	-0.03	0.46	-0.01*	-0.01
3x4	1.34	-0.11**	-0.11**	3.73	-0.33**	-0.19**	1.00	0.09**	-0.03	0.43	-0.03**	-0.03**
3x5	1.21	0.10*	0.07*	3.99	0.02	-0.01	1.00	-0.16**	-0.06	0.42	-0.02*	-0.02*
4x5	1.32	0.18**	0.17**	2.97	0.03	-0.14**	1.00	-0.18**	-0.17**	0.47	0.04**	0.04**
2x1	1.26	0.04*	0.04	2.79	-0.09*	0.25**	1.00	0.01	0.01	0.45	-0.02*	-0.02*
3x1	1.28	0.07*	0.07	3.78	-0.32**	0.19*	1.00	0.01	0.01	0.45	-0.03*	-0.03**
3x2	1.34	0.07*	0.07	4.21	-0.35**	0.01	1.06	0.00	-0.03	0.41	0.01	0.01
4x1	1.47	-0.06	-0.03	3.64	0.37**	0.16	1.00	0.01	0.01	0.45	0.01	0.01
4x2	1.11	0.06	0.06	3.94	0.18	-0.04	2.16	0.01	-0.58**	0.47	0.03	0.03**
4x3	1.11	-0.20**	-0.02	3.97	-0.15	0.05	1.00	0.01	0.01	0.51	-0.04**	-0.04**
5x1	1.59	0.06*	0.06*	4.06	-0.49**	0.36*	1.00	-0.14**	0.44**	0.42	-0.04**	-0.04**
5x2	1.03	0.17**	0.17**	3.00	-0.19	-0.11*	1.00	0.00	0.00	0.37	0.05**	0.05**
5x3	1.12	0.08*	0.07	3.60	0.34**	0.01	1.00	0.03	0.00	0.42	0.01	0.01
5x4	1.30	0.02	0.01	4.10	-0.31**	-0.15	1.00	0.00	0.00	0.5	0.01	0.01
SE (S_{ij})	1.30[1]	0.03	0.04	3.61[1]	0.08	0.11	1.11[1]	0.02	0.07	0.44[1]	0.01	0.01
SE (S_{ij}-S_{kl})	0.23[2]	0.11	0.11	0.60[2]	0.24	0.31	0.23[2]	0.08	0.08	0.11[2]	0.03	0.02
SE (R_{ij})		0.06	0.10		0.22	0.29		0.07	0.07		0.03	0.02

Parent: 1=Chigoma mafia, 2=Mulaleia, 3=MZ 89186, 4=IMM 30025, 5=Macia 1,PH=plant height, ARN=average root number, FRW=average fresh root weight, CBSDr= cassava brown streak necrosis, RTST= root taste, RPH=root pulp hardness, CM=cassava mealybug Y= year, HI=harvest index, SE=standard error, [1]=average, [2]= LSD (5%), **P<0.05,**P<0.01*

3.6.3 Phenotypic correlation

Nineteen agronomic and morphological characters were combined for this analysis (Table 3.6). Plant height was significantly positively correlated with average root number, fresh root weight, shape of the central lobe and petiole length. Average root number increased with average fresh root weight (r=0.26), as shown by the significant and positive correlation found in this study. Fresh root weight was positively correlated with the harvest index (r=0.17). This trait also presented the highest positive correlation with petiole length (r=0.84), indicating a high probability to select for yield using this trait.

Hershey (2005) cited data from an experiment of Ramanujan and Birade (1987) where they correlated a wide range of traits and concluded that yielding ability in a given variety is governed by the total biomass production and balanced proportioning of biomass, where petiole length contributed significant values. These results were supported by Jones (1970) and Kanju (2000) who have also found significant and positive correlation between petiole length and fresh root weight in sweet potato, suggesting therefore that petiole length might be of value in selection for yield in the breeding programme. However, the appropriated selection strategy in improvement programmes depend on the environment where it takes place and the gene pool used (Hershey, 2005).

Cassava brown streak root necrosis was significantly and positively correlated with root pulp hardness (r=0.19) and mature stem colour (r=0.21). CBSD affect all plant parts and the root symptoms are described as brown and corky (Calvet and Thresh, 2002), thus affecting the root pulp hardness. The leaf pubescence was significantly and positively correlated with mature leaf colour (r=0.34) and stem habit (r=0.23), but negatively correlated with unexpanded leaf colour (r=-42) and mature stem colour (r=-0.24).

Central leaf vein colour had high and significant correlation with petiole colour (r=0.79) and apical stem colour (r=0.64). There was also significant correlation

Table 3. 6 Estimates of phenotypic correlation for various characters evaluated during two seasons in Mogincual, 2004 and 2005

Trait	PH	ARN	FRW	CBSRr	RTST	DM	HI	PBs	NL	SHL	ULC	MLC	CNC	PLC	PTP	PTL	MSC	AST
ARN	0.25*																	
FRW	0.26*	0.26*																
CBSDr	0.12	0.05	0.034															
RTST	0.17	0.19*	0.09	-0.12														
DM	-0.15	-0.036	-0.17	0.19*	-0.04													
HI	0.12	0.09	0.17*	0.08	0.01	0.03												
PBs	0.11	0.06	0.24**	-0.70	0.33**	0.04	0.05											
NL	0.17	0.02	0.05	0.20	-0.4*	0.04*	0.06	-0.59										
SHL	0.02	-0.03	0.29	-0.17	0.29**	-0.18	0.04	0.57	-0.28									
ULC	-0.17	0.02	-0.12	0.03	-0.17	-0.08	-0.11	-0.42*	0.15	-0.29**								
MLC	0.02	-0.02	0.20	-0.01	0.06	-0.06	0.12	0.34**	-0.08	0.13	-0.06							
CNC	-0.06	0.15	-0.11	-0.16	0.21*	-0.25*	-0.2**	0.12	-0.47**	-0.15	0.23*	-0.14						
PLC	-0.05	0.16	-0.11	-0.09	0.2*	-0.14	-0.18*	0.08	-0.47*	-0.11	0.22*	-0.13	0.79**					
PTP	-0.01	-0.5	-0.24	0.06	0.07	-0.3	-0.01	0.01	0.11	-0.05	-0.12	-0.13	0.09	0.17				
PTL	0.08	-0.08	0.84*	0.03	0.05	-0.03	0.17	-0.04	0.04	0.08	-0.16	0.11	-0.17	-0.04	-0.04			
MSC	0.08	-0.14	-0.07	0.21*	0.22*	0.27*	-0.01	-0.24*	0.25	0.02	0.27**	0.03	-0.34*	-0.15	-0.11	0.12		
AST	-0.02	0.14	0.02	0.09	0.29**	-0.17	-0.15	-0.04	-0.37**	-0.03	0.16	-0.19	0.64**	0.63**	0.09	0.04	-0.21	
StH	-0.02	0.25	0.05	-0.12	-0.06	-0.01	0.01	0.23**	-0.09	0.06	-0.24*	0.12	0.09	0.02	-0.56	-0.01	-0.09	-0.01

PH=plant height, ARN=average root number, FRW=average fresh root weight, CBSDr= cassava brown streak necrosis, RTST= root taste, RPH=root pulp hardness; HI=harvest index, PBs=pubescence, NL= number of leaf lobe, SHL=shape of central lobe, ULC=unexpanded leaf colour, MLC=Mature leaf colour, CNC=central leaf vein colour, PLC=petiole colour, PTL=petiole length, MSC=mature stem colour, AST=apical stem colour, StH=stem habit, *$P<0.05$; ** $P<0.01$

between petiole colour and apical stem colour (*r*=0.63). These plant parts are all located in the shoot part of the plant and Elias *et al.* (2001) suggested that the pigmentation pathway in cassava plant parts may be correlated.

3.6.4 Estimates of heterosis

Mid-parent heterosis (MPH) analyses of seven characters are presented in Table 3.7. The largest heterosis value was observed for root pulp hardness (66.05) followed by average root number (34.69) and fresh root weight (32.33). Heterosis for plant height and harvest index was rarely observed. Only the cross Mulaleia x MZ 89186 (2.57) had a positive percentage of heterosis. Average root number was observed with significant heterosis values that varied from 2.09 to 34.69. MZ 89186 x Mulaleia (34.69), MZ 89186 x IMM 30325 (32.09), and MZ 89186 x Chigoma mafia (23.09) had the highest values.

Four combinations were observed with positive and significant heterosis for fresh root yield: Chigoma mafia x Mulaleia (32.33), MZ 89186 x IMM 30025 (12.74), MZ 89186 x Macia 1 (14.01) and the reciprocals IMM 30025 x Mulaleia (12.80).

Cassava brown streak necrosis is the most important biotic constraint in the country. Negative but therefore desirable, and significant heterosis values were observed for this characteristic and the combinations were variable, compared to the others already reported in this study. The best four mid-parent values observed were Macia 1 x MZ 89185 (-38.29), MZ 89186 x Macia 1 (-36.12), Chigoma mafia x Macia 1 (-29.75), MZ 89286 x IMM 30025 (-28.75) and Macia 1 x Chigoma mafia (-23.94).

Lower root taste values identifies the sweet tasting roots and were observed for Macia 1 x Mulaleia (-20.77), while the most bitter was Macia 1 x Chigoma mafia (22.31). In the small-scale farms it is common to find mixtures of varieties grown by farmers in Africa (Jones, 1959; Chiwona-Karltun *et al*., 2004). Sweet types are commonly used fresh and as snack, while the bitter ones are processed into flour prior to consumption and they are usually correlated to high levels of cyanogenic glucosides (Chiwona-Karltun *et al*., 2004).

Table 3. 7 Mean performance and percentage of mid-parent heterosis (MPH) for various characters evaluated during the 2004 and 2005 seasons

Parents and crosses	PH		ARN		FRW		CBSDr		RTST		RPH		HI	
	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH	Mean	MPH
P1	176.8		6.98		3.22		1.55		1.56		2.27		0.50	
P2	133.5		5.46		2.07		1.34		1.13		3.04		0.55	
P3	145.8		5.32		3.32		2.59		1.12		3.32		0.63	
P4	169.4		7.55		2.96		2.32		1.30		4.06		0.47	
P5	183.7		7.87		2.82		2.92		1.04		2.62		0.39	
1x2	141.3	-8.91*	6.35	2.09**	3.50	32.33**	1.63	12.80*	1.20	-10.78**	2.77	4.33	0.40	-23.81*
1x3	132.5	-17.83	6.11	-0.65*	2.26	-30.89	2.13	2.90	1.58	17.91*	3.26	16.64	0.39	-30.97*
1x4	147.8	-14.60	6.94	-4.47*	2.69	-12.94*	2.32	19.90	1.44	0.70*	3.90	23.22	0.47	-3.09*
1x5	132.6	-26.42**	5.59	-24.71*	1.59	-47.35	1.57	-29.75**	1.29	-0.77**	3.34	36.61	0.34	-23.60*
2x3	142.2	2.57**	6.97	1.58**	2.07	-0.63**	2.02	0.06	1.29	0.17*	3.77	18.55	0.41	-0.18
2x4	135.4	-8.02	5.21	-0.65*	3.10	0.29*	1.93	0.05	1.29	0.04*	3.79	6.76	0.53	0.01**
2x5	154.6	-14.19**	6.43	-13.40*	2.99	-0.99	2.08	-6.94	1.42	9.23*	3.68	50.51*	0.46	3.37**
3x4	140.9	-10.58	8.50	32.09**	3.54	12.74**	1.75	-28.72*	1.34	10.74**	3.73	1.08	0.43	-21.82
3x5	142.8	-13.31*	7.73	17.21**	3.50	14.01*	1.76	-36.12*	1.21	12.04*	3.99	34.34	0.42	-17.65
4x5	141.1	-20.07*	5.22	-32.30*	2.09	-27.68*	1.87	-28.63*	1.32	12.82	2.97	-11.08**	0.47	9.30**
2x1	152.4	-1.79*	1.07	-82.80*	2.70	2.08	2.01	39.10*	1.26	-6.32*	2.79	5.08*	0.45	-14.29
3x1	128.6	-20.23*	7.57	23.09**	2.58	-21.10	1.74	-15.94**	1.28	-4.48**	3.78	35.24	0.45	-20.35*
3x2	135.0	-3.32*	7.26	34.69**	2.31	-14.29	2.41	22.65*	1.34	19.11*	4.21	32.39*	0.41	-30.51*
4x1	135.0	-21.99*	6.94	-4.47*	2.53	-18.12	1.83	-5.43	1.47	2.80	3.64	15.01	0.45	-7.22*
4x2	169.4	-4.04*	6.60	-14.40*	3.26	12.80**	2.68	2.29	1.11	-5.13	3.94	17.96**	0.47	9.30**
4x3	150.4	-4.55*	7.36	14.37**	2.70	-14.01	2.06	-16.09**	1.11	-8.26*	3.97	7.59*	0.51	-7.27*
5x1	166.7	-7.50*	5.47	-26.33*	2.72	-9.93	1.70	-23.94**	1.59	22.31**	4.06	66.05**	0.42	-5.62*
5x2	153.0	-15.10**	4.89	-34.14*	2.14	-29.14	2.10	-6.04*	1.03	-20.77**	3.00	22.70*	0.37	-16.85*
5x3	158.5	-3.78*	4.12	-37.53*	1.09	-64.50*	1.70	-38.29**	1.12	3.70*	3.60	21.21**	0.42	-17.65*
5x4	160.8	-8.91*	4.43	-42.54*	3.13	8.30**	2.70	3.05	1.30	11.11*	4.10	22.75*	0.50	16.28**

Parent: 1=Chigoma mafia, 2=Mulaleia, 3=MZ 89186, 4=IMM 30025, 5=Macia 1, PH=plant height, ARN=average root number, FRW=fresh root weight; CBSDr=cassava brown streak necrosis, RTST= root taste, RPH=root pulp hardness; HI=harvest index, **P<0.05; ** P<0.01*

Root pulp hardness is also one of the traits of commercial importance in cassava. The only combination observed with highly significant and negative values for root pulp hardness was IMM 30025 x Macia 1 (-11.8). The classes used in this study, varied from 1 to 5, where the lowest was better, which means that for among all hybrid combinations for MPH, the cross between IMM 30025 x Macia 1 (-11.8) was the best.

Harvest index had only four combinations that had positive and significant mid-parent heterosis. They were Mulaleia x Macia 1 (3.317), IMM 30025 x Mulaleia (9.30), IMM 30025 x Macia 1 (9.30) and its reciprocal Macia 1 x IMM 30025 (16.28).

From this analysis it can be observed that it is difficult to produce hybrids with a combination of the most important economic traits, such as fresh root yield, CBSDr and root pulp hardness.

3.6.5 Estimates of genetic parameters

The general combing ability variances (δ^2_{gca}) were low, in general, compared to SCA variances (δ^2_{sca}). Plant height had higher values of δ^2_{gca} in both years than δ^2_{sca} (Table 3.8). When δ^2_{gca}, is higher than δ^2_{sca}, it indicates the contribution of both additive and non-additive variability for inheritance of traits in this study. For the characters with δ^2_{gca} higher than δ^2_{sca}, it is an indication that they should respond favourably to direct selection. This is more reliable in cases when parents are selected randomly. In this case, cassava is a highly heterozygous crop and in particular for this study the parents were selected for their performance in a high pressure CBSD environment in Mozambique, capacity of seed production and the capacity of their F_1 progeny to produce five stem cuttings. The relative amount of GCA variance measured in this case, might have been under-estimated. However, significant SCA effects were also observed, which Stuber (1970) suggested were probably the result of additive x additive epistatic effects. Negative variances are not subject to analysis in this study, but their calculations were represented in the table.

The broad sense (h^2b) and narrow sense (h^2n) heritability were estimated for all the characteristics and were also presented in Table 3.8. Broad sense heritability varied from 95.2 (HI) to 38.6 (FRW). Among all the characters, plant height, root taste, root pulp hardness, CM and harvest index, had the highest heritability values. Score scales are subject to change depending on the objective, for example using different scales, the results may differ to that obtained in this study. The heritability of CBSDr was lower due to its poor GCA:SCA ratios, which consequently produced negative δ^2_{gca} and δ^2_A , a clear indication that it is a polygenic trait. The SCA variance was higher than the GCA variance, thus, the SCA is more important in predicting progeny performance for expression of resistance to CBSDr.

High broad sense heritability indicated that the characteristics had high genetic variance, both additive and non-additive. In this study, most of the characteristics measured 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 this case, the narrow sense heritability of all characteristics was relatively low except for harvest index. Falconer and Mackay (1996) reported that the lower narrow sense heritability was caused by low additive effects and high dominant gene action.

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, as 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 hybridisation mainly broad-sense in nature.

The average degree of dominance ($\sqrt{H/D}$) for the characters was less than unit for average root number and harvest index (Table 3.8), suggesting partial dominance. The opposite suggests the presence of dominance. The degree of

dominance was greater than unit for plant height, fresh root yield, root taste, root pulp hardness and cassava mealybug, indicating the presence of over-dominance for these characters.

The predictability ratio (PR) is important to estimate the relative importance of progeny performance. 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 components of variance to predict the progeny performance. The closer the ratio is to a unit, the greater the predictability based on GCA alone. The predictability ratio in this study varied from the lowest 0.13 to 1.0. The character with the ratio closest to 1 was harvest index (0.9 to 1.0). Jaramillo *et al.* (2005), using a different approach for analysis, found similar results for harvest index and suggested that GCA effects were more important than SCA for harvest index.

