Characterisation of Malawian cassava germplasm for diversity, starch extraction and its native and modified properties

By

IBRAHIM ROBENI MATETE BENESI

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SOUTH AFRICA

Supervisor: Prof. Maryke T. Labuschagne

Co-supervisors: Dr. Liezel Herselman
Prof. John K. Saka
Dr. Nzola M. Mahungu

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DECLARATION

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Ibrahim Robeni Matete Benesi Date

DEDICATION

This piece of work is dedicated to my mother Makungula Swaleyi, my father Benesi Robeni, my wife Hawa Rajabu Benesi, my brother Yahaya Benesi and my children Swaleyi, Adamu, Bwanali, Atupele and Rolini for the suffering they have gone through in the course of my studies. Yahaya passed away while I was still at primary school, while my beloved mother passed away while I was struggling with my PhD. I will miss them forever since they suffered a lot for my studies but did not enjoy fully the fruits of their efforts. May their souls rest in peace.

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ADD	Agricultural development division
AFLP	Amplified fragment length polymorphism
AMMI	Additive Main effects and Multiplicative Interaction
ANOVA	Analysis of variance
ATP	Adenosine 5'-triphosphate
BC	Before Christ
bp	Base pairs
BSA	Bovine serum albumin
C	Carbon
^{o}C	Degrees Celsius
CAD	Cassava anthracnose disease
CBB	Cassava bacterial blight
CBSD	Cassava brown streak disease
cDNA	Complementary DNA
CGIAR	Consultative Group on International Agricultural Research
CGM	Cassava green mite
CIAT	International Centre for Tropical Agriculture
CM	Cassava mealybug
cM	Centimorgans
cm	Centimetre
cm^3	Cubic centimetre
CMD	Cassava mosaic disease
cmol	Centimolar
CRP	Colour of root pulp
CRS	Colour of root surface
CTAB	Cetyltrimethylammonium bromide
CTS	Colour of tip shoots
CUAL	Colour of unexpanded apical leaves
CV	Coefficient of variation
DARS	Department of Agricultural Research Services
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate

df	Degrees of freedom
DM	Dry matter content
DNA	Deoxyribonucleic acid
dNTP	2'-deoxynucleoside 5'- triphosphate
DRC	Democratic Republic of Congo
DSC	Differential scanning calorimetry
dTTP	2'-deoxythymidine 5'-triphosphate
DWB	Dry root weight basis
ΔH_G	Enthalpy of gelatinisation
EDTA	Ethylene-diaminetetraacetate
EPA	Extension planning area
EST	Expressed sequence tags
FAO	Food and Agricultural Organisation
fmol	Femtomole
FW	Fresh root weight
FWB	Fresh root weight basis
g	Gram
G	Genotype
GD	Genetic distance
GD	Genetic dissimilarity
GDP	Gross domestic product
GP	Gene pool
GS	Dice similarity coefficient
GxE	Genotype by environment interaction
HCl	Hydrochloric acid
HCN	Hydrogen Cyanide
HFB	Height of first branch
HUL	Hairiness of unexpanded apical leaves
IITA	International Institute of Tropical Agriculture
IPCA	Interactive principle component analysis
IPGRI	International Plant Genetic Resources Institute
IPICS	International Programme in the Chemical Sciences
ISC	Root inner skin colour
ISI	International Starch Institute
ISP	International Science Programme

J	Joules
K	Potassium
KCl	Potassium chloride
kg	Kilogram
KK	Nkhota kota
l	Litre
L	Location
LAMP	Latin America Maize Project
LCLS	Leaf central lobe shape
LSD	Least significant difference
m	Metre
M	Molar
Mac	Macintosh
MAP	Months after planting
MAS	Marker-assisted selection
masl	Metre above sea level
MBS	Malawi Bureau of Standards
MC	Moisture content
mg	Milligram
Mg	Magnesium
$MgCl_2$	Magnesium chloride
µg	Microgram
µl	Microlitre
µm	Micrometre
µM	Micromolar
min	Minute
ml	Millilitre
MLC	Mature leaf colour
mm	Millimetre
mM	Millimolar
MoALD	Ministry of Agriculture and Livestock Development
MPL	Malawi Pharmacies Limited
MSC	Mature stem colour
MSG	Monosodium glutamate
MW	Molecular weight

N	North
NaCl	Sodium chloride
NCSS	Number cruncher statistical system
ng	Nanogram
NGO	Non-governmental organisation
NH_3	Ammonia
NIL	Near isogenic line
nm	Nanometre
NS	Not significant
NSO	National statistical office
NTSYS	Numerical taxonomy multivariate analysis system
P	Phosphorus
PC	Petiole colour
PCA	Principle component analysis
PCR	Polymerase chain reaction
pH	Power of hydrogen
QTL	Quantitative trait loci
R	Round
RAPD	Random amplified polymorphic DNA
RDP	Rural Development Project
RFLP	Restriction fragment length polymorphism
rpm	Revolution per minute
RVA	Rapid visco-analyser
S	South
SADC	Southern Africa Development community
SARRNET	Southern Africa Root Crops Research Network
SD	Standard deviation
SDS	Sodium dodecyle sulphate
SE	Standard error
SS	Sums of squares
SSA	Sub-Saharan Africa
ssp	Subspecies
SSR	Simple sequence repeat
Taq	*Thermus aquaticus*
TDF	Transcript-derived fragments

Tris-HCl	Tris[hydroxymethyl]aminomethane hydrochloric acid
TSS	Total soluble solutes
U	Unit
USA	United States of America
UPGMA	Unweighted pair group method of arithmetic averages
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

GENERAL INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub with an edible root, which grows in tropical and subtropical areas of the world. The starchy tuberous roots of cassava provide more than half of the calories consumed by more than 800 million people in Sub-Saharan Africa (SSA), Latin America and Asia (Shore, 2002). Cassava has become the most important source of dietary energy in SSA (Scott *et al*., 2000) as it provides more dietary energy per hectare and working hours than any other staple crop (Akoroda, 1995; Fregene *et al*., 2000; Nassar, 2005). Other advantages of cassava include flexibility in planting and harvesting time, and drought tolerance. The ability of cassava to grow and produce on low nutrient soils, where cereals and other crops do not grow well, and suitability for incorporation in various cropping systems are the other advantages of cassava (Onwueme, 1978; Fregene *et al*., 2000; Nassar, 2005). Leaves of cassava are used as a vegetable in Africa and are a cheap but rich source of proteins, vitamins A, B and C, and other minerals (Hahn, 1988; FAO, 1993; Moyo *et al.*, 1998; Fregene *et al*., 2000; IITA, 2001). These attributes make cassava a mainstay of smallholder farmers in the tropics with limited access to agricultural inputs (Fregene *et al*., 2000). Most smallholder farmers grow a number of cultivars, each with locally preferred characteristics such as taste, early maturity, pest and disease resistance, and/or processing characteristics (Salick *et al*., 1997; Chiwona-Karltun *et al.,* 2000; Mkumbira *et al*., 2001).

Cassava is the most important root crop in Malawi. It is grown across the country and is a staple food crop for more that 30% of the people along the central and northern lake shore areas of Lake Malawi and the Shire highlands. Cassava is used as an important food supplement, a main part of breakfast and a snack in the rest of the country (FAO, 1993; Sauti *et al*., 1994; Moyo *et al.*, 1998). Cassava is becoming an important cash crop for smallholder farmers, middlemen as well as sellers in various markets and is increasingly becoming an important industrial crop (Sauti *et al*., 1994; Moyo *et al.*, 1998; Benesi *et al.*, 2001a; 2001b; Benesi, 2002; Benesi *et al.*, 2004). Cassava tuberous roots are an excellent source of carbohydrates but contain very little protein. By contrast, fresh cassava leaves contain 17-18% protein and are used extensively as a vegetable in many areas in Malawi. They are especially useful in the dry season when other green vegetables are in short supply (FAO, 1993).

One of the cassava products of economic value for farmers as well as various industries is cassava starch. Industries in Malawi import starches, dextrins and cassava substitutes from Zimbabwe, South Africa, the Netherlands, United Kingdom and Tanzania. Starches largely constituted those from maize, potato and wheat (NSO, 1994-1999; Fungulani and Maseko, 2001; Itaye, 2001; Munthali, 2001; Masumbu, 2002). The importation of starch, dextrins, and cold setting adhesives leads to loss of large amounts of foreign currency, and to increased unemployment (NSO, 1994-1999; Masumbu, 2002).

Starch is a valuable ingredient for the food industry, being widely used as a thickener, gelling, bulking and water retention agents (Niba *et al*., 2001; Singh *et al*., 2003). Cassava starch is used directly in different ways or as a raw material for further processing in the production of paper, textiles, as monosodium glutamate (MSG), and as an important flavouring agent in Asian cooking (FAO, 2001; IITA, 2001; Benesi, 2002). Cassava starch use has a high potential for growth, both in industry and for human consumption. The unique properties of cassava starch suggest its use even for speciality markets such as adhesives, baby foods, non-allergenic products and food for hospitalised persons (Moorthy, 1994; Thomas and Atwell, 1999; Masumbu, 2002).

Starch is the most abundant reserve for carbohydrate in plants (Singh *et al*., 2005). Moorthy (2001) pointed out that starch functional properties such as viscosity, gelatinisation temperature, and solubility need to be given attention. Numfor and Walter (1996) considered amylose content, average granule diameter, solubility and swelling power, enthalpy of gelatinisation (ΔH_G) and profile texture as important starch functional properties. These insights on pasting and granular characteristics are relevant in quality assessment of cassava starch-based products and processing variables.

Results of a study conducted by Benesi (2002) showed that native cassava starch from elite Malawian cassava genotypes met the requirements of the major industries using starch in Malawi. However, tablet making for long storage time in the pharmaceutical industry needs more specialised starch properties. Some researchers reported differences in starch functional properties from different genotypes (Moorthy, 1994) which affect specialised use in various sectors. It is imperative to evaluate the functional properties for cassava starch of the prominent released varieties and the most promising cassava clones in Malawi. Cassava starch modification should be investigated to meet the needs of the more specialised markets. In addition, modified starches fetch better prices than native starches.

Significant interaction between genotypes and environment for starch yield has been observed by Benesi *et al*. (2004). Ngendahayo and Dixon (2001) reported that no universal maturity time could be recommended for cassava because the optimum harvest time for cassava depends on the cultivar, rainfall distribution pattern, and soils. They recommended that the optimum harvest time of cassava genotypes has to be determined at the target agro-ecologies where they will be grown.

Efficient utilisation of germplasm involves exploration, conservation and characterisation of germplasm. The greatest genetic diversity of cassava exists in Latin America, although substantial diversification has taken place in Africa since the crop was introduced (Hershey, 1987; Second *et al.*, 1997) because cassava in Africa is mostly produced by smallholder farmers in marginal environments. They use a relatively large number of crops and crop varieties in trying to reduce risks in terms of food security and balancing their diet (Brush, 1995; Chiwona-Karltun *et al*., 1998; Elias *et al*., 2001).

Farmers distinguished genotypes by examining plant morphology better than did the investigators using standard botanical keys (Nweke *et al*., 1994). Mkumbira (2002) observed that cassava farmers in Malawi could examine and differentiate with ease 167 (92%) of 181 cassava plants. Malawian farmers accurately classified cassava into sweet and bitter varieties, and linked them to safety levels of cyanogenic glucoside (Chiwona-Karltun, 2001; Mkumbira *et al*., 2001). The accuracy of this classification was confirmed by simple sequence repeat (SSR) studies. Processing methods are adopted based on toxicity levels of cassava. Studies using botanical taxonomy did not recognise any morphological signs of genetic division which supported the idea that morphological characters did not relate to agronomical characters (Mkumbira, 2002).

Economical, political, and technological integration of farming systems is generally seen as a positive step that enables development since it leads to increased production, income, and wellbeing (Brush, 2000). Nevertheless, this integration has several negative impacts, which include: farmers relinquish personal and local control of the production system as they become subjected to market and political systems, which are not always stable or positive for particular locations or commodities; the increasing growing of uniform crops may lead to vulnerability to diseases and pests; and local knowledge and crop diversity may be lost (Brush, 2000). It has been observed that farmers in Malawi continually replace and update varieties (Benesi *et al*., 1999; Moyo *et al*., 1999).

A prerequisite for evolution, adaptation and genetic improvement is the existence of genetic variation (Beeching *et al*., 1993; Jarvis and Hodgkin, 2000). If the continued use of local cultivars by farmers is to form part of the conservation strategy and/or the planning and establishment of a core collection, knowledge of levels of genetic variation is a must (Jarvis and Hodgkin, 2000). This knowledge needs to be linked to how farmers perceive and value diversity, which leads to decision making according to their preferences and needs (Jarvis and Hodgkin, 2000; Elias *et al*., 2001).

Studies carried out in South America and Africa by various researchers (Kapinga *et al*., 1997; Cardoso *et al*., 1998; Chiwona-Karltun *et al*., 1998; 2000; Elias *et al*., 2001; Narváez-Trujillo *et al*., 2001) revealed the importance of indigenous knowledge in germplasm collection, conservation and genetic improvement. Ignoring indigenous knowledge has led to wrong sampling of germplasm during explorations, and recommendation of varieties, which did not solve farmer and consumer problems. As a result, most of the technologies which have been developed are on the shelf, since farmers are not ready to adopt them as they do not address their preferences and needs (Nweke *et al*., 1994; Spencer, 1994; Chiwona-Karltun *et al*., 1998).

Plant breeders relied on phenotypic traits as markers for cultivar identification before the development of molecular markers (Hershey and Ocampo, 1989; Elias *et al*., 2001; Zacarias *et al*., 2004). Even now, using simple breeder observations, which is a low technology conventional approach, is still important. These markers are readily available for use on cassava, especially in Africa, where the capacity to use molecular markers is not yet fully developed (Mkumbira, 2002).

The estimation of plant genetic resource diversity has become much more simple and reliable since the advent of molecular marker technology. In contrast to morphological or biochemical marker techniques, DNA-based methods are independent of environmental factors and give rise to a high number of polymorphic loci (Karp *et al*., 1997). This holds particularly true for DNA fingerprinting or 'DNA-profiling' methods based on the polymerase chain reaction (PCR).

A number of DNA fingerprinting techniques are available and are important tools for genetic identification in plant breeding and germplasm management (McGregor *et al*., 2000). When planning DNA fingerprinting, one of the most important decisions is the marker system and technique to be used. Mueller and Wolfenbarger (1999) reported that no single technique is

universally ideal. Each available technique exhibits strengths and weaknesses, so the choice of technique often depends on the question being pursued, the genetic resolution needed as well as available expertise and finances.

Attempts to customise amplified fragment length polymorphism (AFLP) analysis for cassava in genetic diversity studies have not been conclusive (Roa *et al*., 1997; Wong *et al*., 1999). Previous studies concentrated on primer combinations which are not commercially available for researchers in Africa. There is therefore a need to identify the best AFLP primer combinations that are commercially available on the market for use in cassava fingerprinting. Due to the reliability and reproducibility of AFLP analysis, the use of common primer combinations will allow comparison of studies carried out by different researchers (Robinson and Harris, 1999).

This study, therefore, aimed at making collections and conserving Malawian cassava germplasm. In addition, the Malawian cassava germplasm was analysed for genetic diversity. Customisation of the AFLP technique for cassava fingerprinting was one of the aims of this study. Appropriate genotypes, locations and best season to harvest cassava for starch extraction for optimum starch yield were also studied. In addition, characterisation and modification of cassava starch from elite genotypes from Malawi were considered.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

One of the great scientific success stories of the 20th century is the Green Revolution. Cooperative efforts of different stakeholders over three decades led to breakthroughs in wheat and rice production. Unfortunately, we still face the challenge of mass hunger in a world of plenty (Rockefeller Foundation, 1999).

People need enough food to perform normal activities at all times. This will continue to be a central challenge for millions of households, numerous countries and at least one continent, Africa, over the next half century. Of the 5.1 billion people living in developing countries, 3 billion live in rural areas, most of them dependent on agriculture for livelihoods. Currently, about 800 million people remain undernourished and roughly 24000 people die each day from hunger and hunger-related causes. Most of those who remain undernourished, live in regions bypassed by the agricultural advances of the Green Revolution that contributed to dramatic improvements in food security for the majority of the world's people. Living on land with low natural agricultural potential, having few formal educational opportunities and little access to technology, these farming families, concentrated in SSA, and less-favoured parts of Asia and Latin America, remain in poverty (Rockefeller Foundation, 2002).

2.2 Cassava

2.2.1 Taxonomy of cassava

Cassava (*Manihot esculenta* Crantz), a single species, belongs to the family Euphorbiaceae. 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). Cassava cultivars have been classified according to morphology, e.g. leaf shape and size, plant height, stem and petiole colour, inflorescence and flower colour, root shape and colour, and content of cyanogenic glucoside in the roots (Onwueme, 1978; Mkumbira, 2002; Nassar, 2005).

Cyanogenic glucoside has been used to place cassava cultivars into two major groups: bitter cultivars, in which the cyanogenic glucoside is distributed throughout the tuberous root, at levels higher than 100mg/kg fresh root weight, and sweet/cool varieties, in which the cyanogenic glucoside at low levels is confined mainly to the peel. The flesh of sweet/cool varieties is therefore relatively free of cyanogenic glucoside (Mkumbira, 2002; Nassar, 2005). Early literature on cassava therefore described the genus as having two edible species, *Manihot utilissima* Phol and *Manihot aipi* Phol delineating cultivars with high and low cyanogenic glucoside concentration respectively. Cassava has recently been classified as being one species, *Manihot esculenta* Crantz (Onwueme, 1978).

2.2.2 Cytology and reproductive biology of cassava

Cassava has a sporophytic chromosome number of 2n=36 (Onwueme, 1978; Nassar, 2005) which shows regular bivalent pairing at meiosis. However, there is evidence of polyploidy, from studies of pachytene karyology (Mkumbira, 2002). Nassar (1978) and Nassar *et al*. (1996) reported some aneuploids for certain genotypes. Magoon *et al*. (1969) reported that polyploids are rare, though cassava could be an allotetraploid. Cassava is proposed to be an allotetraploid since there are three nuclear chromosomes, which is high for a true diploid. *Manihot* species are probably segmental allotetraploids derived from crossing between taxa whose haploid complements had six chromosomes in common but differed in the other three (Magoon *et al*., 1969). Studies using biochemical markers supported this interpretation, in that disionic inheritance was seen at 12 loci, with evidence of gene duplication (Jennings and Hershey, 1985; Charrier and Lefevre, 1987).

Cassava is highly heterozygous and monoecious. Female flowers normally open 10 to 14 days before male flowers on the same inflorescence, promoting cross pollination. Self pollination can occur from male and female flowers from different branches or plants of the same genotype that open simultaneously. Seed obtained from self pollination is considered partially inbred due to reduced heterozygosity (Onwueme, 1978; Hahn *et al*., 1979; Kawawo, 1980; IITA, 1990; Nassar, 2005). Insects, particularly bees and wasps, are the main pollination agents (Onwueme, 1978; IITA, 1990; Mkumbira, 2002; Nassar, 2005). Pollen varies in fertility from almost sterile to 95% fertile. 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).

Apomixis (viable seed formation without fertilisation) has been reported in cassava by Nassar (1994; 2000). In cassava, apomixis is an alternative to reproduction through cuttings, which is normally practised by farmers. Propagation using cuttings leads to accumulation of viral and bacterial diseases that reduces productivity and may lead to extinction of superior genotypes. The use of apomitic plants for propagation could avoid accumulation of systemic pathogens and exclude genetic segregation of good characters in the progeny. Use of apomitic seeds can ensure preservation of superior genotypes as opposed to extinction (Nassar, 2005).

2.2.3 Morphology, agronomy and climatic requirements of cassava

Cassava is a perennial woody shrub of one to three metres high with edible tuberous roots arising from the stem cutting, but farmers mostly grow it as an annual crop (Onwueme, 1978; Lozano *et al*., 1980; IITA, 1990; 2001; Benesi, 2002; Nassar, 2005). It is propagated mainly from stem cuttings but during plant breeding and under natural conditions, propagation is by sexual seed in the first cycle (Onwueme, 1978; IITA, 1990; Nassar, 2005). Cassava seeds germinate slowly and normally display dormancy. The germination period can be shortened by filing the micropylar end until the white embryo is just visible. A wet treatment of cassava seed has also been reported to improve seed germination (Onwueme, 1978). The best scarifying method is thermal treatment, by exposing seeds to temperatures of 18^{o}C for 16 hours or 26^{o}C for 8 hours (Nassar, 2005). Whether cassava seeds are scarified or not the most essential factor for cassava seed germination is temperature which should range between 30 and 35°C Onwueme, 1978. Cassava plants arising from sexual seeds are normally weaker than those from cuttings due to the genetic heterozygosity structure of cuttings, while sexual seeds are homozygous for recessive and prejudicial genes (Nassar, 2005).

Cassava tuberous roots are composed of a peel which represents about 10-20% of the tuberous root. The cork layer represents 0.5-2.0% of the total tuberous root weight. The fleshy edible portion makes up 80-90% of the tuberous root and is composed of 60-65% water, 30-35% carbohydrate, 1–2% protein, 0.2-0.4% fat, 1.0-2.0% fibre, and 1.0-1.5% mineral matter (Nassar and Costa, 1976; Onwueme, 1978; Nassar, 1986). Most of the carbohydrate fraction contains starch which makes up 20-25% of the tuber flesh (Purseglove, 1968). The tuber is relatively rich in vitamin C (35mg/100g fresh weight), and contains traces of niacin and vitamins A, B1 and B2 but the amounts of thiamine and riboflavin are negligible (Onwueme, 1978).

Cassava grows in tropical and subtropical areas of the world between latitudes 30^{o} N and S of the equator under diverse ecological and agronomical conditions (Onwueme, 1978; Lozano *et al*., 1980; IITA, 2001; Benesi, 2002; Nassar, 2005). Cassava is a lowland tropical plant and needs a warm moist climate with mean temperature of 24-30^{o}C (Onwueme, 1978; IITA, 1990; Nassar, 2005). The ideal soils for cassava are light sandy loam with medium fertility. Cassava has the ability to grow on marginal lands where cereals and other crops do not grow well, it can tolerate drought and can grow in low nutrient soils but does not tolerate high concentrations of salts with a pH above 8, excess soil moisture, and temperatures of 10^{o}C and below (Onwueme, 1978; Lozano *et al*., 1980; IITA, 2001; Benesi, 2002; Mkumbira, 2002; Nassar, 2005).

Cassava tuberous root formation commences by the end of the second month after planting. With time, the tuberous roots continue to increase in size by swelling due to the deposition of large amounts of starch within the tuberous root tissues. Hence, very young tuberous roots contain much less starch than old ones, so harvesting must be delayed until an appreciable amount of starch has accumulated in the roots. However, as the tuberous roots become older, it tends to become more lignified and fibrous, so that the starch content, as a percentage of the total dry weight of the tuberous root, tends to decrease or remain constant (Onwueme, 1978; ISI, 1999-2001). It is therefore best to harvest cassava at the time when the tuberous roots are old enough to have stored sufficient starch, but not too old to have become woody or fibrous (Onwueme, 1978). The exact time in terms of months after planting, when it is best to harvest cassava depends on the cultivar. Some cultivars are ready for harvest at seven months after planting (MAP) while others require up to 18 MAP (Onwueme, 1978). Corbishley and Miller (1984) reported that starch content of cassava tuberous roots depends on many factors such as variety, soil type and climate, in addition to the age of the plant.

Cassava tuberous roots formation is photoperiodically controlled. Under short day conditions tuberisation occurs readily, but when the day length is 12 hours or longer, growth is delayed, and yield reduced (Bolhuis, 1966).

2.3 Origin of cassava

Cassava is an ancient crop species. Purseglove (1968) reported that cassava was grown as a crop in Peru some 4000 years ago and 2000 years ago in Mexico. It is estimated that domestication of cassava started 5000 to 7000 years BC and archaeological findings using starch particles from the Amazon supported this idea (Towle, 1961; De Boer, 1975; Gibbons, 1990; Chiwona-Karltun,

2001). By the time the first Europeans reached the New World, the crop was already cultivated in all neotropical America (Purseglove, 1968; Allem, 2002).

The question of botanical origin, i.e. the wild species from which cassava originated, was unresolved until recently. Purseglove (1968) and Umanah and Hartmann (1973) reported that cassava was unknown in the wild state, which qualified it to be a cultigen, known only in cultivation, since no wild cassava had been found. Recent studies in Brazil, using molecular genetics, have shown that Brazilian *M. esculenta* subspecies (ssp) *flabellifolia* is the most likely source, and the Amazon Basin as the site of domestication (Allem, 1994; Haysom *et al*., 1994; Second *et al*., 1997; Olsen and Schaal, 1999). Further studies suggested that in addition to the domesticated *M. esculenta* Crantz ssp *esculenta* (all cultivated genotypes), two wild plants, *M. esculenta* ssp *flabellifolia* (Phol) Ceferri (the likely ancestor) and *M. esculenta* ssp *peruviana* (Muell. Arg) Allem, are said to form the primary gene pool (GP-1) (Allem *et al*., 2001; Allem, 2002). These studies therefore classified cassava as an indigen, i.e. a plant known to encompass both wild and cultivated populations.

2.4 Introduction and spread of cassava to Africa

The Portuguese first brought cassava to Africa in the form of flour or 'farinha'. The Tupinamba Indians of eastern Brazil taught the Portuguese techniques of *manioc* preparation and production, and they developed a liking for the various processed forms (Ross, 1975). The first mention of cassava cultivation in Africa dates back to 1558 (Mauny, 1953; Silvestre and Arraudeau, 1983). At first, it was cultivated with a sole purpose of providing chips to slaves, until about 1600 when it became an important part of African subsistence farming (Carter *et al.,* 1992). Jones (1959) and Ross (1975) proposed that multiple, and more-or-less simultaneous introductions took place at Portuguese trading stations (Figure 2.1; Carter *et al.,* 1992).

Knowledge of the diffusion of cassava into the interior of Africa during the next 250 years is extremely sparse. From the writings of the European explorers who penetrated central Africa in the late 19th century, it is seen that cassava had by then been successfully incorporated into many farming systems (Jones, 1959).

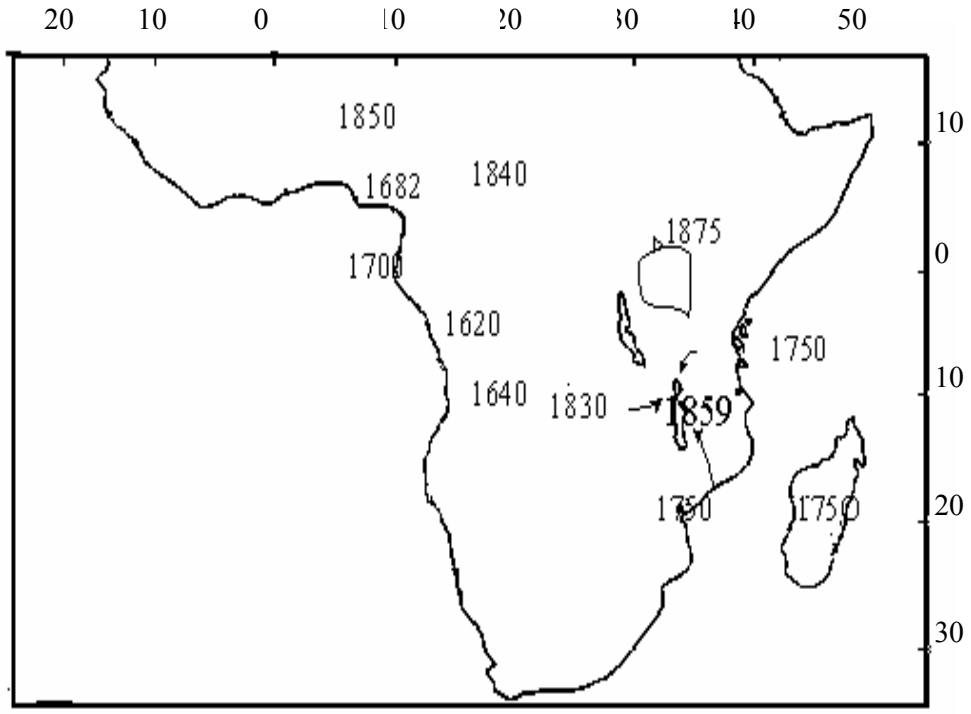


Figure 2.1 Map of Africa showing when cassava was first reported in various parts (Carter *et al.,* 1992)

2.5 Importance of cassava

Cassava (*M. esculenta*) is the most important tropical root crop (Onwueme, 1978, Roa *et al*., 1997; Mkumbira, 2002) and is primarily grown for its starchy tuberous roots, which are a major source of dietary energy (Onwueme, 1978; Cock, 1985; Lynam, 1993; Nassar, 2005). It was estimated that in 2002, more than 700 million people consumed cassava in one form or the other (Dixon *et al*., 2003). Cassava accounts for approximately one-third of the total staples produced in SSA and is grown exclusively as food in 39 African countries stretching through a wide belt from Madagascar in the south-east to Senegal in the north-west (Raji *et al*., 2001a). Cassava is grown throughout Malawi and is used as staple food in the densely populated lakeshore districts (Sauti *et al.,* 1994). Cassava leaves are an important vegetable rich in protein, minerals and vitamins (Jones, 1957; Onwueme, 1978; Hahn, 1988, FAO, 1993; Nweke, 1994; Chiwona-Karltun *et al*., 1998; Fregene *et al*., 2000; IITA, 2001; Benesi *et al*., 2001a; 2001b).

