Quantitative and molecular analyses of agronomic traits in cassava (*Manihot esculenta* Crantz)

By

HENRY FRED OJULONG

Submitted in accordance with the requirements for the Philosophiae Doctor degree in the Department of Plant Sciences: Plant Breeding, in the Faculty of Natural and Agricultural Sciences at the University of the Free State

UNIVERSITY OF THE FREE STATE
BLOEMFONTEIN
SOUTH AFRICA

Supervisor: Prof. Maryke T. Labuschagne

Co-supervisors: Dr. Martin A. Fregene
Dr. Liezel Herselman

November 2006

Declaration

"I declare that the thesis hereby submitted by me for the degree of Philosophy 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.

I further more cede copyright of the thesis in favour of the University of the Free State."

……..... ……....

Henry Fred Ojulong Date

Dedication

This work is dedicated to Mary Teresa Akiteng for being a good mother; Tracy, Lilian, Casy and Marvin, thank you for being there for me.

Acknowledgements

My sincere gratitude to Dr. Martin Fregene for giving me the chance to work with you. Thanks to you and family for providing me a family in Colombia. Thank you to the Rockefeller foundation with special thanks to Dr. Joe DeVries for sponsoring the study. Professor Maryke Labuschagne, thank you for accepting to supervise me, the guidance, valuable discussions and all the help while at CIAT and here at the university. It has been of great help and much appreciated. I will always be grateful to Dr. Liezel Herselman for accepting to co-supervise me.

May I thank the people at CIAT, for making my stay their enjoyable. Special appreciation to Dr. H. Ceballos for providing the material for the study and for your useful comments and discussion, I benefited a lot. Cassava breeding programme for such excellent working atmosphere, especially J. Perez; N. Morante, Calle, Teresa and the pollination group, thank you. People of Genetica Yuca lab, I appreciated working with you. Jaime thank you for walking me through molecular work: Edgar, Caeser, Paula, Danilo, Adriana, Isabel, Janet, Charles, Wilson, Ana Maria, Olalekan you are such a wonderful people. Dr. Egesi thank you for going through parts of this work for me. To people in training office, Dr. A. Caldas, Marcela and Andres I am already missing you. *Para todos muchas gracias.*

To Dr. Jim Whyte, for introducing me to the cassava club and for the mentorship while in IITA. I will always be grateful. I am indebted to the ESARC-EARRNET breeding team, Drs. B. Khizzah and P. Ntawuruhunga, and F. Okello for being wonderful workmates.

To my colleagues and friends I am very grateful for your friendship and support throughout this time. Jose and Godwin thank you for being housemates in Cali, and Oscar and Wako in South Africa. Philip Ragama, Peter Takan, Fred Ssango, I guess it is finally done, appreciation to you, now what? Gorettie Ssemakula, thank you for being a big sister, for reading through most of this work, for those wonderful emails and fun, believe me you have made a difference. To Elizabeth Okai, Elizabeth Kizito, Anabela, thank you for being more than friends.

I wish to thank the Department of Plant breeding, for housing me and for such nice people, especially Mrs. Sadie for you excellent coordination, Fred, Scott and Oscar for being wonderful officemates.

Special thank you to my wife Apese, daughers Tracy and Lily, thank you for being there for me, for enduring all that time when I was not there. Appreciation is extended to my sister Helen, brothers Albert and Charles and Dad Henry senior, for helping out on my family and for being always there for me to lean on, eyalama. Last but not least, I thank all those I have not been able to mention, deep in heart you are appreciated.

Above all I thank God Almighty for giving me the strength to go through this and for surrounding me with wonderful people, may Your name always be praised.

Contents

List of tables

List of figures

Chapter 1

General introduction

Cassava (*Manihot esculenta* Crantz) is a perennial crop native to tropical America with its center of origin in north-eastern and central Brazil (Allem, 2002). It has spread to all tropical and subtropical regions where it is grown from sea level up to altitudes of 1800 m.a.s.l. (Cock, 1985). Cassava is one of the most important food energy sources in many tropical countries (Cock, 1982; 1985; Henry and Hershey, 2002; Hillocks, 2002; Onwueme, 2002).

Cassava was disseminated from South America to Africa by the Portuguese (Charrier and Lefevre, 1987). First place of entrance was West Africa (Ross, 1975), where successful introduction to other parts of the continent was made, probably in the later half of the 16th century. In east Africa the crop was first reported in Zanzibar in 1779. Cassava was not greatly valued and was a minor crop throughout eastern Africa until 1885, except around Lake Tanganyika (Carter *et al*., 1992). Cassava use increased in the second half of the 19th century after its value as a famine reserve crop was discovered (Charrier and Lefevre, 1987).

Cassava is a staple food crop for over 800 million people around the world (Nweke, 1996; FAO, 1996) and as a low cost carbohydrate source it plays a food security role in Africa. There is an estimated 70 million people, particularly in Africa and north-east Brazil, who obtain more than 500 cal/day from cassava (Iglesias *et al*., 1997). Area under cassava has been continuously expanding into marginal environments, particularly in regions with poor soils and lengthy dry seasons (El-Sharkawy, 1993). Cassava offers the advantage of flexible harvesting which permits farmers to keep the storage roots in the ground until needed (Benesi, 2005).

Cassava, which was earlier considered to be a poor man's food crop, has become important as a source of income as well as an industrial raw material (Nweke, 1995). Findings from the collaborative study on cassava in Africa (COSCA) showed that it is potentially more of a cash crop than a subsistence crop (Nweke, 1996). In south-east Asia and South America the crop has taken on more importance as a source of starch for industry and food processing, and as animal feed (Ceballos, 2002).

Africa produces more cassava than the rest of the world combined. In 2005, the largest producing nations were Nigeria (40%), Democratic Republic of Congo (DRC; 19%), Ghana (10%), Tanzania (7%) and Mozambique (6%; FAO, 2006). Uganda on the other hand, produced only five million tonnes representing 5.0% of the total African production. Total production of cassava in Africa increased from 35 million tonnes in 1965 to over 100 million tonnes in 2005 (FAO, 2006). Increases in cultivation of cassava during the 1990s occurred, at least partly, in response to declining soil fertility and increased cost of inorganic fertilisers (FAO, 1998). In a number of countries the increase has been at the expense of major food crops. The productivity per unit area in Africa (8.2 t/ha) is still low compared to the world average (9.8 t/ha; FAO, 1998). Low yields have been attributed to many production constraints such as the use of late bulking varieties, poor in-ground storability, disease and pest susceptibility, use of poor planting material and low yielding potential of many varieties (Nweke, 1996).

Cassava is an open pollinated crop and on farmers' fields recombines with itself and related wild species, creating greater variability for different traits. Farmers select desired clones with agronomic traits suitable for particular ethnic requirements. In the Americas, Africa and Asia, progress towards improvement, adaptation and quality occurred first through subconscious selection by farmers (Kizito *et al.,* 2005). These new genotypes, referred to as landraces, provide a wealth of exotic genes for some traits and form part of the crop's genetic resource (Gulick *et al.,* 1983; Hershey, 1987). A high level of genetic diversity has been generated through centuries of farmer selection (Bonierbale *et al*., 1995).

The potential to increase cassava yields through genetic improvement has been demonstrated with considerable progress and success (Hahn *et al*., 1980b; IITA, 1982; 1993). However, despite the proven record in cassava improvement, many challenges remain. Lawson (1988) noted that cassava genotypes find optimum physiological expression of their genetic potential within narrow ranges of biophysical conditions. Cock (1987) found that few cassava cultivars were stable over a wide range of ecological conditions. There exists growing consensus that stable productivity in cassava depends on a number of factors acting synergistically: abiotic factors (soils, temperature, photoperiod and latitude), biotic elements (diseases, pests and nematodes) and management practices (Allem and Hahn, 1991).

Genetic control mechanisms and environmental influences on important characteristics of cassava are largely unknown. Carter (1986) reported that 19% of cassava in Africa is found in mid-altitudes where trends in socio-economic and physical environment favour increased cassava production. This has stimulated considerable interest in increasing cassava production within this ecology since earlier research focused on the lower altitudes of the tropics where cassava finds its most suitable growth environments (IITA, 1993; FAO, 1996). Cooper and Hammer (1996) suggested that the analysis of variation in plant adaptation is inextricably linked with understanding environmental factors that influence the differential yield performance of genotypes. Understanding the nature of the influence of the environment is therefore a critical component of improving efficiency of plant breeding programmes.

Molecular markers are not affected by environmental conditions and are insensitive to gene interactions, allowing geneticists and plant breeders to locate and follow the numerous interacting genes that determine a complex trait as well as tagging those controlled by single genes (Botstein *et al*., 1980). Two of the main strategies used to identify molecular markers associated with traits of interest are genetic linkage mapping and bulk segregant analysis (BSA; Tanksley *et al*., 1989; Giovannoni *et al.,* 1991; Michelmore *et al.,* 1991). Genetic linkage mapping as a tool for localising both simple and complex traits can provide a more direct method for selecting desirable genes via

their linkage to easily detectable molecular markers (Tanksley *et al*., 1989). Bulk segregant analysis is a rapid method for identification of markers in specific regions of the genome (Giovannoni *et al*., 1991; Michelmore *et al*., 1991), in that pools of deoxyribonucleic acid (DNA) of extreme phenotypes or marker alleles are screened to determine molecular markers associated with the trait of interest.

Both BSA and genetic linkage mapping have been used to identify markers linked to loci for a number of traits in cassava, for example resistance to cassava mosaic disease (CMD) in populations segregating for resistance to CMD (Fregene *et al*., 1997). Once the trait is identified and mapped, marker-assisted selection can be used to introduce the trait into other populations. Marker-assisted selection (MAS) can reduce breeding population sizes, continuous recurrent testing and time required to develop a superior line (Koga-Ban *et al*., 1999; Okogbenin, 2004).

Genetic linkage maps of cassava are being constructed at the Centro International de Agricultura Tropical (CIAT) in Colombia and the International Institute of Tropical Agriculture (IITA) in Nigeria (Fregene *et al*., 1997; Jorge *et al.*, 2000; 2001; Mba *et al*., 2001; Akano *et al*., 2002; Okogbenin and Fregene, 2002; 2003; Okogbenin *et al*., 2006). Fully saturated maps will allow cassava breeders and geneticists to identify and clone genes and quantitative trait loci (QTLs) associated with different traits. The map can be used to develop a consensus map of cassava to design experiments, identify QTLs, and generate a genomic database for comparative mapping with other species. To facilitate the saturation of maps with molecular markers, more segregating populations need to be developed, evaluated in different environments to accumulate quantitative data, and BSA and linkage analysis employed to place markers on the maps. This study aimed to:

(a) employ and evaluate the diallel mating system as a method for selecting parents
(b) investigate the cause-effect relationships of yield components
(c) estimate the magnitude of genotype x environment interaction (G x E)
(d) identify molecular markers linked to genes controlling dry matter content.

Chapter 2

Literature Review

2.1 Importance of cassava

Cassava is a perennial shrub of the family Euphorbiaceae, cultivated mainly for its starchy roots. It is one of the most important food staples in the tropics, where it is the fourth most important energy source (Alves, 2002). On worldwide basis it is ranked as the sixth most important source of calories in the human diet (FAO, 1999). It has been reported that cassava, among tropical crops, has the highest potential production of calories per hectare per year (deVries *et al*., 1967). Given the crop's tolerance to poor soil and harsh climatic conditions, it is generally cultivated by small-scale farmers as a subsistence crop in a diverse range of agricultural and food systems (Alves, 2002). Although cassava is a perennial crop, storage roots can be harvested from six to 24 months after planting (MAP), depending on cultivar and growing conditions (El-Sharkawy, 1993). Roots can be left in the ground without harvesting for a long period of time, making it a useful crop as security against famine.

2.2 Taxonomy of the genus *Manihot*

Manihot esculenta (cassava) is placed in the fruticosae section of the genus *Manihot*, which is a member of the Euphorbiaceae family. The fruticosae section contains low-growing shrubs adapted to Savannah, grassland or desert and is considered less primitive than the Arboreae section, which contains the tree species. Of the 98 species that belong to the genus *Manihot*, cassava is the only species that is widely cultivated for food production (Rogers and Appan, 1973; Onwueme, 1978; Mkumbira, 2002; Nassar, 2005).

All *Manihot* species have 2n=36 chromosomes and are regarded as polyploids with n=18, have regular bivalent pairing and behave as diploids (Jennings, 1976). Studies on the

pachytene karyology of *M. esculenta* suggested that the species is probably a segmental allotetraploid derived from a combination of two diploid taxa whose haploid complement has six common and three different chromosomes (Jennings, 1976). Inheritance of several isoenzymes indicated disomic heredity confirming the diploid behaviour (Hussain *et al*., 1987; Lefevre and Charrier, 1993). Current research towards development of a molecular genetic linkage map is likely to provide a better structural definition of the cassava genome (Fregene *et al*., 1997).

Spontaneous hybrids between cassava and other *Manihot* species have been reported to occur naturally in Africa and Brazil (Nassar, 1994). In earlier hybridisation studies in the late 1930s Doughty suggested that tree cassava is a natural hybrid between cassava and *M. glaziovii* Allem (Fregene, 1996). Doughty's suggestion was later confirmed by other workers who observed normal pairing at meiosis in an F_1 between cassava and *M. glaziovii* and in an F_1 between cassava and arborescent cassava (Magoon, 1967; Bai, 1982; IITA, 1988). Farmers sometimes take cuttings from the spontaneous seedlings for subsequent planting (Lefevre and Charrier, 1993; Kizito *et al*., 2005).

2.3 Cassava botany and physiology

Cassava is a perennial shrub, cultivated mainly for its starchy roots and is mainly propagated from stem cuttings (IITA, 1990; Hallack, 2001). Propagation from true seed occurs under natural conditions and is widely used in breeding programmes (Iglesias *et al.,* 1994a). Plants generated from true seed take longer to become established, and are smaller and less vigorous than plants from cuttings (Alves, 2002). Seedlings are genetically segregated into different types due to reproduction by cross-pollination (Osiru *et al*., 1996), as opposed to plants obtained from cuttings.

2.3.1 Flowering

Cassava is monoecious and predominantly out-crossing (Fregene *et al.,* 1997). Out-crossing is mediated by protogyny and results in high levels of heterozygosity (Bryne,

1984; Hershey and Jennings, 1992). Flowering of cassava plants may begin as early as six weeks after planting, although the actual time of flowering depends upon cultivar, time of planting and the environment (Jennings and Iglesias, 2002). Flowering is frequent and regular in some cultivars, while in others it is rare or non-existent (Onwueme, 1978; IITA, 1990). The availability of flowers is influenced by plant habit, because branching always occurs when an inflorescence is formed (Jennings and Iglesias, 2002). Tall un-branched plants are less floriferous than highly branched, low-growing ones. Based on the flowering habit, varieties are classified as non-flowering, poor flowering, moderate flowering, profuse flowering with poor fruit setting and profuse flowering with high fruit setting (Indira *et al*., 1977). Cassava flowers are borne on terminal panicles, with the axis of the branch being continuous with the panicle inflorescence. Male flowers occur near the tip, while female flowers occur close to the base. Each female or male flower has five yellowish or reddish perianths. The female flower has an ovary mounted on a 10 lobed glandular disc. The stigma has three locules and six ridges. The female flowers normally open 10 to 14 days before the males on the same branch, encouraging cross-pollination, but self-fertilisation can occur because male and female flowers on different branches or on different plants of the same genotype open simultaneously (Onwueme, 1978; Osiru *et al*., 1996; Jennings and Iglesias, 2002). Insects, particularly bees and wasps, are the main pollination agents (Onwueme, 1978; IITA, 1990; Mkumbira, 2002; Nassar, 2005). Prolific production of readily disseminated pollen grains suggests that wind may be an important pollinating agent (Buerno, 1987). Female flowers open by 11 to 12 o'clock in the morning and the stigma becomes receptive six hours before flower opening. Pollen viability is reduced to about 50% one day after opening, and looses viability two days after opening (Nassar, 1978).

2.3.2 Fruit and seeds

After pollination and fertilisation, the ovary develops into a fruit within 70 to 90 days. The fertility of clones is variable and can be very low. An average of one seed per fruit is commonly achieved through controlled pollination from a maximum of three seeds from the tri-locular ovary (Jennings and Iglesias, 2002). The genotype of the female parent is

more important in determining success than that of the pollen parent (Jennings, 1963). The mature fruit is a globular capsule, 1.0 to 1.5 cm in diameter with six narrow longitudinal wings along which it naturally splits explosively to release the seed (Onwueme, 1978; IITA, 1990; Osiru *et al*., 1996). Fruit maturation generally occurs 75 to 90 days after pollination (Ghosh *et al*., 1988).

Newly harvested seeds are dormant and require 3 to 6 months storage at ambient temperatures before germination. Seeds take about 16 days to germinate. Germination can be hastened by carefully filing the sides of seed coats at the radicle end and by temperature management. Ellis *et al*. (1982) found that few seeds germinated unless the temperature exceeded 24^0C; the best rates occurred at 30 to 35^0C. A dry treatment of 14 days at 60^0C is beneficial for newly harvested seeds. Seeds for storage should be kept at 5^0C and 60% relative humidity (IITA, 1978), as they tend to loose viability rapidly during a year's storage at ambient temperatures (Kawano, 1978).

2.3.3 Roots

Roots are the main storage organ of cassava. Anatomically, the cassava root is not a tuberous root, but a true root, which cannot be used for vegetative propagation (Alves, 2002). Root size and shape depend on cultivar and environmental conditions. Variability in root size within a cultivar is greater than that found in other root crops (Wheatley and Chuzel, 1993). Cassava roots have the shortest post-harvest life compared to any of the major root crops (Ghosh *et al*., 1988). Roots are highly perishable and usually become inedible within 24 to 72 hours after harvest due to a rapid physiological deterioration process, in which synthesis of simple phenolic compounds that polymerise occurs, forming blue, brown and black pigments (condensed tannins) (Wheatley and Chuzel, 1993).

In plants propagated from true seeds a typical tap root system is developed, similar to dicot species. The radicle of the germinating seed grows vertically downwards and develops into a taproot, from which adventitious roots originate. Later, the taproot and

some adventitious roots become storage roots. In plants grown from stem cuttings, the roots are adventitious and arise from the basal cut surface of the stake and occasionally from the buds under the soil. These roots develop into a fibrous root system. Only a few fibrous roots (between three and 10) start to bulk and become storage roots (Alves, 2002). Most of the other fibrous roots remain thin and continue to function for water and nutrient absorption. Once a fibrous root becomes a storage root, its ability to absorb water and nutrients decreases considerably. Storage roots result from secondary growth of the fibrous roots. The soil is penetrated by thin fibrous roots, and their enlargement begins only after penetration has occurred (Alves, 2002).

The difference in the root system between the seedling and clonal stages causes a dilemma for most breeders. In the seedling stage the taproot tends to dominate other roots creating non-uniformity in size. The taproot tends to have a large wooden "neck", which affects the dry matter content (DMC). In the seedling stage, roots tend to develop from one point as opposed to several in the case when the plant is propagated from cuttings, leading to fewer roots. These differences have led most breeders to believe that yield at seedling stage will not be representative of the later stages since most yield components (DMC and root size and number) are likely to change (Ceballos *et al*., 2004). Most breeders therefore restrict selection in the seedling stage to eliminating "obvious bad plants", in the process prolonging the cycle (personal experience).

Cassava produces potentially toxic levels of cyanogenic glucosides (Linamarin [95%] and lotaustralin [5%]) which are synthesised in the leaves (Koch *et al.*, 1992; Conn, 1994) and translocated to all other parts of the plant including the edible tuberous roots (McMahon *et al.*, 1995). The breakdown of cyanogenic glucosides results in hydrogen cyanide (HCN) production when cassava tissues are mechanically damaged. HCN in cassava tissues has been medically proven to be a potential health hazard for consumers if the plant is inadequately processed (Tylleskär *et al.*, 1992; McMahon *et al.*, 1995). There exists considerable variation in the root content of cyanogenic glucosides among genotypes but the level also depends on the growth environment (Bokanga, 1994; Mkumbira, 2002). Environmental factors during the growing season contribute

significantly to variation in cyanogenic potential (CNP) among genotypes as well as within genotypes and in various parts of the plant (Dixon *et al.*, 1994). The growth stage of the plant appears to have an effect on the cyanogenic glucoside build up. A high level occurring 120 days after planting (DAP) drops dramatically by 180 DAP coinciding with the beginning of the active root-bulking phase (Bokanga, 1994). Cassava varieties with high cyanogenic glucoside content (>1000 mg HCN equivalent kg/dry weight) are said to be toxic while cassava with low content of cyanogenic glucosides ($\leq$200 mg HCN equivalent kg/dry weight) are said to be safe for consumption without processing (Iglesias *et al.*, 2002). Traditionally cassava roots are processed by a variety of methods into many different products and used in diverse ways according to local custom and preference. However, some basic steps are followed. After peeling of the roots, processing steps consist of grating, crushing, microbial fermentation, enzymic action or a combination of these. This is usually followed by either heating or drying to reduce moisture content. The final stage in the processing of the roots is to make cassava flour (Ugwu and Ay, 1992).

2.4 Agronomy and cropping systems

The genus *Manihot* occurs naturally only in the western hemisphere, between south-west USA (33^0N) and Argentina (33^0S). Highest levels of diversity occur in two areas, namely north-eastern Brazil extending towards Paraguay, and in western and southern Mexico. Cassava is grown in areas with annual rainfall higher than 750 mm and annual mean temperature higher than 18^0C to 20^0C. Small quantities of cassava are grown near the equator in South America and Africa at altitudes up to 2000 m.a.s.l., under annual mean temperatures as low as 16^0C to 17^0C, but with minimal seasonal fluctuations (Cock, 1982).

Cassava is often grown under low-input/low-output production systems, particularly when it is grown as a food crop (Leihner, 2002) and is highly tolerant to low nutrient levels. Under zero-input conditions and poor soils, cassava can yield closer to its potential total biomass than most other food crops. Unlike many other crops, cassava,

once established, has no critical period when drought will cause a disastrous decrease in yield (Oliveira *et al.,* 1981). Although the crop is affected by a number of arthropod pests, diseases and weed competition, it generally requires little attention once established. Nevertheless, attention to a few simple aspects of agronomic management can result in a doubling or tripling of output at low cost (Leihner, 2002).

Cassava is propagated vegetatively with stem cuttings or stakes. The size and quality of the stakes are of fundamental importance to yield (Lozano *et al*., 1977). The length of stakes commonly used by farmers is 15 to 25 cm. Jennings (1970) suggested that long, moderately thick stakes, taken from the basal part of the plant result in higher yields. Optimum plant density is highly dependent on adaphic and climatic factors, variety, soil fertility, cultural practices, and the end use of roots (Toro and Atlee, 1985). However, the most commonly used plant population for cassava is 10 000 plants/ha.

2.5 Cassava growth and development

A limited number of studies have reported on growth and development of cassava (Cours, 1951; Cock *et al*., 1979; Connor *et al*., 1981; Keating *et al*., 1982). During the initial growth phase, which lasts about six weeks, auxiliary shoots and adventitious roots regenerate. The first leaves appear by the 10^{th} day of growth and photosynthesis starts after three weeks, contributing positively to all plant parts, including storage roots between the 6^{th} and 16^{th} week (Cours, 1951; Simwambana, 1988). Development of storage roots starts with the initiation of secondary thickening of the adventitious roots, a process observed as early as three weeks after planting (WAP; Veltkamp, 1986, IITA, 1990). Onwueme (1978) and Vine (1979) reported that the relatively thin root accomplishes the initial penetration through the soil, and the increase in girth or growth occurs after this penetration. However, soil physical conditions, such as soil hardness, are important factors which affect storage root yield (Ntawuruhunga, 2000).

Storage roots are arbitrarily distinguished from others when their thickness surpasses 0.5 cm, which is generally reached between one to four MAP (Boerboom, 1978; Veltkamp,

1986). Storage root bulking is affected by assimilate supply to the roots which is competitively influenced by shoot growth and hormonal changes (Williams, 1972). The number, shape, size and angle at which storage roots penetrate the ground, the colour of the outer cork, and internal tissues vary greatly among varieties. There are usually five to 10 storage roots per plant, which are cylindrical, 15 to 100 cm long, three to 15 cm in diameter, and occasionally branched. The final yield is related to the storage root number and size (Williams, 1972; Simwambana, 1988; Ntawuruhunga, 1992).

2.5.1 Dry matter partitioning and source-sink relationship

During cassava growth, the carbohydrates from photosynthesis have to be distributed to assure good development of the source (active leaves) and provide dry matter (DM) to the sink (storage roots, stem and growing leaves; Alves, 2002). Cassava DM is translocated mainly to stems and storage roots, and DM accumulation in the leaves decreases during the crop cycle. Until 60 to 75 DAP, cassava accumulates DM mainly in the leaves compared to stems and storage roots, not including stem cuttings. After 75 days storage roots increase rapidly, reaching 50 to 60% of the total DM around 120 DAP (Howeler and Cadavid, 1983; Tavora *et al*., 1995). After the fourth month, higher levels of DM are accumulated in the storage roots compared to the rest of the plant. At harvest (12 MAP) DM is present mainly in roots, followed by stems and leaves (Howeler and Cadavid, 1983). During the growth cycle, DM distribution to the different parts is constant with a high positive linear correlation of the total DM with shoot and root DM (Veltkamp, 1985). The period of maximum rates of DM accumulation depends on genotypes and growth periods (Oelsligle, 1975; Lorenzi, 1978; Howeler and Cadavid, 1983).

The distribution of DM to economically useful plant parts is measured using harvest index (HI). In cassava, HI represents the efficiency of storage root production and is usually determined by the ratio of storage root weight to total plant weight. Significant differences in HI have been reported among cultivars, indicating that it can be used as a selection criterion for higher yield potential in cassava (Kawano *et al*., 1998; Kawano,

2003). Harvest index values of 0.49 to 0.77 have been reported 10 to 12 MAP (Lorenzi, 1978; Cavalcanti, 1985; Pinho *et al*., 1995; Tavora *et al*., 1995; Peressin *et al*., 1998). Although DM distribution is constant, its accumulation depends upon photo-assimilate availability (source activity) and sink capacity of storage parts. Sink capacity is determined by the number of storage roots and their mean weight. The significant positive correlation of photosynthetic rate with root yield and total biomass, as well as correlations between leaf area index (LAI), interception of radiation and biomass production, indicate that demand for photo-assimilates by roots increases photosynthesis (Williams, 1972; El-Sharkawy and Cock, 1990; Ramanujam, 1990).

2.5.2 Genetic variability and interrelationships of growth and storage root yield characteristics in cassava

Varma and Mathura (1993) investigated genotypic and phenotypic relationships among plant characteristics and the relative contribution of yield components to cassava storage yield under rain-fed conditions in India. Cultivar and year interactions were significant ($P \leq 0.05$) for all yield components studied except for mean storage root weight which showed little variation. Correlation coefficients between yield and mean storage root weight were high and significant ($P \leq 0.01$). Storage root weight was positively correlated with storage root girth (0.73), which had a high broad-sense heritability estimate of 0.88, suggesting that the weight and girth of storage roots were effective indirect selection criteria for yield. Kawano *et al*. (1998) also found cultivar interactions within locations and years to be significant, but their actual influence on the genotype mean was proportionally small in all traits. Varma and Mathura (1993) further suggested that effective direct selection for yield through clump characteristics via storage root weight per clump, number of marketable storage roots per clump and indirect selection through storage root weight, length and girth, was possible. Storage root weight in upland conditions in India had a high heritability and coefficient of genetic advance and could therefore be a useful character for improving cassava storage root yield (Varma and Mathura, 1993). Williams (1972) reported that root size contributed most to differences in storage root yield and that the diameter of the storage root yield was the major

component rather than length. Kawano (2003) observed that yield is a mathematical product of biomass and HI and concluded that indirect selection for fresh root yield (FRY) through HI was very effective.

Mahungu (1983) reported that storage root yield was highly correlated with the number of storage roots and indicated a good fit between expected and observed values for genetic progress. Mahungu *et al*. (1994), studying the correlated response and use of the selection index in cassava, observed that the merit of indirect selection depended on the ratio of the expected correlated response of a trait indirectly selected to the expected direct response of that trait. Estimates of merit of indirect selection for storage roots yield showed that selection for number of storage roots per plant was in the order of 0.82, followed by HI (0.74), storage root size (0.61), stem girth (0.61), total number of branches (0.55), canopy width (0.50), and plant height (0.39). On the other hand, selection for DM in storage roots and number of stems per stand exhibited the least merit, 0.22 and 0.17 respectively (Mahungu *et al*., 1994).

2.6 Genotype by environment (G x E) interactions and stability statistics in cultivar assessment programmes

2.6.1 Concept of stability

Successful cultivars need to possess high performance for yield and other essential agronomic characters over a wide range of environmental conditions. The basic cause for differences between genotypes in yield stability is a wide occurrence of G x E interactions. Genotype refers to a set of genes possessed by an individual that is important for the expression of the traits under investigation. The environment is usually defined as all non-genetic factors that influence the expression of traits. Environment may include all sets of biophysical factors like water, nutrition, temperature, and diseases that influence the growth and development of individuals and thereby influence the expression of traits (Basford and Cooper, 1998).

The knowledge of G x E interactions can help to reduce the cost of extensive genotype evaluations by eliminating unnecessary testing sites and by fine tuning breeding programmes (Shafii *et al*., 1992; Kang and Magari, 1996; Basford and Cooper, 1998). G x E interaction relates to sustainable agriculture as it affects efficiency of breeding programmes and allocation of limited resources. According to Kang and Magari (1996) G x E interaction is a major concern in plant breeding since it can reduce progress from selection and may make cultivar recommendation difficult as it is statistically impossible to interpret the main effects.

For varietal trials, which are tested using the same locations (L) and genotypes (G) over years (Y), G x E analysis of variance may be partitioned into components due to G x L, G x Y, and G x L x Y. If G x L is the main portion of the G x E interaction, the specific adaptation is exploitable by subdividing regions into homogenous sites that minimise G x E interactions within regions. Accumulation of tolerance to a number of stresses is the key to stable genotypes (Ramagosa and Fox, 1993). Successes for crops like wheat, in combining high yield potential and wide adaptation, have involved a large number of crosses, testing advanced lines internationally and continuously alternating selection cycles in various environments (Eisemann, 1981; Getinet and Balcha, 1989; Ramagosa and Fox, 1993). These environments, which differ in altitude, latitude, photoperiod, temperature, rainfall, soil-type and disease incidence allow the expression of high yield potential. Choice of selection sites is particularly relevant in the case of production areas with variable levels of abiotic stress (Ramagosa and Fox, 1993).

Different concepts and definitions of stability have been developed for application in crop breeding programmes and evaluation of yield trials (Lin *et al*., 1986; Becker and Leon 1988; DeLacy *et al*., 1996). According to Becker and Leon (1988), two concepts of stability exist, namely static and dynamic, both of which are useful, although their application depends on the traits under consideration. Under static stability, stable genotypes possess unchanged or constant performance regardless of variation in environmental conditions. In contrast, the dynamic concept allows a predictable response to environments and a stable genotype has no deviation from this response to

environments. Stable yield plays a major role in developing countries, where small-scale farmers, particularly those living in marginal areas, are working towards risk-minimisation (Adugna and Labuschagne, 2002). Farmers are basically interested in a constantly superior performance of cultivars on their own farms, specifically adapted to their conditions and needs, and which have a high degree of stability over time (Ceccarelli, 1989; 1994).

2.6.2 Concept of adaptation

Plant adaptation is a fundamental process, which is not clearly defined but widely used in genetics and plant breeding literature (Cooper and Byth, 1996). In an attempt to provide a definition, Byth (1981) and Clement *et al*. (1983) suggested that adaptation applied to both a 'condition' and a 'process'. The condition or level of adaptation possessed by an individual or genotype refers to how the genetic constitution of the genotype matches the genotype to the environment it occupies. It is a function of genes possessed by the genotype, biochemical and physiological processes controlled by these genes during growth and development, and how these are matched with the available resources and possible hazards (Bidinger *et al*., 1996). With regard to process, adaptation is regarded as a change in the genetic constitution of individuals as they accumulate genes or a change in the genetic constitution (Cooper and Byth, 1996).

Evaluation of adaptation has been approached in different ways depending on the researcher's background. Quantitative geneticists and plant breeders rely on the analysis of variance and G x E interactions (Pérez de la Vega, 1997). Crop performance is a function of the genotype of the crop and the nature of the production environment (Cooper and Byth, 1996). Expression is dependent on the test environment and the relative performance may vary in different environments, reflecting G x E interactions. G x E interaction is the change in the relative performance of cultivars resulting from their differential response to various edaphic, climatic, and biotic factors (Dixon *et al*., 1994). It constitutes a challenge to plant breeders because it causes difficulty in selecting genotypes evaluated in different environments, inhibits the genetic analysis of the

performance and reduces the efficiency of crop improvement via plant breeding (Cooper and Byth, 1996). An understanding of the nature, relative magnitude, and consequences of G x E interaction will aid the breeder in formulating an efficient breeding strategy for improving crops.

In Brazil, Bueno (1986) found FRY to be more influenced by environmental variation than HI and starch content. Cock (1985), on the other hand, reported macro-spatial stability in some cassava genotypes for FRY and starch content across adapho-climatic zones in Colombia. This implied that location played a less dominant role in variation of these traits. Cock (1985) discovered a high correlation between yields of the same set of genotypes across years at different sites, suggesting stability of genotypes.

Storage DMC had a highly significant correlation among selection stages within sites and between some sites in Colombia (CIAT, 1975), implying insignificant G x E effects. Hahn *et al*. (1979) nevertheless, found a high genotype x season interaction for storage root matter in Africa. Bueno (1986) reported important G x L and G x L x Y interactions for FRY when testing a number of genotypes in the humid tropics of Brazil. By contrast, Rodriquez and Garcia (1990), in their studies on clonal stability over two locations and two years in Cuba, were unable to detect significant G x L interaction for this trait. They detected strong effects due to G, Y, and L instead.

Tan and Mak (1995), studying the relative influence of genotype, environment and G x E effects on six agronomic traits of cassava, in peninsular Malaysia, reported that G effects were strong in controlling HI and DMC while E was the main source of variation for commercial storage root number and FRY. Location x season effects were the most prominent of the environmental components. Tan and Mak (1995) detected that G x E effects were significant for FRY, commercial storage root number, HI, starch and cyanide content. Although significant, their effects were smaller than G effects, except for storage root number and FRY. However, unlike Bueno (1986), Tan and Mak (1995) did not observe a significant G x L x S interaction, and suggested that differences in their results were due to different sets of genotypes tested.

Irikura *et al*. (1979) and Hahn *et al*. (1980a) reported that the DMC of cassava storage roots showed cultivar x year and cultivar x temperature interaction. CIAT (1975), Irikura *et al*. (1979) and Kawano *et al*. (1987) reported that the highly significant clonal effect on DMC reflected the relative stability of the character and that selection in one environment would be effective for other environments. Final selection, however, has to be done at each location due to the existence of the small magnitude in G x L interaction. Selections conducted in different environments indicated that the fresh weight of storage roots were more sensitive to differential environments than the storage root number. Storage root number and weight were significantly correlated to dry root yield (DRY) but not to percentage DMC (IITA, 1993). Similar trials conducted in Cameroon with genotypes selected in mid-highland conditions (1000 to 2000 m.a.s.l.), indicated that storage root number was more sensitive to differential environments than storage root weight (Whyte, 1987).

Environments used to test breeding materials often differ widely in their effects on crop yield. The extremes are generally referred to as stress and non-stress environments. Requirements for productive agriculture constrain us to this scenario, therefore we must find a way to live with G x E interaction, or better, to take advantage of it. In a more uniform arena of production, G x E interaction should have more identifiable underlying causes. We need to identify and understand the pattern of the G x E interaction to be able to use it constructively in genetic manipulation (Zobel, 1990).

2.7 Cassava breeding

2.7.1 Breeding for yield

Efforts to improve cassava yield are generally not geared towards the highest possible yield under favourable conditions, but rather towards obtaining stable yields in marginal conditions where cassava is grown at present and is likely to expand in future (Cock, 1984; El-Sharkawy, 2003). High yield is achieved firstly by selecting plants that have both a genetic and a plant structure which maximises performance, and secondly by

adding resistances or tolerances to factors which limit yield (Ellis *et al*., 1982). Hybrid vigour through heterozygosity is the main requirement for the genetic structure of new varieties and a major objective of breeding programmes (Nassar *et al*., 2004).

2.7.2 Models for high yield: the significance of plant habit and leaf longevity

Cassava plant habits are so variable that efforts have been made to discover which is best equipped for giving high yields, which essentially is the ability to convert solar energy into starch and storage in roots (Ellis *et al*., 1982). To obtain high production levels, it is necessary for the plant to intercept light as much as possible, use it efficiently in photosynthesis (Simwambana, 1988), and favourably distribute synthates to storage roots. As physiological information became available, computer modeling was used to estimate the effects of the many variables, including those associated with stress and disease (Hunt *et al*., 1977; Cock *et al*., 1979). Based on this, a theory was developed that root growth rate, which is the difference between the total growth rate and that of the tops, increases up to a certain level and then decreases. There exists an optimum LAI for yield, and manipulation of the components of LAI can bring LAI closer to this optimum and maximum yield.

Leaf and stem growth have preference over root growth and the latter only receives the carbohydrates remaining after the requirements of the tops have been met (Gilzen *et al*., 1990). The size of the roots rarely limits yield, and it is the LAI and not the root sink that determines yield. Roots can accept much more carbohydrates than is normally available (Tan and Cock, 1979; Pellet and El-Sharkawy, 1994). However, since LAI and growth of the roots develop simultaneously there is continuous competition between the two for available photosynthates. The balance between distribution of assimilate and nutrients to the maintenance of LAI and to the formation of starch in the roots is closely related to HI (Cock and El-Sharkawy, 1988). Kawano *et al*. (1998) demonstrated the effectiveness of including an optimum HI as a criterion for selection for high yield.

2.7.3 Breeding for root quality: starch and dry matter content

Since cassava is used for diverse purposes, most of the criteria for quality are also diverse, but high starch content and quality is always required (Ellis *et al*., 1982; Moorthy, 1994; Benesi, 2002; 2005). Starch content is usually estimated from DMC, to which it is highly correlated (R=0.810; IITA, 1974; CIAT, 1975), but a quicker method is to determine the root's specific gravity, which is related to both DMC and starch content. A calculation can be obtained from the specific weight of a sample (3 to 5 kg) of unpeeled roots in air and water (Ellis *et al.*, 1982).

Growing conditions, especially temperature and rainfall patterns, can have a strong influence on DMC. However, it is a relatively highly heritable character, apparently multi-genetically controlled with predominantly additive gene effects. Consequently, selection for DMC can be highly effective in cassava breeding (Hershey, 1987).

High DMC is not necessarily ideal because, for reasons unknown, it is associated with post-harvest deterioration (Ellis *et al.*, 1982; Van Oirschot *et al*., 2000; Chavez *et al*., 2005). This can be serious for commercial outlets, but not where roots are immediately used, for example in subsistence agriculture. Dry matter content is not associated with FRY and it is still uncertain whether a high level of DMC can be maintained when yields are high: progress in one may require sacrifice in the other (CIAT, 1981; Iglesias *et al*., 1994b).

Similarly, substantial progress towards a capacity for prolonged post-harvest storage may be difficult, but genetic differences have been identified (Kawano and Rojanaridpiched, 1983). More recently, Iglesias *et al*. (1996) indicated that it was possible to break the association between high DMC and high post-harvest deterioration, and that heritability of the trait is high enough for considerable progress through conventional breeding.

2.8 Mating designs

Presently, the main features of the breeding methodology in cassava involve crossing, via controlled or open pollination, phenotypic selection of parents and selection of superior genotypes, followed by clonal perpetuation of selected genotypes. Rajendran (1989) suggested that selection of parents based on their *per se* performance is not reliable in breeding for root yield in cassava and that it is necessary to estimate the combining ability of the parents before formulating specific breeding programmes.

The diallel and North Carolina design II (NCD II) mating designs provide genetic interpretations including combining abilities on the inheritance of quantitative traits (Kang, 1994). The concept of general and specific combining ability in a diallel analysis was first defined by Sprague and Tatum (1942). Knowledge on the relative importance of general combining ability (GCA) and specific combining ability (SCA), which represent two major modes of gene action for quantitative traits, is essential in formulating an efficient breeding strategy. General combining ability of a line refers to the average value of the line based on its performance when crossed with other lines and is due largely to additive gene effects. Specific combining ability is the deviation of a cross from the average GCA of the parent lines and is due to non-additive gene effects (Sprague and Tatum, 1942; Falconer and Mackay, 1996).

The NCD II mating scheme is a cross-classification design that was first proposed by Comstock and Robinson (1948). It differs from the diallel in that different sets of parents are used as males and females. It accommodates more parents in determining combining abilities than a diallel and provides the same type of genetic information (Hallauer and Miranda, 1988). Main effects of males and females are equivalent to GCA and the female x male interaction is equivalent to SCA (Calle *et al.*, 2005; Jaramillo *et al*., 2005; Cach *et al*., 2006).

Both the diallel and NCD II mating designs have been used to obtain genetic information on morphological and agronomical traits of importance in cassava (Hahn *et al.,* 1989;

Rajendran, 1989; Amma *et al*., 1995; Calle *et al*., 2005; Jaramillo *et al*., 2005; Cach *et al*., 2006). Rajendran (1989) reported additive gene action for storage root yield and non-additive gene action for yield components (HI, storage root number and storage root weight). Amma *et al.* (1995) reported that root quality traits, namely starch, DM and HCN content are predominately non-additive. Specifically, diallel crosses were devised to investigate GCA of parents and to identify superior parents for use in hybrid and cultivar development (Ortiz *et al*., 2001; Yan and Hunt, 2002).

2.9 Cassava breeding in future: the role of biotechnology

Classical breeding methods have produced large advances in root yields of cassava. Hershey and Jennings (1992) reported improvements of over 200% during the period from 1976 to 1990 at CIAT. Similar advances have been made at IITA (Jennings and Iglesias, 2002). However, the rate of improvement in average national cassava yields in the most important producing countries has not paralleled progress at experimental level, except for some Asian countries (Kawano, 1978).

Progress in future will be aided by new biotechnology tools such as gene transfer from other species and marker assisted selection (MAS). Genetic engineering has a special role to play for improving heterozygous, clonally propagated crops such as cassava, because genes can be introduced into popular varieties without changing their positive attributes (Ellis *et al*., 1982; DeVries and Toenniessen, 2001). All quality combinations which make these varieties preferred by farmers could be maintained, allowing a higher rate of adoption of improved genotypes (Taylor *et al*., 2004).

In the last few years, molecular markers have made an immense contribution to cassava breeding and genetics. Areas covered include the development of genetic maps (Fregene *et al*., 1997; Mba *et al*., 2001; Okogbenin and Fregene, 2006), the assessment of genetic diversity (Beeching *et al*., 1993; Lefevere and Charrier, 1993; Bonierbale *et al*., 1997; Mignouna and Dixon 1997; Fregene *et al*., 2000), taxonomy studies (Second *et al*., 1997), understanding the phylogenetic relationships in the genus (Calvalho *et al*., 1993; Roa *et*

al., 1997; 2000; Olsen and Schaal, 1999), confirmation of ploidy (Lefevre and Charrier, 1993; Fregene *et al.,* 1994) and cultivar identification (Ocampo *et al*., 1992; Wanyera, 1993; Laminski *et al*., 1997).

When planning a molecular experiment, one of the most important decisions is the marker system and technique to be used. This problem arises since various systems and related techniques are currently available (McGregor *et al*., 2000). Molecular markers, which include biochemical (isozymes and storage proteins) and DNA markers, exist in every genotype and can be exploited to improve breeding programmes.

2.9.1 Isozyme markers

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

Scandalios, 1974). Nevertheless, isozymes have been successfully applied in cassava breeding and genetics.

2.9.2 DNA-based markers

A DNA marker is basically a small region of DNA showing sequence polymorphism in different individuals within and between species (Liu, 1998a). These markers are based on the enormous variation or polymorphism in DNA sequences of organisms. DNA markers eliminate the limitations in genome investigation using morphological and isozyme markers, such as gene expression and environmental interaction, heritability, and low map resolution (Vogel *et al.,* 1996). In addition to identifying and discriminating closely related cultivars, DNA markers can be applied in assessing taxonomic and phylogenetic relationships, pedigree analysis and linkage mapping. DNA-based marker systems can be used for indirect selection of tagged loci affecting qualitative or quantitative traits and to monitor loci during introgression or selection programmes, thus reducing the number of backcross generations (Baird *et al*., 1996).

Polymerase chain reaction (PCR) based fingerprinting involves the *in vitro* amplification of particular DNA sequences using specific or arbitrary primers and a thermostable DNA polymerase. Amplification products are separated by electrophoresis and detected by staining or the use of labeled primers in the amplification reaction (Ehrlich *et al*., 1989). Techniques in this category include random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990), DNA amplification fingerprinting (DAF) (Caetano-Anolles *et al*., 1991), arbitrarily primed-PCR (AP-PCR; Welsh and McClelland, 1990; Welsh *et al*., 1991), microsatellites or simple sequence repeats (SSR) (Hamada *et al*., 1982) and amplified fragment length polymorphism (AFLP; Vos *et al*., 1995).

DNA markers vary in level of detected polymorphism and the amount of information generated. The most informative DNA markers are characterised by high polymorphism information content (PIC), indicating relatively large numbers of alleles with similar frequencies in each locus (Botstein *et al*., 1980). Polymorphism may be a result of single

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

2.9.2.1 Restriction Fragment Length Polymorphism (RFLP)

The first DNA markers to be used were fragments produced by restriction enzyme digestion. Restriction fragments from a given chromosome locus often vary in size in different individuals. The differences are what are referred to as RFLP markers (Botstein *et al.*, 1980; Wyman and White, 1980). The development of RFLP technology represented an important contribution to breeding programmes (Burr *et al.*, 1983; Young *et al*., 1988). RFLP was developed in the 1980s to overcome problems encountered with isozymes and phenotypic markers (Botstein *et al.*, 1980; Helentjaris *et al*., 1986). Since RFLPs represent the entire genome and are both co-dominant and multi-allelic (Brettschneider, 1998), RFLPs have been and are still used in cassava. The RFLP technique generates relatively high levels of detectable loci and alleles, 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).

The most important advantage of RFLP markers is that they are co-dominantly inherited, being able to distinguish between homozygous and heterozygous loci (Rafalski and Tingey, 1993). RFLP markers have contributed to DNA marker technology in cassava. Angel *et al*. (1993) initiated work on a detailed genetic map of cassava for tagging

agronomically important traits and to clone cassava genes. Fregene *et al*. (1997) constructed a linkage map using 132 RFLP, 30 RAPD, three microsatellite and three isozyme markers from a heterozygous female parent of an inter-specific cross. The map consisted of 20 linkage groups spanning 931.6 cM. A second map was constructed from the segregation of 107 RFLP, 50 RAPD, one microsatellite and one isozyme marker from the male parent.

RFLP has been used to assess the genetic diversity within cassava and between *Manihot* species. Beeching *et al*. (1993) assessed the genetic diversity within a collection of cassava germplasm using RFLPs and recommended the use of RFLPs in the genetic diversity analysis within collections of cassava. Beeching *et al*. (1994) compared RFLPs and RAPDs in assessing genetic diversity within cassava and between *Manihot* species and found that RFLPs and RAPDs were comparable in revealing genetic diversity but that at least 30 probes or primers should be used to achieve these relationships. RFLPs have been applied in studies of analysis for phylogenetic relationships of species within the genus *Manihot* (Haysom *et al*., 1994).

The disadvantages of RFLP analysis are that it is time consuming, costly, labour intensive (Marsan *et al*., 1993), and requires specific probes (Tommerup *et al*., 1995). The complexity in performing RFLP analysis, coupled with the widespread use of short lived radio-isotopes, has led to its limitation for routine application in large scale crop improvement programmes (Yamamoto *et al*., 1994). In addition, the RFLP technique requires a substantial amount of DNA and involves special manipulations to come up with pure DNA which requires high levels of expertise and skill (Beeching *et al*., 1994).