Table 3.8 Estimates of genetic parameters for various characters evaluated during two seasons in Mogincual, 2004 and 2005

Character	Years	Genetic parameter								
		δ^2_{gca}	δ^2_{sca}	δ^2_e	δ^2_A	δ^2_D	$H^2_{(b)}$ (%)	$h^2_{(n)}$ (%)	PR	√□(H/D)
PH	2004	214.333	371.310	142.690	428.667	371.310	84.9	45.47	0.54	1.32
	2005	45.047	232.630	222.780	90.093	232.630	59.2	16.52	0.28	2.27
ARN	2004	-0.122	0.545	0.412	-0.245	0.545	42.2	-34.35	-0.81	
	2005	-0.241	-3.116	4.574	-0.481	-3.116	-36.8	-49.28	0.13	0.31
FRW	2004	0.054	0.134	0.227	0.108	0.134	51.6	23.03	0.45	1.58
	2005	0.041	0.075	0.251	0.083	0.075	38.6	20.23	0.52	1.35
CBSDr	2004	-0.037	0.241	0.074	-0.074	0.241	69.3	-30.71	-0.44	-
	2005	-0.030	0.300	0.260	-0.060	0.300	48.0	-12.00	-0.25	-
RTST	2004	0.023	0.041	0.019	0.047	0.041	82.2	43.75	0.53	1.33
	2005	0.030	0.030	0.020	0.060	0.030	81.8	54.55	0.67	1.00
RPH	2004	0.140	0.350	0.100	0.280	0.350	86.3	38.36	0.44	1.58
	2005	0.068	0.710	0.166	0.135	0.710	83.6	13.38	0.16	3.24
CM	2004	0.015	0.117	0.009	0.030	0.117	94.2	19.23	0.20	2.79
	2005	-0.017	0.070	0.010	-0.033	0.070	78.6	-	-	-
HI	2004	0.001	0.001	0.001	0.003	0.001	78.6	57.14	1.00	0.00
	2005	0.009	0.001	0.001	0.019	0.001	95.2	90.32	0.95	0.33

PH=plant height, ARN=average root number, FRW=average fresh root weight, CBSDr= cassava brown streak necrosis, RTST= root taste, RPH=root pulp hardness; CM=cassava mealybug, HI=harvest index, h^2 (b)= broad sense heritability, h^2(n)=narrow sense heritability

3.7 Conclusions and recommendations

The present study generated relevant information for planning a more efficient cassava breeding programme for Mozambique. The analysis of variance and the GCA: SCA ratio indicated that the GCA was larger than SCA for average root number, average fresh root yield, root taste, root pulp hardness yield and harvest index, indicating the presence of additive gene effects and a possibility for improvement for this characters.

The parental genotypes were selected from a particular region where CBSD is the main economic constraint. Chigoma mafia had the best GCA effect for plant height, average root number and cassava brown streak root necrosis, while the clone IMM 30025, had the best GCA effect for fresh root weight and root pulp hardness. However, the combination Chigoma mafia x Mulaleia, had the best mean performance for fresh root weight and cassava brown streak root necrosis. While the combination with parents Macia 1 x Chigoma mafia had the best SCA for fresh root weight, cassava brown streak root necrosis and root pulp hardness. With considerations to the cassava brown streak root necrosis constraint, the combinations MZ 89186 x IMM 30025 and the reciprocal IMM 30025 x MZ 89186, Macia 1 x Chigoma mafia and MZ 89186 x Mulaleia were the best for resistance to cassava brown streak root necrosis. The two sets are important for different traits and should be considered as parents in the next hybridisation scheme.

In terms of mid-parent hybrid performance, three had combinations with traits of interest for the breeding programme. The hybrid combination, their respective characters and mid-parent performances were as follows: (i) Chigoma x Mulaleia: average root number (2.09), fresh root weight (32.33), root pulp hardness (4.33); (ii) MZ 89185 x IMM 30025: average root number (32.09), fresh root weight (12,74), root taste (10,74), cassava brown streak root necrosis (-28.75), but root pulp hardness 1.08; (iii) MZ 89186 x Macia 1: average root number (17.21), fresh root weight (14.01), cassava brown streak root necrosis (-36.12), root taste (12.04).

The broad sense heritability estimates were moderate to high. It varied from 38.9 observed for fresh root weight to as high as 95.52 for harvest index, as clear indication that the phenotypic variance was additive. However some characters showed non-additive gene action. Harvest index had a GCA variance higher than SCA variance its predictability ratio was close to unit, confirming that this character is highly heritable and under additive gene action.

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CHAPTER 4

GENETIC DIVERSITY ANALYSIS OF 17 ORIGINAL DIALLEL CROSS GENOTYPES BY MEANS OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) ANALYSIS

4.1 Introduction

The knowledge of genetic distance of gene pools in a breeding programme is useful because it permits the organization of germplasm and provides information for more efficient parental selection. It may also help the breeders to concentrate their efforts on the most promising combinations (Carpentieri-Pípolo *et al.,* 2000). The choice of parents is an important step in a breeding programme and it can be achieved by the knowledge of their performance and genetic relatedness. Genetic distance estimates have been widely used for descriptive analysis in crop plants as well as in assigning lines to heterotic groups (Cheres *et al.,* 2000).

Genetic diversity can be revealed by a number of methods including pedigree data, morphological data, agronomic performance, biochemical data, and recently molecular (DNA-based) data (Mohammadi and Prasanna, 2003). The DNA-based molecular markers reveal polymorphisms at 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, 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; Melchinger *et al.,* 1994) and give rise to a higher number of polymorphisms (Karp *et al*., 1997). Molecular marker techniques include restriction fragment length polymorphisms (RFLPs) (Beckman and Soller, 1983), simple sequence repeats (SSRs) or microsatellites

(Tautz, 1989), random amplification of polymorphic DNA (RAPDs) (Williams *et al.,* 1990; Welsh and McClelland, 1990; Karp *et al.,* 1997) and amplified fragment length polymorphisms (AFLPs) (Vos *et al.,* 1995). DNA markers have been successfully used in cassava and contributed to cassava breeding and genetics in understanding the phylogenetic relationship in the genus (Roa *et al.*, 1997; Olsen and Schaal, 1999; 2004), assessing the genetic diversity (Beeching *et al*., 1993; Mkumbira *et al*., 2003; Elias *et al*., 2000; 2001), helping with the development of genetic maps and identification of quantitative loci (QTL) for some traits of importance (Fregene *et al.,* 1997; Jorge *et al*., 2001; Okogbenin and Fregene, 2003)

AFLP provide a high level of resolution, allowing for the delineation of complex genetic structures (Powell *et al.,* 1996). Two restriction endonucleases are used to produce restriction fragments, *Eco*RI as rare, and *Mse*I as frequent cutter enzyme. These allow the researcher to manipulate the number of fragments generated for amplification and produce a fingerprint of desired complexity. Its analysis allows detection of polymorphisms (Krauss, 1999) that are distributed across the genome, as it has a high multiplex ratio. Each fragment is assumed to originate from a different area of the plant genome (Rafalski *et al.,* 1996).

AFLP markers have been utilised in evaluating hybrids and in parentage assignment in many species (VanToai *et al.,* 1996; Krauss, 2000; Thottapilly *et al.*, 2000; Lima *et al.,* 2002). AFLP studies carried out in soybean revealed that it is possible to assess, with adequate precision and reasonable cost, the parental contributions to subsequent progeny generations (VanToai *et al.,* 1996). AFLP markers stand out as one of the most effective methods to detect polymorphism in cassava (Weising *et al.*, 2005).

The aim of this study was to analyse the genetic distance and parental potential of 17 cassava accessions, which represents the first set of parents of the cassava breeding programme in Mozambique, by AFLP markers.

4.2 Material and methods

4.2.1 Planting material

Fresh leaf samples of the 17 entries were collected from the cassava gene bank in Nampula, Mozambique. The entries comprised of 13 landraces, and four introduced clones from IITA (Table 4.1). All accessions are part of the field gene bank and recently were selected as parents for the breeding nursery, after being identified by farmers for their good root quality, resistance and tolerance to cassava brown streak disease (CBSD) and lastly, their capacity to produce usable flowers for hybridization. The IITA clones were identified as the best parents for introgression of cassava mosaic disease (CMD) resistance within the gene bank. Leaf samples were collected in the cassava field in the Nampula province, packed between moist tissue paper and sent to the University of the Free State, South Africa, where the samples were freeze-dried at -60^{0}C for 3 days and preserved at -70^{0}C.

4.2.2 DNA extraction

The DNA extraction was done using a modified method of Dellaporta *et al.* (1983). The extraction buffer composed of 100 mM Tris-HCL, 50 mM ethylene-diaminetetraacetate (EDTA), 500 mM NaCl, 700 µl of β-Mercaptoethanol and 1% (w/v) Polyvinylpirrodine (PVP-40). Lyophilised leaf material (0.2 g) was ground to a fine powder using a TissueLyser (Qiangen). Subsequently, 800 µl of extraction buffer, at 65^{o}C, containing 2% (w/v) sodium dodecyl sulphate (SDS) were added to the microfuge tubes containing the ground tissue. The mixture was incubated at 65^{o}C for 15 minutes and vortexed frequently. Ice-cold potassium acetate (5 M) was added to the homogenate and incubated for 20 minutes. The phases were separated by centrifugation for 10 minutes at 12000 rpm. The supernatant was transferred to a new tube and 700 µl (v/v) of ice-cold iso-propanol was added and incubated at -80^{o}C for one hour, followed by centrifugation at 12000 rpm for 10 min. The pellet was resuspended in 500 µl of 50 mM Tris-HCl and 10 Mm EDTA and incubated at 65^{o}C. Ice-cold iso-propanol (500 µl) was added to the tube and incubated again at -80^{o}C

for one hour. The supernatant was removed after centrifugation at 12000 rpm for 10 min, and the resulting pellet was suspended in 200 ul TE buffer (10 mM Tris-HCl, pH8.0; 1 Mm EDTA, pH 8.0) containing 10 mg/ml RNAse.

Table 4.1. List of entries used for the AFLP study

Entry	Origen	Main use
Nikwaha	Nampula	Fresh and flour
TMS 30001	IITA improved clone	Breeding line
Chigoma mafia	Cabo Delgado	Fresh
Mulaleia	Zambezia	Flour
Mocuba	Zambezia	Fresh and flour
Nachinaya	Cabo Delgado	Fresh and flour
Mucudo muevia	Zambezia	Flour
IMM 30025	IITA improved clone	Breeding line
Munhaca	Maputo	Fresh
Namuhiripwi	Nampula	Flour
Munamwahula	Nampula	Flour
MZ 89001	IITA improved clone	Breeding line
MZ 89186	IITA improved clone	Breeding line
Likonde	Cabo Delgado	Flour
Macia1	Zambezia	Fresh and flour
N'xinkole	Cabo Delgado	Fresh and flour
Baadje	Cabo Delgado	Fresh

4.2.3 DNA concentration, quality and integrity determination

DNA concentration and purity were determined using a UV spectrophotometer (Hitachi U-2000) by measuring absorbance at 260 and 280nm. The DNA concentration was calculated as:

[DNA] = optical density (OD_{260})*dilution factor*constant (50µg/ml)

The DNA purity was estimated by the ratio between A_{260} and A_{280}. The purity and quality of the DNA was verified by electrophoresis on a 0.8% (w/v) agarose gel in 1 x UNTAN running buffer (40 mM Tris-HCl, 2 mM EDTA, pH adjusted to 7.4 with acetic acid) containing ethidium bromide, for 60 min at 80 volts. Gel Doc 100 was used to visualise DNA under UV light with the aid of Molecular Analyst software. DNA

samples were diluted to a final concentration of 200 ng/µl in 0.1xTE buffer pH 8.0 and stored at 4^{o}C, until further use.

4.2.4 AFLP analysis

AFLP analysis was performed following the method of Vos *et al.* (1995) modified by Herselman (2003) using primers and adaptors synthesised by Integrated DNA Technologies, Inc, USA. DNA was digested using *Eco*RI and *Mse*I as described by Vos *et al.* (1995). The primer combinations *Eco*-RI and *Mse*I were represented as E- and M- respectively and are given in Table 4.2.

Table 4.2 Adapter and primer sequences used for AFLP pre-amplification and selective amplification

Enzyme	Type	Sequence (5'-3')
*Eco*RI	Adaptor-F	CTCGTAGACTGCGTACC
	Adaptor-R	AATTGGTACGCAGTCTAC
*Mse*I	Adaptor-F	GACGATGAGTCCTGAG
	Adaptor-R	TACTCAGGACTCAT
*Eco*RI	Primer +1	GACTGCGTACCAATTCA
*Eco*RI + ACT	Primer + 3	GACTGCGTACCAATTCACT
*Eco*RI + ACA		GACTGCGTACCAATTCACA
*Eco*RI + ACC		GACTGCGTACCAATTCACC
*Eco*RI + ACG		GACTGCGTACCAATTCACG
*Mse*I	Primer +1	GATGAGTCCTGAGTAAC
*Mse*I + CAA	Primer + 3	GATGAGTCCTGAGTAACAA
*Mse*I + CAT		GATGAGTCCTGAGTAACAT
*Mse*I + CTT		GATGAGTCCTGAGTAACTT
*Mse*I + CGT		GATGAGTCCTGAGTAACGT
*Mse*I + CAG		GATGAGTCCTGAGTAACAG

4.2.4.1 Double digestion and ligation of genomic DNA

Genomic DNA (200ng) was digested at 37°C for five hours using 1 x *Mse*-Buffer and 4 U *Mse*I in a final volume of 40 µl. Thereafter it was further digested with 5 U EcoRI and 100 mM NaCl and incubated overnight at 37°C. Adaptor ligation of digested DNA was performed by adding a solution containing 50 pmol of *Mse*I-adaptor, 5 pmol *Eco*RI-adaptor, 1U T4 DNA Ligase [91 unit/ µl in 10 mM Tris-HCl pH 7.5, 1 mM DTT, 50 mM KCl and 50% glycerol (v/v)], 0.4 mM Adenosine triphosphate (ATP) and 1 x Ligase buffer (66 mM Tris-HCl pH 7.6, 6.6 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 66 µM ATP) followed by overnight incubation at 16°C.

4.2.4.2 Pre-selective amplification reactions

Pre-selective amplification reactions were performed in 50µl reaction mixtures containing 5 µl template DNA (undiluted ligation mixture), 1 x GoTaq® Flexi buffer (colourless), 2 mM $MgCl_2$, 0.02 U GoTaq ® Flexi DNA polymerase (Promega, Madison, USA) 200 µM of each dNTP and 30 ng of each pre-selective primer (*Eco*RI and *Mse*I. Reactions were performed using the following programme: 30 cycles of 30 seconds at 94°C, 60 seconds at 56°C and 60 seconds at 72°C (Herselman, 2003). Quality and quantity of pre-selective reactions were determined by electrophoresis in 1.0% (w/v) agarose gels, at 60 volts for 45 minutes. Pre-selective amplification products were diluted accordingly (1:30 times), prior to selective amplification.

4.2.4.3 Selective amplification reactions

The selective amplification was conducted in a final volume of 20 µl, containing 5 µl diluted pre-amplification product, 30 ng *Mse*I-primer, 3,30 ng *Eco*RI Primer, 1 x GoTaq® Flexi buffer (colourless) and 0.75 U GoTaq ® Flexi DNA Polymerase. The following cycling programme was used for selective amplification: one cycle of denaturation at 94°C for five minutes followed by one cycle of 30 seconds at 94°C, 30 seconds at 65°C, and 60 seconds at 72°C. The annealing temperature was lowered by 1°C per cycle during the next eight cycles after which 25 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 60 seconds were performed, followed by one last elongation of five minutes at 72°C. Primer combinations are listed in Table 4.2.

4.2.4.4 Polyacrylamide gel electrophoresis and silver staining

Prior to loading, amplification products were mixed with an equal volume of formamide loading buffer [98% (v/v) de-ionised formamide, 10 mM EDTA pH 8.0, 0.05% (w/v) bromophenol blue, and 0.05% (w/v) xylene cyanol] and denaturated at 95^0C for five minutes. The mixture was immediately placed on ice. Aliquots of 5 µl of each sample were separated on 5% denaturating polyacrylaminde gels [19:1 acrylamide:bis-acrylamide, 7 M urea and 1 X TE buffer (89 mM Tris-HCl, 89 mM Boric acid, 20 mM EDTA)] at a constant power of 80 W for two hours.

AFLP gels were stained according to the instructions of Silver Sequence ™ DNA Sequencing System protocol supplied by Promega (Madinson, WI, USA). Gels were left to air-dry overnight, and photographs were taken by exposing photographic paper (Ilford Multigrade IV RC de Luxe) placed under the gel, to dim light for approximately 20 seconds. This produced a negative image of the same size and scale as the gel. AFLP fragment lengths were determined by comparison with a 100 bp DNA ladder (Promega).

4.3 Data analysis

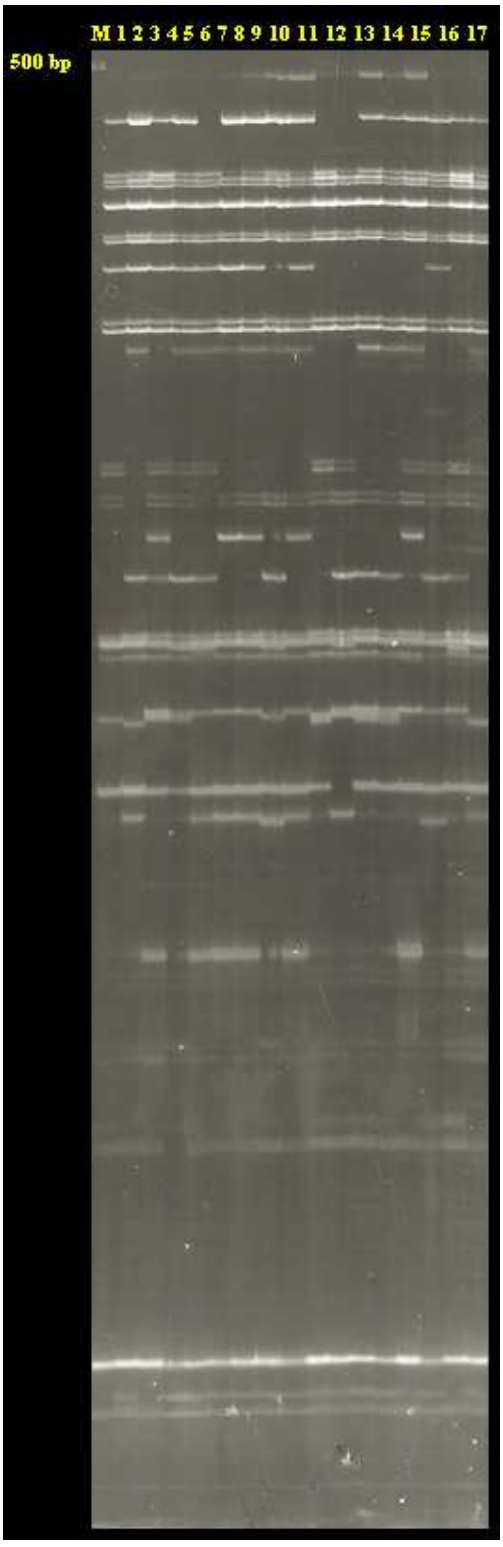
All unambiguous AFLP fragments were scored manually using binary unit characters for presence (1) or absence (0) across the 17 accessions for the nine primer combinations utilised. Fragments, smaller than 40 bp, were excluded from the data matrix. The binary data matrix was used to calculate the genetic similarity matrix using Dice Similarity coefficient (Dice, 1945) with the help of the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc), version 2.02i (Rohlf, 1993). The similarity matrix was subjected to UPGMA (unweighted pair group method analysis; Sokal and Michener, 1958) clustering and utilized to construct the dendrogram using the SAHN programme of NTSYS. For each dendrogram the cophenetic correlation coefficient between the genetic similarity matrix (original distances) and the cophenetic distances were computed using the appropriate routines of the COPH and MXCOMP programme of NTSYS-pc. The significance was tested using the Mantel correspondence test (Mantel, 1976) to test the goodness of fit between the similarity and the cophenetic matrices (Sneath and Sokal, 1973).