Shore (2002) said that cassava has all indicators to be a possible salvation for Africa from the famines that have spread through the continent. This is because of its high calorie production, year-round availability, and tolerance to extreme environmental stress conditions. In Africa,

people are starting to use cassava in industries like textile, wood, as binding agent, and partial substitution for wheat flour. This provides income to resource-poor farmers and saves foreign exchange for nationals. Opportunities for product and market diversification are excellent in several countries, such as Nigeria, Uganda, Malawi, and of late South Africa (CGIAR Research, 2001; Benesi *et al.,* 2004).

Although cassava has a wide range of uses, it is mainly used as a food crop in Africa and the rest goes to waste. In most cases cassava is used as a fresh product at homesteads and by other users. Sale or use of fresh cassava for processing effectively reduces downward pressure on producer prices at harvest caused by the often abundance of supply, thereby raising farm incomes or enabling the market to absorb greater surpluses without causing farm gate prices to fall. In Africa, there exists a need for increased production of cassava to meet food requirements and have surplus for industry, feed and even export. Processing adds value at farm level and reduces perishability and bulkiness, thereby facilitating the sale of cassava products in the off-season and in distant markets (Chiwona-Karltun, 2001). Processing can help improve food security by generating employment and income for non-growers, thereby enhancing purchasing power to gain more ready access to food (Benesi, 2002).

Cassava is the most important root crop in Malawi (FAO, 1993; Moyo *et al.*, 1998). It is a staple food for over 30% of the population especially those living along the Lakeshore districts of Karonga, Rumphi, Nkhata Bay, Nkhotakota and Salima as well as in the Shire highlands. The importance of cassava as a food security crop became more apparent with climatic, physical and socio-economic environmental changes in the early to mid 1990s. Increases in prices of farm inputs due to devaluation of the Malawian Kwacha and removal of subsidies highlighted the importance of cassava (Minde *et al.*, 1997). The fact that cassava tolerates drought, poor quality soil, and less elaborate management practices has made it the best candidate crop to be promoted by the Government of Malawi in crop diversification for achieving food security as well as a commercial crop (FAO, 1993; Benesi, 2002). Even farmers themselves now agree that cassava is a salvation crop for their livelihood. When there is mass starvation, in predominantly maize growing areas, the hunger situation is much less where cassava is grown as a main staple. Despite all the advantages and uses of cassava, it still lags behind in terms of production and technology since it has been neglected and considered as a primitive crop, food for the poor and a crop with poor nutritional value for a long time. It has been realised that cassava is the crop for food security. Climatic changes which is causing erratic rains and socio-economic problems has had negative impacts on

the production of the crop in the Southern Africa Development Community (SADC) countries, leading to mass starvation in most of the SADC countries.

2.6 Starch

Utilisation of any crop as an industrial raw material depends on a number of factors such as growing conditions, availability, price and ease of use (Jarowenko, 1977). In many cases, availability becomes the determining factor since this affects the price (Moore *et al*., 1984; Fabiano *et al.*, 2001). This explains why the USA uses maize starch, Canadians, Australians and New Zealanders use mostly wheat starch, while Europeans use potato and maize starch. Tropical countries like Brazil and the East Indies (in Asia) use cassava starch (Radley, 1976; Jarowenko, 1977; Wurzburg, 1986a).

Although cassava starch has been in use for a long time in many parts of the world, maize starch has almost exclusively been used in Malawi. Cassava has the highest starch content among root and tuber crops. Cassava starch extraction is easy since it settles rapidly and gives a good yield. The resulting starch is free from any colour or impurities, in contrast to other plant starches which are contaminated with proteins or fats and are hence discoloured (Moorthy, 1994).

Maize is the main staple crop for most of the people in Malawi, and it is used in the feed industry. At the same time maize is facing serious challenges in production due to climatic changes, and increase in input costs like fertilisers (Minde *et al*., 1997). Moreover, Malawi has been unable to produce enough maize for food in recent years. Therefore, use of maize for starch production in Malawi would increase demand for maize and most likely its price. On the other hand cassava is high yielding and gives high return per unit energy input into cultivation (Agboola *et al.*, 1990; Rickard *et al.*, 1991). Production of cassava starch in Malawi would promote cassava production.

Native and modified starches can be used to influence physical properties of many foods like gelling, thickening, adhesion, moisture retention, stabilising, texturising, and anti-staling applications (Thomas and Atwell, 1999). Starch and its products are important in the paper, pharmaceutical, wood, packaging and textile industries, in ethanol and alcohol production, battery making, and in the production of explosives like matches (Whistler, 1984; Moorthy, 1994; Benesi *et al*., 2004).

Modified cassava starch can compete with other starches for the production of alcohol, starch for sizing paper and textiles, glues, MSG, sweeteners, bio-degradable products, butanol and acetone, manufacturing of explosives, and coagulation of rubber latex (FAO, 2001). Despite the competition from synthetic polymer adhesives (Phibbs, 1997; Central Science Laboratory, 2002), the use of starch adhesives continues to increase world wide (Kennedy, 1989). Starch has several advantages as a raw material for the production of adhesives. These include re-newability, biodegradability, abundance, cheapness and stability in price (Kennedy, 1989; Masamba *et al*., 2001). Starch adhesives can be applied at ambient temperatures or moderately low temperatures and are usually re-wettable and have little or no odour and taste (Radley, 1976). As a polymeric polyhydroxy compound, starch yields adhesives with excellent affinity to polar substrates including cellulose (Jarowenko, 1977).

Starch is a primary source of stored energy and consists primarily of D-glucopyranose polymers linked by α-1,4 and α-1,6 glucosidic bonds called amylose and amylopectin, respectively (Figure 2.2) (Wurzburg, 1986a; Thomas and Atwell, 1999). These bonds are formed when carbon number 1 (C1) on a D-glucopyranose molecule reacts with carbon number 4 (C4) or carbon number 6 (C6) from the adjacent D-glucopyranose molecule. Since the aldehyde group on the end of the starch polymer is always free, starch polymers have at least one reducing end (Figure 2.2; Wurzburg, 1986a; Thomas and Atwell, 1999). Starch polymers contain only α-linkages which allow some starch polymers to form helical structures unlike the β configuration of cellulose which forms the sheeted ribbon-like structure (Thomas and Atwell, 1999).

Amylose is essentially a linear polymer in which the anhydroglucose units are predominantly linked through α-1,4 glucosidic bonds (Figure 2.2). The molecular weight (MW) for amylose ranges between 243000 and 972000. Although amylose from potato starch has been reported to have a MW of up to 1000000, the MW for amylose is typically less than 500000. The average MW of amylose from cassava starch seems to vary greatly, possibly due to the variety of cassava from which starch is extracted and extraction methods. For instance, three MWs of 232000 (Ciacco and D'Applonia, 1977), 431000 (Takeda *et al.*, 1984) and 522000 (Suzuki *et al.*, 1985) for cassava amylose have been reported in literature. The average MW for maize amylose was reported to be 250000 (Zobel, 1984). The average degree of polymerisation is 960 for maize, 3280 for cassava, 2000 for potato and 2600 for sweetpotato (Jarowenko, 1977; Takeda *et. al*, 1984; Wurzburg, 1986b).

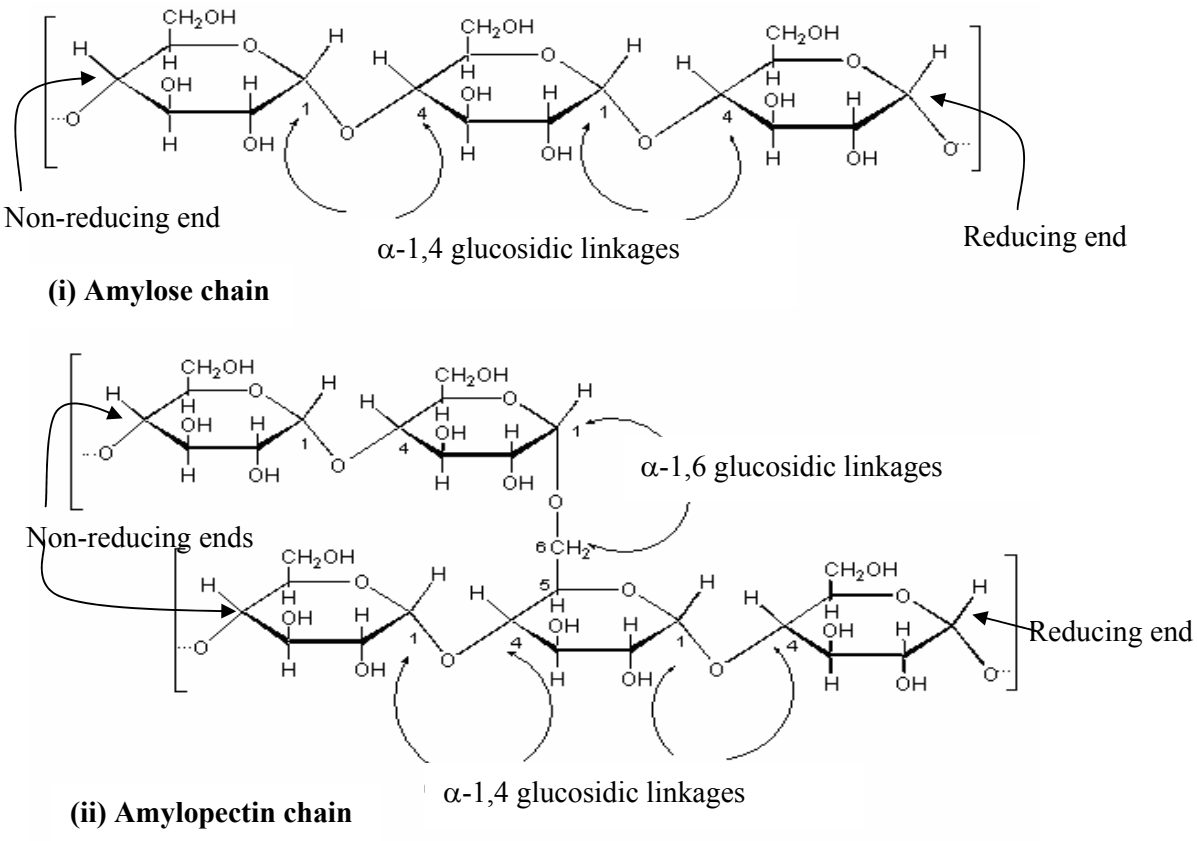


Figure 2.2 Amylose (i) and amylopectin (ii) chains showing the α-1,4 and α- 1,6 glucosidic linkages (Wurzburg, 1986a; Thomas and Atwell, 1999).

Amylopectin, like amylose, is a polymer with α-1,4 glucosidic bonds. However, unlike amylose, it has periodic branches linked to C6 by α-1,6 glucosidic bonds (Figure 2.2[ii]). The MW of amylopectin ranges from 10 million to 500 million (Thomas and Atwell, 1999). The relatively high amylopectin content of cassava probably accounts for the high MW. The average degree of polymerisation is 1450 for maize amylopectin, 1300 for cassava amylopectin and 2000 for potato amylopectin (Jarowenko, 1977; Wurzburg, 1986a).

The level of amylose and amylopectin found in starch depends upon crop and variety from which starch was extracted (Wurzburg, 1986a). Maize and wheat starch have an average amylose content of 28% and 26%, respectively, while potato**,** sweet potato and cassava have 20%, 18% and 17%, respectively (Onwueme, 1978; Young, 1984).

Amylose and amylopectin do not exist free in nature, but as components of discrete, semi-crystalline aggregates called starch granules. The diameters of starch granules generally range from 1μm to more than 100μm, and shapes can be regular (spherical, ovoid or angular) or quite

irregular. The diameter for cassava starch granules ranges from 4-35μm (Onwueme, 1978; Moorthy, 1994; Thomas and Atwell, 1999). The study of Moorthy and Ramanujam (1986) revealed that cassava starch granules increase in size two to six months after planting, then remain steady for the rest of the growing cycle of the plant. Cassava starch granules are mostly round or oval with a flat surface on one side containing a conical pit which extends into a well (Moorthy, 1994; Thomas and Atwell, 1999) which Moorthy (1994) described as an eccentric hilum, while Thomas and Atwell (1999) described it as truncated or kettledrum. Some granules appear perfectly round (Moorthy, 1994; Thomas and Atwell, 1999). Although the major components of all types of starch granules are amylose and amylopectin polymers, there is great diversity in the structure and characteristics of native starch granules depending on environment and source in terms of the biochemistry of the chloroplast or amyloplast and the physiology of the plant, as shown in Figure 2.3 (Snyder, 1984; Thomas and Atwell, 1999; Singh *et al*., 2005).

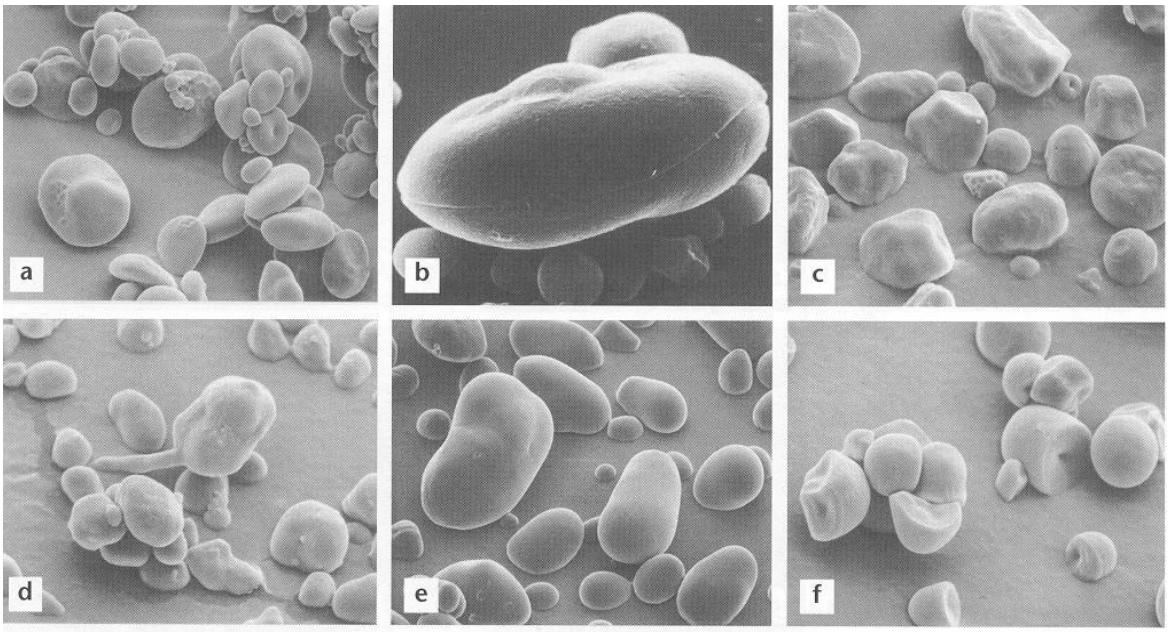


Figure 2.3 Scanning electron micrographs of starches from: (a) wheat (1000x); (b) wheat (2000x); (c) dent maize (2000x); (d) high-amylose maize (2000x); (e) potato (600x); and (f) cassava (2000x) (Evers, 1971; Thomas and Atwell, 1999)

Physicochemical properties, such as percentage light transmittance amylose content, swelling power and water-binding capacity are significantly correlated with the average granule size of starches extracted from different plant sources (Zhou *et al*., 1998).

Gelatinisation temperatures and enthalpies associated with gelatinisation endotherms vary between different starches. Sandhu *et al*. (2004) observed that for normal maize starches peak

onset temperature was in the range of 64.0–68.9^{o}C, peak maximum temperature 68.9–72.1^{o}C and peak end temperature in the range of 73.2–76.8^{o}C. According to Singh *et al*. (2003), the high transition temperatures for maize starch might be a result of more rigid granular structure and the presence of lipids. Peak maximum temperature gives a measure of crystallite quality (double helix length) (Singh *et al*., 2003). Waxy maize starches showed higher values of transition temperatures in the range of 70.8–80.9^{o}C, compared to sugary maize (66.5–76.1^{o}C; Singh *et al*., 2003). The B-type crystal form of amylopectin in high amylose starches results in higher gelatinisation temperatures than normal maize starches (Richardson *et al*., 2000). Enthalpy of gelatinisation (ΔH_G) reflected the loss of double helical rather than the crystalline order (Cooke and Gidley, 1992). The ΔH_G values of starches were reported to be affected by factors such as granule shape, percentage of large and small granules, and the presence of phosphate esters (Stevens and Elton, 1971; Yuan *et al*., 1993). The ΔH_G was highest for high amylose starch and lowest for sugary maize starch. Noda *et al.* (1998) postulated that low peak onset, peak maximum and peak end temperatures reflect the presence of abundant short amylopectin chains in starch. The variation in transition temperatures and ΔH_G from different maize starches might be due to differences in amount of longer chains of amylopectin. The longer chains were reported to require a higher temperature to dissociate completely than that required for shorter double helices (Yamin *et al*., 1999)

Granular starch molecules within the ungelatinised granules are tightly bonded to one another. Starch granules absorb water and swell to a limited extent when suspended in water or exposed to high humidity. This absorption is a reversible swelling, as the original volume is retained when humidity is reduced or the starch is dried (Wurzburg, 1986a). In order for starch molecules to become detached from one another, they must be gelatinised by heating in water or other solvents (Thomas and Atwell, 1999).

Cassava starch adhesives are tackier, more viscous, and smoother in working and more easily prepared, and their joints exhibit higher tensile strength than those from potato and cereal starches, including maize. This is advantageous because for similar viscosities and tack, cassava-based adhesives require less starch and tackifiers compared to adhesives prepared using starches from other sources (Radley, 1976).

The colour of starch depends on the processing method. Cassava starch extracted under perfect conditions is pure white in colour. Colour is an important criterion for starch quality especially in some foods, and the pharmaceutical and textile industries. The starch paste should be clear and

free from colour for better acceptability. The transparency of starch paste varies tremendously among different starches. Cassava starch is quite transparent and hence has good clarity, making it an excellent starch for food applications. In addition, cassava and sweetpotato starches have low retrogradation tendency and therefore are high in paste stability (Moorthy, 1994).

Generally, root and tuber crops' starches contain less lipid and protein compared to cereals. Although minor amounts of residual lipid and protein can influence gelatinisation, the most dramatic effect of these compounds is on the flavour profile of starch. Compared to most starches, cassava and potato starches are considered to be very bland in flavour because of the small amount of lipid and protein content (Thomas and Atwell, 1999). Studies by Moorthy (1994) and Benesi *et al*. (2004) did not detect any protein in cassava starch.

Starches contain trace amounts of mineral elements and inorganic salts. These minerals are collectively referred to as ash. Ash content can vary depending upon the source of raw material, agronomic practices, extraction and milling procedures, and types of chemical modifications. The ash content of starch is typically less than 0.5% of the dry mass (Thomas and Atwell, 1999).

Benesi *et al*. (2004) reported that environment played an important role in starch yield, in addition to genotypes. It is therefore important to study the effect of environment and genotypes on starch extraction in detail by including more sites. The effect of season on starch yield by varying starch extraction times in relation to onset of the rainy season need to be investigated. An appropriate and user friendly method for determining the best time for harvesting cassava for starch extraction in Malawi should be considered.

2.7 Constraints to cassava research, production and utilisation

2.7.1 Policies towards cassava research and production

Cassava farming is hampered by several constraints, which include: shortage of high yielding varieties addressing end-users' needs (in terms of quantity and quality); high incidence of pests and diseases; use of inappropriate cultural practices; post harvest losses; limited modes of utilisation; the negative attitude of consumers towards cassava products; and shortage of clean and healthy planting material (Sauti *et al.*, 1994; Benesi *et al.*, 2003).

Breeding for high dry matter content, early bulking, and better peeling have been the major objectives of cassava breeding programmes for the past 30 years in SSA and at global level. Starch percentage and starch yield are closely related to dry matter percentage. Dry matter content, leaf retention and accumulation of starches in the roots have an important impact on dry matter yield (Pérez *et al*., 2001).

2.7.2 Biotic and abiotic constraints to cassava production and expansion

In SSA, a number of biotic (diseases, insects, mites, and weeds) and abiotic (soil, climate, and agronomic factors) constraints militate against increased production of cassava. Cassava is a long season crop and the diverse agro-ecologies in which it is grown contribute largely to its exposure to a number of these constraints (Dixon *et al*., 1992; Mahungu *et al*., 1994).

Nichols (1950), Storey (1936) Onwueme (1978) Sauti (1981), IITA (1985) and Raji *et al*. (2001a) pointed out that the economically most important diseases include cassava mosaic disease (CMD), cassava bacterial blight (CBB) and cassava anthracnose disease (CAD), while the most important arthropod pest is cassava green mite (CGM), variegated grasshopper, whiteflies, termites. The important mammal pests are rodents, wild pigs and monkeys. Diversification of resistance to diseases and pests is necessary because diseases and pests may continue to evolve into new races or biotypes which can increase in prevalence and economic losses (Raji *et al*., 2001a).

There exist differences in the types of diseases or even strains in Africa and America. Even within Africa, the diseases which are problematic in east and south Africa are not the same as those in west Africa. Cassava brown streak disease (CBSD) is a serious problem in the coastal areas of south-east Africa and of late CBSD-like symptoms were observed in 2002 in the western part of Democratic Republic of Congo (DRC), namely Bas-Congo and Kinshasa provinces, which were previously unaffected areas such as DRC and the whole West Africa (Mahungu *et al*., 2003). In the case of pests, cassava mealy bug is the most important pest in cassava production in the east and south Africa as complemented by Sauti *et al*. (1994) and Nassar (2005) compared to CGM which is a big problem in west Africa as highlighted by Raji *et al*. (2001a). This emphasises the need to use local germplasm in breeding programmes since landraces are already adapted to local conditions. Only useful exotic genes should be introgresed into local cultivars to stabilise yield and add value to the local varieties. Unfortunately as pointed

out by Raji *et al.* (2001a) local cultivars of cassava have been used to a lesser extent in African breeding programmes.

2.7.3 Limitations in amount of knowledge available on local germplasm

The diversity of African local genotypes is yet to be fully exploited. There exists a need to exploit genes of resistance to major diseases and pests as well as preferred food quality traits of local cultivars in tropical Africa (Raji *et al*., 2001a).

The study of Raji *et al*. (2001a) showed that out of 11 selected landraces, some of the local cultivars were superior to improved checks in terms of pest and disease resistance as well as quality traits. Some of the cultivars were comparable to the improved checks in terms of yield in addition to combining resistance and quality traits, although it was widely reported that local Nigerian genotypes were low yielding (Raji *et al.*, 2001a).

2.7.4 Environmental constraints

Rainy season for most of Malawi is between October/November and March. There are few areas where rain continues for more than six months, and most of the areas have up to nine months of dry weather. The majority of the areas where cassava is the most important crop happen to be prevalent drought areas. Although cassava is drought tolerant, inception of drought at time of planting and establishment, influences performance (Sauti, 1981; Sauti *et al*., 1994). Although cassava grows under diverse ecological and agronomical conditions, it favours a warm moist climate with mean temperature of 24-30^{o}C. Cassava can grow in low nutrient soils but does not tolerate high concentrations of salts with a pH above 8, excess soil moisture, and temperatures of 10^{o}C and below (Onwueme, 1978; Lozano *et al*., 1980; IITA, 1990; IITA, 2001; Benesi, 2002; Mkumbira, 2002; Nassar, 2005). Since cassava has proved to be a key to food security by farmers and income by various players, it is grown even in areas where it can not do well like cool and /or water logging areas which leads to poor yields.

2.8 Management of cassava genetic diversity

2.8.1 Availability of cassava genetic diversity

Cassava genetic diversity and its origin should play an important role in unmanaged ecosystems. Although human and natural selection work independently within a certain ecological system, they

may act as similar agents of cassava genetic diversity. For example, human selection for productivity may reinforce natural selection (Nassar, 2005). In certain environmental conditions, cassava genetic diversity may be affected, for example, by unfavourable climatic conditions (floods), and diseases and pests may lead to losses of certain varieties (Nassar, 2005). A variety may be lost accidentally due to lack of suitable space or planting material but may be recovered since there is a wide exchange of planting materials by farmers of different villages (Salick *et al.,* 1997; Chiwona-Karltun, 2001).

Cassava genetic diversity exists due to the occurrence of occasional sexual propagation through use of volunteer seedlings by farmers, incorporation of varieties or seedlings of cassava that have similar phenotypes but of different genetic constitutions in the farming system, and exchange of planting materials by farmers within and between areas. These processes lead to the creation, evolution and conservation of gene pools that may be useful should conditions change (Chiwona-Karltun *et al*., 1998; Chiwona-Karltun, 2001; Elias *et al*., 2001; Mkumbira, 2002; Nassar, 2005). The role of sexual reproduction in generating diversity is complex. Not only does it directly generate new genotypes, it influences the way in which genetic diversity is distributed across perceived categories. This perception of diversity can lead to further indirect effects on the dynamics of diversity, because it changes the relation between phenotypes that are the object of selection and management, and genotypes, the durable results of selection (Elias *et al*., 2001).

Managed farming ecosystems pay more attention to improved production to respond to the needs for the market and/or uses than genetic diversity (Nassar, 2005). The shift from subsistence to market oriented farming has resulted in a major loss of genetic diversity of many crops in the world. Farmers replace many folk varieties with one or a few high yielding varieties that have high market demand. This has led to the idea of collecting and conserving crop genetic resources (Berthaud, 1997; Mkumbira, 2002).

In management of genetic resources, a variety is often considered to be the unit of conservation. In the case of traditional farming systems, the concept of a variety can encompass diverse genetic entities subjected to evolution. Factors that contribute to such a situation are: traditional naming and classification systems are often based on traits that are perceived subjectively and are thereby subjected to inter-individual variations in use or interpretation, confusion between varieties or use of different names for the same variety, and different varieties often co-exist within a single field, which could promote gene flow between varieties through hybridisation (Chiwona-Karltun *et al*., 1998; Elias *et al*., 2001; Mkumbira, 2002).

There is relatively less genetic information available on cassava compared to other important food crops of the world (Mkumbira, 2002) although it is a prerequisite for any cassava genetic improvement programme (Beeching *et al*., 1993; Benesi, 2002).

2.8.2 Methods of germplasm conservation

Current questions being addressed by investigators of genetic resources are related to management. It is necessary to successfully conserve collected material as long as possible, regenerate this material when needed, and establish a more systematic utilisation of genetic resources by breeders. Genetic resources are largely an untapped reservoir of diversity (Berthaud, 1997).

Three models of conservation of genetic resources namely, linear, triangular and circular are proposed (Ericksson *et al*., 1993; Brush, 1995; Berthaud, 1997). The linear model is seen as classic, as it is the most widely used, especially in the international centres of Consultative Group on International Agricultural Research (CGIAR). This model relies on *ex situ* conservation and emphasises stability of conserved material, and ease of availability to breeders who are in charge of utilisation (Berthaud, 1997). *Ex situ* collections reached impressive sizes (IPGRI, 1993). The problem with management of large quantities of accessions is worse for vegetatively propagated crops like cassava. Due to this problem, researchers in the 1980s started to work with collections of reduced size, the core collections. Core collections maintain a level of diversity representative of the reference collection but with a limited number of accessions (Berthaud, 1997). The major advantage of the linear model is its flexibility, which allows the use of core collections. Organisation of a core collection relies on available information, either morphological and adaptive traits, or on neutral traits such as molecular markers (Berthaud, 1997).