2.9.2.2 Random Amplified Polymorphic DNA (RAPD)

RAPD primers usually have 10 bases that are used to amplify unknown and arbitrarily regions of a genome. The short sequences of the primers allow a multitude of possible primer binding sites throughout the genome. Efficient amplification of DNA fragments may occur when two primer-binding sites occur in close proximity. RAPD markers are

dominant since the polymorphism is detected as a failure of one allele to amplify due to mutations in the primer-binding site (Williams *et al*., 1990; Welsch *et al*., 1991) or due to size differences of the amplified fragments due to insertions or deletions.

RAPD markers have been the most extensively used markers in cassava biotechnology, especially in determination of phylogenetic relationships and genetic diversity in *Manihot* species (Marmey *et al*., 1994; Laminski *et al.,* 1997; Schaal *et al.,* 1997). The RAPD methodology is simple and rapid and requires only small amounts of DNA. Michelmore *et al*. (1991) were the first to report three RAPD markers linked to major disease resistance genes using contrasting DNA bulks composed of F_2 individuals of known genotype. Marmey *et al*. (1994) demonstrated genetic diversity among African cassava accessions using RAPD markers. Mignouna and Dixon (1997) demonstrated genetic differences among several African landraces with varying levels of resistance to CMD using RAPD analysis. Zacarias *et al*. (2004) assessed genetic diversity of cassava germplasm from Mozambique using RAPDs. Results showed that the cassava germplasm had wide genetic diversity, and accessions did not group according to geographical distribution. Raji *et al*. (2001) assessed the diversity of 500 African landraces of cassava using RAPD and AFLP analysis. Results showed that both markers provided similar genetic relationship of the population. However, the AFLP technique detected a much higher level of polymorphism giving a better diversity structure than RAPD analysis.

However, reproducibility of RAPDs between runs and/or laboratories is a problem (Weeden *et al*., 1992). The homozygous presence of a fragment is not distinguishable from its heterozygote, since polymorphisms detected by RAPDs are inherited in a dominant fashion (Williams *et al*., 1990; Welsch *et al*., 1991). Buso *et al*. (1994) suggested that the RAPD technique had serious limitations for use in cassava, due to cassava's high level of heterozygosity.

2.9.2.3 Microsatellite or simple sequence repeat (SSR)

Simple sequence repeats or microsatellites, are tandem repeats of short (2 to 5) sequences such as $(GT)_n$ or $(CAC)_n$. Hamada *et al.* (1982) demonstrated the large number and widespread occurrence of short tandem repeats in eukaryotic genomes. This finding was verified by Tautz and Renz (1984). The fragment polymorphism is due to variation in the total sequence length as determined by the number of repeat units. Such differences are detected on polyacrylamide or agarose gels, where repeat lengths migrate different distances according to size (Robinson and Harris, 1999).

SSRs have a high level of allelic diversity as a result of the variable number of repeat units within their structure, making them valuable as genetic markers (Hamada *et al*., 1982; Morgante and Olivieri, 1993). SSRs are often multi-allelic and can be multiplexed and automated for high-throughput genotyping. SSR analysis is easy and convenient to exchange between laboratories (Powell *et al*., 1996; Chen *et al*., 1997). Although the procedure for obtaining microsatellites is laborious and expensive, their conversion to PCR markers allows screening of large numbers of alleles at defined loci. SSR markers are co-dominant meaning that they can identify heterozygotes. This makes SSRs a suitable option for mapping and molecular characterisation of cassava, given the fact that cassava is a highly heterozygous crop (Agyare-Tabbi *et al.,* 1997; Chavarriaga-Aguirre *et al*., 1998).

Chavarriaga-Aguirre *et al*. (1998) isolated and characterised 14 highly heterozygous GA-rich microsatellite DNA regions in cassava. A total of 521 accessions from the cassava core collection at CIAT were successfully screened using SSR primers in order to determine genetic diversity (Chavarriaga-Aguirre *et al*., 1999). The study revealed between-country allele number and frequency variation, which agreed with between-country allele size variation at the same loci. Unique alleles were present in countries such as Brazil, Colombia and Guatemala. Cassava microsatellites were used to assess genetic diversity among cassava accessions and between cassava and its wild relatives (Roa *et al*., 2000). Fregene *et al*. (1997) placed 77 SSR markers on the cassava linkage

map. Mba *et al*. (2001), developed and characterised 172 new SSR markers and placed 36 of these on the cassava linkage map. A SSR marker linked to CMD resistance was identified with the aid of BSA (Akano *et al*., 2002). Fregene *et al*. (2001) assessed the SSR diversity at 67 unlinked loci in 303 accessions of cassava land races from Tanzania, Nigeria, Brazil, Colombia, Peru, Venezuela, Guatemala, Mexico and Argentina. Results revealed that more than 90% of the loci were polymorphic in all samples, and estimates of genetic diversity and differentiation ranged widely from locus to locus. It was observed that factors that contributed to differences in allele frequency at SSR loci in this predominantly vegetatively propagated crop appeared to be spontaneous recombination (Fregene *et al*., 2001).

Mkumbira *et al.* (2001) used SSR markers to study the traditional way farmers in Malawi classify cassava varieties as sweet/cool (safe for direct consumption) and bitter (toxic and needs to be processed before consumption). Results showed that farmers' classification into bitter and sweet cultivars corresponded to genetic subdivision with four-fold difference in cyanogenic glucoside levels. The necessity to differentiate between bitter and cool based on cyanogenic glucoside levels seem to have influenced the genetic structure of cassava in this area (Mkumbira *et al*., 2001).

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 entire genome at once (Robinson and Harris, 1999). Maughan *et al*. (1996) found that AFLPs produced more polymorphic loci than SSRs.

2.9.2.4 Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism is based on PCR amplification using selective oligonucleotide primers of restriction fragments generated by specific restriction enzymes (Vos *et al*., 1995). Genomic DNA is digested with two restriction enzymes, usually a rare and frequent cutter. Double stranded oligonucleotides, known as adapters, are ligated to the ends of the genomic DNA at the specific restriction sites. Adapters have a nucleotide

hangover known as a "sticky end", complementary to that of the restriction site. Separate adapters are needed for each of the different restriction enzymes. The ligated DNA is used as template for PCR reactions. Primers are complementary to the sequences of the two adapters. The AFLP method generates a large number of restriction fragments which are selectively reduced by primers that have one or three different nucleotides at the 3' end, facilitating the detection of polymorphisms. Selection of different selective nucleotide numbers and composition of nucleotides can control the number of DNA fragments which are amplified. PCR products are separated on denaturing polyacrylamide gels. Caution is needed in scoring AFLP fragments because of the large number of fragments. AFLP fragments are usually scored as dominant markers, but occasionally polymorphisms can be distinguished as co-dominant markers (Liu, 1998a). When scoring AFLPs as co-dominant markers, a mixture distribution model can be used to fit the band intensity for three possible genotypes such as in a di-allelic model. The co-dominant marker approach is very useful in saturation mapping and for discrimination between varieties. Lin *et al*. (1996) compared three different DNA mapping techniques i.e. RFLP, RAPD, and AFLP, for efficiency in detecting polymorphism in soybean and found AFLP to be the most efficient technique. High reproducibility, rapid generation and high frequency of identifiable polymorphisms make AFLP analysis an attractive technique for identifying polymorphisms and for determining linkages by analysing individuals from a segregating population (Vos *et al*, 1995; Winter and Kahl, 1995; Powell *et al*., 1996; Blears *et al*., 1998).

The AFLP technique has been applied in cassava in various studies. 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. *Manihot aesculifolia* (Kunth) Pohl, *M. brachyloba* Mull. Arg. and *M. carthagenensis* (Jacq.) Mull.Arg. 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 fragments which could be useful for identification and classification of germplasm, and introgression studies (Bonierbale *et al*., 1997).

Second *et al.* (1997) assessed the numerical taxonomy and genetic structure of 358 clones representing the geographic and ecological range of distribution of *Manihot* species along with classical botany and ecology. They used 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* (Pohl) Cif. and *peruviana* Mull. (Arg.) Allem, it seemed that some other species also contributed. Results suggested the importance of genetic recombination at the origin of 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. Results indicated that AFLP and SSR fragments that appeared in some varieties of cassava and not in *M. esculenta* ssp. *flabellifolia*, the presumed ancestor of cassava, were considered as introgressed fragments. Findings of this study showed evidence of introgression from *M. glaziovii* in some genotypes.

Narváez-Trujillo *et al*. (2001) used AFLP and SSR markers to study the traditional cassava varieties from various Amerindian communities from French Guiana and Ecuador. They found that recently bred varieties tended to be hybrids derived between sweet and bitter varieties. Benesi (2005) characterised Malawian cassava germplasm using AFLP markers. Results suggested that there existed diverse cassava germplasm among the landraces in Malawi and that diversity was mainly due to introduction of improved varieties, mainly from IITA.

2.10 Identification of molecular markers associated with traits of interest

Strategies available to facilitate detection of molecular markers associated with specific traits of interest include BSA (Giovannoni *et al.,* 1991; Michlemore *et al.,* 1991) linkage mapping and QTL analysis (Dudley, 1993; Tanksley, 1993).

2.10.1 Bulk segregant analysis

Bulk segregant analysis is a rapid method for identification of markers in specific regions of the genome (Giovannoni *et al.,* 1991; Michlemore *et al.,* 1991). The method is especially useful when there are no other markers available within the region of interest. It involves comparing two pooled DNA samples of individuals from a segregating population. The two bulks contrasting for a trait (e.g. resistant and susceptible) or a marker allele in a previously mapped population is analysed to identify markers distinguishing between bulks. Individuals in each bulk are identical for the trait or gene of interest but arbitrary for other genes. The targeted genomic region is studied against a randomised genetic background of unlinked loci. Due to selection, bulks have completely random genotypes for most of the genome, except in the region around the gene conferring the characteristic of interest. Markers polymorphic between bulks should be genetically linked to loci determining the trait used to contract the bulks (Giovannoni *et al*., 1991).

Giovannoni *et al*. (1991) demonstrated the use of DNA pooling to saturate specific chromosomal regions with additional markers, based on DNA pools from an existing tomato mapping population. Kiehne and Neale (1998) adapted this strategy in the out-crossing species loblolly pine, and identified nine new RAPD markers linked to a chromosomal interval containing the previously mapped QTL for wood specific gravity. Reamon-Buttner *et al*. (1998) detected nine AFLP markers, tightly linked to the sex locus of the dioecious plant *Asparagus officinalis* L., which is important to plant breeding in developing sex specific PCR primers. Bulk segregant analysis has been explored in tagging molecular markers linked to major disease and pest resistance genes (Michelmore

et al., 1991; Haley *et al*., 1993; Miklas *et al*., 1993; Garcia *et al*., 1996; Seo *et al*., 1997; Fritz *et al*., 1999; Correa *et al*., 2000).

2.10.2 Linkage mapping

Linkage maps are based on recombination frequencies. The distance between points on a genetic map is a reflection of recombination frequencies between points. Recombination is the process by which new combinations of parental genes or characters arise. It can occur by independent segregation of unlinked loci or by crossover between loci that are linked on a chromosome. The percentage of segregating progeny that are recombinants for a pair of linked loci is the recombination frequency. The recombination frequency gives an estimate of the distance between two loci on a chromosome, on the assumption that the probability of crossover is proportional to the distance between loci (Liu, 1998b). Many statistical procedures have been used to detect linkage and to estimate recombination fraction at two point or multi-point levels (Ott, 1991). These procedures are the fundamentals of linkage map construction. Recombination fraction is not additive along a chromosome and the departure from additivity increases with distance between loci (Liu, 1998b). Additivity is based on the assumption that the average number of crossovers per chromatid occurring between two loci is directly proportional to the distance between the two loci. Estimates of the frequency of crossing over are most reliable when genes are closely linked i.e. 1 to 10 map units (centi-Morgans or cM), where a centi-Morgan is the chance of a one percent recombination (GeneticEngineering, 2006).

Genetic linkage map construction requires that the researcher: 1) select the most appropriate segregating mapping population(s); 2) calculate pair-wise recombination frequencies using these population(s); 3) establish linkage groups and estimate map distances, and 4) determine map order (Stuber and Edwards, 1986). Different marker systems may be included in the analysis of populations. Computer packages such as Mapmaker (Lander and Botstein, 1986; Lander *et al*., 1987), MapManager (Manly *et al.,* 2001) and JoinMap (Van Ooijen and Voorrips, 2001) have been developed to aid in the

analysis of genetic data for map construction. These programmes use data obtained from segregating populations to estimate recombination frequencies that are then used to determine the linear arrangement of genetic markers by minimising recombination events (Tuberosa *et al.*, 2002).

Genetic linkage mapping is used for localising and isolating both simple and complex traits. Molecular markers placed on genetic maps allow the development and efficient use of indirect selection schemes for germplasm improvement, thereby increasing precision in the manipulation of both qualitative and quantitative traits (Baird *et al.,* 1996). This, in turn, is the basis for MAS, where markers closely associated with a trait(s) of interest can be used to introgress a specific gene(s) of interest into a desired background (Taylor *et al*., 2004).

Genetic linkage maps can provide a more direct method for selecting desirable genes via their linkage to easily detectable molecular markers (Tanksley *et al*., 1989). Once a trait is identified and mapped, MAS could be used to introduce the trait into a wide variety of populations. MAS can reduce breeding population sizes, continuous recurrent testing and the time required to develop a superior line. In cassava, the application of MAS has been developed more recently compared to other major crops, with the construction of genetic linkage maps using RFLP, isoenzymes and SSR markers at CIAT (Fregene *et al.*, 1997; Mba *et al.*, 2001). Despite the low saturation of loci in the genetic maps of cassava, marker loci are randomly distributed over linkage groups and the information from these maps has been utilised in cassava genetics. Genes for resistance to CMD have been mapped including a major one (*CMD2*) (Akano *et al.*, 2002). MAS for breeding CMD resistance has successfully been applied for introducing resistance into elite gene pools at CIAT (CIAT, 2003; Fregene and Mba, 2004) and to introgress resistance to cassava green mite (CGM) and CMD in local Tanzanian varieties (Kullaya *et al.*, 2004).

2.10.3 Quantitative trait loci

Molecular markers are being utilised in studying quantitative trait loci that affect traits and have been suggested as tools to enhance the efficiency of selection of quantitative traits in breeding programmes (Dudley, 1993; Young, 1996). The theory of QTL mapping was first described in 1923 by Sax and later elaborated by Thoday in 1961 who suggested that if the segregation is simply inherited, monogenes could be used to detect linked QTLs (Thoday, 1961). Young (1996) suggested that defined sequences of DNA, in modern day QTL mapping act as the linked monogenic markers. Mapping QTLs with molecular markers require analysis of large segregating populations (Tanksley, 1993). A population derived from distinctively different parents (e.g. resistant and susceptible) is analysed with the marker to test for linkage of markers to a QTL based on its position on the map from flanking markers (Liu, 1998a; 1998b).

Construction of a molecular map of cassava has led to identification of QTLs in cassava. Jorge *et al*. (2000) identified eight QTL for resistance to cassava bacterial blight (CBB). Okogbenin and Fregene (2002; 2003) identified several QTLs for early root bulking, productivity and plant architecture. Kizito (2006) identified two QTLs on two different linkage groups controlling CNP and six QTLs on four different linkage groups controlling DMC. One QTL for CNP and one QTL for DMC mapped near each other, suggesting pleiotrophy or linkage of QTLs.

Very little research has been done on the genetics of yield related traits in cassava. This is because classic breeding is difficult in a heterozygous crop, and cassava's long gestation period does not allow for quick results. Though some progress has been made on cassava through molecular markers, little has been done compared to other crops. Yet, based on its heterozygous state and long gestation period, it is a crop which stands to gain a lot from molecular markers. Work done so far has been targeted towards constructing of a linkage map (Fregene *et al*., 1997; Mba *et al*., 2001; Okogbenin and Fregene, 2006), analysis of genetic diversity (Fregene *et al*., 2000), mapping QTLs of mainly qualitative traits for example, disease resistance (Jorge *et al.*, 2001; Akano *et al*., 2002) and

morphological characteristics (Okogbenin and Fregene 2002; 2003). The only research regarding yield related quantitative traits has just been reported by Kizito (2006). It is known that traits which stand to benefit most from molecular work are quantitative in nature as they are usually influenced by environmental factors.

In cassava, DMC in particular stands to gain from molecular techniques, because it is evaluated at harvest (nine to 12 MAP), is usually affected by time of harvest and is now being threatened by cassava frogskin disease (CFSD). It is because of these problems that this study was undertaken, to try and shed light onto some phenotypic and genotypic characteristics of the crop from the yield point of view.

Chapter 3

Diallel mating model as a means of developing research material

3.1 Introduction

Diallel crosses have been used in genetic research to investigate the inheritance of important traits among a set of genotypes (Yan and Hunt, 2002). Specifically, diallel crosses were devised to investigate GCA of parents and to identify superior parents for use in hybrid and cultivar development (Ortiz *et al*., 2001; Yan and Hunt, 2002). Two types of analysis are commonly used in a diallel cross; a genetic analysis devised by Hayman (1954a;b) and Jinks (1954), and Griffings' (1956) combining ability approach (Hill *et al*., 1998). The former, which requires the inclusion of the parents, provides information on the genetic architecture of a quantitative character, whereas Griffings' analysis splits the contribution of each parent into GCA, SCA and if reciprocal crosses were made, allows the investigation of reciprocal effects (Ortiz *et al*., 2001).

Cassava has benefited from technological inputs in the area of breeding (Kawano, 2003; Kawano *et al*., 1998) to satisfy the needs of farmers and processors (Calle *et al*., 2005). Little progress in understanding the inheritance of agronomic traits in cassava has been achieved and articles regarding the inheritance of quantitative traits have been published (Easwari Amma *et al*., 1995; Easwari Amma and Sheela, 1998; Calle *et al*., 2005; Jaramillo *et al*., 2005; Cach *et al*., 2006). In this regard, cassava is unique because a molecular map has already been developed (Fregene *et al*., 1997; Mba *et al*., 2001; Okogbenin *et al*., 2006) and yet it is complemented with limited knowledge regarding traditional genetics. In order to address the situation, the CIAT cassava breeding programme embarked on a project to provide information regarding relevant yield related attributes. Genotypes from the three major cassava growing agro-ecologies in Colombia were crossed in a diallel fashion. These agro-ecologies are representative of the major cassava growing agro-ecologies worldwide (Ceballos, personal communication),

allowing extrapolation of information obtained. The objective of this study was to develop the material to be used for the research chapters of this study.

3.2 Materials and methods

A quantitative genetic study was initiated in 2000 to provide information on the genetics of traits of agronomic interest. Three sets of parents (Table 3.1), that represent clones adapted to the three major agro-ecologies most important for cassava in the tropics (lowland semi-arid, acid-savannas and mid-altitude), were crossed in a diallel fashion to produce F_1 families used in the study (Calle *et al*., 2005; Jaramillo *et al*., 2005; Cach *et al.* 2006). These were planted in September 2001 and evaluated in May 2002. Crosses were established in two locations each in lowland semi-arid (Pitalito and St. Thomas) and mid-altitude (Jamundi and Palmira) and one location in acid-savannah (Villavicencio). Locations within each agro-ecology had contrasting soil conditions (Calle *et al*., 2005; Jaramillo *et al*., 2005).

The experiment consisted of 36 F_1 crosses (nine parents) each in the lowland semi-arid and mid-altitude and 45 F_1 (ten parents) in acid savannah agro-ecologies. Each cross was represented by at least 30 plants. A randomised complete block design with three replications per location was used. The evaluation was similar to a split-plot design. Plants were hand-harvested individually and results averaged across the 30 plants of each F_1 cross. All roots, as well as the above-ground biomass (stems and foliage) produced by each plant were weighed. Harvest index was measured as the ratio of root weight to total biomass. Root dry matter content was estimated using the specific gravity methodology (Kawano *et al*. 1987). The analysis of variance was performed according to method 4 proposed by Griffing (1956). Genotypes and environments were considered fixed and random effects, respectively. Analysis was performed using Microsoft Excel (Microsoft Corporation, 2004; Nelson, 2000; Cach *et al*., 2006).

Data from each agronomic zone were analysed separately as it represented a different set of parents. Dry matter content, DRY, HI and FRY were used in the analysis due to their importance to yield (Bryne, 1984). General combining ability and SCA estimates were used for selecting parents for generating larger families and SCA estimates for selecting families to be used in the study.

3.3 Results and discussion

High positive and negative values were estimated for GCA (Table 3.1) for all traits. Estimates varied for the different locations within agro-ecologies and this might be attributed to influences due to local conditions. Higher GCAs were generally estimated for DMC, followed by FRY and the lowest for HI in all agro-ecologies. In the lowland semi-arid agro-ecology, GCA for HI ranged from 0.00 in CM 6754-8, MTAI 8 and SM 1411-5 to 0.05 in SM 1565-17. MECU 72 recorded -0.06 for HI in the mid-altitude agro-ecology followed by SM 1741-1 with 0.05 (Table 3.1). The highest HI GCA of 0.05 was recorded in SM 2219-11 in the acid-savannah. General combining abilities for DMC ranged from 0.02 in SM 1219-9 to -2.04 SM 1565-17 in the lowland semi-arid area, -0.01 in HMC 1 to 1.86 in SM 1278-2 in the mid-altitude and -0.07 to -4.66 in CM 7033-3 and MPER 183 respectively in the acid-savannah agro-ecologies. Fresh root yield GCA ranged from 0.00 in CM 8027-3 to 0.55 in SM 1565-17 in the lowland semi-arid, -0.04 in SM 1741-1 to 0.75 in MPER 183 in the mid-altitude and 0.00 in SM 2058-2 to -0.60 in MPER 183 in the acid-savannah agro-ecology.

Analysis of variance was performed for locations and across locations within each agro-ecology. The analyses followed a similar pattern and only across location results are presented. Analysis across locations indicated that locations were not significant (Tables 3.2 and 3.3). For the mid-altitude ecology genotype was highly significant ($P \leq 0.001$) for HI and FRY, significant ($P \leq 0.01$) for CFSD, DMC and DRY and was not significant

Table 3.1 General combining ability (GCA) estimates of yield related traits evaluated in three locations in Colombia during the 2001-2002 season

	General combining ability							
Parents	HI[a]	DMC[b]	FRY[c]	DRY[d]	HI	DMC	FRY	DRY
i) Low-land semi-arid								
	Pitalito				**St. Thomas**			
MTAI 8	-0.02	0.33	-0.05	-0.00	0.00	-0.17	0.03	-0.01
CM 6754-8	0.00	-0.18	-0.34	-0.10	0.00	0.68	-0.43	-0.09
CM 8027-3	-0.03	0.54	-0.25	-0.05	0.02	1.19	0.00	0.06
SM 805-15	-0.03	-0.28	-0.39	-0.12	-0.03	0.37	-0.48	-0.10
SM 1565-17	0.05	-0.89	0.55	0.12	0.03	-2.04	-0.03	-0.07
SM 1411-5	-0.04	0.63	-0.05	0.00	0.00	1.30	0.33	0.15
SM 1219-9	-0.01	0.02	0.17	0.06	-0.03	-0.21	0.27	0.07
SM 1657-12	0.03	-0.28	0.03	-0.00	-0.01	-0.39	0.02	-0.01
SM 1665-2	0.03	0.05	0.33	0.09	0.03	-0.74	0.27	0.06
ii) Mid-altitude, Andeans valley								
	Jamundi				**Palmira**			
CM 6740-7	-0.01	0.49	0.21	0.09	-0.01	0.74	-0.22	-0.06
SM 1219-9	0.02	-1.04	0.30	0.05	0.03	-0.13	0.42	0.15
SM 1278-2	0.02	1.86	-0.29	-0.01	0.00	-0.03	-0.58	-0.20
SM 1636-24	-0.02	-0.89	-0.46	-0.17	-0.02	-0.04	-0.18	-0.05
SM 1673-10	0.00	0.59	-0.22	-0.04	0.01	0.72	-0.37	-0.11
SM 1741-1	0.03	1.45	-0.04	0.05	0.05	0.70	0.12	0.07
HMC 1	0.02	-0.01	-0.05	-0.02	0.01	-0.88	-0.57	-0.23
MECU 72	-0.01	-1.79	0.04	-0.08	-0.06	-0.37	0.63	0.20
MPER 183	-0.02	-0.65	0.52	0.13	-0.01	-0.69	0.75	0.22
iii) Acid-savannah								
	Villavicencio							
CM 4574-7	-0.01	1.89	0.17	0.07				
CM 6740-7	0.00	0.34	0.04	0.02				
CM 7033-3	-0.01	-0.07	-0.06	-0.02				
SM 1219-9	0.03	0.83	0.05	0.02				
SM 1565-15	0.02	1.33	0.18	0.07				
SM 2058-2	-0.00	-0.46	-0.00	-0.01				
SM 2219-11	0.05	0.94	-0.28	0.09				
HMC 1	0.03	-0.40	0.08	0.02				
MPER 183	-0.10	-4.66	-0.60	-0.22				
MTAI 8	-0.00	0.27	-0.14	-0.04				

[a]Harvest index; [b]Dry matter content (%);[c]Fresh root yield (t/ha); [d]Dry root yield (t/ha)

Table 3.2 Analysis of variance (ANOVA) table of means of the variables evaluated on a diallel cross at harvest in two mid-altitude locations, Palmira and Jamundi, Colombia in 2002

Source of variation	Df[a]	Mean squares					
		CFSD[b]	ComRt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
Location (L)	1	1.75	24.67	0.504	562.84	3.65	0.30
Rep/location	4	0.05	20.62	0.028	18.41	17.45	2.70
Genotypes (G)	35	0.13**[k]	4.10	0.009***	9.83**	3.27***	0.34**
GCA[h]	8	0.18	5.16	0.033***	27.08	5.86*	0.47
SCA[i]	27	0.11**	3.78*	0.002	4.72***	2.50***	0.30***
G x L	35	0.04**	2.45**	0.001*	3.15**	0.67*	0.11*
GCA x L	8	0.12**	4.52*	0.001	11.20***	1.42*	0.28*
SCA x L	27	0.01*	1.84	0.001*	0.77	0.44	0.06
Error	140	0.01	1.17	0.001	0.89	0.44	0.06
% SS[j] for GCA			***28.79***	***81.04***	***62.95***	***40.96***	***32.17***
% SS for SCA			***71.21***	***19.27***	***37.05***	***59.04***	***67.83***

[a]Degrees of freedom; [b]Cassava frogskin disease; [c]Number of commercial roots; [d]Harvest index; [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]Dry root yield (t/ha); [h]General combining ability; [i]Specific combining ability; [j]genotype sum of squares; [k]*=significant at P≤0.05, **=significant at P≤0.01, ***=significant at p≤0.001

for number of commercial roots (ComRt; Table 3.1). GCA was highly significant (P≤0.001) for HI, significant (P≤0.05) for FRY and not significant for the other variables. Specific combining ability was highly significant for DMC, FRY and DRY, significant (P≤0.01) for CFSD and ComRt (P≤0.05) and not significant for HI. This indicated that additive effects were more important for HI, while none additive effects were more important for DMC, FRY, DRY, CFSD and ComRt. Studies have shown the importance of both additive and non-additive gene action in the inheritance of yield traits in other crops (Mishra *et al*., 1994; Khan *et al*., 1995; El-Hennawy, 1996; Uma-Menon *et al*., 1996).

Genotype x location interaction was recorded for all the traits, GCA x location for all except HI, while interaction for SCA x location was recorded only in CFSD and HI. Interactions indicated that the estimation of GCA was greatly influenced by location in all traits except HI. This agreed with previous findings that cassava genotype performance is greatly influenced by G x E interactions, and that HI is more reliable for selection in the earlier stages of selection (Kawano, 2003, Jaramillo *et al*., 2005). It is important to note that the highest GCA x location interaction was recorded in the mid-altitude agro-ecology for DMC, where CFSD is prevalent. Other studies indicated that CFSD greatly influenced the genetic estimation of DMC. Ortiz *et al*. (2001), observed that the GCA effects interacted significantly with environments being most pronounced at the location where the parental genotypes were selected for their wide response to yellow rust (Wagoire *et al*., 1998), an important disease affecting grain yield in the east African highlands (Wagoire *et al*., 1998).

Table 3.3 Analysis of variance (ANOVA) table of means of the variables evaluated on a diallel cross at harvest in two lowland semi-arid locations, Pitalito and St. Thomas, Colombia in 2002

		Mean squares				
Source of variation	df[a]	ComRt[b]	HI[c]	DMC[d]	FRY[e]	DRY[f]
Location (L)	1	1.19	0.22	161.13	4.51	1.09
Rep/location	4	0.64	0.02	14.38	0.53	0.10
Genotypes (G)	35	1.51*[k]	0.01*	11.74	1.11**	0.08
GCA[g]	8	3.71*	0.02	26.50	2.86*	0.18
SCA[h]	27	0.86	0.01*	7.37	0.59	0.05
G x L	35	0.78	0.01**	6.81*	0.46*	0.05**
GCA x L	8	1.16	0.01*	9.54	0.82*	0.09*
SCA x L	27	0.67	0.00	6.01	0.35	0.03*
Error	140	0.53	0.00	3.63	0.24	0.02
% SS[j] for GCA		***56.10***	***50.00***	***51.58***	***58.88***	***52.71***
% SS for SCA		***43.90***	***50.00***	***48.42***	***41.09***	***47.29***

[a]Degrees of freedom; [b]Number of commercial roots; [c]Harvest index; [d]Dry matter content (%); [e]Fresh root yield t/ha); [f]Dry root yield (t/ha); [g]General combining ability; [h]Specific combining ability; [j]Genotype sum of squares; [k]*=significant at P≤0.05, **=significant at P≤0.01, ***=significant at p≤0.001

Analysis of lowland semi-arid crosses (Table 3.1) revealed genotype to be significant for FRY ($P \leq 0.01$), ComRt and HI ($P \leq 0.05$). General combining ability was only significant ($P \leq 0.05$) for ComRt and FRY and SCA for HI. Lower levels of significance for genotype, GCA and SCA at the lowland semi-arid locations compared to the mid-altitude locations suggest that there was less genetic variability among the lowland semi-arid genotypes evaluated. The lack of significance between locations despite contrasting soil conditions indicated that genotypes were well adapted to the agro-ecologies and that soil types had little effect on yield within an agro-ecology.

The sum of squares due to crosses was partitioned in two orthogonal components represented by the general (GCA) and specific (SCA) combining ability effects (Tables 3.2, 3.3 and 3.4). The proportion of the sum of squares for crosses explained by GCA and SCA effects is an estimation of the relative importance of additive and non-additive effects in the expression of each variable (Calle *et al*., 2005). The contribution due to different traits differed between locations and agro-ecologies (Table 3.4). In mid-altitude locations (Palmira and Jamundi) GCA contribution to HI was the highest (Table 3.2) while the opposite was true for lowland semi-arid locations (Pitalito and St. Thomas). In acid-savannah (Villavicencio), GCA and SCA contributed about equal sum of squares for HI (Table 3.4). General combining ability contributed more variation to DMC in mid-altitude and acid-savannah and less in lowland semi-arid locations. General combining ability contributed more to FRY in all locations except Jamundi and more to DRY except in Pitalito (Table 3.4). This indicated that gene action was influenced by environment. The study showed that DRY, FRY and DMC were under the control of additive gene action in most of the locations. This is in agreement with what is stated in literature that these are quantitative and under additive gene action (Losado Valle, 1990; Kawano, 2003).

Sum of squares were also partitioned in the combined analysis (Tables 3.2 and 3.3). General combining ability contributed more to HI (81.04%) and DMC (62.95%) in the mid-altitude agro-ecology and contributed more to all traits in the lowland semi-arid agro-ecology, except for HI where it contributed half of the sum of squares.

Table 3.4 ANOVA sum of squares for agronomic yield traits evaluated in three locations in Colombia during the 2001-2002 season

Source of Variation	**Sum of squares**			
	HI[a]	DMC[b]	FRY[c]	DRY[d]

Low land semi-arid				
Pitalito				
Rep[e]	0.05	7.12	0.74	7.12
Genotype (G)	0.29	83.10	29.56	83.10
GCA[f]	0.06	35.21	16.38	35.21
SCA[g]	0.15	47.89	13.17	47.89
SS[h] for GCA	***28.57***	***42.37***	***55.43***	***42.37***
SS for SCA	***71.43***	***57.63***	***44.57***	***57.63***
St Thomas				
Rep	0.02	50.39	1.32	0.34
Genotype (G)	0.25	566.38	25.58	2.23
GCA	0.10	253.06	12.92	1.13
SCA	0.15	313.31	12.66	1.10
SS for GCA	***40.40***	***44.68***	***50.51***	***50.67***
SS for SCA	***60.00***	***55.32***	***49.49***	***49.33***
Mid-altitude				
Jamundi				
Rep	0.00	64.11	8.48	1.12
Genotype (G)	0.17	113.70	60.77	6.47
GCA	0.12	62.01	15.79	3.62
SCA	0.04	51.69	44.97	2.85
SS for GCA	***75.00***	***54.54***	***25.98***	***55.95***
SS for SCA	***25.00***	***45.46***	***74.02***	***44.05***
Palmira				
Rep	0.11	9.62	61.30	9.62
Genotype (G)	0.21	9.17	77.12	9.17
GCA	0.15	4.64	42.46	4.64
SCA	0.06	4.53	34.66	4.53
SS for GCA	***71.43***	***50.50***	***55.06***	***50.60***
SS for SCA	***28.57***	***49.40***	***44.64***	***49.40***
Acid-savannah				
Villancencio				
Rep	0.12	150.72	1.97	0.32
Genotype (G)	0.53	835.78	18.55	2.30
GCA	0.36	701.00	12.84	1.71
SCA	0.17	134.78	5.71	0.60
SS for GCA	***50.67***	***67.92***	***83.87***	***69.22***
SS for SCA	***49.33***	***32.08***	***16.13***	***30.78***

[a]Harvest index; [b]Dry matter content (%); [c]Fresh root yield (t/ha); [d]Dry root yield (t/ha); [e]Replication; [f]General combining ability; [g]Specific combining ability; [h]Genotype sum of squares; [d]Genotype sum of squares

The combined analysis seems to be more accurate. Harvest index and DMC have been shown to be highly heritable and under additive gene action (Mahungu, 1987; Kawano, 2003) and have been preferred for selection at earlier stages of selection (Kawano, 2003). Number of commercial roots, FRY and DRY, on the other hand, have been shown to be greatly influenced by the environment (Ntawuruhunga, 2000). From the results it is evident that the magnitude of the influence of GCA or SCA on a trait in cassava will depend on the environment and genotypes being tested. This agrees with previous findings in literature (Ntawuruhunga, 2000).

Based on the above results, it was decided that for the development of markers associated with QTLs for use in breeding programmes emphasis should be placed on DMC given its stability across environments within agro-ecologies. Genotypes were selected based on whether they had contrasting GCA values as was the case with SM 1741-1 (1.48) being crossed with MPER 183 (-0.65; Table 3.1), whether they combined well or because they appeared more often as parents in crosses with high SCA values. Parents selected for the generation of larger sized progeny for QTL analysis included: SM 1741-1 crossed to MPER 183 and SM 1219-9 to MECU 72 for the mid-altitude agro-ecology. For the lowland semi-arid agro-ecology SM 1411-5 was crossed to MTAI 8, SM 8027-3 to SM 6754-8 and SM 1665-2 to SM 805-15, while crosses for the acid-savannah agro-ecology included SM 1219-9 crossed to SM 1565-15 and SM 4574-7 to SM 1565-15. These parents were planted in a crossing block in Palmira and crosses made (Chapter 5). Specific combining ability values for DMC for the mid-altitude agro-ecology (Table 3.5) were used for selecting families for initiating BSA (Chapter 8). Twenty one families with the highest mean for SCA for the two locations were selected (Table 3.6). Families GM 312 and GM 313 were selected to initiate BSA (Chapter 8). Both these families had MECU 72, a frequently used genotype in CIAT programmes, as one parent (high DMC) and either SM 1673-10 or SM 1741-1, two genotypes which did well in the diallel analysis, as the other parent. The other 19 families were selected for confirmation of markers identified via BSA.

Table 3.5 Specific combining ability (SCA) values for percent dry matter estimated at harvest in different mid-altitude locations of Colombia during the 2001-2002 season (Jamundi, upper, Palmira, lower)

Parent	CM 6740-7	SM 1219-9	SM 1278-2	SM 1636-24	SM 1673-10	SM 1741-1	HMC 1	MECU 72	MPER 183
CM 6740-7		-1.522	0.683	-0.004	-0.157	-0.623	0.137	-0.344	1.848
SM 1219-9	-0.566		0.666	-2.164	0.828	1.750	0.037	-0.563	0.958
SM 1278-2	0.018	0.500		-0.092	-0.612	-0.838	0.139	0.804	-0.743
SM 1636-24	0.187	-1.509	0.275		0.781	0.309	-1.250	1.850	0.576
SM 1673-10	-0.580	0.525	0.176	0.519		1.089	0.052	-0.349	-1.633
SM 1741-1	-0.659	-0.086	-0.509	0.454	0.803		0.123	-1.392	-0.411
HMC 1	-0.887	1.234	-0.139	-1.010	-0.144	0.617		0.679	0.090
MECU 72	0.607	-0.403	0.149	0.893	0.065	-0.560	0.028		-0.677
MPER 183	1.865	0.307	-0.467	0.193	-1.362	-0.057	0.300	-0.782	

Table 3.6 **List of mid-altitude agro-ecology families selected for bulk segregant analysis and their respective dry matter content specific combining ability**

Family	Mother	Father	SCA at Jamundi	SCA at Palmira
CM 9642	CM 6740-7	MPER 183	1.85	1.87
CM 9733	HMC 1	MPER 183	0.09	0.30
CM 9001	CM 6740-7	SM 1219-9	-1.52	-0.57
GM 257	SM 1219-9	SM 1636-24	-2.16	-1.51
GM 260	SM 1219-9	SM 1673-10	0.83	0.53
GM 265	SM 1219-9	MPER 183	0.96	0.31
GM 267	SM 1278-2	SM 1636-24	-0.09	0.28
GM 268	SM 1278-2	SM 1673-10	-0.61	0.18
GM 269	SM 1278-2	SM 1741-1	-0.84	-0.51
GM 283	SM 1636-24	SM 1673-10	0.78	0.52
GM 284	SM 1636-24	SM 1741-1	0.31	0.45
GM 286	SM 1636-24	MPER 183	0.58	0.19
GM 293	SM 1673-10	HMC 1	0.05	-0.14
GM 294	SM 1673-10	MPER 183	-1.63	-1.36
GM 306	MECU 72	MPER 183	-0.68	-0.78
GM 309	SM 1219-9	MECU 72	-0.56	-0.40
GM 310	SM 1278-2	MECU 72	0.80	0.15
GM 311	SM 1636-24	MECU 72	1.85	0.89
GM 312	SM 1673-10	MECU 72	-0.35	0.07
GM 313	SM 1741-1	MECU 72	-1.39	-0.56
GM 314	HMC 1	MECU 72	0.68	0.03

3.4 Conclusions

Diallel mating designs have proved informative in determining the inheritance of quantitative traits of interest to plant breeders. From the practical point of view, diallel mating designs provided a simple and convenient method for the estimation of genetic parameters. The diallel mating design is of interest, in that ANOVA uses the concepts of GCA and SCA to distinguish between the average performance of parents in crosses (GCA) and the deviation of individual crosses from the average of the parents (SCA). Among various diallel forms, the half diallel techniques have certain advantages over others, giving maximum information about genetic architecture of a trait, parents and allelic frequency.

Diallel crosses were evaluated to select parents and families to initiate a phenotypic and genetic study for DMC and related yield traits. The crosses were evaluated in three major cassava agro-ecologies in Colombia, mid-altitude, lowland semi-arid and acid-savannah. The agro-ecologies are representative of the main cassava growing agro-ecologies. High positive and negative GCA and SCA values were estimated for yield agronomic traits. Results indicated that HI and DMC were under the influence of GCA, while ComRt, FRY and DRY were under the influence of both additive and non-additive effects. Since genetic estimates varied across locations and agro-ecologies it seemed to be affected by environment and test genotypes. Genetic studies on yield related studies in other crops have stated that traits are controlled by both additive and non-additive gene action. Results furthermore indicated that biotic factors like CFSD influenced the estimation of DMC.

Results indicated that a programme to generate material for a root quality study was successfully initiated using a diallel analysis. Parents and families could be selected based on their GCA estimates and used for initiating BSA as reported later. The obtained GCA and SCA estimates increasing our knowledge of cassava genetics and yield related traits.

Chapter 4

Genotype by environment interaction influence on cassava performance

4.1 Introduction

Cassava is one of the most important calorie-producing crops in the tropics. It is efficient in carbohydrate production, adapted to a wide range of environments, and tolerant to drought and acid soils (Jones, 1959; Rogers and Appan, 1970; Kawano *et al.,* 1978; Cock, 1982). Throughout the tropics, small-scale farmers grow cassava in areas with poorer soils using traditional methods (Kawano, 2003). The importance of cassava as a security crop became more apparent with changes in climatic, physiological and socio-economic environments in a number of African countries in the 1990s (Minde *et al*., 1997). These are forcing farmers to plant cassava, which is a hardy crop.

Expansion of a crop to new areas requires basic understanding of its performance in relation to the environment, and to determine whether G x E interactions are important (Tan and Mak, 1995). Such information is often limited in a food crop mainly associated with subsistence small-scale farming. Bueno (1986) reported that FRY in Brazil was more influenced by environmental variation than HI and starch content. Cock (1985) on the other hand, reported partial stability in some cassava clones for yield and root starch content across adapho-climatic zones in Colombia. This indicated that location played a less dominant role in these two traits. Cock (1985) reported generally high correlations between yields of the same set of genotypes across years in different sites, suggesting that a genotype with high yield in one year will continue to be superior to the others over time. Root DMC had highly significant correlations among selection stages within sites and between some sites in Colombia (CIAT, 1988), indicating an absence of G x E effects.

Plant breeders invariably encounter G x E interactions when testing varieties across a number of environments (Kaya *et al*., 2002). In yield trials, standard statistical methods

that have been applied include analysis of variance (ANOVA), principal component analysis (PCA), linear regression (LR), and additive main effects and multiplicative interaction (AMMI). A combined analysis of variance can quantify interactions and describe the main effects, however it is not informative for explaining G x E. The AMMI model (Kaya *et al*., 2002) is a hybrid analysis that incorporates both additive and multiplicative components of the two-way data structure. AMMI analysis has been shown to be effective because it captures a large portion of the G x E sum of squares, it clearly separates main and interaction effects that present agricultural researchers with different kinds of opportunities, and often provides agronomical meaningful interpretation of the data (Gauch, 1992).

In AMMI, the additive portion is separated from the interaction by ANOVA. Then the PCA, which provides a multiplicative model, is applied to analyse the interaction effects from the additive ANOVA model. The biplot display of PCA scores plotted against each other, provides visual inspection and interpretation of G x E components. Integration of biplot display and genotypic stability statistics enables genotypes to be grouped based on similarity of performance across diverse environments (Thillainathan and Fernandez, 2001). The objective of this study was to assess the contribution of genotype, environment and the G x E interaction on different cassava yield traits.

4.2 Materials and methods

Trials were conducted in three mid-altitude valley locations in the Departamento de Valle del Cauca, Colombia. They included Jamundi, Palmira and Santander de Quilichao located between $3^0$06' N and $76^0$32' W. Jamundi is 889 m.a.s.l., has acid soils with a pH of 5.1 and low P content (3.88 ppm). Palmira is 965 m.a.s.l., and has contrasting soil conditions with a pH of 7.8 and adequate P availability (>6.00 ppm; Jaramillo *et al.,* 2005). Santander de Quilichao is 990 m.a.s.l., and has amorphous, isohiperthermic oxicdystropept soil with a low pH (Leihner *et al.,* 1999). The stations represent semi-humid, mid-altitude agro-ecologies, representing the main cassava growing regions. The mid-altitude agro-ecology was selected for logistic reasons, being where the CIAT station

is located. Materials used in this study were selected from a diallel experiment reported by Jaramillo *et al.* (2005). A diallel experiment comprising nine parents and planted in two locations, Jamundi and Palmira, was evaluated at harvest in 2002 and used to select entries for this experiment. Dry matter content was used to select families with high levels of variation. Twenty-one out of the 36 families constituting the diallel were selected (Chapter 3). Individuals with enough cuttings to plant six stands were selected within the families for continuing the study (Table 4.1).

Experiments were conducted between 2001 and 2004. The diallel experiment was conducted in Palmira and Jamundi, hereafter referred to as Palmira2002 and Jamundi2002. Trials were planted in August 2001 in a randomised complete block design. Three stakes each were planted in three replications at each of the three locations. The spacing was 1 m between plants and rows. Plants were hand harvested individually in May 2002, 10 MAP. Roots produced by each plant, as well as the aboveground biomass (stem and foliage), were weighed. Roots were separated into marketable and non-marketable, where marketable represented roots viewed as acceptable for sale in the local supermarkets. Root weight was derived by dividing fresh root yield with total number of roots. Harvest index was measured as the ratio between fresh root yield and total biomass. Root DMC in the roots was estimated using the specific gravity methodology (Kawano *et al.,* 1987). Approximately 3 kg of root were weighed in a hanging scale (W_A). The same sample was weighed with roots submerged in water (W_W). Dry matter content was estimated using the formula:

$$\%DMC = (158.3 \times (W_A/(W_A - W_W) - 142)$$

Where W_A = weight in air and W_W = weight in water (Jaramillo *et al.,* 2005).

Dry root yield was derived by multiplying FRY with DMC.

Data was analysed and 21 families selected (Table 4.1). These were used to establish the next trial. Only data from the selected families will be reported here.

Table 4.1 List of families, genotypes per family and standard deviation of dry matter content within families used in the study

Family	Number of entries	Standard deviation
CM 9642	27	3.50
CM 9733	22	3.14
CM 9001	22	4.66
GM 257	20	5.60
GM 260	26	3.64
GM 265	28	3.17
GM 267	4	2.56
GM 268	20	3.74
GM 269	19	2.86
GM 283	23	3.54
GM 284	24	3.74
GM 286	27	3.47
GM 293	23	3.60
GM 294	26	3.76
GM 306	27	3.56
GM 309	21	4.15
GM 310	24	3.07
GM 311	26	4.16
GM 312	25	4.25
GM 313	25	4.55
GM 314	19	3.55

The Quilichao trial was planted in July 2003 and harvested in June 2004, and will hereafter be referred to as Quilichao2004. A completely randomised block design with 1 x 10 plants and four replications, with a spacing of 1 x 1 m between plants and plots was used. A large plot was used because of higher availability of planting material at harvest.

Irrigation and fertiliser were applied as required. Plots were sprayed against CGM, which is endemic in the region. Manual weeding was done until the crop was established, thereafter an appropriate herbicide was routinely applied depending on the type of weeds present.

Agrobase (2000) and SAS (2002) statistical programmes were used for data analysis. From the originally selected 478 genotypes used during the 2002 analysis, 254 were selected for the 2004 based trial on the availability of enough planting material. In the G x E analysis only genotypes which had three replications throughout the three environments (total of 175), were used in order to take advantage of the Agrobase software, that needs balanced data. The 2002 data was used for location analysis, while the 2002 and 2004 data were used as environments (locations within years). Since roots per plant, root weight and fresh and dry root yield data were not normally distributed, data were transformed by the square root method using the formula: $y = \text{sqrt}(x+0.5)$, where y is the resulting transformation and x the data point.

The SAS correlation (proc corr) and regression (proc reg) procedures were used to estimate correlation and regression coefficients between different parameters. Yield and yield components were subjected to combined ANOVA for each location, across locations and across environments to test the significance of variation due to genotypes, genotype x location and genotype x environment. The following model was used for the combined data:

$$Yij = \mu + G_i + E_j + GE_{ij} + e_{ij}$$

Where μ is the general mean, G_i, E_j and GE_{ij} represent the effect of the genotype, environment and G x E interaction respectively, and e_{ij} is the average of random errors associated with r^{th} plot that receives the i^{th} genotype in the j^{th} environment (Crossa, 1990). Agrobase (2000) was used for estimating broad-sense heritability.