4.4 Results and discussion

4.4.1 Primer combination and fragments

The primer combinations M-CAA x E-ACA, M-CTT x E-ACT, M-CTT x E-ACC and M-CAT x E-ACA were among those recommended by Benesi (2005). The primer combination M-CAT x E-AAC, was discarded due to poor amplification. Thus, data from eight individual primer combinations were analysed, separately with successive addition of data from different primer combinations (Figure 4.1).

A total of 425 fragments were produced by the eight primer combinations, with an average of 61.88% polymorphic fragments (Table 4.3). The fragments ranged from 50 bp to 500 bp with an average of 53.13 fragments produced per primer combination. A total of 263 fragments were polymorphic with an average of 32.88 fragments per primer combination. The primer combination M-CTT x E-ACT produced the lowest number of total amplified fragments, 37, followed by M-CAA x E-ACT with 42 fragments. The polymorphic fragments produced ranged from 22 to 48. Primer combination M-CAA + E-ACT produced the lowest number of polymorphic fragments (22) and the highest was observed with primer combination M-CGT x E-ACT (48). Primer combination M-CTT x E-ACT scored with highest percentage of polymorphic fragments (81.1%). The primer combination M-CAA x E-ACA produced the lowest percentage polymorphic fragments (46.8%)



Legend:

M	100 bp DNA Ladder
1	Nikwaha
2	TMS30001
3	Chigoma mafia
4	Mulaleia
5	Mocuba
6	Nachinhaya
7	Mucudo moeviha
8	IMM30025
9	Munhaça
10	Namuhiripwe
11	Mwnamwhuua
12	MZ89001
13	MZ89186
14	Likonde
15	Macia1
16	Nxinkole
17	Baadje

Figure 4.1 Photograph of a silver stained, 5% denaturating polyacrylamide gel. AFLP fragments were amplified using the primer combination M-CGTXACT

Table 4.3. Number of fragments and polymorphisms detected by AFLP primer combinations of 17 accessions

Primer combination	Total fragments	Monomorphic fragments	Polymorphic fragments	Percentage of polymorphic fragments
M-CAA x E-ACT	42	20	22	52.38
M-CAT x E-ACA	46	11	35	76.09
M-CTT x E-ACC	49	21	28	57.14
M-CTT x E-ACT	37	7	30	81.08
M-CAA x E-ACA	79	42	37	46.84
M-CGT x E-ACG	46	15	31	67.39
M-CAT x E-ACT	43	11	32	74.42
M-CGT x E-ACT	83	35	48	57.83
Total fragments	425	162	263	61.88
Average fragments	53.13	20.25	32.88	64.15

4.4.2 Estimates of genetic distance

Genetic distance estimates for all pairwise combinations of the 17 genotypes are presented in Table 4.4. Highest and lowest genetic distances were observed within and between clusters, respectively. The highest Dice similarity coefficient was observed between the clones MZ89001 and MZ 89186 (0.928). Both of these were introduced cassava genotypes and were selected from botanical seed in Mozambique. Within the local germplasm, high similarities were observed between the parents Nachinaya and Mucudo muevia (0.917), followed by Mwanawahula and Mucudo mevia (0.886). The average genetic distance was 0.836 across the five parents.

Table 4.4 Dice similarity coefficients for AFLP characterisation of 17 analysed accessions

Parent	Nikwa	TMS	Chigo	Mulal	Mocuba	Nachi	Mucudo	IMM	Munh	Namuh	Munam	MZ891	MZ896	Likonde	Macia1	Nxink
Nikwa	1.000															
TMS	0.816	1.000														
Chigo	0.828	0.845	1.000													
Mulal	0.790	0.829	0.834	1.000												
Mocuba	0.730	0.739	0.756	0.790	1.000											
Nachi	0.770	0.802	0.818	0.811	0.819	1.000										
Mucudo	0.773	0.819	0.813	0.818	0.779	0.917	1.000									
IMM	0.784	0.822	0.809	0.821	0.742	0.816	0.832	1.000								
Munh	0.756	0.812	0.817	0.811	0.760	0.855	0.886	0.841	1.000							
Namuh	0.742	0.792	0.801	0.787	0.694	0.768	0.787	0.806	0.814	1.000						
Munam	0.801	0.837	0.832	0.812	0.733	0.789	0.817	0.836	0.821	0.833	1.000					
MZ891	0.789	0.827	0.815	0.803	0.705	0.763	0.797	0.852	0.811	0.795	0.887	1.000				
MZ896	0.789	0.833	0.841	0.811	0.718	0.791	0.815	0.856	0.830	0.781	0.853	0.928	1.000			
Likonde	0.781	0.833	0.831	0.814	0.750	0.798	0.812	0.818	0.827	0.792	0.831	0.846	0.874	1.000		
Macia1	0.798	0.822	0.827	0.814	0.729	0.801	0.807	0.793	0.808	0.799	0.820	0.823	0.839	0.865	1.000	
Nxink	0.793	0.796	0.808	0.756	0.709	0.803	0.820	0.781	0.803	0.786	0.834	0.808	0.799	0.813	0.827	1.000
Baadje	0.770	0.759	0.750	0.723	0.693	0.736	0.768	0.732	0.734	0.720	0.772	0.749	0.757	0.789	0.788	0.820

Nikwa=Nikwaha, TMS=TMS 30001, Chigo=Chigoma mafia, Mulal=Mulaleia, Nachi=Nachinaya, IMM=IMM 30015, Munh=Muhaça, Namuh=Namuhiripwi, Munam=Mwanamwahula, MZ891=MZ 89001, MZ896=MZ 89186; N'xink= N'xinkole

The lowest genetic similarity was observed between local varieties Mocuba and Namuhiripwi (0.694), followed by Mocuba and Baadge (0.693). They were collected in different places and they represented the most dissimilar material in this study. The classification of the best parental combinations, based on distance analysis, could provide a basis for selecting parents for the hybridisation programme (Zhong-hu, 1991). Moreover, Mocuba was collected in Zambezia, while the variety Namuhiripwi was collected in Nampula and Baadge in Cabo Delgado. The variety Baadge showed an average genetic distance coefficient of 0.734, suggesting good performance as parent. Baadge has some distinct morphological characteristics such as larger and thicker leaf lobes and very sweet roots. CBSD root necrosis has not been recorded so far in this variety. However, small scale farmers reported high incidences of theft for this variety due to very tasty roots, both fresh and boiled, although it is difficult to peel the roots.

The lowest dissimilarity between introduced and local cultivars was found between MZ 89001 and Mocuba with a genetic distance of 0.704, showing some dissimilarity with local varieties, although, not lower than the genetic distance between the most dissimilar cultivars, Mocuba and Baadge, which was 0.685. The information regarding genetic distance is useful as it helps to organize the germplasm and guarantee more efficient decision making regarding the choice of parents to use in hybrid production, and to maximize the expression of heterosis (Smith *et al.,* 1990). The choice of parents and crosses should be those with larger genetic distances such as Nikwaha, Baadge, Mocuba, Nachinaya and Namuhiripwe. These are local varieties and have a wide range of adaptation to the local environments and have desired quality characters, which are important in cassava. The information regarding genetic distance should be combined with cultivars' performance of important traits (Carpentieri-Pípolo *et al.,* 2000; Miranda *et al.,* 1988; Rangel *et al.,* 1991; Destro, 1991) for a successful decision of parental selection in a breeding programme (Carpentieri-Pípolo *et al.,* 2000).

Another important fact was that the five parents that were analysed in Chapter 3, were also included in this study. It was observed that parent 4 (IMM 30025) and parent 5 (Macia 1) were the most dissimilar (0.793) and the average genetic distance between the five parents was 0.803. At the time when the hybridization was

conducted for the diallel trial, very little was known about the flowering period of some varieties, including Mocuba and Nikwaha, which significantly reduced the ability of producing enough seeds. Recent documentation by Matoso *et al*. (*in press*) briefly discussed the flowering period of cassava in Northern Mozambique.

4.4.3 Cluster analysis

The dendrogram based on UPGMA cluster analysis constructed from the AFLP markers revealed two major clusters, A and B at a genetic similarity of 0.78 (Figure 4.2). Cluster A was the smallest cluster and contained only variety, Mocuba, while cluster B contained 16 entries. Mocuba was grouped separately at a genetic similarity (GS) of 0.74. The distinctiveness of Mocuba from the rest of cultivars includes good root traits and resistance to CBSD reported by farmers which also contributes to the high adoption rate of the variety among farmers in the newly introduced production areas (Cuambe *et al.,* 2007).

Cluster B was divided into two sub-clusters, I and II, with sub-cluster I consisting of two varieties, Baadge and N'xinkole, with a genetic similarity of 0.82. Both were collected in the Cabo Delgado province. Sub-cluster II contained 14 varieties and was subdivided in two main groups i and ii. Group ii had one variety, Nikwaha, with 77% dissimilarity to the rest of the group. Nikwaha is an important variety in the community due to tolerance to the main biotic constraints and was recently described as horizontally resistant to CBSD (Cuambe *et al.,* 2007). However, data so far compiled on flowering have shown that Nikwaha produces abundant flowers, but a high number of flowers are aborted when controlled crosses are made.

Sub-group i comprised of 13 entries and was also divided into three main groups ia, ib and ic, with 81.1% dissimilarity between ia and ib, 82.2% dissimilarity between ib and ic. Sub-group ic had one variety, Namuhiripwe. This local variety was collected in Nampula. Contrary to other varieties, Namuhiripwi has a bitter taste, and farmers usually process it into flour. Another characteristic of this variety is late flowering, compared to the rest of the 16 varieties in the breeding nursery, suggesting that flower synchronization should be done to get as much full-sib seeds as possible. Sub-group ib comprised of local varieties from different origins of collection,

Munhaça collected in Maputo, in the south of the country, Mucudo muevia from Zambezia and Nachinaya was collected in Cabo Delgado; sub-group ia comprised of nine entries and only the varieties Chigoma mafia and Macia 1, have a sweet taste. Within this sub-group, local varieties and introduced clones were clustered together and sub-dived into four smaller groups.

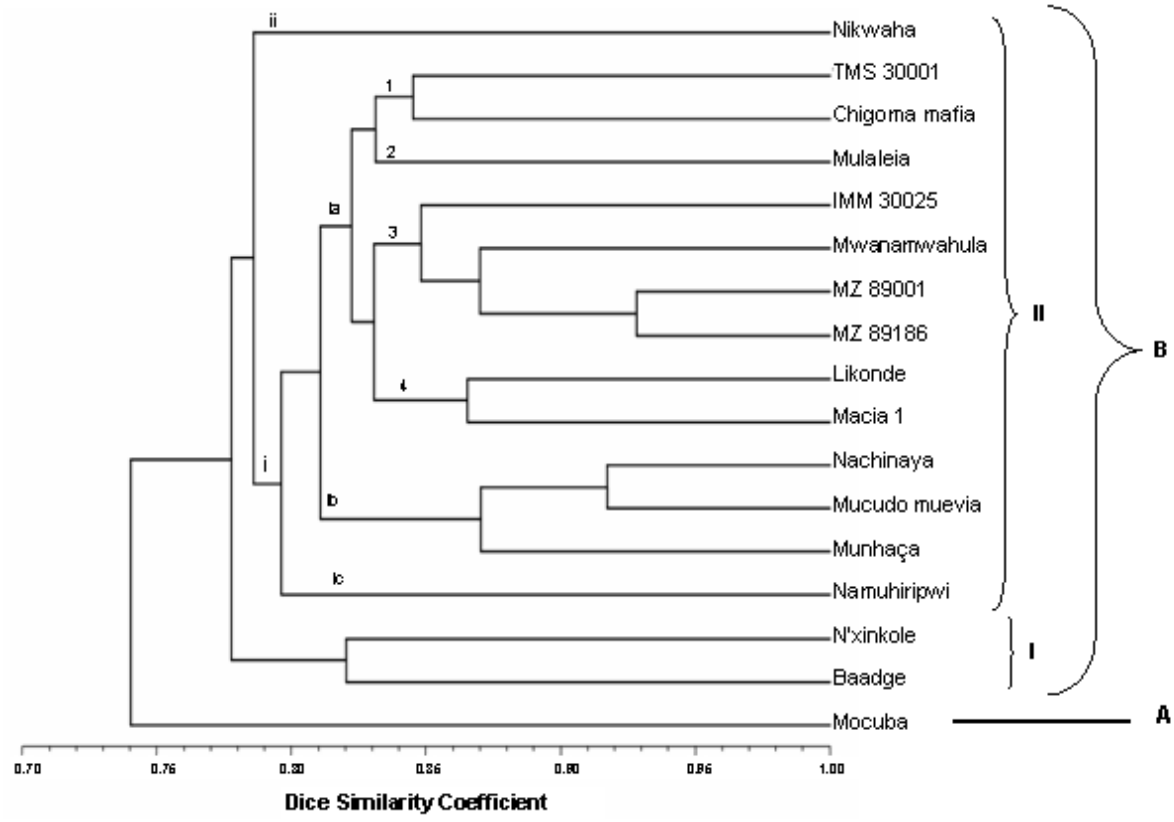


Figure 4.2. Dendogram based on the UPGMA cluster analysis of genetic similarity estimates using Dice similarity coefficient

Many authors agree with selection of parents for hybridization purposes from inter-cluster materials (Aruanachalam *et al.,* 1984; Bhatt, 1970; Chauhan and Singh, 1982; Zhong-hu, 1991; Benesi, 2005), hybrid variability can then be maximized. Thus, crosses within the same cluster group should be avoided (Destro, 1991).

Heterotic groups are very important in maize and from this study, two heterotic groups with utility for the Mozambican breeding programme for hybridization, are suggested, based on the genetic distance and clustering:

- Heterotic Group A (HGA), comprised of all genotypes from Cluster B II ia and ib;
- Heterotic Group B (HGB), comprised of genotypes from Clusters A, B I, B II ic and B II ii.

The HGB is coincidently the one already proposed as containing the best parents due to the large genetic distances observed, as proposed by Bhatt (1970). It is expected that by crossing genotypes from divergent groups increases the probability to maximize heterosis, and as a consequence show superior advances in segregant progenies and widen the genetic basis. Taking into consideration the breeding objectives, the crosses involving material from this HGB, which are local varieties, are more likely to have a high frequency of CBSD resistant genes. Again, the adaptation of the material from HGB to the local condition plays an important role in the success of the expected results. In wheat breeding, in most cases, parents from inter-cluster material is used, to develop high yielding varieties. However, Solomon *et al.* (2007) reported that in the case of heterozygosity *per se*, diversity is not the best predictor of F_1 performance. It is also important to note that parent performance is also required for efficient and useful implementation of the heterotic groups in the breeding programme.

The analysis permitted differentiation of all varieties, indicating that samples used did not contain genetic duplicates. It yielded high cophenetic correlation (r=0.83) (Rohlf, 1992), suggesting that the methods applied in this analysis was appropriate for this analysis.

The clustering patterns of cassava genotypes in this study did not indicate any relationship between genetic distance and eco-geographical distribution, contradicting findings of Benesi (2005) in Malawi, Elias *et al.* (2001) in Guyana, Kizito *et al.* (2005) in Uganda and many others. However, it is important to note that previous conclusions, using accession from the Mozambican gene bank (Zacarias and Cuambe*,* 2005) agreed with present results. In fact this continuous observation can be due to three main reasons; the first is the dispersal and movement of the population during the years of the civil war. There was also a historical reason.

Cassava programmes, since the colonial era, have reportedly been moved within research stations from one place to another and as a precaution, the programme leaders took the main varieties along. Thus the possibility for the occurrence of natural hybridization and selection was increased throughout the years, principally in the northern part of the country, where cassava flowers abundantly. Elias *et al.* (2001) reported that the exchange of cuttings between farmers sometimes lead to homogenisation of varieties. However, it is also important to note that the set of germplasm in this study does not represent the gene bank accessions of Mozambique, but the set of germplasm used as parents in the breeding nursery where the main objective was to breed for CBSD resistance. Under these circumstances it is not appropriate to make an overall judgement on the diversity of the existing cassava germplasm in the country.

4.5 Conclusions and recommendations

From this study it was concluded that AFLP is a powerful molecular tool to study genetic diversity for utilisation of germplasm in a breeding programme. A total of 425 fragments were produced by eight primer combinations. A total of 263 bands were polymorphic with an average of 32.88 fragments per primer combination, representing 61.8% polymorphism.

The highest Dice similarity coefficient was observed between the clones MZ89001 and MZ 89186 (0.929) followed by parents Nachinaya and Mucudo muevia (0.917). The lowest genetic distance was observed between local varieties Mocuba and Namuhiripwi (0.694). The average genetic distance was 0.803 across all the parents indicating a narrow genetic basis within the studied parents, but using AFLP based markers, it was possible to distinguish between them.

The accessions were grouped into two main clusters and subdivided further, but the parents Mocuba and Nikwaha were the most distinct among the accession. They were placed in a divergent position within the clusters. This result suggests that these accessions should be exploitated more in breeding program for traits with high combining ability.

Two heterotic groups for possible utility in the Mozambican breeding programme for hybridization are suggested, based on the observed genetic distances and clustering:

- Heterotic Group A, comprised of 12 genotypes from Cluster B II ia and ib: TMS 30001, Chigoma mafia, Mulaleia, Mucudo muevia, IMM 30025, Munhaça, Mwanamwahula, MZ 89105, MZ 89186, Likonde, Macia 1 and N'xinkole;
- Heterotic Group B, comprised of five parents from Clusters A, B I, B II ic and B II ii: Nikwaha, Baadge, Mocuba, Nachinaya and Namuhiripwe

The molecular clusters have also shown that the existing introduced clones are not distinct from the local landraces, apart from the well known TMS 30001, carrier of ACMV resistance. Introduction of improved germplasm should take into consideration specific traits to incorporate into the breeding programme, that can alleviate the main production constraints, or traits that respond to the emerging markets, such as high dry matter content.