A characteristic of the linear model is the gene by gene use, while the triangle model is based on a reflection to utilise the wide range of diversity available in gene banks for building new varieties while minimising the loss of diversity at each step of improvement. This model is called triangular due to its multi-step procedure of progressive selection and restriction of variability from the original accessions maintained in the base gene banks to the released varieties (Berthaud, 1997). This model has been applied by the Latin America Maize Project (LAMP) that was established on a large scale covering all of Latin America (Sevilla *et al*., 1994; Berthaud, 1997). This conservation model is dynamic. New genetic structures are created by grouping

accessions from various origins in pools. It has been reported that when intra-accession diversity is large, it gives a reason to organise the pools according to specificity of utilisation and not on the general inter-genetic diversity which is low. Evolution is favoured by recurrent selection within pools. The genetic material proceeding from these various steps of improvement is available for inclusion in other improvement projects and is conserved on the same basis as the base collection. This conservation-utilisation system could become quite complex if it is organised at a global level but is well adapted to projects of a limited size, that is, for the needs of a country or a region (Berthaud, 1997).

In the linear and triangle models, the relation between conservation and utilisation is a one way process. Conservation is followed through utilisation which is linked to a reduction of diversity and when new needs appear they will be satisfied by returning to the base collection. In this case, the key stage is the static conservation of the broadest diversity available in genetic material. Hence, a circular model was proposed by Ericksson *et al*. (1993) to take into account the evolution and evolutionary potential of genetic material. Evolution is favoured by conserving plants *in situ* where they face challenges of diseases and pests prevailing in the area, and participate in gene flow between populations. For crops, *in situ* conservation would be experienced on-farm, as landraces have been created and maintained by farmers themselves, with their own selection pressure and process, and with controlled gene flow between landraces (Berthaud, 1997). Very often, it has been reported that modern varieties, with higher productivity, are replacing traditional landraces, reducing the available diversity in the process as modern varieties have lower genetic diversity (IPGRI, 1993; Berthaud, 1997). On-farm conservation has been proposed as a response to the genetic erosion phenomenon but it freezes the evolution of agricultural activities. In this sense, it is not desirable as there is no reason to impose the maintenance of genetic diversity by farmers at the expense of their income and food security (Iltis, 1974; Berthaud, 1997). However, Brush (1995) showed that there are some examples where farmers maintained modern and traditional varieties in the same field. This situation may be true for smallholder subsistence farmers who grow their crops under marginal environments. However, commercialisation of crops and recommendation of specific varieties for specific uses in the industry force farmers to optimise production and maximise profit (Mkumbira, 2002). These issues pose challenges to the circular model of conservation.

Ex situ is not a unique way of conserving genetic material for improvement of future varieties. *In situ* and on-farm conservation led to introduction of new relationships between farmers and breeders, and between users and managers of genetic resources (Berg, 1993; Hardon and De Boef, 1993). Since the proposed circular model faces a lot of challenges, it leaves the field of conservation of genetic resources wide open for new investigation and experiments, new

relations between scientists and users, and different distribution of responsibilities in conservation of genetic resources (Berthaud, 1997).

2.8.3 Contributions of indigenous knowledge in the status quo of cassava

Studies carried out by Kapinga *et al*. (1997), Chiwona-Karltun *et al*. (1998), Chiwona-Karltun *et al*. (2000), Chiwona-Karltun (2001), Elias *et al.* (2001), Narváez-Trujillo *et al*. (2001), Mkumbira *et al*. (2001) and Mkumbira (2002) agreed that farmers have elaborate knowledge of the cassava cultivars they grow. Knowledge is based on folk taxonomy that uses the botanical appearance and taste of plant parts. The extensive local knowledge about cassava cultivars in Malawi was in agreement with findings of a survey across Africa (Chiwona-Karltun *et al*., 1998; 2000; Nweke and Bokanga, 1994). This knowledge on cassava has evolved since its cultivation and the knowledge is used by farmers in classification and safety of the crop. Hence, cassava breeding and extension services in Africa should be based on existing knowledge and farmers' preferences regarding cassava cultivars (Richards, 1985).

2.8.4 Management of bitterness and toxicity by cassava farmers

Several plants used as food crops like beans, peas, sorghum, lupine, potatoes and cassava contain natural toxins. Our ancestors that domesticated these plants solved this problem by selecting for varieties with negligible toxin levels or developing processing methods that reduced toxins to negligible levels (Nordenskiold, 1924). During domestication and evolution of cassava, both ways were employed leading to the availability of both bitter and sweet/cool types.

Quantitative interviews in Tanzania and Malawi, on preferences of cassava cultivars by farmers in areas where cassava is predominantly consumed as a staple crop, showed that for food security cassava farmers regard bitterness and toxicity of tuberous roots as a protector against theft, spoilage by animals and unplanned harvesting by family members. Farmers reported no problems regarding toxicity and classified cassava as sweet/cool or bitter (Kapinga *et al*., 1997; Chiwona-Karltun *et al*., 1998). Tuberous roots from bitter cultivars need to be processed before consumption while tuberous roots from sweet/cool cultivars could be eaten fresh or directly cooked (Chiwona-Karltun *et al*., 1998). A further study carried out by Chiwona-Karltun *et al*. (2000) assessed the preferences and safety of farmers and consumers in predominantly bitter cassava growing and eating communities. Results showed that there was a strong positive correlation (r=98) between taste and safety scores by farmers. Cyanogenic glucoside potential of these varieties using an enzymatic assay method showed that there was a strong positive correlation (r=87) between cyanogenic glucoside potential levels and mean taste scores by the

sensory panel. Farmers are rich in knowledge and have the ability to differentiate between toxic and safe cultivars. The traditional processing methods adopted by farmers reduce bitterness and toxicity to negligible levels (Chiwona-Karltun *et al*., 2000).

The government of Malawi was right to develop and distribute bitter varieties to communities which are predominantly cassava eating. Recent studies clarified the confusion surrounding the toxic potential of cassava and the mechanisms for removal of cyanogenic compounds from cassava during processing (Bokanga, 1994; Chiwona-Karltun *et al*., 2000).

The farmers' knowledge has been useful for management of cassava for both safety and characterisation. Grouping of cassava varieties as sweet and bitter received support from studies of Elias *et al.* (2001), Narváez-Trujillo *et al*. (2001) and Mkumbira *et al*. (2001) using morphological and molecular markers. For example, Mkumbira (2002) showed that from 170 accessions collected from farmers, SSR analysis revealed 161 genotypes. This represented a 95% accuracy for the use of indigenous knowledge for characterisation of cassava.

2.9 Characterisation of cassava germplasm

2.9.1 Morphological characterisation

Phenotypic identification of plants is based on morphological traits recorded in the field. It has been used as a powerful tool in the classification of genotypes and to study taxonomic status. Certification of new varieties is based on the genetic purity of a particular crop. However, traditionally these assessments depended on botanical traits (Stegemann, 1984; Zacarias, 1997). Most characteristics of agronomic importance are controlled by multiple genes and are subjected to varying degrees of environmental modifications and interactions, hence are ambiguous and have limited use for cultivar identification. Natural specific hybridisation and introgression in plants aggravate this problem. Many of these traits are difficult to analyse because they do not have the simple genetic control assumed by many in genetic models (Liu and Furnier, 1993) and are of very little use (Tanksley *et al*., 1989). Morphological characterisation has been used for various purposes including identification of duplicates, studies of genetic variation patterns, and correlation with characteristics of agronomic importance. These methods involve a lengthy survey of plant growth that is costly, labour intensive and vulnerable to environmental conditions (CIAT, 1993).

Cassava cultivars are generally distinguished on the basis of morphological traits and have a wide variability of botanical characteristics. These features are not very distinct and sometimes quite variable, but have to be used by local farmers for basic identification of plant material. Generally, a large number of accessions and sometimes cultivars are not clearly identified which is further complicated by the influence of the changing environment (Waycott and Fort, 1994). Picking of cassava leaves for use as vegetable causes morphological changes of the cassava plant (Onwueme, 1978; Simwambana *et al*., 1996). Therefore, from reports it is imperative to employ a better approach to resolve the issues of duplication, and genetic diversity.

Morphological traits of plants are grouped as either variable (polygenic) or constant (monogenic). Variable characteristics are associated with large genotype by environment interaction. Constant characteristics are salient, thus typifying the species or genotype, for example, petiole colour, root skin and pulp colour, and stem colour (Onwueme, 1978; Zacarias, 1997; Elias *et al*., 2001). Due to the influence of different ecological environments on cassava morphology, morphological classification based on variable traits is difficult. Hence, phenotypic variance in cassava is higher than genotypic variance for traits of agronomic importance like tuberous roots weight (Mathura *et al*., 1986).

Characters which are being used for morphological characterisation of cassava include: hairiness of unexpanded apical leaves, colours of unexpanded apical leaves, tip shoots, mature leaves, veins of mature leaves, mature stems, petioles, root surface, inner root skin, root pulp, flowers and seeds, leaf shape and size, number of leaf lobes and branching levels, plant and first branching height, petiole and root peduncle lengths, root constrictions, root shape, and root position (Benesi, 2002; Nassar, 2005).

Manihot species have been extensively classified using morphology. Bonierbale *et al.* (1997) assessed the genetic diversity of 105 genotypes using morphological and AFLP methods in order to estimate genetic similarities among taxa and evaluate intra- and inter-specific variability. Results of that study grouped individuals according to previous taxonomic classification. A large percentage of duplicates were identified at the International Centre for Tropical Agriculture (CIAT) using passport, morphological and isozyme characterisation (Ocampo *et al*., 1995). Such redundancy makes it more expensive to maintain and manage existing collections and slows down the introduction of new germplasm.

2.9.2 DNA fingerprinting

Morphological characters have been in use for a long time (Hershey and Ocampo, 1989; Elias *et al*., 2001; Zacarias *et al*., 2004). Many phenotypic markers have been useful to demonstrate that leaf lobe width, root surface and pulp colour, stem growth habit and male sterility are controlled by single genes and that most agronomically important traits are under multigenetic control, with a high proportion of additive genetic effects (Hershey and Ocampo, 1989; Iglesias and Hershey, 1994). Phenotypic markers are still playing an important role in conventional plant breeding as well as in identification of specific markers and quantitative trait loci (QTLs) using molecular markers (Fregene *et al*., 2000; Akano *et al*., 2002, Mkumbira, 2002).

DNA fingerprinting for cultivar or varietal identification has become an important tool for genetic identification in plant breeding and germplasm management. When planning DNA fingerprinting, 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).

2.9.2.1 *Isozymes*

Isozymes are protein markers based on 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 multilocus genotypes for each analysed individual. Isozymes were among the earliest markers used for plant analysis (Brewbaker *et al*., 1968; Mäkinen and Brewbaker, 1976). Isozymes were useful tools for genetic fingerprinting and studies of genetic diversity in cassava (Hussein *et al*., 1987; Ramirez *et al*., 1987; Ocampo *et al*., 1992; Lefevre and Charrier, 1993). The alpha beta esterase system was informative, providing 22 alleles, which complemented morphological descriptors for the identification of duplicates in the collection at CIAT (Ocampo *et al*., 1995). However, isozymes are difficult to work with due to a limited amount of polymorphism, 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).

2.9.2.2 *Restriction fragment length polymorphism*

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 is referred to as restriction fragment length polymorphism (RFLP) (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 the problems encountered with isozymes and phenotypic markers (Botstein *et al.*, 1980; Helentjaris *et al*., 1986). RFLPs were superior to isozymes and phenotypic markers since they represent the entire genome and are both co-dominant and multi-allelic (Brettschneider, 1998). RFLPs have been and are still used in cassava. The RFLP technique generates more detectable loci and alleles, is not sensitive to environmental factors, and can be used at any developmental stage of the organism (Kelley, 1995). This has allowed the extensive use of RFLP analysis in genetic studies (Tanksley *et al*., 1989), in the exploration of evolutionary relationships among different species (Song *et al*., 1990), and populations (Bonierbale *et al*., 1988; Miller and Tanksley, 1990), for identification of genotypes (Smith *et al*., 1990; Melchinger *et al*., 1991; Livini *et al*., 1992), and for mapping genes that control quantitative as well as qualitative traits (Beavis and Grant, 1991).

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 has been particularly useful in mapping species that display high levels of intraspecific variation. A preliminary linkage map of cassava was drawn from F_1 segregation data of a single dose of polymorphisms of RFLP and random amplified polymorphic DNA (RAPD) markers. The map comprised of 200 loci corresponding to genomic clones selected from *Pst*I, *Hin*dIII and *Eco*RI random genetic libraries (RFLP markers; Fregene *et al*., 1994; 1997). Several maps have been reported in different crops, for example, maize (Burr *et al*., 1983; Helentjaris *et al*., 1986; Gardiner *et al*., 1993), barley (Garmer *et al*., 1993), sorghum (Xu *et al*., 1994), sunflower (Berry *et al*., 1995), rice (McCouch *et al*., 1988) and wheat (Chao *et al*., 1989).

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

to achieve these relationships. RFLPs have been applied in studies of analysis for phylogenetic relationships of species within the genus *Manihot* (Haysom *et al*., 1994).

The disadvantages of RFLP are that it is lengthy, 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 radioisotopes, 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).

During the last decade, there has been a revolution of various PCR based DNA molecular marker techniques (McGregor *et al*., 2000; Mkumbira, 2002). PCR based molecular markers are most commonly used. They are preferred since they are generally quick and straightforward to perform, use small amounts of genomic DNA, allowing analysis of single seeds or young seedlings or plantlets (McGregor *et al*., 2000). PCR based methods include: RAPD, AFLP, SSR, expressed sequence tags (EST), and their derivatives.

2.9.2.3 *Random amplified polymorphic DNA*

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

There are several advantages of RAPD compared to RFLP and isozymes techniques. RAPD analysis is relatively simple, rapid, does not involve radioactive isotopes and is less expensive than RFLPs (Welsh and McClelland, 1990; Williams *et al*., 1990; Barua *et al*., 1993; Varghese *et al*., 1997; Benesi, 2002). RAPDs do not require target DNA sequence information (Welsh and McClelland, 1990), and can provide markers in the genomic regions not accessible by RFLP analysis (Williams *et al*., 1990). Another advantage of the RAPD method is that a universal set of primers can be used for genomic analysis of any organism (Welsch and McClelland, 1990). These advantages make the RAPD method suitable for genetic studies.

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

RAPD has been used to explore genetic diversity in cassava collections. 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*. (2001b) assessed the diversity of 500 African landraces of cassava using RAPD and AFLP. 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.

Fregene *et al*. (1997) constructed a linkage map using 132 RFLP, 30 RAPD, three microsatellite and three isozyme markers from a heterozygous female parent of an interspecific cross. The map consisted of 20 linkage groups spanning 931.6cM. A second map was constructed from the segregation of 50 RAPD, 107 RFLP, one microsatellite and one isozyme markers from the male parent.

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 RAPD technique has serious limitations for use in cassava, due to cassava's high level of heterozygosity.

2.9.2.4 *Expressed sequence tags*

Boventius and Weller (1994) suggested using ESTs as candidate loci of quantitative traits to increase the accuracy of mapping complex traits. ESTs are generated by sequencing random cDNA clones from libraries obtained from different tissues at various stages of an organism's development (Suárez *et al.*, 2000). A method is needed for selecting and mapping suitable ESTs. The application of the AFLP technique to cDNA libraries proved to be a highly effective tool for displaying genes that are differentially expressed during the life cycle of an organism (Bachem *et al*., 1996). Constructing cDNA libraries from different tissues and developmental stages are

important to studying certain traits, and combined with AFLP analysis, it yields highly informative transcript-derived fragments (TDF) for mapping the trait in question. Sequencing of differentially expressed TDFs converts them into ESTs (Suárez *et al.*, 2000).

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

The recommendation of Suárez *et al.* (2000) for use of cDNA-AFLP approach in mapping ESTs at different developmental stages of the crop using several enzyme pairs and all possible primer combinations makes the method expensive and complicated.

2.9.2.5 *Amplified fragment length polymorphism*

Amplified fragment length polymorphism, a PCR based assay for plant DNA fingerprinting, combines the specificity of restriction analysis with PCR amplification (Zabeau, 1992; Zabeau and Vos, 1993; Vos *et al*., 1995). AFLP involves digestion of genomic DNA with restriction endonucleases followed by ligation of terminal adapter sequences to generate template DNA for amplification. Selective PCR primers are modified by adding two or three selective nucleotides (Vos *et al*., 1995; McGregor *et al*., 2000).

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

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

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

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

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

and *peruvian*, it seemed that some other species also contributed. Results suggested the importance of genetic recombination at the origin of the diversity of cassava, which was postulated as being a favourable perspective for various strategies of genetic mapping and gene tagging since this crop is multiplied vegetatively.

Morillo *et al*. (2001) used mapped AFLP and SSR markers as evidence of introgression in a set of 60 plants. Results indicated that AFLP and SSR bands that appeared in some varieties of cassava and not in *M. esculenta* ssp. *flabellifolia*, the presumed ancestor of cassava, were considered as introgressed bands. 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. Amerindian cassava varieties from French Guiana and Ecuador were at the two extremes of the first axis of variation in a multivariate analysis of the polymorphism. The Caboclo's cassava varieties and the collection assembled in modern times were intermediate, which expressed a progressive historical merging of sweet and bitter varieties in a continuum. The recently bred varieties tended to be hybrids derived between sweet and bitter varieties.

As in the case of RAPDs, AFLPs are dominant markers but technical refinements to distinguish homozygous and heterozygous genotypes have recently been mooted (Vos and Kuiper, 1998). The technique is more reliable than RAPD (Vos *et al*., 1995), but more laborious and time consuming (McGregor *et a*l., 2000; Powell *et al*., 1996).

2.9.2.6 *Simple sequence repeats*

Simple sequence repeats or microsatellites are tandem repeats of short (2-5) 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 sizes (Robinson and Harris, 1999).

SSR markers appear to be hypervariable, reproducible (Dayanandan *et al.*, 1998) and are co-dominant, thus the heterozygote for different fragments in diploid genomes can generally be distinguished (Wang *et al*., 1994). Microsatellite co-dominance increases the efficiency and

accuracy of population genetic measures based on these markers compared with other markers, such as AFLPs and RAPDs. Furthermore, the identity of heterozygotes in the F_1 generation makes gene-flow, hybridisation and paternity analyses simpler (Schlötterer and Pemberton, 1994). Rossetto *et al*. (1999) studied the partitioning of variation within and between populations of *Melaleuca alternifolia* (Myrtaceae) to facilitate the identification of genetic resources and assist in the conservation of genetic diversity. Chase *et al.* (1996) studied the gene-flow and mating patterns of *Pithecellobium elegans* (Leguminosae) in a forest fragment in Costa Rica.

SSR markers are usually species specific, costly to develop, and prior sequence information is a requirement. Once primers have been developed, the system becomes relatively inexpensive. Furthermore, cross-species amplification of SSRs means that identification of suitable SSR primers may not be necessary in closely related taxa. For example, three sets of microsatellite primers have been designed in *Malus domestica* (Rosaceae), which yielded 35 loci, some of which may amplify other *Malus* taxa (Guilford *et al*., 1997; Gianfranceschi *et al*., 1998; Hokanson *et al*., 1998). Some studies indicated that SSR primers may amplify the same SSR region in closely related taxa. For example, White and Powell (1997) amplified DNA from seven of the 11 microsatellite loci in other *Swietenia* species, six loci in other genera of the same tribe, and four to six loci in species of the same family. Wang *et al.* (2005) evaluated 210 SSR markers developed from maize, sorghum, wheat and rice (major cereals) for transferability to minor grass species like finger millet (*Eleusine coracana*), seashore paspalum (*Paspalum vagnatum*) and Bermudagrass (*Cynodon dactylon*). Results indicated that 412 cross-species polymorphic amplifications were identified.

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

In cassava, the SSR technique has been applied in various studies. For example a SSR marker linked to CMD resistance was identified with the aid of bulk segregant analysis (Akano *et al*., 2002). Fregene *et al*. (2001) assessed the SSR diversity at 67 unlinked loci in 303 accessions of cassava land races from Tanzania, Nigeria, Brazil, Colombia, Peru, Venezuela, Guatemala, Mexico and Argentina. Results revealed that more than 90% of the loci were polymorphic in all samples, and estimates of genetic diversity and differentiation ranged widely from locus to locus.

It was observed that factors that contributed to differences in allele frequency at SSR loci in this predominantly vegetatively propagated crop appeared to be spontaneous recombination.

Mkumbira *et al.* (2001) used SSR markers to study the traditional way farmers in Malawi classify cassava varieties 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 sub-division 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.

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

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

2.10 Study area

Malawi is a country of 12.3 million hectares and is located in the south-east of Africa, and between latitudes 9^o and 17^o south of the Equator, and longitudes 33^o and 36^o east. It shares boundaries with Mozambique to the east, south and south-west, Zambia to the west, and Tanzania to the north (Moyo *et al*., 1998). Approximately 85% of about 11 million people live in rural areas and are engaged in agriculture. This sector contributes about 35% of the Gross Domestic Product (GDP) and 92% of the country's foreign exchange earnings (MoALD, 1995). The main cash crops are tobacco, sugar, cotton and tea. Maize is the main food crop and occupies 70% of cultivated land. A survey done in 1997 revealed that according to the farmers themselves cassava ranks second as a food crop after maize, and third as a cash crop after tobacco and cotton (Moyo *et al*., 1998).

2.11 Justification of research project

Literature demonstrated that cassava is a very important crop in terms of returns, ease and flexibility in management, adaptability to various micro-environments and multiplicity in uses as food and in various industries. The misunderstanding of cassava cyanogenesis and negative attitude towards cassava products led to its negligence in research and development. Economic and climatic hardships have forced governments and donors to pay attention to cassava. Although a lot of work is being done on cassava, there exists a big gap in researchers' knowledge of cassava. On the other hand, farmers have been managing the crop for a very long time, hence are knowledgeable in the crop, including the areas of classification and cyanogenesis. However, there has been very little or no interaction among researchers, farmers and users, which hampers progress in promotion of the crop. Commercialisation of cassava, and biotic and abiotic constraints led to genetic erosion which called for germplasm exploration and conservation. Knowledge of genetic diversity is a must for use of germplasm in a breeding programme, therefore cassava germplasm need to be characterised.

Cassava starch is superior to cereal starches in many aspects. Native starch is useful in many industries but because of some specialised uses of starch, native starch needs to be modified to suit the specialised uses. Farmers and starch producers can benefit if starch extraction is optimised in terms of choice of appropriate varieties, locations and correct timing of doing the starch extraction.

This study was aimed at looking into these aspects and tried to come up with some solutions or recommendations which could lead to sustained conservation of cassava germplasm, efficient utilisation of local germplasm as well as cassava products in industries.

CHAPTER 3

EXPLORATION AND CHARACTERISATION OF MALAWIAN CASSAVA GERMPLASM USING ETHNOBOTANY AND MORPHOLOGY

3.1 Introduction

Cassava is a favoured root crop among all the root and tuber crops of the tropics (Fregene *et al*., 2000). The starchy tuberous roots of cassava have become the most important source of dietary energy in SSA (Scott *et al*., 2000) as they provide more returns per unit input than any other staple crop (Akoroda, 1995; Fregene *et al*., 2000; Nassar, 2005). Other advantages of cassava include adaptability to various cropping systems, ability to do well in diverse environments with minimal inputs, and diversity in uses (Onwueme, 1978; Fregene *et al*., 2000; Nassar, 2005). Cassava is grown across Malawi for food, as a cash crop and for industrial uses (FAO, 1993; Sauti *et al*., 1994; Moyo *et al.*, 1998; Benesi, 2002; Benesi *et al.*, 2004). Cassava leaves are rich in proteins, vitamins and minerals and are extensively used in Malawi and in the whole of Africa (Hahn, 1988; FAO, 1993; Moyo *et al.*, 1998; Fregene *et al*., 2000; IITA, 2001). Cassava leaves are an important vegetable, especially in the dry season when other vegetables are in short supply (FAO, 1993). Most smallholder farmers grow a number of cultivars with specific characteristics which they prefer (Salick *et al*., 1997; Chiwona-Karltun *et al.,* 2000; Mkumbira *et al*., 2001).

Chiwona-Karltun *et al*. (1998) reported that African smallholder farmers mainly acquire new cultivars from their neighbours or by collecting volunteer seedlings from sexually propagated cassava found in fields left fallow for several years. A study in the northern region of Malawi showed that just in one village farmers have descriptive names for up to 50 cultivars (Chiwona-Karltun *et al.,* 2000). Local cultivar names denote phenotypic attributes, place of origin, the person that introduced it or that it originated from a volunteer seedling. The performance of a cultivar within a local environment and farming system determines whether it will be adopted and continue to be cultivated (Chiwona-Karltun *et al.,* 1998).

One of the main obstacles to the expansion of cassava has been the limited understanding of cassava cyanogenesis. As a result, scientists and governments including CGIAR centres have

been busy promoting sweet cassava (Fresco, 1986; Chiwona-Karltun, 2001). This has been evidenced by the fact that cassava collections established at CIAT and in Southern Brazil largely underrepresented bitter cassava (Narváez-Trujillo *et al.*, 2001), while the predominantly cassava eating (as staple) communities continue growing bitter cultivars (Essers *et al*., 1992). Due to this confrontation between farmers versus researchers and governments, Kapinga *et al*. (1997), Cardoso *et al.* (1998) and Chiwona-Karltun *et al*. (1998) carried out quantitative interviews in Tanzania, Malawi and Mozambique, respectively, on preferences of cassava cultivars by farmers in predominantly cassava eating areas. Results clearly showed that cassava farmers regarded bitterness and toxicity of tuberous roots as a protector of cassava against theft, spoilage by animals and unplanned harvesting by family members. Tuberous roots from bitter cultivars need to be processed before consumption while roots from sweet/cool cultivars could be eaten fresh or directly cooked (Kapinga *et al*., 1997; Cardoso *et al.*, 1998; Chiwona-Karltun *et al*., 1998). Cassava farmers are rich in knowledge with regard to the crop including cyanogenesis. exclusion of cassava growers and ethnobotany of the crop in setting research and promotion objectives resulted in low adoption of formally improved varieties. This has been evidenced from several parts of Africa where few cassava cultivars actually originated from formal breeding programmes (Nweke *et al*., 1994; Spencer, 1994; Chiwona-Karltun *et al*., 1998).