Principal component analysis (Iezzoni and Pritts, 1991) was used to investigate the relevant traits contributing to the phenotypic variation among genotypes. The AMMI model was used to estimate interaction components. The model used was:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + E_{ger}$$

Where Y_{ger} is the observed yield of genotype g in environment e for replicate r. The additive parameters are: μ is the grand mean, α_g is the deviation of genotype g from the grand mean, and β_e is the deviation of environment e. The multiplicative parameters are: λ_n the singular value for interaction principal component axis (IPCA) n, γ_{gn} the genotype eigenvector for axis n, and δ_{en} the environment eigenvector. The ρ_{ge} is the residual and E_{ger} is te error. The eigenvectors are scaled as unit vectors and are unit-less, whereas λ has yield units.

4.3 Results and discussion

Simple correlations were performed among traits for individual sites, between locations and among environments. All correlations followed the same trend and only those among environments are presented (Table 4.2). Dry root yield was highly correlated ($P \leq 0.001$) with storage roots per plant (RtPlt), HI, FRY and DMC, and storage root weight (RtWt) at $P \leq 0.01$, indicating that all traits were important. All yield traits were positively inter-correlated, showing interdependency. Environment (E) was highly correlated ($P \leq 0.001$) with all yield related traits except HI. It was positively correlated with FRY, DRY, and CFSD and negatively correlated with the other yield traits.

It is interesting to note that although CFSD was negatively correlated with all other yield traits, it had no significant influence on FRY, which in turn influenced DRY. It is known that diseases and pests reduce storage root yield in cassava (Hahn *et al*., 1980b). According to Kawano *et al*. (1998), total photosynthesis of the crop sets the ceiling for the dry biomass, which is to be shared among HI, FRY and DMC. Fresh root yield and root DMC constitute DRY, the final goal of field production and compete for sink. It is

probable that in a situation where one is reduced and there is no competition, the other can actually compensate. This seems to be the case here.

Table 4.2 Phenotypic correlation of environment, cassava frogskin disease and yield related traits in three environments in Colombia

	Env[a]	CFSD[b]	RtPlt[c]	RtWt[d]	HI[e]	DMC[g]	FRY[f]
CFSD	0.24***[h]						
RtPlt	-0.39***	-0.07**					
RtWt	-0.57***	-0.14***	0.11***				
HI	-0.02 ns	-0.10***	0.39***	0.21***			
DMC	-0.20***	-0.09***	0.26***	0.30***	0.34***		
FRY	0.13***	0.01 ns	0.51***	0.02 ns	0.42***	0.11***	
DRY[i]	0.10***	0.00 ns	0.53***	0.07**	0.44***	0.26***	0.98***

[a]Environment; [b]Cassava frogskin disease; [c]Roots per plant; [d]Root weight (kg); [e]Harvest index; [f]Dry matter content (%);[g]Fresh root yield (t/ha); [h]ns=not significant; *=significant at P≤0.05, **=significant at P≤0.01, ***=significant at P≤0.001; [i]Dry root yield (t/ha)

Analysis of variance (ANOVA) for Palmira2002, Jamundi2002 and Quilichao2004 indicated that genotype was highly significant (P≤0.001) for all traits being evaluated except for RtWt (Table 4.3). This indicated high variability among genotypes for RtPlt, HI, DMC, FRY and DRY and suggested that conventional crossing and selection should be sufficient for improving the traits under evaluation. The low significance (P≤0.05) at Quilichao2004 or lack in Palmira2002 and Jamundi2002 for RtWt indicated that there was little variability among crosses for RtWt and that improvement of this trait through conventional methods is likely to prove difficult. Tan (1985) reported that RtWt is less heritable. Replication was highly significant for all traits evaluated in all three sites except for DMC and RtWt in Quilichao2004. In conventional cassava breeding programmes, initial stages are not replicated because of the low seed multiplication rate of the crop. The nature of cassava necessitates that a breeder starts with a huge number of genotypes. In order to be practical, the breeder has to reduce the number of genotypes by about 95% during clonal

Table 4.3 Analysis of variance (ANOVA) table of yield parameters evaluated at harvest at three sites over two years at CIAT, Colombia

Source of Variation	df[a]	MEAN SQUARES					
		RtPlt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
Palmira2002							
Cross	20	30.3***[h]	0.10***	0.14***	81.06***	2.88***	0.77***
Genotype(Cross)	457	0.87***	0.04 ns	0.02***	14.92***	0.90***	0.28***
Rep	2	7.98***	0.72***	0.91***	180.18***	13.66***	4.86***
Genotype x Rep	58	0.28ns	0.03ns	0.01ns	5.24ns	0.23ns	0.07ns
Error	896	0.28	0.03	0.01	5.81	0.23	0.07
CV		21.78	15.84	17.38	6.81	21.30	18.42
Heritability		0.71	0.23	0.68	0.67	0.77	0.76
Jamundi2002							
Cross	20	3.05***	0.08***	0.10***	224.11***	2.55***	0.60***
Genotype(Cross)	457	0.99***	0.03 ns	0.02***	20.56***	0.89***	0.24***
Rep	2	4.03***	0.39***	0.06**	54.98***	2.30***	0.75***
Genotype x Rep	58	0.39ns	0.03ns	0.01ns	9.76ns	0.20ns	0.06ns
Error	896	0.37	0.03	0.01	7.23	0.23	0.06
CV		24.37	14.46	23.26	8.54	21.28	17.76
Heritability		0.66	0.22	0.63	0.75	0.77	0.75
Quilichao2004							
Cross	20	0.54***	0.05***	0.11***	83.68***	1.56***	0.53***
Genotype(Cross)	234	0.36***	0.03*	0.04***	29.93***	1.69***	0.51***
Rep	2	2.80***	0.04ns	0.25***	27.86ns	17.80***	3.94***
Genotype x Rep	130	0.22ns	0.02ns	0.01ns	11.26ns	0.59ns	0.17ns
Error	378	0.20	0.02	0.01	10.76	0.51	0.14
CV[i]		23.42	63.57	26.60	10.87	28.82	24.82
Heritability[j]		0.45	0.35	0.70	0.68	0.69	0.71

[a]Degrees of freedom; [b]Number of roots per plant; [c]Root weight (kg); [d]Harvest index; [e]Dry matter content (%); [f]Fresh root yield (t/ha);, [g]Dry root yield (t/ha); [h]ns=not significant, *=significant at P≤0.05, **=significant at P≤0.01, ***=significant at P≤0.001; [i]Coefficient of variation; [j]Broad-sense heritability

evaluation (Kawano *et al*., 1998). Thus selection is crucial during this stage, as a large proportion of genotypes are eliminated without a proper evaluation setup (Ceballos *et al*., 2004). Replications with fewer plants have been suggested as a way to avoid this (Kawano *et al*., 1998, Pérez *et al*., 2001). However, because of the large number of genotypes involved, it is difficult to get a uniform plot for a trial and emphasis is usually put on attaining uniformity within a replication. This was confounded in the present trial by the non-uniform nature of soil in trial sites (we at times had to establish trials in different fields within a station to attain uniformity within a replication). It is therefore inevitable to obtain significant differences among replications. It is, though, interesting to note that there were no significant interactions between genotype and replication in all trials for all traits (Table 4.3).

Findings indicated that the genotypes' relative performance across replications in all three trials was consistent for all traits evaluated, suggesting that replications were actually different. It furthermore suggested that genotype performance for all traits was stable among replicates across trials. If this is true, then results suggested that replication was successful, as it enabled us to increase our data points from one to three per individual, thus improving selection at an earlier stage. Pérez *et al*. (2001), reporting on a new breeding scheme at CIAT where replication is being introduced at the early stage reported similar findings. They observed that the new scheme shortened the breeding cycle, improved the heritability of traits to be selected for and allowed estimation of GCA from genotypes that were used as parents. The non-significant replication effect for DMC in Quilichao2004, despite the apparent replication differences, tended to confirm earlier findings that replication might not be necessary for DMC trials planted as single rows.

Combined ANOVA results over two locations (Table 4.4) indicated that there were highly significant differences ($P \leq 0.001$) in the main effects for genotype, with the exception of RtWt. This indicated that selection can be effective for RtPlt, HI, DMC, FRY and DRY between locations. Lack of significance for RtWt gave an indication of the difficulty likely to be encountered when improving this trait. Location was highly significant for all traits evaluated except FRY, an indication that locations were different.

Interaction between genotype and location (G x L) was not significant except for RtPlt and FRY (Table 4.4). Considering the highly significant location effect, it is an indication that genotypes' performance for RtWt, HI, DMC, FRY and DRY were stable. Root number and FRY, though, did show signs of not being stable in the two locations. Hay and Walker (1989) and IITA (1990), indicated that although roots per plant in cassava is primarily under genotypic control, expression is controlled by a number of crop and environmental factors.

Table 4.4 Combined analysis of variance (ANOVA) table of yield parameters evaluated in two locations in CIAT, in 2002

Source of Variation	df[a]	MEAN SQUARES					
		RtPlt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
Location (L)	1	5.45***[h]	0.33**	7.79***	10835.48***	0.00ns	2.71***
Cross	20	3.79***	0.08***	0.20***	241.08***	4.51***	1.05***
Genotype (G)	457	1.42***	0.03ns	0.03***	24.75***	1.40***	0.40***
Rep	2	9.58***	1.07***	0.31***	216.40***	13.49***	4.64***
G x L	29	0.56*	0.03	0.01	6.79	0.41*	0.11
Error	2358	0.36	0.03	0.01	7.88	0.26	0.08
CV[i]		24.55	15.26	21.50	8.40	22.98	19.60
Heritability[j]		0.66	0.30	0.67	0.62	0.69	0.67

[a]Degrees of freedom; [b]Storage roots per plant; [c]Storage root weight (kg); [d]Harvest index; [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]Dry root yield (t/ha). [h]ns=not significant; *=significant at P≤0.05, **=significant at P≤0.01, ***=significant at p≤0.001; [i]Coefficient of variation; [j]Broad-sense heritability

Due to the highly significant difference attributed to location effects, its contribution to the total sum of squares was considered (Table 4.5). For RtPlt, RtWt, FRY, and DRY genotype contributed at least 75% of the total trait sum of squares. Genotype contributed twice as much sum of squares as location for HI, while for DMC, genotype and location contributed an equal sum of squares. For all traits there was G x L interactions, although small. The low contribution of the locations to the total sum of squares, coupled with

non-significant G x L interactions, and the main contribution of replications (Table 4.5), suggested that traits evaluated were not as much influenced by location as by genetic differences, and are thus stable. For the environment analysis, E, G, and G x E interactions were significant for all traits except G x E for RtWt (Table 4.6). Based on these results, the effects were partitioned using AMMI analysis.

Table 4.5 Sum of squares table of yield parameters taken at two locations in CIAT, Colombia, in 2002

Source of Variation	Df[a]	MEAN SQUARES					
		RtPlt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
Trait	511	764.47	19.16	26.96	27597.99	767.65	218.42
Location (L)	1	5.44	0.33	7.79	10835.48	0.00	2.71
Cross (C)	20	75.85	1.55	4.09	4821.52	90.27	21.04
Genotype (G)	457	647.90	14.31	14.12	11311.36	638.37	182.25
Rep	2	19.17	2.16	0.63	432.81	26.98	9.27
G x L	29	16.10	0.81	0.34	196.83	12.02	3.13
Error	2358	854.90	73.97	23.64	18580.30	619.74	185.62
Total	2867	1619.37	93.13	50.61	46178.29	1387.39	404.05
% SS[h] due to L		0.70	1.72	28.89	39.26	0.00	1.24
% SS due to G		84.75	74.69	52.37	40.99	83.16	83.44
% SS due to G x L		2.11	4.23	1.26	0.71	1.57	1.43

[a]Degrees of freedom; [b]Roots per plant; [c]Root weight (kg); [d]Harvest index; [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]Dry root yield (t/ha). [h]Traits sum of squares

Moderate to high heritability estimates were obtained for the different traits (Tables 4.3, 4.4 and 4.6). Estimates for FRY ranged from 0.54 to 0.77, 0.52 to 0.76 for DRY and 0.62 to 0.75 for DMC. Heritability for RtWt was the lowest (0.22 to 0.35). Estimates for RtPlt ranged from 0.45 to 0.71 and for HI from 0.53 to 0.70. Trait estimates obtained for individual sites, between locations and among environments were not significantly

different from each other (Tables 4.3, 4.4 and 4.6). High heritability estimates obtained for most of the traits suggested that their improvement could be effected by conventional breeding. This is in agreement with Kawano (2003), who noted that the physiological yield basis of cassava is uncomplicated and selection using simple components (HI, biomass, and DMC) is highly efficient. Heritability estimates of 0.62 to 0.75 for DMC among the environments are within those reported by Kawano *et al.* (1987) of 0.87 and Kawano (2003) of 0.51 to 0.67. Values furthermore agreed with the suggestion of Pérez *et al.* (2001) that DMC in cassava roots is likely to be controlled by one or a few major genes.

Table 4.6 Analysis of variance (ANOVA) table of yield parameters evaluated in three environments in Colombia, between 2002 and 2004

Source of		MEAN SQUARES					
variation	Df[a]	RtPlt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
Environment	2	72.9***[h]	16.5***	2.13***	4045.3***	5.37***	0.58**
Rep(E)	6	2.55***	0.18***	0.23***	44.0***	6.53***	1.75***
Genotype	175	0.99***	0.02***	0.04***	42.8***	1.66***	0.47***
G x E	350	0.50***	0.02 ns	0.02***	13.7***	0.76***	0.23***
Error	1050	0.30	0.02	0.01	6.97	0.33	0.09
CV[i]		23.3	11.4	21.2	8.11	24.1	20.6
Heritability[j]		0.49	0.22	0.53	0.68	0.54	0.52

[a]Degrees of freedom; [b]Roots per plant; [c]Root weight (kg); [d]Harvest index; [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]Dry root yield (t/ha). [h]ns=not significant; *=significant at P≤0.05, **=significant at P≤0.01, ***=significant at p≤0.001; [i]Coefficient of variation; [J]Broad-sense heritability.

Ntawuruhunga (2000), evaluating cassava genotypes for adaptation to different altitudes in Uganda, estimated high heritability for DMC (0.54), moderate for RtPlt (0.40) and FRY (0.35) and low for RtWt (0.14) and DRY (0.17). Estimates for DMC, RtPlt and RtWt were in the range of those obtained in this study, though lower for FRY and DRY.

Iglesias and Hershey (1994) on the other hand reported a DMC range of 0.4 to 0.6 and 0.2 to 0.4 for FRY. They noted that heritability estimates varied with the trait, the genetic background of genotypes being evaluated, environmental conditions and experimental design.

Principle component analysis was used to explain the relative contribution of the various traits to the genotypes' performance. To reduce redundancy, DRY was left out of the analysis due to its high correlation with FRY (Iezzoni and Pritts, 1991). The first four principal components (PCs) explained most of the variation and accounted for 97.5% of the total variation (Table 4.7). The first PC had an eigenvalue of 2.24 and accounted for 44.9% of the total variation. All variables were positively correlated indicating that they

Table 4.7 Principle component coefficient of the various traits with principles of the various yield related traits evaluated on 21 families in three environments in Colombia

Trait	PC1[a]	PC2	PC3	PC4	PC5
RtPlt[b]	0.58[g]	0.03	0.38	0.32	-0.64
RtWt[c]	0.20	-0.67	-0.66	-0.08	-0.28
HI[d]	0.47	0.26	-0.01	-0.84	0.01
DMC[e]	0.13	0.68	-0.65	0.29	-0.09
FRY[f]	0.62	-0.14	0.00	0.30	0.71
Eigenvalue	2.24	1.21	0.82	0.69	0.13
Percent total Variance	44.9	24.1	16.6	11.9	2.5
Cumulative	44.9	69.0	85.6	97.5	100.0

[a]PC=Principal component; [b]Roots per plant; [c]Root weight (kg); [d]Harvest index; [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]underlined are loadings of contributing traits

all contributed. Based on the PC coefficients, at least three variables had major contributions to PC1, namely FRY, RtPlt and HI. Principal component two, explaining

24.1% of the total variation had major contributions from DMC and RtWt. Principal component three (16.6%) had major contributions from RtWt and DMC and PC4 (11.9%) from HI. The contributing variables in the four PCs should have large eigenvalues to have a biological meaning. The major contributors to PC1, FRY, RtPlt, and HI, are used as indices by cassava breeders during the initial stages of selection. Major contributors to PC2, PC3 and PC4, DMC, RtWt (mainly assessed as root size or commercial roots) and HI are used during advanced stages. It seems therefore that PC1 is useful for seedling selection, while PC2 to PC4 are useful for selection in advanced stages. Three of the traits, DMC, RtWt and HI contributed significantly to two of the four significant PCs, making them relatively more important. Of these, only RtWt is not used directly in the selection index. Results indicated that it is important that RtWt should be included in the selection index. Ntawuruhunga (2000) also noted that root weight was important.

Insight can often be obtained by plotting the PC scores for individual observations in relation to the important PC axes (Iezzoni and Pritts, 1991). Clustering along PC axes suggests that some relationship exists among individuals within a cluster. Principal component scores were plotted with respect to PC1 and PC2 (Figure 4.1) to determine if the resulting pattern revealed genetic relationships among the 21 families. Families with high positive values along the PC axes will provide more desirable progeny. Quadrants with PCs greater than zero (2 and 3) are considered good. Figure 4.1 represents plotting of FRY, RtPlt and HI against RtWt and DMC. At seedling level, selections will be made mainly from families in quadrants 2 and 3. In the advanced stages, most of the selections made from families in quadrant 3 will be discarded. Assuming PCA was employed and selection was done simultaneously, selection would mainly be done only in quadrant 2, thus saving time and resources. Overall, the best performing families were CM 9733, GM 257, GM 260 and GM 265, while the worst were CM 9901 and GM 312.

The AMMI analysis of DRY showed that 0.7% of the total sum of squares was attributed to environmental effects, 50.6% to genotype and 48.7% to G x E effects (Table 4.8). The low sum of squares for environments indicated that environments were uniform for this trait, with small differences among environmental means causing little variation in DRY.

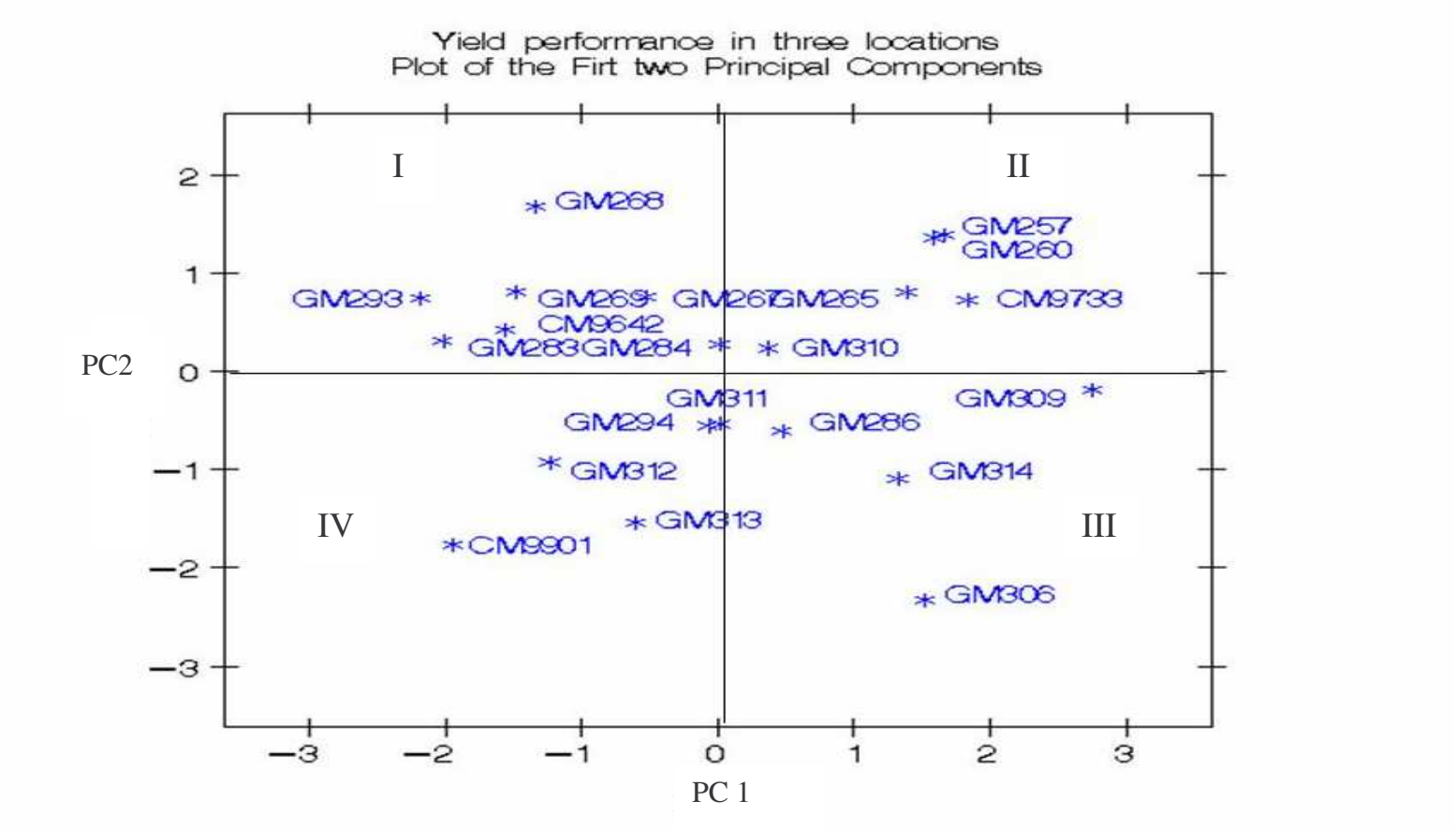


Figure 4.1 **Plot of first and second principal components of yield performance evaluated on 21 families in three environments in Colombia**

Table 4.8 AMMI analysis of variance for the various yield related traits evaluated on 21 families in three environments in Colombia

Source of variation	Df[a]	SUM OF SQUARES					
		RtPlt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
Traits	527	493.9	42.6	19.1	20308.5	569.2	161.6
Genotype	175	173.1	3.7	7.69	7486.9	291.1	81.7
Environment	2	145.9	33.1	4.26	8090.5	10.7	1.17
GxE Interaction	350	175.0	5.8	7.17	4811.1	267.3	78.7
IPCA1	176	92.7	3.8	4.94	3206.5	204.7	60.6
IPCA2	174	82.3	1.9	2.34	1604.5	62.6	18.0
Error	1050	312.9	15.1	9.74	7231.7	342.1	98.5
Total	1583	822.1	58.7	30.2	27973.9	950.4	270.5
% SS[h] due to G		35.1	8.7	40.3	36.9	51.1	50.6
% SS due to G x E		35.4	13.6	37.5	36.7	47.0	48.7
% SS due to PCA1		18.8	9.0	25.9	15.8	36.0	37.5

[a]Degrees of freedom; [b]Roots per plant; [c]Root weight (kg); [d]Harvest index; [e]Fresh root yield (t/ha); [f]Dry matter content (%); [g]Dry root yield (t/ha); [h]Traits sum of squares

G x E sum of squares was almost equal to the genotypes' sum of squares, indicating that there were no differences in genotypic response across environments. Results indicated that the first principal component axis (PCA1) of the interaction captured 77.1% of the interaction sum of squares in 50.3% of the interaction degrees of freedom. Results indicated that genotypes were stable for DRY across environments. AMMI components followed the same pattern for FRY (Table 4.8).

For HI analysis, environment attributed 22.2% to the variation, genotype 40.3% and G x E 37.5% (Table 4.8). Both PCA1 and PCA2 had sum of squares lower than genotypes sum of squares suggesting that the genotypes were stable across environments for the trait. Environment, genotype and G x E effects contributed 26.6%, 36.9% and 36.7% to variability in this characteristic. Genotype sum of squares were higher than both PCA1 and PCA2, suggesting that DMC was relatively stable.

AMMI analysis for RtPlt partitioned sum of squares into 29.54% for environment, 35.1% for genotype and 35.4% for G x E (Table 4.8). This in agreement with Ntawuruhunga (2000) who showed that environment, genotype and G x E accounted for 21.6%, 39.6% and 38.8% of the treatments sum of squares for cassava storage root number. He concluded that root numbers were less affected by the environment. He also quoted IITA (1993), Gauch and Zobel (1996) and Dixon and Nukenine (1997), as having shown in many G x E interactions, that the proportion of sum of squares due to differences among sites for RtPlt were in the range of 40% and that variation due to G x E is usually larger than G main effects. Results of the current study indicated that RtWt had 77.6% of the sum of squares accounted for by environment, 8.7% by genotype and 13.6% by G x E (Table 4.8). The environment mean of squares were highly significant ($P \leq 0.001$), while genotype and G x E were significant ($P \leq 0.05$), indicating that RtWt was not stable across these environments.

AMMI statistics presented in biplots can be used to provide insightful interpretation of data (Gauch, 1992; Ebdon *et al*., 1998). Biplots for the different traits are presented in Figures 4.2 to 4.6. Due to the large number of entries (175) the programme could not

place all of them in the graph, and most are represented as stars or signs. These have been left in the table to give an indication of the distribution. A number of individuals which could be identified have been placed to give the nature of distribution. Displacement along the vertical axis indicates interaction differences between genotypes or environments. Interaction scores for vertical axis, close to zero indicate little or no interaction (Ebdon and Gauch, 2002). Displacement along the horizontal axis indicates differences in genotype or environment main effects. Individuals from families CM 9642 and CM 9733, the first in chronological order, could be identified and a few were fixed to show the trends.

For all traits (Figure 4.2 to 4.6) genotypes were spread along the vertical axis around zero, indicating stability along the three environments for all traits. There is usually a biological explanation for such a reaction. Crosses were all adapted to the three environments, because they are progeny from parents adapted to mid-altitude agro-ecologies, where these environments are located. Environments interacted with genotypes for most of the traits. Genotype interaction to the environment was similar in Jamundi2002 and Palmira2002, but different from Quilichao2004 for DRY, FRY and DMC. For RtPlt, Jamundi2002 and Quilichao2004 had contrasting interactions, while Palmira2002 was more stable. Genotypes interacted differently to Jamundi2002 and Palmira2002 for RtWt, while no interaction was observed for Quilichao2004. Since all environments had contrasting soil types the only explanation as to why genotypes had similar interactions for Palmira2002 and Jamundi2002 but different for Quilichao2004 was year effects which were as a result of differences in weather conditions.

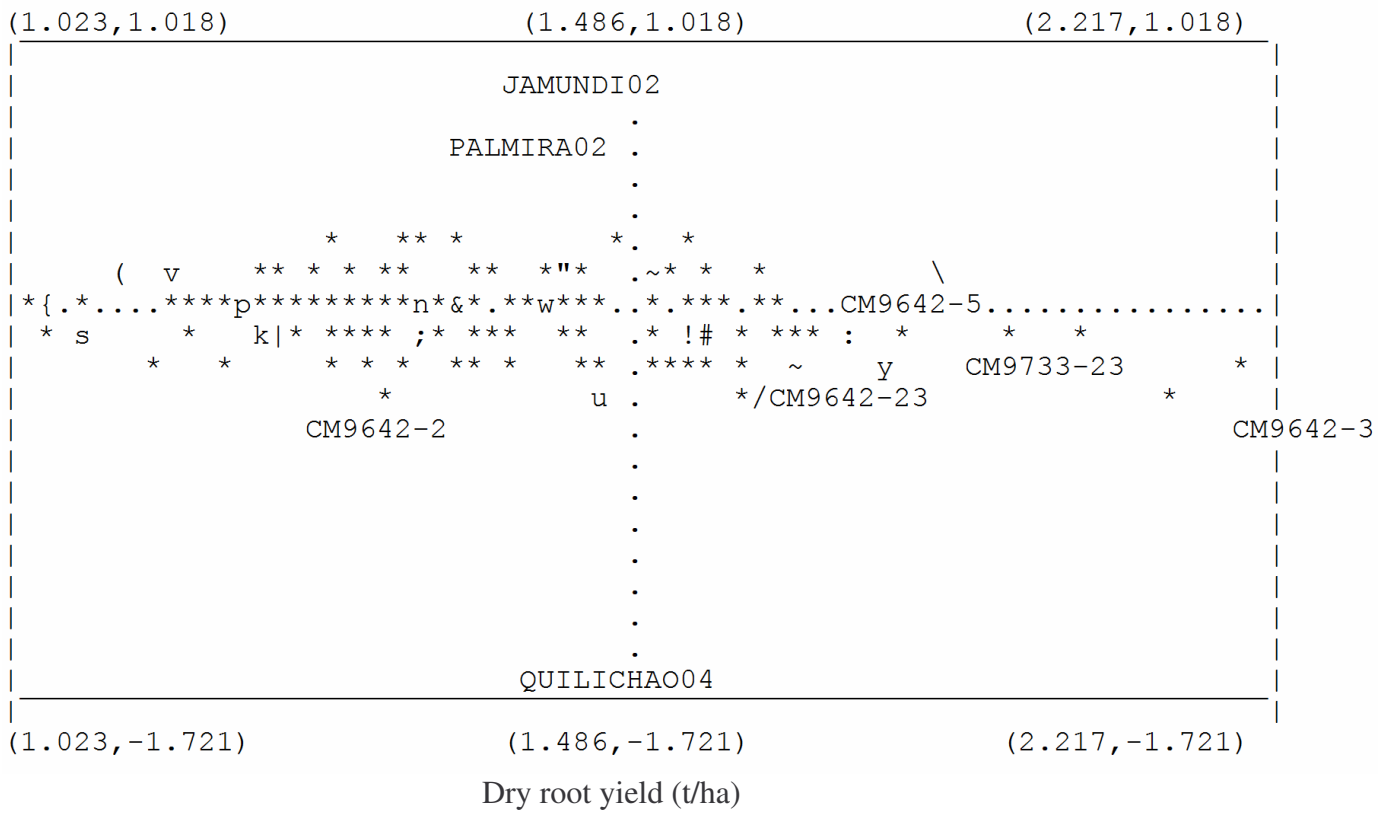


Figure 4.2 **Biplot for AMMI IPCA axis 1 scores against means of dry root yield (DRY) for genotype by environment for genotypes evaluated in three environments in Colombia between 2002 and 2004**

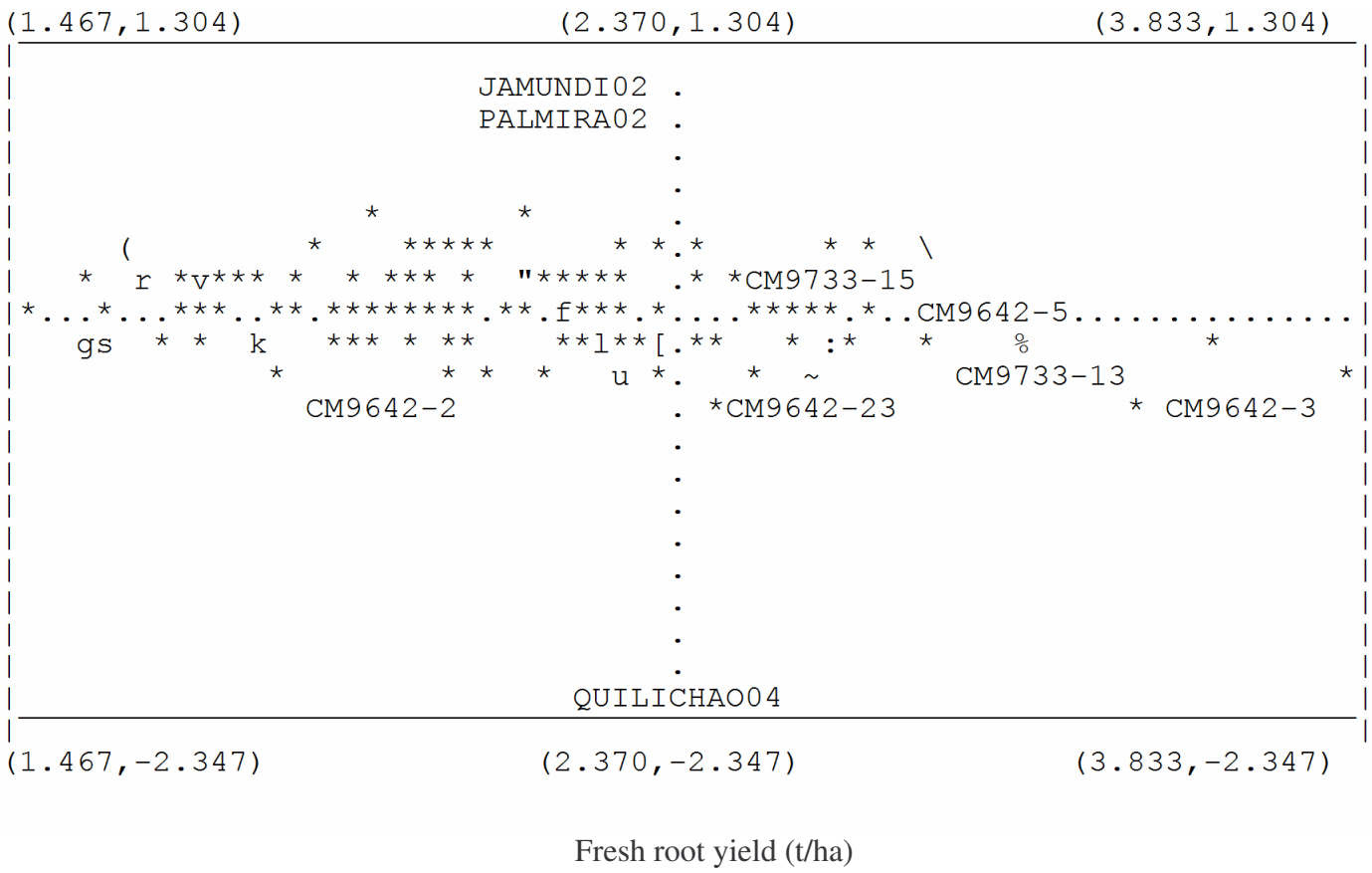


Figure 4.3 **Biplot for AMMI IPCA axis 1 scores against means of fresh root yield (FRY) for genotype by environment for genotypes evaluated in three environments in Colombia between 2002 and 2004**

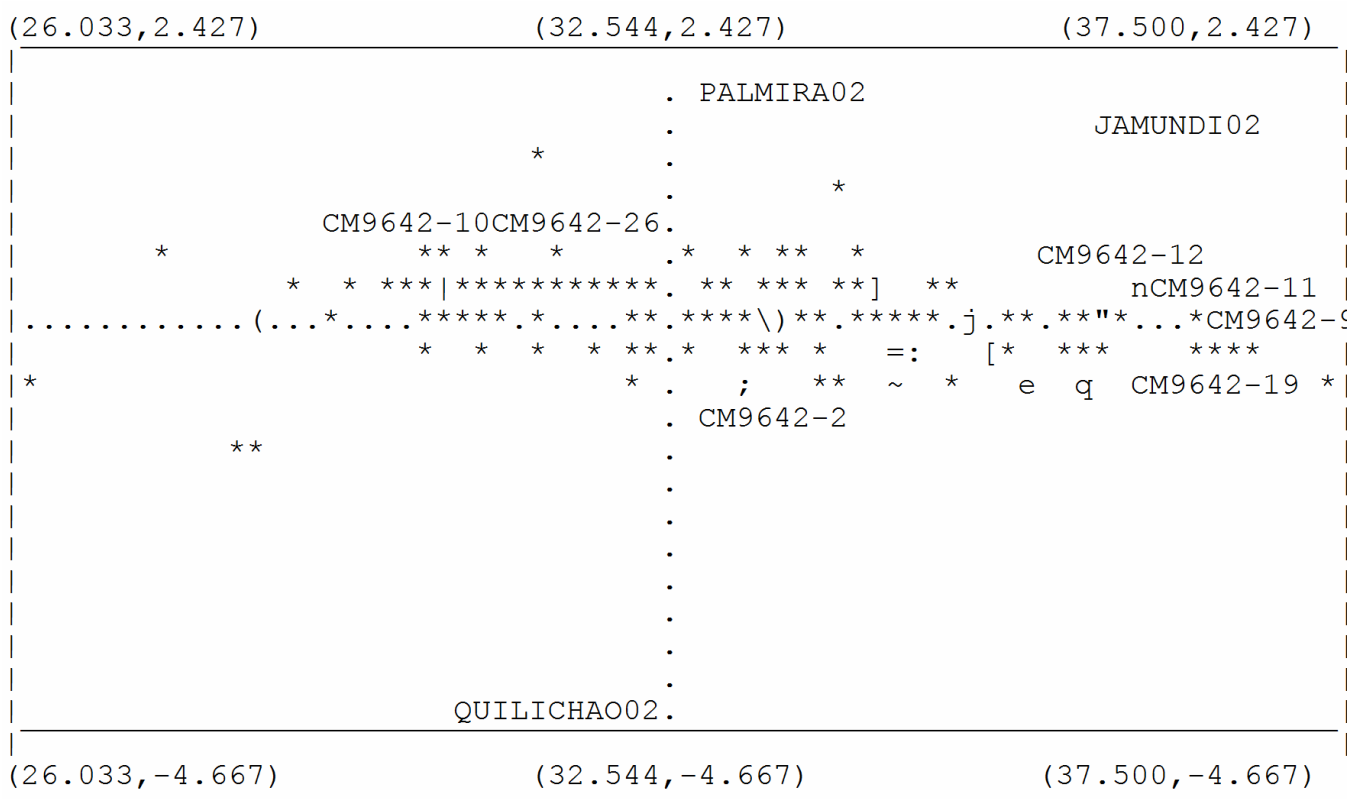


Figure 4.4 **Biplot for AMMI IPCA axis 1 scores against means of percentage dry matter content (DMC) for genotype by environment for genotypes evaluated in three environments in Colombia between 2002 and 2004**

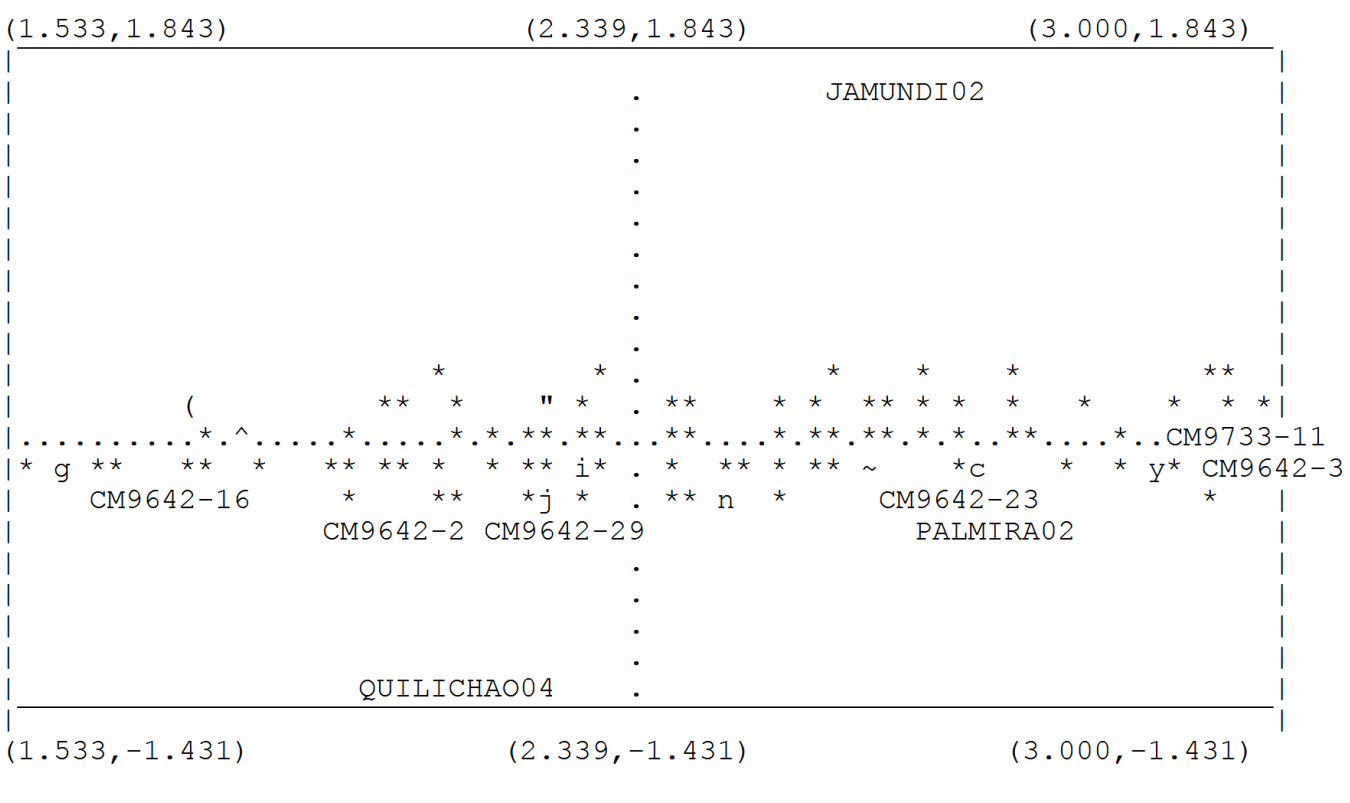


Figure 4.5 **Biplot for AMMI IPCA axis 1 scores against means of root per plant (RtPlt) for genotype by environment for genotypes evaluated in three environments in Colombia in 2002 and 2004**

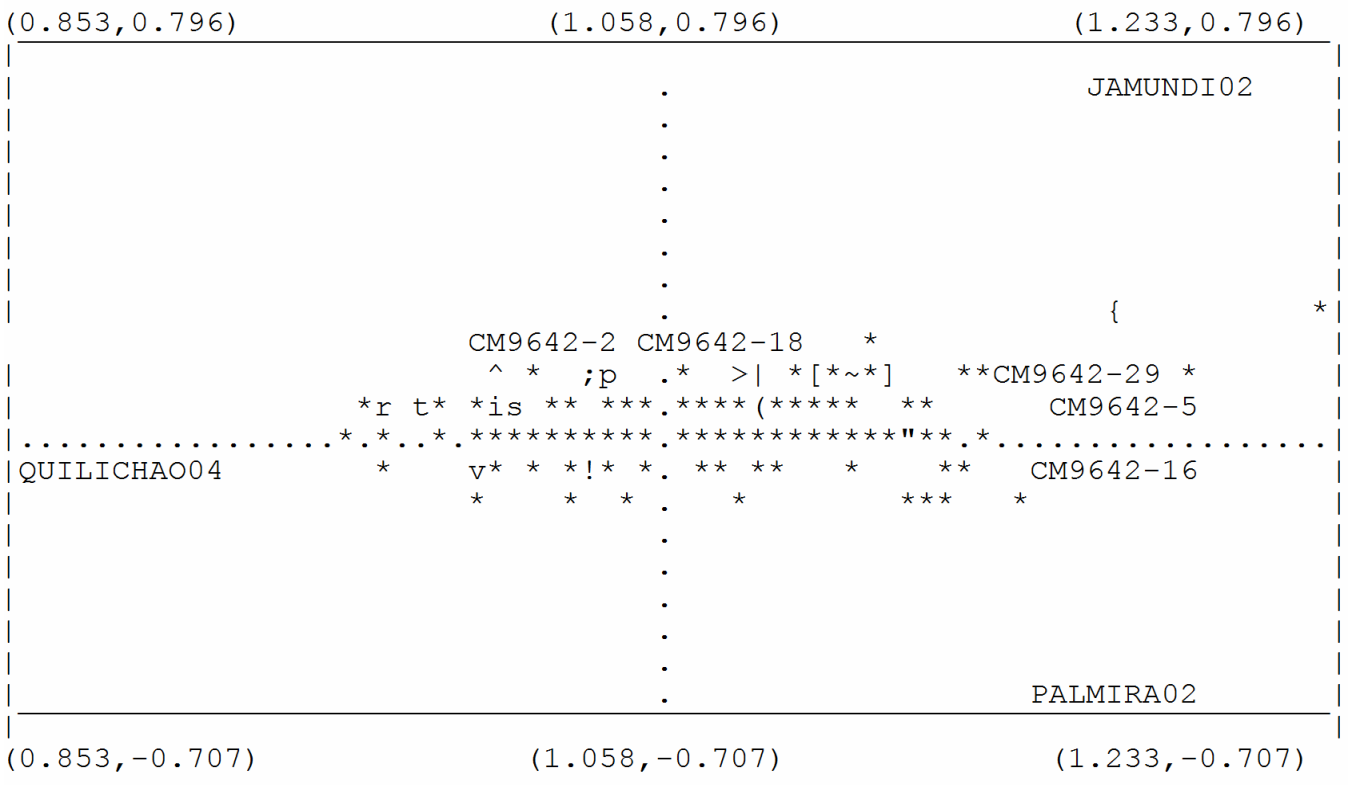


Figure 4.6 **Biplot for AMMI IPCA axis 1 scores against means of root weight (RtWt) for genotype by environment for genotypes evaluated in three environments in Colombia in 2002 and 2004**

The spread of genotypes across the horizontal axis indicated differences among genotypes for different traits. This is an indication that there exists a potential for improving traits by use of conventional breeding. The identifiable best performers in the three environments for DRY were CM 9642-3, CM 9733-23 and CM 9642-5 (Figure 4.2), CM 9642-3, CM 9733-13 and CM 9642-5 (Figure 4.3) for FRY, and CM 9642-9, CM9642-11, CM 9642-19 and CM9642-12 for DMC (Figure 4.4). Genotypes CM 9642-3 and CM 9642-23 generally performed well for a number of traits (Figures 4.2 to 4.6). Genotypes CM 9642-23, CM 9733-23 and CM 9642-3 performed better at Quilichao2004 for DRY, FRY and DMC as shown by their association with this particular environment (Figures 4.2 to 4.4). The plot of root number indicated that CM 9642-16, CM9642-2 and CM 9642-29 performed better in Quilichao2004, while CM9642-23, CM9733-11 and CM 9642-3 performed better in Palmira2002 for this trait. From the location of identifiable clones, families CM 9642 and CM 9733 performed better in Quilichao2004.

4.4 Conclusions

Highly significant genotype effects indicated that the traits were relatively stable within environments, which reflected the fact that the population was developed from parents adapted to the specific agro-ecology. Selection at one environment would be largely effective for other environments within the agro-ecology. However, the existence of genotype x location interaction, though small in magnitude, suggested that final selection has to be done at each location. Kawano *et al.* (1998), while reviewing 30 years data of the CIAT breeding programme found that cultivar interactions within locations and years were significant, but that their actual influence on the genotype mean was proportionally small for all traits. They noted that given the comparatively large variations in genotypes and locations, the results justified doing early selections in one location and advancing selections on target environments. Cassava breeders usually find themselves faced with the task of developing cultivars for different ago-ecologies, yet cultivars are sensitive to G x E interaction. The finding that evaluation of material can start in one location and advance at later stages, when genotype numbers are reduced, is of significance for breeders.

High heritability estimates were obtained, indicating that simple phenotypic selection would be effective for all traits evaluated, with exception of RtWt. Dry matter content, RtWt and HI were singled out by PCA as the main contributors to yield. This confirmed the fact that the CIAT breeding programme focused on HI when improving its germplasm (Kawano, 2003; Ceballos *et al*., 2004), and both HI and DMC are included in the selection index of the programmes' advanced selection stages (CIAT, 2004). Root weight, singled out by PCA, is currently not included in the selection index (CIAT, 2004). It is important that cassava breeders should include RtWt into their selection indices.

AMMI analysis ranked FRY, DRY, HI, and RtPlt as stable, DMC as relatively stable and RtWt as unstable across the three environments. The stability of FRY and DRY which usually are the most affected by the environment (Ntawuruhunga, 2000) confirmed that these genotypes are well adapted to the specific agro-ecology. Interaction patterns revealed by AMMI plots indicated that genotypes were well adapted to the environments. Environment interaction was more influenced by year rather than location effects. This again would be because locations were all in the same agro-ecology. It should be noted that the three locations where the trials took place have contrasting soil types (Leihner *et al.,* 1999; Jaramillo *et al*., 2005). This would suggest that soil type is not very important in determining yield in cassava.