The clustering patterns of cassava genotypes in this study did not indicate any relationship between genetic distance and eco-geographical distribution, although these accessions did not represent the entire cassava gene bank of Mozambique.

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CHAPTER 5

COMBINED GENETIC DISTANCE ANALYSIS OF CASSAVA (*Manihot esculenta* Crantz) USING MORPHOLOGICAL AND AFLP MARKERS

5.1 Introduction

Cassava (*Manihot esculenta* Crantz) is the fourth most important starch crop grown in the world (FAO, 1993). Unlike many other crops, cassava can be grown with minimal inputs and it is able to produce reasonably well under unfavourable conditions, such as, low soil fertility, acidic soils or drought. It is a staple, food security, cash crop and thrives where most other crops fail (Nweke, 1994; Fregene *et al.*, 2000; Colombo *et al.*, 1998). Cassava is also an industrial crop used for starch, flour and animal feed (Benesi, 2005; Colombo *et al.*, 1998).

Native to South America (Rogers, 1972; Olsen and Schaal, 1999), cassava was first introduced to Africa in the 1500s and later by Portuguese traders in the 1700 (Jones, 1959). Since then, natural cross-pollination followed, through selection by farmers and the exchange of seeds with neighbours. These are most probably the causes for the existing large number of morphologically distinct local varieties (Beck, 1982; Chiwona-Karltun *et al.*, 1998; Fregene *et al.*, 2000; Benesi, 2005), resulting in the accumulation of genetic diversity in this crop.

Traditional methods for identifying different crop plants are based on conventional phenotypic characteristics which are subject to environmental influences. This leads to a low accuracy of quantitative genetic parameter estimates (Vieira *et al.*, 2007). However, these methods remain effective (Gepts, 1993; Geleta *et al.*, 2006).

Genetic diversity can be revealed by a number of methods, including morphological data, agronomic performance, biochemical and DNA-based data (Mohammadi and Prasanna, 2003). Knowledge of genetic distance not only generates a better understanding of germplasm organization and efficiency during genotypic sampling, but also has implications on the results of choice of crosses and gene introgression

from exotic germplasm. It can also be used to recommend cultivars for a given region (Vieira *et al.*, 2007).

Genetic diversity studies using a combination of techniques, such as morphological and molecular markers, RAPD and AFLP analysis, have been conducted in cotton (Wu *et al.*, 2001; Bie *et al.*, 2001; Lukonge, 2005), perennial ryegrass (Roldan-Ruiz *et al.*, 2001), linseed (Adugna *et al.*, 2002), wheat (Cox and Murphy, 1990; Vieira *et al.*, 2007), alfalfa (Riday *et al.*, 2003), maize (Diers *et al.*, 1996; Riaz *et al.*, 2003; Betran *et al.*, 2003) and pepper (Geleta *et al.*, 2004). Combined morphological and molecular markers have also been used to access genetic diversity in cassava (Zambrano *et al.*, 2007; Elias *et al.*, 2000; 2001, Benesi, 2002; 2005; Colombo *et al.*, 2000).

Both morphological and molecular analysis matrices are very informative tools for the estimation of genetic distance (Vieira *et al.*, 2007). The objective of this study was to compare the use of morphological characters and AFLP analysis to assess genetic diversity analysis between cassava genotypes of Mozambique.

5.2 Material and methods

5.2.1 Morphological characterisation using descriptors

Cassava collection was conducted during the 2002/03 and 2003/04 seasons in Mozambique. Information regarding passport data for each accession was also collected, which included: accession code, name of cultivar, sample status, name of farmer, ethnic group, village, district, collection institution, names of individuals forming the collection team, collection date, taste, maturity period, target use, period that cultivar has been with the farmer and preferred characteristics of the cultivar. Collected accessions were planted at an *ex-situ* gene bank with a total of 59 accessions in 2004.

Seventeen cassava accessions (Table 5.1), that formed the breeding parents, were used in this study. The trial consisted of single rows, 12 plants per row, with one meter spacing between and within rows. The field was planted at the Northeast Centre Zone,

Nampula, situated in the Nampula province. Morphological characteristic data of the above and below ground parts were collected for 10 plants per accession, using a modified cassava descriptor of Fukuda and Guevara (1998) and Benesi (2005) (Table 5.2). The 20 qualitative and quantitative morphological characters scored were: unexpanded leaf colour, mature leaf colour, frequent number of lobes, leaf lobe wide, leaf lobe length, leaf lobe shape, shoot pubescence, petiole colour, petiole length, mature stem colour, branching habit, height of first branch, plant height, apical stem colour, storage root surface colour, storage root cortex colour, root taste, storage root pulp colour, root shape, root peduncle, and root peeling. The average morphological data for 17 analysed accessions was converted into a binary matrix using the binary transformation function of NTSYSpc version 2.11c computer package (Rohlf, 2000). The binary transformation converts continuous variables into binary data, using the following formula: 1 if yij > p, else 0.

Table 5.1 Accession name and some passport data for the Mozambican cassava germplasm which was included in this study

Accession code	Accession name	Village	District	Region	Place of origin
GBMZ 22	Nikwaha	Corrane	Meconta	Norte	Mozambique
GBMZ 41	TMS30001	Maputo	Maputo	-	IITA-Nigeria
GBMZ 03	Chigoma mafia	Nango	Mocimboa da praia	Norte	Mozambique
GBMZ 40	Mulaleia	Maneia	Maganja da Costa	Centre	Mozambique
GBMZ 46	Mocuba	Mugeba	Mocuba	Centre	Mozambique
GBMZ 47	Nachinhaya	Mute	Palma	Norte	Mozambique
GBMZ 20	Mucudo muevia	Licoa	Morrumbala	Centre	Mozambique
GBMZ 32	IMM30025	Namapa	Namapa	Norte	IITA-Nigeria
GBMZ 14	Munhaça	Maputo	Maputo	South	Mozambique
GBMZ 48	Namuhiripwe	Mutivaze	Rapale	Norte	Mozambique
GBMZ 08	Mwnamwhula	Mutivaze	Rapale	Norte	Mozambique
GBMZ 49	MZ89001	Maputo	Maputo	South	IITA-Nigeria
GBMZ 50	MZ89186	Maputo	Maputo	South	IITA-Nigeria
GBMZ 51	Likonde	Nango	Mocimboa da Praia	Norte	Mozambique
GBMZ 11	Macia1	Macia	Bilene	Centre	Mozambique
GBMZ 52	Nxinkole	Mkumbi	Palma	Norte	Mozambique
GBMZ 53	Baadge	Mkumbi	Palma	Norte	Mozambique

Table 5.2 Morphological characteristics of 17 accessions

Accession	MLC	ULC	PUB	NLB (5-7)	LCLS	LBW (cm)	LOBL (cm)	PTL (cm)	PC	MSC	BH	NBST (1-2)
Nikwaha	Green	Dark green	Present	5	Lanceolate	5.06	19.34	29.26	Red	Silvery green	Trichotomous	2
TMS30001	Green	Light green	Absent	5	Lanceolate	3.28	13.96	16.68	Green	Silvery green	Tetrachotomous	3
Chigom mafia	Green	Purple	Absent	5	Lanceolate	4.06	15	14.92	Red	Orange	Trichotomous	2
Mulaleia	Green	Green	Absent	5	Lanceolate	2.6	9.76	8.52	Pale green	Light brown	Tetrachotomous	2
Mocuba	Green	Green	Present	7	Lincar	4.36	17.12	26.78	Green	Silvery green	Trichotomous	2
Nachinhaya	Green	Green	Absent	7	Lanceolate	3.38	14.44	15.6	Green	Silvery green	Trichotomous	2
Mucudo moevia	Green	Green	Absent	5	Lanceolate	3.06	12.92	13.62	Green	Silvery green	Trichotomous	1
IMM30025	Green	Light green	Absent	7	Lanceolate	4.46	17.2	30.34	Green	Silvery green	Tetrachotomous	3
Munhaca	Green	Green	Present	7	Lanceolate	3.32	14.1	15.4	Green	Silvery green	Trichotomous	2
Namuhiripwe	Green	Green	Absent	5	Lincar	5.48	17.74	29.62	Red	Dark brown	Trichotomous	2
Munamuhuwa	Dark green	Green	Absent	7	Elliptic	4.06	18.8	19.62	Green	Reddish	Trichotomous	2
MZ89001	Green	Light green	Absent	7	Lanceolate	3.7	14.35	23.7	Green	Silvery green	Tetrachotomous	2
MZ89186	Dark green	Light green	Absent	5	Lincar	4.16	16.5	24	Green	Silvery green	Tetrachotomous	2
Likonde	Dark green	Green	Present	7	Lincar	4.6	14.96	18.64	Mainly red	Silvery green	Dichotomous	2
Macia1	Green	Green	Absent	7	Lincar	3.94	16.82	24.6	Green	Silvery green	Tetrachotomous	2
Nxinkole	Green	Dark green	Absent	7	Lincar	4.72	17.64	31.14	Purple	Silvery green	Trichotomous	2
Baadje	Green	Green	Absent	5	Elliptic	4.44	13.02	17.1	Pale Red	Silvery green	Dichotomous	2

MLC=mature leaf colour; ULC=unexpanded leaf colour; PUB=pubescence; NLB=number of lobes; LCLS=central lobe shape; LBW=central lobe width, LOBL=lobe length; PTL=petiole length; PC=petiole colour; MSC=mature stem colour; BH=branch habit; NBST=level of ramifications; HFB=height of first branch;; PH=plant height; AST=apical stem colour; RCXC=cortex colour; RPC=colour root pulp; RSC=colour surface root; RSp=root shape; PED=root peduncle; PL=root peeling.

Table 5.2 Cont.....

Accession	HFB (cm)	PH (cm)	AST	PES (1-7)	RSC	RCXC	RTX	RPC	RSp	PED	PL
Nikwaha	113.6	254	Green	7	Brown	White	Sweet	White	Conical-cylindrical	Pedunculate	Moderate easy
TMS30001	73.2	154	Light green	3	White	White and pink spots	Bitter	White	Irregular	Sessile	Moderate easy
Chigoma mafia	65.2	184	Purple	7	Dark brown	Purple	Sweet	White	Cylindrical	Pedunculate	Easy
Mulaleia	64	151	Green	7	Brown	White and pink spots	Bitter	White	Irregular	Pedunculate	Easy
Mocuba	105	210	Dark green	3	White	White and pink spots	Sweet	White	Conical-cylindrical	Both	Moderate easy
Nachinhaya	83	174	Dark green	3	White	Pink	Sweet	White	Conical	Both	Easy
Mucudo moevia	65.4	173	Green	7	White	Pink	Bitter	White	Conical-cylindrical	Sessile	Moderate easy
IMM30025	114.2	259	Light green	7	Brown	Pink	Bitter	White	Conical-cylindrical	Sessile	Easy
Munhaca	93.33	198	Green	3	White	White and pink spots	Sweet	White	Conical	Sessile	Easy
Namuhiripwe	140.67	272	Dark green	7	Brown	Pink	Bitter	White	Irregular	Both	Moderate easy
Mwnamhula	112.5	263	Green	7	Brown	Purple	Bitter	White	Conical-cylindrical	Both	Easy
MZ89001	80.2	187	Light green	1	White	White	Bitter	White	Conical	Pedunculate	Easy
MZ89186	66	187	Light green	3	White	White	Bitter	White	Irregular	Sessile	Easy
Likonde	112.6	250	Dark green	7	Brown	White	Bitter	White	Conical-cylindrical	Both	Moderate easy
Macia1	126	247	Green	7	Brown	White pinkish	Sweet	White	Conical	Both	Easy
Nxinkole	82	203	Dark green	3	Brown	White pinkish	Sweet	White	Conical-cylindrical	Pedunculate	Moderate easy
Baadje	73.6	174	Green	7	White	White	Sweet	Light Yellow	Conical-cylindrical	Both	Easy

MLC=mature leaf colour; ULC=unexpanded leaf colour; PUB=pubescence; NLB=number of lobes; LCLS=central lobe shape; LBW=central lobe width, LOBL=lobe length; PTL=petiole length; PC=petiole colour; MSC=mature stem colour; BH=branch habit; NBST=level of ramifications; HFB=height of first branch; PH=plant height; AST=apical stem colour; RCXC=cortex colour; RPC=colour root pulp; RSC=colour surface root; RSp=root shape; PED=root peduncle; PL=root peeling.

5.2.2 DNA extraction

DNA extraction was done using a modified Dellaporta *et al.* (1983) method as described in section 4.2.2. DNA quality, concentration and integrity determination were described in section 4.2.3

5.2.3 AFLP analysis

AFLP analysis, that included double digestion and ligation, pre-selective amplification (Herselman, 2003), selective amplification and resolution, were done as described in section 4.2.4.

5.2.4 Genetic similarities and clustering analysis

The transformed morphological data were subject to analysis using NTSYSpc version 2.11c computer package (Rohlf, 2000). Similarity matrices were compiled for all pairs of varieties using Dice similarity coefficient (Dice, 1945; Nei and Li, 1979). Cluster analysis was done using UPGMA (unweighted pair-group method of arithmetic averages; Sokal and Michener, 1958) and dendrograms were constructed using the SAHN programme. Cophenetic analysis was done as described in 4.4. The similarities calculated and the cophenetic distances obtained from the tree, were compared using the Mantel correspondence test (Mantel, 1967; Sneath and Sokal, 1973). Mantel (1967) developed a test to determine the goodness of fit between two matrices. The relatedness between two matrices is measured by '*r*', a product moment correlation coefficient. A higher *'r'* - value indicates a higher degree of similarity and vice versa. Similar analysis was done on combined AFLP and transformed morphological data. The different dendrograms were then compared using consensus tree analysis. Estimation of the consensus fork index (CI_c) (Rohlf, 1982) is an equivalent of the method of Duarte *et al.* (1999), which provides an indication of the similarity of the dendograms. CI_c was also calculated using NTSYS software (Rohlf, 2000).

Principal Coordinate Analysis (PCoA) biplots for morphological, AFLP and combined morphological and AFLP methods were performed by calculating Eigenvectors and Eigen values, and PCoA projections were constructed to show the broad multiple dimension contribution of the cultivars, using NTSYS software (Rohlf, 2000). Comparison of morphological, AFLP and combined AFLP and morphologic analysis were done by correlating Dice similarity confidents for each, using Agrobase (2000).

5.3 Results and discussion

5.3.1 Estimates of morphologic genetic similarity

Estimates of genetic similarity for morphological data ranged from 0.105 to 0.833 (Table 5.3). The average genetic distance for all pairwise comparisons (N=136) was 0.456. The lowest genetic distance was observed between TMS 30001 and Mwanamwahula (0.105), followed by MZ 89001 and Mwanamuahula (0.118). The accessions TMS 30001 and MZ 89001 were introduced from IITA, while Mwanamwahula and Chigoma mafia are local varieties. The similarities between TMS30001 and Mwanamwahula are obvious when the following morphological traits are compared: unexpanded leaf colour, mature leaf colour, number of lobes, petiole length, mature stem colour, petiole length, branch habit, plant height, apical stem colour, storage root surface colour and root cortex colour. Local varieties, like Mwanamwahula, are typically taller and the first branch is usually high (112.5 cm). The increased plant height (263 cm) helps to facilitate the crop management and intercropping system. The same observations were made for leaf and root characteristics. About 62.5% of the mentioned morphological characteristics contribute to differentiate these accessions.

The highest genetic distance was found between the local varieties Likonde and Nikwaha (0.833) followed by Mocuba and Nikwaha (0.824). The morphological characteristics had a minimal contribution to differentiate between these accessions, contributing 17% and 31% respectively, showing that they are quite similar The accessions Likonde and Nikwaha were differentiated by the apical stem colour, root peduncle, mature stem colour, mature leaf colour, branch habit and central lobe

shape. The plant height (PH) varied from 250 to 254 cm, and the height of the first branch was between 112.6 and 113.6 cm. This clearly indicates morphological patterns preferred by farmers, and that morphologically they are quite similar. The above ground characteristics were also analyzed and the main difference was that accession Likonde presents sessile and pedunculate root peduncules while Nikwaha only has pedunculate. The accessions Likonde, Nikwaha and Mocuba, were the varieties selected by farmers, due to their resistance to the main diseases and pests in the northern part of the country. These varieties were collected in different provinces and villages (Table 5.1).

The average genetic distance for morphological characteristics ranged between 0.105 to 0.833, compared to distances obtained from AFLP data, ranging from 0.694 to 0.917, with averages of 0.456 and 0.803, respectively (Table 5.3). The first 50 pairwise combinations for morphological and AFLP genetic distances, varied from 0.105 to 0.400 and 0.685 to 0.782, respectively. Baadge had seven repeated pairwise combinations (in bold) with both morphologic and AFLP genetic distances that ranked among the lowest 50 (Table 5.4) suggesting the relative importance of this accession within the breeding programme. Furthermore, Baadge is among the varieties that have not been accessed with CBSD, leaf and root symptoms, in farmers' fields and the experimental station, as well.

The morphological analysis provided a larger range of genetic dissimilarity compared to AFLP analysis. The genetic distances based on morphology for the three accession, Nikwaha, Likonde and Mocuba, were the closest (0.833 to 0.813), but according to AFLP analysis were more dissimilar (0.784 to 0.735) (Table 5.3). This indicates that the selection through the years affected the morphologically linked and adaptive characteristics (Camussi *et al.*, 1985) and demonstrates the sensitivity of molecular technique, especially AFLP, due to the fact that it represents almost complete genome coverage (Ajmone Marsan, 1998). Large numbers of morphological traits were used to distinguish among the studied accession, which were indicated as parents in the breeding nursery for disease resistance.