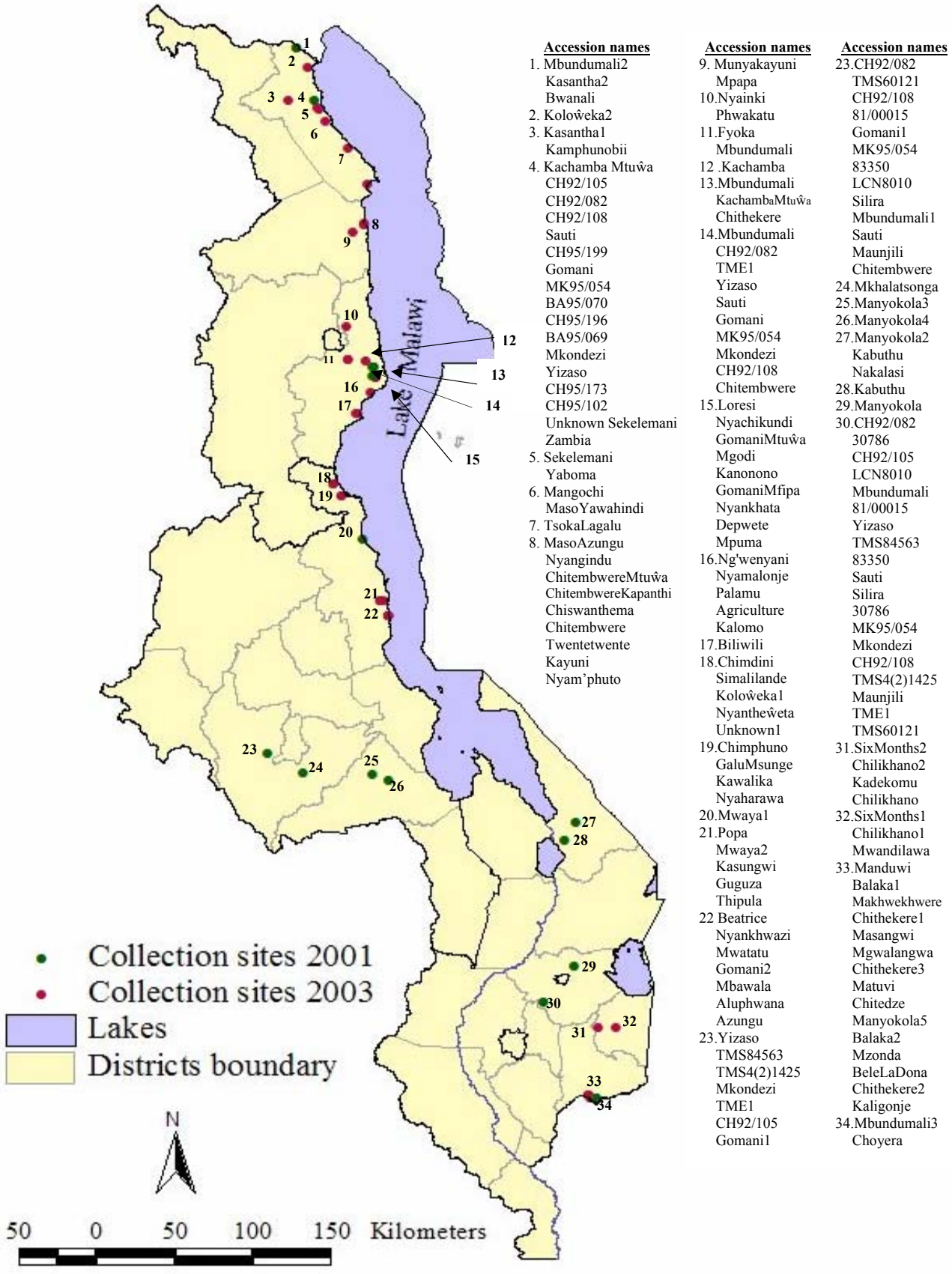
Commercialisation of cassava and adoption of improved varieties increases production and income but encourages genetic erosion (Moyo *et at*., 1999; Brush, 2000; Raji *et al.*, 2001a; 2001b; Mkumbira, 2002; Benesi *et al*., 2004). This calls for the collection, characterisation and conservation of local germplasm for use in crop improvement programmes in Malawi, the region and at global level.

The objectives of this study were to (1) explore cassava germplasm in Malawi with the aid of indigenous knowledge, (2) asses the diversity of farmer preferences on cassava varieties, and (3) assess the genetic diversity of Malawian cassava accessions using ethnobotany and morphological markers.

3.2 Materials and methods

3.2.1 Exploration of Malawian cassava germplasm and gathering of ethnobotany data

Cassava clones and some recommended varieties were collected from Baka, Mkondezi, Chitedze and Makoka research stations (Figure 3.1). Accessions were morphologically characterised at each site at the age of 12 months followed by planting at Chitedze research station, Malawi.



Accession names

1. Mbundumali2
   Kasantha2
   Bwanali
2. Kolowêka2
3. Kasantha1
   Kamphunobii
4. Kachamba Mtuŵa
   CH92/105
   CH92/082
   CH92/108
   Sauti
   CH95/199
   Gomani
   MK95/054
   BA95/070
   CH95/196
   BA95/069
   Mkondezi
   Yizaso
   CH95/173
   CH95/102
   Unknown Sekelemani
   Zambia
5. Sekelemani
   Yaboma
6. Mangochi
   MasoYawahindi
7. TsokaLagalu
8. MasoAzungu
   Nyangindu
   ChitembwereMtuŵa
   ChitembwereKapanthi
   Chiswanthema
   Chitembwere
   Twentetwente
   Kayuni
   Nyam'phuto

Accession names

9. Munyakayuni
   Mpapa
10. Nyainki
    Phwakatu
11. Fyoka
    Mbundumali
12 .Kachamba
13. Mbundumali
    KachambaMtuŵa
    Chithekere
14. Mbundumali
    CH92/082
    TME1
    Yizaso
    Sauti
    Gomani
    MK95/054
    Mkondezi
    CH92/108
    Chitembwere
15. Loresi
    Nyachikundi
    GomaniMtuŵa
    Mgodi
    Kanonono
    GomaniMfipa
    Nyankhata
    Depwete
    Mpuma
16. Ng'wenyani
    Nyamalonje
    Palamu
    Agriculture
    Kalomo
17. Biliwili
18. Chimdini
    Simalilande
    Kolowêka1
    Nyanthewêta
    Unknown1
19. Chimphuno
    GaluMsunge
    Kawalika
    Nyaharawa
20. Mwaya1
21. Popa
    Mwaya2
    Kasungwi
    Guguza
    Thipula
22 Beatrice
    Nyankhwazi
    Mwatatu
    Gomani2
    Mbawala
    Aluphwana
    Azungu
23. Yizaso
    TMS84563
    TMS4(2)1425
    Mkondezi
    TME1
    CH92/105
    Gomani1

Accession names

23. CH92/082
    TMS60121
    CH92/108
    81/00015
    Gomani1
    MK95/054
    83350
    LCN8010
    Silira
    Mbundumali1
    Sauti
    Maunjili
    Chitembwere
24. Mkhalatsonga
25. Manyokola3
26. Manyokola4
27. Manyokola2
    Kabuthu
    Nakalasi
28. Kabuthu
29. Manyokola
30. CH92/082
    30786
    CH92/105
    LCN8010
    Mbundumali
    81/00015
    Yizaso
    TMS84563
    83350
    Sauti
    Silira
    30786
    MK95/054
    Mkondezi
    CH92/108
    TMS4(2)1425
    Maunjili
    TME1
    TMS60121
31. SixMonths2
    Chilikhano2
    Kadekomu
    Chilikhano
32. SixMonths1
    Chilikhano1
    Mwandilawa
33. Manduwi
    Balaka1
    Makhwekhwere
    Chithekere1
    Masangwi
    Mgwalangwa
    Chithekere3
    Matuvi
    Chitedze
    Manyokola5
    Balaka2
    Mzonda
    BeleLaDona
    Chithekere2
    Kaligonje
34. Mbundumali3
    Choyera

Figure 3.1 Map of Malawi showing collection points for accessions of 2001/02 and 2003 cassava germplasm explorations

Over the same period of time, 15 local cultivars were morphologically characterised and collected from farmers' fields from Mulanje, Zomba, Mangochi, Dedza, Lilongwe, Nkhota kota, Nkhata Bay and Karonga districts (Figure 3.1).

Collection of Malawian cassava germplasm with the aid of indigenous knowledge continued in January 2003 (Figure 3.1) with detailed passport data, based on farmers' knowledge, preferences and special attributes for particular varieties. Passport data included: accession code, name of cultivar, sample status, name of farmer, ethnic group, village, traditional authority, district, rural development project (RDP), agricultural development division (ADD), collection institution, names of individuals forming the collection team, collection date, taste, maturity period, target use, period that cultivar been with the farmer and preferred characteristics of the cultivar. The location of collection in terms of latitude, longitude and altitude were captured. In addition, scoring for diseases CMD, CBSD and CBB and pests like CGM and cassava mealybug (CM) for all collected cultivars was done in the farmers' fields at the time of germplasm exploration. Scoring included average severity score and incidence in terms of percentage infected plants in the field. Collected accessions were planted at Chitedze and Baka research stations in January 2003.

3.2.2 Morphological characterisation using descriptors

Eighteen cassava accessions of the 2001 and 75 accessions of the 2003 collections (Figure 3.1 and Appendix 1) were planted at Chitedze research station, Malawi in January 2003. Morphological characterisation of the above and below ground parts was conducted using a modified cassava descriptor of Nweke *et al.* (1994) and Benesi (2002) (Appendix 2). The 12 morphological characters scored were: shoot colour, shoot pubescence, leaf lamina colour, leaf lobe shape, petiole colour, tender shoot colour, mature stem colour, branching habit, root outer skin colour, root inner skin colour, root pulp colour, and taste. Morphological data for 93 analysed accessions was converted into a binary matrix using the procedure of Benesi (2002) as follows:

3.2.2.1 Traits with two categories of description

Traits that had only two categories of description were scored normally in the binary matrix. Tuberous root pulp colour was scored as 1 for white pulp and 0 for yellow pulp.

3.2.2.2 *Traits with more than two categories of description*

Traits concerning colour and shape

All traits concerning colour and shape were coded by considering the entire range of diversity of that trait and scored against that particular class. For example, root surface colour ranged from 1=white/cream, 2=light brown to 3=dark brown (Appendix 2). If a genotype's root skin colour was white, it was scored as 1 against CRS1 (white/cream), and 0 for CRS2 (light brown) and CRS3 (dark brown). If a genotype's root skin colour was dark brown, it was scored as 1 against CRS3 (dark brown), and 0 for CRS1 (white/cream) and CRS2 (light brown; Appendix 2).

Pubescence of apical unexpanded leaves

Hairiness of apical unexpanded leaves was classified as 1=none, 2=little, 3=moderate and 4=high (Appendix 2). If a genotype's unexpanded apical leaves had no hairs it was scored as 1 against HUL1 (none), and 0 for HUL2 (little), HUL3 (moderate) and HUL4 (high). If a genotype's unexpanded apical leaves had moderate amount of hairs it was scored as 1 against HUL3 (moderate), and 0 for HUL1 (none), HUL2 (little), and HUL4 (high; Appendix 2).

Branching habit

First branching height was classified as low (for first branches of 50cm and below), medium (for first branches of between 50cm and 100cm), and high (for first branches over 100cm). The means of branching heights of 10 plants were recorded as branching heights for each accession, and fitted into respective groups as low, medium or high. If a genotype's mean branching height was 60cm, it was scored as 1 against a column for medium, and 0 for low and for high.

3.2.3 Data analysis

Similarity coefficients for morphological data were calculated using Dice coefficient **(**Dice**,** 1945**;** Nei and Li, 1979) and the NTSYSpc version 2.11c computer package (Rohlf, 2000). Dendrograms were constructed using the Unweighted Pair Group Method of Arithmetic Averages (UPGMA) in SAHN programme parameters (Rohlf, 2000).

3.3 Results and discussion

3.3.1 Exploration and conservation of cassava germplasm

A total of 78 accessions were collected from December 2001 to January 2002. Sixty three accessions represented a Malawian working collection while 15 accessions were local land races (from farmers fields). The working collection comprised of eight locally recommended, 23

introduced between 1990 and 2000 in tissue culture form, and 32 locally screened (whose open pollinated seeds came from International Institute of Tropical Agriculture (IITA) but were sprouted and screened in Malawi) accessions. Of the 78 accessions, 72 sprouted clean. Five accessions sprouted with primary CMD infection and were among those collected from farmers' fields. A total of 87 accessions were collected from farmers' fields in January 2003 of which 76 sprouted clean at Chitedze research station. Chitedze is a low pressure area for CMD and CBSD, hence no single accession was lost or re-infected by viruses in the field gene bank by 2005. Some of the passport data for all the accessions included in this study are presented in Appendix 1.

The 1990 collection was conserved at Mkondezi research station, which is a high pressure area for CMD and CBSD, and crop hygiene was not employed during collection as well as in the field gene bank. As a result, most of the accessions were infected, some got lost and agronomic evaluation of those accessions could not be done due to CMD and CBSD infections. Another problem with the 1990 exploration was that indigenous knowledge, which is crucial in exploration, conservation and utilisation of germplasm, was disregarded (FAO, 1993; Chiwona-Karltun *et al*., 1998; 2000; Mkumbira, 2002). More collections were done in one extension planning area (EPA) in Nkhata Bay district in the north of Malawi, and one EPA in Zomba and Mulanje districts in the south (Chiwona-Karltun *et al*., 2000; Mkumbira, 2002). By the time Mkumbira (2002) characterised the germplasm using SSR, only 79 of the 1990 collections were available which were later lost due to red spider mite and mealybug attack. In contrast, the 2001/02 and 2003 collections were carefully conducted, employing crop hygiene at points of collection as well as in the field gene bank coupled with establishing the gene bank at a low pressure area for the prevalent virus diseases in Malawi. This allowed maintenance of clean accessions from 2002 to 2005. Results suggested that people with experience on the crop should be involved in exploration of germplasm. Detailed passport data should be collected to enable recollection of lost or infected accessions at exact points of previous collections. Enforcement of crop hygiene and conserving germplasm at low pressure areas ensure maintenance of clean germplasm and avoid unnecessary losses.

3.3.2 Indigenous knowledge, preferences of cassava varieties by farmers, cyanogenesis and cassava improvement

Exploration of the 2003 collection revealed that preferences of cassava varieties by farmers in Malawi in terms of taste varied according to the intended use and modes of utilisation of cassava in that particular area (Table 3.1). In the north where farmers rely on cassava as a staple crop,

they preferred very bitter cassava varieties. Although the percentages for sweet to very sweet varieties was 23% each, areas planted to sweet varieties in the field were very small. In the centre, farmers generally preferred sweet cassava varieties (Table 3.1), but preferences varied according to area and intended use of cassava within the region. Along the lake shore (towards the northern region), vast areas of very bitter cassava varieties were grown as a staple crop. In the upland and the rest of the central region, vast areas of sweet cassava varieties were grown for the fresh market, snack or food security. Hence, in the centre both sweet and bitter varieties were preferred depending on areas and uses.

In the south, farmers preferred very sweet cassava varieties (Table 3.1) intended for the fresh market, as a snack or processed into *makaka* (scraped or peeled cassava tuberous roots, cut and split into chips, and directly sun dried) for food security or sale. Since submerged fermentation is not practiced, bitter varieties can not be used to make *makaka* . The few farmers who grow bitter cassava in the south practice heap fermentation for one week before drying.

Table 3.1 Taste and maturity periods as preferred by farmers at points of collections

Collection areas	Collected Acc.		Farmers' preferences of sweet and bitter cassava varieties												Farmers' preferences of maturity period of cassava varieties											
			V.Sweet			Sweet			Bitter			V.Bitter			Early			Medium			Late			V.Late		
	No.	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)
North	44	46	10	23	10	10	23	10	9	21	9	15	34	16	23	52	24	18	41	19	2	5	2	1	3	1
Centre	25	26	10	40	10	1	4	1	7	28	7	7	28	7	8	32	8	14	56	15	2	8	2	1	4	1
South	27	28	17	63	18	7	26	7	1	4	1	2	7	2	5	19	5	20	74	21	0	0	0	2	7	2
Total	96	100	37	-	38	18	-	18	17	-	18	24	-	25	36	-	37	52	-	54	4	-	4	4	-	4

acc. = accessions; No. = number of collected accessions; [%] = percentage collected accessions within region; (%) = percentage collected accessions across the country; V.Sweet = very sweet; V.Bitter = very bitter; V.Late = very late

Results agreed with previous studies of Kapinga *et al.* (1997), Cardoso *et al.* (1998) and Chiwona-Karltun *et al.* (1998; 2000). This is because bitterness is directly related to toxicity or cyanogenic potential levels (Chiwona-Karltun *et al.*, 2000). Hence, the use of bitterness by the farmers for safety against thieves, monkeys and unplanned harvests in order to guarantee food security has proven to be right (Chiwona-Karltun *et al.,* 1998; 2000). In addition, cyanogenesis is no longer an issue under farmers' conditions since they are dealing with a specific micro-environment, have reliable detection methods for distinguishing toxic and safe cassava, and have processing methods for turning toxic cassava to safe levels (Chiwona-Karltun *et al.,* 2000; Mkumbira *et al*., 2001).

The issue of cyanogenesis is still a problem for researchers dealing with crop improvement for heterogeneous micro-environments under farmers' field conditions (Mkumbira, 2002). The

complexity of the cyanogenesis issue is coupled with the progressive merging of bitter and sweet varieties due to the continuous breeding efforts by both farmers and scientists (Narváez-Trujillo *et al*., 2001).

The study of Benesi *et al*. (2003) revealed that bitter roots are high in cyanogenic potential while sweet roots were low in cyanide. Bitter or sweet taste of tuberous roots of cassava is not only a varietal factor, but it is more environmentally than genotypically controlled. Varieties regarded as bitter like Maunjili (TMS91934), Silira (TMS60142A) and Gomani tasted very sweet and had very low cyanogenic potential levels under farmers' fields in the Nkhunga EPA in Nkhota kota district. Similar results were observed at Makoka research station, except that Gomani tasted slightly bitter and had intermediate levels of cyanogenic potential. Mbundumali which is widely known as a very sweet variety, tasted slightly bitter and had medium levels of cyanogenic potential at Chitedze, Chitala and Baka research stations. It was noted that site means for cyanogenic potential varied extensively from one site to the other (Table 3.2). These results agreed with the finding of Sanni and Olubamiwa (2003) who reported cyanogenic content in the dry season being more than twice for wet season values. Classification of cyanogenic potential levels is: 5mg or less hydrogen cyanide (HCN) per 100mg fresh root weight as safe or low or negligible; between 5 and 10mg HCN as intermediate or medium, and over 10mg HCN per 100mg as toxic or poisonous or very high, according to the classification of Koch (1933).

Table 3.2 Total cyanogenic potential (mg HCN/100g) in fresh root pulp, flour and *nsima* of promising cassava clones (Benesi *et al*., 2003)

Genotype	Sites							Product		
	Mkondezi	Bvumbwe	Chitala	Baka	Makoka	Chitedze	KK	Mean SD	Flour	*Nsima*
Mbundumali	2.7	3.7	6.6	7.3	2.6	5.4	2.7	**4.4 ± 1.75**	0.067	0.040
Gomani	14.5	13.2	27.1	19.1	8.9	18.5	3.6	**15.0 ± 5.53**	0.070	0.040
TMS91934	8.3	8.1	19.8	17.8	3.4	12.2	2.7	**10.3 ± 5.58**	0.097	0.046
TMS60142B	8.0	9.2	14.7	19.5	2.7	9.4	3.6	**9.6 ± 5.09**	0.040	0.026
Site mean	**8.4**	**8.6**	**17.1**	**15.9**	**4.4**	**11.4**	**3.2**			
SD ±	4.83	3.91	8.63	5.80	3.02	5.51	0.52			

KK = on-farm trials in Nkhota kota rural development project; HCN = total cyanogenic potential; SD = standard deviation; *nsim*a = stiff porridge prepared using cassava or maize or other cereals and is normally consumed together with relish (meat/vegetables/fish/eggs)

Results suggested that cassava breeding programmes need to be strategised to address specific needs of particular areas. Where cassava is used as a staple crop, emphasis should be on development of very bitter varieties. Where cassava is grown for the fresh market, for consumption as a snack, direct boiling, or for preparation of *makaka*, emphasis should be on

development of very sweet varieties. Thus, no universal cassava improvement programme can address the needs of all farmers in Malawi, the region and beyond.

All farmers across the country concurred on early maturing (Table 3.1 and 3.3), good storage in ground, more roots per plant, pest and disease resistance, tuberous roots with no fibres, leaves suitable as a good vegetable, and varieties with high multiplication ratio in terms of production of planting materials as important and positive characteristics (Table 3.3). Since the crop is vegetatively propagated, ideal varieties need to have many, long, healthy and quality stems to avert shortage of planting materials and to withstand dry spells soon after planting (IITA, 1990).

Table 3.3 Preferred cassava characteristics by farmers in Malawi

Preferred characteristics	Frequencies of desired traits as mentioned by farmers at collections points							
	General		North		Centre		South	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
High yielding	43	45	16	17	14	15	13	14
Early maturing	35	37	14	15	10	10	11	12
Resistant to diseases and pests	16	17	4	4	2	2	10	10
High dry matter	15	16	1	1	4	4	10	10
No fibres in the roots	13	14	6	6	3	3	4	4
More roots per plant	7	7	2	2	1	1	4	4
Leaves make very good vegetable	10	10	3	3	3	3	4	4
Good storage of chips (*makaka*)	2	2	0	0	0	0	2	2
Mealy	3	3	1	1	2	2	0	0
Stable yields	2	2	2	2	0	0	0	0
Good in ground storage	1	1	0	0	1	1	0	0
Rapid multiplication of planting materials (profuse branching)	3	3	1	1	1	1	1	1
Good *nsima*	1	1	1	1	0	0	0	0
Good for beer brewing	1	1	0	0	0	0	1	1
Long shelf life for fresh roots	1	1	0	0	0	0	1	1
Fast fermentation	1	1	1	1	0	0	0	0
No lodging	2	2	2	2	0	0	0	0
Total (n)	**-**	**(n=96)**	**-**	**(n=96)**	**-**	**(n=96)**	**-**	**(n=96)**

Farmers in the central and southern regions preferred varieties which were very sweet, high in dry matter and with mealy tuberous roots (Tables 3.1 and 3.3). Farmers in the south additionally preferred varieties whose *makaka* stored well without being attacked by weevils (Table 3.3). Due to land pressure in the south, farmers harvest cassava and make *makaka* to release the land for other crops and cassava for the following season. The *makaka* is stored for a long period of time until required in food supplementation when maize supplies go low. Farmers in the northern region and along the lake shore in the centre preferred varieties which made good *nsima*, which were very bitter, whose tuberous roots ferment fast when submerged in water, and varieties with stable yields to ensure food security (Tables 3.1 and 3.3).

Results suggested that breeding for sweet cassava can meet the needs of many people over a wide area since people who use sweet cassava have similar preferences. On the other hand people who use bitter cassava for a staple have diverse preferences of the product as reflected in the diversity of their landraces. Hence, it is not easy to set up and achieve breeding objectives of developing an ideal variety which capture the preferences of most consumers who use cassava as staple crop. This was clearly demonstrated when executing on-farm trials in Malawi. In Nkhata Bay (northern region), farmers preferred Yizaso and Maunjili while in Karonga (northern region) they preferred Sauti and Mkondezi despite the fact that all these varieties are bitter and in both areas cassava is used as staple crop (Benesi *et al*., 1999; Moyo *et al*., 1999).

Farmers keep a wide range of varieties in their fields. During the exploration of 2003 up to nine accessions were collected from one field in the southern region (Appendix 1). When moving from one field to another, similar cultivars were found, while cultivars differed considerably from one field to another in the north. Farmers were maintaining old varieties, and at the same time were flexible in accepting or introducing new cultivars in their farming system. Some farmers have been growing some cultivars for over 50 years while others did not even know when some cultivars were introduced, as they have been passed on from one generation to the next. Other varieties were newly introduced in their fields from neighbours, friends, relatives, government institutions, non-governmental organisations (NGOs), and unknown sources (probably from volunteer seedlings).

3.3.3 Cassava diseases and pests in Malawi

Figure 3.2 shows that CMD is widely distributed throughout the country. Infection was serious in all three regions along the rift valley but mild in upland areas. The northern region had the highest and most serious infection rates followed by the southern region. Farmers in the south indicated that CMD was a serious problem and associated it with CM. These findings agreed with those of Moyo *et al.* (1998). Farmers in the north did not realise the impact of CMD as they were confusing it with the effects of onset of the cold season on cassava leaves. Hence, farmers were not managing the disease resulting in further spreading of the disease. Fields in the upland of the central region were clean since it is a low pressure area for CMD, and farmers practice crop hygiene as it is regarded as a cash crop targeting the fresh market in the capital city (Lilongwe).

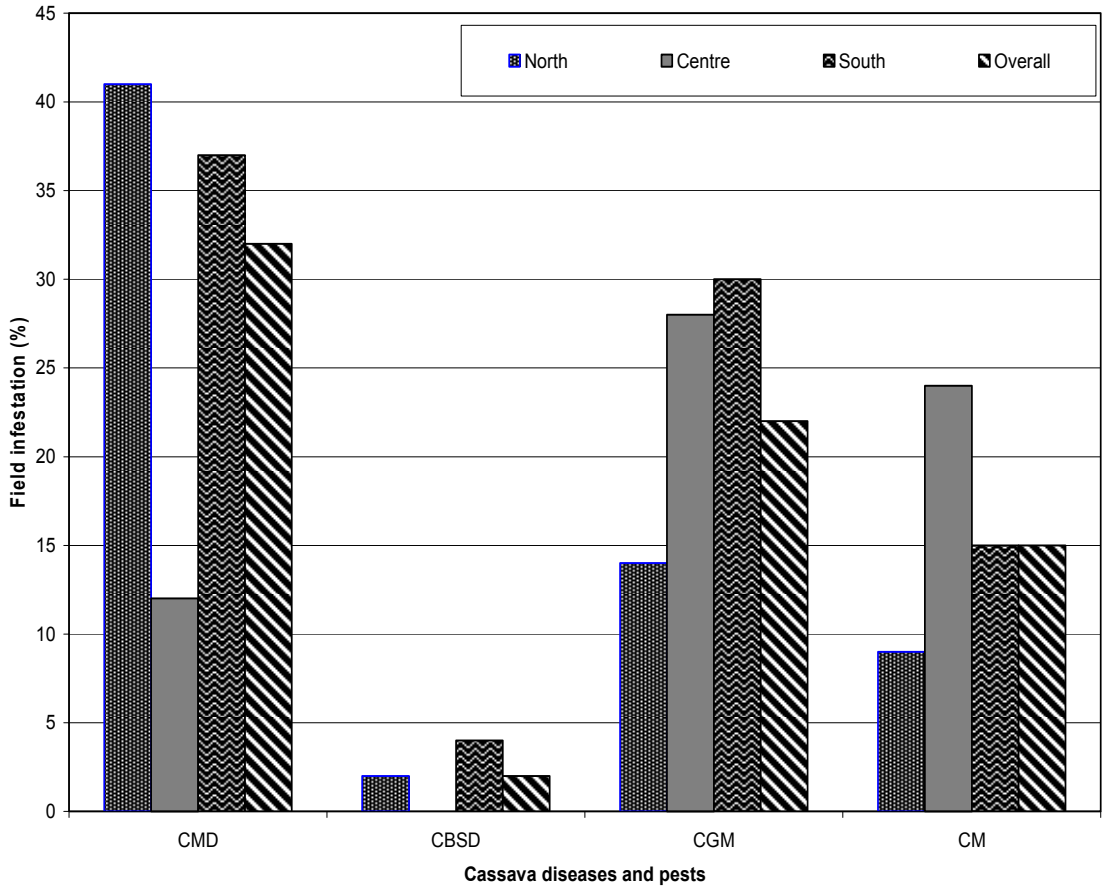


Figure 3.2 Field infection of cassava diseases and pests in Malawi (CMD = cassava mosaic disease; CBSD = cassava brown streak disease; CGM = cassava green mite; CM = cassava mealybug)

Cassava brown streak disease was observed along the rift valley in the north and south of Malawi (Figure 3.2). CBSD symptoms are shown both in the foliage and tuberous roots. Above ground CBSD symptoms are better expressed in the early stages of plant growth, while root symptoms come in at advanced stage of plant growth (commonly from seven months after planting). In addition, when cassava flush new leaves after the long dry season, above ground CBSD symptoms are not shown on the new leaves. During exploration (January), scoring for CBSD was done only for above ground symptoms, and normally, the period of November to January is when cassava has just flushed in Malawi. Scoring for CBSD root symptoms would have been better since exploration was done in January at 12 months of crop age, but it is not easy to carry out destructive sampling in farmers' fields. The fact that CBSD was observed in the leaves during exploration in January, although at low rates (Figure 3.2) suggested that the disease is prevalent and serious in that area.

Cassava green mite (*Mononychellus tanajoa*) infestation was wide spread across the country, although infestation was mild at the time of exploration (Figure 3.2). January was not the best time for assessing CGM severity since they favour hot and dry conditions while January was hot and wet (Akinlosotu, 1982). CGM can be controlled biologically using *Tryphlomaleus alipo* which is said to be taking shape in Malawi (Phiri, G., personal communication).

Cassava mealybug (CM) (*Phenacoccus manihoti*) was observed across the country (Figure 3.2). Infestation was serious in the northern and central regions especially along the lake shore, and mild in the south. CM was prevalent along the lakeshore. This could be due to the long hot and dry conditions which prevail in this area. The impact of CM during the dry season is aggravated by the fact that the plant is already experiencing moisture stress and CM infection worsens the productivity of the crop and even lead to loss of germplasm.

During the 2003 exploration, it was observed that the Malawian cassava germplasm was diverse in terms of resistance to various pests and diseases prevalent in the country. There exists a possibility of exploiting local germplasm to develop varieties with pest and disease resistance.