Chapter 5

Evaluation of yield traits in seedling populations of cassava

5.1 Introduction

In cassava improvement, the breeder's primary concern is to increase the root yield of varieties that are resistant to major diseases and pests (Mahungu, 1987; Ceballos *et al.,* 2004). Root yield is usually assessed visually as the number, size and shape of roots. Selection for root quality is normally carried out at advanced stages of yield trials when clones being evaluated are reduced in number (Mahungu, 1987). Root quality and yield related characteristics often considered are cyanogenic glycosides content, percentage DMC and HI. Percentage DMC determination is very useful because of its association with other root quality characteristics (Mahungu, 1987; Lenis *et al.,* 2006) and its economic relevance. Harvest index is used because it is highly correlated with root yield (r=0.76) and has a high heritability (Kawano and Thung, 1982; Kawano *et al.*, 1998).

The breeding scheme described above, extends the breeding cycle by many years. To reduce the cycle, it might be necessary to simultaneously select for yield and root quality during the earliest stages of selection. Moderate to high heritability values have been reported for DMC in roots (52%), while a high broad-sense heritability of 80% was reported by IITA (1980), indicating that selection of clones can be effectively carried out at an early stage (Mahungu, 1987). Kawano (1978) has shown that selecting for high HI in seedling plants as well as clones in single-row evaluation (clonal evaluation) is more effective in identifying high-yielding genotypes than using root yield itself as selection criterion. Furthermore, root number, a trait affecting yield, is known to be fixed early on during the plant's breeding cycle (Wholey and Cock, 1974), and can be used as an early selection criterion. Quality is, however, difficult to evaluate as such, since it is a composite of many root characters (Wheatley *et al*., 1985).

Principal component analysis is one of the most frequently used multivariate techniques (Crossa, 1990; Purchase, 1997), and has been used to examine association among sets of traits (Ntawuruhunga, 2000). In this study, PCA and stepwise regression, among others, were used to determine yield related traits that can be used in selecting genotypes at seedling stage. The specific aims of the study were to evaluate a seedling nursery specifically developed for yield characteristics, and assess the different yield components for use as selection indices at seedling stage.

5.2 Materials and methods

Clones adapted to the three main cassava growing agro-ecologies (mid-altitude, lowland semi-arid and acid savannah soils) were crossed in a diallel fashion (Jaramillo *et al.,* 2005). Progeny were evaluated, data analysed, and used to select parents for this study. Genotypes with high GCA for DMC or yield were selected as parents and controlled pollinations performed following the standard procedures described by Kawano (1980).

Parents for crossing were planted in a crossing block in CIAT, Palmira. Entries were planted in single rows of 1 m between plants and 2 m between rows, to facilitate movement. Genotypes were monitored for start of flowering. At the onset of flowering, daily visits to the plots were made. Each morning, plants were inspected for flowers about to open, and promising flowers enclosed with muslin bags, to prevent contact with stray pollen on opening. Pollen was collected in plastic bottles, from matching male parents. At around 11.00 am when flowers open, the muslin bags were removed, and pollen from the matching parent dusted on the stigma. All non-mature flowers were removed from the flower batch. The flower batch was tagged with a label containing the pedigree, mother first, number of flowers in that batch pollinated, and date of pollination. Flowers were again enclosed. Two days later the muslin bag was removed to allow the fruit to develop freely. Three weeks after pollination fruits were covered with netting bags to catch the seed when the ripe fruits dehisce explosively (Alves, 2002). Seed was collected from the field after 60 days, cleaned and labelled, ready for planting.

Seed was planted in a screen house in pots filled with sterilised soil, mixed with sand in a ratio of 1:1. Plantlets were watered regularly to required soil moisture. Fungicide was applied occasionally to prevent fungal infection arising from the humid conditions. When seedlings were 20 to 25 cm tall, they were transplanted to a well-prepared seedbed in the field, in CORPOCA-Palmira, Colombia. Seedlings were planted on ridges 1.5 m apart, with spacing of 0.6 m between plants. Daily irrigation was done during the first week, after which rainfall served as water supply. Hand weeding was done three times, and thereafter weeds were controlled using herbicide.

Immediately after planting, nitrogen fertiliser was applied around plants to boost them after the long period in the screen house. Thereafter, fertiliser was selectively applied to weak plantlets. Pesticide was periodically applied against the prevalent pest, CGM depending on the incidence.

Six MAP seedlings were genotyped and families assigned following the CIAT naming system. Seedlings within a family were assigned numbers, with the first seedling assuming number one and the rest subsequent numbers. Parents were not included as they were from stem cuttings and could not be used for comparison purposes. At harvest, individual plants were harvested, the harvestable biomass divided into storage roots, and vegetative biomass, comprising leaves and stems. Roots were weighed to obtain fresh root yield. Roots which pass for sale in the local supermarkets were selected and counted to give number of commercial roots. Harvest index was calculated by dividing fresh root yield by total biomass. Percentage DMC of the roots was estimated using the standard CIAT procedure (Kawano *et al*., 1987; Jaramillo *et al*., 2005). Dry yield was derived as a product of fresh root yield and DMC.

Simple statistics were performed using the Microsoft Excel programme (Microsoft, 2004). Because of the large number of progeny from each parent, progeny from a given parent was taken to represent a random population and its mean performance was used to estimate the parents' breeding value. Chi-square was used to test the reciprocal crosses for presence of maternal effects. The relative contribution of the different traits to the

genotype performance was estimated by PCA and stepwise regression performed using Agrobase programme (Agrobase, 2000).

5.3 Results and discussion

A total of 2935 seeds from eight crosses were produced, of which 1885 plantlets representing 64.2% germination were established in the field (Table 5.1). Of the transplanted plants, 1453 (77.1%) resulted in plants vigorous enough to produce sufficient biomass for evaluation.

Table 5.1 Seed generated and resulting plantlets from eight crosses and their respective reciprocals

Cross	Pedigree	Seeds generated		Plants in the field		Target area
		Direct	Reciprocal	Direct	Reciprocal	
GM 901	SM 1741-1 x MPER 183	605	3	355	2	Mid-altitude
GM 9953	SM 1741-1 x SM 1219-9	150	198	121	152	Mid-altitude
GM 9958	SM 1411-5 x MTAI 8	19	151	19	90	Low-land semi-arid
GM 853	CM 8027-3 x CM 6757-8	41	2	29	2	Low-land semi-arid
GM 252	SM 1665-2 x SM 805-15	831	364	520	310	Low-land semi-arid
GM 847	SM 1411-5 x CM 4574-7	0	10	0	7	"out cross"
GM 256	SM 1219-9 x SM 1565-15	116	0	95	0	Acid-savannah soils
GM 536	CM 4574-7 x SM 1565-15	101	344	63	120	Acid-savannah soils

At the seedling stage a relatively high number of RtPlt was obtained (average 6.67), with clone GM 901-192 having the highest number of 19 (Table 5.2). The average commercial sized storage roots (ComRt) was 2.17, with clone GM 901-263 having the highest number of 15 roots. Clones with the highest number of roots, both total and commercial, were from families GM 901 and CM 9953, both of which had SM 1741-1 as a common parent. Highest RtWt was recorded for GM 252B-159, with the top five clones all coming from the same family. Harvest index estimates ranged from 0.05 (GM 252B-215) to 0.90 (GM 853-13). Recorded DMC ranged from 16.3% in GM 252-307 and CM 9953B-030 to 69.1% in GM 901-270 (Table 5.2). This range was higher than what was reported by Magoon *et al.* (1973) of 20.0% to 47.2% in crosses between one female parent and three different high yielding male parents, but fell within the range later reported by Rajendran and Hrishi (1982) of 19.2% to 66.4% among four high, medium and low DMC parents. Highest yield was recorded in GM 536-146 (14.67 t/ha) followed by GM 536-79 (8.6 t/ha) and GM 901-304 (13.33 t/ha), while highest DRY was recorded in GM 536-79 (4.33 t/ha) and GM 901-329 (4.27 t/ha). Only genotypes with measurable biomass were evaluated, others were cloned to provide planting material for further studies.

Chi-square tests of reciprocal cross means were not significant for any of the traits, indicating a lack of maternal effects. Data was averaged per family to give an indication of a family's performance (Table 5.3). Family GM 256 recorded the highest average number of roots (7.6) and GM 853 the lowest (5.9). Families GM 901 and GM 847 had the highest average HI estimates, 0.53 and 0.50 respectively, while GM 256 had the lowest (0.37). Highest DMC was estimated in GM 901 (33.5%), GM 256 (33.3%) and GM 847 (33.2%) and the lowest in GM 9958 (28.6%). Average FRY ranged from 1.31 to 2.01 t/ha in families GM 9958 and GM 536 respectively. Families GM 536 and GM 853 out yielded others, with DRY of 0.61 and 0.56 t/ha respectively, while the lowest, GM 9958, yielded 0.39 t/ha.

Average performance of progeny from a parent was used to estimate its breeding value (Table 5.4). Dry and fresh root yield, RtWt, and ComRt ranked the parents in the same

direction. Dry matter content and HI ranked parents the same but in the opposite direction for other yield characteristics. Jaramillo *et al.* (2005) noted that HI had the highest correlation with DMC and that in some parents there was a negative association between DMC and yield potential. Roots per plant, on the other hand, ranked parents

Table 5.2 Simple statistics of agronomic variables evaluated on the seedling nursery (1453 genotypes) in CIAT-Palmira in April, 2005

Variable	Minimum	Maximum	Average	Standard deviation
RtPlt[a]	1.00	19.00	6.67	3.08
ComRt[b]	0.00	15.00	2.17	1.79
RtWt[c]	0.01	1.33	0.25	0.15
HI[d]	0.05	0.90	0.48	14.4
DMC[e]	16.30	69.10	32.10	4.80
FRY[f]	0.10	14.67	2.69	0.86
DRY[g]	0.03	4.33	0.86	0.28

[a]Roots per plant; [b]Commercial roots per plant; [c]Root weight (kg); [d]Harvest index (0-1); [e]Dry matter content (%); [f]Fresh root yield (t/ha) ; [g]Dry root yield (t/ha)

two groups of low and high, indicating that visual evaluation alone could be enough for selection. SM 1411-5 and MTAI 8 were ranked the poorest parents for almost all traits. Overall rating ranked MPER 183 and CM 4574-7 as best parents followed by SM 1565-15 and SM 1665-2. Jaramillo *et al.* (2005) reported that MPER 183 had the highest GCA for several traits. Most breeders, when selecting, do not take into consideration all traits but use a selection index which in most cases consist of FRY, HI and DMC.

For example the selection index used by CIAT (2004) is:

Selection index = [FRY ∗ 10] + [DMC ∗ 8] - [PT ∗ 3] + [HI ∗ 5]

where, PT = plant type using a 1(excellent) to 5 (very poor) visual scale and the rest as described earlier.

When parents were ranked based on FRY, DMC, and HI, the best parent was MPER 183 followed by SM 1741-1, SM 1665-2 and SM 805-15. Jaramillo *et al*. (2005) observed that SM 1741-1 had a good performance as a parent across the different variables evaluated. Ranking by selection index followed the same ranking as for HI and DMC, while ranking by all traits followed the ranking by yield and storage root numbers. From this, it is apparent that by using the current selection index, breeders may not be maximising the potential of the genotype.

Relative importance of the contribution of various yield-related traits to yield improvement was assessed using frequency of distribution in the progeny (Figures 5.1 to 5.6). To be able to improve a trait by conventional breeding, breeding populations should result in a number of individuals that depart from the mean, expressed as positive skewness, the skewned the greater the potential. Distribution frequencies of FRY, DRY, RtWt and RtPlt indicated that genotype improvement can be achieved by crossing and selecting superior individuals. The potential of improvement differed between crosses as shown by difference in the level of skewness among crosses, suggesting that it might be important to select particular parents for particular traits (Figures 5.1 to 5.6). Dry matter content (Figure 5.2) and HI (Figure 5.3) seem to offer limited chances for improvement in these crosses. This is in agreement with a report by Lenis *et al.* (2006) who came to the conclusion that most breeding populations now have a large number of clones with HI close to the optimum and there is little scope to further increase yield by selecting solely for HI. It is important to note that DMC and HI are yield traits which breeders have been using extensively in their selection indices, and it is therefore logical that their use has been maximised. It is therefore rational that breeders should change to other traits, or develop non-conventional methods for yield improvement in cassava.

Table 5.3 Means and standard deviations of root quality characteristics of eight families evaluated at harvest in CIAT-ICA, Palmira in April, 2005

FAMILY	PEDIGREE	VARIABLE						
		RtPlt[a]	ComRt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
GM 901	SM1741-1 x MPER183	6.7±3.0	2.2±1.7	0.25±0.13	0.53±0.13	33.5±5.0	1.61±1.2	0.54±0.4
GM 9953	SM1741-1 x SM1219-9	6.3±2.7	1.7±1.5	0.23±0.14	0.48±0.16	31.7±5.5	1.42±1.2	0.45±0.4
GM 9958	SM1411-5 x MTAI 8	6.7±2.9	1.5±1.2	0.20±0.11	0.46±0.17	28.6±6.7	1.31±0.9	0.39±0.3
GM 853	CM8027-3 x CM6754-8	5.9±2.8	2.3±1.5	0.35±0.21	0.46±0.17	29.2±3.1	1.90±1.2	0.56±0.4
GM 252	SM1665-2 x SM805-15	6.5±3.1	2.1±1.7	0.25±0.18	0.48±0.14	32.2±4.4	1.64±2.0	0.53±0.7
GM 847	SM1411-5 x CM4574-7	7.2±1.9	1.7±1.6	0.24±0.23	0.50±0.18	33.2±3.7	1.58±1.3	0.55±0.5
GM 256	SM1219-9 x SM1565-15	7.6±3.2	2.1±1.5	0.20±0.12	0.37±0.15	33.3±3.6	1.45±0.9	0.48±0.3
GM 536	CM4574-7 x SM1565-15	7.1±3.3	2.9±2.4	0.28±0.18	0.47±0.13	30.4±3.8	2.01±1.6	0.61±0.5
Total		***6.7±3.1***	***2.2±1.8***	***0.25±0.16***	***0.48±0.15***	***32.0±4.8***	***1.64±1.6***	***0.53±0.5***

[a]Roots per plant; [b]Commercial roots per plant; [c]Root weight (kg); [d]Harvest index (0-1); [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]Dry root yield (t/ha)

Table 5.4 Means and rankings of root quality characteristics of progeny from nine parents evaluated at harvest in CIAT-ICA, Palmira in April, 2005

PARENT	VARIABLES									
	Number	**RtPlt**[a]	**ComRt**[b]	**RtWt**[c]	**HI**[d]	**DMC**[e]	**FRY**[f]	**DRY**[g]	**Total Ranking**	**Ranking by index selection**
MPER 183	309	6.7 (3)[h]	2.2 (3)	0.246 (4)	0.53 (1)	33.5 (1)	1.61 (3)	0.54 (3)	***1***	**1**
SM 1741-1	524	6.5 (7)	2.0 (5)	0.237 (6)	0.51 (2)	32.8 (2)	1.53(6)	0.50 (6)	***6***	**2**
SM 1665-2	536	6.5 (7)	2.1 (4)	0.248 (3)	0.48 (4)	32.2 (3)	1.57(5)	0.51 (5)	***4***	**3**
SM 805-15	520	6.6 (7)	2.0 (5)	0.240 (5)	0.49 (3)	32.0 (5)	1.58(4)	0.53 (4)	***5***	**3**
CM 4574-7	236	7.1 (2)	2.9 (1)	0.280 (1)	0.47 (5)	30.4 (7)	2.00 (1)	0.61 (1)	***1***	**5**
SM 1565-15	315	7.2 (1)	2.7 (2)	0.258 (2)	0.44 (9)	31.2 (6)	1.86 (2)	0.58 (2)	***3***	**6**
SM 1219-9	300	6.7 (3)	1.8 (7)	0.217 (7)	0.45 (8)	32.1 (4)	1.43 (7)	0.46 (7)	***7***	**7**
SM 1411-5	55	6.7 (3)	1.5 (8)	0.205 (8)	0.46 (6)	29.1 (8)	1.34 (8)	0.40 (8)	***8***	**8**
MTAI 8	49	6.7 (3)	1.5 (8)	0.200 (9)	0.46 (6)	28.6 (9)	1.31 (9)	0.39 (9)	***9***	**9**
Total	***2844***	***6.7±3.1***	***2.2±1.8***	***0.248+0.16***	***0.48±0.15***	***32.0+4.8***	***1.6±1.6***	***0.5±0.5***		

[a]Roots per plant; [b]Commercial roots per plant; [c]Root weight (kg); [d]Harvest index (0-1); [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]Dry root yield (t/ha); [h]Numbers in brackets are the rankings

Simple correlation analysis showed DRY to be highly correlated (P≤0.001) to all traits evaluated (Table 5.5), signifying that all contribute to economic yield. Fresh root yield was correlated (P≤0.001) to all traits except DMC. No association was detected between FRY and DMC. Kawano *et al*. (1998) also observed the lack of association between FRY and DMC at earlier stages of selection and concluded that FRY and DMC can be handled largely as independent characters. This is of interest especially to national breeding programmes. In cassava breeding, large numbers of genotypes are handled at the seedling stage. Most of the programmes do not have the resources to evaluate DMC for such high numbers. Evaluation for FRY can be done at seedling stage and DMC included at a later stage, since selecting for yield alone at seedling stage would not affect DMC.

Table 5.5 Simple correlation table of yield related traits evaluated on a seedling nursery in 2005, at CIAT-Palmira, Colombia

	ComRt[a]	RtPlt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]
RtPlt	0.61***[h]					
RtWt	0.34***	-0.06*				
HI	0.18***	-0.001 ns	0.32***			
DMC	-0.001 ns	0.09***	0.002 ns	0.11***		
FRY	0.68***	0.53***	0.55***	0.24***	-0.04 ns	
DRY[g]	0.66***	0.49***	0.14***	0.25***	0.21***	0.96***

[a]Number of commercial roots; [b]Roots per plant; [c]Root weight (kg); [d]Harvest index; [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]Dry root yield (t/ha); [h]ns=not significant; *=significant at P(t/ha)≤0.05, **=significant at P≤0.01, ***=significant at p≤0.001

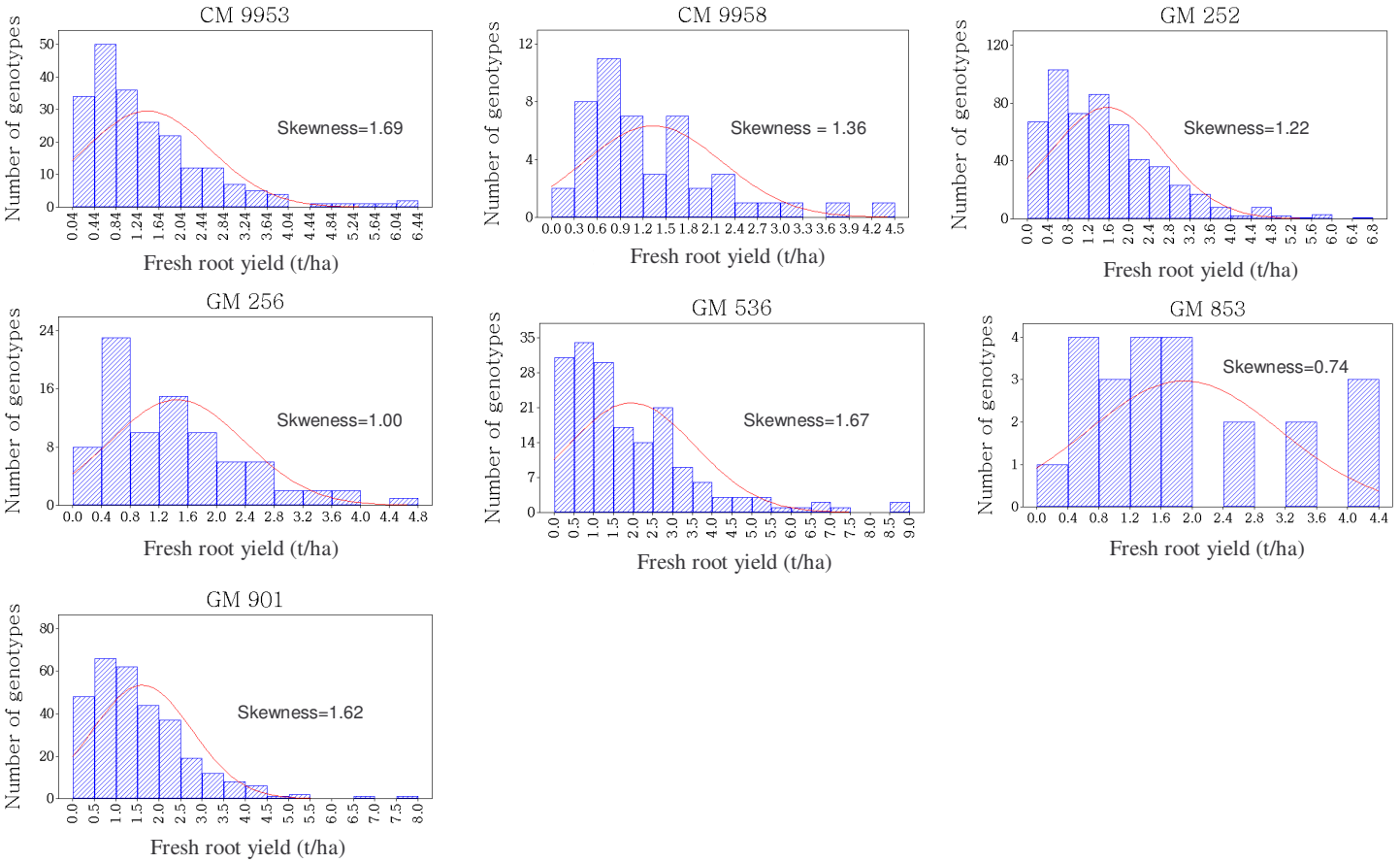


Figure 5.1 Fresh root yield distribution in seven seedling families

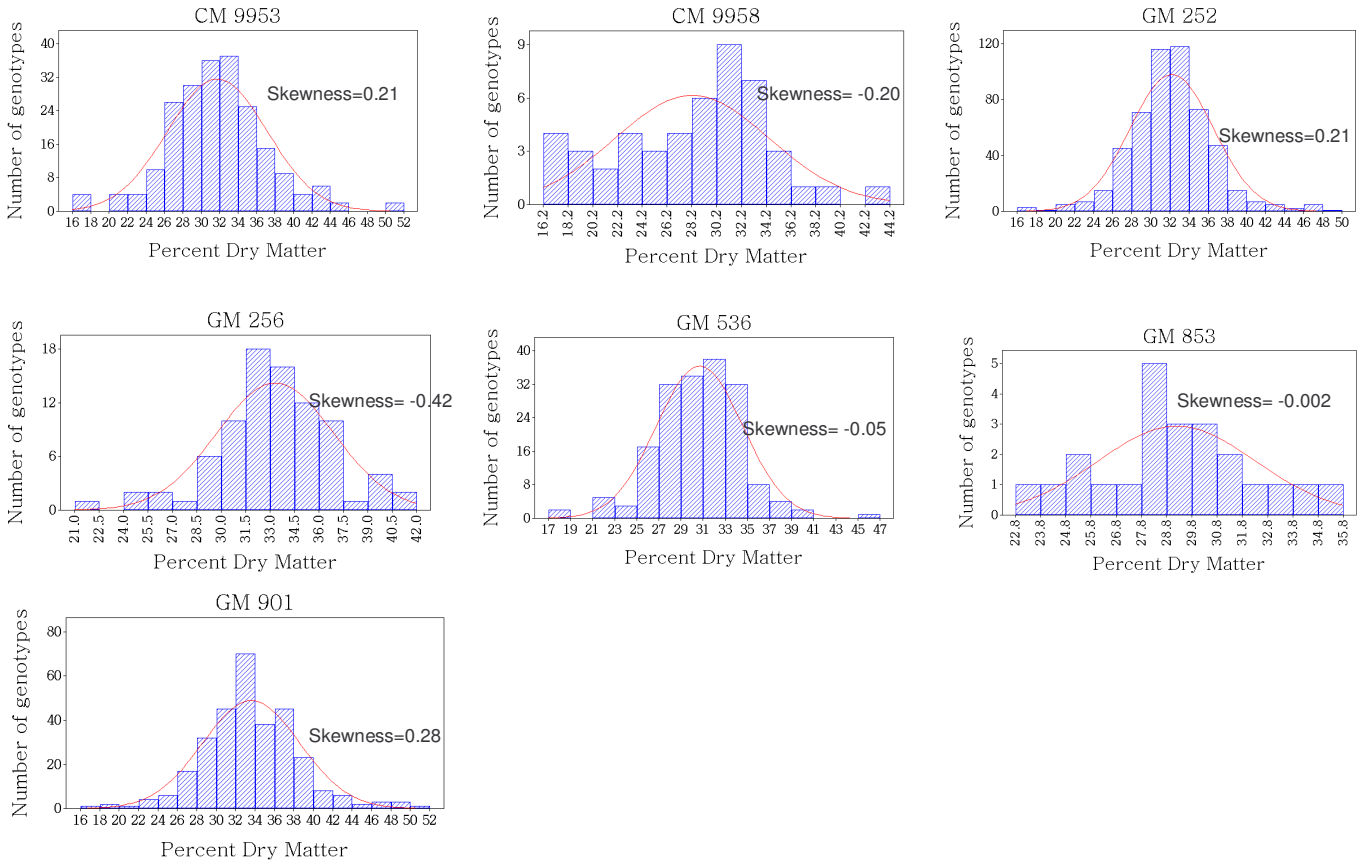


Figure 5.2 Percent dry matter content distribution in seven seedling families

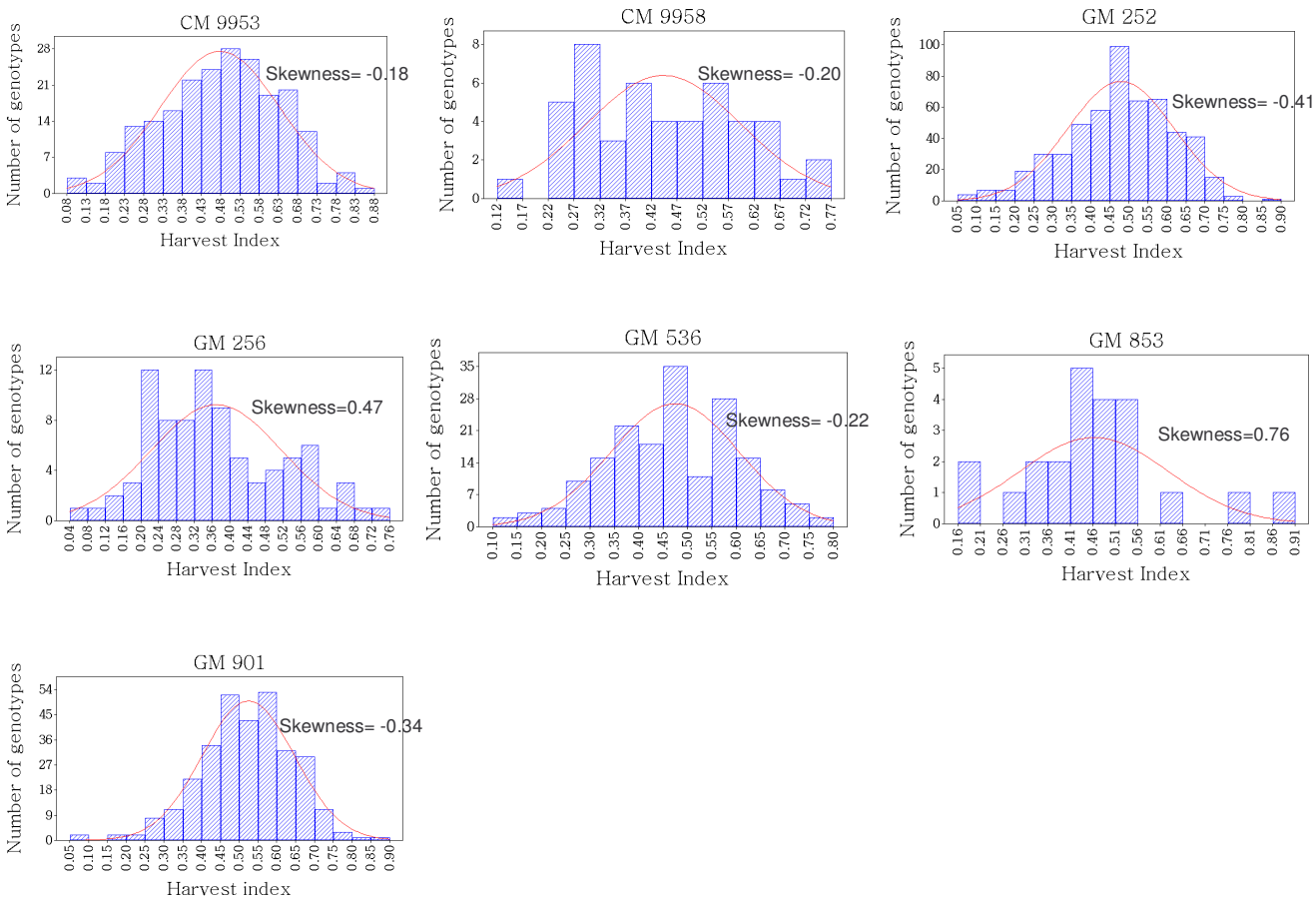


Figure 5.3 Harvest index distribution of seven seedling families

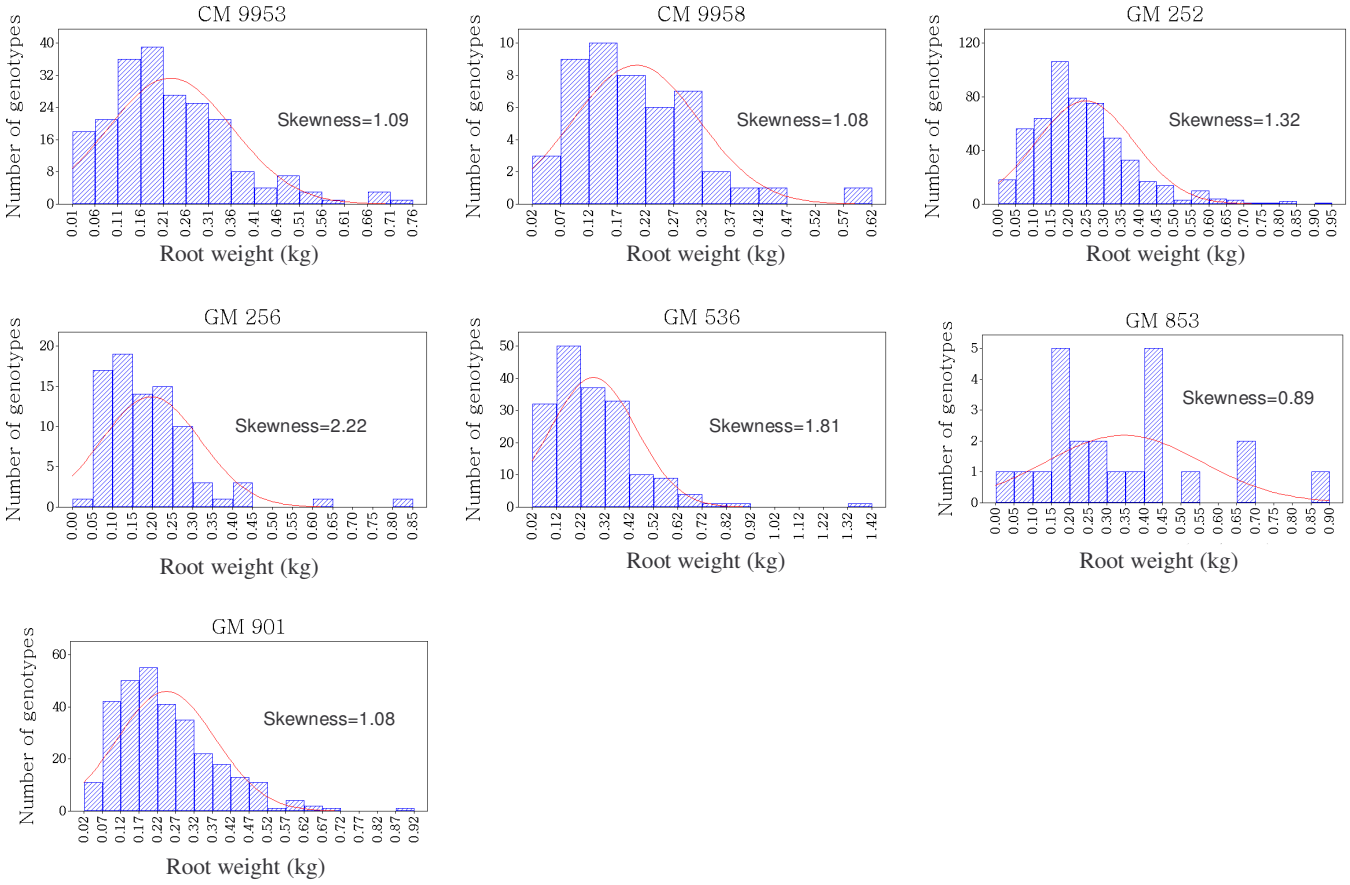


Figure 5.4 Root weight distribution of seven seedling families

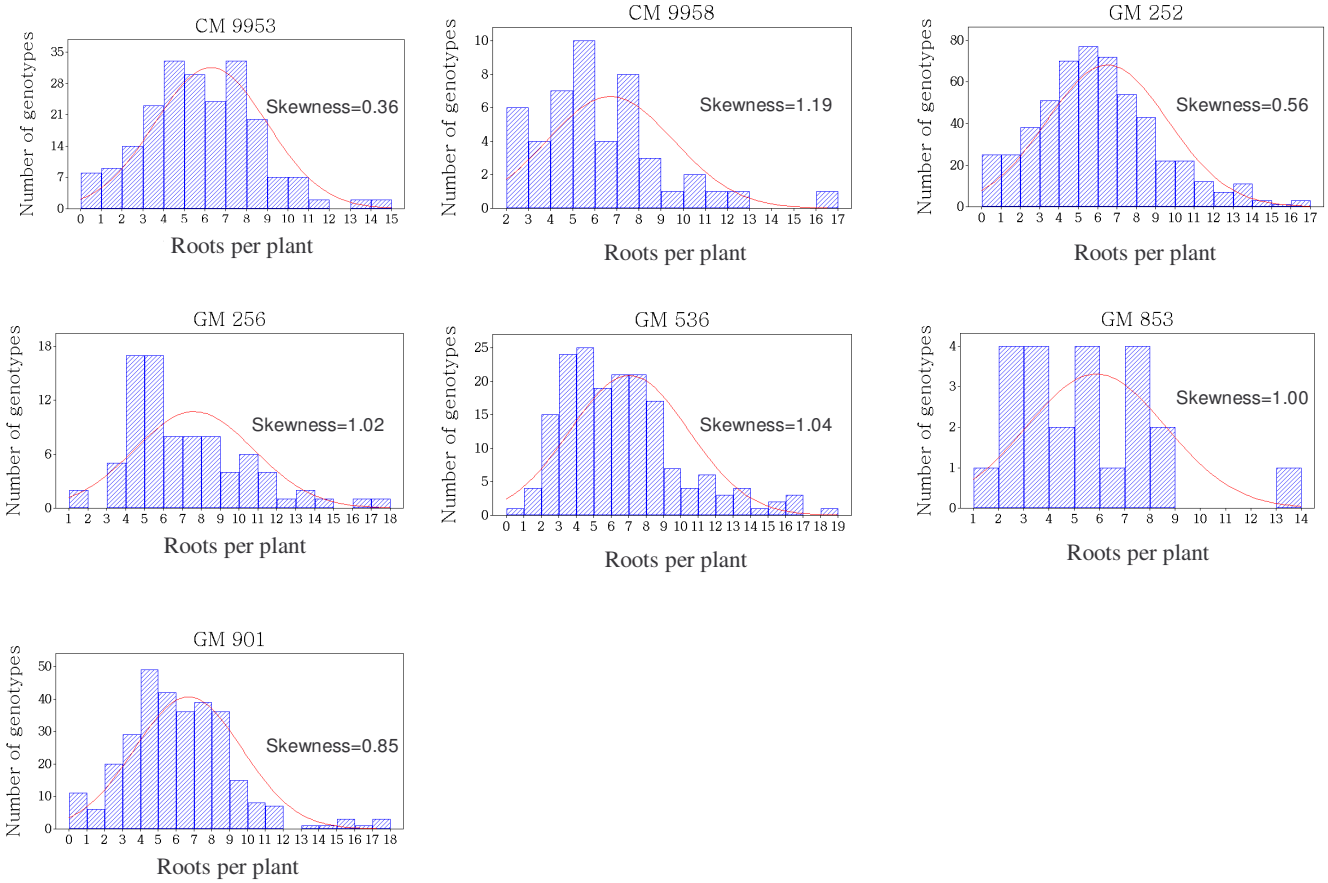


Figure 5.5 Root number distribution of seven families

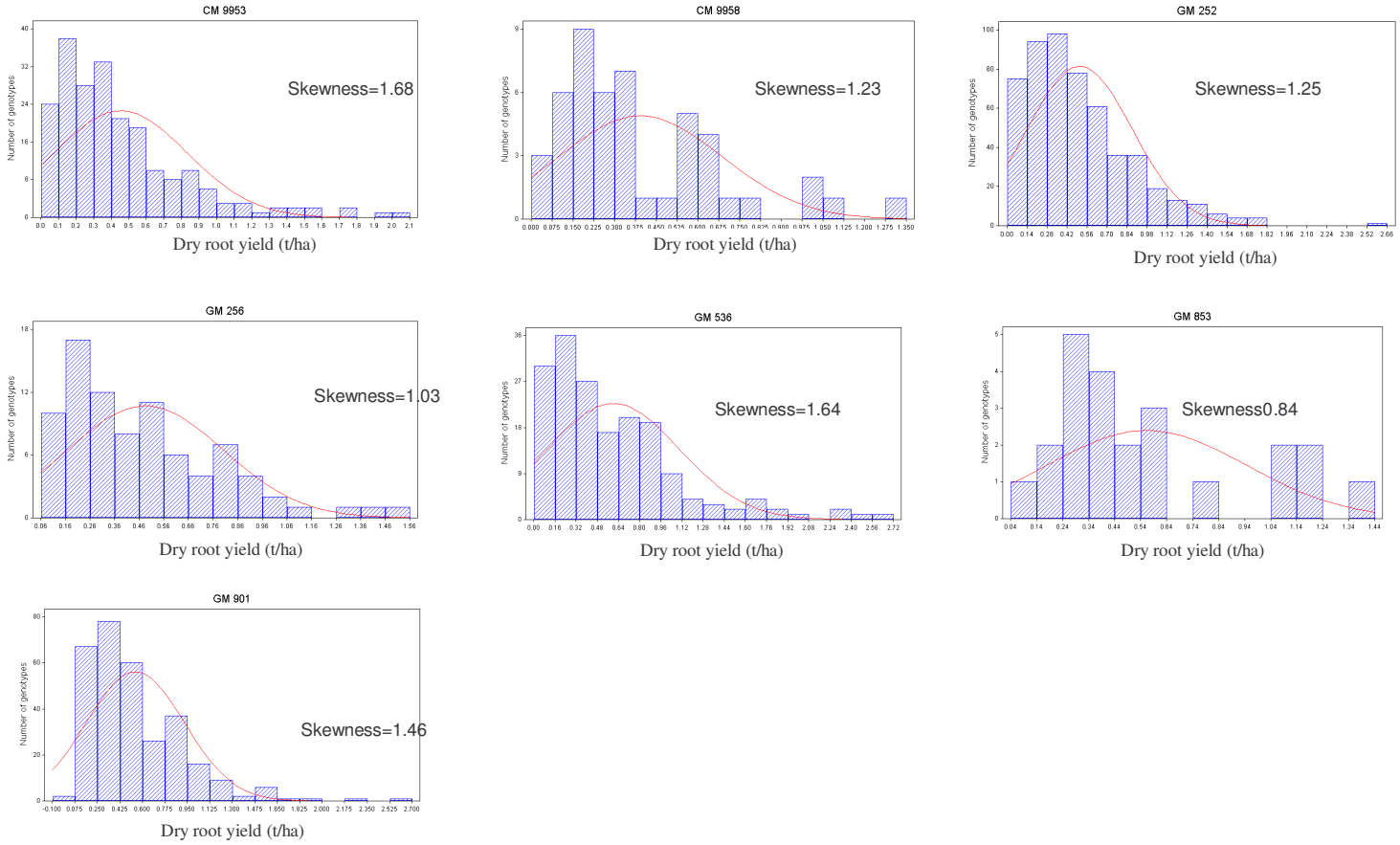


Figure 5.6 Dry root yield distribution of seven seedling families

Percentage DMC was highly correlated ($P \leq 0.001$) with RtPlt, HI and DRY and was not associated with ComRt, RtWt, and FRY. Harvest index was highly correlated with all traits evaluated except RtPlt, an indication that it is suitable for use in indirect selection for yield. Kawano *et al*. (1998) observed that indirect selection for yield through HI at earlier stages of selection was more effective than direct selection using yield itself. Roots per plant was negatively correlated ($P \leq 0.05$) with root weight. This suggested that during selection a breeder has to keep a balance between the amount he/she increases each trait with.

Principle component analysis was used to help explain the relative contribution of the various traits to the genotypes' performance. Eigenvalues of the first four PCs are presented in Table 5.6. The first four PCs accounted for 94.8% of the total variation. PC1 accounted for 51.0% of the total variance and had an eigenvalue of 3.56 indicating that it had at least four major contributing factors. Main factors for PC1 were FRY, ComRt, RtWt and RtPlt. All factors were positively correlated. PC2 accounted for 19.0% of the total variance, and indicated HI, RtPlt and RtWt as the next set of factors contributing to variation. PC3 accounted for 14.1% indicating DMC and RtWt as the next factors while PC4 accounted for 10.7% with HI and RtWt as the main factors. The main contributors to PC1, FRY, ComRt, RtWt, and RtPlt, are traits used by breeders in the seedling trial stage. Major contributors in the other PC stages, HI, DMC, RtPlt and RtWt are used in seedling and advanced trial selection. Selection based on all these traits at the earliest stage would therefore save a lot of time and resources. Storage root weight was a major contributor to all PCs, while RtPlt was a major contributor in PC1 and PC2 indicating their relative importance to root yield. Varma and Mathura (1993), while investigating genotypic and phenotypic relationships of cassava in India, reported significant correlation ($P \leq 0.01$) between yield and mean storage root weight, and between yield and number of marketable storage roots. They suggested that effective direct selection for yield would be obtained through clump characteristics via storage root weight per clump, number of marketable storage roots per clump and indirect selection through storage root weight, length and girth. Williams (1972) reported root size as the major contributor to differences in storage root yield. Mahungu (1983) reported that

storage root yield was highly correlated to the number of storage roots and suggested the existence of a good fit between expected and observed values for genetic progress. The negative correlation between storage root number and weight indicates that a comprise has to be made, since improving both is unlikely. Where cassava is consumed boiled most consumers prefer medium sized tubers, indicating that by fixing tuber size, a breeder can exploit the increase in root number.

Stepwise regression using $P \leq 0.05$ entry and exit included DMC, RtWt, RtPlt and FRY in the model and rejected ComRt and HI. Of the included traits, FRY had the highest coefficient (0.31), followed by RtWt (0.04), DMC (0.01) and RtPlt (0.002). Since DRY is directly derived from FRY and DMC and as such they are expected to be highly correlated, the regression showed the importance of RtWt and RtPlt in yield determination. This agrees with the findings from PCA above.

Table 5.6 Principle component coefficients of the various traits with principles of the various yield related traits evaluated on eight seedling families in Colombia in 2005

Trait	PC[a]1	PC2	PC3	PC4
RtPlt[b]	0.339[h]	-0.555	0.113	0.388
ComRt[c]	0.458	-0.156	0.048	0.112
RtWt[d]	0.357	0.479	-0.237	-0.456
HI[e]	0.099	0.619	-0.068	0.775
DMC[f]	0.063	0.234	0.956	-0.114
FRY[g]	0.517	-0.005	-0.097	-0.079
Eigenvalue	3.56	1.33	0.99	0.74
Percent total				
Variance	51.0	19.0	14.1	10.7
Cumulative	51.0	70.0	84.1	94.8

[a]Principal component; [b]Roots per plant; [c]Commercial roots per plant; [d]Root weight (kg); [e]Harvest index (0-1); [f]Dry matter content (%); [g]Fresh root yield (t/ha); [h]Underlined values are the loadings of contributing traits

5.4 Conclusions

Results from this study indicated that it is possible to select simultaneously for yield and quality characteristics at seedling stage as most of the quality characteristics have been shown to be fixed at earlier stages. Since DMC is independent of yield, the two can concurrently be selected for, with no detrimental effect on either. Use of a selection index should include RtWt and RtPlt, in addition to FRY, HI and DMC since these are important to root yield. Based on the relative contribution of the traits, the heaviest weight should be given to RtWt and RtPlt. Breeders should shift emphasis from HI and DMC as the primary selection indices to other yield components like root numbers and weight or look for alternatives to the above. For example, Lenis *et al.* (2006) suggested selecting for stay green as an alternative to selecting for high HI. Since total photosynthesis of the crop sets the ceiling for the dry biomass (Kawano *et al.*, 1998), increasing the leaf lifecycle by selecting stay green genotypes is likely to increase yield. Selecting for genotypes with low HI and high LAI will increase the amount of biomass translocated for DMC and FRY. From the performance of the families it is apparent that different families perform better for specific yield traits. This indicates that a breeder has to make specific crosses for the different yield traits.

Seedling nursery results and the diallel analysis (Jaramillo *et al*., 2005) both identified MPER 183 and SM 1741-1 as good parents for a number of traits. This implies that a breeder does not necessary have to make a diallel cross to select good parents, but can actually select from ordinary cross data, if the number of genotypes per crosses is high. This study therefore concludes that cassava breeders should design breeding programmes where proper selection starts at the seedling nursery. The selection index should include, in addition to the traditional traits, RtWt and RtPlt.

Chapter 6

Clonal evaluation trial

6.1 Introduction

Cassava along with maize, sugarcane and rice, constitute the most important sources of energy in the diet of most tropical countries of the world (Ceballos *et al*., 2004). Basically every part of the cassava plant can be utilised, but the starchy roots are by far the most commonly used product. Cassava roots are not tubers and therefore cannot be used for reproductive purpose and have a short shelf life (Beeching *et al*., 1998). Within one or two days after harvest there is rapid initiation of post-harvest physiological deterioration (PPD; Ceballos *et al*., 2004). To date, little useful genetic variation to delay or reduce PPD has been found, and the solution to this problem remains one of the most important goals for cassava research.

The vegetative multiplication rate of cassava is low. From a single plant, five to ten cuttings can typically be obtained, resulting in a lengthy process to arrive at the point where replicated evaluations across several locations can be conducted. It takes about five to six years from the time the botanical seed is germinated until the evaluation/selection cycle reaches the regional trial stage, when several locations can be included (Kawano, 2003; Ceballos *et al*., 2004). One further complication in a cassava breeding programme is the number of factors that can affect the quality of planting material. For example, the original positioning of the vegetative cutting along the stem considerably affects the performance of the plant which is propagated. Cuttings from the mid-section of stems usually produce better performing plants than those at the top or bottom (Ceballos *et al*., 2004). Variation in the performance of the plant depending on the physiological status of the vegetative cutting, results in larger experimental errors and undesirable variation during the evaluation process.

The first selection on plants derived from botanical seed is conducted in the seedling nurseries. Due to low correlations between performance at this early stage of selection and when genotypes reach replicated trials, early selections are based on highly heritable traits like plant type and reaction to certain diseases (Hahn *et al*., 1980a; 1980b; Hahn, 1984; Hershey, 1984; Iglesias *et al*., 1994a). The second stage of selection is called the clonal evaluation trial (CET). Genotypes from single-plant selections conducted during the F_1 stage produce six to ten vegetative cuttings required for this second stage. The capacity to produce this number of cuttings is another selection criteria utilised during the F_1 stage. The CET usually contains between 2000 to 3000 clones. Because competition between neighbouring genotypes in the CET may favour more vigorous plant architectures, selection at this stage will rely heavily on highly heritable traits such as HI (Kawano *et al*., 1998; Kawano, 2003). Important selection criteria at these early stages of selection are plant type, high DMC and CNP (Iglesias and Hershey, 1994). Between 100 and 300 clones will be selected from the CET.