Table 5.3. Genetic distances for morphological (above diagonal) and a combination of AFLP and morphological (bellow diagonal) based on Dice similarity coefficients for 17 characterised accessions

	Nikwa	TMS	Chigo	Mulal	Mocuba	Nachi	Mucudo	IMM	Munh	Nam	Muna	MZ891	MZ896	Likonde	Macia1	Nxink	Baad
Nikwa		0.462	0.467	0.357	0.824	0.370	0.519	0.765	0.519	0.737	0.581	0.417	0.370	0.833	0.706	0.750	0.571
TMS	0.801		0.333	0.375	0.455	0.400	0.533	0.364	0.400	0.308	0.105	0.500	0.400	0.417	0.273	0.400	0.375
Chigo	0.810	0.829		0.500	0.308	0.526	0.421	0.539	0.421	0.600	0.435	0.125	0.316	0.500	0.385	0.500	0.400
Mulal	0.770	0.816	0.822		0.167	0.353	0.471	0.500	0.235	0.500	0.286	0.286	0.353	0.308	0.500	0.273	0.333
Mocuba	0.735	0.727	0.735	0.762		0.435	0.435	0.667	0.522	0.706	0.519	0.400	0.435	0.813	0.667	0.714	0.500
Nachi	0.751	0.791	0.808	0.797	0.802		0.625	0.522	0.500	0.444	0.300	0.308	0.375	0.400	0.348	0.381	0.353
Mucudo	0.762	0.811	0.800	0.807	0.765	0.909		0.609	0.625	0.444	0.300	0.462	0.250	0.480	0.348	0.476	0.471
IMM	0.783	0.805	0.797	0.808	0.738	0.804	0.823		0.522	0.765	0.667	0.500	0.522	0.625	0.733	0.571	0.417
Munh	0.745	0.801	0.804	0.794	0.750	0.844	0.878	0.828		0.296	0.300	0.462	0.125	0.480	0.435	0.381	0.353
Namu	0.742	0.770	0.790	0.772	0.695	0.752	0.771	0.803	0.789		0.710	0.250	0.444	0.722	0.706	0.625	0.429
Munam	0.790	0.814	0.817	0.794	0.723	0.773	0.799	0.829	0.803	0.827		0.118	0.300	0.621	0.667	0.320	0.286
MZ891	0.775	0.821	0.798	0.791	0.695	0.753	0.790	0.841	0.803	0.774	0.867		0.308	0.273	0.400	0.333	0.286
MZ896	0.770	0.822	0.824	0.797	0.707	0.779	0.799	0.843	0.810	0.766	0.835	0.916		0.320	0.348	0.476	0.353
Likonde	0.784	0.816	0.815	0.792	0.754	0.780	0.797	0.808	0.812	0.788	0.821	0.827	0.851		0.625	0.667	0.615
Macia1	0.793	0.801	0.808	0.801	0.726	0.782	0.789	0.790	0.793	0.793	0.813	0.810	0.820	0.852		0.500	0.417
Nxink	0.791	0.783	0.795	0.738	0.709	0.788	0.807	0.772	0.788	0.778	0.814	0.795	0.788	0.806	0.811		0.546
Baadje	0.762	0.749	0.739	0.711	0.685	0.725	0.760	0.719	0.723	0.707	0.756	0.739	0.746	0.782	0.774	0.820	

Nikwa=Nikwaha, TMS=TMS 30001, Chigo=Chigoma mafia, Mulal=Mulaleia, Nachi=Nachinaya, IMM=IMM 30015, Munh=Munhaça, Nam=Namuhiripwi, Muna=Mwanamwahula, MZ891=MZ 89001, MZ896=MZ 89186; N'xink= N'xinkole; Baad=Baadge

Table 5.4 Rank of first 50 combination pairs with lowest morphologic and AFLP GD

Morphological genetic distance			AFLP genetic distance		
Pair wise		Rank	Pair wise		Rank
Mwamanwahula	TMS 30001	0.105	Baadje	Mocuba	0.685
MZ 89001	Mwamanwahula	0.118	MZ 89001	Mocuba	0.695
MZ 89001	Chigoma mafia	0.125	Namuhiripwe	Mocuba	0.695
MZ 89186	Munhaca	0.125	Baadje	Namuhiripwe	0.707
Mocuba	**Mulaleia**	0.167	MZ 89186	Mocuba	0.707
Munhaca	Mulaleia	0.235	N'xincole	Mocuba	0.709
MZ 89001	**Namuhiripwe**	**0.250**	**Baadje**	**Mulaleia**	**0.711**
MZ 89186	Mucudo muevia	0.250	Baadje	IMM30025	0.719
Likonde	MZ 89001	0.273	**Baadje**	**Munhaca**	**0.723**
Macia1	TMS 30001	0.273	Mwamanwahula	Mocuba	0.723
N'xincole	**Mulaleia**	**0.273**	**Baadje**	**Nachinaya**	**0.725**
Baadje	**Mwamanwahula**	**0.286**	Macia1	Mocuba	0.726
Baadje	**MZ 89001**	**0.286**	Mocuba	TMS 30001	0.727
Mwamanwahula	Mulaleia	0.286	**Mocuba**	**Chigoma mafia**	**0.735**
MZ 89001	Mulaleia	0.286	Mocuba	Nikwaha	0.735
Namuhiripwe	Munhaca	0.296	IMM30025	Mocuba	0.738
Mwamanwahula	Mucudo muevia	0.300	**N'xincole**	**Mulaleia**	**0.738**
Mwamanwahula	Munhaca	0.300	Baadje	Chigoma mafia	0.739
Mwamanwahula	**Nachinaya**	**0.300**	**Baadje**	**MZ 89001**	**0.739**
MZ 89186	Mwamanwahula	0.300	Namuhiripwe	Nikwaha	0.742
Likonde	Mulaleia	0.308	Munhaca	Nikwaha	0.745
Mocuba	**Chigoma mafia**	0.308	**Baadje**	**MZ 89186**	**0.746**
MZ 89001	**Nachinaya**	**0.308**	**Baadje**	**TMS 30001**	**0.749**
MZ 89186	MZ 89001	0.308	Munhaca	Mocuba	0.750
Namuhiripwe	**TMS 30001**	**0.308**	**Nachinaya**	**Nikwaha**	**0.751**
MZ 89186	Chigoma mafia	0.316	Namuhiripwe	Nachinaya	0.752
Likonde	MZ 89186	0.320	**MZ 89001**	**Nachinaya**	**0.753**
N'xincole	Mwamanwahula	0.320	Likonde	Mocuba	0.754
Baadje	**Mulaleia**	**0.333**	**Baadje**	**Mwamanwahula**	**0.756**
Chigoma mafia	TMS 30001	0.333	Baadje	Mucudo muevia	0.760
N'xincole	MZ 89001	0.333	Baadje	Nikwaha	0.762
Macia1	Mucudo muevia	0.348	**Mocuba**	**Mulaleia**	**0.762**
Macia1	MZ 89186	0.348	Mucudo muevia	Nikwaha	0.762
Macia1	**Nachinaya**	**0.348**	Mucudo muevia	Mocuba	0.765
Baadje	**Munhaca**	**0.353**	MZ 89186	Namuhiripwe	0.766
Baadje	**MZ 89186**	**0.353**	**Mulaleia**	**Nikwaha**	**0.770**
Baadje	**Nachinaya**	**0.353**	**MZ 89186**	**Nikwaha**	**0.770**
MZ 89186	Mulaleia	0.353	**Namuhiripwe**	**TMS 30001**	**0.770**
Nachinaya	Mulaleia	0.353	Namuhiripwe	Mucudo muevia	0.771
Mulaleia	**Nikwaha**	**0.357**	Namuhiripwe	Mulaleia	0.772
IMM30025	TMS 30001	0.364	N'xincole	IMM30025	0.772
MZ 89186	**Nikwaha**	**0.370**	**Mwamanwahula**	**Nachinaya**	**0.773**
Nachinaya	Nikwaha	0.370	Baadje	Macia1	0.774
Baadje	**TMS 30001**	**0.375**	**MZ 89001**	**Namuhiripwe**	**0.774**
Mulaleia	TMS 30001	0.375	MZ 89001	Nikwaha	0.775
MZ 89186	**Nachinaya**	**0.375**	N'xincole	Namuhiripwe	0.778
N'xincole	Munhaca	0.381	**MZ 89186**	**Nachinaya**	**0.779**
N'xincole	Nachinaya	0.381	Likonde	Nachinaya	0.780
Macia1	Chigoma mafia	0.385	Baadje	Likonde	0.782
Nachinaya	TMS 30001	0.400	**Macia1**	**Nachinaya**	**0.782**

5.3.2 Morphological cluster analysis

Two main clusters were obtained, A and B (Figure 5.1). Cluster A sub-cluster II, had one entry, clone MZ 89186, at a genetic similarity of 0.39. Cluster B I, had 11 entries, subdivided into two sub-groups at a genetic similarity (GS) of 0.401. Sub-group ii contained two accessions, Chigoma mafia and Mulaleia (GS of 0.500), both local varieties and collected in Cabo Delgado and Zambezia, respectively. Chigoma mafia has purple to red pigmentation for petiole colour, mature stem colour, apical stem colour and root cortex colour, while for accession Mulaleia, the pigmentation varied from pale green, light brown to brown. Sub-group i, was subdivided in two sections. Section 1 consisted of one introduced accession and seven local entries. Within the local accessions, the varieties Nikwaha and Likonde clustered together with a genetic distance of 0.833, indicating high morphological similarity. Furthermore, they clustered with Mocuba at 0.824 and with N'xincole at 0.72. The accession N'xincole had the lowest genetic similarity, in this section. This could be explained by the fact that it has the highest PTL (31.14 cm), purple PC and it branches very low (82 cm), when compared to these characteristics of the other entries under study. Varieties Nikwaha, Mocuba and Likonde are in high demand for production due to their adaptation to the environment, suitable plant architecture for the farmer production system and root quality preferred by farmers. The subgroup i 2, contained a single accession, the local variety Baadge (GS of 0.466). This might be due to the fact that it is the only accession that had oblanceolate leaf shape and light yellow flesh colour.

Cluster B contained two sub-clusters. Sub-cluster I comprised of two accessions that were introduced from IITA, TMS 30001 and MZ 89186 with a genetic distance of 0.500. They had a tetrachotomous branching habit, a characteristic predominantly observed in the introduced clones. The accessions Nachinaya, Mucudo muevia and Munhaça, from cluster B I, had genetic similarities of 0.632 and 0.560, respectively. These are local varieties and collected in different places with dark green AST. The PH varied from 173 to 198.3 cm. The cophenetic correlation coefficient indicated a poor fit (r=0.78). This signifies that die clusters generated do not accurately represent the distances between the accessions as determined by the similarity

coefficient. This may be as result of distortions that might have occurred during the analysis and transformation process (Mohammandi and Prasanna, 2003).

The accessions clustered regardless of the geographic origin. However, more accessions should be added to improve the analysis of the diversity of germplasm in the country.

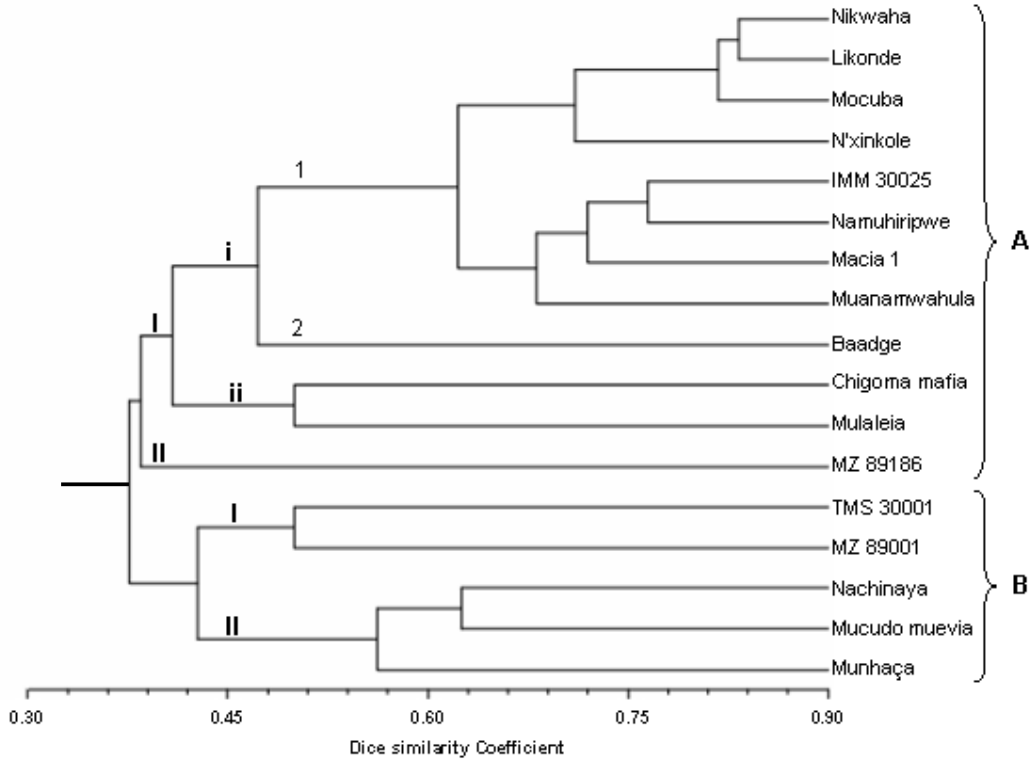

Figure 5.1 Dendrogram for morphological characterization of 17 analyzed accessions using NTSYS computer package, Dice similarity coefficient and UPGMA clustering

5.3.3 Genetic distance and cluster analysis based on AFLP analysis

AFLP cluster analysis using NTSYS was discussed in Chapter 4 in terms of genetic distance and clustering patterns (4.2.1, 4.2.2)

5.3.4 Comparison of morphological versus AFLP dendrograms

Morphological and AFLP dendrograms were produced from binary data using the Dice similarity coefficient and the UPGMA clustering method (Figure 5.1 and 5.2). The dendrograms and similarity matrices were similar for many clusters and genetic distances. The resulting dendrograms revealed two main clusters and most accession clustered together for both analyses. The accessions Nachinaya, Mucudo muevia, Munhaça clustered together in the morphological (B II) and the AFLP dendrograms (B II i b). The accession Mwanamwahula and IMM 30025 was also observed positioned in the same cluster of the morphological (A I i 1) and AFLP dendrograms (B II i a 3), the same for the accessions Chigoma mafia and Mulaleia, that remained clustered together for both the morphological (cluster A I ii) and the AFLP dendrogram (cluster B II i a 3). These results suggest that most of the accessions had similar grouping patterns for both dendrograms and that the morphological clusters were confirmed by AFLP analysis.

However, between the dendrograms some discrepancies were found. Accessions Nikwaha, N'xincole and Mocuba were observed in the same morphological cluster (A I i), with accession Baadge clustered separately (A I i 2). This cluster is the largest in the morphological dendrogram. Accessions Nikwaha, N'xincole and Mocuba were among the most similar accessions according to the morphological genetic distance. The AFLP dendrogram analysis, however, showed that accessions N'xincole and Baadge (B I) clustered together and that Nikwaha was (B II ii) isolated within cluster B, while the accession Mocuba clustered separately from the rest of the group (A). The genetic distances, for these accessions, were among the most dissimilar in the group.

The genetic distance ranged from 0.105 to 0.833 and 0.685 to 0.916 for morphological and AFLP analysis, respectively. These results, suggest that morphological analysis showed wider genetic diversity within accessions, but molecular markers used, were more reliable to detect genetic differences among cassava accessions. Accordingly, molecular analysis provides a wider range of genome sampling than morphological analysis. Thus, the morphological similarities detected between the accessions were not necessarily the product of genotypic similarity, but due to a combination of genes.

In this study 23 morphological, quantitative and qualitative markers and six AFLP primer pairs were used to investigate the level of genetic diversity within 17 cassava parents. The results obtained agree with the findings of Zacarias (1997), who found similar grouping patterns using morphological and RAPD analysis in cassava, although the clusters where different. Similar results were also reported by Adugna (2002) in linseed and Lukonge (2005) in cotton. The morphological traits used might play an important role to improve the results when morphological molecular markers are used in the analysis. Vieira *et al*. (2007) studies on wheat, suggested an increase in the number of morphological markers. Benesi (2005) did a detailed study on cassava and suggested the use of morphological traits that are not influenced by the environment, while Zambrano *et al.* (2007) suggested increasing the percentage of botanical traits that are less influenced by the environment. Furthermore, the format of morphological descriptors is nominal data that are transformed into binary data, whereas the AFLP analysis uses binary data. The transformation of nominal morphological data to a binary matrix, may lead to a flawed and biased interpretation. Conversely, the AFLP dendrogram was based on 425 polymorphic bands, whereas the morphological dendrogram only had 23 data points.

The morphological traits are related to the main selection features of the individual and these traits influence the grouping of the accessions into different clusters. The existence of unique traits in some accession, also influences the positioning of the accession in a cluster, for example, the accession Baadge, that clustered separately (A I i 2). In vegetatively propagated crops, such as cassava, the seed stems are exchanged from farmer to farmer. Apart from that, the occurrence of natural hybridization and the fact that the process of small selections affects some

characteristics may be the result of somatic mutation and not always detectable by morphological means.

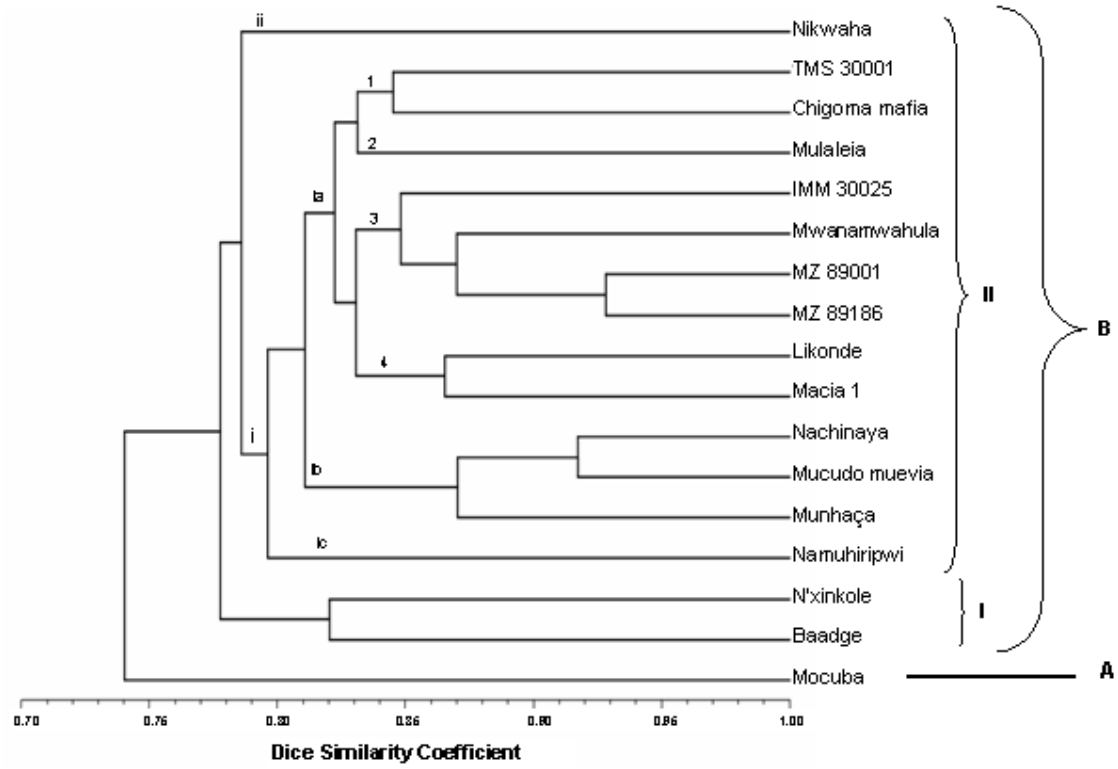

Figure 5.2. Dendrogram for characterization of 17 analyzed accessions using eight AFLP primer pairs with the aid of NTSYS computer package, Dice similarity coefficient and UPGMA clustering

5.3.5 Combined morphological and AFLP cluster analysis

A combined dendrogram, of morphological and AFLP data, is represented in Figure 5.3. Two main clusters were found. Cluster B was represented by a unique cultivar, Mocuba, while Cluster A, comprised of 17 accessions. Furthermore, Cluster A was sub-divided into Sub-cluster A I, with three entries and AII comprised of Group AII ii also with unique accession, Namuhiripwe and Group A II i , represented by i 1 and i 2. The Sub-group A II i 2 represent the same cluster in the morphological and AFLP

cluster analysis, while the Sub-group A II i 1 represents also the same cluster in AFLP analysis, but some similar grouping were found with morphological analysis.