3.3.4 Morphological description of 93 characterised accessions

This study considered only morphological traits and not agronomical traits since morphological traits typify varieties and are used in characterisation of germplasm. Agronomical traits are used for evaluation purposes with the aim of recommending a variety for farmers. Agronomical evaluation has to be done in several environments due to genotype by environment interaction of agronomical traits since they are multigenetically controlled. Since the aim of this study was morphological characterisation only constant traits were used except for taste. The characteristics of 93 accessions are given in Table 3.4.

More than 70% of the 93 characterised accessions had hairless apical unexpanded leaves rendering them susceptible to CGM attack (Table 3.4). However, some had low amounts of hair and 17% of the accessions had a moderate amount of hair. Hence, the germplasm was diverse in pubescence of apical unexpanded leaves.

About 60% of analysed accessions' leaves had lanceolate shaped central lobes but diverse shapes were available in the collection, including lanceolate, elliptic, linear and oblanceolate (Table 3.4). Leaf shape has an impact on photosynthesis and evapotranspiration (Veltkamp, 1986),

Table 3.4 Morphological description of 93 characterised accessions

Accession name	CUAL	HUL	MLC	LCLS	Petiole colour	MSC	HFB	CRS	ISC	CRP	CTS	Taste
Balaka1	Light purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
Makhwekhwere	Purple	None	Dark green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Chithekere1	Light green	None	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	Sweet
Masangwi	Light purple	None	Dark green	Lanceolate	Green	Silvery green	Medium	White to cream	White to cream	White	Green	V.Bitter
Chithekere2	Light green	None	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	Sweet
Kaligonje	Green with red/purple tinge	Little	Light green	Elliptic	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	Sweet
Mgwalangwa	Light purple	Moderate	Light green	Lanceolate	Mainly red to dark	Silvery green	High	White to cream	White to cream	White	Green	Bitter
Chithekere3	Green with red/purple tinge	Little	Green	Elliptic	Green with pale red sllading	Dark brown	Medium	Dark brown	White to cream	White	Green	Sweet
Matuvi	Purple	None	Dark green	Elliptic	Red/purple	Silvery green	High	White to cream	White to cream	White	Green	V.Bitter
Chitedze	Light purple	None	Green	Elliptic	Green	Silvery green	Low	Dark brown	White to cream	White	Reddish	Sweet
Manyokola5	Light purple	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
Balaka2	Light purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
Mzonda	Green with red/purple tinge	None	Light green	Lanceolate	Green	Silvery green	Medium	White to cream	Pink	White	Green	V.Sweet
BeleLaDona	Light green	Moderate	Dark green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
SixMonths1	Green with red/purple tinge	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Chilikhano1	Light green	Little	Green	Elliptic	Green	Dark brown	Low	Dark brown	White to cream	White	Green	V.Sweet
Mwandilawa	Light green	Little	Green	Elliptic	Mainly red to dark	Dark brown	Medium	Dark brown	White to cream	White	Green	V.Sweet
SixMonths2	Green with red/purple tinge	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Chilikhano2	Light green	Little	Green	Elliptic	Green	Dark brown	Low	Dark brown	White to cream	White	Green	V.Sweet
Kadekomu	Green with red/purple tinge	None	Green	Lanceolate	Green with pale red sllading	Silvery green	Low	White to cream	White to cream	White	Green	V.Sweet
Azungu	Purple	None	Green	Oblanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	Pink	White	Green	V.Bitter
Aluphwana	Light purple	None	Green	Lanceolate	Mainly red to dark	Silvery green	Low	White to cream	White to cream	White	Green	V.Bitter
Mwatatu	Purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	Pink	White	Green	V.Bitter
Gomani2	Purple	None	Dark green	Lanceolate	Mainly red to dark	Light brown/orange	High	Dark brown	White to cream	White	Green	V.Bitter
Beatrice	Light purple	None	Green	Linear	Green with pale red sllading	Silvery green	Low	Light brown	Pink	White	Green	Bitter
Nyankhwazi	Purple	None	Dark green	Elliptic	Red/purple	Silvery green	Low	White to cream	White to cream	White	Green	V.Bitter
Mbawala	Light purple	None	Green	Elliptic	Green	Silvery green	High	White to cream	Pink	White	Green	Bitter
Mwaya2	Green with red/purple tinge	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
Kasungwi	Purple	None	Light green	Elliptic	Red/purple	Light brown/orange	Medium	Dark brown	Yellow	White	Reddish	V.Sweet
Guguza	Purple	None	Green	Elliptic	Red/purple	Silvery green	High	White to cream	Yellow	White	Green	Sweet
Thipula	Light green	Moderate	Light green	Lanceolate	Red/purple	Silvery green	N	White to cream	Pink	White	Green	V.Bitter
Popa	Green with red/purple tinge	None	Light green	Elliptic	Mainly red to dark	Silvery green	N	Light brown	Pink	White	Green	V.Sweet
Kawalika	Purple	None	Green	Lanceolate	Green	Silvery green	Medium	White to cream	Pink	White	Green	Bitter
Nyaharawa	Purple	None	Green	Oblanceolate	Red/purple	Silvery green	High	Light brown	White to cream	White	Green	Bitter
Chimphuno	Purple	None	Green	Oblanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Bitter
Simalilande	Purple	None	Light green	Pandurate	Mainly red to dark	Silvery green	Medium	White to cream	Pink	White	Purple	Bitter
Kolowẽka1	Purple	None	Dark green	Elliptic	Green with pale red sllading	Silvery green	Low	White to cream	White to cream	White	Green	V.Bitter
Chimdini	Green with red/purple tinge	None	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	Light brown	White to cream	White	Green	Bitter
Unknown1	Light purple	None	Green	Lanceolate	Green	Dark brown	High	Light brown	Yellow	White	Green	V.Bitter
Biliwili	Purple	None	Dark green	Elliptic	Mainly red to dark	Silvery green	High	White to cream	White to cream	White	Red	V.Sweet
Ng'wenyani	Purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Bitter
Nyamalonje	Purple	None	Dark green	Elliptic	Green with pale red sllading	Silvery green	Medium	White to cream	Pink	White	Green	V.Bitter
Kalomo	Purple	None	Dark green	Lanceolate	Red/purple	Silvery green	Medium	White to cream	White to cream	White	Green	V.Bitter
Depwete	Purple	None	Dark green	Elliptic	Red/purple	Silvery green	High	White to cream	White to cream	White	Green	V.Bitter
Mpuma	Purple	None	Dark green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Bitter
Mgodi	Purple	None	Dark green	Elliptic	Mainly red to dark	Light brown/orange	High	Light brown	White to cream	White	Purple	V.Bitter
Kanonono	Purple	None	Light green	Lanceolate	Red/purple	Light brown/orange	Medium	Light brown	White to cream	White	Red	Bitter
GomaniMfipa	Purple	None	Green	Lanceolate	Green with pale red sllading	Light brown/orange	High	Dark brown	Yellow	White	Green	Sweet

Table 3.4 Morphological description of 93 characterised accessions (continued)

Accession name	CUAL	HUL	MLC	LCLS	Petiole colour	MSC	HFB	CRS	ISC	CRP	CTS	Taste
Loresi	Purple	None	Green	Oblanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	Pink	White	Green	Sweet
Nyachikundi	Purple	None	Light green	Elliptic	Red/purple	Light brown/orange	Low	Light brown	White to cream	White	Green	V.Sweet
GomaniMtuŵa	Purple	None	Light green	Lanceolate	Mainly red to dark	Silvery green	High	White to cream	White to cream	White	Green	V.Bitter
Kachamba	Light green	Little	Green	Pandurate	Green with pale red sllading	Dark brown	High	Light brown	White to cream	White	Green	Sweet
Fyoka	Purple	None	Dark green	Lanceolate	Red/purple	Light brown/orange	Medium	White to cream	Pink	White	Green	V.Sweet
Mbundumali3	Light purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
Nyainki	Purple	None	Dark green	Lanceolate	Red/purple	Silvery green	High	White to cream	White to cream	White	Red	V.Bitter
Munyakayuni	Purple	None	Dark green	Elliptic	Mainly red to dark	Silvery green	Medium	White to cream	White to cream	White	Green	V.Bitter
Twentetwente	Light purple	None	Dark green	Lanceolate	Green	Silvery green	Medium	White to cream	White to cream	White	Green	Bitter
ChitembwereKa.	Purple	None	Light green	Elliptic	Red/purple	Silvery green	Medium	White to cream	White to cream	White	Green	V.Bitter
Chiswanthema	Light purple	None	Green	Elliptic	Mainly red to dark	Light brown/orange	Medium	Dark brown	White to cream	White	Green	V.Sweet
MasoAzungu	Purple	None	Light green	Elliptic	Red/purple	Silvery green	Medium	Dark brown	Yellow	White	Reddish	V.Sweet
Mangochi	Green with red/purple tinge	None	Green	Elliptic	Green with pale red sllading	Light brown/orange	Medium	Dark brown	Yellow	White	Green	Sweet
Sekelemani	Light purple	None	Green	Linear	Green with pale red sllading	Silvery green	Low	White to cream	White to cream	White	Green	Bitter
Yaboma	Light purple	None	Green	Linear	Green with pale red sllading	Silvery green	High	Light brown	Pink	White	Green	Sweet
UnknownSe.	Purple	None	Dark green	Pandurate	Green with pale red sllading	Silvery green	N	White to cream	White to cream	White	Green	Bitter
Kasantha1	Purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	N	White to cream	White to cream	White	Green	Bitter
Kamphunobii	Light purple	None	Green	Lanceolate	Red/purple	Silvery green	High	White to cream	White to cream	White	Green	Sweet
Bwanali	Purple	None	Green	Elliptic	Green	Silvery green	High	Light brown	Pink	White	Green	V.Bitter
Kasantha	Purple	None	Green	Elliptic	Red/purple	Silvery green	High	White to cream	White to cream	White	Green	V.Bitter
Kolowêka2	Light purple	None	Light green	Linear	Mainly red to dark	Silvery green	N	White to cream	White to cream	White	Green	V.Bitter
Agriculture	Purple	None	Light green	Elliptic	Red/purple	Silvery green	N	White to cream	Yellow	White	Green	V.Bitter
Unknown2	Light green	Little	Dark green	Lanceolate	Red/purple	Silvery green	N	Dark brown	White to cream	White	Green	V.Bitter
Yizaso	Green with red/purple tinge	Little	Light green	Lanceolate	Red/purple	Light brown/orange	High	Dark brown	White to cream	White	Reddish	V.Bitter
TMS4(2)1425	Light green	Moderate	Green	Elliptic	Green with pale red sllading	Silvery green	Low	White to cream	White to cream	White	Green	V.Sweet
Mkondezi	Purple	None	Green	Lanceolate	Green with pale red sllading	Light brown/orange	Medium	Dark brown	White to cream	White	Green	V.Bitter
CH92/082	Light purple	None	Green	Lanceolate	Green with pale red sllading	Dark brown	Medium	Dark brown	White to cream	White	Green	V.Bitter
81/00015	Light purple	None	Green	Lanceolate	Green with pale red sllading	Dark brown	Low	Dark brown	White to cream	White	Green	Bitter
Gomani1	Purple	None	Dark green	Lanceolate	Mainly red to dark	Light brown/orange	High	Dark brown	White to cream	White	Green	V.Bitter
MK95/054	Light purple	None	Light green	Lanceolate	Mainly red to dark	Light brown/orange	Low	Dark brown	White to cream	White	Reddish	V.Sweet
83350	Light purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Bitter
LCN8010	Purple	Little	Dark green	Lanceolate	Green with pale red sllading	Light brown/orange	Medium	Pink	White to cream	White	Green	V.Bitter
Mbundumali1	Light purple	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
Sauti	Green with red/purple tinge	Moderate	Green	Elliptic	Green with pale red sllading	Light brown/orange	Medium	Dark brown	White to cream	White	Green	V.Bitter
Maunjili	Light purple	Little	Green	Elliptic	Green with pale red sllading	Light brown/orange	Medium	Dark brown	White to cream	White	Green	Bitter
Silira	Purple	None	Light green	Lanceolate	Red/purple	Light brown/orange	Medium	Dark brown	White to cream	White	Green	Bitter
Manyokola1	Green with red/purple tinge	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Manyokola2	Green with red/purple tinge	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Kabuthu	Green with red/purple tinge	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Nakalasi	Green with red/purple tinge	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Mbundumali2	Light purple	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
Mwaya1	Green with red/purple tinge	Moderate	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet
Manyokola3	Light purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Manyokola4	Light purple	None	Green	Lanceolate	Green with pale red sllading	Silvery green	Medium	White to cream	White to cream	White	Green	V.Sweet
Mkhalatsonga	Light purple	Little	Green	Lanceolate	Green with pale red sllading	Silvery green	High	White to cream	White to cream	White	Green	V.Sweet

CUAL = colour of unexpanded apical leaves; HUL = hairiness of unexpanded apical leaves; MLC = mature leaf colour; LCLS = Central lobe shape; MSC = mature stem colour; HFB = First branching height; CRS = Root surface colour; ISC = Root inner skin colour; CRP = root pulp colour; CTS = tip shoot colour; N = non-branching; V.Sweet = very sweet; V.Bitter = very bitter

hence the availability of diverse leaf shapes in the germplasm can be exploited for improved root bulking and drought resistance.

More than 72% of the accessions had silvery green mature stems and more than 65% had white to cream root outer skin. Colour of both mature stems and root outer surfaces were diverse in the collection (Table 3.4). Stem colour is associated with outer skin colour of cassava roots (Elias *et al*., 2001). Root surface colour is a preference attribute for sweet cassava buyers in Malawi. They have the notion that white skinned cassava roots are sweet and mealy although there are many very bitter and/or watery but white skinned cassava varieties. A trader who brings brown skinned, very sweet and mealy cassava to the market will not sell until the white skinned cassava varieties are sold out.

About 52% of the accessions were low to medium branching (Table 3.4), which is problematic in incorporation in intercropping systems as they tend to grow bushy and suppress the other crop. Branching habit is associated with flowering ability of a variety. Low branching varieties flower readily (Fregene *et al*., 2000), which gives opportunity for use in a breeding programme using conventional approaches.

3.3.5 Clustering of 93 morphologically characterised accessions

Morphological data was converted into a binary matrix, which was used to generate a Dice similarity coefficient matrix followed by clustering the accessions using the UPGMA algorithm. The resulting dendrogram revealed two major clusters (I and II), while the third cluster (III) contained only two accessions (Figure 3.3). Cluster I consisted of most of the genotypes (Figure 3.3). Within cluster I, there were different sub-clusters and groups (Figure 3.3).

Cluster I sub-cluster A group i contained 23 accessions which were local cultivars from all three regions of Malawi except for TMS4(2)1425, which was an introduction from IITA (Figure 3.3). Most accessions which were morphologically similar in the collection were found in this group, indicating low levels of morphological diversity for most of the accessions which belonged to this cluster. The most distant accessions in this cluster were Kamphunobii and TMS4(2)1425 with a Dice similarity coefficient (GS) of 0.434. The rest of accessions in this group were closely related to each other, which was evidenced by the range of GS being between 0.696 and 1.000 (Figure 3.3 and Appendix 3). Accessions in this cluster were characterised by lanceolate shaped

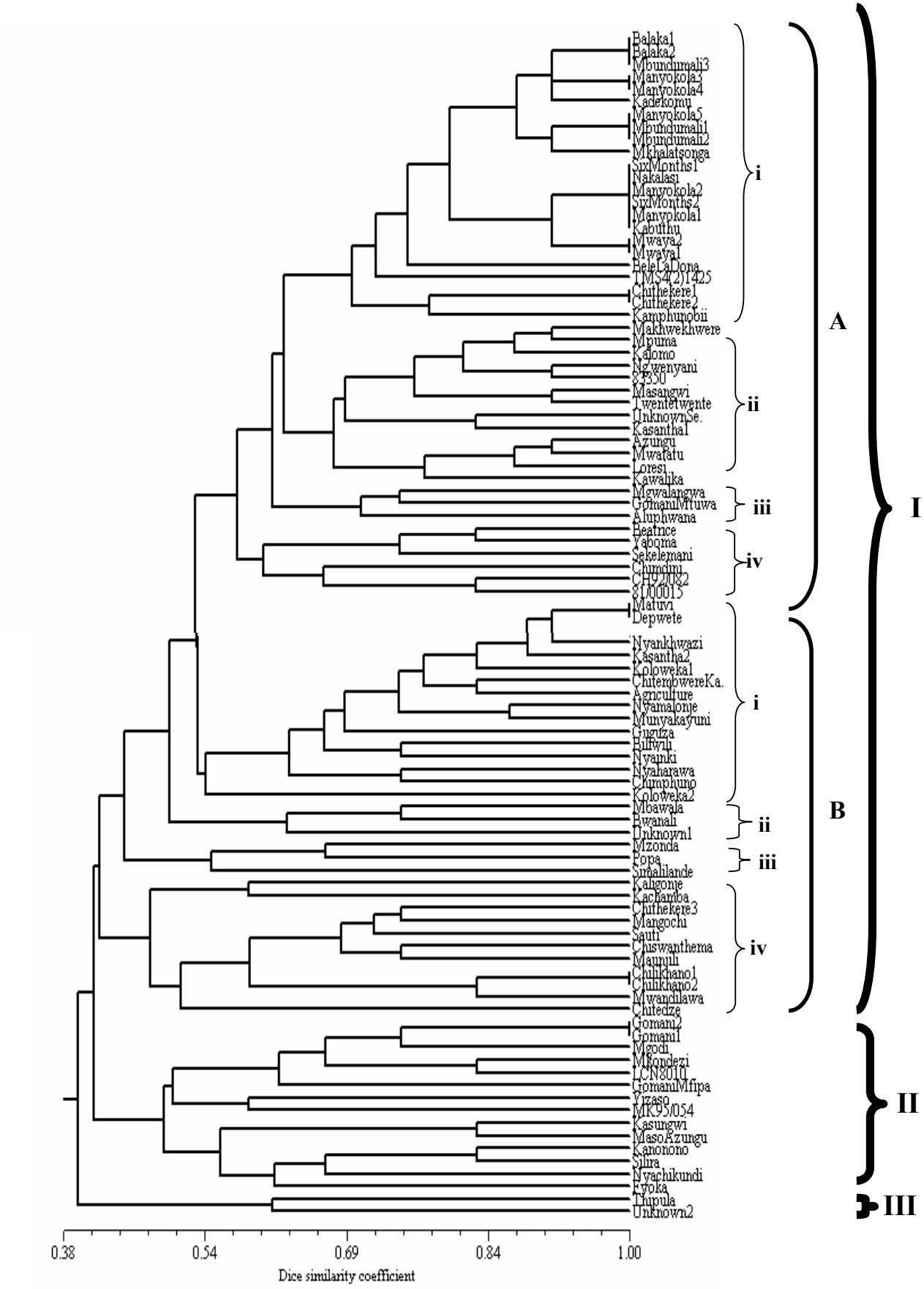


Figure 3.3 Dendrogram for morphological characterisation of 93 accessions included in this study

central leaf lobes, silvery green mature stems, white outer and root inner skin colours, and green shoot tips. In addition, all accessions in this cluster were sweet.

Cluster I sub-cluster A group ii contained 13 accessions which were local cultivars from all three regions of Malawi except for 83350, which was an introduction from IITA (Figure 3.3). The closest accessions in this cluster were Azungu and Mwatatu, Ng'wenyani and 83350, Masangwi and Twentetwente, and Makhwekhwere and Mpuma with GS values of 0.917 between accessions in each pair (Figure 3.3 and Appendix 3). Accessions in this cluster were characterised by glabrous (hairless) unexpanded apical leaves, silvery green mature stems, medium branching heights, white root outer skin colour and green shoot tips.

Cluster I sub-cluster A group iii contained three local cultivars, Mgwalangwa from the south, Aluphwana from the centre and GomaniMtuŵa from the north of Malawi (Figure 3.3). Accessions in this cluster were characterised by lanceolate shaped central leaf lobes, silvery green mature stems, white outer and inner skin colours, and green shoot tips. All accessions in this cluster were bitter.

Cluster I sub-cluster A group iv contained six accessions of which four were local cultivars. Chimdini and Beatrice were from the centre, while Sekelemani and Yaboma were collected in the north of Malawi. CH92/082 was locally screened (whose open pollinated seeds came from IITA but were sprouted and screened in Malawi) while 81/00015 was an introduction from IITA in tissue culture form (Figure 3.3). The closest accessions in this cluster were Beatrice and Yaboma, and CH92/082 and 81/00015 with a GS of 0.833 each (Figure 3.3 and Appendix 3). Accessions in this cluster were characterised by glabrous (hairless) unexpanded apical leaves, green mature leaves and shoot tips. Accessions in this cluster had petioles which were mainly green but with some pale red sllading.

Cluster I sub-cluster B group i contained 15 local cultivars from all three regions of Malawi (Figure 3.3). The GS values in this cluster ranged from 0.609 to 1.000. There was one pair of morphologically similar accessions in this cluster (Matuvi and Depwete with a GS of 1.000). Matuvi was collected in the southern region while Depwete was collected in the northern region, which implicates that farmers in Malawi use multiplicity of nomenclature. The most distant accessions were Matuvi or Depwete and Kolowêka2 with a GS of 0.609, followed by Matuvi or Depwete and Nyaharawa with a GS of 0.667. The GS value between Kolowêka2 and Nyaharawa

was 0.435 (Figure 3.3 and Appendix 3). All accessions in this cluster were characterised by purple and glabrous (hairless) unexpanded apical leaves and silvery green mature stems.

Cluster I sub-cluster B group ii contained Mbawala and Unknown1 from the central, and Bwanali from the northern regions of Malawi (Figure 3.3). Accessions in this cluster were characterised by glabrous unexpanded apical leaves, green mature leaves (lamina) and petioles, high branching heights and green shoot tips. All accessions in this cluster were bitter.

Cluster I sub-cluster B group iii contained Popa and Simalilande from the central, and Mzonda from the southern regions of Malawi (Figure 3.3). Accessions in this cluster were characterised by glabrous unexpanded apical leaves, light green mature leaves, silvery green mature stems and pink inner root skin colour.

Cluster I sub-cluster B group iv contained 11 accessions, of which nine were local cultivars. Sauti (CH92/077) was locally screened while Maunjili (TMS91934) was an introduction from IITA (Figure 3.3). There was one pair of morphologically similar accessions in this cluster (Chilikhano1 and 2 with a GS of 1.000), which suggested that Chilikhano1 and 2 were duplicates. The most distant accessions were Kachamba and Kaligonje with a GS of 0.583. Accessions in this cluster were characterised by white root outer skin colour and green shoot tips.

Cluster I A was characterised by having accessions with green shoot tips, silvery green mature stems and white root outer skin colour. Cluster I B contained accessions which were characterised by hairless unexpanded apical leaves (Figure 3.3).

Cluster II contained 14 accessions. Nine of them were local cultivars from central and northern regions of Malawi (Figure 3.3). Mkondezi (MK91/478), Yizaso (CH92/112), and MK95/054 were locally screened while LCN8010 and Silira (TMS60142A) were introductions from IITA (Figure 3.3). The diversity of accessions belonging to this cluster was wide as GS values ranged from 0.333 to 1.000. There was one pair of morphologically similar accessions in this cluster (Gonani1 and 2 with a GS of 1.000), which suggested that Gonani1 and 2 were duplicates. The most distant accessions were Gomani and MasoAzungu, and Fyoka and Yizaso with GS values of 0.333 between each pair of accessions (Figure 3.3 and Appendix 3). The unique trait for cluster II was light brown to orange mature stems of accessions belonging to this cluster.

Cluster III contained Thipula and Unknown2 with a GS of 0.609. These accessions were characterised by green and hairless unexpanded apical leaves, lanceolate shaped central leaf lobes, purple petioles and green shoot tips. Green unexpanded apical leaves, purple petioles and non-branching growth habit made them unique from the rest of the accessions. Their uniqueness was also captured in the ethnobotany since farmers indicated that tuberous roots of these accessions were not fibrous and good for *Chiphuwa* (fermented and roasted cassava). Results suggested that the two accessions are good candidates to be used as parents in a breeding programme.

Morphological markers failed to uniquely distinguish all 93 accessions which were characterised in this study. This might be due to the limited number of morphological makers since 12 morphological traits were used which generated 41 markers. Morphological characterisation resulted in several accessions which were morphologically similar being clustered together. Balaka1 and Balaka2 and Mbundumali3 were similar. Manyokola5, Mbundumali1 and Mbundumali2, Mwaya1 and Mwaya2, and Chithekere1 and Chithekere2 were similar. Six months1, Six months2, Kabuthu, Nakalasi, Manyokola1 and Manyokola2 were similar. These accessions were found in the same cluster (IAi) with narrow genetic diversity. This implied that most of these accessions were closely related and one cannot expect to make much genetic gain if parents are selected within this cluster. The other morphologically similar accessions were Matuvi and Depwete in cluster IBi, Chilikhano1 and 2 in cluster IBiv, and Gomani1 and 2 in cluster II (Figure 3.3).

Although some morphologically similar accessions were found, morphological markers revealed wide genetic diversity for Malawian cassava germplasm. The germplasm covered a wide range of GS from 0.083 to 1.000. The most distant accessions were Yizaso and Simalilande, Beatrice and Loresi, and Simalilande and Chilikhano 1 and 2 with GS values of 0.083 each. Kachamba was distant from MasoAzungu and Kasungwi with a GS of 0.083 but the GS between MasoAzungu and Kasungwi was 0.833 (Figure 3.3 and Appendix 3). Accessions did not group according to geographic distribution (Figure 3.3).

Colour traits, shape of central lobe shapes and branching habit typified many accessions, hence were important salient characters used by farmers to identify varieties. These observations were in agreement with the findings of Elias *et al*. (2001) who reported that colour variables played a crucial role in differentiating cassava varieties. Morphologically close varieties have a greater possibility of being confused. The confusion can happen either when one farmer acquires cuttings from another farmer or even in the farmer's own field as he or she selects plants to be

planted in the next field (Elias *et al*., 2001). Such confusion resulted in assigning individuals from one variety being considered to belong to another one. This could be one of the reasons for the high number of morphologically similar accessions observed in this study.

No relationship was found between genetic structure and taste (sweet and bitter cassava varieties; Figure 3.3). Findings of this study in terms of lack of relationship between bitterness and genetic structure agreed with those of Narváez-Trujillo *et al*. (2001) and Benesi (2002) which confirmed the polygenetic control of cassava root taste, hence being influenced by both genotype and environment, but disagreed with those of Mkumbira (2002).

Cassava cultivars collected in the northern region were more and diverse compared to the central and southern regions (Tables 3.1 and 3.3, and Appendix 1). Most of the accessions collected in the central and southern regions were closely related as revealed by morphological characterisation, while those collected along the lake shore in the central and northern regions were genetically diverse. Results agreed with earlier studies by Chiwona-Karltun *et al*. (2000) and Mkumbira (2002) who observed that in the north even between two adjacent villages, varieties differed greatly, while in the southern and upland of the central region, except for the northern part of the lakeshore, varieties grown across the region were more uniform.

3.4 Conclusions and recommendations

A total of 167 accessions were collected in 2001/02 and 2003 explorations. Of these 151 accessions sprouted clean and were being conserved at Chitedze research station by December 2005. The involvement of experienced farmers and scientists in the exploration significantly contributed in gathering of indigenous knowledge, reduction in numbers of accessions which were to be sampled while covering the diverse germplasm, and ensured collection of clean cuttings for conservation, evaluation and use in cassava improvement programmes.

Strict implementation of crop hygiene and setting up of a gene bank in a low pressure area for pests and diseases eased management of the germplasm, and reduced the risk of loosing accessions through secondary infections. Detailed passport data will assist in recollection of lost accessions. Passport data furthermore gave the background of the agro-ecology under which cultivars evolved. There exists a need to recollect and characterise accessions which sprouted with primary virus infections.

Cassava mosaic disease (CMD) is widespread along the rift valley across all three regions in Malawi. Most farmers were not aware of the impact of virus diseases (CMD and CBSD) especially in the north of Malawi. There exists an urgent need for campaigning so that extension

staff and farmers should know the diseases and their impact on cassava production and quality, and management strategies for those diseases need to be disseminated. Farmers were aware of cassava pests but were helpless since they were not sure which chemicals they could use to control pests. There exists therefore a need to teach farmers that CM and CGM were being managed through biological control in addition to crop hygiene. However, the effort of rearing, releasing and monitoring the predators and parasitoids for CM stopped some time back. This needs to be continued as many farmers and extension staff reported CM being a serious problem in some areas.