A common feature in most cassava programmes is that the first two stages of selection are not replicated. In order to manage the large number of materials at low cost, selection is frequently visual with no data taking (Ceballos *et al*., 2004). It is during these stages that the highest number of genotypes is discarded (more than 95%; Kawano, 2003; Ceballos *et al.*, 2004). A large proportion of genotypes are therefore eliminated without a proper evaluation set-up. The change of emphasis of selection from highly heritable to polygenic or lower heritable traits such as yield only takes place with the initiation of replicated trials when proper data taking occurs (Ceballos *et al*., 2004).

Based on these reasons, there exist clear opportunities to further improve the efficiency and effectiveness of cassava breeding programmes. Some modifications for overcoming this drawback have recently been introduced at the breeding project at CIAT-Colombia (Pérez *et al.*, 2002b, Ceballos *et al*., 2004). Modifications include: All clones from a given family are separated into three groups. Each group of a family is randomly allocated to one of the three “blocks”. Data are taken and recorded for every genotype, regardless of whether or not they are selected to pass to the following stage. Clones

comprising each group will obviously be different. However, replicated information can be derived for each family. This information can be used to derive the relative values of parents that generated these families.

The objectives of this study were to conduct a clonal evaluation trial using the CIAT-Colombia cassava breeding system and to assess the effectiveness of the CIAT-Colombia cassava breeding system on clonal evaluation.

6.2 Materials and methods

Stems from the seedling nursery evaluation described in Chapter 5 were cloned and used to establish a CET experiment in July 2005. The trial was established in CIAT-Palmira (described in Chapter 4). No selection was done on the seedlings. The number of planting material (cuttings) which could be obtained from a seedling was highly variable among and within families. To avoid the confounding effects between number of plants and genotypic differences, the same number of plants was used within a plot. Genotypes within a family were thus separated into groups based on the number of cuttings which could be obtained. Genotypes with nine to 11 cuttings were planted in a 3 x 3 replicated trial and those with 12 cuttings in a 4 x 3 replicated trial. The rest of the clones could, due to low numbers, not be replicated and were planted as single row plots of three, four or six plants depending on the availability of cuttings. Of the eight families used, three (GM 252, GM 901 and CM 9953) had sufficiently high numbers of genotypes with enough cuttings which were replicated.

Cuttings were planted on ridges with a 1.6 m between and 0.7 m along row spacing, spacing being selected to reduce inter-specific and increase intra-specific competition. Genotypes within each family were randomly divided into three groups (blocks). A lattice square design was used for the replicated trial. A total of 261, 211 and 200 genotypes of GM 252, GM 901 and CM 9953 respectively were established in the replicated trials. A total of 979 clones were established as replicated or single row plots. Irrigation was applied in the first two weeks before regular rainfall periods. Two hand weedings were

done and after the trial was established, herbicide was used to control weeds. No fertiliser was applied as the soil in the trial plots was generally fertile.

The experiment was evaluated in March 2006 eight MAP. Plants were harvested and bulked per genotype for measurement of yield related agronomic traits, as described in Chapter 4. In addition, plants were rated for incidence of CFSD, colour of root pulp and signs of PPD. Roots were examined during weighting for signs of CFSD and rated as absent (0) or present (1). The colour of the root pulp was assessed as described by Iglesias *et al.* (1997). A 1 to 4 scale for visual estimation of root colouration was used, where white = 1; cream = 2; yellow = 3 and orange = 4. Signs of PPD were assessed about 20 hours after harvesting. Due to the heavy rains which usually happened at night during harvesting, harvesting was usually done in the afternoon and roots left intact on the plant overnight. The next morning roots were separated from the plants, weighed and samples taken for DMC. After DMC assessment two to three of the roots used were cut across in the middle section and signs of PPD (streaking or discolouration) noted, 1 for present and 0 for absent.

Data was subjected to analysis as described in Chapter 4. Data from all genotypes was used in descriptive statistics, correlation and PCA, while data from replicated trials was used in generalised linear model (GLM) analysis.

6.3 Results and discussion

Low incidence of CFSD was recorded (Table 6.1). This was because cuttings used were from a seedling nursery established in an area with no cassava cultivation history and were CFSD clean. The plot where the CET was established had not been under cassava for a long time. Post-harvest physiological deterioration rate was low with a skewness of 2.81, the highest skewness for all traits. Chavez *et al.* (2005) working on landraces and improved clones in CIAT germplasm, observed that the distribution of PPD was asymmetrical with a longer tail to the right, and concentration of frequencies around low-PPD values. Most roots were white or cream as shown by the average of 1.43, with a

skewness of 1.55. Chavez *et al*. (2005), visually scoring root colour based on a sample of 788 landraces and improved CIAT clones, recorded a higher frequency of roots with light or white colouration, with fewer cases of roots with intense colouration (skewness = 1.73). They recorded a phenotypic correlation between total carotene content in the roots and root colour of P=0.86. They noted that identification of cassava clones with high carotene density in the roots can easily and effectively be done through visual evaluation of their parenchyma colour. The higher the colour intensity, the higher the amount of carotene (Iglesias *et al*., 1997; Graham *et al*., 1999). Results of both studies therefore indicated that germplasm at CIAT is low in carotene content.

Table 6.1 Simple statistics of disease and agronomic variables evaluated on 979 genotypes of a clonal evaluation trial (CET) evaluated in CIAT-Palmira in April 2006

Variable	Minimum	Maximum	Average	StdDev[k]	Skewness
Pulp Colour[a]	1.00	4.00	1.43	0.68	1.55
PPD[b]	0.00	1.00	0.09	0.28	2.81
CFSD[c]	0.00	1.00	0.17	0.34	1.82
ComRt[d]	0.00	19.00	5.19	2.56	0.44
RtPlt[e]	2.00	25.00	10.81	3.16	0.53
RtWt[f]	0.03	1.71	0.36	0.16	1.69
HI[g]	0.08	0.92	0.52	0.12	-0.41
DMC[h]	20.65	45.33	34.37	3.22	-0.33
FRY[i]	0.20	67.19	19.14	11.09	0.75
DRY[j]	0.10	25.61	6.68	4.02	0.80

[a]Pulp colour assessed on a scale of 1-4; [b]Post-harvest deterioration assessed as absent=0 and present=1; [c]Cassava frogskin disease assessed as absent=0 or present=1; [d]Commercial roots per plant; [e]Roots per plant; [f]Root weight (kg); [g]Harvest index (0-1); [h]Dry matter content (%); [i]Fresh root yield (t/ha); [j]Dry root yield (t/ha); [k]Standard deviation

An average of 10.8 RtPlt was obtained with GM 252-309 having the highest number of 25, which is a high value for RtPlt, especially for an unselected population. According to Alves (2002) three to ten fibrous cassava roots develop into tuberous roots.
Harvest index of 0.08 to 0.92 with a mean of 0.52 was obtained. This mean is higher than that reported by Jaramillo *et al*. (2005) of 0.47 from the diallel cross used for selecting parents for this experiment. Percentage dry matter content ranged from 20.65% to 45.33% (Table 6.1) in GM 252B-254 and CM 9958B-006 respectively, with the best four clones coming from GM 9958B. Jaramillo *et al*. (2005) reported a lower average of 33.63% compared to 34.37% recorded here. Since parents were selected specifically to study DMC, it is an indication that selecting parents for high DMC based on the diallel analysis improved the DMC of the resultant population.

Best yielders (both fresh and dry) were CM 9953-088, GM 901-091, CM 9953-082 and GM 901-317, with FRY ranging from 0.20 to 67.19 t/ha and DRY from 0.10 to 25.61 t/ha. Mean FRY was 19.14 t/ha and 6.68 t/ha for DRY. Jaramillo *et al*. (2005), reporting on the diallel used for selecting parents for this crosses recorded a higher FRY mean of 46.7 t/ha. Cach *et al*. (2006), reporting on another diallel set used for selecting the parents from a sub-humid agro-ecology reported a DRY range of 0.8 t/ha to 28.3 t/ha, falling within the range reported in this study. Calle *et al*. (2002) reported an average yield of 38.8 t/ha among a group of 15 elite clones in the same agro-ecology. They recorded FRY ranges of 18.4 t/ha to 52.8 t/ha and 18.5 t/ha to 27.8 t/ha from the best clones in sub-humid and acid-soil savannah respectively. The range in the data presented here is larger than that reported by Calle *et al*. (2002), because this data is from unselected material. The difference between Jaramillo *et al*. (2005) and the present study mean may be because in this study no selection was done as opposed to Jaramillo *et al*. (2005) where vigorous seedlings and plants capable of producing good quality vegetative cuttings were selected. Results indicate that cassava clones are capable of producing high yields and show the high potential of cassava as a source of raw material for different purposes.

All traits, except HI and DMC, had a positive skewness indicating improvement potential through conventional crossing and selection. Of the yield related agronomic traits, the largest skewness was observed in RtWt. This indicates that the greatest improvement should be achieved by concentrating on RtWt. Distribution of HI and DMC were negatively skewed, an indication that not much improvement can be expected for HI and DMC from conventional breeding.

Genotypes within a family were averaged to determine the families' performance (Table 6.2). All families had colour ratings ranging from white (1) to cream (2). Family GM 536 had the highest PPD rate (0.41), with none being observed in GM 853 (Table 6.2). Highest incidence of CFSD was observed in GM 256 and GM 9953 while no CFSD incidence was observed in GM 9958. Family GM 901 recorded the highest HI followed by GM 252 and GM 9953 of 0.56, 0.55 and 0.55, respectively. Dry matter content estimates ranged from 33.1 to 38.3 in GM 901 and GM 9958 respectively. Fresh root yield ranged from 10.0 to 23.2 in GM 853 and GM 901. Highest dry yielders were GM 256, GM 9953 and GM 901 with 8.3, 8.0 and 7.9 t/ha respectively. The best overall family was GM 256 followed by CM 9953. Means for the families were not significantly different from each other except for HI and DRY.

Pearson correlations are presented in Table 6.3. Cassava frogskin disease was negatively correlated to all traits, and highly significant for DMC and HI. Dry matter content and HI are usually used during the early stages of selection programmes because of their relative stability (Kawano *et al*., 1998). Interference by a biotic stress like CFSD, whose biology is not yet even fully understood, is going to be a big problem. Cassava frogskin disease causes a problem in accurate estimation of DMC phenotypic data, which is crucial in molecular marker analysis.

Pulp colour was negatively correlated to all agronomic traits except DMC. Yellow colour was not associated with other agronomic traits because breeders previously selected against it. In the past, CIAT, IITA and the cassava national programmes, breeding

Table 6.2 Means and standard deviations of root quality related characteristics estimated on 979 genotypes of a clonal evaluation trial of eight families evaluated at harvest in CIAT, Palmira in March l, 2006

FAMILY	VARIABLE									
	Colour[a]	PPD[b]	CFSD[c]	ComRt[d]	RtPlt[e]	RtWt[f]	HI[g]	DMC[h]	FRY[i]	DRY[j]
GM 252	1.6±0.79	0.02±0.10	0.2±0.3	11.7±7.9	10.2±3.4	0.37±0.16	0.55±0.12	33.4±2.7	15.5±9.5	5.3±3.4
GM 901	1.1±0.28	0.04±0.12	0.1±0.3	14.6±8.7	10.8±2.8	0.44±0.18	0.56±0.11	33.1±2.9	23.2±12.9	7.9±4.6
GM 9953	1.3±0.70	0.01±0.09	0.3±0.4	15.8±10.1	11.3±3.0	0.36±0.12	0.55±0.11	34.7±3.2	22.6±11.8	8.0±4.4
GM 536	1.7±0.49	0.41±0.49	0.1±0.3	20.4±11.7	11.0±3.4	0.27±0.10	0.43±0.09	35.8±2.9	17.3±8.1	6.2±3.1
GM 9958	2.0±1.13	0.09±0.29	0.0±0.2	15.9±11.3	12.0±3.2	0.21±0.07	0.45±0.09	38.3±3.5	15.9±8.9	6.1±3.4
GM 853	1.1±0.24	0.00±0.00	0.1±0.3	10.0±8.1	8.5±2.5	0.23±0.10	0.43±0.11	35.4±3.1	10.0±5.9	3.6±2.3
GM 256	1.2±0.36	0.18±0.39	0.3±0.5	25.1±13.7	11.5±2.9	0.35±0.17	0.51±0.10	35.9±2.8	23.0±10.4	8.3±3.8
GM 847	1.6±0.48	0.03±0.16	0.1±0.4	20.0±10.5	10.9±3.2	0.31±0.08	0.41±0.08	35.2±3.0	16.3±7.2	5.8±2.8
Total	***1.4±0.73***	***0.06±0.25***	***0.2±0.4***	***14.8±10.3***	***10.9±3.7***	***0.38±0.18***	***0.53±0.13***	***34.1±3.4***	***20.6±12.8***	***7.1±4.6***

[a]Pulp colour assessed on a scale of 1-4; [b]Post-harvest deterioration assessed as absent=0 and present=1; [c]Cassava frogskin disease assessed as absent=0 or present=1; [d]Commercial roots per plant; [e]Roots per plant; [f]Root weight (kg); [g]Harvest index (0-1); [h]Dry matter content (%); [i]Fresh root yield (t/ha); [j]Dry root yield (t/ha)

Table 6.3 Correlation for yield related traits and biotic stress recorded on 979 genotypes of a clonal evaluation trial (CET) at harvest in CIAT-Palmira, Colombia in April 2006

	VARIABLES								
	Pulp Colour[a]	PPD[b]	CFSD[c]	ComRt[d]	RtPlt[e]	RtWt[f]	HI[g]	DMC[h]	FRY[i]
PPD	0.03								
CFSD	-0.04	0.02							
ComRt	-0.08*	0.17***	-0.07*						
RtPlt	-0.091**	0.02	-0.05	0.36***					
RtWt	-1.13***	-0.13***	-0.03	0.13***	-0.01				
HI	-0.10***	-0.20***	-0.15***	0.16***	0.24***	0.43***			
DMC	0.03	0.19***	-0.26***	0.37***	0.25***	-0.15***	0.09**		
FRY	-0.16***	0.00	-0.03	0.72***	0.41***	0.44***	0.32***	0.25***	
DRY[j]	-0.16***	0.03	-0.06	0.74***	0.42***	0.39***	0.32***	0.37***	0.999***

[a]Pulp colour assessed on a scale of 1-4; [b]Post harvest deterioration assessed as absent=0 and present=1; [c]Cassava frogskin disease assessed as absent=0 or present=1; [d]Commercial roots per plant; [e]Roots per plant; [f]Root weight (kg); [g]Harvest index (0-1); [h]Dry matter content (%); [i]Fresh root yield (t/ha); [j]Dry root yield (t/ha), *P≤0.05, ** P≤0.01, ***P≤0.001

objectives were geared towards human consumption which frequently emphasised cooking quality or starch characteristics as the determining factor. Good cooking quality is usually associated with other morphological traits such as the colour of the peel of the roots, the leaf petiole or the shoot (Ceballos *et al*., 2004). Farmers frequently reject any change in such morphological traits, although little or no correlation with actual cooking quality exists. Due to these farmer and consumer preferences, participatory research and breeding approaches had to be developed for cassava breeding (Goncalvez Fukuda *et al*., 2000; DeVries and Toenniessen, 2001; Goncalvez Fukuda and Saad, 2001).

Personal experience from working in cassava breeding indicated that farmers who consume cassava as 'boil and eat' will always reject a variety with yellow roots. This is because yellow roots will turn black on cooling. Usually roots are boiled and left to cool to be eaten as a snack. Likewise, in areas where roots are processed to flour for making porridge or "enzima", yellow rooted varieties will be rejected because they produce "dirty" flour. In order for farmers to accept yellow rooted varieties, breeders need to educate farmers on the advantage of yellow rooted varieties which have higher concentrations of carotenes.

Harvest index was positively correlated ($P \leq 0.001$) to ComRt, RtPlt, RtWt, FRY and DRY and negatively correlated to pulp colour, PPD and CFSD. It was positively correlated ($P \leq 0.01$) to DMC. The high positive correlation between HI and FRY agrees with results described in literature (Kawano *et al*., 1998; Kawano, 2003). The high positive correlation of HI to other agronomic traits indicates that HI can be used for selection at early stages of the programme. Correlation between HI and FRY was higher than is usually encountered during earlier stages of selection. Kawano *et al*. (1998) obtained a low correlation (-0.19) between HI and FRY at the early stages of selection compared to a high correlation (0.93) at the later stages of selection. They observed that correlation at early evaluation was mostly caused by non-genetic factors. Replicating and blocking of clones in a family in the present study should have greatly reduced the non-genetic factors. Ceballos (personal communication*)* agrees that replication during the early stages improves correlation between HI and FRY. Kawano and Thung (1982)

observed that genotypes showed contrasting yield performances between single row trials (SRT) and plot trials due to inter-genotypic competition. In SRT or single-plant trial (ST), genotypes with high HI are weak competitors, while those with greater biomass are strong competitors. In plot trials where inter-genotypic competition is absent and yield is expressed per area rather than per plant or row, weaker competitors with high HI tend to perform better than stronger competitors with low HI (Kawano and Jennings, 1983). In the present study spacing was increased between genotypes and reduced within genotypes to reduce inter-genotypic competition and yield was expressed per area. These allowed better genotypic expression, thus expressing the true HI and FRY correlation.

DMC was positively correlated with PPD, ComRt, RtPlt, FRY and DRY at $P \leq 0.001$, HI at $P \leq 0.01$, negatively correlated to CFSD and RtWt and not associated with colour. Dry matter content is known to be positively associated with PPD (Jennings and Hershey, 1985; Van Oirschot *et al*., 2000). Chavez *et al*. (2005) noted that this was an unfortunate situation because, in general, breeding projects look for higher DMC, which leads to a faster or more serious PPD.

Dry root yield was highly correlated to all agronomic traits, negatively correlated to pulp colour and CSFD and not associated with PPD. This indicated that all agronomic yield traits - ComRt, RtPlt, RtWt, HI, DMC and FRY make important contributions toward economic yield. The negative association between DRY and pulp colour is discouraging though. Since colour is highly correlated to carotene content (Chavez *et al*., 2005), selecting for high FRY is likely to be detrimental to carotene levels and *vice versa*. Fresh root yield followed the same association pattern as DRY. It is interesting to note that although CFSD was negatively correlated to DMC no significant association was observed between FRY and CFSD, which influenced the relation between DRY and CFSD. This was also observed in another population evaluated over a two year period (Chapter 4). It seems we have a similar situation where a reduction in DMC leads to compensation in FRY, in that case leading to constant DRY.

The different variables were regressed against DRY. Stepwise regression at entry and exit point of P=0.01 included DMC, RtWt and yield and excluded the rest. This indicated that DMC, RtWt and yield were the most important determinants of DRY. Since DRY is directly derived from FRY and DMC it indicates that RtWt is the most important independent trait for DRY. This is in agreement with findings from the seedling evaluation (Chapter 5) trial evaluated earlier. This confirmed the importance of RtWt in DRY yield determination and re-enforces the argument that cassava breeders should include RtWt as an index in their breeding programmes.

Generalised linear model analysis showed clones to be highly significant for all traits (Table 6.4). Family was significant for all traits except PPD and HI. High significance shows variability within the population. Lack of significance for HI in the family shows that CIAT clones' ability to act as parents for HI has been fully exploited. Ceballos *et al*. (2004) observed that cassava improvement in the past in CIAT was mainly based on improving HI and this trait might have been over exploited by now. Lack of significance for PPD also signified that CIAT clones, when used as parents, lack variability to be exploited for improving PPD. Ceballos *et al*. (2004) noted that genetic variability for PPD is lacking in *M. esculenta* and suggested making inter-specific crosses with other *Manihot* species to introgress useful genes to solve the problem.

Replication was significant at $P \leq 0.01$ for PPD, RtPlt, RtWt and DMC and at $P \leq 0.05$ for FRY and DRY. It was not significant for CFSD, ComRt and HI. In cassava breeding, experiments at this stage of selection are extremely large and obtaining uniformity among replications is difficult. In this particular trial, the experimental area was more than 0.5 ha, with a down–slope nutrient gradient. Differences in replications were therefore expected. Another reason for differences is that in establishing a replicated trial in cassava, usually all cuttings for the three replications are put together in a bag which are then placed along plots in the first replication. After picking cuttings for the first replication, bags are collected, randomised and placed in the next plots where cuttings for the second replication are picked and the process repeated for the third replication.

Table 6.4 Analysis of variance (ANOVA) table of yield related parameters evaluated at harvest in CIAT, Palmira, Colombia in March, 2006

Source of variation	Df[a]	MEANS								
		PPD[b]	CFSD[c]	ComRt[d]	RtPlt[e]	RtWt[f]	HI[g]	DMC[h]	FRY[i]	DRY[j]
Clone	306	0.01***	0.07***	0.61***	0.39**	0.014***	0.03***	18.82***	3.74***	1.30***
Family	2	0.01	0.25***	3.74***	2.39***	0.15***	0.03	157.64***	72.59***	25.54***
Rep[k]	2	0.03**	0.02	0.18	1.72**	0.03**	0.01	42.58**	4.67*	2.14*
Block	3	0.01	0.10*	1.39**	1.74***	0.01	0.02	17.45	5.18*	2.21**
Block x Rep	6	0.01*	0.10**	0.83*	0.13	0.01*	0.01	24.39	2.67	1.12*
Clone x Rep	530	0.01***	0.02	0.28	0.26	0.01**	0.01	5.46	1.12	0.38
Clone x block	150	0.01	0.07***	0.61***	0.37*	0.12***	0.03***	21.97***	3.09***	1.09***
Family x Block	5	0.00	0.02	0.69	0.63	0.00	0.01	6.35	4.96**	1.65**
Error	351	0.01	0.03	0.31	0.28	0.00	0.01	8.22	1.44	0.50
CV[l]		9.82	21.80	23.83	16.23	7.37	19.80	8.51	26.93	26.84
Heritability[m]		0.02	0.79	0.60	0.43	0.61	0.80	0.75	0.73	0.73

[a]Degrees of freedom; [b]Signs of post-harvest physiological deteroriation, evaluated as present=1 or absent=0; [c]Cassava frogskin disease incidence evaluated as absent=0 or present=1; [d]Commercial roots per plant; [e]Roots per plant; [f]Root weight (kg); [g]Harvest index (0-1); [h]Dry matter content (%); [i]Fresh root yield (t/ha); [j]Dry root yield (t/ha); [k]Replication; [l]Coefficient of variation; [m]Broad-sense heritability, [ki]ns=not significant; *P≤0.05, ** P≤0.01, ***P≤0.001

Experience has shown that planters tend to pick the best cuttings first. In advanced trials with plenty of planting material this is offset by selecting uniform cuttings. In a situation like this where all cuttings from the stem are used, the first replication will contain the best cuttings and the third the worst. Lack of significance in HI and ComRt implied that the two traits are not significantly influenced by the environment and the type of planting material. Kawano *et al*. (1998) noted that regressions for HI were highly significant throughout all stages of evaluation. Block was highly significant for RtPlt, ComRt and DRY and significant ($P \leq 0.05$) for FRY and CFSD and not significant for the other agronomic yield traits.

It is interesting to note that there was no significant clone x replication interaction in the yield related traits except for RtWt. This suggested that the performance of clones for ComRt, RtPlt, HI, DMC, FRY and DRY was relatively similar across replications. This indicates that the genotypes' performance was different among replications but consistent. Replication was therefore successful in improving accuracy of the trial. Significance of PPD could have been as a result of experimental error caused by differences in time of assessment. Due to the heavy rain during evaluation, evaluation was delayed at times. Chavez *et al*. (2005), quoting Wheatley *et al*. (1985) and Zapata (2001), reported that evaluation of PPD is prone to large experimental errors.

Clone x block interaction was highly significant ($P \leq 0.001$) for CFSD, ComRt, RtWt, HI, DMC, FRY and DRY and significant ($P \leq 0.05$) for RtPlt. This was expected as different clones were used in the different blocks. It is interesting to note that apart from FRY and DRY, no family x block interactions were significant. This indicated that blocking was effective and should be used in early cassava breeding programmes as a way of reducing environmental effects and for acquiring additional data.

High heritability estimates were obtained. The highest was for HI (0.80), followed by CFSD (0.79), DMC (0.75) FRY and DRY (0.73), RtWt (0.61), ComRt (0.60) and RtPlt (0.43). A very low value for PPD (0.02) was obtained. Pérez *et al*. (2002a) evaluating 38 elite clones in a uniform regional trial across thirteen locations in northern Colombia

recorded high heritabilities. A range from 0.30 to 0.88 with a mean of 0.56 was recorded for FRY, 0.62 to 0.88 for HI, while DMC ranged from 0.42 to 0.92. Results obtained from the present study fall within this range. Okogbenin (2004), evaluating an F_1 population, recorded broad-sense heritability for HI of 0.87, DMC of 0.62, DRY of 0.60, FRY of 50, RtPlt of 0.36 and PPD of 0.07. Although these values are lower compared to the present study (except for HI), they fall within the range and follow the same trend. Okogbenin (2004), recording low heritability for PPD (0.07) concluded that there were no real genetic differences for the trait. Low heritability for PPD agreed with Ceballos *et al*. (2004), who stated that to date low levels of useful genetic variation to delay or reduce PPD has been found. Heritability is defined as the percentage of total phenotypic variability for a trait that is due to genes, and their interaction with other genes. It is an indication of the ease with which a trait is transferred to the progeny (Kang, 1994). The high heritability values for agronomic traits indicated the great possibilities of improvement that can be achieved through breeding.

Principal component analysis revealed that the first four PCs were important and explained 89.57% of the total variation (Table 6.5). Variables in PC1 were positively correlated, indicating that all contributed to yield. PC1 had an eigenvalue value of 2.63 and accounted for 43.85% of the variation. This represented an equivalent of at least three variables and indicated that ComRt, FRY and HI were important contributing variables. PC2 had an eigenvalue of 1.28, contributing 21.40% of the variation and had DMC, RtWt and RtPlt as the main contributing factors. Principal components three and four had eigenvalues less than one indicating that most likely a single variable was contributing in each case. In PC3 DMC was the main factor while HI was important in PC4.

Harvest index and DMC were important in at least two PCs, while ComRt, FRY, RtWt and RtPlt were important in one of the PCs (Table 6.5), indicating their relative importance to yield. These results confirmed those earlier obtained from the correlation analysis which indicated that HI and DMC were important in early stages of selection. Results furthermore confirmed the regression results that FRY, DMC and RtWt were the most important determinant variables for DRY.

Table 6.5 Principle component coefficients of the various traits with principles of the various yield related traits evaluated on 979 genotypes at a clonal evaluation trial in Colombia in 2006

Trait	PC[a]1	PC2	PC3	PC4	PC5
ComRt[b]	0.52	-0.05	-0.32	-0.03	0.51
RtPlt[c]	0.38	0.46	-0.57	0.14	-0.08
RtWt[d]	0.40	-0.54	0.24	-0.25	0.33
HI[e]	0.41	-0.19	0.29	0.79	-0.29
DMC[f]	0.14	0.67	0.63	-0.00	0.36
FRY[g]	0.48	0.09	0.17	-0.53	-0.64
Eigenvalue	2.63	1.28	0.80	0.66	0.40
Percent total Variance	43.85	21.40	13.28	11.05	6.75
Cumulative	43.85	65.25	78.52	89.57	96.32

[a]Principal component; [b]Commercial roots per plant; [c]Roots per plant; [d]Root weight (kg); [e]Harvest index (0-1); [f]Dry matter content (%); [g]Fresh root yield (t/ha); Underlined values are loadings of contributing traits

Insight can often be obtained by plotting the PC scores for individual observations in relation to the important PC axes. Figure 6.1 shows the plot of PC1 against PC2 and Figure 6.2 PC1 against PC3. Usually clustering along PC axes suggests that some relationship exists among individuals. In Figure 6.1, GM 901 and CM 9953, crosses made for the mid-altitude agro-ecology (where the experiment was conducted) were the best performers for FRY, ComRt and HI. They had DMC and RtWt values about equal to average (CM 9953) or slightly lower than average (GM 901). This is an indication that these families were well adapted to this agro-ecology. Families developed for the acid-savannah soils agro-ecology, GM 536 and GM 256, had higher DMC and RtWt values, but lower FRY, ComRt and HI values, compared to the mid-altitude families. The lowland semi-arid agro-ecology families had lower FRY, ComRt and HI (CM 9958 and

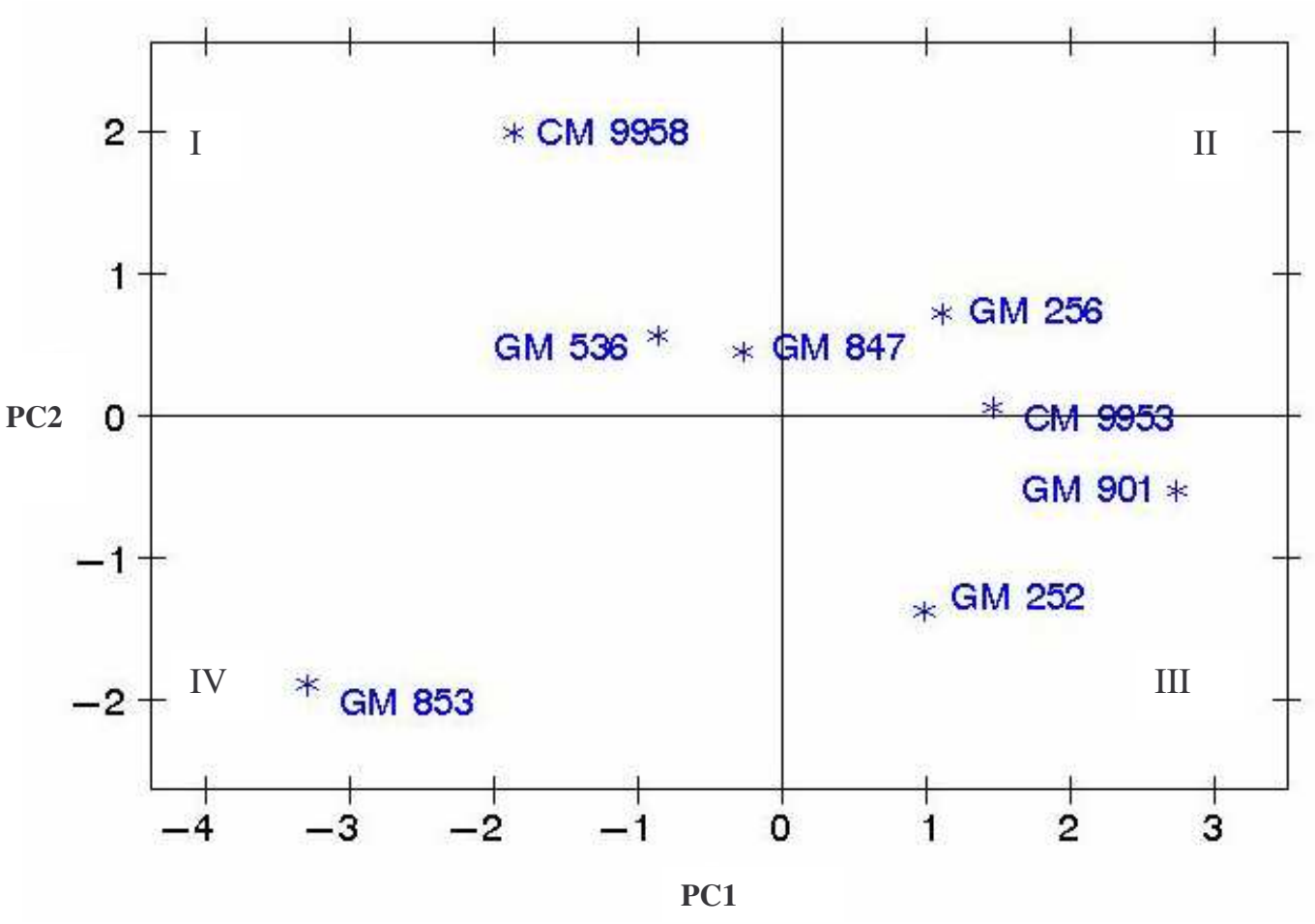


Figure 6.1 **Plot of PC1 against PC2 for eight families of a clonal evaluation trial evaluated in Palmira-CIAT in 2006**

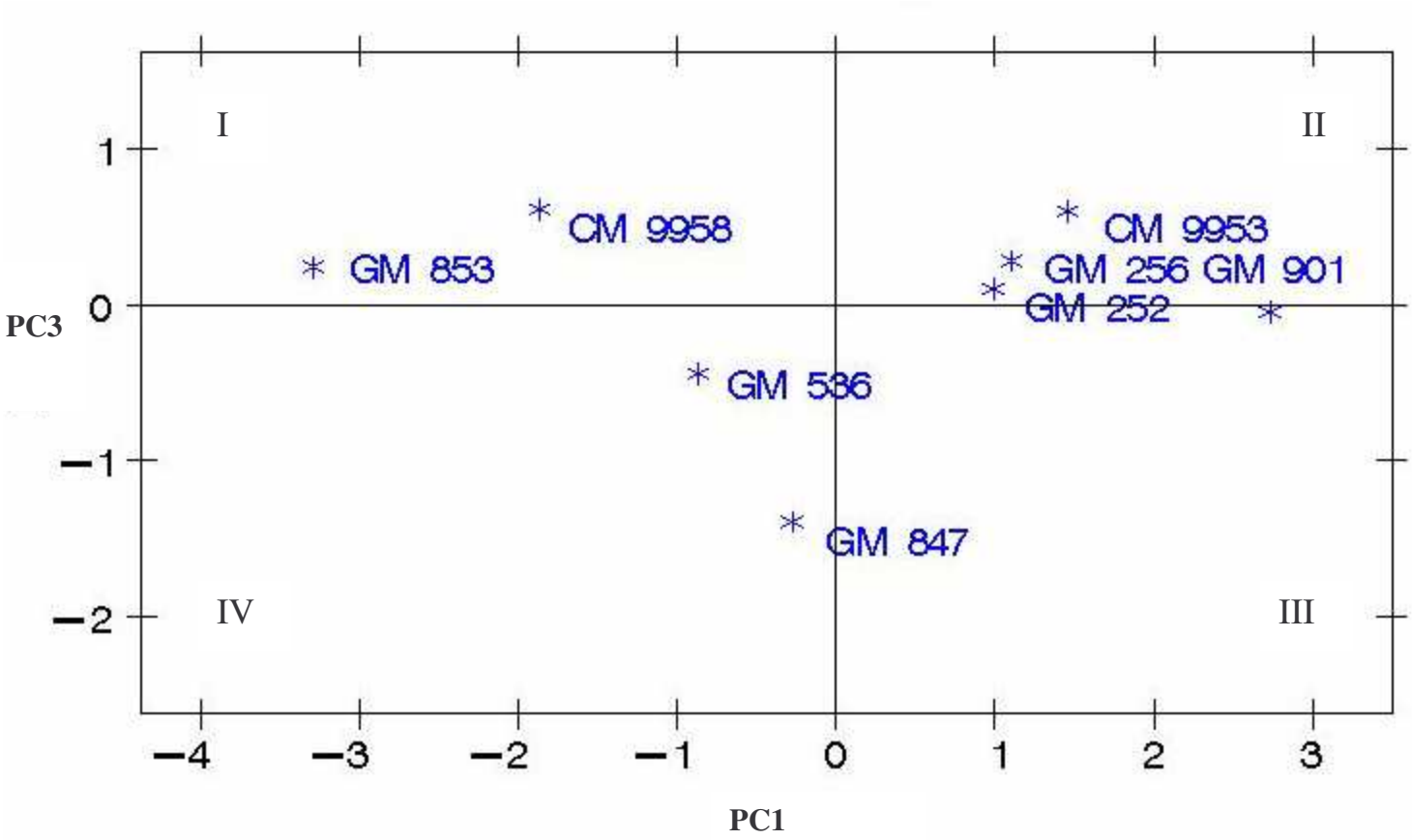


Figure 6.2 Plot of PC1 against PC3 for eight families of a clonal evaluation trial evaluated in Palmira-CIAT in 2006

GM 853) or DMC and RtWt (GM 853 and GM 252) compared to the mid-altitude families. The outcross GM 847 was average in all aspects. Figure 6.2 plotted the mid-altitude families CM 9953 and GM 901 in quadrant two or on the line separating quadrant two and three, indicating that as a group, their clones were superior and have better chances of getting selected. Family GM 256, developed for acid-savannah soils and GM 252 for the lowland semi-arid soils, clustered in quadrant two. However, the other families GM 853 and CM 9958 for the lowland semi-arid agro-ecology clustered in quadrant one and GM 536 for acid-savannah in quadrant four. This implied, as a group, clones developed for the lowland semi-arid and acid-savannah soils agro-ecologies had lower chances of selection success compared to the mid-altitude group. Principal component analysis was therefore able to separate families based on their adaptation. Families developed for lowland semi-arid and acid-savannah soils where not able to fully express themselves for proper evaluation. These families need be evaluated in their respective ecologies.

6.4 Conclusions

Clones crossed for three agro-ecologies were evaluated at clonal evaluation trial level. Evaluation of clones indicated that CIAT cassava germplasm is low in carotene content. There exists a need to improve the carotene level of the germplasm, especially in the present wake of increasing protein levels in cassava. Farmers and end-users need to be educated regarding the advantage yellow cassava tubers have over white ones, which have higher carotene levels. In this way breeders will be able to formulate appropriate participatory research programmes which will result in clones, which will be adopted by end-users. A high number of roots per plant (average of 10.8) was obtained compared to the botanically recognised three to ten. This study identified RtPlt as important in determining DRY and manipulation of the trait should increase yield. It also means that the germplasm at CIAT has been greatly improved for this trait. The negative correlation between DMC and CFSD is of concern, since currently breeding efforts are focusing on the development of markers linked to DMC and CFSD makes it especially difficult to obtain accurate phenotypic data.

The use of the improved cassava breeding programme employing replication and blocking of clones at early stages improved the accuracy of the data obtained. This was shown by the highly significant correlation values among agronomic traits, especially between FRY and HI. It was possible to do an ANOVA analysis which is not always possible with clonal evaluation. High heritability values, comparable to those at advanced selection stages, were obtained since environmental effects were minimised. During SRT it is important to reduce inter-genotypic and increase intra-genotypic competition to allow full expression of genotypes. Kawano *et al*. (1998) stated that the yield performance of the same individuals in SRT and plot trials is usually different because of border effects caused by inter-genotypic competition. In SRT, those genotypes with higher biomass tend to dominate others with less biomass in competition for light (Kawano and Thung, 1982). Increasing the distance between rows reduces the inter-specific competition and reducing intra-specific distance will help keep the plant density constant.

Principal component analysis singled DMC, HI and RtWt as important variables in selection and determination of economic yield at early stages of selection. This confirmed correlation and regression findings. Plots of PCs were able to separate families based on their target agro-ecologies.

Cassava breeders should design programmes which enable them to collect data as early as at the clonal stage and use the data for proper selection of clones and for selecting parents for future crosses. The trials should be replicated and where planting materials doesn't permit, clones within a family can be "replicated" in a lattice square fashion. Although the replications will be containing different clones within a family, data for the families will still be obtained. There is a need to investigate the smallest possible plot size which should give meaningful results in a replication. Ceballos (personal communication) for example is of the view that if you had three cuttings you would be better of planting a 1 x 3 replicated trial than a single row of three plants.

Chapter 7

Introgression of genes for dry matter content from wild cassava species

7.1 Introduction

Cassava is one of the most important sources of food energy in many tropical countries (Cock, 1982; Henry and Hershey, 2002; Hillocks, 2002; Onwueme, 2002). An estimated 70 million people in the tropics obtain more than 500 cal/day from cassava. In many areas of Africa, north east Brazil and in parts of Indonesia, it is a major source of dietary calories (Cock, 1985). The crop performs better than most other crops on low fertility soils, in areas with uncertain rainfall patterns and prolonged dry periods. Of particular importance, and unlike most other staple crops, cassava almost never fails due to drought (Ceballos *et al*., 2004). Crop productivity plays a major role in industrial uses of cassava (i.e. starch production and dried roots for animal feed; Ceballos *et al*., 2004), whereas stability of production is fundamental in many areas where cassava is the main subsistence crop. Industrial uses of cassava require high DMC as the main root quality trait, whereas human consumption will frequently emphasise cooking quality or starch characteristics over productivity, as the determining trait. Cooking quality and starch characteristics have been shown to be related to DMC (Mahungu, 1987).

Cassava cultivars lack many economically important characters such as resistance to biotic stress and good quality traits (Nassar and Dorea, 1982; Nassar and Grattapaglia, 1986). This can be attributed to the mode of evolution of the species and modifications of the allogamy system of the plant (Nassar and O'Hair, 1985). Most cassava farmers are resource-poor, lacking the ability to purchase and apply agro-chemicals on a regular basis (Taylor *et al*., 2004). Development and deployment of enhanced germplasm therefore remains the most important method for ensuring improved cassava production. Conventional breeding programmes at CIAT, Colombia and IITA, Nigeria, have been successful in developing and delivering cassava varieties with enhanced biotic stress resistance, DMC and improved processing quality in Africa (Manyong *et al*., 2000; Nweke *et al*., 2002), Asia and the

Americas (Jennings and Iglesias 2002). It is recognised that traditional cassava breeding will be problematic and, on its own, unlikely provide all solutions for improving the crop to suit the varying needs of small-scale farmers and commercial production in the tropics (Jennings and Iglesias, 2002; Kawano, 2003).

There is a growing consensus that traditional means of increasing crop productivity are reaching their limits and that new means are required for future improvements in crop yields (Beadle *et al*., 1985; Kawano, 2003; Taylor *et al*., 2004; Lenis *et al*., 2006). These traditional approaches have principally relied on major improvements in HI and the support of changes in cultural practices, plant nutrition, and pest and disease control that allowed yield improvement (El-Sharkawy *et al*., 1990). Wild species of cultivated crops have been frequently used as an important source of genetic diversity and have been employed effectively in a variety of breeding programmes (Nassar, 2003). Cassava breeders are becoming increasingly interested in incorporating genes of wild relatives (Nassar, 2003). Wild relatives of cassava, *M. esculenta* ssp *flabellifolia* (Pohl) Cif., *M. esculenta* ssp *peruviana* (Mull.Arg.) Allem, *M. tristis* Mull. Arg., *M. carthagenensis*, *M. chlorosticta* Standl. & Goldman and *M. pseudo cyaziovii* are important sources of genes for improvement of root quality, pest and disease resistance, and some have been known to possess more than 50% DMC in their roots (CIAT, 2003). Aggressive evaluation of crosses with related *Manihot* species is one of the approaches being used currently to try and overcome the limited variations for starch, root and nutritional traits in cassava (Ceballos *et al*., 2004). In 2000 CIAT initiated a programme to introgress genes for several yield quality traits from wild cassava relatives present in its germplasm collection. The objectives of this study were to evaluate one resulting inter-specific cross with high variability for DMC and to assess the effect of such a cross on other yield related traits.

7.2 Materials and Methods

In 2000 CIAT initiated a programme to introgress genes for several yield traits from the wild relatives of cassava to cassava. Wild accessions present in the germplasm collection which include *M. esculenta* ssp *flabellifolia*, *M. esculenta* ssp *peruviana*, *M. tristis*, *M.*

carthagenensis, *M. chlorosticta* and *M. pseudo cyaziovii* were crossed to a number of elite CIAT cultivars (Fregene and Morante, 2002). More than 1000 resulting crosses were evaluated for protein and DMC, waxy starches and resistance to CGM. The best F_1 for each yield trait were backcrossed to the respective improved parent to form the backcross one generation (BC_1). These were evaluated for the above mentioned traits, the best chosen and backcrossed to form BC_2. The BC_2 were evaluated and the family CW 208 with the highest diversity for DMC (Figure 7.1) was selected. Family CW 208 was developed from a cross between the improved variety MTAI-8 and a wide relative *M. tristis*. Segregation of DMC in this family may be one of the highest found to date (CIAT, 2004). The original family comprised of 185 genotypes. Due to difficulty in establishment, a number of entries were lost and enough planting material was obtained from only 37, which were used for the trial.

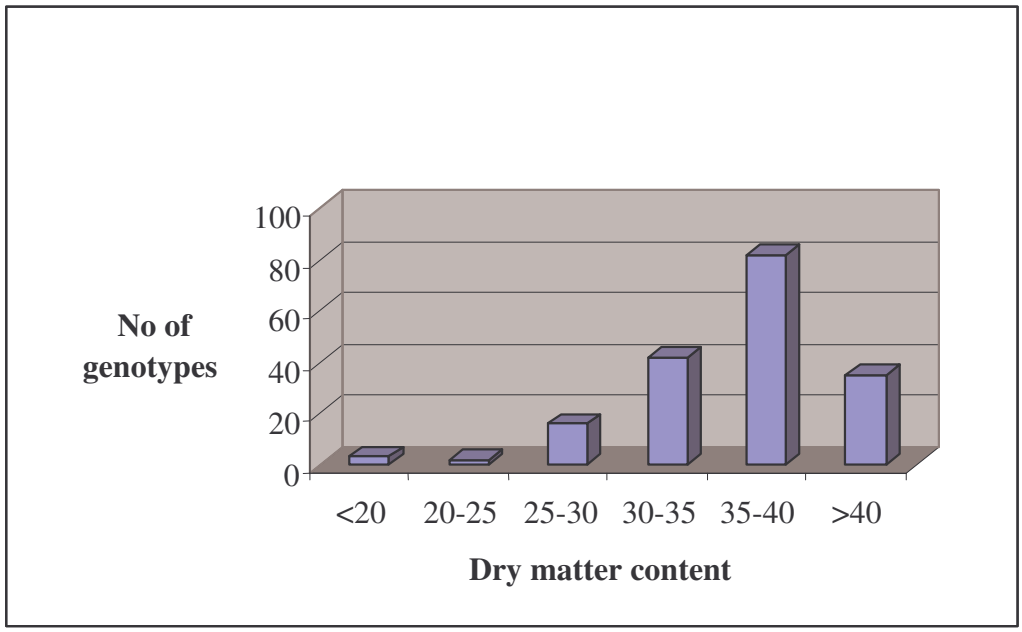


Figure 7.1 Frequency distribution of different classes of dry matter content in an inter-specific family CW 208 obtained from a cross between MTAI-8 and *M. tristis*

These trials were evaluated at CIAT-Palmira in 2005 and 2006. Entries were planted as single rows 5 m long with spacing of 1 x 1 m between plots and plants. Maintenance and evaluation of plots were done as described in Chapter 4. Data obtained was subjected to simple analysis, ANOVA and PCA. Agrobase (2000) and SAS (2002) programmes were used for analysis.

7.3 Results and discussion

Simple statistics of the clones of family CW 208 evaluated over a two year period for the different traits are presented in Table 7.1. Clone CW 208-15 had the highest number of ComRt per plot of 23.6. Roots per plant ranged from 4.6 in CW 208-135 to 16.1 in CW 208-07. Root weight was 0.12 kg in CW 208-114 and CW 208-145 to 0.34 kg in CW 208-38. Clone CW 208-108 had the lowest HI of 0.17 and CW 208-40 the highest of 0.55. High DMC was estimated, ranging from 34.39 in CW 208-12 to 42.73 in CW 208-114. Clones CW 208-07 and CW 208-115 out competed others in FRY with 38.43 t/ha and 38.38 t/ha respectively, while CW 208-114 had the lowest value of 3.98 t/ha. It is interesting to note that the best clone for DMC, CW 208-114, was the worst performer for FRY and furthermore had one of the lowest HI values, and ended being the worst performer for DRY. It was also one of the lowest clones for ComRt, RtPlt and RtWt. Likewise, the second best performer for DMC, CW 208-108, had the lowest HI and FRY values, much lower than average and was among the worst DRY performers. These results suggest that selecting for DMC *per se* might be detrimental to total yield. Cassava root growth is normally more limited by the source or LAI, than the sink strength of the roots (Tan and Cock, 1979). El-Sharkawy *et al*. (1990) noted that yield of crops that have not been subjected to improvement for high-input agricultural systems depends on HI. Hay and Walker (1989) reported that a series of sequentially determined yield components, each of which is the result of source-sink relationships during plant development contributes to storage root yield. The relationship has to be in balance for a productive and adaptable genotype in a given environment (Cock *et al*., 1979; Cock, 1984).