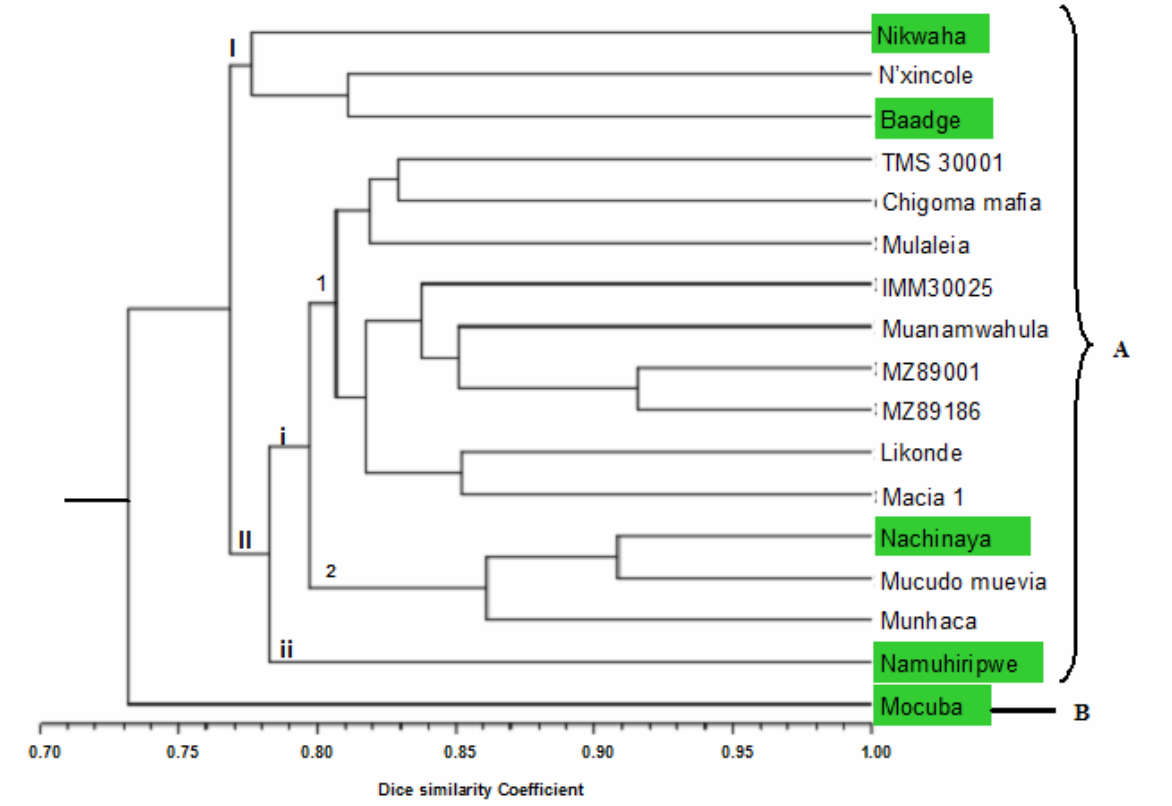

Figure 5.3 Combined AFLP and morphological characterisation of 17 cassava accessions with the aid of NTSYS computer package, Dice similarity coefficient and UPGMA clustering

Most distinct varieties of the accession

5.3.6 Comparison of morphological versus AFLP versus combined dendrograms

The combined AFLP and morphological analysis (Figure 5.3) resembled the AFLP dendrogram (Figure 5.2) more than the morphological dendrogram (Figure 5.1)clustering patterns for most of the accessions. Cluster B I (N'xincole and Baadge) in the molecular analysis, was incorporated into cluster A I of the combined

analysis and they clustered in the same group for the morphological analysis. Accessions Mocuba and Nikwaha maintained their distinct positioning, for both the AFLP and the combined dendrograms. Once again the accessions, Nachinaya, Mucudo, Munhaça, clustered together in the morphological (B II), AFLP (B II i b) and combined (A II i 2) dendrograms. The accessions, Chigoma mafia, Mulaleia, IMM 30025 and Mwanamwahula, also had the same clustering in all dendrograms analysed. Based on the dendrograms produced, the results indicate some relationship among these characterization methods of genetic relationship.

Some differences were found when comparing the combined, AFLP and morphological analysis, regarding the positions of Nikwaha, Likonde, N'xincole, Baadge and Mocuba. Baadge clustered separately within cluster A in the morphological dendrogram (A I i 2) while in the AFLP analysis, Baadge clustered with accession N'xincole (B I) and also in the combined analysis (A I), showing similarity between AFLP and combined versus morphological analysis. When the accessions Nikwaha, Likonde and Mocuba were compared, they are observed in the same cluster in the morphological dendrogram (A I i 1), but clustered randomly in the AFLP analysis and in the combined dendrogram, as well. These accessions indicated a high similarity based on the morphological analysis, while in the AFLP and combined analysis they were placed in different clusters and they were among the most dissimilar accessions. This suggests the importance of molecular markers to complement morphological characterization to improve the differentiation of some accessions. The positioning of the accessions Nikwaha, Likonde, Mocuba, Baadge and Namuhiripwe, when combined morphological and AFLP analysis are used, suggest clear confirmation of findings on the Chapter 4, section 4.2 and 4.3, as HGB. These results also suggest that combined analysis resembles the AFLP analysis rather than morphological analysis. This is also due to the number of data points generated by AFLP analysis versus that of morphological data. Conversely, the AFLP dendogram was based on 425 polymorphic bands where the morphological only had 23 data points. The association between dendrograms were influenced by the fact that variations detected by AFLP markers are not adaptive, therefore, not subject to be influenced by natural or artificial selection. Thus, AFLP analysis provided a greater degree of discrimination than the morphological analysis and this provides further explanation for the increased resemblance between the combined

AFLP and morphological dendrogram and the AFLP dendrogram. Besides, the format of morphological descriptors is nominal data that are transformed into binary data, whereas the AFLP analysis uses binary data. The transformation of nominal morphological data to a binary matrix, may lead to a flawed and biased interpretation.

Results of this study are similar to findings of Nemera (2003) and Benesi (2005) and they have suggested the use of a combination of morphological and molecular markers as a major source of information in separating closely related accessions in different crops.

Morphological characterization has been traditionally used (Hershey and Ocampo, 1989; Elias *et al.*, 2001) despite their limitation. Its usefulness is still valid for farmers, breeder and curators, as well as for variety registration and release. However, genetic markers have received extensive attention in the last decade as a tool to improve knowledge about the genetics of various traits, and to enhance breeding efficiency (Soller and Beckmann, 1983; Beckmann and Soller, 1983). DNA-based molecular markers can facilitate the precise identification of genotypes without the confounding effect of the environment, thus increasing heritability. They also contribute to the efficient reduction of large breeding populations at the seedling population, particularly important in long growth cycle crops such as cassava.

The genetic diversity analysis using association of AFLP and morphological data, is more powerful because both analysis are incorporated and a large proportion of variation detected by molecular markers are not subject to the environment. Conventional breeding methods in combination with molecular markers have been advantageously reported in development of elite productive varieties in cotton (Abdukarimov *et al.*, 2003).

5.3.7 Principal coordinate analysis based on morphological analysis

The principal coordinate analysis (PCoA) biplot for morphological analysis was performed to study the inter-relationship between all varieties (Figure 5.4). PCoA

clustered varieties similarly to the morphologic dendogram. As observed in Cluster A I i 1 and 2 (Figure 5.1), most of the cultivars with similar morphology also grouped together with PCoA. Varieties Nikwaha, Likonde, Mocuba, N'xincole and IMM 30025, Namuhiripwe, Macia 1 and Muanamwahula grouped together. The variety Baadge, clustered separately, this corresponds to the results obtained from the dendrogram. However, the varieties Mulaleia and Chigoma mafia (GS of 0.625) clustered together according to the dendrogram, but with PCoA they seemed to be further apart, and closer to the average.

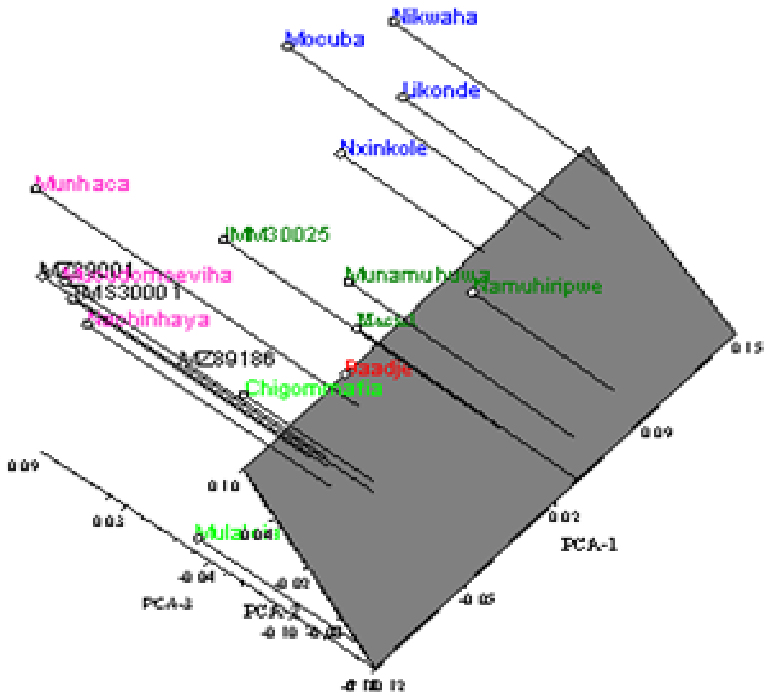

Figure 5.4 PCoA plot for characterisation of 17 analysed cassava accessions using morphological markers with the aid of NTSYS computer package

5.3.8 Principal coordinate analysis based on AFLP analysis

The PCoA for AFLP analysis is presented in Figure 5.5. The accessions clustered closely, but still in discrete groups. The distinctiveness of the varieties such as

Mocuba, Nachinaya, Baadge, Namuhiripwe, Nikwaha and N'xincole, were apparent with both the genetic distance analysis (Table 5.1) and cluster analysis (Figure 5.3). This was similar to the results obtained with the PCoA biplot (Figure 5.5). These varieties were also found with prominent height when compared with other varieties analysed. The clusters previously defined as A and B I and B II were similar to that observed using the plot.

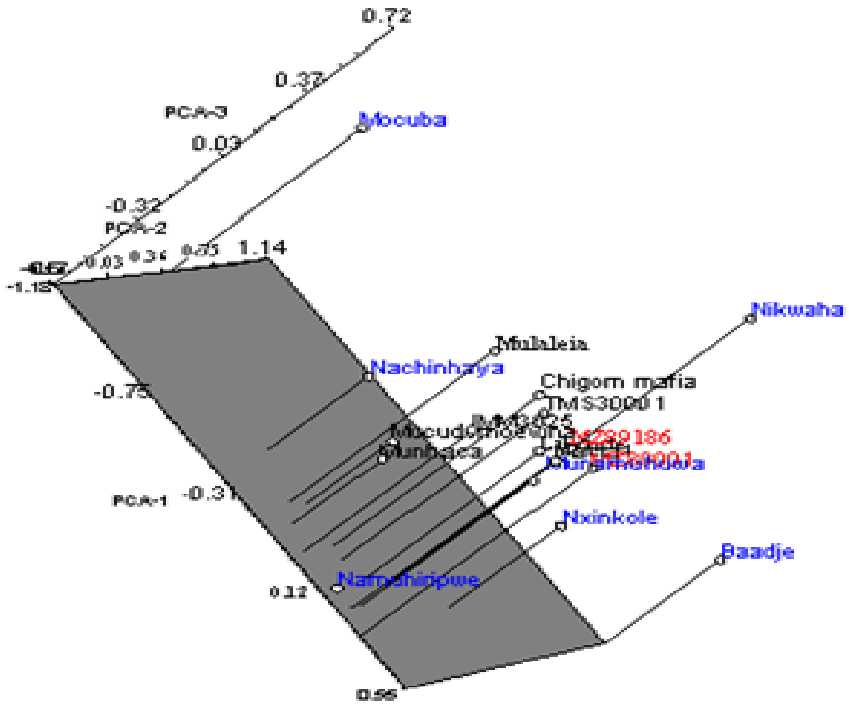

Figure 5.5 PCoA plot for characterisation of 17 analysed cassava accessions using AFLP markers with the aid of NTSYS computer package

The PCoA biplot has shown that the varieties clustered in distinct groups (Figure 5.6). Cluster A I (Figure 5.3), although scattered, formed a group with three varieties, Nikwaha, N'xincole and Baadge. These varieties had higher dissimilarity based on the genetic distance, cluster analysis and also the separation from the rest of the group as observed from the PCoA plot. The uniqueness of variety Mocuba, was confirmed by both cluster analysis and PCoA. Dendrogram analysis revealed that it

clustered (cluster B, consisting of a single variety) and plotted (Figure 5.6) independently. The variety Mocuba was notably one of the most dissimilar varieties, according to the genetic distances. Again the PCoA more closely resembled the clusters obtained with AFLP analysis (Table 5.3, Figure 5.2 and 5.3).

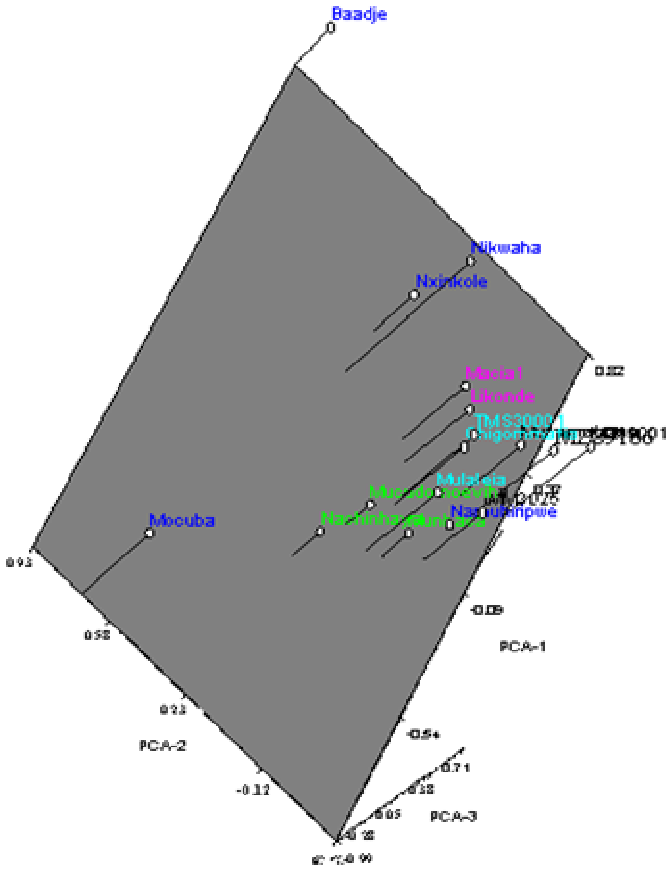

Figure 5.6 PCoA plot for characterisation of 17 analysed cassava accessions using combined analysis with the aid of NTSYS computer package

5.3.10 Correlation analysis

Cophenetic correlation coefficients were used to objectively compare matrices generated from AFLP and morphological data, using the Mantel test. The cophenetic correlation coefficient of the combined dendogram (r=0.835) showed good

agreement between the graphical representation of the distances and the original matrices, which enables more accurate visual inferences to be drawn (Figure 5.3).

Consensus tree analysis (*CIc*) was used to compare the different dendrograms. Dendrograms are considered identical when the consensus fork index *CIc* equals one. Dendrograms obtained from binary transformed and untransformed morphological data was 60% identical (*CIc*= 0.60). The consensus fork index value of 0.666 indicated high resolution of the dendrograms obtained from morphological and AFLP data. The correlation coefficient was significant (*r*=0.998) between AFLP and combined AFLP and morphological analysis. Zambrano *et al.* (2007) observed different levels of significance between qualitative and quantitative morphological traits and concluded that a high percentage of neutral botanical characteristics greatly influenced the distribution of subspace using a consensus matrix.