Preferences of cassava varieties by farmers in Malawi are diverse according to areas and uses. Two major groups of farmers were identified, those who use cassava as a staple crop and those who grow cassava for the fresh market or snack. Cassava breeding programmes need to be strategised to address specific needs in different areas and uses.

Most of the 93 characterised accessions had hairless and light purple to purple unexpanded apical leaves, green and lanceolate shaped leaves, green shoot tips, green with pale red sllading petioles, silvery green stems and white root outer and inner skin colour. Although these attributes dominated in the germplasm, each trait was diverse in the collection providing an opportunity for selection and exploitation in a breeding programme. The only exception was that pulp of tuberous roots of all accessions was white.

Some morphological traits like branching height, root pulp colour, leaf shape and hairiness of unexpanded apical leaves have economic importance. There exists a need for further research to delineate the relationship of the rest of morphological traits with agronomical traits. The claims of some consumers of associating some morphological traits with taste of the product like the case of petiole colour and taste of vegetable need to be established.

The absence of distribution of accessions according to areas of origin and the high number of morphologically similar accessions identified in this study suggested that farmers exchanged varieties across the country and might be due to the successful implementation of the government of Malawi, Southern Africa Root Crops Research Network (SARRNET) and other stakeholders' initiative for multiplication and distribution of cassava planting material. Results suggested that local cassava germplasm had a lot to offer in the crop improvement programme but has not been exploited. The wide genetic diversity suggested that the local germplasm can be evaluated and superior genotypes identified and recommended for farmers. This study therefore emphasises the use of local cassava germplasm in crop improvement to meet the needs of farmers, consumers and industries.

CHAPTER 4

CUSTOMISATION AND APPLICATION OF AFLP IN THE ASSESSMENT OF GENETIC DIVERSITY WITHIN MALAWIAN CASSAVA GERMPLASM

4.1 Introduction

An insight into the magnitude of variability present in a crop species is of utmost importance, as it allows effective selection (Allard, 1988; Beeching *et al*., 1993; Jarvis and Hodgkin, 2000). Traditionally, genetic diversity estimates, and segregation of genes and hybrids in crop species were based on differences in morphological characters and quantitative traits (Kochert, 1994; Schut and Stam, 1997). However, use of morphological traits for the study of genetic relationships has been criticised, since it is perceived as being lengthy, and largely affected by the environment and developmental stage of the plant (Kumar, 1999).

Advances in biology and biotechnology, especially the advent of molecular marker technology, complemented conventional approaches for genetic studies and enabled scientists to dissect biochemical pathways to isolate genes of interest and study genetics with more precision (Mkumbira, 2002). DNA markers are reliable and are not influenced by environmental factors and give rise to a high number of polymorphic loci (Karp *et al*., 1997).

Amplified fragment length polymorphism (AFLP) is a marker technique that can be used for DNA of any origin or complexity (Zabeau, 1992; Zabeau and Vos, 1993; Vos *et al*., 1995; Blears *et al*., 1998; Mueller and Wolfenbarger, 1999). Restriction fragments are produced using two restriction endonucleases, one frequent and one rare cutter. The high degree of specificity of restriction enzymes results in production of reproducible sets of DNA fragments. The complexity of the genome and factors such as the methylation status of the DNA influence the choice of restriction enzymes. The use of two different enzymes allows the researcher to manipulate the number of fragments generated for amplification and produce a fingerprint pattern of desired complexity. The generally used restriction enzymes for AFLP are *Eco*RI as a rare cutter and *Mse*I as a frequent cutter.

Apart from the choice of restriction enzymes, the number of amplified fragments is determined by the complexity of the genomic DNA, as well as the number and type of selective nucleotides of the PCR primers. There is an almost linear correlation between the number of amplified fragments and genome size. As the number of selective nucleotides is increased, the complexity of DNA fragments decreases. The number of amplified fragments is reduced approximately four-fold with each additional selective base. For small genomes of 10^6-10^7 base pairs (bp), one or two selective nucleotides on the 3' end of each primer may be sufficient to reveal polymorphism (Vos *et al*., 1995; Blears *et al*., 1998). More complex genomes ranging from 10^8-10^9 bp require additional selective nucleotides to yield the desired number of amplified fragments. Typically, the ideal number of amplified restriction fragments ranges from 50 to 100 (Vos *et al*., 1995). Although the complexity of the fingerprint is reduced with each additional selective nucleotide, selectivity is maintained at a maximum of three bases. Selectivity is lost with a 4-base extension (Vos *et al*., 1995; Blears *et al*., 1998). Krauss and Peakall (1998) emphasised the need for initial screening of primer combinations for each crop after which it has a high throughput and up to 100 polymorphic loci for 100 individuals can be analysed per week. Although AFLP requires genomic DNA of high quality, it is insensitive to the template DNA concentration since the protocol is optimised such that the amplification reaction ceases when the labelled primer is consumed (Vos *et al*., 1995; Krauss and Peakall, 1998; Robinson and Harris, 1999).

The above mentioned issues need to be addressed for results to be reliable and reproducible across runs and laboratories. Hence, having a customised AFLP analysis procedure for cassava will enable comparison of results from studies done in different laboratories and times. Comparison can only be done if the same restriction enzymes and primer combinations have been used. Commercially available primers need to be given priority in screening AFLP primers. Most of the countries involved in cassava research, especially in SSA, do not have the ability to generate primers but depend on purchases of commercially available primers.

Attempts to customise AFLP analysis for cassava in genetic diversity studies have been fragmented, and no concrete nor unanimous conclusions were reached (Roa *et al*., 1997; Robinson and Harris, 1999; Wong *et al*., 1999). There exists a need to identify the best AFLP primer combinations that are commercially and readily available on the market for cost effective use in cassava fingerprinting.

The objectives of this study were to (1) identify informative AFLP primer combinations for fingerprinting cassava germplasm, and (2) assess the genetic diversity within Malawian cassava germplasm using AFLP markers.

4.2 Materials and methods

4.2.1 Plant material and DNA isolation

DNA was extracted from 28 cassava accessions. Of the 28 accessions, 19 were local cultivars, four were locally screened clones (whose open pollinated seeds came from IITA but were sprouted and screened in Malawi), and five were introductions from IITA in tissue culture form. A modified method of Edwards *et al*. (1991) was used for DNA extraction as follows: Fresh young leaves were collected and kept on ice. A well-chilled mortar and pestle were used to grind the plant material to a fine powder in the presence of liquid nitrogen. Ten millilitres of extraction buffer [0.5M NaCl, 0.1M Tris-HCl (pH 8.0), 0.5M eththylene-diaminetetraacetate (EDTA) and 1.5% (w/v) sodium dodecyle sulphate (SDS)], preheated to 65°C, 1ml cetyltrimethylammonium bromide (CTAB) buffer [0.2M Tris-HCl (pH8.0), 0.5M EDTA and 10% (w/v) CTAB] and 2ml NaCl (5M) were added. The homogenate was vortexed and incubated at 65°C for one hour, inverting every 10 minutes. Ten millilitres chloroform-isoamylalcohol [24:1 (v/v)] was added and phases were separated by centrifugation for 15 minutes at 10000 rpm at room temperature. The supernatant was transferred to a new tube and DNA was precipitated overnight at 4°C with 100% cold ethanol in the ratio of 1:2 (v/v). The DNA pellet was scooped out with a sterile hooked Pasteur-pipette, washed three times with 70% (v/v) ethanol, and dissolved in 250µl sterile water.

4.2.2 DNA concentration, quality and integrity determination

DNA concentration and purity were determined using a UV spectrophotometer (U-2000) by measuring absorbances at 260 and 280nm. The DNA concentration was calculated using the formula [DNA = optical density (OD_{260})*dilution factor*constant (50µg/ml)]. DNA purity was estimated by dividing A_{260} by A_{280}. DNA samples were diluted to a working concentration of 250ng/µl in sterile water and stored at 4^{o}C. The integrity, purity and concentration of the DNA was confirmed by visualisation of genomic DNA (250ng) through electrophoresis for 90 minutes at 80 volts in a 1% (w/v) agarose gel stained with ethidium bromide. Gel Doc 100 was used to visualise DNA under UV light with the aid of Molecular Analyst software.

4.2.3 AFLP analysis

AFLP analysis was performed according to Vos *et al.* (1995) as modified by Herselman (2003) using the AFLP Plant Mapping Kit for regular genomes (Promega, Madison, USA). *Eco*RI-primers were screened in combination with *Mse*I-primers. *Eco*RI and *Mse*I primers were given names beginning with E and M, respectively. The code following E and M refers to the selective nucleotides at the 3'-end of the primer. This coding system will be used throughout this thesis.

4.2.3.1 *Double digestion of genomic DNA and ligation of adaptors*

Genomic DNA (250ng) was digested for two hours at 37°C using 0.1U *Eco*RI/*Mse*I and 1x reaction buffer [10mM Tris-HCl (pH 7.5), 10mM Mg-acetate, 50mM K-acetate] in a final volume of 25µl. The reaction mixture was further incubated at 70°C for 15 minutes to inactivate restriction endonucleases. Adaptor ligation was achieved by adding 24µl adaptor/ligation solution [*Eco*RI/*Mse*I adaptors, 0.4mM ATP, 10mM Tris-HCl (pH 7.5), 10mM Mg-acetate, 50mM K-acetate] and 1U T4 DNA ligase in a final volume of 50µl. The reaction mixture was incubated at 20°C for two hours.

4.2.3.2 *Pre-selective amplification reactions*

*Eco*RI+A and *Mse*I+C pre-selective primers (Table 4.1) were used with the Promega AFLP plant mapping kit. Pre-selective amplification reactions were performed in 50µl reaction mixtures containing template DNA (undiluted restriction/ligation mixture), Promega pre-amp primer mix I, 1x Promega PCR buffer [10mM Tris-HCl (pH 9.0), 50mM KCl, 0.1% (v/v) Triton X-100], 2mM $MgCl_2$ and 0.02U *Taq* DNA polymerase (Promega, Madison, USA). Reactions were performed using the following cycling programme: 30 cycles of 30 seconds at 94°C, 60 seconds at 56°C and 60 seconds at 72°C (Herselman, 2003). Quality and quantity of pre-selective reactions were determined by electrophoresis in a 1.5% (w/v) agarose gel at 60 volts for 45 minutes. Pre-selective amplification products were diluted accordingly (1:5, 1:10 or 1:20 times) prior to selective amplification.

4.2.3.3 *Selective amplification reactions*

DNA from the variety Mbundumali was used for screening 32 AFLP primer pairs (Table 4.1). Primers were screened based on the number and ability to produce unambiguous fragments. During the second step, six pre-selected primer pairs (M-CAA/E-ACA, M-CAA/E-AAC, M-CAT/E-ACA, M-CAT/E-AAC, M-CTT/E-ACT and M-CTT/E-ACC) were screened on 28

Malawian cassava accessions for polymorphism and resolution. *Eco*RI primers were either FAM (E-ACA and E-ACT) or NED (E-AAC and E-ACC) labelled.

Table 4.1 Sequences and selective nucleotides of AFLP primers screened and applied on cassava

Enzyme	Type	Sequence (5'-3')
*Eco*RI	Adapter-F	CTCGTAGACTGCGTACC
	Adapter-R	AATTGGTACGCAGTCTAC
*Mse*I	Adapter-F	GACGATGAGTCCTGAG
	Adapter-R	TACTCAGGACTCAT
*Eco*RI	Primer+1	GACTGCGTACCAATTCA
	Primer+3	GACTGCGTACCAATTCANN ANN = ACA, AAC, ACT ACC
*Mse*I	Primer+1	GATGAGTCCTGAGTAAC
	Primer+3	GATGAGTCCTGAGTAACNN CNN = CAA, CAC, CAG, CAT, CTA, CTC, CTG, CTT

Selective amplification reactions were performed in a total of 20µl reaction volumes containing 5µl of five, 10 or 20-fold diluted pre-selective product, 1x Promega *Taq* buffer, 2mM $MgCl_2$, 200µM of each dNTP [dATP, dCTP, dGTP and dTTP], 100µg/ml bovine serum albumin (BSA), 30ng *Mse*I-primer+3, 30ng *Eco*RI-primer+3 and 0.75U Promega *Taq* DNA polymerase. The following cycling programme was used for selective amplification: one cycle of denaturation at 94^{o}C for five minutes followed by one cycle of 30 seconds at 94^{o}C, 30 seconds at 65^{o}C, and 60 seconds at 72^{o}C. The annealing temperature was lowered by 1^{o}C per cycle during the next eight cycles after which 25 cycles of 94^{o}C for 30 seconds, 55^{o}C for 30 seconds and 72^{o}C for 60 seconds were performed, followed by one last elongation of five minutes at 72^{o}C.

PCR products were prepared for capillary electrophoresis by mixing equal amounts of FAM and NED labelled reactions, 99.5% (v/v) formamide and 6fmol GENESCAN-1000 Rox^{TM} size standard. Samples were denatured at 95^{o}C for 10 minutes. Mixtures were immediately put on ice for five minutes before resolving on an ABI Prism 310 Automated Capillary Sequencer (Applied Biosystems, 2001). DNA fingerprint analysis was done on a Macintosh (iMac) computer using GeneScan 3.1 (Perkin-Elmer Corporation, 1997). Fragments were scored into a binary matrix as present (1) or absent (0).

Similarity coefficients for AFLP data were calculated using Dice similarity coefficients **(**Dice**,** 1945**;** Nei and Li, 1979) using NTSYSpc version 2.11c computer package (Rohlf, 2000). Dendrograms were constructed using UPGMA clustering in SAHN programme parameters under

NTSYSpc computer package (Rohlf, 2000). The goodness of fit of clustering to data matrices was calculated using COPH and MXCOMP programmes. Comparison of primer pairs and combinations of primer pairs was done using correlation with the aid of Agrobase (2000).

4.3 Results and discussion

4.3.1 Customisation of AFLP for cassava diversity analysis

GeneScan and Invitrogen (Perkin-Elmer Corporation, 1997) instruction manuals have given AFLP primer pairs that give informative DNA fingerprints for Sunflower, Pepper, Barley, Maize, Sugar beat, Tomato, Lettuce, Arabidopsis, Cucumber and Rice among the primers that are commercially available on the market, but cassava is not on that list. In addition, Vos *et al*. (1995), Blears *et al*. (1998), and Robinson and Harris (1999) highlighted the need to screen AFLP primer pairs for use in any study. There existed a need to customise AFLP for cassava genetic diversity analysis. Standardisation results based on AFLP analysis using 32 primer pairs on the cassava variety Mbundumali are given in Table 4.2. Wong *et al*. (1999) suggested the implementation of primer pairs that revealed 40 and more fragments. Results showed that *Mse*I primers M-CTG, M-CTA and M-CTC did not produce a large number of unambiguous fragments while *Eco*RI primers E-ACA, E-AAC and E-ACT were efficient on cassava.

Table 4.2 Number of unambiguous fragments generated by AFLP primer pairs used for standardisation on the cassava variety Mbundumali

***Mse*I Primers**	***Eco*RI Primers**			
	E-ACA	**E-AAC**	**E-ACT**	**E-ACC**
M-CAA	41	50	40	31
M-CAT	55	41	43	13
M-CTG	22	9	23	16
M-CTT	12	25	50	46
M-CTA	29	6	24	18
M-CAG	44	26	37	26
M-CAC	36	42	40	19
M-CTC	33	34	26	25

A summary of the overall performance of the 32 screened primer pairs are presented in Table 4.3. Eleven AFLP primer pairs (A and B; Tables 4.2 and 4.3) amplified more than 40 fragments. Eight of the tested primer pairs (x) are not recommended for AFLP analysis on cassava (Tables 4.2 and 4.3) since they amplified few unambiguous fragments. The large number of ambiguous

fragments made it difficult to score. The difficulty in scoring complex and ambiguous fragments can lead to low repeatability, which is not desired.

Table 4.3 Guidelines for AFLP primer pair selection for cassava characterisation

*Mse*I Primers (codes)	*Eco*RI Primers (codes)			
	E-ACA (E1F)	E-AAC (E1N)	E-ACT (E2F)	E-ACC (E2N)
M-CAA (M1)	A	A	B	C
M-CAT (M2)	A	A	B	x
M-CTG (M3)	C	x	C	x
M-CTT (M4)	x	C	A	A
M-CTA (M5)	C	x	C	x
M-CAG (M6)	B	C	C	x
M-CAC (M7)	C	B	B	x
M-CTC (M8)	C	C	C	C

A = primer pairs whose FAM and NED (different labelled primers) both worked and amplified more than 40 fragments. B = Only one set of primer labelling (i.e. either FAM or NED) produced more than 40 fragments; C = Primer pairs which amplified less than 40 unambiguous fragments but reactions worked to some extent; x = Not recommended primer combination.

Results showed that inclusion of E-ACA and E-AAC in primer pairs amplified informative fragments, which agreed with the recommendation of Roa *et al*. (1997). Wong *et al.* (1999) used E-C, E-CCC and E-CT which did not work nor are commercially available. The choice of *Eco*RI primers by Wong *et al*. (1999) was questionable since one and two selective nucleotides were used on cassava which is against the recommendation that addition of two nucleotides is suitable for organisms with small genome size (Vos *et al*., 1995; Blears *et al*., 1998). Narváez-Trujillo *et al.* (2001) did not use AFLP primers as per recommendation of Roa *et al*. (1997) or Wong *et al*. (1999) but used E-AGA, E-AAT and E-AGT which are not commercially available. E-AGT was among primers tested by Roa *et al*. (1997) that produced informative fragments. Narváez-Trujillo *et al.* (2001) did not indicate whether primer combination screening was done before applying primer pairs. If primer combination screening was not done it does not make sense to use primer pairs which did not work in earlier studies, and leave out those which were informative. Results of the current study showed that E-ACT and E-ACC were efficient on cassava. This is a new innovation since previous studies did not include these primers in screening AFLP primer combinations for cassava. E-ACT and E-ACC are commercially available, hence convenient for acquisition and use.

Among the *Mse*I primers which were efficient in the current study included M-CAA, M-CAT and M-CTT which are commercially available. Roa *et al*. (1997) screened the following *Mse*I primers: M-GTA, M-GCG, M-GAC and M-GGT which are not commercially available while Wong *et al*. (1999) did not mention *Mse*I primers used in their study. Therefore, *Mse*I primers

which amplified more than 40 unambiguous fragments in the current study are recommended since they are commercially available and convenient for use in most SSA countries.

Based on standardisation results, six primer pairs (primer pairs A, Table 4.3) were chosen to characterise 28 cassava accessions. Selection of 28 accessions for AFLP genetic analysis was based on ethnobotanical and morphological data. The 28 accessions presented morphological and agronomical traits preferred by farmers and their cropping systems and represented all major clusters of Figure 3.3. Apart from genetic analysis of the 28 accessions, this stage further evaluated the six pre-selected primer pairs by considering polymorphism and resolution in addition to number of fragments amplified on 28 cassava accessions.

Comparison of information content and resolution of amplification products of the six pre-selected primer pairs on 28 cassava accessions are given in Table 4.4. Single primer pairs amplified between 94 and 118 fragments, and polymorphism ranged between 58 and 81%. Primer pair M-CTT/E-ACC amplified the least number of fragments (94) and gave the lowest percentage of polymorphism (58%; Table 4.4). The highest number of fragments (118) as well as percentage polymorphism (81%) on the 28 accessions were realised from M-CAA/E-AAC (Table 4.4). The number of fragments amplified by each primer pair were higher than those obtained by Fregene *et al*. (2000), while percentage polymorphisms obtained in both studies were within the same range.

Resolution of primer pairs and combinations were measured by their ability to differentiate analysed accessions, and by correlating genetic distances of primer pairs or combinations of primer pairs with a combination of all six primer pairs. The single primer pairs and combinations of primer pairs are given in Table 4.4. Single primer pairs, and combinations of the primer pairs differentiated all 28 analysed accessions. Roa *et al*. (1997) recommended two primer pairs but it was not clear whether they should be used as substitutes or whether they complement each other. Narváez-Trujillo *et al.* (2001) used three primer pairs but how the combination of those three primer pairs were arrived at, was not explained.

There existed weak or no correlation between single primer pairs (Table 4.4), which was in disagreement with previous studies since comparison of primer pairs recommended by Roa *et al*. (1997) indicated strong correlation of r=0.90 when tested by Fregene *et al*. (2000). The difference could be due to the method employed in screening of primer pairs. If only the number of fragments and percentage polymorphism are considered, it eliminates information on

Table 4.4 Correlation matrix, number of fragments, percentage polymorphism and ability of primer pairs and combinations to differentiate 28 cassava accessions for AFLP using Dice similarity coefficients from NTSYSpc

	Single primer pairs												Primer pair combinations																	
	M1E1F		M1E1N		M2E1F		M2E1N		M4E2F		M4E2N		M1E1		M2E1		M4E2		M1E1M2E1		M1E1M4E2		M2E1M4E2		M1E1M2E1M4E2	
M1E1N	0.2051	*																												
M2E1F	-0.0322	NS	0.2113	*																										
M2E1N	0.1533	*	0.2067	*	0.1478	NS																								
M4E2F	0.2225	*	0.2213	*	0.1336	NS	0.1596	*																						
M4E2N	0.1288	NS	0.1451	NS	0.1202	NS	0.0334	NS	0.3706	**																				
M1E1	0.7942	***	0.7569	***	0.1069	NS	0.2339	*	0.2830	*	0.1761	*																		
M2E1	0.0748	NS	0.2657	*	0.7817	***	0.7128	***	0.1673	*	0.0899	NS	0.2149	*																
M4E2	0.2230	*	0.2331	*	0.1566	*	0.1320	NS	0.9146	***	0.7136	***	0.2913	**	0.1656	*														
M1E1M2E1	0.5758	***	0.6691	***	0.5522	***	0.5933	***	0.2935	**	0.1754	*	0.7999	***	0.7576	***	0.2987	**												
M1E1M4E2	0.6223	***	0.6124	***	0.1704	*	0.2268	*	0.7530	***	0.5600	***	0.7941	***	0.2406	*	0.8120	***	0.6800	***										
M2E1M4E2	0.2010	*	0.3254	**	0.5888	***	0.5271	***	0.7383	***	0.5504	***	0.3347	**	0.7279	***	0.7963	***	0.6722	***	0.7122	***								
M1E1M2E1M4E2	0.5291	***	0.6049	***	0.4823	***	0.4963	***	0.6730	***	0.4855	***	0.7276	***	0.6348	***	0.7194	***	0.8775	***	0.9026	***	0.8896	***						
No. of fragments	98		118		100		98		117		94		216		198		211		414		427		409		625					
% Polymorphism	72.3		81.4		72.0		66.3		72.7		57. 5		76.9		69.2		65.1		73.0		71.0		67.1		70.4					
Ability to differentiate all acc.	Yes		Yes		Yes		Yes		Yes		Yes		Yes		Yes		Yes		Yes		Yes		Yes		Yes					

M1E1F, M1E1N, M2E1F, M2E1N, M4E2F and M4E2N are as defined in Table 4.3; M1E1 = M1E1F+M1E1N; M2E1 = M2E1F+M2E1N; M4E2 = M4E2F+M4E2N; *** = P≤0.001; ** = P≤0.01; * = P≤0.05; NS = not significant; acc. = accessions

whether they reveal similar regions on the genome or not. There exists a need to compare primer pairs and select those which are informative and reveal different regions of the genome. This can be achieved through correlation or clustering. The lack or weak correlation of single primer pairs in the current study confirms the efficiency in selecting primer pairs with the aid of cluster analysis which was done in the current study (data not shown) in addition to number of fragments and percentage polymorphism. Pre-selected single primer pairs were compared with a combination of all six primer pairs. It was found that M-CTT/E-ACT (r=0.673) and M-CAA/E-AAC (r=0.605) had strong correlation with the combination of all six primer pairs in terms of revealing genetic distances among the analysed 28 accessions (Table 4.4).

There existed weak correlations when combinations containing two primer pairs, were compared among themselves as was the case with single primer pairs. Combinations of two primer pairs had strong correlations with the six pair primer combination. The strongest correlation was obtained between a six pair primer combination and a combination of M-CAA/E-ACA+M-CAA/E-AAC (r=0.728) followed by M-CTT/E-ACT+M-CTT/E-ACC (r=0.719; Table 4.4).

There existed strong correlations when four pair primer combinations were compared among themselves. There were very strong correlations between the four pair primer combinations and a six pair primer combination. The strongest correlation was realised between the six pair primer combination and a combination of M-CAA/E-ACA+M-CAA/E-AAC+M-CTT/E-ACT+M-CTT/E-ACC with r=0.903 (Table 4.4). Previous studies never evaluated resolution of different combinations of primer pairs.

The correlation of each primer pair and each combination of primer pairs, with a combination of all six primer pairs were significant (Table 4.4). As the combinations of primer pairs increased from single primer pairs to four primer pairs, there was a corresponding increase in the correlation coefficient (r) which was an indication of resolution. The resolution increased from the range r=0.482-0.673 for single primer pairs, to r=0.635-0.728 for two pair primer combinations, to r=0.878-0.903 for four pair primer combinations. Improvement in resolution with the increase of primer pairs in a combination of primer pairs could be due to the corresponding increase in number of fragments. Since different primer pairs reveal different regions on the genome, the genome is better explored by a combination of different pairs if they were properly chosen.

4.3.2 Clustering of 28 accessions characterised by AFLP analysis

Dice similarity matrix (Table 4.5) was used to cluster accessions using the UPGMA algorithm. The resulting dendrogram revealed two clusters 1 and 2 (Figure 4.1). Only Fyoka belonged to cluster 2, while the rest were in cluster 1. However, the major clusters were I and II. Cluster I consisted of most of the genotypes.

Cluster 1 sub-cluster IAi group a contained four local accessions from all three regions of Malawi (Figure 4.1). The closest accessions in this cluster were Mbundumali1 and Manyokola5 with Dice genetic similarity (GS) of 0.946 (Figure 4.1 and Table 4.5). Accessions in this cluster were characterised by lanceolate shaped central leaf lobes, silvery green mature stems, and white root outer skin colour.

Cluster 1 sub-cluster IAi group b contained five local accessions from the central and northern regions of Malawi, and Yizaso, which was a locally screened clone from IITA open pollinated (OP) seeds. The closest accessions in this cluster were Gomani1 and 2 with GS of 0.938 (Table 4.5 and Figure 4.1). Accessions in this cluster were characterised by white root inner skin colour.

Only Beatrice belonged to cluster 1 sub-cluster IAi group c. Although this accession belonged to a different sub-group, it was closely related to accessions in cluster IAi with a GS range of 0.830-0.863 (Table 4.5 and Figure 4.1). These results suggested that accessions belonging to cluster IAi had narrow genetic diversity with respect to AFLP characterisation.

Cluster 1 sub-cluster IAii contained Masangwi, Mgwalangwa and Matuvi from the southern and the other three accessions from the northern regions of Malawi (Figure 4.1). The closest accessions in this cluster were Masangwi and Mgwalangwa with a GS of 0.889 (Table 4.5 and Figure 4.1). The GS range of 0.860-0.889 revealed narrow genetic diversity within this cluster (Table 4.5). Accessions in this cluster were characterised by silvery green mature stems, and white root outer and inner skin colours.

Cluster 1 sub-cluster IB contained two local accessions (Twentetwente and MasoAzungu with a GS of 0.862), both from the northern region of Malawi (Table 4.5 and Figure 4.1). These accessions were characterised by glabrous unexpanded apical leaves and silvery green mature stems.