Table 7.1 Table of means of clones for the BC_2 generation of an inter-specific cross evaluated over two years in CIAT-Palmira

Clone	MEANS						
	ComRt[a]	RtPlt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
CW 208-01	14.60	7.02	0.31	0.46	40.59	11.84	5.15
CW 208-03	15.58	9.72	0.25	0.38	36.47	17.19	6.33
CW 208-06	7.20	7.21	0.18	0.45	39.16	8.91	3.50
CW 208-07	16.83	16.06	0.33	0.40	37.07	38.43	14.84
CW 208-12	17.89	10.21	0.24	0.47	34.39	20.23	7.07
CW 208-14	3.00	6.08	0.15	0.37	40.25	5.50	2.24
CW 208-15	23.60	10.22	0.30	0.33	36.41	22.40	8.33
CW 208-18	19.75	9.66	0.29	0.37	38.52	18.50	7.21
CW 208-20	6.67	7.19	0.28	0.39	40.46	11.33	4.66
CW 208-29	7.75	7.33	0.19	0.48	42.06	12.75	5.40
CW 208-33	9.90	10.26	0.21	0.23	37.52	14.81	5.47
CW 208-34	10.80	7.69	0.25	0.50	37.31	14.98	5.72
CW 208-36	10.00	8.67	0.23	0.47	38.78	11.33	4.30
CW 208-38	17.11	10.22	0.34	0.42	39.59	28.04	11.12
CW 208-40	7.60	6.80	0.14	0.55	39.23	6.35	2.52
CW 208-58	20.33	9.16	0.29	0.43	42.36	23.73	10.16
CW 208-64	4.60	6.50	0.20	0.26	38.61	9.58	3.71
CW 208-65	9.00	6.72	0.27	0.27	39.43	12.28	4.94
CW 208-90	7.60	9.57	0.24	0.41	36.54	10.93	4.03
CW 208-92	17.75	12.27	0.33	0.46	42.52	23.55	10.34
CW 208-95	4.00	5.38	0.27	0.26	42.24	10.63	4.51
CW 208-106	8.92	7.88	0.26	0.41	39.04	17.78	6.95
CW 208-108	4.20	5.73	0.22	0.17	42.62	9.87	4.24
CW 208-111	3.00	5.89	0.20	0.44	41.03	8.78	3.67
CW 208-113	4.00	5.33	0.21	0.30	38.80	8.81	3.40
CW 208-114	1.75	5.08	0.12	0.28	42.73	3.98	1.70
CW 208-115	7.75	15.75	0.25	0.45	40.70	38.38	15.67
CW 208-119	2.50	7.75	0.13	0.45	40.87	8.46	3.40
CW 208-120	3.67	7.89	0.26	0.39	41.17	9.00	3.79
CW 208-124	7.50	8.08	0.24	0.37	42.04	10.18	4.39
CW 208-135	6.17	4.56	0.25	0.31	37.18	10.53	3.97
CW 208-141	6.00	6.17	0.24	0.48	40.99	10.29	4.22
CW 208-145	5.83	12.13	0.12	0.25	39.53	8.44	3.48
CW 208-152	7.58	6.90	0.18	0.31	38.65	10.69	4.02
CW 208-161	9.42	6.94	0.26	0.36	37.05	17.60	6.51
CW 208-176	8.25	9.14	0.29	0.38	41.92	17.88	7.44
CW 208-179	11.25	14.92	0.25	0.34	39.40	21.77	8.55
Mean	***9.36***	***8.48***	***0.24***	***0.38***	***39.55***	***14.75***	***5.86***
StdDev[h]	***5.71***	***2.90***	***0.06***	***0.09***	***2.11***	***8.04***	***3.20***

[a]Number of Commercial roots; [b]Roots per plant; [c]Root weight (kg); [d]Harvest index; [e]Dry matter content (%); [f]Fresh root yield (t/ha); [g]Dry root yield (t/ha); [h]Standard deviation

The extent to which either source (HI) or sink (DMC and FRY) limitations predominate at any particular stage is an indication of imbalances in the system. Based on results of the study, it might be necessary to select for DMC and HI or DMC, HI and FRY concurrently. Overall, clones CW 208-115 and CW 208-07 out-competed the rest with DRY of 15.67 t/ha and 14.84 t/ha respectively. Clones CW 208-07 and CW 208-115 also had among the highest values for all seven traits, except for ComRt in CW 208-115.

The overall mean for DRY was 5.86 t/ha, 14.75 t/ha for FRY, 39.55% for DMC, 0.38 for HI and 8.48 for RtPlt. Jaramillo *et al*. (2005), evaluating F_1s from a diallel cross of elite CIAT cultivars in the same locations reported mean DMC of 33.63% and HI of 0.47. Mean DMC reported was lower than the one reported here, while HI was higher. Cach *et al*. (2006), reporting on another F_1 cross from another set of elite CIAT cultivars in sub-humid conditions in Colombia, obtained a much lower DMC of 27.6%, a higher HI of 0.52 and a higher FRY of 37.2 t/ha. Based on results of this study, crossing with wild cassava led to an increase in average DMC. It is known that crossing with wild relatives tend to lower yield and related quality traits. Continued backcrossing to the elite parent can eliminate this problem.

Simple correlation analysis revealed DRY to be highly correlated ($P \leq 0.001$) to ComRt, RtPlt and FRY and not associated with colour of the pulp and DMC (Table 7.2). It was significantly ($P \leq 0.05$) associated with RtWt and HI. Fresh root yield followed a similar association pattern to DRY. Percentage DMC was not significantly correlated to colour of pulp, ComRt, RtWt, HI and DRY and correlated to RtPlt (P=-0.35) and FRY (P=-0.26). This indicates that selecting for DMC will have no effect on ComRt, RtWt, HI and DRY, while it is likely to have a detrimental effect on RtPlt and FRY. Phenotypic correlation between total carotene content in roots and root colour score based on the visual scale has been found to be high (P=0.86; Chavez *et al*., 2005). Results indicate that selecting for DMC will not have any effect on the carotene concentration present in roots. This is encouraging since emphasis is now being placed on increasing the protein percentage in cassava roots through selection of high carotene levels.

Table 7.2 Phenotypic correlation (means of two years) of yield traits evaluated for the BC_2 generation of an inter-specific cross evaluated in mid-altitude Valleys in CIAT-Palmira, Colombia

	Colour[a]	ComRt[b]	RtPlt[c]	RtWt[d]	HI[e]	DMC[f]	FRY[g]
ComRt	0.12 ns						
RtPlt	-0.01ns	0.50***[i]					
RtWt	0.05ns	0.60***	0.18ns				
HI	0.12ns	0.25*	0.21ns	0.23*			
DMC	-0.10ns	-0.22ns	-0.35**	-0.18ns	-0.17ns		
FRY	0.06ns	0.76***	0.69***	0.63***	0.27*	-0.26*	
DRY[h]	0.05ns	0.76***	0.66***	0.26*	0.26*	-0.15ns	0.99***

[a]Colour of the pulp; [b]Number of Commercial roots; [c]Roots per plant; [d]Root weight (kg); [e]Harvest index; [f]Dry matter content (%); [g]Fresh root yield (t/ha); [h]Dry root yield (t/ha); [i]ns=not significant; *P≤0.05, ** P≤0.01, ***P≤0.001

Negative correlation between DMC and FRY shows that the photosynthetic assimilation capacity of this population may have reached the physiological ceiling. Fresh root yield and root DMC constitute DRY, the final goal of field production (Kawano *et al*., 1998). Total photosynthesis of the crop sets the ceiling for the biomass, which is shared among HI, FRY and DMC. When there is ample genetic variation present in dry biomass, FRY and DMC can be handled largely as independent characters. As breeding advances, the capacity of the breeding population may approach the physiological ceiling of photosynthetic assimilation resulting in FRY and DMC competing for the same resource (Kawano and Takahashi, 1968). A positive association was observed between HI and DRY, FRY, ComRt, and RtWt. This indicated that HI can be used to indirectly select for yield. Strong positive correlation was noted between RtPlt and ComRt as well as RtWt and ComRt. No association was observed between RtPlt and RtWt, contrary to the negative association usually observed in crosses from elite cultivars. The root sizes in this cross were small and to increase yield selection both for increased size and number of roots is needed.

Results of linear regressions of the different yield traits against DRY are presented in Table 7.3. Dry matter content and FRY were highly significant with coefficients of 0.10 and 0.39. Root weight was significant ($P \leq 0.01$) with a coefficient of 1.31, while the rest were not significant. Stepwise regression fitted DMC, FRY and RtWt into the model at entry and exit of P=0.01 and rejected the rest, indicating that DMC, FRY and RtWt were the most important determinant of DRY. Since DRY is directly derived from DMC and FRY, RtWt could be considered as the most important independent variable. These results agree with results obtained from different populations evaluated in the same agro-ecology (Chapters 4 and 6). Results furthermore continue to show the important role of RtWt in the economic yield of cassava.

Table 7.3 Regression coefficients of yield traits regressed against dry root yield (DRY) for the BC_2 generation of an inter-specific cross evaluated in mid-altitude CIAT-Palmira, Colombia

Variable	Coefficient	Standard error	Probability
Colour	-0.01	0.03	0.60
Commercial roots	0.00	0.00	0.75
Root per plant	0.01	0.01	0.40
Root weight (kg)	1.31	0.51	0.01
Harvest index (0-1)	0.16	0.27	0.56
Dry matter content (%)	0.10	0.01	0.00
Fresh root yield (t/ha)	0.39	0.01	0.00

Analyses of variance are presented in Tables 7.4 to 7.6. Genotype was highly significant for all traits evaluated in 2005 except DMC (Table 7.4). Replication was highly significant for RtWt ($P \leq 0.001$) and significant for DMC ($P \leq 0.05$) in 2005. High significance of RtWt indicated that RtWt was highly affected by the environment. Higher broad-sense heritabilities were recorded in 2005 compared to 2006 except for DMC. The highest estimate in 2006 of 0.66 was recorded in FRY, followed by DRY with 0.64, HI

Table 7.4 Mean squares of yield related traits evaluated on a BC_2 generation of an inter-specific cross evaluated in mid-altitude CIAT-Palmira, Colombia

Source of Variation	df[a]	MEAN SQUARES						
		ComRt[b]	RtPlt[c]	RtWt[d]	HI[e]	DMC[f]	FRY[g]	DRY[h]
2005								
Genotype	36	3.49***	0.49***	0.01***	0.03***	12.10	3.42***	1.36***
Rep[i]	2	2.00	0.60	0.01***	0.01	36.32*	0.76	0.56
Error	64	0.85	0.21	0.001	0.01	8.98	0.51	0.21
CV[i]		28.01	14.73	3.54	19.45	7.23	17.86	17.55
Heritability		0.51	0.31	0.51	0.58	0.10	0.66	0.64
2006								
Genotype	36	1.36**	0.82**	0.01*	1.36**	18.83***	2.21	0.72*
Rep	2	0.11	0.35	0.01	0.11	6.75	0.45	0.11
Error	64	0.62	0.36	0.003	0.62	7.84	1.11	0.38
CV[j]		33.21	22.08	6.17	33.21	7.55	32.63	30.28
Heritability[k]		0.28	0.30	0.20	0.28	0.31	0.25	0.23

*P≤0.05, **P≤0.01, ***P≤0.001; [a]Degrees of freedom; [b]Number of commercial roots; [c]Roots per plant; [d]Root weight (kg); [e]Harvest index; [f]Percentage dry matter content; [g]Fresh root yield (t/ha); [h]Dry root yield (t/ha); [i]Replication; [j]Coefficient of variation; [k]Broad-sense heritability

Table 7.5 Mean squares from the ANOVA, combined across years for the BC_2 generation of an inter-specific cross evaluated in mid-altitude CIAT-Palmira, Colombia

Source of Variation	df[a]	MEAN SQUARES						
		ComRt[b]	RtPlt[c]	RtWt[d]	HI[e]	DMC[f]	FRY[g]	DRY[h]
Genotype (G)	36	3.50***	0.99***	0.01***	0.04***	23.63***	4.19***	1.54***
Rep[i]	2	0.46	1.42**	0.01***	0.00	24.50	0.07	0.07
Year (y)	1	44.40***	4.52***	0.26***	0.01	1026.64***	20.54***	13.34***
G x Y	36	1.13	0.38	0.004**	0.01*	8.97	1.48**	0.54**
Error	112	0.77	0.26	0.002	0.01	8.41	0.76	0.29
CV[j]		29.34	17.45	5.11	20.33	7.37	23.25	22.02
Heritability[k]		0.41	0.39	0.13	0.61	0.14	0.49	0.46

*P≤0.05, ** P≤0.01, ***P≤0.001; [a]Degrees of freedom; [b]Number of commercial roots; [c]Roots per plant; [d]Root weight (kg); [e]Harvest index; [f]Percentage dry matter content; [g]Fresh root yield (t/ha); [h]Dry root yield (t/ha); [i]Replication; [j]Coefficient of variation; [k]Broad-sense heritabilty

Table 7.6 **Analysis of variance sum of squares, combined across years, for the BC_2 generation of an inter-specific cross evaluated in mid-altitude Valleys in Valle del Cauca Department, Colombia**

Source of variation	Df[a]	SUM OF SQUARES						
		ComRt[b]	RtPlt[c]	RtWt[d]	HI[e]	DMC[f]	FRY[g]	DRY[h]
Traits	75	212.11	56.52	0.61	1.78	2249.39	224	88.41
Genotype (G)	36	123.80	36.16	0.20	1.36	834.42	137.97	50.42
Rep[i]	2	4.22	1.90	0.04	0.01	86.38	2.41	1.33
Year (Y)	1	32.52	5.06	0.24	0.004	735.87	21.51	12.84
G x Y	36	41.18	13.67	0.13	0.34	327.47	52.88	19.49
Error	110	82.99	29.66	0.20	0.65	936.23	83.48	31.22
Total	187	297.92	86.19	0.83	2.45	3192.39	310.05	120.47
SS[j] due to G		***58.37***	***63.98***	***32.79***	***76.40***	***37.10***	***64.41***	***57.03***
SS due to Y		***15.33***	***8.95***	***39.34***	***0.22***	***32.71***	***9.57***	***14.52***
SS due to G x Y		***19.41***	***24.19***	***21.31***	***19.10***	***14.56***	***23.54***	***22.05***

[a]Degrees of freedom; [b]Number of commercial roots; [c]Roots per plant; [d]Root weight (kg); [e]Harvest index; [f]Dry matter content (%); [g]Fresh root yield (t/ha); [h]Dry root yield (t/ha); [i]Replication; [j]Traits sum of squares

with 0.58, ComRt and RtWt with 0.51, with the lowest recorded in DMC of 0.10. Dry root yield and FRY estimates fell within the range obtained in Chapter 4 of 0.52 to 0.71 for DRY and 0.54 to 0.69 for FRY. The estimate for DMC was lower than obtained in Chapter 4 of 0.62 to 0.76. Dry matter content is known to have high heritability (Mahungu, 1987). However, backcrossing the F_1s from the inter-specific cross, high in DMC, to the cultivated mother, with a lower DMC, might have lowered the heritability of the trait in this particular trial. Exceptionally low heritability estimates were obtained for 2006. This could be due to unfavourable weather conditions which could have increased the influence of environmental effects.

Combined analysis over years indicated that genotype was highly significant for all traits (Table 7.5). Replication was highly significant for RtWt ($P \leq 0.001$) and RtPlt ($P \leq 0.01$). This confirmed the unstable nature of RtWt and the high environmental interference of genetic expression for RtPlt earlier observed in Chapter 4. Year was highly significant for all yield traits except HI, indicating that years had a major effect on all these traits. It furthermore suggested that HI was stable at this level of selection across years. This is in agreement with Jaramillo *et al*. (2005) who stated that at early stages of selection which are based on single plants or single-row plots, selection based on FRY is drastically affected by the environment, whereas HI is not. Indirect selection through HI is more accurate than selecting directly for FRY. Genotype by year interaction was significant at $P \leq 0.01$ for RtWt, FRY and DRY and significant at $P \leq 0.05$ for HI. No G x Y interaction was observed for ComRt, RtPlt and DMC, an indication that these traits were stable across the two years.

To weigh the relative importance of genotype, environment and G x E interaction effects in the expression of the different traits, the proportion of the crosses sum of squares explained by each effect is provided in Table 7.6. More than a half of the variation in sum of squares for ComRt, RtPlt, HI, FRY and DRY was explained by genotype, while 32.79% and 37.10% of the sum of squares were explained by genotype in RtWt and DMC. This implied that ComRt, RtPlt, HI, FRY and DRY are mainly controlled by genetic factors, and highly heritable. Root weight and DMC are moderately heritable, as

genotype explained a larger sum of squares that G x E, though the difference was smaller. This tends to agree with heritability estimates obtained earlier. Genotype by year interaction was present in all traits at low levels. The highest G x Y interaction was observed for RtPlt (24.19%) followed by FRY (23.54%) and DRY (22.05), while DMC had the lowest interaction (14.56%). The low G x Y interaction in DMC indicated that it was the most stable trait of the yield parameters evaluated. High G x Y interaction in RtPlt despite the high genotype effect suggested that although this character was mainly under genetic control its expression was also controlled by environmental factors. IITA (1990) reported that the number of storage roots per cassava plant is primarily under genetic control. However, Hay and Walker (1989) and IITA (1990) indicated that the expression of the genetic potential is in turn, controlled by a number of genetic and environmental factors like assimilate supply.

Principal component analysis was used to deduce general and detailed statements about the relative performance of genotypes. To reduce redundancy, DRY was eliminated from analysis since it is highly correlated to FRY (Iezzoni and Pritts, 1991). Eigenvalues of the first five PCs and the correlation coefficients of the yield traits with the PCs are presented in Table 7.7. Biological meaning is determined by examining the eigenvectors (weights) and correlations of original variables with PC scores (loadings; Iezzoni and Pritts, 1991). Generally, variables with large weights and loadings, either positive or negative, are considered to be contributors to the PC. In many cases, there exists a biological reason why these particular variables are related. The first four PCs had variances high enough to be considered as the main contributors and accounted for 93% of the total variation. Principal component one accounted for 50% of the variation and indicated that FRY, ComRt, RtPlt and RtWt were the main contributors and all were positively correlated. Principal component two, accounting for 16% of the variation had DMC and RtWt as the main factors, while PC3, accounting for 15% of variation had HI and RtPlt as main factors. PC4 contributed 12% of the variation and indicated that DMC, RtPlt and RtWt were the main factors. PC1 represented traits used by breeders in the initial stages of selection (seedling and clonal evaluation), while main traits in PC2 to PC4 are used in

advanced stages of selection. Combining the two sets of traits at the earliest selection stage with the use of PCA would shorten the time required to complete a breeding cycle.

Table 7.7 Principle component coefficient of the various traits with principles of the various yield related traits evaluated BC_2 generation of an inter-specific population

Trait	PC1[a]	PC2	PC3	PC4	PC5
ComRt[b]	0.49	0.25	-0.10	0.02	-0.80
RtPlt[c]	0.42	-0.39	-0.31	0.51	0.30
RtWt[d]	0.41	0.49	0.11	-0.49	0.46
HI[e]	0.24	-0.14	0.92	0.25	-0.00
DMC[f]	-0.26	0.71	0.01	0.64	0.10
FRY[g]	0.53	0.11	-0.17	0.15	0.20
Eigenvalue	2.98	0.98	0.88	0.72	0.30
Percent total					
Variance	0.50	0.16	0.15	0.12	0.05
Cumulative	0.50	0.66	0.81	0.93	0.98

[a]Principal component; [b]Number of commercial roots; [c]Roots per plant; [d]Root weight (kg); [e]Harvest index; [f]Dry matter content (%); [g]Fresh root yield (t/ha); Underlined values are loadings of the contributing traits

It would also rapidly reduce the usually huge number of genotypes cassava breeders start with to manageable sizes. Root weight and RtPlt were major contributors in three of the four main PCs while DMC was present in two PCs indicating their relative importance to yield. Results are in agreement with results obtained from a different population evaluated in the same agro-ecology (Chapter 4). It also indicated that breeders should seriously consider including RtWt and RtPlt in their selection indices when evaluating at the advanced selection stages.

Insight can often be obtained by plotting the PC scores for individual observations in relation to important PC axes. Clustering along PC axes suggests that relationships exist within individuals within a cluster. Principal component plots are shown in Figures 7.2

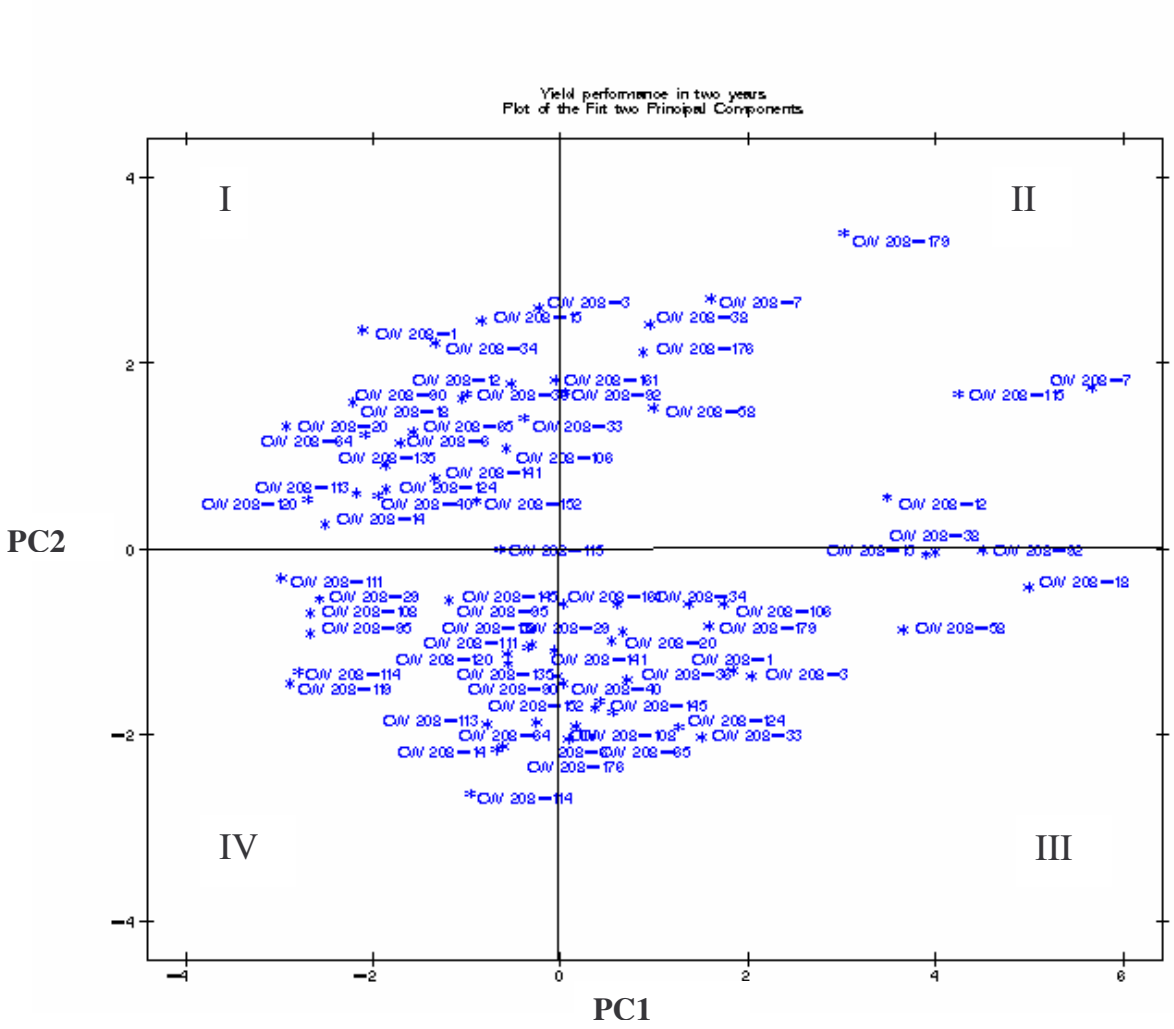


Figure 7.2 Plot of PC1 against PC2 of a BC_2 generation of an inter-specific cross between MTAI-8 and *M. tristis*

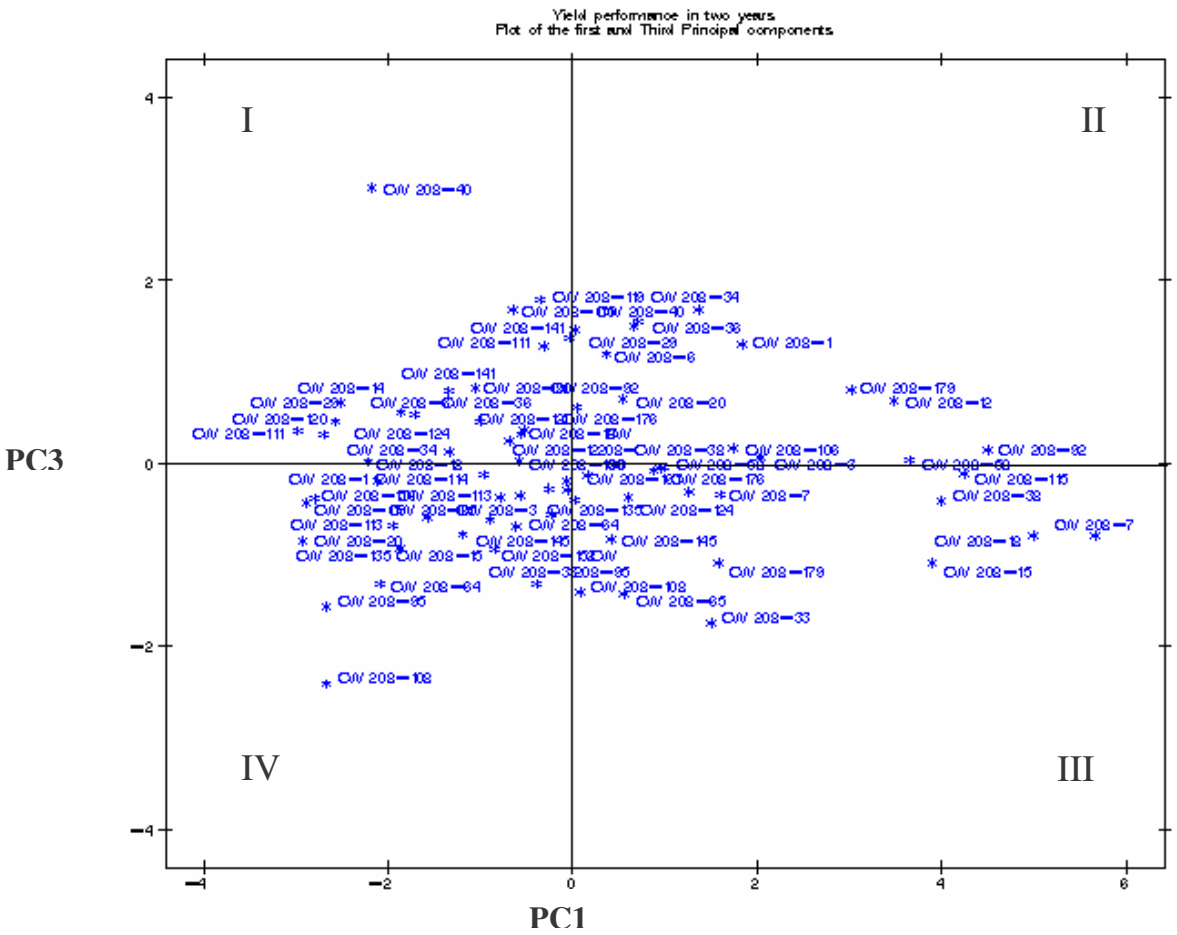


Figure 7.3 Plot of PC1 against PC3 of a BC_2 generation of an inter-specific cross between MTAI-8 and *M. tristis*

and 7.3. Figure 7.2 shows a plot of ComRt, RtPlt, RtWt and FRY (PC1) against DMC and RtWt (PC2; Table 7.7). Figure 7.3 on the other hand is a plot of ComRt, RtPlt, RtWt and FRY (PC1) against HI and RtPlt (PC3). The heavy weight of 0.92 for HI in PC3 compared to the second contributor, RtPlt with -0.31, suggested that PC1 could actually be plotted only against HI. The figures indicated that genotypes were not evenly distributed, but clustered together, a clear sign of a segregating population. In Figure 7.2 clones fell into two main groups, one above and the other below the PC2 zero point. Clones located above the zero point are of main interest, as they possess high DMC. Clones best for selection are those in quadrant two, which include CW 208-07, CW 208-115, CW 208-179 and CW 208-12. Others include CW 208-38, CW 208-176, CW 208-58, CW 208-82 and CW 208-181. These clones have high DMC and yield related traits and backcrossing to the cultivated mother should not be necessary. Clones in quadrant three and near the PC2 zero axis like CW 208-10, CW 208-12 and CW 208-18 could also be selected. Since the average of the population is above the usual DMC average, these clones could still be having DMC higher than those of cultivated clones. The rest of the clones in quadrant two could still be backcrossed to the wild relative to increase the DMC and then backcrossed to the cultivated parent. Clones in quadrant one have high DMC values but low ComtRt, RtPlt, RtWt and DRY. These clones should be backcrossed to the cultivated parent to restore these traits. Quadrant four clones low for all traits should be discarded.

Most clones clustered together, but a few like CW 208-17, CW 208-115, CW 208-179, CW 208-12, CW 208-38, CW 208-10, CW 208-32 and CW 208-18 were out-lying. It is important to note that some sort of grouping was evident, suggesting that they might be under influence of a different genetic action. It is also interesting to note that all the outliers were in quadrant two or in quadrant three, immediately below PC2 = 0, indicating their superiority; maybe under influence of “hybrid vigour”. It is an indication that after a few backcrosses it is possible to get clones with high DMC, which have retained the good yield qualities of the cultivated parent. The same pattern was observed in Figure 7.3. These clones should be monitored closely for future use.

Figure 7.3 shows clones segregating for HI and RtPlt or as earlier noted just HI. Yield of foods crops is genetically improved through the improvement of either total biomass yield or HI. In cassava improvement, emphasis was placed on HI as HI holds universal importance to yield over a wide range of environmental conditions (Kawano, 1990). During the past 30 to 40 years, significant progress has been achieved in the initial phase of the genetic improvement of cassava. This process involved assembling major traits such as improved yield mainly through a higher HI (Ceballos *et al*., 2004). Crossing of the improved variety to wild cassava, apart from introducing good DMC genes, did introduce genes with negative HI effects. However, since HI is highly heritable, backcrossing to the cultivated cassava clone should improve it. Clones in quadrant two, possessing high HI and ComRt, RtPlt, RtWt and FRY should be selected. Clones in quadrant three, having high ComRt, RtPlt, RtWt and FRY but low HI should be backcrossed to the cultivated cultivar to improve on the HI. Clones in quadrant one having high HI but performing poorly in others should be discarded as well as genotypes in quadrant four.

7.4 Conclusions

Crossing of the elite cultivar MTAI-8 to the wild relative *M. tristis* greatly improved the DMC. High DMC values ranging from 34.39 to 42.73 were recorded. The cross generated variability not only in DMC but also in other traits, which will allow selection for these traits. The crosses, however, were accompanied by some detrimental effects, most noticeable the reduction in HI. Backcrossing to the improved parent is needed to concentrate the desired traits accumulated through years of selection. It is apparent that when selecting for DMC, caution should be taken and HI and FRY should be monitored. Results indicated that it might be necessary to start considering improvement of the sink-source relationship, if a big increase in yield improvement, especially through increase in DMC is to be expected. This might be the case because the photosynthetic ceiling seems to have been reached. Cassava root growth is normally more limited by the source than the sink strength of the roots (Tan and Cock, 1979). Most breeding populations now have a large number of clones with HI close to optimum and there is little scope for further

increasing yield by selecting solely for HI (Ceballos *et al*., 2004). One possible way is to reduce HI, which has been the main target for improvement and direct emphasis to longer leaf retention, through the stay green approach (Lenis *et al*., 2006) or concentrate on improving photosynthetic efficiency through improving LAI (El-Sharkawy *et al*., 1990). It is obvious that if a substantial increase in yield has to be achieved through DMC increase, ways of improving on the source have to be reached.

Regression analysis singled RtWt, DMC and FRY as the most important contributors to DRY. Principal component analysis indicated that RtWt, RtPlt and DMC contributed most to yield, which confirmed earlier results obtained in previous chapters.

Chapter 8

Identification of molecular markers linked to dry matter content

8.1 Introduction

Cassava is a perennial starchy root crop that is widely grown in the tropical regions of the world. Half of the 16 million hectares of cultivated cassava worldwide is devoted to small-scale cassava cultivation in Africa, 30% is grown in Asia and 20% in Latin America (CIAT, 2001). Small-scale farming is characterised by cultivation using traditional methods with little or no inputs and frequent intercropping practices (Taylor *et al*., 2004).

As a major staple food crop across the tropics, cassava can serve as a cheap means of deploying adequate protein requirements amongst the poor and for feeding animals. Unfortunately cassava's starchy roots are low in protein compared to other crops. Roots contain less than 2% on dry matter basis of protein compared to 9.1% in potato. Therefore there exists a need to increase the protein content in cassava roots (Fregene and Morante, 2002). Cassava is an important source of starch, and 70-90% of cassava dry root matter consists of starch, the rest being fibre. Raw or unmodified cassava starches are increasingly important raw materials in textile, alcohol, animal and human food industries worldwide and this is expected to increase (Henry and Gottret, 1995). An increase therefore in starch DMC (equivalent to starch content) translates into higher income per unit land, and per unit labour (investment) for farmers growing cassava (Fregene and Morante, 2002).

The genetics of cassava are the least understood of any of the major staple crops that feed mankind. This is due to the heterozygous nature of cassava (Fregene *et al*., 1997; CIAT, 2004), long growing cycle of nine to 18 months (Fregene *et al*., 1997; Fregene *et al*., 2001; Kizito, 2006), and the low multiplication rate of cassava (Ceballos *et al*., 2002).

These are confounded by the limited funding the crop attracts for research (Fregene *et al*., 1997; Ceballos *et al*., 2002). Genetic markers have become fundamental tools for understanding the inheritance and diversity of natural variation. The earliest genetic markers in cassava were morphological (Graner, 1942; Hershey and Ocampo, 1989) followed by biochemical markers, such as isozymes (Hussain *et al.*, 1987; Ocampo *et al.*, 1992; Lefevre and Charrier, 1993). Over the last decade, a number of DNA markers have been developed and used in the study of cassava genes, genome and genetic diversity. Marker systems such as RFLPs, RAPDs, Sequence Tagged Sites (STS), Expressed Sequence Tags (ESTs), AFLPs, Single Nucleotide Polymorphisms (SNPs), SSRs also called microsatellites and others have been developed and applied. However, RFLP, AFLP and SSR markers stand out as the most effective in detecting polymorphism in cassava (Weising *et al.*, 2005). Due to the large amount of DNA required for RFLP detection and difficulties in automating RFLP analysis, and the dominance of AFLP markers and their requirement of high quality DNA to ensure complete restriction (Weising *et al.*, 2005), SSR analysis is the marker system of choice for cassava. SSR's are co-dominant, which is very important for a heterozygous crop like cassava.

In crops where highly heterozygous parents are used in breeding, classical genetic analysis is not sufficiently developed to explain complex genetic models (Tabor *et al*., 2000). However, technologies such as BSA enable breeders to rapidly identify genetic markers based on minimal classical genetic information (Goue-Mourier *et al*., 1996). Bulk segregant analysis has been developed as a rapid procedure for identifying markers in specific regions of the genome (Michelmore *et al*., 1991). It was initially proposed for screening qualitative traits known to express variation at a single locus of large effect (Giovannoni *et al*., 1991; Michelmore *et al*., 1991; Lynch and Walsh, 1997; Hill *et al*., 1998). However, the simplicity and low cost of BSA have led to its use for more complex traits, including traits whose genetic control is unknown (Mackay and Caligari, 2000). This method is often restricted to segregating populations, which are simplest and cheapest to produce (Mackay and Caligari, 2000). The aim of the study was to identify markers linked to DMC in different cassava populations using BSA.

8.2 Materials and methods

Three sets of populations were used for marker identification:

8.2.1 Families from the diallel experiment

A diallel experiment described in Chapter 3 was analysed and used for selecting genotypes to be used for this trial. Twenty-three families with high DMC diversity were selected for G x E analysis and DMC marker identification using BSA. Of these 23 families, two with the highest SCA (signifying highest diversity; Johnson and King, 1998) for DMC were selected for BSA. The two families GM 312 and GM 313 both had MECU 72 as parent crossed to high DMC genotypes, SM 1673-10 and SM 1741-1, respectively. Genotypes with the lowest DMC within each family were selected for the low DMC bulks and genotypes with highest DMC for the high DMC bulks (Table 8.1). Based on the distribution of DMC within the families, cut-off values for low and high DMC bulks was determined, emphasis being placed on dry matter range rather than numbers. This resulted in different sample sizes within and between families for low and high bulks. Genotypes were planted in the screen house to obtain leaf tissue for DNA extraction. Total genomic DNA was isolated using the DNA mini-prep extraction protocol based on a modified Dellaporta extraction procedure (Dellarporta *et al.*, 1983). DNA samples were prepared by drying young leaves in the oven at 55^0C overnight, followed by grinding 100 mg in an eppendorf tube with sterile white sand using a power drill fitted with a small pestle until a fine powder was obtained. The powder was suspended in 800 µl extraction buffer [100 mM Tris-HCl (tris-hydroxymethyl) amonimethane), 50 mM EDTA (Ethylenediaminetetraacetate) and 500 mM NaCl] and 1.25% (w/v) SDS (Sodium dodecylsulphate). The mixture was shaken vigorously in the buffer and placed in a waterbath at 65^oC for 15 min, samples were removed during this 15 min and vortexed intermittently. To this solution, 1.5 M of ice-cold Potassium Acetate was added and homogenised by gentle inversion 5 to 6 times. The mixture was incubated

on ice for 20 min and centrifuged at 12 000 rpm for 10 min. The aqueous solution was transferred to a new tube and the nucleic acids was precipitated by adding one volume of

Table 8.1 Individuals from the diallele experiment used to construct each bulk and their dry matter content

Family GM 312: Mother SM 1673-10, Father MECU 72			
High bulk	**DMC**	**Low-bulk**	**DMC**
GM 312-10	34.5	GM312-26	24.7
GM 312-25	34.5	GM312-29	25.5
GM 312-2	34.6	GM312-15	29.5
GM 312-28	35.0	GM312-20	30.2
GM 312-7	35.1	GM312-22	30.7
GM 312-14	35.1	GM312-11	30.9
GM 312-19	35.3	GM312-1	31.0
GM 312-3	35.7	GM312-13	31.1
GM 312-30	35.7		
GM 312-8	35.9		
GM 312-9	36.0		
GM 312-21	36.4		
Family GM 313: Mother SM 1741-1, Father MECU 72			
High bulk	**DMC[a]**	**Low bulk**	**DMC**
GM 313- 21	34.3	GM 313- 26	29.0
GM 313- 2	34.4	GM 313- 27	29.1
GM 313- 16	34.4	GM 313- 7	29.8
GM 313- 5	35.1	GM 313- 13	29.9
GM 313- 20	35.2	GM 313- 12	30.0
GM 313- 30	36.8	GM 313- 19	30.2
GM 313- 6	37.7	GM 313- 8	31.5

[a]Dry matter content (%)

ice-cold isopropanol (approximately 700 µl), and mixed by gently inverting it 8 to 10 times before incubation at –80°C for one hour, followed by centrifugation at 12 000 rpm for 10 min. The resulting supernatant was discarded and the collected pellet was re-

suspended in 500 µl of 50 mM Tris-HCl/10mM EDTA (pH 8.0). The isopropanol precipitation process was repeated and the pellet dissolved in 200 µl TE buffer (10 mM Tris-HCl/1 mM EDTA, pH 8.0) and treated overnight at 4^{o}C with 0.4 mg/ml DNase free RNase. Agarose gel electrophoresis and fluorometry were used to determine DNA quality and concentration, respectively.

After dilution to 10 ng/ml, equal volumes of DNA from each individual in a bulk was mixed and used for PCR amplification using SSR primers. Amplification reactions were carried out in 25 µl volumes containing 25 ng of target DNA, 0.25 mM of each primer, 1 X of *Taq* DNA polymerase buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.5), and 1mg/ml gelatine), 1.0 mM of $MgCl_2$, 0.5 mM of dNTPs and 0.25 U of *Taq* DNA polymerase enzyme. Temperature cycling was done on a GeneAmp PCR System 9600 (Perkin Elmer) using 1 s ramp rates. The amplification profile for all PCR reactions consisted of one polymerase activation cycle at 94^{o} C for 10 min; one DNA denaturation cycle at 95^{o}C for 4 min; 25 amplification cycles at 95^{o} C for 1 min, 55^{o}C for 2 min and 72^{o}C for 2 min and a final elongation cycle at 72^{o}C for 10 min.

Prior to loading, PCR products were mixed with 10 µl formamide dye [98% (v/v) de-ionised formamide; 10 mM EDTA, pH 8.0; 0.05% (w/v) bromophenol blue, 0.05% (w/v) xylene cyanol] and denatured by incubation for 5 min at 95^{0}C. Mixtures were immediately placed on ice. PCR products (3.0 µl) were separated on 4 % (w/v) denaturing polyacrylamide gels [19:1 acrylamide:bis-acrylamide; 7 M urea; 1 X TBE buffer (0.89 M Tris-HCl; 0.89 M Boric acid; 2.0 mM EDTA)]. Electrophoresis was performed at constant power of 120 W for approximately 2 hours. DNA was visualised using silver staining according to the manufacturer's guide (Promega).

Simple sequence repeat markers developed for cassava by Mba *et al*. (2001) were used in this study. Another 132 SSR markers were obtained from a cassava root and leaf cDNA library (Mba *et al*., unpublished data). A third set of 154 SSR markers was generated from a genomic library by Fregene *et al.* (unpublished data). A total of 600 SSR markers now exist for cassava and were the source of markers for genotyping the bulks and

respective genotypes. Bulks and parents were screened using all 600 SSR markers. Markers polymorphic and informative between parents and bulks were used to screen individuals from the bulks. Polymorphic and linked markers in the individuals from the bulks were screened on the entire population. To identify putative markers, data from screening these markers on the entire population of 45 individuals was regressed against the phenotypic percentage DMC data. Regression was done by the proc reg. function of SAS (2002). Identified putative markers SSRY 150 and SSRY 160 were screened on the other 19 families.

8.2.2 Wild crosses

Wild crosses between elite varieties and accessions of the wild progenitor of cassava *M. esculenta* ssp *flebellisbia* were made in the year 2000. The resulting F_1 were backcrossed to the elite cassava lines to form backcross 1 generations (BC_1) which were again backcrossed to form BC_2. The BC_2 families were evaluated for root quality traits and family CW 208 with high diversity for DMC was selected for BSA. Distribution of DMC in this family (Chapter 5) revealed a non-bimodal distribution suggesting that major QTLs might be involved. Dry matter was assessed on seedlings in 2003 followed by clonal evaluation in 2004. The low DMC bulk was constituted of genotypes with DMC values ranging from 19.51 to 23.88 while the high DMC bulk ranged from 35.02 to 41.94 (Table 8.2). Differences were seen between the 2003 and 2004 phenotypic data. The 2004 data was used to constitute bulks because it contained replicated data. Bulk constitution, DNA extraction, screening of SSR markers and selection of polymorphic markers were done as described earlier. A population size of 60 individuals was used.

8.2.3 Mapping population

After evaluation of the seedling nursery described in Chapter 5, two families, GM 901 and CM 9953, were selected to initiate QTL analysis using BSA. Families were selected based on high levels of diversity for DMC and each had SM 1741-1, a variety earlier found to be associated with high levels of DMC, as a parent. The crosses resulted in a

large number of individuals and were intended for mapping DMC QTLs in cassava. Bulks were constituted (Table 8.3) and analysis done as described earlier. Due to the large number of individuals per cross there was a significant difference between the DMC values in the low and high bulks in both crosses. Family GM 901's low bulk ranged from 17.7 to 24.6% and the high bulk from 36.1 to 69.1%. Family CM 9953's low bulk ranged from 16.3 to 26.9% and the high from 40.4 to 54.6%.

Table 8.2 Composition of the low and high bulks of the BC_2 population CW 208 developed from a wild cross

Low bulk		High bulk	
Genotype	DMC[a]	Genotype	DMC
CW 208-75	19.51	CW 208-106	35.02
CW 208-15	22.91	CW 208-01	35.80
CW 208-29	23.21	CW 208-115	36.03
CW 208-52	23.22	CW 208-108	37.59
CW 208-88	23.82	CW 208-09	38.94
CW 208-28	23.83	CW 208-176	39.73
CW 208-49	23.88	CW 208-182	40.38
		CW 208-92	41.94

[a]Dry matter content (%)

8.3 Results and discussion

8.3.1 Diallel families

None of the primers tested were polymorphic between the bulks and parents of family GM 312. Thirty-one primers were polymorphically linked in the bulks and parents from GM 313 and were tested on all genotypes of the bulks. Of these, 11 primers, NS 701, NS 717, NS 781, NS 80, NS 9, NS 909, NS 917, NS 955, SSRY 150, SSRY 160, SSRY 88 and NS 371 detected linked polymorphisms. These primers were screened on 60 individuals of the family. A simple regression was done between the respective DMC and polymorphic fragment patterns to detect associations. Only SSRY 150 (R^2=18.1) and

Table 8.3 Composition of bulks used for marker identification in families GM 901 and CM 9953

GM 901				CM 9953			
Low bulk		High bulk		Low bulk		High bulk	
Clone	**DMC[a]**	**Clone**	**DMC**	**Clone**	**DMC**	**Clone**	**DMC**
GM 901-03	**21.2**	**GM 901-01**	**36.9**	**CM 9953-33**	**24.6**	**CM 9953B-12**	**40.5**
GM 901-40	**22.9**	**GM 901-17**	**39.0**	**CM 9953-83**	**26.8**	**CM 9953B-14**	**42.8**
GM 901-44	**24.6**	**GM 901-23**	**36.2**	**CM 9953B-03**	**22.9**	**CM 9953B-34**	**54.6**
GM 901-121	**17.7**	**GM 901-59**	**36.1**	**CM 9953B-06**	**26.3**	**CM 9953-38**	**40.4**
GM 901-152	**22.9**	**GM 901-67**	**38.0**	**CM 9953B-18**	**23.9**	**CM 9953-71**	**40.7**
GM 901-158	**24.5**	**GM 901-72**	**36.3**	**CM9953B-28**	**26.4**	**CM 9953-79**	**42.1**
GM 901-173	**22.2**	**GM 901-86**	**36.5**	**CM 9953B-30**	**16.3**	**CM 9953-80**	**44.2**
GM 901-180	**23.5**	**GM 901-92**	**36.1**	**CM 9953B-33**	**26.7**	**CM 9953-81**	**48.0**
GM 901-193	**22.0**	**GM 901-94**	**36.3**	**CM 9953B-37**	**26.4**	**CM 9953-87**	**43.8**
GM 901-195	**22.7**	**GM 901-97**	**52.1**	**CM 9953B-52**	**22.5**	**CM 9953-88**	**40.7**
GM 901-202	**23.3**	**GM 901-104**	**37.1**	**CM 9953B-58**	**25.4**	**CM 9953-94**	**43.6**
GM 901-234	**22.9**	**GM 901-106**	**37.9**	**CM 9953B-62**	**20.2**	**CM 9953-95**	**49.1**
GM 901-258	**18.3**	**GM 901-119**	**39.0**	**CM 9953B-66**	**26.9**	**CM 9953-98**	**41.2**
GM 901-299	**21.9**	**GM 901-120**	**36.9**	**CM 9953B-67**	**25.5**	**CM 9953-103**	**40.6**
GM 901-301	**24.3**	**GM 901-123**	**39.1**	**CM 9953B-76**	**19.5**	**CM 9953-104**	**40.5**
GM 901-313	**25.0**	**GM 901-125**	**38.5**	**CM 9953B-78**	**19.5**	**CM 9953-105**	**42.7**
GM 901-315	**17.7**	**GM 901-129**	**36.5**	**CM 9953B-83**	**25.4**	**CM 9953-106**	**46.7**
GM 901-322	**22.8**	**GM 901-270**	**69.1**	**CM 9953B-90**	**25.8**	**CM 9953-107**	**50.5**
GM 901-333	**24.6**	**GM 901-282**	**46.5**			**CM 9953-108**	**49.1**
		GM 901-283	**39.7**			**CM 9953-109**	**44.0**
		GM 901-297	**37.3**			**CM 9953-118**	**46.0**
						CM 9953-120	**47.6**

[a]Dry matter content (%)

SSRY 160 (R^2=29.3) showed association with the trait in family GM 313 with values high enough for practical use in a breeding programme. These two markers explained 47.4% of the total phenotypic variation in GM 313. Since both parents for GM 313 had high DMC values, detection of low levels of polymorphism using BSA was expected. Markers with smaller R^2 values might therefore be linked to smaller or minor QTLs, which might determine the difference in DMC values between parents.