Correlations between genetic distance matrices based on AFLP, morphological and combined AFLP and morphological traits were highly significant (Table 5.4). Correlations were weak and not significant between morphological and AFLP analysis and the combination of AFLP and morphological traits, supporting the discrepancy found with some cluster groups and the range of genetic diversity. The high correlation between AFLP and combined AFLP and morphologic matrices might be due to the fragile contribution of morphological analysis to the combination. On the other hand, the morphological descriptors use raw nominal data that are transformed into binary, while the AFLP analysis uses raw binary data. The process of data transformation, may lead to a bias and weak interpretation. The combined cluster analysis was similar to the observed AFLP clusters for most of the accessions. Similar clusters observed were: TMS 30001, Chigoma mafia and Mulaleia, followed by IMM30025, Mwanamuahula, MZ 89001 and MZ 89186, and at last Nachinaya, Mucudo muevia and Namuhiripwi. The accession Mocuba, maintained a distinct position, clustering apart from any group and PCoA confirmed this (Figure 5.2, 5.3, 5.5, 5.6). This suggests that there was significant contribution of AFLP binary information for the construction of the combined AFLP and morphological dendrogram, supported by the high and significant correlation found between the matrices (Table 5.5). The apparent disagreement between the

morphological and AFLP analysis in this study, already mentioned, might be also due to continuous natural hybridization between the existing varieties. The natural selection conducted by farmers, affects morphological traits and it is important to note that the morphological traits relate to the main features of the entry and are sometimes more strictly correlated with and widely influenced by the environment (Camussi *et al.*, 1985).

Adugna (2002) on linseed, Lukonge (2005) on cotton, Ben-Har *et al.* (1995) and Swanepoel (1999) on maize inbred line collections and Roldan-Ruiz *et al.* (2001) on perennial ryegrass, did similar studies. Their results indicated that these two methods were different and highly variable and might be due to the inclusion of more polygenic compared to salient traits found as descriptors. Ryegrass studies conducted by Meléndez-Ackerman *et al.* (2005) reported not significant and quite low correlation between the morphologic and AFLP dendrograms, while Roldán-Ruiz *et al.* (2001) reported negative correlation between AFLP and morphologic characteristics. Elias *et al.* (2000) found weak correlation between inter-varietal distances assessed using morphologic, agronomic and AFLP markers, while for this reason Benesi (2005) suggested the combination of both AFLP and morphological characterisation on cassava germplasm for more accurate results. Colombo *et al.* (2000) studying genetic relatedness between cultivated cassava and two naturally occurring species, *M. Flabellifolia* and *M. peruviana* concluded that RAPD and AFLP markers were unable to differentiate between the two wild species, which confirms their botanical similarity.

However, Vieira *et al*. (2007) on wheat, and Nemera (2003) on sorghum reported a significant correlation between AFLP and morphological genetic diversity analysis. Tatineni *et al.* (1996) reported a high correlation between RAPD and morphological characters on cotton. Benesi (2002, 2005) reported high correlation and a high degree of relationship between AFLP and morphological genetic diversity analyses methods.

It is known that cassava is influenced by the environment and this influences the morphological variables. When morphological traits are compared to DNA markers, it is found that they are relatively less reliable and sometimes inefficient for precise

discrimination of closely related genotypes and analysis of their genetic similarity. Morphological traits are limited in number and interact with the environment. They should be used as a general approach and preliminary tests of varietal identification. AFLP analysis has several advantages over other methods, including morphological methods. They are numerous, genetic variation is observed at DNA level and they are reproducible (Janssen *et al.*, 1996; Jones *et al.*, 1997) and it has a widespread application across all phyla (Janssen *et al.*, 1996; 1997; Folkertsma *et al.*, 1996; Mueller *et al.*, 1996; Otsen *et al.*, 1996; Travis *et al.*, 1996).

Table 5.5 Correlation matrix for AFLP, morphological genetic diversity analysis

	AFLP	Morphological
Morphological	0.013	
Combined morphological and AFLP analysis	0.998**	0.0144

**$p \leq 0.05$

5.4 Conclusions and recommendations

Relatively low levels of correlation were found between the morphological versus AFLP and combined analysis, but strong correlation was found between AFLP and combined analysis. AFLP analysis has several advantages over other methods including morphological methods. They are numerous, genetic variation is observed at DNA level and it provides more accurate estimation of genetic diversity. In practice, morphological traits have been used traditionally for the characterization of genetic diversity. It forms the bases for the distinction of germplasm at farm level and also for breeders and curators. At certain stages, the morphological traits used were able to distinguish between cultivars and the molecular markers complemented the data obtained to distinguish and separate the most distinct accessions. These results support the idea of combining both methods, to obtain a more accurate idea of the accession.

Morphological and molecular data matrices are very informative tools for the establishment of genetic distance. Both types of information appear to be complementary and useful to determine distinctiveness among accessions.

The use of molecular markers allowed the identification of the unique accessions to be used in the breeding programme. The AFLP and morphological analysis did not group the accessions according to geographical origin. The PCoA provided a diversity structure similar to the observed clustering patterns of the dendrograms, suggesting the effectiveness of PCoA analysis in the genetic diversity studies.

Further studies should involve larger numbers of accessions with information about their pedigree for a clear picture of diversity in the country and the role of qualitative and quantitative morphological traits in the combined analysis. Alternatives methods of transforming morphological data to binary data should be identified. In this study we have used PCoA analysis. Additional work is needed to determine the most accurate means of transformation.

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CHAPTER 6

IDENTIFYING SUPERIOR FAMILIES BY CLONAL EVALUATION

6.1 Introduction

Cassava is produced mainly for household consumption as staple diet in most rural areas of Mozambique. It is produced almost exclusively by smallholder farmers (99%) occupying about 17% of the arable land area. Cassava has an average annual yield of 10.5 ton/ha (FAO, 2005), and the main use remains human consumption (FAO/MIC, 2007). In Southern Africa, it is estimated that cassava contributes 25-60% of the national balance sheet (Phiri, 2001).

Fresh roots, dried chips and roasted cassava flour (rale) constitute the main forms of rural and urban markets and thus provide a source of income generation to the producers in Mozambique. Cassava is grown for the food market, with minimum processing, using traditional tools (FAO/MIC, 2007), where root taste and cooking quality are important requirements (Kawano *et al.*, 1998; Ceballos *et al*., 2004). The plant height and type plays an important role in the relative plant density per area, and the cropping system is based on intercropping (Zacarias and Cuambe, 2004).

Walker *et al.* (2006) conducted a study in the agricultural sector in Mozambique and have identified cassava as the crop with the highest production value, followed by maize. The two crops have 55% of potential to contribute to alleviate income poverty in the small-scale sector in the country and an increase in productivity of cassava and maize of 20% is translated into a reduction of income poverty of as much as 19% and leads to a poverty reduction that exceeds 5% in staples.

Cassava faces new challenges and there is a need to provide quick responses to crop development needs where higher root yield per area, high dry matter content, as well as the capacity to produce under biotic and abiotic stresses are the main issues. However, the conventional cassava breeding assessment method has a long

cycle (IITA, 1990). The breeding project at CIAT-Colombia (Pérez *et al.,* 2002; Ceballos *et al.,* 2004), proposed a modified breeding scheme to improve the efficiency and effectiveness of the cassava breeding programme. The modifications includes: (i) the clones under evaluation from a given family are separated into three groups; (ii) each group of a family is randomly allocated to one of the three blocks to allow replication of information. This scheme has some advantages such as that the potential information is gathered at the first clonal evaluation stage, the possibility to shorten the crop evaluation cycle and more efficiently identify superior germplasm, which can easily be maintained by vegetative multiplication.

The objective of this study was the identification of superior cassava families and clones in a clonal evaluation trial and to test the selection procedure developed by CIAT, to screen segregating progeny in a cassava brown streak pressure area.

6.2 Material and methods

The trial was conducted in Nampula, Mogincual Substation (described in the Chapter 3). Seventeen parents (described in the Chapter 4) assigned for this experiment were selected on their ability to produce many flowers. The hybridisation block was planted at PAN, Nampula-Mozambique. Entries were planted in single rows of 1 m between plants and 2 m between rows. Each parent was represented in two consecutive rows. Genotypes were monitored for start of flowering and crosses. Development of progeny followed the procedures described in Chapter 3. The seedling nursery trial was developed during the 2004/05 season. At seedling evaluation, minimum selection was done. The plant growth and the amount of planting material per plant depend to a large extent on the weather conditions (Fig 3.1), consequently the number of clones varied among families. The trial was comprised of 12 families which were divided into three blocks. Only families with more than 21 clones and which had the capacity to produce more than six stem cuttings each plant were incorporated in the first evaluation trial. This permits the reduction of confounding effects between number of plants and genotypic differences (Ojulong *et al.*, 2007). The experiment was established in December 2005, at the beginning of the rain season. A randomised complete block design was

used. Plots comprised of single rows with a 1 m x1 m spacing. Weeding was done, but no irrigation and fertiliser were applied during the course of trial. Harvesting was done in November 2005.

Before harvest, data was recorded on plant height, to the highest terminal stem, and plant type (1=excellent, 5=very undesirable) (Ceballos *et al.*, 2002). Both foliage (leaves and stems) and fresh root weight were recorded. Complementary data was collected on taste, root necrosis caused by cassava brown streak disease (CBSD) and harvest index, which were scored as described in Chapter 3. Harvest Index was derived as a function of root weight and total biomass. Number of roots per plant was calculated as ratio between number of roots and number of plants. Root dry matter was estimated using gravimetric methodology (Kawano *et al.*, 1987), where approximately 3 kg of roots were weighed in a hanging scale (W_A) followed by measuring its weight in water (W_W). Dry matter was estimated using the formula:

%DMC= (158.3 x (W_A/ (W_A-W_W)) – 142); where W_A=weight in air and W_W=is weight in water (Jamarillo *et al.*, 2005).

Dry root yield was derived by multiplying the fresh root weight and DMC. Data was subject to analysis as described in the Chapter 3. Analytical software Statistix 8 was used to perform the correlation analysis of biotic stresses and yield components and stepwise linear regression and Spearman rank correlation. Excel software was used to calculate the selection index (SI) using the available SI for cassava developed by CIAT for sub-humid environments:

SI = (FRY x 10) + (DMC x 8) – (PTS x 5) + (HI x 5) (Ceballos, 2002);

where: FRY, is the fresh root weight; DMC is the dry matter content; PTS is the plant type score and HI the harvest index.

6.3 Results and discussion

Twelve F_1 families were included in the first clonal evaluation trial with a total of 927segregating genotypes (Table 6.1). The number of genotypes evaluated per F_1 family depended largely on the capacity of the genotype to produce viable flowers, seed set at the breeding nursery and the germination of seeds. In this case, the family with the highest number of genotypes under evaluation was the F_1 family F-BBSARMG/05-12 (183), followed by F-BBSARMG/05-16 (157), while a smaller number of genotypes was observed for F-BBSARMG/05-10 (21).

Mean performance and standard deviation of all traits are presented in Table 6.2. The results observed by genotypes within family where averaged to determine the performance of a given family. Plant type varied from 2.05 to as high as 3.22 observed in F-BBSARMG/05-13 and F-BBSARMG/05-10 and F-BBSARMG/05-15 respectively, revealing that more attention should be given to plant type in the breeding programme, where farmers prefer plant type 1 to 2 which is less branching and taller. This is an indication of their adaptation to the intercropping system. This trait is also crucial to facilitate the adoption of new varieties.

The foliage weight varied from 8.58 to 16.55 t/ha, observed in F-BBSARMG/05-15 and F-BBSARMG/05-12, respectively. The fresh root weight mean performance ranged from 9.02 to 16.96 in the families F-BBSARMG/05-17 and F-BBSARMG/05-12. The total biomass ranged from 19.91 to 33.51, observed in the F-BBSARMG/05-15 and F-BBSARMG/05-12, respectively. The lowest dry matter weight was found in F-BBSARMG/05-13 (1.31). Harvest index obtained ranged from 0.36 in F-BBSARMG/05-14 to 0.54 in F-BBSARMG/05-18. CBSD root necrosis, varied from 1.00 in the F-BBSARMG/05-19 to 2.60 F-BBSARMG/05-14. Therefore family F-BBSARMG/05-19 should be considered as one of the sources for the next nursery crosses due to this performance where all 67 entries had no CBSDr but had other poor traits that allowed inclusion of only five entries.

Table 6.1 Pedigree and clones evaluated at clonal evaluation trial in Mogincual in 2006

Pedigree	Family code	Total clones Evaluated
Mocuba x Munhaca	F-BBSARMG/05-10	21
TMS 30001 x Nikwaha	F-BBSARMG/05-11	70
Likonde x MZ 89106	F-BBSARMG/05-12	183
Chigoma mafia x IMM 30025	F-BBSARMG/05-13	53
Macia 1 x Mocuba	F-BBSARMG/05-14	67
Nachinaya x Muanamwahula	F-BBSARMG/05-15	60
Namuhipwi x Macia 1	F-BBSARMG/05-16	157
Macia 1 x Mocuba	F-BBSARMG/05-17	100
Mulaleia x Nikwaha	F-BBSARMG/05-18	77
N'xinkole x Macia 1	F-BBSARMG/05-19	57
Likonde x Macia 1	F-BBSARMG/05-20	43
IMM 30025 x Baadge	F-BBSARMG/05-21	23
Nikwaha x Likonde	F-BBSARMG/05-22	16
Total		927

Sweet and bitter taste were recorded in the tested entries. In general, bitter taste was predominant. However, farmers are wiling to include both sweet and bitter types in their fields (Zacarias and Cuambe, 2004). Chiwona-Karltun *et al.* (1998) reported that farmers prefer the bitter taste varieties as they are less prone to theft when compared to sweet varieties. The dry matter content mean ranged from 27.65% to 33.28% observed with families F-BBSARMG/05-15 and F-BBSARMG/05-18 respectively. A total of ten characters were used in the analysis of the clonal evaluation trial. It is important to note that this area is a CBSD hot spot, which affects the root quality. Only genotypes which did not show any visible CBSDr symptoms (class 1) were selected. The breeding objective at the present stage is focussed on generation of genotypes that are resistant to CBSD, high yielding and mainly for household consumption. Thus, root taste plays an important role in the selection for the final products.

Table 6.2 Mean performance and standard deviations for some characters estimated from the 12 full-sib F_1 families evaluated at first clonal evaluation in Mogincual in 2006

Family code	PTY (1-5)	Fol (ton/ha)	FRY (ton/ha)	BIOM (ton/ha)	DRY (ton/ha)	HI (0-1)	CBSDr (1-5)	RTST (1-2)	PlH (cm)	DMC (%)
F-BBSARMG/05-10	3.22±0.22	16.21±9.36	11.10±7.02	27.32±15.16	1.80±0.57	0.41±0.15	2.55±1.66	1.67±0.43	188.32±44.01	27.65±8.13
F-BBSARMG/05-11	3.03±0.72	17.3±13.09	11.34±8.19	28.67±20.34	1.61±0.54	0.44±0.15	1.26±0.64	1.30±0.44	173.40±48.24	33.65±2.79
F-BBSARMG/05-12	2.80±0.56	16.55±5.6	16.96±5.69	33.51±08.68	2.29±0.75	0.50±0.10	1.50±0.81	1.87±0.31	212.08±38.96	32.62±3.33
F-BBSARMG/05-13	2.05±0.78	14.44±7.23	12.67±7.50	27.11±12.40	1.31±0.44	0.45±0.13	1.41±0.82	1.47±0.48	153.86±69.00	33.22±3.55
F-BBSARMG/05-14	3.22±0.41	15.31±9.55	9.02±7.34	24.34±15.01	1.53±.0.80	0.36±0.14	2.60±1.71	1.79±0.43	191.89±32.16	28.05±7.01
F-BBSARMG/05-15	2.86±0.57	8.58±7.65	11.33±12.09	19.91±16.91	1.62±1.10	0.54±0.10	1.95±1.02	1.55±0.46	164.64±19.70	31.42±4.77
F-BBSARMG/05-16	3.12±0.66	15.01±9.41	14.95±09.49	29.97±12.65	1.77±0.96	0.50±0.18	1.33±0.84	1.78±0.40	172.69±59.36	33.28±3.79
F-BBSARMG/05-17	3.11±0.42	16.38±13.70	14.34±20.07	30.73±29.16	2.88±2.70	0.45±0.13	2.02±1.41	1.50±0.53	177.71±49.25	29.46±7.78
F-BBSARMG/05-18	3.17±0.58	10.78±8.54	13.47±9.49	24.34±14.24	1.98±1.62	0.54±0.10	1.55±1.58	1.40±0.53	168.33±58.60	31.49±6.81
F-BBSARMG/05-19	3.20±0.40	12.16±3.27	14.06±4.74	26.2±37.92	2.16±0.46	0.53±0.02	1.00±0.00	1.66±0.47	194.16±13.67	32.06±2.46
F-BBSARMG/05-20	2.89±0.28	13.37±5.21	12.06±8.55	25.43±11.97	2.12±0.42	0.45±0.13	1.41±0.95	1.58±0.49	204.11±27.12	32.67±2.24
F-BBSARMG/05-21	2.87±0.42	13.97±6.13	13.40±9.22	27.38±12.91	2.54±1.08	0.44±0.16	1.34±0.85	1.52±0.54	191.87±46.12	31.87±6.79
F-BBSARMG/05-22	2.88±0.65	16.72±10.99	12.75±6.81	29.48±15.08	1.50±0.94	0.44±0.12	1.29±0.83	1.41±0.47	207.28±33.17	28.33±3.81

PTY=plant type; Fol=foliage weight; FRY=Fresh root yield; BIOM= Biomass; DRY= dry matter weight; HI= harvest index; CBSDr= cassava brown streak root necrosis; RTST= Root taste; Pl_H= plant height; DMC= Dry matter content

It is known that household cassava consumption in this area is in the form of fresh snacks, boiled and processed into dry chips to produce flour for soft porridge. Surveys to attempt to evaluate the effect of CBSD in the country found that a score of 2 compromised the quality of cassava while roots with a class 3 score were considered uneatable (Zacarias and Cuambe, 2004).

6.3.1 Coefficient of correlation

The Spearman's coefficient of rank correlation was performed on yield components and biotic stress traits, such as CBSD root necrosis in the roots (Table 6.3). Dry matter content was significant and positively correlated with biomass (r=0.328). Dry matter content showed also significant and negative correlation with cassava brown root necrosis (r=-0.964). This is probably due to the fact that cassava roots affected by CBSD have a change in consistency, thus affecting the dry matter content. Foliage positive and highly significant correlated with biomass (r=0.493). Root number per plant was positively and highly correlated with total biomass (r=0.499), dry matter content (r=0.605), fresh root weight (r=0.733) and harvest index (r=0.609), indicating that it is one of the best traits to consider at early selection stage. Similar results were reported by Kawano (2003) and Ojulong *et al.* (2007) analysing first clonal evaluation trials in respect to the correlation with root yield components. Fresh root weight was highly correlated with total biomass (r=0.538) and dry matter content (r=0.600), while harvest index was highly correlated with dry matter content (r=0.531) and fresh root weight (r=0.852).