Table 4.5 Dice similarity coefficients for AFLP characterisation on 28 analysed accessions

Genotypes	Many.5	Mwat.	Gom.2	Beatr.	Nyach.	Kach.	Fyoka	Nyainki	20:20	MasoA.	Masan.	Mgwal.	Matuvi	Thipula	Biliwili	Depw.	Sekel.	Yizaso	TMS4.	CH92/.	81/.	Gom.1	MK95/.	83350	LCN8.	Mbun.1	Sauti
Manyokola5	1.000																										
Mwatatu	0.885	1.000																									
Gomani2	0.840	0.878	1.000																								
Beatrice	0.863	0.850	0.848	1.000																							
Nyachikundi	0.840	0.875	0.877	0.850	1.000																						
Kachamba	0.883	0.872	0.872	0.855	0.894	1.000																					
Fyoka	0.828	0.811	0.815	0.823	0.826	0.838	1.000																				
Nyainki	0.851	0.868	0.861	0.844	0.855	0.883	0.828	1.000																			
Twentetwente	0.827	0.832	0.825	0.818	0.847	0.859	0.825	0.858	1.000																		
MasoAzungu	0.850	0.827	0.828	0.844	0.854	0.878	0.826	0.855	0.862	1.000																	
Masangwi	0.846	0.819	0.809	0.827	0.826	0.855	0.813	0.829	0.822	0.837	1.000																
Mgwalangwa	0.866	0.836	0.821	0.846	0.846	0.862	0.836	0.836	0.816	0.848	0.889	1.000															
Matuvi	0.848	0.820	0.825	0.826	0.832	0.844	0.845	0.842	0.837	0.852	0.860	0.870	1.000														
Thipula	0.860	0.854	0.864	0.856	0.866	0.861	0.818	0.844	0.835	0.828	0.855	0.877	0.859	1.000													
Biliwili	0.847	0.835	0.843	0.837	0.851	0.848	0.823	0.846	0.831	0.837	0.851	0.882	0.877	0.855	1.000												
Depwete	0.852	0.839	0.843	0.822	0.841	0.867	0.837	0.842	0.820	0.847	0.864	0.881	0.858	0.855	0.875	1.000											
Sekelemani	0.846	0.827	0.820	0.882	0.835	0.837	0.826	0.831	0.827	0.846	0.863	0.875	0.863	0.851	0.863	0.882	1.000										
Yizaso	0.857	0.851	0.859	0.863	0.871	0.872	0.837	0.836	0.834	0.843	0.848	0.851	0.837	0.854	0.845	0.843	0.844	1.000									
TMS4(2)1425	0.838	0.851	0.853	0.826	0.846	0.860	0.818	0.838	0.818	0.847	0.812	0.833	0.838	0.836	0.817	0.834	0.835	0.884	1.000								
CH92/082	0.843	0.837	0.839	0.827	0.860	0.863	0.826	0.825	0.826	0.823	0.835	0.850	0.816	0.849	0.833	0.842	0.828	0.855	0.841	1.000							
81/00015	0.833	0.833	0.857	0.824	0.844	0.864	0.805	0.843	0.829	0.835	0.810	0.829	0.821	0.841	0.826	0.826	0.826	0.869	0.885	0.863	1.000						
Gomani1	0.822	0.852	0.938	0.830	0.858	0.863	0.811	0.859	0.822	0.820	0.810	0.826	0.825	0.862	0.857	0.836	0.824	0.866	0.857	0.848	0.870	1.000					
MK95/054	0.833	0.846	0.844	0.819	0.831	0.847	0.778	0.833	0.806	0.849	0.796	0.822	0.821	0.829	0.814	0.814	0.837	0.846	0.884	0.829	0.873	0.859	1.000				
83350	0.828	0.839	0.830	0.826	0.843	0.833	0.801	0.824	0.809	0.829	0.811	0.830	0.816	0.849	0.833	0.828	0.831	0.853	0.844	0.840	0.858	0.836	0.863	1.000			
LCN8010	0.834	0.829	0.836	0.820	0.815	0.841	0.794	0.847	0.812	0.827	0.819	0.820	0.825	0.819	0.820	0.826	0.824	0.836	0.854	0.841	0.856	0.820	0.840	0.848	1.000		
Mbundumali1	0.946	0.884	0.838	0.849	0.851	0.888	0.831	0.865	0.830	0.842	0.838	0.860	0.842	0.859	0.854	0.861	0.845	0.860	0.836	0.845	0.836	0.833	0.825	0.831	0.839	1.000	
Sauti	0.829	0.822	0.817	0.831	0.819	0.829	0.819	0.820	0.782	0.814	0.839	0.842	0.810	0.835	0.842	0.833	0.838	0.844	0.824	0.846	0.828	0.831	0.815	0.834	0.837	0.840	1.000
Maunjili	0.843	0.833	0.832	0.827	0.838	0.842	0.819	0.841	0.821	0.832	0.848	0.861	0.831	0.852	0.852	0.846	0.841	0.863	0.854	0.856	0.851	0.842	0.837	0.871	0.858	0.840	0.880

Many.5 = Manyokola5; Mwat. = Mwatatu; Gom.2 = Gomani2; Beatr. = Beatrice; Nyach. = Nyachikundi; Kach. = Kachamba; 20:20 = Twentetwente; MasoA. = MasoAzungu; Masan. = Masangwi; Mgwal. = Mgwalangwa; Depw. = Depwete; Sekel. = Sekelemani; TMS4. = TMS4(2)1425; CH92/. = CH92/082; 81/. = 81/00015; Gom.1 = Gomani1; MK95/. = MK95/054; LCN8. = LCN8010; Mbun.1 = Mbundumali1

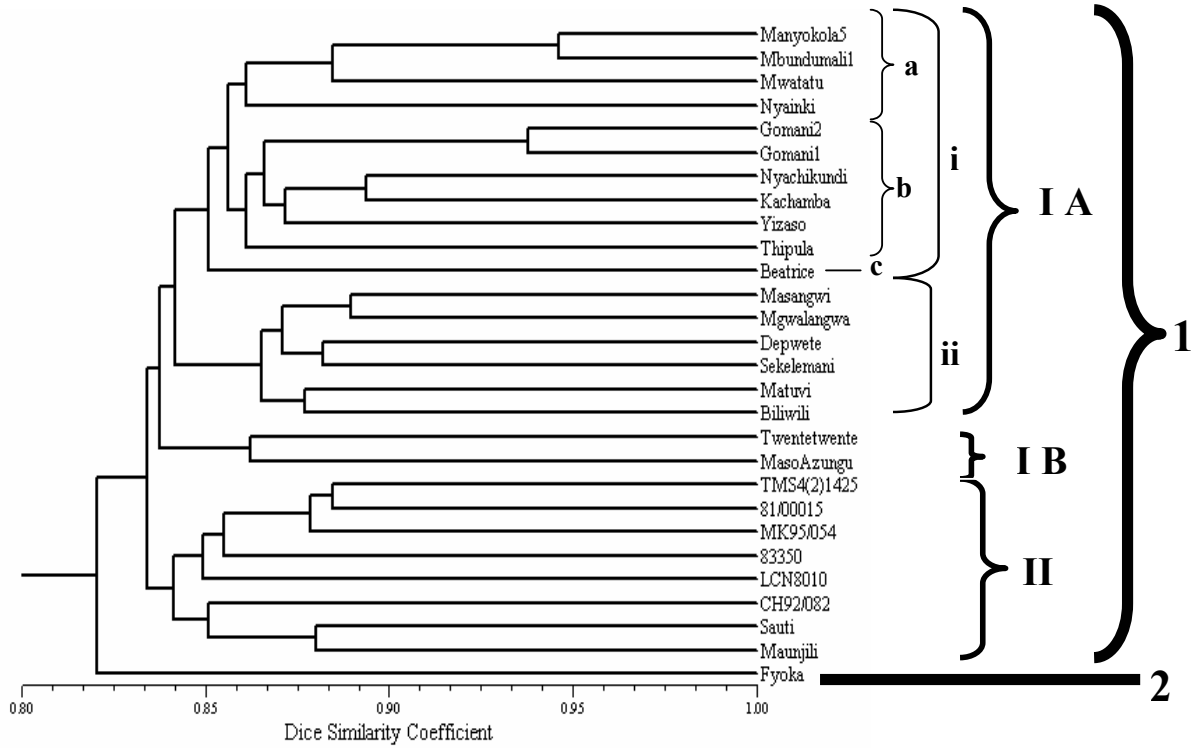


Figure 4.1 Dendrogram for characterisation of 28 analysed cassava accessions using six AFLP primer pairs with the aid of NTSYS computer package, using Dice similarity coefficient and UPGMA clustering

Cluster 1 sub-cluster II had eight accessions, of which accessions Sauti (CH92/077), MK95/054 and CH92/082 were locally screened, and the remaining five were introductions from IITA (Figure 4.1). This cluster contained only locally screened and introduced clones. There could exist a close genetic relationship between the introduced and locally screened clones since seeds for the locally screened clones were obtained from IITA and could have been generated from the same gene pool. The closest accessions in this cluster were TMS4(2)1425 and 81/00015 with a GS of 0.885 (Table 4.5 and Figure 4.1). Accessions in this cluster were characterised by white root inner skin colour.

AFLP analysis showed a narrow range of GS values of 0.778-0.946 between all tested genotypes (Table 4.5 and Figure 4.1). These results were in agreement with previous studies by Fregene *et al*. (2000) who reported a GS range of 0.83-1.00 despite the fact that they characterised accessions which comprised landraces from Nigeria, improved clones from IITA, Africa, and accessions from a core collection of CIAT, South America. However, most accessions clustered according to geographic origin and they concluded that AFLP was efficient and the germplasm was diverse although several accessions were found to be duplicates. Other crops like

groundnuts revealed much closer GS range of 0.977-0.997 (Herselman, 2003) compared to the ones obtained from cassava. Results of the current study (Figure 4.1) clustered accessions according to geographic origin. Introductions were clustered separately from Malawian land races except for Yizaso which was clustered together with local landraces in cluster IAib. No duplicates were found even with single primer pairs. The clustering together of both tissue culture and clones from open pollinated seeds introduced from IITA indicated that they originated from the same gene pool. Malawian cassava landraces formed different clusters suggesting existence of genetic diversity within the local germplasm. Fyoka was separated from the rest of the characterised accessions which was an indication of uniqueness in terms of genetic constitution. The rest of the Malawian landraces were clustered in cluster 1 sub-cluster I where separate and clear heterotic groups were generated by AFLP analysis (Table 4.5 and Figure 4.1). Availability of Yizaso which was an introduction from IITA as OP seed in cluster IAib which comprised of mainly Malawian local cultivars, suggested that cassava working collection from IITA and some Malawian cassava landraces might have evolved from a common gene pool as suggested by Mahungu N.M. (personal communication).

4.4 Conclusions and recommendations

Standardisation and customisation of AFLP analysis on cassava revealed 11 primer pairs which were efficient on cassava. Of these, M-CAA/E-ACA and M-CAA/E-AAC, M-CAT/E-ACA and M-CAT/E-AAC, and M-CTT/E-ACT and M-CTT/E-ACC were sets of primer pairs whose FAM and NED both worked well. Customisation of AFLP analysis on cassava will not only enable comparison of results from different studies but will cut down on time and cost of screening primers each time AFLP analysis is performed on cassava. Screening of commercially available primers will further enhance the convenience in acquisition of those primers for use in future studies. This point is underscored by the fact that Fregene *et al*. (2000) used primers which were recommended by Roa *et al*. (1997). They had the capacity to generate or order *Mse*I-G for pre-selective amplification. However, commercially available primers screened in the current study increases the convenience and minimises cost for those who do not have the ability to generate or order special primers. The review of Robinson and Harris (1999) stressed that the cost of custom and off-the-shelf AFLP primers are different.

In addition to the number of fragments and percentage polymorphism, a measure of resolution for combinations of primer pairs is crucial since it indicates the compatibility of combinations of different types and numbers of primer pairs when it comes to screening AFLP primer

combinations. Results showed that primer pair M-CAA/E-ACA gave the highest number of fragments as well as percentage polymorphism. Loss of resolution with decrease in the number primer pairs was evident but combinations of four primer pairs had highly significant positive correlations with a combination of all six pre-selected primer pairs and M-CAA/E-ACA+M-CAA/E-AAC+M-CTT/E-ACT+M-CTT/E-ACC generated the highest number of fragments with a resolution closest to a combination of all six pre-selected primer pairs. Since the loss in resolution from six to four primer pairs was not high, it is advisable to use a combination of four AFLP primer pairs when analysing cassava for genetic diversity. A reduction in number of primer pairs saves considerable time and money but should not be at the expense of reliability of results. Hence, a combination of four primer pairs is recommended. Use of single primer pairs in analysing cassava genetic diversity is being discouraged due to low resolution. A single primer pair can only be used on special occasions like critical financial constraints but it should be borne in mind that it will be at the expense of resolution, although all pre-selected primer pairs uniquely distinguished all 28 characterised accessions.

AFLP analysis was efficient in generating clear heterotic groups and delineating underlying genetic structure and pedigree of the characterised accessions. Grouping of accessions according to geographic origin especially introductions from IITA suggested that the gene pool for Malawian cassava germplasm was improved through the introductions, which opened the possibility for direct evaluation and selection of introduced materials. This is evidenced by the high number of cassava varieties officially released in Malawi from the introductions. Incorporation of some of the introductions in a breeding programme to develop suitable varieties will benefit Malawi and the whole region.

AFLP analysis showed genetic diversity of Malawian cassava germplasm since the GS range of 0.778-0.946 was larger than the 0.83-1.00 obtained by Fregene *et al*. (2000). However, there exists a need to explore other analytical methods which can present AFLP results better than the cluster analysis.

Results suggested that there exists diverse cassava germplasm among the landraces in Malawi as evidenced by the break away of Fyoka from the rest of the analysed accessions. Hence, it is recommended that Fyoka should be included as a parent in a breeding programme due to high probability of making fast genetic gain through heterosis. Beatrice also broke away from the rest in cluster 1IAi, which indicated that it is another good candidate to be used as a parent in a breeding programme in Malawi. This cultivar showed high levels of resistance in the farmers'

fields. Farmers hailed it for its high yield potential. It is therefore recommend that it should be evaluated for official recommendation/release. Twentetwente and MasoAzungu formed a separate heterotic group, suggesting unique genetics. The rest of the accessions showed genetic diversity as they formed clear heterotic groups. It is therefore concluded that there exists wider genetic diversity among the landraces than within the introductions, hence the need to exploit local cassava germplasm in a breeding programme and evaluation for official release of superior genotypes.

There exists a need to recollect accessions which sprouted with primary virus infection. Extensive exploration should be done especially in the north of the country where diverse cassava cultivars exist. The rest of the existing and newly collected accessions will have to be characterised using AFLP analysis since appropriate AFLP primer combinations have been identified. Characterisation of cassava germplasm using AFLP analysis will give a better genetic structure which will improve the efficiency in management and utilisation of germplasm in Malawi and globally.

CHAPTER 5

EFFECT OF GENOTYPE, LOCATION AND SEASON ON CASSAVA STARCH EXTRACTION

5.1 Introduction

Cassava is produced mainly for household consumption as staple diet, snack and substitute for bread among most households in Malawi including those in the high income category (Mataya, 2001). Over the years, both area and production levels of the crop has significantly increased in Malawi. This crop is now contributing 25-60% of the national food balance sheet in some SARRNET countries (Phiri, 2001). Due to increased cassava production there exists a need to find markets for cassava. Many cassava products have more than one purpose. These products are used for food and in the industry giving cassava a commercial value (Onwueme, 1978; Benesi *et al.*, 2001a; 2001b; Mataya, 2001).

Although demand for cassava products in industries, including bakeries, timber and textile industries, is steadily rising in Malawi, these industries predominantly depend on imports to meet their requirements. This clearly demonstrates that there exists an opportunity for small as well as large scale farmers to invest and profit from this commodity (Benesi *et al*., 2001b; Fungulani and Maseko, 2001; Itaye, 2001; Mataya, 2001; Munthali, 2001).

As a perennial crop, cassava has no definite lifetime or maturity period. However, after full development of the canopy, root growth will gradually decrease and ultimately reach point zero. This moment is sometimes called the maturity period of cassava (De Vries, 1985) and is the point when maximum or near maximum yield is obtained. On the other hand, optimum harvest time is when harvesting is economic, that is when the returns for production and utilisation are optimised. In this case, yield should not necessarily be defined as root yield but rather as starch yield or dry matter yield, which is the combination of yield and starch and/or dry matter content (Ngendahayo and Dixon, 2001).

When cassava is harvested too early, it often leads to reduction in yield, while delayed harvest leads to development of woody and fibrous tuberous roots, and reduction in starch content. Susceptibility to loss in starch content when cassava remains in the ground after maturity,

appears to be a disadvantage for starch production (Ngendahayo and Dixon, 2001). Many researchers tried to define the optimum time for harvesting cassava but there exists variation in their recommendations. Most researchers agreed that the optimum stage of harvest depends on varieties and ecological factors (Ashoka *et al*., 1984; Ngendahayo and Dixon, 2001).

Harvesting parameters such as total dry matter accumulation in the tuberous root and maximum starch content in the storage root have been used but are weak indicators of harvesting time across environmental conditions (Sarmento *et al*., 2000). Hence, there exists a need to evaluate optimum harvest time for different cassava varieties in target environments for optimum economic returns.

Industries in Malawi had a negative attitude towards the use of cassava starch because some suppliers were selling cassava flour in the name of cassava starch which was not as efficient as maize starch or in some industries led to total failure (Fungulani and Maseko, 2001; Itaye, 2001; Benesi, 2002). The study of Benesi *et al*. (2004) in association with the Malawian industries (Nzeru Radio Company, in battery making; Packaging Industries Limited, in the making of corrugated boxes for packaging; David Whitehead and Sons, in the textile industry) proved that native cassava starch was as good as the maize starch being used by industries. Results indicated that suppliers either sold cassava flour in the name of cassava starch or due to ignorance, they thought that cassava flour is the same as cassava starch. It was recommended that the Malawi Bureau of Standards (MBS) should come up with national standards for starch, and should be policing starch suppliers in Malawi to avoid killing the cassava starch market (Benesi, 2002; Benesi *et al*., 2004). MBS (2004) responded positively and are now monitoring starch quality being sold to industries. A pilot factory was established in Malawi to produce cassava starch which is being sold to local industries like Leopard Matches Company Limited and others (Nthonyiwa *et al*., 2005.

The objectives of this study were to (1) determine the effect of genotype, location and season on starch extraction, (2) assess interaction of genotype by environment (GxE) for cassava starch extraction in elite Malawian cassava genotypes, and (3) determine the relationship among starch yield, starch extraction rate on fresh and dry root weight bases, root dry matter content, starch content, and total soluble solutes (TSS).

5.2 Materials and methods

5.2.1 Cassava varieties

The local recommended variety namely Mbundumali, locally screened varieties: Sauti (CH92/077), Mkondezi (MK91/478) and CH92/082, and introduced varieties from IITA: Maunjili (TMS91934), Silira (TMS60142A), 83350, and LCN8010 were included in this study.

5.2.2 Trial sites

Trials were planted at Chitedze, Chitala, Makoka and Mkondezi research stations in December 2002 for set one, and December 2003 for set two of the trials. Weather data for minimum and maximum temperatures, monthly total rainfall and altitudes of trial sites were recorded. Soil samples were collected from trial sites and analysed for texture using hydrometer method as described by Gee and Bauder (1986), pH as described by Eckert and Thomas Sims (1995), and phosphorus and potassium using Mehlich 3 method (Mehlich, 1984). Trials were replicated over two years, and in each year, starch extraction was done at three different times (three rounds of starch extraction), between October and November, in January, and between March and April.

5.2.3 Design of trials

A randomised complete block design was used with four replicates, three rounds of starch extractions, in four locations over two years. Plot sizes were 10 ridges, each with 10 plants. Ridges were 0.9m apart and plants were spaced 0.9m apart along the ridge. The length of cassava planting stakes were 25cm and were planted in a slanting position of about 60^{o}. Starch yield, starch extraction rate on fresh and dry root weight bases, starch content on fresh root weight basis, root dry matter content, and total soluble solutes were among the recorded data.

5.2.4 Storage root dry matter content

Cassava storage root dry matter content was determined within 12 hours after harvest to avoid post harvest changes through physiological deterioration or moisture loss of the root. About five undamaged roots were randomly selected. The medial sections of selected roots were shredded into thin slices, mixed thoroughly and a duplicate of 200g samples (w_1) were dried at 65^{o}C for 72

hours. After removal from the oven, samples were weighed immediately (w_2). Dry matter content percentage (DM %) was calculated as follows: DM % = 100 * (w_2 / w_1).

5.2.5 Starch content on fresh root weight basis

Starch content on fresh root weight basis was determined using a density method of Brautlecht (1953) and modified by the Danish EU-directorate (1996). A stainless steel rod with two hooks was suspended from a hanging balance. A weighing basket was placed on the upper hook of the rod. A few centimetre below the basket, a vessel filled with clean water (≤18^{o}C) was placed. The weight (T_o) of the rod and empty basket was determined. The basket was moved to the lower hook (hooks were adjusted so that the basket was completely immersed in water while on the lower hook) and weight (T_u) of the rod and empty basket was determined. Clean tuberous roots (5kg; W_o) were put in the basket on the upper hook. The basket with the sample was moved to the lower hook (completely immersed) and weighed again (W_u; Figure 5.1). Starch content on fresh root weight basis was calculated as follows:

Starch content (%) = 5050 * 0.051738 [(W_u - T_u) / (0.98 (W_o - T_o)] - 5.2463 (Danish EU-directorate, 1996)

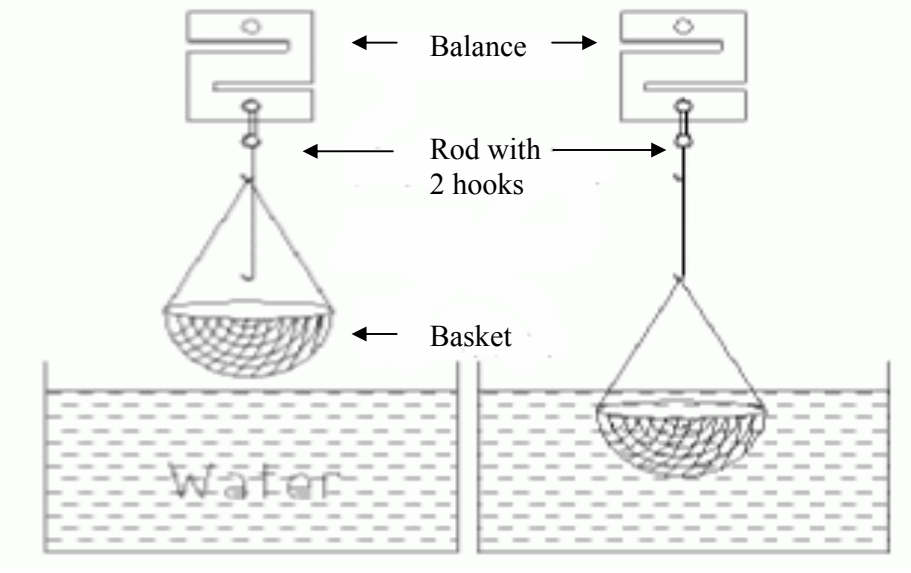


Figure 5.1 Diagram of setup of the weighing procedure for determination of starch content on fresh root weight basis in cassava tuberous roots (Danish EU-directorate, 1996)

5.2.6 Native starch extraction

Native cassava starch extraction was done using methods used by Numfor and Walter (1996) and Masumbu (2002) and modified by Benesi *et al.* (2004). Fresh tuberous roots were washed, peeled, washed again and chopped to about $1cm^3$ cubes. One litre water was added to 500g of the chopped tuberous roots. Roots were pulverised in a high speed industrial blender (Croydon Industrial Blender, Model: TI4AL Set/00) for 5min. The pulp was suspended in 10x its volume water, stirred for 2min and filtered using a double cheese (muslin) cloth. The filtrate was allowed to stand for four hours to facilitate starch sedimentation and the top liquid was decanted and discarded. The sediment was broken, water added as in the first step, and the whole process was repeated. The sediment was washed and then dried in the sun (open air) for two days followed by determination of starch yield and extraction rates.

5.2.7 Total soluble solutes (TSS)

Cassava tuberous roots from which starch was to be extracted at each harvest were analysed for total soluble solutes (TSS) using a refractometer (PR32). Cassava tuberous roots were harvested, washed, peeled, and washed again. Five roots were randomly taken from each variety in each replicate. A disc of about 5mm thick was cut out of the middle of each tuberous root, broken into small cubes, wrapped in muslin cloth and crushed with a hammer on a clean wooden board. The Brix% was determined from one or two drops of the crushed cassava using a refractometer. The mean of the five tuberous roots was used as TSS value.

5.2.8 Data analysis for correlations, analysis of variance (ANOVA) and additive main effects and multiplicative interaction (AMMI)

Parameters were statistically compared by applying linear correlations using Agrobase (2000). Analysis of variance (ANOVA) was performed on starch yield, starch extraction rate on fresh root weight basis, starch content, root dry matter content, starch extraction rate on dry root weight basis and TSS of each of the individual trials, using Agrobase (2000). Thereafter, combined ANOVA was performed on the pooled data of all trials for Chitedze, Chitala, Mkondezi and Makoka research stations across two years and three rounds of starch extraction. Genotype by environment interactions for the traits which were significant were managed using additive main effects and multiplicative interactions (AMMI). AMMI model was performed using Agrobase (2000).

5.3 Results and discussion

5.3.1 Environmental conditions, edaphic status and altitude of trial sites

5.3.1.1 *Weather and climate of trial sites*

Temperature and rainfall data of the trial sites are given in Figure 5.2. Chitedze and Chitala research stations had about five months of effective rain per season. Annual rainfall for Chitedze was 1040mm in 2002/03, 774mm in 2003/04, and 784mm in 2004/05 growing seasons, while annual rainfall for Chitala was 927mm in 2002/03, and 788mm in 2003/04 and 869mm in 2004/05 seasons (Figure 5.2). Makoka had about six months of rain, with annual rainfall of 954mm in 2002/03, 701mm in 2003/04 and 805mm in 2004/05 growing seasons. Mkondezi had much more wide spread rain with a rainfall of 1766mm over a period of nine months for the 2002/03 season, 1747mm over a seven month period of the 2003/04 season and 827mm over a five month period until March 2005 for the 2004/05 season (Figure 5.2).

As for most parts of Malawi, trial sites had monomodal type of rainfall, with effective rain starting between November and December. Chitedze, Chitala and Makoka research stations generally received more rainfall but within a short period (few months), while Mkondezi received a moderate amount of rain well spread within the growing season, except for the month of April in 2004, when a very high amount of rainfall was received. Chitedze and Chitala received high amounts of rainfall during the rainy season, but from April received no rain until the onset of the following rainy season. Makoka received high amounts of rainfall during the rainy season but continued to receive little rainfall or showers during the winter period (Figure 5.2).

Monthly mean maximum temperatures ranged between 24 and 31^{o}C for Chitedze, 26 and 36^{o}C for Chitala, 22 to 30^{o}C for Makoka, and 26 to 35^{o}C for Mkondezi. Monthly mean minimum temperatures ranged between 9 and 19^{o}C for Chitedze, 2 and 17^{o}C for Chitala, 11 to 17^{o}C for Makoka, and 13 to 22^{o}C for Mkondezi (Figure 5.2).

Highest temperatures were recorded at the onset of the rainy season (between November and December), and fell in winter (between June and July; Figure 5.2). The biggest range of temperatures was recorded at Chitala. Chitala and Mkondezi with high monthly temperature means of up to 36^{o}C are situated along the great rift valley floor, having low altitudes compared to Chitedze and Makoka (Figure 5.2 and Table 5.1).