Screening the other 19 selected families from the diallel experiment with markers SSRY 160 and SSRY 150, earlier observed to be associated with DMC in the family GM 313, revealed association with the trait in other families that had SM 1741-1 as parent. This revealed a strong genetic background-specific association between marker SSRY 160 and DMC (Table 8.4). Family GM 269, a cross between SM 1278-2 and SM 1741-1, had a R^2 of 25.7% for marker SSRY 160, and GM 284, a cross between SM 1636-24 and SM 1741-1, had a R^2 of 28.9%. This suggests that this marker is associated with a favourable allele for DMC found in SM1741-1, and can be used in MAS for DMC in crosses having a common background with SM 1741-1. It might be worth noting that although low correlation values were obtained from screening SSRY 150 on all families, the highest R^2 value was obtained in GM 313 having SM 1741-1 as a parent. This further confirms that SM 1741-1 has favourable QTLs for DMC.

Although sample sizes used for this experiment were not large enough to give conclusive results, a number of issues were identified. Selection of crosses based on performance *per se* or traditional breeding methods like diallel analysis alone does not necessarily result in an ideal population for DMC molecular marker analysis. Families GM 312 and GM 313 both segregated for DMC with big differences in segregation values but this segregation did not translate to genotypic segregation. Families GM 312 and GM 313 had phenotypically contrasting parents but genotypic analysis only detected polymorphism between parents of family GM 313. During a MAS study, possible parental lines should be genotyped first before parents are selected for crosses. A marker linked to the trait in one population can not necessarily be applied to another population, especially if the background is different. This problem is usually encountered when working with quantitative traits like DMC.

Table 8.4 **Simple regression coefficients of dry matter content against SSR markers SSRY 150 and SSRY 160 in 20 families obtained from a diallel cross**

Family	Mother	Father	R^2 (SSRY[a] 150)	R^2 (SSRY 160)
GM 257	SM 1219- 9	SM 1636- 24	17.2	5.3
GM 260	SM 1219- 9	SM 1673- 10	ND [b]	12.0
GM 265	SM 1219- 9	MPER 183	4.8	0.5
GM 268	SM 1278- 2	SM1673- 10	4.2	12.6
GM 269	SM 1278- 2	SM 1741- 1[c]	3.2	25.7
GM 283	SM 1636- 24	SM 1673- 10	1.5	ND
GM 284	SM 1636- 24	SM 1741- 1	9.8	28.9
GM 285	SM 1636- 24	HMC 1	ND	0.6
GM 286	SM 1636- 24	MPER 183	12.1	11.7
GM 293	SM 1673- 10	HMC 1	0.3	2.8
GM 294	SM 1673- 10	MPER 183	0.1	5.3
GM 306	MECU 72	MPER 183	0	8.1
GM 309	MECU 72	SM 1219- 9	6.0	2.3
GM 310	MECU 72	SM 1278- 2	0.8	11.4
GM 311	MECU 72	SM 1636-24	0.6	0.4
GM 313	MECU 72	SM 1741- 1	18.1	29.3
GM 314	MECU 72	HMC 1	0.4	10.2
CM 9642	CM 6740- 7	MPER 183	6.3	1.8
CM 9733	HMC 1	MPER 183	6.6	0.5
CM 9901	CM 6740- 7	SM 1219- 9	7.7	ND

[a]Simple sequence repeat yucca; [b]Not determined; [c]Parent of interest

Results confirmed those obtained from the diallel, seedling and clonal analysis which indicated that clone SM 1741-1 is associated with favourable genes for a number of traits. It should in future be used more often as a parent. Unfortunately it is being phased out of the CIAT breeding programme because of its susceptibility to CSFD. There exists a need for CIAT to preserve this clone, if only for breeding purposes.

8.3.2 Wild crosses

Fifty-five primers amplified putatively polymorphic fragments between the extreme bulks in the wild population. Of these, 17 detected informative polymorphisms between genotypes of the bulks. To determine putative markers, phenotypic DMC data was regressed on marker fragment patterns from evaluating the 17 markers on 60 individuals of the CW 208 family. However, due to apparent differences between the 2003 (seedling data) and the 2004 (clonal data), both data sets were used for regression analysis (Table 8.5). The 2003 phenotypic data identified SSRY 99, SSRY 141 and NS 169 with R^2 values of 22.68, 35.89 and 20.01, respectively and the 2004 data SSRY 11 (R^2=26.85) as putative markers. The phenotypic variance explained by these markers based on their regression coefficient, is high enough to be considered as markers for MAS. Total phenotypic variation explained by putative markers for 2003 was 78.6% and for 2004 26.9%. This is a high value and indicates that the markers may be associated with major QTLs. Figure 8.1 shows a typical PCR amplification pattern separated on a polyacrylamide gel obtained from screening the population with the polymorphic marker SSRY 11. The polymorphic fragment detected between the parents and bulks also segregated in the individuals constituting the bulks.

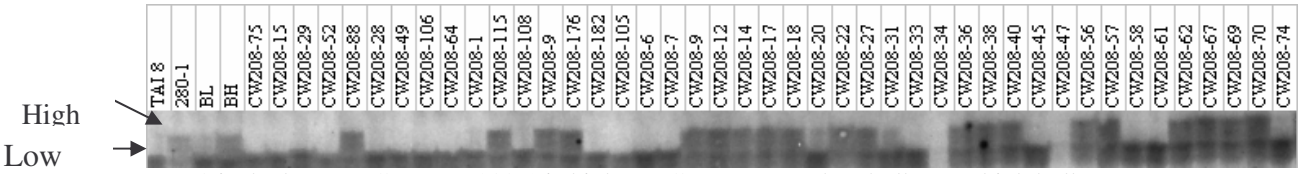
Phenotypic data from the two years resulted in different ranking of the markers. Simple correlation analysis between the two data sets revealed a low value of 7.8%. These different rankings might be either due to the fact the 2003 data was from seedlings which have a tap root system, which is likely to differ in DMC composition from the normal tuberous roots from cuttings of the clones in 2004 or due to the presence of CFSD in 2004 and not in 2003. However, correlation of data from a bigger population of 979 genotypes (Chapters 6 and 7) has shown that seedling and clonal DMC data were highly correlated. The differences might therefore be attributed to CFSD. Cassava frogskin disease, a disease whose biology is yet to be understood, is a real threat to cassava production in Latin America. The disease mainly affects the roots, greatly reducing and at times entirely destroying the whole tuber. Obtaining accurate phenotypic DMC data in a CFSD affected trial is difficult. The disease pressure builds with advancement of the selection stages, usually with the seedlings being almost free, since the main transmission is due to infected vegetative planting material. The other transmission media is soil, so

Table 8.5 Regression coefficients of polymorphic markers' dry matter content phenotypic data of the BC_2 population CW 208

Marker	R^2 values (2003 data)	R^2 value (2004 data)
SSRY 298	0.34	7.42
SSRY 306	2.7	7.74
C306R	0.6	7.64
C313	5.72	3.02
SSRY 99	22.68	0.07
SSRY 141	35.89	0.09
SSRY 11	1.19	26.85
SSRY 23	10.22	0
SSRY 27	7.1	16.6
SSRY 36	10.52	5.15
SSRY 47	1.91	9.97
SSRY 49	2.89	3.18
SSRY 54	6.9	15.37
SSRY 57	5.7	0.11
SSRY 60	0	6.66
SSRY 66	6.7	4.2
SSRY 75	1.5	7.9
NS 169	20.01	7.40

stations where cassava is continuously grown become hot spots for the disease. The main reason for transferring the seedling trial (Chapter 5) to ICA-Palmira and the clonal trial (Chapter 6) to Palmira-CIAT was to avoid the hotspot in Quilichao. Phenotypic data analysis has shown that the effect of CFSD is affected by year (Chapter 4). Developing markers for CFSD would be of great help to breeders in obtaining the true phenotypic nature of genotypes being evaluated. DMC marker trials should be conducted, if possible, in CFSD-free areas to obtain accurate phenotypic data.

Few traits in cassava hold potential for increasing cost-effectiveness of introgressing useful traits from wild relatives of cassava via MAS as DMC (CIAT, 2003). Dry matter content is measured at the end of the growth cycle and influenced by the time of evaluation. It is generally high before the on-set of rain but drops after rain begin since the plant mobilises



TAI 8 is the low DMC parent; 280-1 is high DMC parent; BL=low bulk; BH=high bulk

Figure 8. 1 Silver stained polyacrylamide gel showing PCR amplification using primer SSRY 11 on parents, bulks and individuals constituting the bulks in the BC_2 population CW 208.

starch from the roots for re-growth of leaves (Bryne, 1984). A tool to early and accurately evaluate DMC can increase the cost-effectiveness of breeding for DMC. For several years now it has been shown that the "tremendous genetic potential" locked up in germplasm banks can be released by shifting the paradigm from searching for phenotypes to searching for superior genes using molecular genetic maps and an advanced backcross QTL (ABC-QTL) mapping scheme (Tanksley and McCouch, 1997). Several wild relatives of cassava are known to possess up to 15% protein and more than 50% DMC in their roots. These germplasm resources are a useful source of genes for the improvement of protein in cassava. Reports of crosses between cassava and *M. tristis* revealed root protein content of higher than 8% in F_1 hybrids (Bolhuis, 1953; Asiedu *et al*., 1992). Unfortunately the high protein content was lost during backcrossing trying to recover the desired agronomic characteristics and high root yield of cassava (Asiedu *et al.,* 1992). With the advancement of molecular markers, the genes can be tagged after it has been transferred to the desired genotype through genetic transformation or conventional breeding. Results from this study indicated that markers SSRY 11 and SSRY 141 would be useful in tracing high DMC content in backcrossing programmes using *M. esculenta* ssp *flebellisbia* as a parent. It might also be necessary to screen other inter-specific populations produced from other wild relatives with markers SSRY 11 and SSRY 141 to see if they can be applicable to them too.

8.3.3 Mapping population

Screening of SSR markers on the parents and bulks revealed that 110 markers were informative for family GM 901 and 13 for CM 9953. These markers were screened on the individuals comprising the bulks. None was informative among the constitutes of the bulks in family CM 9953 and 10 in family GM 901. These 10 primers were screened on the entire population of 325 genotypes. The genotypic data was regressed against the phenotypic data (Table 8.6) and three markers which could qualify for MAS were obtained, explaining 113.9% of the total phenotypic variation. Marker SSRY 62 explained 50.21% of the variation, SSRY 11 34.57% and NS 644 29.09%. Since these markers possess high regression values they should be promising for MAS. The next step will be to map these markers to the existing cassava map, with the objective of tagging the genes/QTLs responsible for high DMC and to determine if these markers are linked QTLs on different chromosomes or one the same chromosome and the cM distance from these genes/QTLs. In the past decade many research institutes and breeding companies started using molecular markers and MAS to increase the effectiveness of selection in breeding to shorten the development time of varieties (Ribaut and Hoisington, 1998).

Due to the lack of detected polymorphism in family CM 9953, the phenotypic information of the parents was investigated. It is important to note that selection of parents for the present crosses was based on the offspring performance and not on genotype *per se.* This was because the approach was from the breeding point of view, and diallel analysis was used for selecting parents (Chapter 3) as part of CIAT's breeding programme. Phenotypic DMC data of the parents for CM 9953 revealed no differences. Parent SM 1741-1 had a DMC of 35.0% while parent SM 1219-9 had a DMC of 34.2% as opposed to parents from the family GM 901, which had parent SM 1741-1 with 35.0% and MPER 183 with 30.1% DMC. This is clearly illustrated in Figure 2. Although SM 1741-1 and SM 1219-9 produced siblings highly segregating for DMC, as individuals they did not differ for DMC. This emphasises the fact that parents should be screened for polymorphisms before they are used in crosses for molecular marker work, and that they should be phenotypically different for the trait of interest. Results confirm the

effectiveness of the BSA strategy. Parents might have been genetically different for other traits, but this was not detected, since the bulks were constructed to target DMC genes/QTLs.

Table 8.6 Simple regression coefficients of polymorphic markers' dry matter content phenotypic data of the mapping population GM 901

Marker	R^2
SSRY 11	**34.57**
SSRY 62	**50.21**
SSRY 68	**4.76**
NS 207	**4.11**
NS 347	**0.68**
NS 531	**0.70**
NS 644	**29.09**
NS 664	**0.66**
NS 905	**3.34**
NS 909	**1.15**

8.4 Conclusions

Cassava is an ideal crop to demonstrate the beneficial impact of biotechnology tools. Due to its long gestation period biotechnology would help in shortening the breeding cycle. Use of BSA would help in understanding cassava's genetics, where classical breeding is proving difficult because of the crops heterozygous nature. Traditionally envisioned as a subsistence crop with little potential as a cash crop for industrial purposes, cassava now offers a wide range of industrial uses (Ceballos *et al*., 2002).

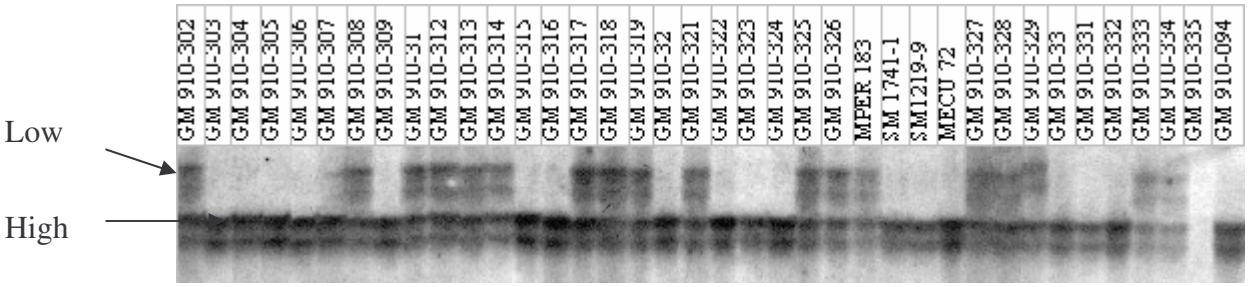


Figure 8. 2 Silver stained polyacrylamide gel showing PCR amplification using primer SSRY 11 on parents of families GM 901 (SM 1741-1 high and MPER 183) and CM 9953 (SM1741-1 and SM 1219-9 both high) and individuals of GM 901, with MECU 72 as check.

Governments and processing sectors are showing increased interest in cassava. The crop offers some remarkable advantages, namely its ability to grow in marginal environments along with its competitive and reliable productivity. Unfortunately it has limitations such as low multiplication rate, short shelf life, and a long breeding cycle. Biotechnology tools offer great benefits for overcoming some of the inherent biological limitations of this crop. Low multiplication rates can be overcome by tissue culture techniques**,** and molecular markers are fundamental for speeding up the otherwise slow breeding process. Genetic transfer using traditional breeding methods from one clone to another is cumbersome and does not allow the full recovery of the recipient variety. However, genetic transformation will greatly facilitate transferring genes between *M. esculenta* and its wild relatives or other species' gene pools. Because of low level involvement of the private sector and its relevance in poor areas, cassava is a perfect crop for improving the public awareness on the potential benefits of biotechnology tools. Marker-assisted selection, used for breeding CMD resistance, has successfully been applied for introducing resistance into elite gene pools at CIAT (Fregene *et al.*, 2004; CIAT, 2003) and to introgress resistance to CGM and CMD in local Tanzanian varieties (Kullaya *et al.*, 2004).

With the discovery of markers, it is inherent that these should be mapped to the cassava linkage map and verified in different genetic backgrounds. There also exists a need to test

these markers in other populations outside the CIAT germplasm and in different environments to see if they are applicable. Currently markers developed at CIAT for CMD are being tested in a number of national programmes in Africa. It would be interesting to do the same for the putative DMC markers. Marker SSRY 11 is of interest since it has been found to be associated with DMC in two different populations of completely different backgrounds.

The discovery of different markers associated with DMC in different population suggests that several genes/QTLs may be controlling DMC in the different populations. Furthermore the discovery of different markers in the same population may also indicate that several genes/QTLs may be in play within a given population. Kizito *et al*. (2006), working with CIAT lines discovered six QTLs on two different linkage groups associated to DMC. It would be interesting to investigate this further. The high R^2 values detected in this study indicate that may be a few major genes/ QTLs are involved. If this is true, then fixing them would be possible. From this study it is apparent that the size of the population greatly affects the results of the QTL experiments. Larger population sizes detected putative markers with higher R^2 values compared to smaller populations. Although there is still a lot to be done, results are promising.

Chapter 9

General conclusions and recommendations

Cassava, being a crop of the tropics, has not benefited from advances in science as temperate crops like wheat or rice. The nature of the crop also complicates research. It is heterozygous and takes up to 10 years to complete a breeding cycle. The crop is lacking in many nutrients and this is compounded by the fact that germplasm lack diversity for major traits like HI, DMC, root carotene, and PPD required for crop improvement. There is therefore a need to improve the existing breeding programme and/or resort to non-conventional methods like inter-specific crosses or use of molecular markers for sustained improvement of the crop.

Diallel crosses were evaluated to generate material for the study of a QTL identification programme for root yield quality and to select families for BSA. At the same time an inter-specific cross was evaluated to assess the effect of introgressing DMC genes from a wild relative. High positive and negative GCA and SCA values were estimated for the agronomic yield traits. Harvest index and DMC were under the influence of additive gene action, while number of commercial roots, FRY and DRY were influenced both by additive and non-additive effects. This implies that HI and DMC are highly heritable and should be used early in the cassava breeding programme for indirect selection of yield.

Evaluation over environments indicated that traits under evaluation were relatively stable within environments, reflecting the fact that populations were developed from parents adapted to the specific agro-ecologies. High heritability estimates were obtained indicating that simple phenotypic selection would be effective for DMC, HI, FRY, DRY and RtPlt, with the exception of RtWt.

Results indicated that it is possible to select simultaneously for yield and quality characteristics at early stages of a cassava breeding programme. This can be done using a

selection index which should include additional to traits like FRY, HI and DMC already included in the selection index, RtWt and RtPlt. Based on results obtained from the seedling, CET and wild cross trials indicating the relative importance of Rtwt, it should be given a bigger weight. Results from seedling, CET and wild cross trials furthermore indicated that DMC and HI have reached their optimum potential in existing germplasm and cannot be improved further through conventional breeding. Breeders should shift emphasis from HI and DMC as the primary selection indices to other yield components like RtWt and RtPlt. Selection for stay green as an alternative to selecting for high HI has been suggested. Lack of variability in carotene levels and PPD among elite germplasm is worrying, especially since commercialisation is currently emphasising value addition, especially addition of carotene levels and increased shelf life. The alternative is to revert to wild relatives. Fortunately, selection for carotene should be easy since it is highly correlated with yellow colour, though this presents a problem of acceptability. CIAT is involved in an aggressive programme to incorporate higher levels of carotene from wild relatives. It is imperative that as this is going on, farmers and end-users are sensitised on the advantages of yellow cassava.

Application of the improved cassava breeding programme, employing replication and blocking of clones at early stages, improved the accuracy of the data. Cassava breeders should design programmes which enable them to collect data as early as at seedling or clonal stage and use the data for proper selection of clones and selecting parents for future crosses. Breeders should at early stages opt for replicated trials with smaller sample sizes. Future research though should investigate the smallest plot size which will give meaningful results in a replication.

High correlation values between seedling and clonal data were contrary to what is reported in literature. This could be as a result of replication or of planting seeds in pots, where development of a taproot is inhibited. The other explanation could be the reduction in inter-genotypic and increase intra-genotypic competition to allow full expression of genotypes, affected by increasing between row and reducing within row spacing which was practiced during the SRT trial. There is need to investigate this further.

Crossing of the elite cultivar MTAI-8 to the wild relative *M. tristis* greatly improved DMC. The cross generated variability not only in DMC but also in other yield related traits. Crosses were, however, accompanied by some detrimental effects, specifically noted in HI. Backcrossing to the improved parent will be needed to concentrate the desired traits accumulated through years of selection. The alternative is to use molecular markers to tag and introduce genes/QTLs to them to create desired genotypes. It is interesting to note that a few of the crosses did not show normal segregation for yield related traits, where outliers were superior. It is possible that some gene action may be in play and we may identify some genotypes not requiring backcrossing. These genotypes should be investigated further.

Cassava is an ideal crop for showing the beneficial impact of biotechnology tools. Due to cassava's long gestation period, biotechnology can help in shortening the breeding cycle. With the discovery of markers linked to DMC- SSRY 160, SSRY 11, SSRY 62, SSRY 141, SSRY 99, NS 169 and NS 644, it is inherent that these should be mapped to the cassava genome and verified in different genetic backgrounds. There also exists a need to test these markers in other populations outside the CIAT germplasm and in different environments to see if they are applicable in breeding programmes.

The discovery of different markers associated with DMC in different populations suggests that several genes/QTLs maybe controlling DMC in the different populations. Furthermore, the discovery of different markers in the same population may also indicate that several genes/QTLs may be involved within a given population. The high R^2 values detected during this study indicate that a few major genes/ QTLs are involved. If this is true, fixing using traditional breeding should be possible.

Results from this study indicated that cassava breeders at CIAT and breeders worldwide should include RtWt and RtPlt during early selection stages if cost and time-effective advances in improving yield are to be made. Furthermore, results indicated that higher demands placed on breeding programmes by developing end-users for improved DMC/yield can be met through the application of both inter-specific crosses and markers linked to DMC genes/QTLs.

References

Adugna, W. and T. Labuschagne, 2002. Genotype-environment interactions and phenotypic stability analyses of linseed in Ethiopia. *Plant breeding* **121**: 66-71.

Agrobase, 2000. Agronomix, 71Waterloo St. Winnipeg, Manitoba R3NNoS$, Canada.

Agyare-Tabbi, A., L.F. Pereira and L.R. Erickson, 1997. Isolation and characterisation of repetitive and microsatellite DNA sequences in cassava. *African Journal of Root and Tuber crops* **2**: 135-137.

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

Allem, A.C., 2002. The origin and taxonomy of cassava. In: Hillocks, R. J., M.J. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, production and utilisation.* CABI International, Oxford. pp. 1-16.

Allem A.C. and S.K. Hahn, 1991. Cassava germplasm strategies for Africa. In: Ng. N.Q., P. Perrino, F. Attere and H. Zedan (eds.). *Crop genetic resources of Africa* vol. II. IITA, Ibadan pp. 127-149.

Alves, A.A.A., 2002. Cassava botany and physiology. In: Hillocks, R. J., M.J. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, production and utilisation.* CABI International, Oxford. pp. 67-89.

Amma, C. S. E., M.N. Sheela and P.K.T. Pillai, 1995. Combining ability heterosis and gene action for three major quality traits in cassava. *Journal of root crops* **21**: 24-29.

Angel, F., F. Giraldo, R. Gomez, C. Iglesias, J. Thome and W. Roca, 1993. Construction of a detailed map of cassava. In: *Proceedings CBN*. CIAT 1992, Colombia. pp. 90.

Asiedu, R., K.V. Bai, R. Terauchi, A.G.O. Dixon and S.K. Hahn, 1992. Status of wide crosses in Cassava and Yam. In: Thotttapily, G. (ed.). *Biotechnology; enhancing*

research on tropical crops in Africa: Proceedings of an international conference held at the International Institute of Tropical Agriculture, 26-30 November 1990, IITA, Ibadan, Nigeria.

Bai, K.B.V., 1982. Cytogenetics of tuber crops. In: IITA (International Institute of Tropical Agriculture) Annual report for 1982, Ibadan Nigeria.

Baird, W.V., R.E. Ballard, S. Rajapakse and A.G. Abbott, 1996. Progress in *Prunus* mapping and application of molecular markers to germplasm improvement. *Horticultural Science* **31**: 1099-1106.

Basford, K.E. and M. Cooper, 1998. Genotype x environmental interactions and some considerations of their implications for wheat breeding in Australia. *Australian Journal of Agricultural Research* **49**: 154-174.

Beadle, C.L., S.P. Long, S.K. Imbaba, D.O. Hall and R.J. Olembo, 1985. Photosynthesis in relation to plant production in terrestrial environments. Natural Resources and the environmental series. Vol.18. United Nations environment program/Tycooly publishing, Oxford, Great Britain.

Beavis, W.D. and D. Grant, 1991. A linkage map based on information from four F_2 populations of maize (*Zea mays* L.). *Theoretical and Applied Genetics* **82**: 636-644.

Becker, H.C. and J. Leon, 1988. Stability analysis in Plant Breeding. *Plant Breeding* **101**: 1-23.

Beeching, J.R., H. Yuanhuai, R. Gomez-Vazquaz, R.C. Day and R.M. Cooper, 1998. Wound and defense responses in cassava as related to post-harvest physiological deterioration. In: Romeo, J.T., K.R. Downum and R.Verpporte (eds.). *Recent Advances in Phytochemistry Vol. 32. Phytochemical Signals in Plant-microbe Interactions***.** Plenum Press, New York. pp. 231-248.

Beeching, J.R., P. Marmey, M.A. Hughes and A. Charrier, 1994. Evaluation of molecular markers and approaches for determining genetic diversity in cassava germplasm. In: Roca, W. and A.M. Thro (eds.). Working Document No. 150. *Proceedings of the Second International Scientific Meeting for the Cassava Biotechnology Network,* 22-26 August 1994. Bogor, Indonesia. **1:** 80-89.

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

Benesi, I.R.M., 2002. *Native starch evaluation, and analysis of genetic distance using AFLP of elite cassava (Manihot esculenta Crantz) genotypes from Malawi*. MSc Thesis in Plant Breeding, Department of Plant Sciences (Plant Breeding) Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa. 148 pp.

Benesi, I.R.M., 2005. *Characterisation of Malawian cassava germplasm for diversity, starch extraction and its native and modified properties.* PhD Thesis in Plant Breeding, Department of Plant Sciences (Plant Breeding) Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa. 217 pp.

Bidinger, F.R., G.L. Hammer and R.C. Muchaw, 1996. The physiological basis of genotype by environment interaction in crop adaptation. In: Cooper M. and G.L. Hammer (eds.). *Plant adaptation and crop improvement*. Wallingford, UK, CABI. pp. 329-347.

Blears, M.S., S.A. De Grandis, H. Lee and J.T. Trevors, 1998. Amplified fragment length polymorphism (AFLP): A review of the procedure and its applications. *Journal of Industrial and Microbial Biotechnology* **21**: 99-114.

Boerboom, B.W.J., 1978. A model of dry matter distribution in cassava. *Agricultural Science* **26**: 267-277.

Bokanga, M., 1994. Distribution of cyanogenic potential in the cassava germplasm. *Acta Horticulturae* **375**: 117-123.

Bolhuis, G.G., 1953. A survey of some attempts to breed Cassava with a high content of proteins in the roots. *Euphytica* **2**: 107-112.

Bonierbale, M., C. Guevara, A.G.O. Dixon, N.Q. Ng, R. Asiedu and S.Y.C. Ng, 1997. Cassava. In: Fuccillo, D., L. Sears and P. Stapleton (eds.). *Biodiversity in trust conservation and use of plant genetic resources in CGIAR centres.* Cambridge University press. pp. 1-20.

Bonierbale, M., C. Iglesias and K. Kawano, 1995. Genetic resources management of cassava at CIAT. In: MAFF, International Workshop on Genetic Resources: Root and Tuber Crops. MAFF, Tsukuba, Japan, pp. 39-52.

Bonierbale, M.W., R.L. Plaisted and S.D. Tanksley, 1988. RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics* **120**: 1095-1103.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic map in man using restriction fragment length polymorphism. *American Journal of Human Genetics* **32**: 314-330.

Brettschneider, R., 1998. RFLP analysis. In: Karp, A., P.G. Isaac and D.S. Ingram (eds.). *Molecular tools for screening biodiversity*. Chapman and Hall, London. pp. 83-95.

Brewbaker, J.L., M.D. Upaddhya, Y. Mäkinen and T. McDonald, 1968. Isozyme polymorphism in flowering plants. III. Gel electrophoretic methods and applications. *Physiologia Plantarum* **21**: 930-940.

Bryne, D., 1984. Breeding cassava. In: Janick, J. (ed.). *Plant Breeding Reviews*. Westpoint. pp. 73-134.

Buerno, A., 1986. Evaluation of cassava cultivars to select parents for cross breeding. *Revista Brasileira de Mandioca* **5**: 23-54.

Buerno, A., 1987. Hybridization and breeding methodologies appropriate to cassava. In: Hershey, C.H. (ed.). *Cassava breeding: a multidisciplinary review*. Proceedings of a workshop in Philippines, 4-7 March, 1985. CIAT, Cali, Colombia. pp. 51-66.

Burr, B., S.V. Evola, F.A. Burr and J.S. Beckmann, 1983. The application of restriction fragment length polymorphism to plant breeding. In: Setlow, J.K. and A. Hollaender (eds.). *Genetic engineering principles and methods*. Plenum Press, London. pp. 45-49.

Buso, G.S.C., L.J.C.B. Carvalho and N.M.A. Nasser, 1994. Analysis of genetic relationships among *Manihot* interspecific hybrids and their parental species, using RAPD assay. In: Roca, W. and A.M. Thro. (eds.). Working Document No.

150. *Proceedings of the Second International Scientific Meeting for the Cassava Biotechnology Network*, 22-26 August 1994. Bogor, Indonesia **1**: 101-105.

Byth, D.E., 1981. Genotype x environment interaction and environmental adaptation – an overview. In: Nyth, D.E. and V.E. Mungomery (eds.). *Interpretation of plant response and adaptation to agricultural environments*. Australian Institute of Agricultural Sciences, Queensland Branch, pp. 2-10.

Cach, N.T., J.I. Lenis, J.C. Pérez, N. Morante, F. Calle and H. Ceballos, 2006. Inheritance of useful traits in cassava grown in sub-humid conditions. *Plant breeding* **125**: 177-182.

Caetano-Anolles, D., B.J. Bassam and P.M. Gresshoff, 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnology* **9**: 553-557.

Calle F., H. Ceballos, G. Jaramillo, J.I. Lenis, J. López, N. Morante and J.C. Pérez. 2002. Results in yield improvement of Cassava at CIAT**.** In: Taylor, N.J., F. Ogbe and C.M. Fauquet (eds.). *Cassava, An Ancient Crop for Modern Times Food, Health, Culture*. Proceedings of Fifth International Scientific Meeting of the Cassava Biotechnology Network (Abstracts). Danforth Plant Science Center St Louis, Missouri USA S6-05.

Calle, F., J.C. Pérez, W. Gaitan, N. Morante, H. Ceballos, G. Llano and E. Alvarez, 2005. Diallel inheritance of relevant traits in cassava (*Manihot esculenta* Crantz) adapted to acid-soil savannas. *Euphytica* **144**: 177-186.

Calvalho, I.J.M.B., J.C.M. Cascardo, P.S. Cimera, M.C.M. Ribeiro, A.C. Allem and J.J. Fialho, 1993. Study of DNA polymorphism in *Manihot esculenta* and related species. In: *Proceedings of the first international meeting of cassava biotechnology network*, 25-28 Aug. 1992. CIAT. pp. 56-58.

Carter, S.E., 1986. *Climatic and adaphic classification at a continental scale (1:5 000 000) for cassava in Latin America*. Working Document, Agro-ecological studies Unit, CIAT., Cali, Colombia.

Carter, S.E., L.O. Freso, P.G. Jones and J.N. Fairbain, 1992. An atlas of cassava in Africa: Historical, agroecological and demographical aspects of distribution, CIAT, Cali, Colombia. 86 pp.

Cavalcanti, J., 1985. *Desenvolvimento das raizes tuberosas em mandioca (Manihot esculenta Crantz).* MSc thesis, Universidade Federal do Ceara, Fortaleza, Brazil, 66 pp.

Ceballos, H., 2002. La yucca en Colombia y el mundo; nuevas perspectives para un Cultivo milenario. In: Ospina, B. and H. Ceballos (eds.). La Yuca en el Tercer Milenio: *sistemas modernos de produccion, procesamiento, utilizacion y comercializacion*. CIAT Publication Number 327, Cali, Colombia.

Ceballos, H., A.C. Igleas, J.C. Pérez and A.G.O. Dixon, 2004. Cassava breeding: Opportunities and challenges. *Plant Molecular Biology* **56**: 506-516.

Ceballos, H., M. Fregene, W. Roca and J. Tohme, 2002. Cassava: An Ideal crop for biotechnology tools. In: Taylor, N.J., F. Ogbe and C.M. Fauquet (eds.). *Cassava, An Ancient Crop for Modern Times Food, Health, Culture*. Proceedings of Fifth International Scientific Meeting of the Cassava Biotechnology Network (Abstracts). Danforth Plant Science Center St Louis, Missouri, USA.

Ceccarelli, S., 1989. Wide adaptation: How wide? *Euphytica* **40**: 197-205.

Ceccarelli, S., 1994. Specific adaptation and breeding for marginal conditions. *Euphytica* **77**: 205-219.

Charrier, A. and F. Lefevre, 1987. The genetic variability of cassava mosaic virus infection on the metabolism of cassava leaves. *Tropical Agriculture (Trinidad)* **48**: 263-270.

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

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

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

Chen, X., S. Temnykn, Y. Xu, Y.G. Cho and S.R. McCouch, 1997. Development of a microsatellite framework map providing genome coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **95**: 553-567.

CIAT, 1975. *Annual Report of the Centro Internacional de Agricultura Tropical,* Cali, Colombia.

CIAT, 1981. *Programa de yuca: informe annual*, 1980. Cali, Colombia. 100 pp.

CIAT, 1988. *Cassava Program Annual Report.* Cali, Colombia.

CIAT, 2001. *Project IP3, Improved cassava for the developing World*. Annual Report 2001. Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT, 2003. *Project IP3, Improved cassava for the developing World*. Annual Report. Cali, Colombia.

CIAT, 2004. *Improved cassava for developing world*. Annual report from IP3 project, CIAT, Apdo Aereo 6713, Cali, Colombia. pp. 26.

Clement, R.J., M.D. Hayward and D.E. Byth, 1983. Genetic adaptation in pasture plants. In: McLvor, J.G. and R.A. Bray (eds.). *Genetics resources of forage plants*. CRISO, East Melbourne, pp.101-115.

Cock, J.H., 1982. Cassava: a basic energy source in the tropics. *Science* **218**: 755-762.

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

Cock, J.H., 1985. *Cassava. New potential for a neglected crop*. Westview Press, Boulder, CO, USA. 191 pp.

Cock, J.H., 1987. Stability of performance of cassava genotypes. In: Proceedings of Workshop on Cassava Breeding, Philippines, March, 1985.

Cock, J.H. and M.A. El-Sharkawy, 1988. Physiological characteristics for cassava selection. *Experimental Agriculture* **24**: 443-448.

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

Comstock R.E. and H.F. Robinson, 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average dominance. *Biometrics* **4**: 254-266.

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

Connor, D.J., J.H. Cock and J. Palta, 1981. Response of cassava to water shortage. I. Growth and yield. *Field Crops Research* **4:** 181-200.

Cooper, M. and D.E. Byth, 1996. Understanding adaptation to achieve systemic applied crop improvement. A fundamental challenge. In: Cooper, M. and H.L. Hammer (eds.). *Plant Adaptation and Crop Improvement.* CAB International 1996, Wallingford, UK. pp. 5-23.

Cooper, M. and G.L. Hammer, 1996. Synthesis of strategies for crop improvement. In: Cooper, M. and G.L. Hammer (eds.). *Plant Adaptation and Crop Improvement.* CAB International 1996, Wallingford, UK. pp. 591-623.

Correa, R.X, M.R. Costa, P.I. Good-God, V.A. Ragagnin, F.G. Faleiro, M.A. Moreira and E.G. de Barros, 2000. Sequence characterised amplified regions linked to rust resistance genes in common bean. *Crop Science* **40**: 804-807.

Cours, G., 1951. Le Manioc a Madagascar. *Memoires de L'institut Scientifique de Madagascar Serie B, Biologie Vegetale* **3**: 203-339**.**

Crossa, J., 1990. Statistical analysis of multi-location trials. *Advances in Agronomy* **44**: 55-85.

DeLacy, I.H., M. Cooper and K.E. Basford, 1996. Relationships among analytical methods used to study genotype-by-environment interactions and evaluation of their impact on response to selection. In: Kang, M.S. and H.G. Zobel Jr (eds.). *Genotype-by-environment interaction*. CRC Press, Boca Raton, Florida. pp. 51-84.

Dellarporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter* **1**: 19-21.

DeVries, C.A., J.D. Ferwerda and M. Flack, 1967. Choice of food crops in relation to actual and potential production in the tropics. *Netherlands Journal Agricultural Science* **15**: 241-248.

DeVries, J. and G. Toenniessen, 2001. *Securing the harvest: biotechnology, breeding and seed systems for African crops*. Chapter 13: Cassava. CABI Publishing Oxon, UK. pp. 147-156.

Dixon A.G.O. and E.N. Nukenine, 1997. Statistical analysis of cassava yields with the Additive Main Effects and Multiplicative Interaction (AMMI) model. *African Journal of Root and Tuber crops* **3**: 46-50.

Dixon, A.G.O., R. Asiedu and M. Bokanga, 1994. Breeding of cassava for low cyanogenic potential: problems, progress and perspectives. *Acta Horticulturae* **375**: 153-161.

Dudley, J.W., 1993. Molecular markers in plant improvement - manipulation of genes affecting quantitative traits. *Crop Science* **33**: 660-668.

Easwari Amma, C. S. and M. N. Sheela, 1998. Genetic analysis in a diallel cross of inbred lines of cassava. *Madras Agricultural Journal* **85**: 264-268.

Easwari Amma, C. S., M. N. Sheela and P. K. Thankamma Pillai, 1995. Combining ability analysis in cassava. *Journal of Root Crops* **21**: 65-71.

Ebdon, J.S. and H.G. Gauch, 2002. Additive main effect and multiplicative interaction analysis of national turfgrass performance trials: I. Interpretation of genotype x environment interaction. *Crop Science* **42**: 489-496.

Ebdon, J.S., A.M. Petrovic and R.W. Zobel, 1998. Stability of evapo-transpiration rates in Kentucky bluegrass cultivars across low and high evaporative environments. *Crop Science* **38**: 135-142.

Ehrlich, H.A., R. Gibbs and H.H. Kazazian, 1989. *Polymerase chain reaction*. Cold Spring Habour Press. Cold Spring Harbour.

Eisemann, R.L., 1981. Interpretation of plant response and adaptation to agricultural environments. University of Queenslands, St Lucia Brisbane. pp: 40-90.

El-Hennawy, R.L., 1996. Heterosis and combining ability in diallel crosses of eight bread wheat varieties. *Bulletin of Faculty of Agriculture University of Cairo* **47**: 379-392.

Ellis, R.H., T.D. Hong and E.H. Roberts, 1982. An investigation of the influence of constant and alternating temperature on the germination of cassava seed using a two-dimensional temperature gradient plate. *Annuals of Botany* **49**: 41-246.

El-Sharkawy, M.A. and J.H. Cock, 1990. Photosynthesis of cassava (*Manihot esculenta). Experimental Agriculture* **26:** 325-340.

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

El-Sharkawy, M.A., 2003. Cassava biology and physiology. *Plant Molecular Biology* **53**: 621-641.

El-Sharkawy, M.A., J.H. Cook, J.K. Lynam, A.P. Hernandez and L.F. Cadavid, 1990. Relationships between biomass, root-yield and single-leaf photosynthesis in field-grown cassava. *Field Crops research* **25**: 183-201.

Falconer, D.S and T.F.C. Mackay, 1996. *Introduction to quantitative genetics*. Fourth edition. Longman Scientific and technical Co., Exssex, England. 464 pp.

FAO, 1996. *Food requirements and population growth*. Technical Background Document. No.4. Rome.

FAO, 1998. *Production Yearbook*. FAOSTAT database. www.apps.fao.org/cgi-bin/nph_db.pl.

FAO, 1999. Crops and Products and Domain www.apps.fao.org/lim500/nph-wrap.pl?FS.

FAO, 2006. www.fao.org/waicent/FAOINFO/Agricult/magazine.

Fregene, M.A., 1996. *Phylogeny of cassava (Manihot esculenta Crantz) and its wild relatives based on restriction fragment length polymorphism (RFLP) analysis.* PhD. Thesis University of Ibadan, Ibadan, Nigeria.

Fregene M. and C. Mba, 2004. Marker-Assisted Selection (MAS) In: *Cassava Breeding*. In: Hershey, C. (ed). FAO, Rome Italy.

Fregene, M.A. and N. Morante, 2002. Prospecting for root quality genes in wild relatives of cassava (*Manihot esculenta* Crantz): Advanced backcross quantitative trait loci (ABC-QTL) mapping and improvement of high protein and dry matter content in cassava (abstract). In: Taylor, N.J., F.O. Ogbe, C.M. Fauquet. (eds.). *Cassava, an ancient crop for modern times food, health, culture.* International Scientific Meeting of the Cassava Biotechnology Network. Cassava Biotechnology Network (CBN), St. Louis, MO, USA. pp. S5-10.

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

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

Fregene, M.A., F. Angel, R. Gomez, F. Rodriguez, M. Mayer, J. Tohme, M. Bonerbale, C. Iglesis and W.M Roca, 1994. A linkage map of cassava (*Manihot esculenta* Crantz) based on RFLP and RADP markers. In: *International Cassava Biotechnology Network: Proceedings of the 2nd International Scientific Meeting.* Bogor Indonesia 22-26 August 1994.

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

Fregene, M., M. Suarez, J. Mkumbira, H. Kulembeka, E. Ndedya, A. Kulaya, S. Mitchel, U. Gullberg, H. Rosling, A. Dixon and S. Kresovich, 2001b. Genetic differentiation in cassava (*Manihot esculenta*) landraces as assessed by simple sequence repeat (SSR) markers. In: Fauquet, C.M. and N.J. Taylor (eds.). *Cassava: An ancient crop for modern times. Proceedings of the fifth International Meeting of the CBN.* Held in November 4-9, 2001 at St Louis Missouri, USA. CD2, CBN-V Video Archives-S4-02.

Fritz, A.K., S. Caldwell and W.D. Worall, 1999. Molecular mapping of Russian wheat aphid resistance from triticale accession PI38156. *Crop Science* **39**: 1707-1710.

Garcia, G.M., H.T., Stalker, E. Shroeder and G. Kochert, 1996. Identification of RAPD, SCAR and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* into *Arachis hypogaea*. *Genome* **39**: 836-845.

Gauch, H.G., 1992. Statistical analysis of regional yield trials. In: Kang, M.S. and H.G. Gauch (eds.). *Genotype-by-environment interaction*. CPC Press, USA.

Gauch, H.G. and W.W. Zobel, 1996. AMMI analyses of yield trials. In: Kang, M.S. and W.W. Zobel (eds.). *Genotype by environment interaction*. CRC Press, Boca Raton, New York. pp. 85-120.

GeneticEngineering, 2006. http://www.geneticengineering.org/dna1/32.html.

Getinet, G. and Y. Balcha, 1989. Performance of bread wheat genotypes grown in three environments in Ethiopia. In: Tanner, D.G., M. Van Ginkel and W. Mwangi (eds). *Sixth Regional Wheat Workshop for Eastern, Central and Southern Africa*. Mexico, CIMMYT. pp. 301-306.

Ghosh, S.P., T. Ramanujam, S. Jos, S.N. Moorthy and R.G. Nair, 1988. *Tuber Crops*. Oxford & IBH Publishing Co., New Dehli. pp. 3-146.

Gilzen, H., H.J. Veltkamp, J. Goudrianaan and G.H. de Brui, 1990. Simulation of dry matter production and distribution in cassava (*Mahihot esculenta* Crantz). *Netherlands Journal of Agricultural Science* **38**: 159-173.

Giovannoni, J.J., R.A. Wing, M.W. Ganal and S.D. Tanksley, 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. *Nucleic Acids Research* **19**: 6533-6558.

Goncalvez Fukuda, W.M., C. Fukuda, C.E. Leite Cardoso, O. Lima Vanconcelos and L.C. Nunes, 2000. Implantacao e evolucao dos trabalhos de pesquisa participativa em melhoramento de mandioca no nordeste Brasileiro. Documento CNPMF No. 92. EMBRAPA, Cruz das Alma, Bahia, Brazil.

Goncalvez Fukuda, W.M. and N. Saad, 2001. Participatory research in cassava breeding with farmers in Northeastern Brazil. Document CNPMF No. 99. EMBRAPA, Cruz das Almas, Bahia, Brazil.

Goue-Mourier, M.C., P. Faivre-Rampant, J.B. Le Guerrou, F. Lefe'vre and M. Villar, 1996. Molecular and genetic approaches to rust resistance (*Melampsora* sp.) in poplar (*Populus sp*.). In: M.R. Ahuja, W. Boerjan, and D. B. Neale (eds.). *Somatic Cell Genetics and Molecular Genetics of Trees*. Kluwer Academic Publishers, Dordrecht, the Netherlands. pp. 249-254.

Graham., R.D., D. Senadhira, S. Beebe, C. Iglesias and I. Monasterio, 1999. Breeding for micronutrient density in edible portions of staple food crops: Conventional approaches. *Field Crops research* **60**: 57-80.

Graner, E.A., 1942. Genetica de Manihot. I. Heriteriada de ca formafotha e da coloracao da pelicula externa das raices en *Manihot utilissima* Pohl. *Bragantia* **2:** 13-22.

Griffing, B., 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Austrian Journal of Biological Science* **9**: 463-493.

Gulick, P., C. Hershey and J. Esquinas-Alcazar, 1983. *Genetic Resources of Cassava and Wild Relatives*. International Board for Plant Genetic Resources Rome, Italy.

Hahn, S.K., 1984. Progress of root and tuber improvement at IITA. In: *Proceedings of the 6th Symposium of the International Society of Tropical Root Crops.* Lima, Peru, 20-25 February, 1983.

Hahn, S.K., A.K. Howland and E.R. Terry, 1980a. Correlated resistance of cassava to mosaic and bacterial blight diseases. *Euphytica* **29**: 305-311.

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

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

Hahn, S.K., J.C.G. Isoba and T. Ikotun, 1989. Resistance breeding in root and tuber crops at the International Institute of Tropical Agriculture (IITA). *Crop Protection* **8**: 147-168.

Haley, S.D., P.N., Miklas, J.R. Stavely, J. Byrum and J.D. Kelly, 1993. Identification of RAPD markers linked to a major rust resistance gene block in common bean. *Theoretical and Applied Genetics* **86:** 505-512.

Hallack, H., 2001. Cassava. Scientific Name: *Manihot esculenta* Family: Euphorbiaceae, Spurge Duration: Short-lived shrubby perennial. www.wam.umd.edu/-mathewsc/cassava.html

Hallauer, A.R. and F.J.B. Miranda, 1988. *Quantitative qenetics in maize breeding*. Iowa State University Press. 468 pp.

Hamada, H., M.G. Pertino and T. Kakunaga, 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionary diverse eukaryotic genomes. *Proceedings National Academy of Science (USA*) **79**: 6465-6469.