Most of the biotic stresses in cassava, such as diseases, have an influence by reducing root yield per area (Mahungu *et al.*, 2003; Benesi, 2005; Hillocks *et al.*, 2002). Despite the fact that the experiments were conducted under high CBSD pressure, the results here show absence of correlation of CBSD root necrosis and root yield traits and yield components. Besides, the loss caused by CBSD in the roots is reflected in the quality of the roots and not in the yield *per se* (Calvet and Thresh, 2002; Hillocks, 2002; Zacarias and Cuambe, 2004; Cuambe *et al.*, 2007).

Plant type is regarded as one of the most important traits to select for in cassava (Ceballos *et al*., 2004; Kawano *et al*., 1978; Hahn *et al*., 1979) and small holder and medium scale farmers normally cultivate cassava under intercropping systems. In this study, plant type had no correlation with CBSDr, although CBSD affects all plant parts, including fruits, leaves, stems and roots, where the storage roots become brown and corky (Calvet and Thresh, 2002). It suggests that plant type and CBSDr, should be considered independent parameters.

Table 6.3 Spearman's coefficient of rank correlation among some traits evaluated in the first clonal evaluation in Mogincual 2006

	BIOM	CBSDr	DM	FOL	FRY	HI	NRPL	PLH	PLT
CBSDr	-0.406								
DM	0.382*	-0.964*							
FOL	0.493**	0.229	-0.214						
FRY	0.538**	-0.642	0.600**	-0.467					
HI	0.106	-0.552	0.531**	-0.769	0.852**				
NRPL	0.499**	-0.691	0.605**	-0.226	0.733**	0.609**			
PLH	0.419*	-0.259	0.262	0.074	0.360	0.218	0.524**		
PTY	0.124	0.257	-0.202	0.127	0.004	-0.009	-0.161	-0.150	
TST	-0.061	0.131	-0.113	-0.145	0.073	0.130	-0.223	-0.326	0.041

BIOM= Biomass; CBSDr= cassava brown streak root necrosis; DMC= Dry matter content; FOL=foliage weight; FRY=Fresh root yield; HI= harvest index; NRPl= root number per plant; PlH= plant height PlT=plant type, RTST= Root taste; * $P < 0.05$; ** $P<0.01$.

6.3.2 Stepwise linear regression

Fresh root yield was highly influenced by most of the yield components but foliage weight and number of roots made the largest contribution to fresh yield (Table 6.4). All the results were significant ($p<0.05$). It indicates that total foliage weight, root number, harvest index, total biomass and dry matter content were important components for fresh root weight. The other components in this study, such as plant

type, cassava brown streak and plant height did not show a significant relationship with fresh root weight and were therefore, not included in the equation.

Table 6.4 Stepwise linear regression for fresh root yield

Fresh root weight	(R^2)
Foliage weight	0.709772
Number of roots	0.703940
Harvest index	0.654025
Total biomass	0.571138
Dry matter content	0.398585

All significant at $P < 0.05$

6.3.3 Selection index under CBSD conditions

From the two analyses presented (Spearman's coefficient of rank and stepwise linear regression correlation) (section 6.3.1 and 6.3.2) it can be concluded that fresh root yield is the most important trait when considering yield and yield component traits. By the Spearman's coefficient rank, FRY had the strongest correlation of 0.852 with harvest index and of 0.732 with number of roots per plant, while by stepwise linear regression FRY had a significant relationship with most other traits. This validates that fresh root weight should be given the highest economic value in the selection index.

CIAT have proposed a selection index with four characters, in which maximum weight (10) was assigned to fresh root weight, followed by dry matter content (8), plant type (5) and harvest index (5). Fresh root weight and dry matter content (which have been given the highest weights) are determinants of starch yield which is the most important economical product. The two traits together with harvest index and plant type have high heritability (Ceballos *et al*., 2004; Kawano *et al*., 1978; 1998; Kawano, 2003; Hahn *et al*., 1979; Iglesias and Hershey, 1994).

Root taste and CBSDr are very important traits for selection at this particular environment because they are highly ranked by farmers for variety adoption for fresh consumption or for processing. However, they were not significantly correlated with any trait in both correlation sets presented here, meaning that they are independent. A selection index appropriate for these conditions should include not only the important traits that are significant for root yield, but also plant type and CBSDr.

Selection at this environment was done based on the following Selection Index (SI):
SI = (FRY x 10) + (DMC x 8) + (HI x 5) + (RTST x 2) – (PTS x 2) – (CBSDr x 5)
where: FRY, is the fresh root weight; DMC is the dry matter content; HI the harvest index; RTST, is root taste; PTS is the plant type score and CBSDr is cassava brown streak disease root necrosis.

The SI proposed here was used to screen the 963 genotypes which were subjected to analysis using Excel software. After screening, about 17% of genotypes were selected using the SI with average selection of 1.47% per family (Table 6.5). The SI was performed per replication and within family in each replication. For each replication the mean values and standard deviations were expected to have the standardised values of zero and unit, respectively for each character (Ceballos *et al.,* 2002). In the SI CBSDr was given the weight of 5, and it was possible to screen most of the genotypes which had CBSD root necrosis. About 1% of clones with CBSDr were among the selected clones, due to their high fresh root yield.

The SI in this study varied from 5.2416 to 4.0447 in the best performing clones. Five families were selected according to their best average performance which was determined using SI. The best families had positive SI, while the poorest had negative SI values. Cassava brown streak had a significant influence on the performance of the families.

The use of a selection index is a superior procedure followed by researchers and may help the breeder to structure the interpretation of complex genetic parameters (Magnussen, 1990). The SI is subject to change depending on the environment or the breeding objectives.

6.4 Conclusions and recommendations

Ten characters were used to assess a clonal evaluation trial comprised of 12 families and 927 segregating progenies. The characters were recorded during plant growth stage and at harvest. By means of correlation analysis, it was found that:

- Root number per plant was positively and highly correlated with total biomass, dry matter content and fresh root weight;
- Fresh root weight was highly correlated with total biomass and dry matter content, while harvest index was highly correlated with dry matter content and fresh root weight;
- CBSDr showed high negative but not significant correlation with dry matter content;
- Plant type is regarded as one of the most important traits to select for in cassava. CBSD affect all plant parts, however, this study concluded that there was not any correlation between plant type and CBSDr. It is recommended that cassava brown streak root necrosis should be considered as independent parameters.

Table 6.5 Mean performance of best and poorest F_1 clones in the evaluation and the average per block

Family code	PTY (1-5)	FoI (t/ha)	FRY (t/ha)	BIOM (t/ha)	HI (0-1)	DRY (t/ha)	CBSDr (1-5)	RTST (1-2)	Pl_H (cm)	DMC %	Selection Index	Selected (%) *
						Best F_1 Clones						
F-BBSARMG/05-15	3	16.4	20.5	36.9	0.56	4.24	1	1	143.3	20.7	5.204070461	0.9
F-BBSARMG/05-10	4	22.3	21.4	43.7	0.49	6.93	1	1	142.5	32.4	4.044776849	1.9
F-BBSARMG/05-22	3	12.7	24.6	37.3	0.66	6.57	1	2	205.0	26.7	5.241677829	2.1
F-BBSARMG/05-18	3	18.5	20.2	38.7	0.52	6.57	1	1	227.0	32.5	4.920755245	1.3
F-BBSARMG/05-20	3	20	31.4	51.4	0.61	9.80	1	1	233.8	31.2	4.623680088	1.3
						Poorest F_1 Clones						
F-BBSARMG/05-17	5	5	2.5	7.5	0.33	0.39	5	1	148.3	15.4	-4.870471347	0.6
F-BBSARMG/05-11	4	5	1.5	6.5	0.23	0.31	5	1	135.0	12.4	-5.48106707	1.7
F-BBSARMG/05-12	4	3	2.5	5.5	0.45	0.33	5	1	110.0	13.2	-12.0885501	3
F-BBSARMG/05-13	5	7	0.5	7.5	0.07	0.39	4	2	45.0	15.4	-7.105147585	1.1
F-BBSARMG/05-14	5	15	2.5	17.5	0.14	0.18	5	1	153.0	13.2	-7.599479336	0.7
						Performance per block						
Replication I	3.1	15.71	11.18	26.87	0.4	3.5	1.94	1.45	174.7	30.27	0.000	
Replication II	3.12	16.06	10.95	27.01	0.4	3.37	1.9	1.48	173.1	30.60	0.000	
Replication III	3.16	16.43	11.28	27.63	0.4	3.48	1.88	1.48	175.4	30.82	0.000	
Mean	3.13	16.07	11.14	27.17	0.4	3.45	1.91	1.47	174.4	30.56	0.000	1.47

Pl_T=plant type; FoI=foliage weight; FRY=Fresh root yield; BIOM= Biomass; DRY= dry matter weight; HI= harvest index; CBSDr= cassava brown streak root necrosis; RTST= Root taste; Pl_H= plant height; DMC= Dry matter content; * = percentage of selected clones

Some traits are influenced by more than one component. The fresh root yield was highly influenced by most of the yield components but foliage weight and number of roots made the largest contribution to fresh yield. It indicates that total foliage weight, root number, harvest index, total biomass and dry matter content were important components for fresh root weight. Besides, fresh root yield is the most important trait when considering yield and yield component traits. This validates that fresh root weight should be given the highest economic value in the selection index.

From this study under Mogincual environmental conditions, a selection index equation was calculated as follows:

SI = (FRY x 10) + (DMC x 8) + (HI x 5) + (RTST x 2) – (PT_S x 2) – (CBSDr x 5)

where: FRY, is the fresh root weight; DMC is the dry matter content; HI the harvest index; RTST, is root taste; PT_S is the plant type score and CBSDr is cassava brown streak disease root necrosis.

Selection index is an important procedure followed by researchers and it helps to structure the interpretation of complex genetic parameters. It is subject to change depending on the environment or the breeding objectives.

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CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The present study generated relevant information to plan a more efficient cassava breeding programme in Mozambique. It was focused on diallel evaluation and genetic diversity studies. The parental genotypes were those with better performance in a particular region where cassava brown streak is the main economic constraint and farmers are willing to have varieties that perform better that those existing in their fields.

The analysis of variance and the GCA: SCA ratio indicated that the GCA was larger than SCA for average root number, average fresh root yield, root taste, root pulp hardness yield and harvest index, indicating the prevalence of additive gene effects and a possibility for improvement of these characters. Parents with superior and positive GCA effects as well as combinations with relatively important positive or negative SCA effects were identified for some of the studied traits. This study demonstrated that reaction to cassava brown streak root necrosis depends strongly on SCA effects, thus SCA effects are more important in predicting progeny performance for expression of resistance to cassava brown streak disease. The heritability estimates also indicate that it is a polygenic trait. Harvest index heritability estimates and its predictability ratio were high, indicating high heritability with additive gene action. The cross Macia 1 x Chigoma mafia had the best SCA for the most important traits in the study; fresh root weight, cassava brown streak and root pulp hardness. Chigoma mafia x Mulaleia, MZ 89186 x IMM 30025 and MZ 89158 x Macia 1, had the best values for mid-parent heterosis.

Cassava is a vegetatively propagated crop and any clone with good traits for specific end use, represents a potential new variety. The other advantage in this study was the fact that the studied accessions were obtained from farmers. They already possess desired traits and they also have adaptation to the specific environment where they are selected and grown. Breeders must take advantage of knowledge

regarding parents identified as good combiners and knowledge on inheritance of cassava traits.

Cassava plant type is regarded as one of the most important traits within farmer's selection criteria. Despite the fact that cassava brown streak disease affects all plant parts, there was no correlation between plant type and this disease. An adequate selection procedure for the best progeny is crucial under these circumstances. A selection index with a combination of characters studied was proposed as follows:

SI = (FRY x 10) + (DMC x 8) + (HI x 5) + (RTST x 2) – (PTS x 2) – (CBSDr x 5)

Genetic diversity was accessed by means of morphological and AFLP markers for parents in the breeding programme. The genetic distance and cluster analysis showed a narrow genetic base within the accessions. AFLP was more accurate to distinguish the accessions. The varieties, Mocuba and Nikwaha, were the most distinct among the accession and were placed in a divergent position within the clusters, suggesting their possible good breeding values when compared to other genotypes and exploitation of their performance should be maximised. The accessions were divided in two heterotic groups which can be used in the breeding programme.

Morphological and molecular data matrices are very informative tools for the establishment of genetic distance. Both types of information appear to be complementary and useful to make distinction among accessions. PCoA provided a diversity structure similar to that observed in the clustering patterns of the dendograms. The accuracy of AFLP analysis was also demonstrated by the correlation coefficient analysis of AFLP, morphological and the combined analysis. Use of molecular markers allowed the identification of unique accessions to be used in the breeding programme. The AFLP and morphological clustering patterns of cassava genotypes in this study did not indicate any relationship between genetic distance and eco-geographical distribution. The use of a combination of both methods, morphological and AFLP markers, to obtain a more accurate idea of the accessions, is recommended.

The information gathered from this study is essential for efficiently formulating a breeding programme strategy. The parents and progeny here identified are potential candidates to produce a new generation of segregating progeny and increase the number of improved cassava varieties in Mozambique. Introduction of improved germplasm from other countries or International Centres should be prioritised in order to broaden the genetic basis for improvement of cassava in the country.

Further studies should be conducted, increasing the number of entries and environments in the diallel study as well as to involve larger numbers of accessions with information about their pedigrees, which was not possible for this study.

CHAPTER 8

SUMMARY

Key words: Cassava, combining ability, heterosis, heritability, morphology, AFLP, selection index, Mozambique

This study represents the first comprehensive genetic study based on local cultivars of Mozambique. Five cassava (*Manihot esculenta* Crantz) parents were crossed in a full diallel. The parents and F_1 progeny were evaluated for eight agronomical and 17 morphological traits in two consecutive seasons. General combining ability (GCA) was higher than specific combining ability (SCA) for number of roots, root taste, fresh root yield, root pulp hardness and harvest index. Positive heterosis was seen for number of roots, fresh root weight and harvest index. Negative heterosis was present for cassava brown streak virus disease. Harvest index showed high broad sense heritability, which indicated that phenotypic variance was additive. The predictability ratio was close to unity, which confirmed that this trait is highly heritable, and determined by additive gene action. Cassava brown streak root necrosis had SCA effects significantly higher than GCA effects indicating the presence of non-additive genetic effects. A selection index comprised of the most relevant characters was compiled.

Amplified fragment length polymorphism (AFLP) analysis showed high genetic similarity among 17 accessions. A total of 425 fragments were produced by eight primer combinations. Mocuba and Nikwaha were the most distinct among the accession and two heterotic groups were suggested. Morphological analysis showed genetic distance ranging from 0.105 to 0.833. The correlation coefficient between AFLP and agronomic clustering was not significant. The AFLP and morphological analysis did not group the accessions according to geographical origin. The principal component analysis provided diversity structure, suggesting the effectiveness of this analysis in genetic diversity studies. The narrow genetic similarity observed, indicates the need to introduce new cassava germplasm.

OPSOMMING

Sleutelwoorde: Cassava, kombineervermoë, heterose, oorerflikheid, morfologie, AFLP, seleksie indeks, Mosambiek

Hierdie studie verteenwoordig die eerste omvattende genetiese studie gebasseer op plaaslike cultivars van Mosambiek. Vyf cassava (*Manihot esculenta* Crantz) ouers is in ‘n volle dialleel gekruis. Die ouers en F_1 nageslag is geëvalueer vir agt agronomiese en 17 morfologiese eienskappe in twee opvolgende seisoene. Algemene kombineervermoë was hoër as spesifieke kombineervermoë vir aantal wortels, wortel smaak, vars wortel opbrengs, wortel pulp hardheid en oesindeks. Positiewe heterose vir aantal wortels, vars wortel gewig en oesindeks is gevind. Negatiewe heterose is gesien vir cassava bruinstreepvirussiekte. Oesindeks het hoë breë sin oorerflikheid getoon, wat gewys het dat fenotipiese variansie additief is. Die voorspellingsverhouding was na aan een, wat bevestig dat hierdie eienskap hoogs oorerflik is en deur additiewe geenaksie bepaal word. Cassava bruinstreepvirussiekte het betekenisvol hoër spesifieke as algemene kombineervermoë getoon wat die teenwoordigheid van nie-additiewe effekte aangedui het. ‘n Seleksie indeks wat bestaan het uit die belangrikste eienskappe, is saamgestel.

“Amplified fragment length polymorphism” (AFLP) analise het hoë genetiese ooreenkoms tussen die 17 genotipes aangetoon. ‘n Totaal van 425 fragmente is met agt priemstuk kombinasies geamplifiseer. Mocuba en Nikwaha was die mees uniek van die genotipes en twee heterotiese groepe is aangedui. Morfologiese analise het ‘n genetiese afstand tussen 0.105 en 0.833 aangetoon. Die korrelasie koeffisiënt tussen AFLP en agronomiese groepering was nie betekenisvol nie. Die AFLP en morfologiese analise het die inskrywings nie volgens geografiese areas gegroepeer nie. Die hoof komponent analise het ‘n diversiteitsstruktuur verskaf, wat die doeltreffendheid van hierdie metode vir genetiese diversiteitsstudies aantoon. Die nou sin oorerflikeidswaardes van die studie het die nodigheid uitgewys om nuwe cassava kiemplasma by die teelprogram in te sluit.

Annexes

Annex 3.1. Morphological descriptors used during two seasons in Mogincual, 2004 and 2005

Morphologic trait	Code	Classes
Plant height	PH	Cm
Leaf pubescence	PBs	1=absence, 2=presence
Number of lobes per leaf	NL	1 to 9
Shape of the central lobe	SHL	1=brad; 2=narrow
Unexpanded leaf colour	ULC	1=light green, 2=dark green, 3=green purple, 4=purple
Mature leaf colour	MLC	1=light green, 2=dark green, 3=green purple, 4=purple
Leaf vein colour	CNC	1=light green, 2= green, 3=green purple, 4=red, 5=dark red; 6=purple
Petiole colour	PLC	1=light green, 2=dark green, 3=red; 4=dark red; 5=green purple, 6=purple;
Petiole position	PTP	1=erect, 2=horizontal, 3=deflex, 4=retorse
Petiole length	PTL	Cm
Mature stem colour	MSC	1=silver green, 2=green, 3=light brown, 4=brown, 5=orange
Apical stem colour	ASC	1=light green, 2=dark green, 3=green purple, 4=purple
Stem habit	StH	1= erect, 2= zigzag