Hay, R.K.M. and A.J. Walker, 1989. An introduction of the physiology of crop yield. Longman. John Wiley. New York, USA. 293 pp.

Hayman, B.I., 1954a. The theory and analysis of diallel crosses. *Genetics* **39**: 789-809.

Hayman, B.I., 1954b. The analysis of variance of diallel tables. *Biometrics* **10**: 235-244.

Haysom, H.R., T.L.C. Chan, S. Liddle and A. Hughes, 1994. Phylogenetic relationships of *Manihot* species revealed by Restriction Fragment Length Polymorphism. In: Roca, W. and A.M. Thro (eds.). Working Document No. 150. *Proceedings of the Second International Scientific Meeting for the Cassava Biotechnology Network*, 22-26 August 1994. Bogor, Indonesia. **1**: 125-130.

Helentjaris, T., D. Weber and S. Wright, 1986. Identifications of the genome locations of duplicate nucleotide sequences in maize by analysis of RFLP. *Genetics* **118:** 353-363.

Henry, G. and C. Hershey, 2002. Cassava in South America and the Caribbean. In: Hillocks, R.J., J.M. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, Production and Utilization*. CABI Publishing Oxon, UK and New York, USA. pp. 17-40.

Henry, G. and V. Gottret, 1995. *Global Cassava Sector Trends: Reassessing the crop's future*. Inter Center Root and Tuber Review. CIAT working document, November 1995.

Hershey, C.H., 1984. Breeding cassava for adaptation to stress conditions: development of a methodology. In: *Proceedings of the 6th Symposium of the International Society of Tropical Root Crops.* Lima, Peru, 20-25 February, 1983.

Hershey, C.H., 1987. Cassava germplasm resources. In: Hershey C.H. (ed.). *Cassava Breeding: A multi-displinary Review*. Proceedings of workshop held in Philippines, 4-7 March 1985. CIAT, Cali, Colombia. pp. 96-151.

Hershey, C.H. and C. Ocampo, 1989. New marker genes found in Cassava. *Cassava Newsletter* **13:** 1-5.

Hershey, C.H. and D.L. Jennings, 1992. Progress in breeding cassava for adaptation to stress. *Plant Breeding Abstracts* **62**: 823-831.

Hill, J., H.C. Becker and P.M.A. Tigerstedt., 1998. Quantitative and ecological aspects of plant breeding. Chapman and Hill, London.

Hillocks, R.J., 2002. Cassava in Africa. In: Hillocks, R.J., J.M. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, Production and Utilization*. CABI Publishing Oxon, UK and New York, USA. pp. 40-54.

Howeler, R.H. and L.F. Cadavid, 1983. Accumulation and distribution of dry matter and nutrients during a 12-month growth cycle of cassava. *Field Crops Research* **7**: 123-139.

Hunt, L.A., D.W. Wholey and J.H. Cock, 1977. Growth physiology of cassava. *Field Crops Abstracts* **30**: 77-91.

Hussain, A., W. Bushuk, H. Ramirez and W.M. Roca, 1987. Identification of cassava (*Manihot esculenta* Crantz) cultivars by electrophorectic patterns of esterase isozyme. *Seed Science Technology* **155**: 19-22.

Iezzoni, F.A. and M.P. Pritts, 1991. Application of principal component analysis to horticulture research. *Horticultural Science* **26**: 334-338.

Iglesias, C.A. and C. Hershey, 1994. Cassava breeding at CIAT: heritability estimates and genetic progress in the 1980's. In: Ofori, F. and S.K. Hahn (eds.). *Tropical Root Crops in a Developing Economy.* ISTC/ISHS, Wageningen, Netherlands. pp. 149-163.

Iglesias, C.A., C. Hershey, F. Calle and A. Bolanos, 1994a. Propagating cassava (*Manihot esculenta* Crantz) by sexual seed. *Experimental Agriculture* **30:** 283-290.

Iglesias, C., F. Calle, C. Hershey and G. Jaramillo, 1994b. Sensitivity of cassava (*Manihot esculenta* Crantz) clones to environmental changes. *Field Crops Research* **36**: 213-220.

Iglesias, C., J. Bedoya, N. Morante and F. Calle, 1996. Genetic diversity for physiological deterioration in cassava. In: Kurup, G.T. (ed.). *Tropical Tuber Crops: Problems, Prospects, and Future Strategies*. Science Publishers Incorporated, Lebanon, New Hampshire pp. 115-126.

Iglesias, C., J. Mayer, L. Chavez and F. Calle, 1997. Genetic potential and stability of carotene in cassava roots. *Euphytica* **94**: 367-373.

Iglesias, C.A., J. Mayer, L. Chavez and F. Calle, 1997. Genetic potential and stability of carotene in cassava roots. *Euphytica* **94**: 367-373.

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

IITA, 1974. Annual report of the International Institute of Tropical Agriculture. IITA, Ibadan, Nigeria.

IITA, 1978. Annual report of the International Institute of Tropical Agriculture. IITA, Ibadan, Nigeria.

IITA, 1980. Annual report of the International Institute of Tropical Agriculture. IITA, Ibadan, Nigeria.

IITA, 1982. Tuber and root crops production manual. Manual series No. 9. IITA, Ibadan, Nigeria.

IITA, 1988. Annual Report of the International Institute of Tropical Agriculture. IITA Ibadan Nigeria.

IITA, 1990. *Cassava in Tropical Africa*: A reference manual. Edited and designed by Chayce Publications Services, United Kingdom. Printed and bound in the United Kingdom by Balding Mansell International, Wisbech.

IITA, 1993. Archival Report (1989-1993). Part 1. *Cassava breeding, cytogenetic and histology. Vol.2. Germplasm enhancement*. Crop Improvement Division, I badan, Nigeria.

Indira, P., T. Kurian and S.B. Maini, 1977. Flowering behaviour in cassava (*Manihot esculenta* Crantz) as influenced by growth regulators. *Indian Jounal of Root Crops* **2**: 37-40.

Irikura, Y., J.H. Cock and B.G. Murria, 1979. The physiological basis of genotype-temperature interactions in cassava. *Field Crops Research* **2**: 227-239.

Jaramillo G., N. Morante, J.C. Pérez, F. Calle, H. Ceballos, B. Aria and A.C. Bellotti, 2005. Diallel Analysis in cassava adapted to the midaltitude valleys environment. *Crop Science* **45**: 1058-1063.

Jennings, D.L., 1963. Variation in pollen and ovule fertility in varieties of cassava, and the effect of interspecific crossing on fertility. *Euphytica* **12**: 69-76.

Jennings, D.L., 1970. Cassava in Africa. *Field Crop Abstracts* **23:** 271-278.

Jennings, D.L., 1976. Breeding for resistance to African cassava mosaic. In*: African cassava mosaic report of an interdisciplinary workshop held at Muguga, Kenya, IDRC-o71e.* pp. 60-87.

Jennings, D.L. and C.H. Hershey, 1985. Cassava breeding: A decade of progress from international programmes. In: Russell, G.E. (ed.). *Progress in Plant Breeding*. Butterworths. London, Boston. pp. 89-116.

Jennings, D.L. and C. Iglesias, 2002. Breeding for crop improvement. In: Hillocks, R. J., M.J. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, production and utilisation.* CABI International, Oxford. pp.149-166.

Jinks, J.L., 1954. The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties. *Genetics* **39**: 767-788.

Johnson, G.R. and J.N. King, 1998. Analysis of half diallel mating designs. 1 - A practical analysis procedure for ANOVA approximation. *Silvae Genetica* **47**: 2-3.

Jones, W.O., 1959. *Manioc in Africa.* Stanford University Press, Stanford, CA.

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

Jorge, V., M. Fregene, C.M. Velez, M.C. Duque, J. Tohme and V. Verdier, 2001. QTL analysis of field resistance to *Xanthomonas axonopodis* pv. *manihotis* in cassava. *Theoretical and Applied Genetics* **102**: 564-571.

Kang, M.S., 1994. *Applied quantitative genetic.* Baton Rouge. 96 pp.

Kang, M. S. and R. Magari, 1996. New developments in selecting for phenotypic stability in crop breeding. In: Kang, M. S. and H.C. Gauch (eds.). *Genotype by environment interaction.* Boca Raton: CRC Press Cap. 1. 1996. p. 1-14.

Kawano, K., 1978. Genetic improvement of cassava (*Manihot esculenta* Crantz) for productivity. *Tropical Agricultural Research*, Series 11, Ministry of Agriculture and Forestry, Japan. pp. 21.

Kawano, K., 1980. Cassava. In: Fehr W.R. and H.H. Hadley (eds.). *Hybridization of crop plants.* ASA and CSSA, Madison, WI. pp. 225-233.

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

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

Kawano, K. and C. Rojanaridpiched, 1983. Genetic study on postharvest deterioration in cassava. *Crop Science* **22**: 59-63.

Kawano, K. and M. Takahashi, 1968. Studies on the interrelationships among plant characters in rice. II. Genotype-Environment interaction as a limiting factor for negative correlation between characters. *Japan Journal of Breeding* **18**: 27-40.

Kawano, K. and M. Thung, 1982. Intergenotypic competition with associated crops in cassava. *Crop Science* **22:** 59-63.

Kawano, K. and P.R. Jennings, 1983. Tropical breeding - Achievements and challenges. In: *Productivity of field crops under different environments*. IRRI, Los Banos, Los Banos, Laguna, Philippines. pp. 81-99.

Kawano, K., A. Amanya, P. Daza and M. Rios, 1978. Factors affecting efficiency of selection in cassava. *Crop Science* **18**: 373-376.

Kawano, K., K. Narintaraporn, P. Narintaraporn, S. Sarakarn, A. Limsila, J. Limsila, D. Suparhan, V. Sarawat and W. Watananonta, 1998. Yield improvement in a multistage breeding program for cassava. *Crop Science* **38**: 325-332.

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

Kaya, Y., C. Palta and S. Taner, 2002. Additive main effects and multiplicative interactions analysis of yield performances in bread wheat genotypes across environments. *Turkish Journal of Agriculture and Forestry* **26**: 275-279.

Keating, B.A., J.P. Evenson and S. Fukai, 1982. Environmental effects on growth and development of cassava (*Manihot esculenta* Crantz) II. Crop growth rate and biomass yield. *Field Crop Research* **5**: 283-292.

Kelley, J.D., 1995. The use of RAPD in breeding for major gene resistance to plant pathogens. *Horticulture Science* **30**: 461-465.

Khan, N.U., M.S. Swati, G. Hassan and B. Ali, 1995. Combining ability analysis for grain yield, flag leaf area and some other morphological characters in wheat (*Triticum aestivum*). *Sarhad Journal of Agriculture* **11**: 635–641.

Kiehne, K. and D.B. Neale, 1998. DNA pooling strategy for saturation mapping in outcrosses. *Molecular Breeding* **4**: 179-185.

Kizito, E.B., 2006. *Genetic and root growth studies in cassava (Manihot esculenta Crantz). Implications for breeding*. PhD Thesis Swedish University of Agricultural Sciences, Uppsala. pp. 47.

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

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

Koga-Ban, Y., T. Kudo, T. Fukasawa-Kada, M. Ishiyama, H. Tanaka, M. Suzuki and T. Kon, 1999. Application of marker-assisted selection (MAS) for selecting hybrid seedlings from a wild apomictic parental line. Plant and Animal Genome VII Conference. San Diego CA USA. January 17-21, 1999.

Kullaya, A., K. Mtunda, H. Kulembeka, M. Ferguson, J. Marin, C. Ospina, E. Barrera, A. Jarvis, N. Morante, H. Ceballos, J. Tohme and M. Fregene, 2004. Molecular marker assisted and farmer participatory improvement of cassava germplasm for farmer/market preferred traits in Tanzania. In: Alves, A. and J. Tohme (eds.). *Adding Value to a Small-Farmer Crop*. Proceedings on the Sixth International Scientific Meeting of the Cassava Biotechnology Network. 8-14 March 2004. CIAT, Cali Colombia. pp. 70.

Laminski, S., E.R. Robinson and V.M. Gray, 1997. Application of molecular markers to describe South African elite cassava cultivars. *African Journal of Root and Tuber crops* **2**: 132-134.

Lander, E.S. and D. Botstein, 1986. Mapping complex genetic traits in humans: New method using a complex RFLP linkage map. *Cold Spring Harbor Symposia on Quantitative Biology* **51**: 49-62.

Lander, E.S., P. Greeb, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newberg, 1987. MAPMAKER: An interactive computer package for constructing

primary genetic linkage maps of experimental and natural populations. *Genomics* **1**: 174-181.

Lawson, T.L., 1988. Targeting cassava breeding and selection to agro-ecological zones for improved clones. Paper presented at the 4th West Africa Root Crops Workshop, Lome, Togo, 12-16 December 1988.

Lefevre, F. and A. Charrier, 1993. Heredity of 17 isozyme loci in cassava (*Manihot esculenta* Crantz) germplasm collections using RAPD markers. *Euphytica* **74**: 203-209.

Leihner, D., 2002. Agronomy and cropping systems. In: Hillocks, R. J., M.J. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, production and utilisation.* CABI International, Oxford. pp. 91-113.

Leihner, D.E, M. Ruppental, J.A. Castillo and K.M. Muller-Samanu, 1999. "Classificacio´n y estudio de la erodabilidad de los suelos andinos en el sur occidente Colombiano", En Muller-Sammann, K.M. (ed.). *Conservacíon de suelos y aguas en la zona audina*. Centro Internacional de Agricultura Tropical, Palmira, Colombia. pp. 41-46.

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

Lin, C.S., Binns, M.R. and Lefkovitch, L.P., 1986. Stability analysis: where do we stand?. *Crop Science* **26**: 894-900.

Lin, J., J. J.M. Kuo, J.A. Saunders, H.S. Beard, M.H. MacDonald, W. Kenworth, G. Ude and B.F. Mathews, 1996. Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. *Plant Molecular Biology Reporter* **14**: 156-169.

Liu, B.H., 1998a. Computational tools for study of complex traits. In: Paterson A.H. (ed.). *Molecular dissection of complex traits.* CRC Press LLC. pp. 43-79.

Liu, B.H., 1998b. *Statistical Genomics: Linkage, Mapping and QTL analysis*. CRC Press Boca Raton, Florida, USA. 611 pp.

Livini, C., M.P. Ajmine, A.E. Melchinger, M.M. Messmer and M. Motto, 1992. Genetic diversity of maize inbred lines within and between heterotic groups revealed by RFLP. *Theoretical and Applied Genetics* **84:** 17-25.

Lorenzi, J.O., 1978. *Absorcao de macronutrients e acumulacao de material seca para duas cultivares de madioca*. MSc thesis, Univesidade de Sao Paulo, Escola Superior de Agricultura Luis de Queiroz, Piracicaba, Brazil, 92 pp.

Losada Valle, T., 1990. *Cruzamentos diale´ licos em mandioca (Manihot esculenta Crantz).* PhD thesis. Escola Superior de Agricultura Luiz de Queiroz, Univ. de Sa˜o Paulo. Piracicaba, Estado de Sa˜o Paulo, Brazil.

Lozano, J.C., J.C. Toro, A. Castro and A.C. Bellotti, 1977. *Production of Cassava Planting Material*. CIAT, Series GE-17, Cali, Colombia.

Lynch, M. and B. Walsh, 1997. Genetics and analysis of quantitative traits. Sinauer Associates, Inc. MA.

Mackay I.J. and P.D.S. Caligari, 2000. Efficiencies of F_2 and backcross generations for bulked segregant analysis using dominant markers. *Crop Science* **40:** 626–630.

Magoon, M. L., 1967. Recent trends in cassava breeding in India. *Proceedings of an International Symposium on Tropical Root and Tuber Crops* **1**: 100–117. St. Augustine, Trinidad: University of the West Indies.

Magoon, M. L., S.B. Maini and R. Krishnan, 1973. Breeding for tuber quality in cassava. *Tropical Root and Tuber Crops Newsletter* **5**: 27-29.

Mahungu, N.M., 1983. *Relationship among selected agronomic characters and their effects on tuberous root yield of cassava* (*Manihot esculenta* Crantz). PhD thesis. University of Ibadan, Ibadan, Nigeria. 193 pp.

Mahungu, N.M., 1987. Selection for improved root quality in cassava. In: Hershey, C.H. (ed.). *Cassava breeding: A multidisplinary review*. Proceedings of a workshop held in the Philippines, 4-7, March, 1985. Centro International de Agricultura Tropical (CIAT), Cali, CO. pp. 89-103.

Mahungu, N.M., A.G.O. Dixon and J. Mkumbua, 1994. Breeding cassava for multiple pest resistance in Africa. *African Crop Journal* **2:** 539-552.

Mäkinen, Y. and J.L. Brewbaker, 1976. Isozyme in flowering plants. I. Diffusion of enzymes out of intact pollen grains. *Physiologia Plantarum* **20:** 477-482.

Manly, K. F., R. H. Cudmore Jr and J. M. Meer, 2001. Map Manager QTX, cross-platform software for genetic mapping. *Mammallian Genome* **12:** 930–932.

Manyong, V.M., A.G.O. Dixon, K.O. Makinde, M. Bokanga and J. Whyte, 2000. The contribution of IITA-improved cassava to food security in sub-Saharan Africa: an impact study. IITA report, IITA, Ibadan, Nigeria.

Marmey, P., J.R. Beeching, S. Hammon and A. Charrier, 1994. Evaluation of cassava (*Manihot esculenta* Crantz) germplasm using RAPD markers. *Euphytica* **74**: 203-209.

Marsan, P.A., G. Egidy, G. Monfredini, S. De Silvestro and M. Motto, 1993. RAPD markers in maize genetic analysis. *Maydica* **38:** 259-264.

Maughan, P.J., M.A. Saghai-Maroof and G.R. Buss, 1996. Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theoretical and Applied Genetics* **93**: 392-401.

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

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

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

Melchinger, A.E., M.M. Messmer, M. Lee, W.L. Woodman and K.R. Lankey, 1991. Diversity and relationships among U.S. maize inbreds revealed by RFLP. *Crop Science* **31:** 669-678.

Michelmore, R.W., I. Paran and R.V. Kesseli, 1991. Identification of markers linked to disease resistant genes by bulked segregant analysis: a rapid method to detect markers in specific genome regions by using segregating populations. *Proceedings of National Academy of Sciences, (USA)* **88**: 9828-9832.

Microsoft Corporation, 2004. http://www.microsoft.com.

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

Miklas, P.N., J.R. Stavely and J.D. Kelly, 1993. Identification and potential use of molecular markers for rust resistance in common bean. *Theoretical and Applied Genetics* **85**: 745-749.

Miller, J.C. and S.D. Tanksley, 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theoretical and Applied Genetics* **80:** 437-448.

Minde, I.J., J.M. Teri, V.W. Saka, K. Rockman and I.R.M. Benesi, 1997. Accelerated multiplication and distribution of cassava and sweetpotato planting material in Malawi. In: Rohrbach, D.D., Z. Bishaw and A.J.G. van Gastel (eds.). *Alternative Strategies for Smallholder Seed Supply*: Proceedings of an international conference on options for strengthening national and regional seed systems in Africa and West Asia. Harare, Zimbabwe. pp. 162-167.

Mishra, P.C, T.B. Singh, O.P. Singh and S.K. Jain, 1994. Combining ability of grain yield and some of its attributes in bread wheat under timely sowing condition. *International Journal of Tropical Agriculture* **12**: 188-194.

Mkumbira, J., 2002. *Cassava development for small-scale farmers.* PhD thesis. Agraria. Swedish University of Agricultural Science. 365 pp.

Mkumbira, J., L. Chiwona-Karltun, U. Lagercrantz, N.M. Mahungu, J. Saka, A. Mhone, M. Bokanga, L. Brimer, U. Gullberg and H. Rosling, 2001. Classification of Cassava into "Bitter" and "Cool" in Malawi: The Farmers' Method. In: Fauquet, C.M. and N.J. Taylor (eds.). *Cassava: An ancient crop for modern times. Proceedings of the fifth International Meeting of the CBN.* Held in November 4-9, 2001 at St Louis Missouri, USA. CD3, CBN-V Video Archive-S6-17.

Moorthy, S.N., 1994. *Tuber Crops Starches*. Central Tuber Crops Research Institute. Technical Bulletin Series: 18. St Josephs Press, Cotton Hill, Thiruwanathapuram, Kerala, India. pp. 40.

Morgante, M. and A. M. Olivieri, 1993. PCR-amplified microsatellites as markers in plant genetics. *Plant Journal* **1**: 175-182.

Morillo, E., P. Fuennayor, C. de Carvalho and G. Second, 2001. AFLP and SSR polymorphism: Evidence of significant levels of introgression from *Manihot glaziovii* and *M. carthagenensis* into traditional varieties of cassava in their area or origin. In: Fauquet, C.M. and N.J. Taylor (eds.). *Cassava: An ancient crop for modern times. Proceedings of the fifth International Meeting of the CBN.* Held in November 4-9, 2001 at St Louis Missouri, USA. CD3, CBN-V Video Archive-S6-18.

Narváez-Trujillo, A., T. Lozada and G. Second, 2001. The dynamics of the Sweet-Bitter differentiation in cassava varieties as unravelled by molecular polymorphism. In: Fauquet, C.M. and N.J. Taylor (eds.). *Cassava: An ancient crop for modern times.* Proceedings of the fifth International Meeting of the CBN. Held in November 4-9, 2001 at St Louis Missouri, USA. CD3, CBN-V file-S6-19.pdf.

Nassar, N.A., 1978. Wild *Manihot* species of central Brazil for cassava breeding. *Canadian Journal of Plant Science* **58:** 257-261.

Nassar, N.M.A., 1994. Development and selection of apomixis in cassava. *Canadian Journal of Plant Science* **74**: 857-858.

Nassar, N.M.A., 2003. Gene flow between cassava, *Mannihot esculenta* Crantz and wild relatives. *Genetics and Molecular Research* **2**: 334-347.

Nassar, N.M.A., 2005. Cassava: Some ecological and physiological aspects related to plant breeding. An article published online with Gene Conserve. URL http:/www.geneconserve.pro.br/.

Nassar, N.M.A. and D. Grattapaglia, 1986. Variabilidade de clones de mandioca em relação a fertilidade e aspectos morfológicos. *Turrialba* **36**: 555-559.

Nassar, N.M.A. and G. Dorea, 1982. Protein content in some cassava cultivars and its hybrid with wild *Mannihot* species. *Turrialba* **32**: 429-432.

Nassar, N.M.A. and S. O'Hair, 1985. Variation among cassava clones in relation to seed germination. *Indian Journal of Genetics and Plant Breeding* **45**: 394-398.

Nassar, N.A., J. Alves and E deSouza. 2004. UnB 033: An interesting interspecific cassava hybrid. *Revista Ceres* **51**: 495-499.

Neilsen, G. and J.G. Scandalios, 1974. Chromosomal location by use of trisomics and new alleles of an endopeptidase in *Zea mays*. *Genetics* **77**: 679-686.

Nelson, S. L., 2000. *Office 2000***.** Manual de referencia. McGraw-Hill/Interamericana de Espan˜ a, Madrid.

Ntawuruhunga, P. 1992. *Assessement of dry matter determination and its' accumulation in cassava (Manihot esculenta Crantz)*. MSc Thesis, University of Ibadan, Nigeria, 97 pp.

Ntawuruhunga, P., 2000. *Evaluation of cassava (Manihot esculenta Crantz) genotypes for adaption for different altitudes*. PhD Thesis, Department of Crop Science, Faculty of Agriculture, Makerere University, Uganda. 156 pp.

Nweke, F.I., 1995. The role of cassava production in poverty alleviation. In: *Proceedings of the 6th Triennial ISTRC-AB Symposium*, 22-28 Oct, Lilongwe, Malawi. pp. 102-115.

Nweke, F.I., 1996. *Cassava: A cash crop in Africa*. Collaborative study of cassava in Africa Working Paper No. 14, International Institute of Tropical Agriculture, Ibadan, Nigeria. 79 pp.

Nweke, F.I., D.S.C. Spenser and J.K. Lynam, 2002. The cassava transformation. Michigan State University Press, East Laning. pp. 101-114.

Ocampo. C., C. Hershey, C. Iglesias and M. Inawaga, 1992. Esterase enzyme fingerprinting of cassava germplasm held at CIAT. In: Roca, W. and A.M. Thro (eds.). *Proceedings of the first International Scientific Meeting of the Cassava Biotechnology Network (CBN),* held at CIAT, Cali, Colombia. pp. 81-89.

Oelsligle, D.D., 1975. Accumulation of dry matter, nitrogen, phosphorus, and potassium in cassava (*Manihot esculenta* Crantz). *Turrialba* **25**: 85-87.

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

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

Okogbenin, E., 2004. *Qualitative trait loci mapping of root quality traits, morphological characters, and early bulking in cassava (Manihot esculenta Crantz)*. PhD Thesis University of Ibadan. 208 pp.

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

Oliveira de, S.L., M.M. Macedo and M.C.M. Porto, 1981. Efeito do deficit na agua na producao de raizes de mandioca. *Report from Centro Naciona le de Pesquisa de Mandioca e Fruticultura.* Cruz das Almas, Bahia, Brazil.

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

Onwueme, I.C., 1978. *The Tropical Tuber Crops: Yams, Cassava, Sweetpotato, Cocoyams*. John Wiley and Sons Ltd. New York.

Onwueme, I.C., 2002. Cassava in Asia and the Pacific. In: Hillocks, R.J., J.M.Thresh and A.C. Bellotti (eds.). *Cassava: Biology, Production and Utilization*. CABI Publishing Oxon, UK and New York, USA, pp. 55-65.

Ortiz, R., S. Madsen, W.W. Wagoire, J. Hill, S. Chandra and O. Stolen, 2001. Additive main effect and multiplicative interaction model for diallel-cross analysis. *Theoretical and Applied Genetics* **102**: 1103-1106.

Osiru, D.S., M.C.M. Porto and I.J. Ekanayake, 1996. *Morphology of cassava plant*. IITA, Research Guide. Ibadan, Nigeria.

Ott, J., 1991. *Analysis of Human Genetic Linkage*. Revised edition. The Johns Hopkins University Press, Baltimore, USA.

Pellet, D. and M.S. El-Sharkawy, 1994. Sink-source relations in cassava: effects of reciprocal grafting and leaf photosynthesis. *Experimental Agriculture* **30**: 359-367.

Peressin, V.A., D.A. Monteiro, J.O. Lorenzi, G.C. Durigan, R.A. Pitelli and D. Perecin, 1998. Acumulo de material seca na presence e na ausencia de plantas

infestantes no cultivar de mandioca SRT 59-Branca de Santa Catarina. *Bragantia* **57**: 135-148.

Pérez de la Vega M, 1997. Plant genetic adaptedness to climatic and edaphic environment. In: Tigerstedt P.M.A. (ed.). *Adaptation in plant breeding*. Proceedings of the XIV EUCARPIA Congress, Jyväskylä, Sweden. pp. 27–38.

Pérez, J.C., H. Ceballos, J.I. Lenis, E. Ortega and N. Morante, 2002a. Heritability of agronomically relevant traits in cassava. In: Taylor, N.J., F. Ogbe and C.M. Fauquet (eds). *Cassava, An Ancient Crop for Modern Times Food, Health, Culture*. Proceedings of Fifth International Scientific Meeting of the Cassava Biotechnology Network (Abstracts). Danforth Plant Science Center St Louis – Missouri – USA.

Pérez J.C., N. Morante, J. López, J.I. Lenis, G. Jaramillo, H. Ceballos and F. Calle, 2002b. Advantages of the New Cassava Breeding Scheme at CIAT. In: Taylor, N.J., F. Ogbe and C.M. Fauquet (eds.). *Cassava, An Ancient Crop for Modern Times Food, Health, Culture*. Proceedings of Fifth International Scientific Meeting of the Cassava Biotechnology Network (Abstracts). Danforth Plant Science Center St Louis, Missouri, USA S6-22.

Pinho, J.L.N. de, F.J.A.F. Tavora, F.I.O. Melo and G.M. Queiroz de, 1995. Yield componets and partitioning characteristics of cassava in the coastal area of Ceara. *Revista Brasileira de Fisiologia Vegetal* **7**: 89-96.

Powell, W., G.C. Machray and J. Provan, 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* **1**: 215-222

Purchase, J.L., 1997. *Parametric analysis to describe G x E interaction and yield stability in winter wheat.* PhD Thesis. Department of Agronomy, Faculty of Agriculture, University of the Free State, Bloemfontein, South Africa.

Rafalski, J.A. and S.V. Tingey, 1993. Genetic diagnostics in plant breeding. *Theoretical and Applied Genetics* **9:** 275-280.

Rajendran, P.G., 1989. Combining ability in cassava. *Journal of Root Crops* **15**: 15-18.

Rajendran P.G. and N. Hrishi, 1982. Variation in dry matter content of tuber in seedling population of three crosses of cassava. In: Belen, E.H. and M. Villanueva (eds.). *International Symposium on Tropical Root and Tuber Crops*, Los Banos, Laguna, Philippines, 1979. Proceedings. Los Banos, Laguna, Philippine Council for Agriculture and Resources Research. pp. 457-460.

Raji, A.A., A.G.O. Dixon, I. Fawole and M. Gedil, 2001. Diversity analysis of African landraces of cassava as assessed with agrobotanical traits and molecular markers. In: Fauquet, C.M. and N.J. Taylor (eds.). *Cassava: An ancient crop for modern times. Proceedings of the fifth International Meeting of the CBN.* Held in November 4-9, 2001 at St Louis Missouri, USA. CD3, CBN-V Video Archive-S6-25.

Ramagosa, I. and P.N. Fox, 1993. Genotype x environment interaction and adaptation. In: Hayward M.D., N.O. Bosemark and L. Romagosa (eds.). *Plant breeding: Principles and Prospects*. Chapman and Hall, London. pp. 373-390.

Ramanujam, T., 1990. Effect of moisture stress on photosynthesis and productivity of cassava. *Photosynthetica* **24**: 217-224.

Ramirez, H., A. Hussein, W. Roca and W. Bushiuk, 1987. Isozyme electropherograms of sixteen enzymes in five tissues of cassava (*Manihot esculenta* Crantz) varieties. *Euphytica* **36**: 39-48.

Reamon-Buttner, S. M., J. Schondelmaier and C. Jung, 1998. AFLP markers tightly linked to the sex locus in *Asparagus officinalis* L. *Molecular Breeding* **4**: 91-98.

Ribaut, J. M. and D. Hoisington, 1998. Marker-assisted selection: new tools and strategies. *Trends in Plant Science* **3**: 236-239.

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

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

Robinson, J.P. and S.A. Harris, 1999. Amplified Fragment Length Polymorphism and Microsatellites: A phylogenetic perspective. In: Gillet, E.M. (ed.). Which DNA marker for which purpose? Final compendium of the research project: Development, optimisation and validation of molecular tools for assessment of biodiversity in forest trees in the European Union. Available at URL: http:/webdoc.sub.gwgd.de/ebook/

Rodriquez, S. and M. Garcia, 1990. Genotype-environment interaction and use of different stability methods on cassava (*Manihot esculenta*). *Ciencia y Tecnica en al agricultura, Vianda Tropicales* (Cuba) **13**: 27-46.

Rogers, D.J. and G. Appan, 1970. *Untapped genetic resources for cassava improvement*. In: Proceedings of second International Symposium on Tropical Root and Root Crops, University of Hawaii, Honolulu. March. University of Hawaii Press, Honolulu. pp. 79-82.

Rogers, D.J. and S.G. Appan, 1973. *Manihot,* Manihotoides (Euphorbiaceae). *Flora Neotropica*, Mongraph 13, Hafner Press, New York.

Ross, H.B., 1975. *The diffusion of the manioc plant from South America to Africa: an essay in ethnobotanical culture history*. PhD thesis, Colombia University, New York, USA. 135 pp.

SAS Institute Inc. 2002. *SAS/STAT software: Changes and Enhancement for Release 9.1.* Cary, NC: SAS Institute Inc. 158 pp.

Sax, K., 1923. The association of size differences with seed-coat patterns and pigmentation in *Phaseolus vulgaris*. Genetics **8**: 552-560.

Schaal, B., L.J.C.B. Carvalho, T. Prinzie, K. Olsen, P. Olson, G. Cabral and M. Hernandez, 1997. Phylogenetic relationships among Manihot species. *African Journal of Root and Tuber Crops* **2**: 147-149.

Second, G.A.C., L. Allem, C. Empérarie, C. Ingram, R.A. Columbo, L.J. Mendes and C.B. Carvalho, 1997. AFLP based *Manihot* and cassava numerical taxonomy and genetic structure analysis in progress: Implications for dynamic conservation and genetic mapping in Africa. *African Journal of Root and Tuber Crops* **2:** 140-146.

Seo, Y.W., J.W. Johnson and R.L. Jarret, 1997. A molecular marker associated with *H21* Hessian fly resistance gene in wheat. *Molecular Breeding* **3**: 177-181.

Shafii, B., K.A. Mahler, W.J. Price and D.L. Auld, 1992. Genotype by Environment interaction effects on winter rapeseed yield and oil content. *Crop Science* **32**: 922-927.

Simwambana, M.S.C., 1988. *Shoot removal studies in cassava (Manihot esculenta Crantz).* PhD Thesis, Faculty of Agriculture, St. Augustine Trinidad. West Indies. 160 pp.

Smith, O.S., J.S.C. Smith, S.L. Bowe, R.A. Temborg and S.J. Wall, 1990. Similarities among a group of elite maize inbreds as measured by F_1 grain yield, grain yield heterosis and RFLP. *Theoretical and Applied Genetics* **80**: 833-840.

Song, K.M., T.C. Osborn and P.H. William, 1990. *Brassica* taxonomy based on molecular restriction fragment polymorphism (RFLP). Genome relationships in *Brassica* and related genera and the origin of *B. oleracea* and *B. rapa*. *Theoretical and Applied Genetics* **79:** 497-506**.**

Sprague, G.F. and L.A. Tatum, 1942. General versus specific combing ability in single crosses of corn. *Journal of the American Society of Agronomy* **34**: 923-932.

Stuber, C. and M.D. Edwards, 1986. Genotypic selection for improvement of quantitative traits in corn using molecular marker loci: *Report of the Annual Corn, Sorghum Research Conferences, Washington, D.C.* **41**: 70-83.

Tabor, G.M., T.L. Kubisiak, N.B. Klopfenstein, R.B. Hall and H.S. McNabb Jr., 2000. Bulked segregant analysis identifies molecular markers linked to *Melampsoramedusae* resistance in *Populus deltoids. Genetic and Resistance* **90**: 1039-1042.

Tan, S.L., 1985. *Selection for yield potential*. Proceedings of Worldwide Cassava Breeding Workshop. PRCRTC, VISCA, Leyte, Phillippines. CIAT/IITA VISCA/UNDP. pp. 133-163.

Tan, S.L. and C. Mak, 1995. Genotype x Environment influence on cassava performance. *Field Crop Research* **42**: 111-123.

Tan, S.L. and J.H. Cock, 1979. Branching habit as a yield determinant in cassava. *Field Crops Research* **2**: 281-289.

Tanksley, S.D., 1993. Mapping polygenes. *Annual Review of Genetics* **27**: 205-233.

Tanksley, S.D. and S.R. McCouch, 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* **277**: 1063-1066.

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

Tautz, D. and M. Renz, 1984. Simple sequences are ubiquitous repetitive components of eukaryote genomes. *Nucleic Acids Research* **12**: 4127-4138.

Tavora, F.J.A.F., F.I.O. Melo, J.L.N. Pinho and G.M. Queiroz de, 1995. Yield, crop growth rate and assimilatory characteristics of cassava at the coastal area of Ceara. *Revista Brasileira de Fisiologia Vegetal* **7**: 81-88.

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

Thillainathan, M. and G.C.J. Fernandez, 2001. SAS applications for Tai's stability analysis and AMMI model in genotype x environmental interaction (GEI) effects. *Journal of Heredity* **92**: 367-371.

Thoday, J.M., 1961. Location of polygenes. *Nature* **191**: 368-370.

Tommerup, I.C., J.E. Barton and P.A. Óbrein, 1995. Reliability of RAPD fingerprinting of three basidiomycete fungi, *Laccaria, Hydnangium* and *Rhizoctonia*. *Mycology Research* **99:** 179-186.

Toro, J.C. and C.B. Atlee, 1985. Agronomic practices for cassava production: A literature review. In: Cock J.H. and J.A Reyes (eds.). *Cassava: Research, Production and Utilization*. CIAT/UNDP. pp. 207-237.

Tuberosa, R., S. Salvi, M.C. Sanguineti, P. Landi, M. Maccaferri and S. Canti, 2002. Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. *Annals of Botany* **89**: 941-943.

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

Ugwu, B.O. and P. Ay, 1992. Seasonality of cassava processing in Africa. COSCA *Working Paper No. 9*. Collaborative study of Cassava in Africa. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Uma-Menon, S., N. Sharma and U. Menon, 1996. Stability analysis for grain yield and associated traits in bread wheat. *Annuals of Agricultural Research* **17**: 33-37.

Van Oirschot, Q.E.A., G.M. O'Brien, D.D. Dufuor, M.A. El-Sharkawy and E. Mesa, 2000. The effect of pre-harvest pruning of cassava upon root deterioration and quality characteristics. *Journal of Science, Food and Agriculture* **80**: 1866-1873.

Van Ooijen, J.W. and R.E. Voorrips, 2001. JoinMap 3.0, software for the calculation of genetic linkage maps. Kyazma B.V, Wageningen.

Varma, S.P. and R. Mathura, 1993. Genetic variability and inter-relation in cassava (Manihot *esculenta* Crantz) under rainfed conditions of Tripura. *Journal of Root Crops* **19**: 77-80.

Veltkamp, H.J., 1985. Partitioning of dry matter in cassava. *Wageningen Agricultural University Papers* **85**: 62-72

Veltkamp, H.J, 1986. *Physiological causes of yield variation in cassava* (*Manihot esculenta* Crantz). Wageningen Agricultural University, Wageningen, the Netherlands. 103 pp.

Vine, P.N., 1979. *Growth and development in cassava (Manihot esculenta Crantz) in relation to soil physical conditions*. PhD Thesis, St Augustine Trinidad University of West Indies.

Vogel, J.M., A. Rafalski, W. Powell, M. Morgante, C. Andre. M. Hanafey and S.V. Tingey, 1996. Application of genetic diagnostics to plant genome analysis and plant breeding. *Horticultural Science* **31**: 1106-1107.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijers, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407-4414.

Wagoire, W.W., O. Stølen, J. Hill and R. Ortiz, 1998. Is there a 'cost' for wheat cultivars with genes for resistance to yellow rust caused by *Puccinia striiformis*? *Crop Protection* **17**: 337-340.

Wagoire, W.W., O. Stølen, J. Hill and R. Ortiz, 1999. Assessment and genetics of host plant resistance to yellow rust in bread wheat germplasm adapted to the East African highlands. In: Scarascia Mugnozza G.T., E. Porceddu and M.A. Pagnotta (eds.). *Genetics and breeding for crop quality and resistance.* Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 67–76.

Wanyera, N.W.M., 1993. *Phylogenetic relationships among cultivated cassava (Manihot esculenta Crantz) and two wild Manihot species*. PhD thesis University of Ibadan, Ibadan Nigeria. 193 pp.

Weeden, N.F., G.M. Timmerman, M. Hemmat, B.E. Kneen and M.A. Lodhi, 1992. Inheritance and reliability of RAPD markers: Applications of RAPD technology to plant breeding. *American Genetic Association* pp. 12-17.

Weising, K., H. Nybom, K. Wolff and G. Kahl, 2005. *DNA Fingerprinting in Plants: Principles, Methods, and Applications*, 2nd edition. CRC Press, London.

Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acid Research* **18:** 7213-7218.

Welsh, J., R.J. Honeycutt, M. McClelland and B.W.S. Sobral, 1991. Parentage determination in maize hybrids using arbitrarily-primed polymerase chain reaction (AP-PCR). *Theoretical and Applied Genetics* **82**: 473-476.

Wheatley, C.C. and G. Chuzel, 1993. Cassava: The nature of the tuber and use as a raw material. In: Macrae, R., R.K. Robinson and M.J. Sadler (eds.). *Encyclopaedia of Food Science, Food Technology, and Nutrtion*. Academic Press, San Diego, California. pp. 734-743.

Wheatley, C.C., C. Lozano and G. Gomez, 1985. Post-harvest deterioration of cassava roots. In: Cock, J.H. and J.A. Reyes (eds.). *Cassava: Research, production and utilization*. UNDP-CIAT, Cali. pp. 655-671.

Wholey, D.W. and J.H. Cock, 1974. Onset and rate of root bulking in cassava. *Experimental Agriculture* **10**: 193-198.

Whyte, J.B.A., 1987. Breeding cassava for adaptation to environmental stress. In: *Cassava breeding-a multidisciplinary workshop*, Philippines 4-5 March 1985. pp. 147-176.

Williams, C.N., 1972. Growth and productivity of tapioca (*Manihot utilissima*). III. Crop ratio, spacing and yield. *Experimental Agriculture* **8**: 15-23.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingley, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531-6535.

Winter, P. and G. Kahl, 1995. Molecular marker technologies for plant improvement. *World Journal of Microbiology and Biotechnology* **11**: 438-448.

Wyman, A.R. and R. White, 1980. A highly polymorphic locus in human DNA. *Proceedings of the National Academy of Sciences (USA)* **77**: 6754-6758.

Yamamoto, T., A. Nishikawa and K. Oeda, 1994. DNA polymorphism in *Oryza sativa* L. amplified by arbitrary primer PCR. *Euphytica* **78:** 143-148.

Yan, W. and A. Hunt, 2002. Biplot analysis of diallel data. *Crop Science*. **42**: 21-30.

Young N.D., 1996. QTL mapping and quantitative disease resistance in plants. *Annual Review of Phytopathology* **34:** 479-501.

Young, N.D., D. Zamir, M.W. Ganal and S.D. Tanksley, 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. *Genetics* **20:** 579-585.

Zacarias, A.M., 1997. *Identification and genetic distance analysis of cassava (Manihot esculenta Crantz) cultivars using RAPD fingerprinting*. MSc thesis. Department of Plant Breeding, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa.

Zacarias, A.M., A.M. Botha, M.T. Labuschagne and I.R.M. Benesi, 2004. Characterisation and genetic distance analysis of cassava (*Manihot esculenta* Crantz) germplasm from Mozambique using RAPD fingerprinting. *Euphytica* **138**: 49-53.

Zapata, G., 2001. *Disminucion de deterioro fisiologico postcosecha en raices de yucca (Manihot esculenta Crantz) mediate almacenamiento controlado*. B.S. Thesis, Universidad de San Buenaventura, Facultad de Ingenieria Agroindustrial, Cali, Colombia.

Zobel, R. W., 1990. A powerful statistical model for understanding genotype-by-environment interaction. In: M.S. Kang (ed.). *Genotype-by-environment interaction and plant breeding*, Department of Agronomy, Louisiana State University, Baton Rouge, Louisiana. pp. 126-140.

Summary

Keywords: *Diallel analysis, stability, seedling nursery, clonal evaluation, inter-specific cross, molecular markers, SSR*

Diallel crosses were evaluated in three agro-ecologies in Colombia and parents and families selected for conventional and molecular breeding trials conducted during the study. At the same time an inter-specific cross was evaluated for the effective introduction of higher DMC from wild cassava relatives. High positive and negative GCA and SCA values were estimated for the agronomic yield traits. Harvest index and DMC were under the influence of additive gene action, while ComRt, FRY and DRY were more influenced by non-additive effects. Moderate to high heritability estimates were obtained for the different agronomic traits. High heritability values were estimated for DRY, FRY, DMC, HI, RtPlt and moderate for RtWt. AMMI analysis ranked FRY, DRY, HI and RtPlt as stable, DMC relatively stable and RtWt as unstable across environments.

In the seedling stage DRY, FRY, RtWt, and ComRt ranked parents in the same direction. Dry matter content and HI ranked parents similar but in the opposite direction for other yield characteristics. Overall rating ranked MPER 183 and CM 4574-7 as best parents followed by SM 1565-15 and SM 1665-2. Seedling nursery and the diallel analysis identified SM 1741-1 and MPER 183 as good parents for a number of traits. Replication and blocking during clonal evaluation greatly reduced the non-genetic factors resulting in high correlation among traits evaluated. Fresh root yield, DMC, HI, RtWt and RtPlt were found to be important in determining DRY. Root weight was found to be the most important indicator of DRY.

Crossing of the elite cultivar MTAI-8 to the wild relative *M. tristis* resulted in high DMC values ranging from 34.39% to 42.73%. Bulk segregant analysis showed that parent SM 1741-1 was associated with favourable QTLs for DMC. Markers SSRY 150 (R^2=18.1%) and SSRY 160 (R^2=28.9%) in the diallel cross, SSRY 99 (R^2=22.68), SSRY 141

($R^2$35.89) and NS 169 (R^2=20.01) in the wild cross and SSRY 11 (R^2=34.57), SSRY 62 (R^2=50.21) and NS 644 (R^2=29.09) in the mapping population showed association with DMC with values high enough for practical use in a MAS breeding programme.

OPSOMMING

Sleutelwoorde: *Dialleel analise, stabiliteit, saailingkwekery, klonale evaluasie, inter-spesifieke kruis, molekulêre merkers, SSR*

Dialleel kruisings is in drie agro-ekologiese areas in Kolombië geëvalueer en ouers en families is vir konvensionele en molekulêre telingseksperimente geselekteer vir hierdie studie. Terselfdertyd is ‘n inter-spesifieke kruising vir die effektiewe oordrag van droë materiaal opbrengs gene geëvalueer. Hoë positiewe en negatiewe algemene en spesifieke kombineervermoë waardes is vir agronomiese opbrengs eienskappe gevind. Oesindeks (OI) en droë materiaal opbrengs (DMO) was onder invloed van additiewe geenaksie, terwyl kommersiële wortels, vars en droë wortelopbrengs meer deur nie-additiewe geeneffekte beheer is. Gemiddeld tot hoë ooreflikheidswaardes is vir die verskillende agronomiese eienskappe gevind. Hoë oorerflikheid is vir droë en vars wortelmassa, DMO, OI en wortels per plant en gemiddelde oorerflikheid vir wortelmassa gevind. AMMI analise het vars en droë wortelopbrengs, OI en wortels per plant as stabiel geklassifiseer, DMO relatief stabiel en wortelgewig as onstabiel oor omgewings.

In die saailingfase was ouers vir droë en vars wortelopbrengs, wortelmassa en kommersiële wortels ongeveer in dieselfde rangorde. Vir DMO en OI was ouers se rangordes weer ongeveer dieselfde, maar in die teenoorgestelde rigting as vir die ander opbrengseienskappe. In die geheel was MPER 183 en CM 4574-7 die beste ouers, gevolg deur SM 1565-15 en SM 1665-2. Die saailingkwekery en dialleel analise het SM 1741-1 en MPER 183 as goeie ouers vir ‘n aantal eienskappe geïdentifiseer. Herhaling en blokke tydens klonale evaluasie het die effek van nie-genetiese faktore grootliks verminder wat tot hoë korrelasies tussen die eienskappe wat geëvalueer is gelei het. Vars wortelopbrengs, DMO, OI, wortelmassa en wortels per plant was belangrik in die bepaling van droë wortel opbrengs. Wortelmassa was die belangrikste faktor in bepaling van droë wortel opbrengs.

Die kruising van die elite cultivar MTAI-8 met die wilde tipe *M. tristis* het tot hoë DMC waardes gelei wat tussen 34.39% en 42.73% gewissel het. Massa segregerende analise het getoon dat ouer SM 1741-1 met goeie kwantitatiewe eienskap lokusse vir DMO geassosieer is. Merkers SSRY 150 (R^2=18.1%) en SSRY 160 (R^2=28.9%) in die dialleel kruis, SSRY 99 (R^2=22.68), SSRY 141 ($R^2$35.89) en NS 169 (R^2=20.01) in die wilde kruising en SSRY 11 (R^2=34.57), SSRY 62 (R^2=50.21) en NS 644 (R^2=29.09) in die karteringspopulasie het ‘n groot genoeg assosiasie met DMC aangetoon om dit prakties bruikbaar te maak in ‘n merker ondersteunde teelprogram.