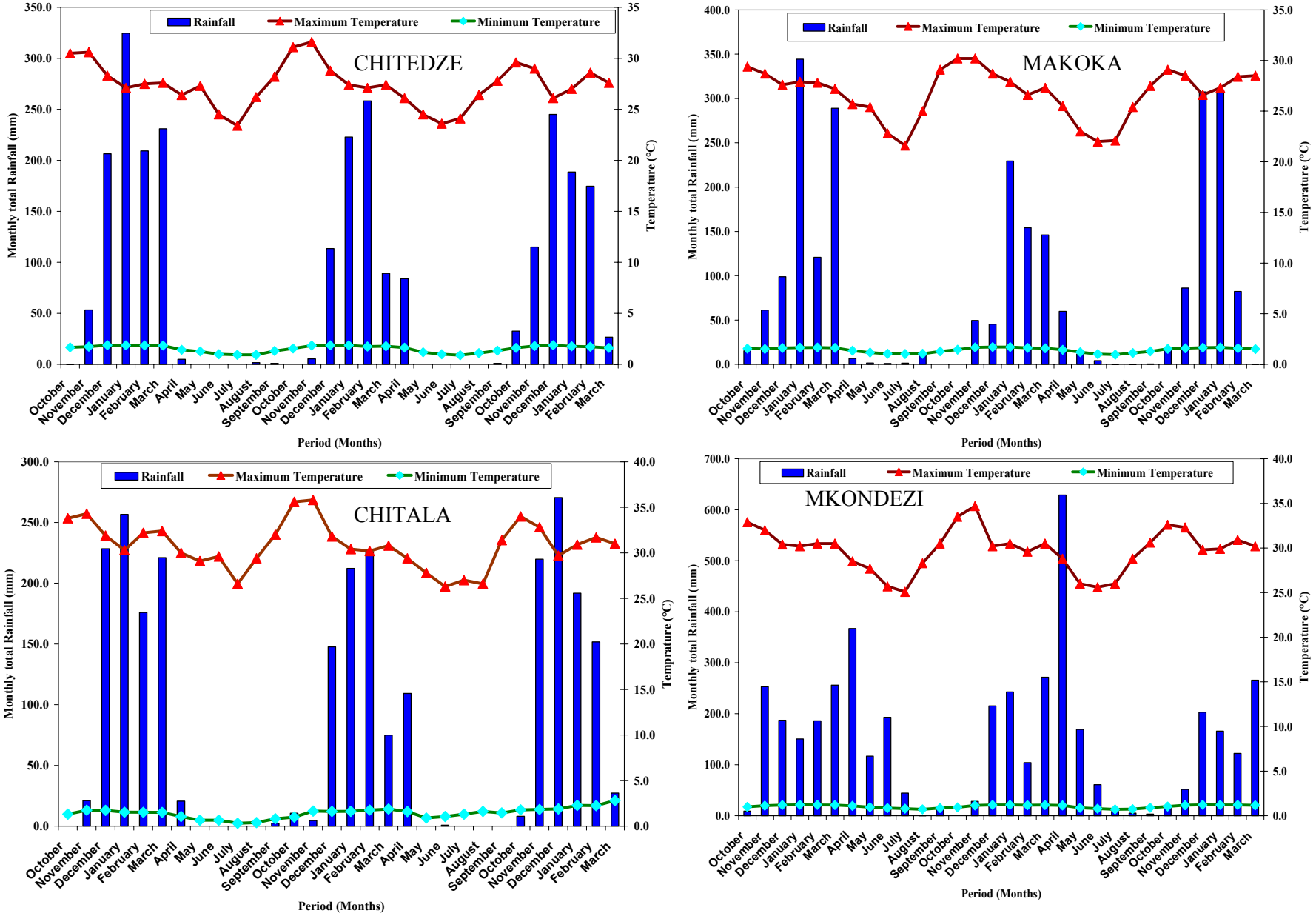


Figure 5.2 Temperature and rainfall data for the four test locations from October 2002 to March 2005

5.3.1.2 *Edaphic description of trial sites*

Soil analysis indicated that Chitala soils were sandy clay and slightly acidic. Soils from Makoka and Chitedze were sandy clay loam, and slightly acidic. Soils from Mkondezi were sandy loam and strongly acidic (Table 5.1). Classification of soils based on acidity (pH) was according to Eckert and Thomas Sims (1995) and Donnan Landscape Services (2005).

Clay soils, as found at Chitala, prevent leaching of nutrients but restrict the expansion of tuberous roots, and are difficult to work with in the field. Sandy clay loam soils, as found at Chitedze and Makoka, are ideal for tuberisation and restrict leaching of nutrients. Sandy loam soils, as found at Mkondezi, are good for expansion of tuberous roots but encourage leaching of nutrients (Table 5.1). Soil pH greatly affects the solubility of minerals or nutrients. Plants obtain 14 of the 17 essential nutrients from soil. Most minerals and nutrients are more soluble or available in acid soils than in neutral or slightly alkaline soils (SUNY-ESF, 2005). Soil pH influences plant growth by affecting the activity of beneficial micro-organisms. For example bacteria that decompose soil organic matter, are hindered by strong acid soils. This prevents the breakdown of organic matter resulting in an accumulation of organic matter and the subsequent tie up of nutrients, particularly nitrogen in the organic matter (SUNY-ESF, 2005; Table 5.1).

Table 5.1 Altitude and edaphic description of trial sites

Trial site	Alt. masl	Textural Class	pH	P (µg/g)	K (cmol/kg)
Chitedze Top (0-45cm)	1178	Sandy clay loam soil	5.8	15.57	0.31
Chitedze Sub (46-60cm)	1178	Sandy clay loam soil	5.2	24.81	0.40
Makoka Top (0-45cm)	1026	Sandy clay loam soil	5.7	31.93	0.41
Makoka Sub (46-60cm)	1026	Sandy clay loam soil	5.4	69.34	0.18
Mkondezi Top (0-45cm)	508	Sandy loam soil	5.0	19.97	0.14
Mkondezi Sub(46-60cm)	508	Sandy clay loam soil	4.8	26.27	0.08
Chitala Top (0-45cm)	606	Sandy clay soil	6.1	16.25	0.29
Chitala Sub (46-60cm)	606	Sandy clay soil	6.1	19.39	0.23

Alt. = altitude; masl = metres above sea level; P = Phosphorus; K = Potassium;.

Phosphorus (P) level was low at Chitedze for top soil and Chitala for sub soil (Table 5.1; Makumba, W., personal communication). Potassium (K) was high at Chitedze for both top and sub soils, and was comparable to the top soil of Makoka. Low levels of K were recorded at Mkondezi (Table 5.1).

IITA (1990) reported that phosphorus is important for development of the root system. Cassava has modest requirement for P but its response to P application in terms of tuberous root yield

under field conditions is low and varies greatly in different soils. It could be that P plays a role in root quality. Although cassava removes large quantities of K from soils, there has been no direct impact on tuberous root yield but low levels of K has an unfavourable effect on tuberous root quality (IITA, 1990).

5.3.2 Comparison of starch extraction parameters

Correlation of all parameters considered in this experiment revealed positive and highly significant correlation between starch yield and starch extraction rate on fresh root weight basis (r=0.999; Table 5.2); starch yield and root starch content on fresh root weight basis (r=0.606); starch yield and root dry matter content (r=0.598); starch content on fresh root weight basis and root dry matter content (r=0.791); starch content on fresh root weight basis and starch extraction rate on fresh root weight basis (r=0.608); and starch extraction rate on fresh root weight basis and root dry matter content (r=0.600).

Table 5.2 Correlation matrix for starch extraction parameters

	Starch Yield		Dry Matter		Starch Extra. DWB		Starch Extra. FWB		TSS	
Dry Matter	0.5977	***								
Starch Extra. DWB	0.4497	**	-0.3008	*						
Starch Extra. FWB	0.9991	***	0.6001	***	0.4488	**				
TSS	0.1890	NS	0.3236	*	-0.1191	NS	0.1915	NS		
Starch Content	0.6058	***	0.7913	***	-0.0828	NS	0.6081	***	0.3035	*

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; NS = not significant; Starch Extra. DWB = starch extraction on dry root weight basis; Starch Extra. FWB = starch extraction on fresh root weight basis; TSS = total soluble solutes.

There were no significant correlations between root dry matter content and starch extraction rate on dry root weight basis (r =-0.301); TSS and starch extraction rate on dry root weight basis (r =-0.119); and starch extraction on dry root weight basis and starch content on fresh root weight basis (r =-0.083; Table 5.2). Weak but significant positive correlations were observed between starch yield and starch extraction rate on dry root weight basis (r=0.450); and starch extraction rate on dry root weight basis and starch extraction rate on fresh root weight basis (r=0.459; Table 5.2). No significant relationships were observed between starch yield and TSS (r=0.189); TSS and starch extraction rate on fresh root weight basis (r=0.192); and TSS and starch content on fresh root weight basis (r=0.304; Table 5.2). A weak but positive correlation was observed between TSS and root dry matter content (r=0.32; Table 5.2).

A similar study conducted by Ngendahayo and Dixon (2001) at two contrasting sites in Nigeria indicated that starch content on fresh root weight basis was closely related to root dry matter

content during the active plant growth period. These findings were in agreement with results of this study. This study included starch extraction rate on fresh and dry root weight basis, which indicated that starch extraction rate on fresh root weight basis is more closely related to starch yield per unit weight basis than starch content on fresh root weight basis and dry matter content, while starch extraction rate on dry root weight basis had no relationship with starch yield.

Results and discussions for starch extraction parameters from ANOVA and AMMI analyses in different trial sites and rounds of starch extraction are split into three parts. The first part is where results of starch yield, starch extraction on fresh root weight basis, starch content on fresh root weight basis and root dry mater content are discussed simultaneously in section 5.3.3. Starch extraction rate on dry root weight basis is discussed in section 5.3.4, while TSS is discussed in section 5.3.5.

5.3.3 Starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content

5.3.3.1 *Analysis of variance (ANOVA)*

ANOVA results for different trial sites for starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis, and root dry matter content indicated highly significant ($p<0.001$; Tables 5.3 and 5.4) differences among genotypes and trial sites. Cassava genotypes that gave the highest starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content at Chitedze were Silira, Mbundumali and Mkondezi. Maunjili, Sauti and CH92/082 did well at Chitedze for starch content on fresh root weight basis and root dry matter content. The lowest starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content were obtained from 83350 (Tables 5.3 and 5.4). Cassava genotypes that gave the highest starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content at Makoka were Silira, Mkondezi, Mbundumali, CH92/082 and Sauti, while 83350 was the poorest performer (Tables 5.3 and 5.4). Cassava genotypes that gave the highest starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content at Chitala were Mkondezi, Mbundumali, CH92/082 and Silira, while 83350 was the worst performer (Tables 5.3 and 5.4). Cassava genotypes that gave the highest starch yield, starch extraction rate on fresh root weight

Table 5.3 Starch yield, starch extraction rate on fresh root weight basis and starch content on fresh root weight basis for Malawian cassava genotypes for different trial sites

Genotypes	Starch weight (g)/ 500g FW					Starch extraction rate (%) on FWB					Starch content (%) on FWB				
	Chitedze	Makoka	Chitala	Mkondezi	Mean	Chitedze	Makoka	Chitala	Mkondezi	Mean	Chitedze	Makoka	Chitala	Mkondezi	Mean
Mbundumali	85.63	101.63	100.71	102.78	**97.61**	17.11	20.36	20.12	20.55	**19.53**	33.65	35.61	34.26	30.90	**33.63**
83350	71.63	92.00	73.50	78.28	**78.20**	14.32	18.36	14.68	15.66	**15.63**	23.48	29.41	19.16	22.17	**23.43**
LCN8010	75.46	96.25	86.25	85.39	**85.87**	15.08	19.34	17.25	17.03	**17.18**	26.54	31.02	26.78	22.19	**26.64**
Mkondezi	85.21	110.04	104.17	103.39	**100.48**	17.03	22.00	20.84	20.69	**20.09**	27.16	32.87	32.55	28.69	**30.08**
Silira	78.92	112.88	98.46	100.17	**99.64**	17.61	22.57	19.67	20.03	**19.93**	27.81	33.16	26.24	28.09	**28.80**
Sauti	76.63	102.92	85.54	65.06	**82.79**	15.33	20.58	17.10	12.99	**16.55**	24.52	32.10	22.17	14.84	**23.38**
CH92/082	79.79	100.71	94.21	96.11	**92.65**	15.95	20.14	18.85	19.19	**18.52**	28.58	35.82	32.76	29.13	**31.67**
Maunjili	82.29	94.00	85.92	85.61	**86.90**	16.44	18.80	17.17	17.11	**17.37**	28.11	28.92	24.84	26.13	**26.99**
Site Means	**79.45**	**101.30**	**91.10**	**89.60**	**90.52**	**16.11**	**20.27**	**18.21**	**17.91**	**18.10**	**27.48**	**32.36**	**27.35**	**25.27**	**28.08**
CV (%)	10.79	7.58	11.77	12.08	**10.65**	10.81	7.53	11.76	12.03	**10.6**	10.07	6.55	8.82	12.11	**8.92**
SE (±) for G	2.520	2.238	3.133	5.149	**1.391**	0.505	0.445	0.625	0.725	**0.277**	1.12	0.872	0.993	1.448	**0.513**
LSD for G	4.17	3.70	5.19	8.55	**2.29**	0.84	0.75	1.04	1.20	**0.46**	1.86	1.45	1.65	2.43	**0.85**
Sign for G	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
SE (±) for L					**0.984**					**0.139**					**0.342**
LSD for L					**1.62**					**0.23**					**0.56**
Sign for L					***					***					***

*** p≤0.001; CV = coefficient of variation; SE = standard error; LSD = least significant difference; Sign = significance; G = genotypes; L = locations; R = rounds of starch extraction; FW = fresh root weight; FWB = fresh root weight basis

Table 5.4 Root dry matter content, starch extraction on dry root weight basis and total soluble solutes (TSS) for fresh cassava roots for Malawian cassava genotypes for different trial sites

Genotypes	Root dry matter content (%)					Starch extraction rate (%) on DWB					Total Soluble Solutes (Brix%)				
	Chitedze	**Makoka**	**Chitala**	**Mkondezi**	**Mean**	**Chitedze**	**Makoka**	**Chitala**	**Mkondezi**	**Mean**	**Chitedze**	**Makoka**	**Chitala**	**Mkondezi**	**Mean**
Mbundumali	44.50	43.38	43.17	42.11	**43.27**	38.82	46.56	46.64	49.21	**45.30**	9.1	8.6	9.9	9.3	9.2
83350	33.83	36.29	28.08	31.17	**32.29**	42.88	50.99	52.51	50.00	**48.78**	7.8	7.4	8.1	8.2	**7.9**
LCN8010	35.67	38.25	33.71	32.11	**34.90**	42.71	50.47	50.95	53.61	**49.51**	7.9	8.5	8.6	7.8	**8.2**
Mkondezi	38.00	41.50	39.46	37.94	**39.08**	45.47	53.40	53.20	54.75	**51.81**	7.5	7.9	8.8	7.8	**8.0**
Silira	39.33	41.75	37.04	37.11	**38.82**	45.40	54.44	53.11	53.83	**51.58**	8.4	8.5	9.0	8.4	**8.6**
Sauti	37.79	40.50	35.50	26.89	**35.10**	40.95	50.88	48.30	48.79	**47.72**	7.8	7.6	8.8	7.6	**7.9**
CH92/082	40.04	42.17	39.92	38.28	**40.06**	40.41	48.13	47.37	50.53	**46.67**	8.0	7.8	8.8	8.0	**8.1**
Maunjili	39.71	38.13	36.46	32.61	**36.75**	41.82	49.82	47.50	52.68	**47.86**	8.1	7.5	8.6	7.9	**8.0**
Site means	**38.61**	**40.25**	**36.67**	**34.78**	**37.53**	**42.31**	**50.59**	**49.95**	**51.68**	**48.65**	**8.1**	**8.0**	**8.8**	**8.1**	**8.2**
CV (%)	6.16	4.49	7.93	9.19	**6.99**	10.88	8.02	12.09	9.88	**10.92**	8.51	9.19	11.80	9.95	**9.86**
SE (±) for G	0.686	0.524	0.849	1.067	**0.379**	1.332	1.178	1.745	1.714	**0.767**	0.281	0.301	0.426	0.377	**0.165**
LSD for G	1.14	0.87	1.41	1.77	**0.62**	2.20	1.95	2.89	2.85	**1.26**	0.87	0.50	0.71	0.63	**0.27**
Sign for G	***	***	***	***	***	***	***	***	***	***	***	***	*	***	***
SE (±) for L					**0.268**					**0.542**					**0.110**
LSD for L					**0.44**					**0.89**					**0.18**
Sign for L					***					***					***

*** p≤0.001; CV = coefficient of variation; SE = standard error; LSD = least significant difference; Sign = significance; G = genotypes; L = locations; R = rounds of starch extraction; Round = rounds of starch extraction; DWB = dry root weight basis

basis, starch content on fresh root weight basis and root dry matter content at Mkondezi were Mbundumali, Mkondezi, Silira and CH92/082, while Sauti and 83350 were the poorest performers (Tables 5.3 and 5.4). Silira, Mbundumali, CH92/082 and Mkondezi gave the highest starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content across four trial sites. Genotype 83350 gave the lowest starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content across all trial sites (Tables 5.3 and 5.4).

Similar studies done by Sriroth *et al*. (2000), Ngendahayo and Dixon (2001) and Sanni and Olubamiwa (2003) found differences in starch content and dry matter content among genotypes. For example, Ngendahayo and Dixon (2001) found large variation of starch content among evaluated clones. Their results agreed with findings of this study where significant differences in starch content on fresh root weight basis and root dry matter among genotypes were observed. Results indicated that there existed variation in starch extraction among genotypes that need to be screened to isolate genotypes which are amenable to starch extraction.

ANOVA results from different trial sites for starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content indicated highly significant ($p<0.001$; Tables 5.3 and 5.4) differences among genotypes. Makoka was the best site for starch yield, starch extraction rate on fresh root weight basis, root dry matter content, and starch content on fresh root weight basis, followed by Chitala. Chitedze performed similar to Chitala in terms of starch content on fresh root weight basis and root dry matter content but was the worst site in terms of starch yield and starch extraction rate on fresh root weight basis. Mkondezi was the poorest site for starch content on fresh root weight basis and root dry matter content (Tables 5.3 and 5.4).

Ngendahayo and Dixon (2001) observed that dry matter content trends were closely related to rainfall during six to 18 months of plant growth. Hence, differences in starch extraction among trial sites in the current study might be due to differences in rainfall distribution. IITA (1990) pointed out that not only the amount of rainfall but the ability of the soil to retain water play a crucial role in cassava production. Makoka had the highest starch extraction rates. This site received enough rainfall during the rainy season and continued to receive rain or showers deep into the dry season. This site had sandy clay loam soils which have the ability to retain soil moisture. Chitedze received more rainfall compared to Makoka during the 2002/03 and 2003/04 seasons, and had similar soil texture as for Makoka. The difference was on rainfall distribution whereby Chitedze received rain within five months followed by a dry season until the onset of

the following rainy season, which might have led to the low extraction rate at Chitedze (Figure 5.2, Tables 5.1 and 5.3). Mkondezi received enough and well distributed rainfall during the growing season but the poor soil water holding capacity of soils at that site might have adversely affected starch extraction. Hence, both amount and distribution of rainfall and soil structure affected starch extraction.

Some relationship was observed between P content in soil and starch extraction. Makoka with the highest P content both in top and sub soil gave the highest starch extraction rate compared to the rest of the sites which had significantly low P content for both in top and sub soils. Although K is associated with tuberous root quality, no relationship was observed between K and starch extraction rate on fresh root basis. Chitedze had high levels of K in both top and sub soils but had the lowest starch extraction rate while the top soil of Makoka had similar levels of K and recorded the highest starch extraction rate (Tables 5.1 and 5.3). High temperatures are known to hasten the growth cycle and tuberisation of cassava (Onwueme, 1978; IITA, 1990; Nassar, 2005) but seemed to have no direct impact on starch extraction rate since Chitedze and Makoka had almost similar temperatures but gave contrasting starch extraction rates.

ANOVA results for different rounds of starch extractions (Tables 5.5 and 5.6) for starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content indicated highly significant ($p<0.001$) differences among genotypes and rounds of starch extraction. Mkondezi, Silira and Mbundumali produced the highest starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content in all three rounds of starch extraction (Tables 5.5 and 5.6). The lowest (significantly) starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content were recorded for 83350 (Tables 5.5 and 5.6) in all three rounds of starch extraction.

ANOVA results from different rounds of starch extraction for starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content indicated highly significant ($p<0.001$ in Tables 5.5 and 5.6) differences among genotypes. Round 1 of starch extraction produced the highest starch yield, starch extraction rate on fresh root weight basis, starch content on fresh root weight basis and root dry matter content followed by round 3 of starch extraction, while round 2 of starch extraction trailed in all the sites (Tables 5.5 and 5.6).

Table 5.5 Starch yield, starch extraction on fresh root weight basis and starch content on fresh root weight basis for Malawian cassava genotypes for different rounds of starch extractions

Genotypes	Starch weight (g)/ 500g FW				Starch extraction rate (%) on FWB				Starch content (%) on FWB			
	Round1	Round2	Round3	Mean	Round1	Round2	Round3	Mean	Round1	Round2	Round3	Mean
Mbundumali	98.54	91.18	102.32	**97.61**	19.71	18.26	20.61	**19.53**	35.89	30.58	34.41	**33.63**
83350	82.68	67.95	85.58	**78.20**	16.50	13.59	16.81	**15.63**	25.42	20.63	24.24	**23.43**
LCN8010	93.84	77.07	87.58	**85.87**	18.83	15.41	17.32	**17.18**	30.30	23.17	26.44	**26.64**
Mkondezi	106.68	94.91	99.40	**100.48**	21.33	18.97	19.98	**20.09**	31.77	27.56	30.90	**30.08**
Maunjili	91.14	78.84	92.14	**86.90**	18.22	15.77	18.13	**17.37**	29.58	24.91	26.47	**26.99**
Silira	101.01	91.52	105.12	**99.64**	20.20	18.32	21.26	**19.93**	28.53	27.67	30.21	**28.80**
Sauti	90.78	70.90	86.64	**82.79**	18.15	14.18	17.33	**16.55**	24.89	21.51	23.76	**23.38**
CH92/082	95.80	88.17	84.83	**92.65**	19.14	17.63	18.79	**18.52**	34.59	29.95	30.48	**31.67**
Round mean	**95.06**	**82.57**	**94.20**	**90.51**	**19.01**	**16.52**	**18.78**	**18.10**	**30.12**	**25.75**	**28.36**	**28.17**
CV (%)	10.51	10.24	10.89	**10.65**	10.44	10.24	10.22	**10.6**	8.69	11.05	9.37	**8.92**
SE (±) for G	2.498	2.113	2.564	**1.391**	0.50	0.423	0.480	**0.277**	0.712	1.006	0.939	**0.513**
LSD for G	4.13	3.50	4.24	**2.29**	0.82	0.70	0.79	**0.46**	1.19	1.67	1.56	**0.85**
Sign for G	***	***	***	***	***	***	***	***	***	***	***	***
SE (±) for R				**0.852**				**0.170**				**0.296**
LSD for R				**1.40**				**0.28**				**0.49**
Sign for R				***				***				***

*** p≤0.001; CV = coefficient of variation; SE = standard error; LSD = least significance difference; Sign = significance; G = genotypes; L = locations; R = rounds of starch extraction; Round = rounds of starch extraction; FW = fresh root weight; FWB = fresh root weight basis

Table 5.6 Root dry matter content, starch extraction on dry root weight basis and total soluble solutes (TSS) of fresh cassava roots for Malawian cassava genotypes for different rounds of starch extractions

Genotypes	Root dry matter content (%)				Starch extraction rate (%) on DWB				TSS (Brix%)			
	Round1	Round2	Round3	Mean	Round1	Round2	Round3	Mean	Round1	Round2	Round3	Mean
Mbundumali	45.38	40.64	43.78	**43.27**	42.88	45.57	47.45	**45.30**	10.4	8.5	8.8	**9.2**
83350	34.16	29.05	33.66	**32.29**	47.69	48.07	50.58	**48.78**	9.3	7.1	7.2	**7.9**
LCN8010	39.59	31.48	33.63	**34.90**	47.60	48.78	52.15	**49.51**	9.3	7.4	7.7	**8.2**
Mkondezi	41.81	35.46	39.97	**39.08**	50.11	55.04	50.28	**51.81**	8.8	7.7	7.5	**8.0**
Maunjili	40.03	33.13	37.09	**36.75**	45.64	48.36	49.56	**47.86**	9.1	7.3	7.6	**8.0**
Silira	40.34	35.11	41.00	**38.82**	48.67	53.89	52.19	**51.58**	9.4	8.0	8.3	**8.6**
Sauti	37.91	31.51	35.88	**35.10**	46.34	47.14	49.67	**47.72**	8.7	7.0	8.0	**7.9**
CH92/082	43.34	37.97	38.88	**40.06**	43.51	47.54	48.96	**46.67**	8.9	7.7	7.8	**8.1**
Round mean	**40.52**	**34.29**	**37.98**	**37.53**	**46.56**	**49.30**	**50.10**	**48.65**	**9.2**	**7.6**	**7.8**	**8.2**
CV (%)	5.68	7.18	7.96	**6.99**	10.42	10.42	10.63	**10.92**	1061	8.52	8.21	**9.86**
SE (±) for G	0.573	0.615	0.756	**0.379**	1.21	1.28	1.33	**0.767**	0.347	0.228	0.227	**0.165**
LSD for G	0.95	1.02	1.25	**0.62**	2.01	2.12	2.20	**1.26**	0.58	0.38	0.38	**0.27**
Sign for G	***	***	***	***	***	***	**	***	***	***	***	***
SE (±) for R				**0.232**				**0.470**				**0.100**
LSD for R				**0.38**				**0.77**				**0.16**
Sign for R				***				***				***

*** p≤0.001; ** p≤0.01; CV = coefficient of variation; SE = standard error; LSD = least significance difference; Sign = significance; G = genotypes; L = locations; R = rounds of starch extraction; Round = rounds of starch extraction; DWB = dry root weight basis

Most of the cultivars evaluated by Ngendahayo and Dixon (2001) reached maximum or near maximum starch content at six months after planting. Thereafter, starch content was dependent on harvesting time, genotype and rainfall pattern. Similar studies by Sriroth *et al*. (2000), and Sanni and Olubamiwa (2003) reported the effect of season on starch content and dry matter content. Hence, the results of this study agreed with the earlier studies. For example, Sanni and Olubamiwa (2003) observed dry matter of 37-45% in the dry season and 28-31% for the rainy season.

5.3.3.2 *Contributions of sources of variation for starch yield*

Combined ANOVA results for starch yield over locations and rounds of starch extraction indicated highly significant differences ($p<0.001$; Table 5.7) for main effects (genotypes, locations and rounds of starch extraction) and their interactions (Table 5.7). Locations contributed 12.12%, and rounds of starch extractions 8.52%, while genotypes contributed 28.74% of variation of the total sum of squares. The interaction of genotype by location contributed 10.39%, genotype by round of starch extraction 2.83%, and genotype by location by round of starch extraction 7.67% (Table 5.7). These results indicated that starch yield was mainly influenced by the genetic constitution of the plant. However, sites, rounds of starch extraction and their interaction had appreciable influence on starch yield. This is in line with the observation of Ngendahayo and Dixon (2001) that after six months, plants starch content is influenced by genotype, harvesting time and rainfall pattern.

Table 5.7 ANOVA for starch yield, starch content and starch extraction on fresh root weight basis for Malawian cassava genotypes

Sources of Variation	df	Starch weight (g)/ 500g FW		Starch extraction (%) on FWB		Starch content (%) on FWB	
		Sum of Squares (SS)	Contribution to total SS (%)	Sum of Squares (SS)	Contribution to total SS (%)	Sum of Squares (SS)	Contribution to total SS (%)
Total	431	115291.14		4588.65		14574.37	
Location (L)	3	13969.75 ***	12.12	562.29 ***	12.25	3004.22 ***	20.61
Round (R)	2	9823.97 ***	8.52	391.17 ***	8.52	1496.10 ***	10.27
L x R	6	2753.19 ***	2.39	111.00 ***	2.42	349.09 ***	2.40
Rep (L x R)	36	8186.76 ***	7.10	321.02 ***	7.00	534.63 ***	3.67
Genotype (G)	8	33136.21 ***	28.74	1325.61 ***	28.89	4593.09 ***	31.51
G x L	24	11979.25 ***	10.39	471.94 ***	10.28	1692.35 ***	11.61
G x R	16	3145.19 ***	2.73	129.80 **	2.83	367.35 ***	2.52
G x L x R	48	8839.26 ***	7.67	347.44 ***	7.57	720.72 ***	4.95
Residual	288	23457.56		928.39		1816.82	

*** $p\leq0.001$; ** $p\leq0.01$; G = genotypes; L = locations; R = rounds of starch extraction; FW = fresh root weight; FWB = fresh root weight basis; df = degrees of freedom

5.3.3.3 *AMMI for starch yield*

Since interactions of genotypes by locations, genotype by rounds of starch extractions, and genotypes by locations by rounds of starch extraction were highly significant ($p<0.001$; Table 5.7), further analysis was needed as recommended by Crossa (1990). Stability analysis using AMMI with the help of Agrobase (2000) was performed.

AMMI analysis of cassava starch yield for interactions of genotype by location and round of starch extraction revealed that interactive principle component analysis (IPCA) axis 1 explained 81.03% of the GxE interaction. IPCA scores of a genotype by environment interaction in AMMI analysis are an indication of the stability of a genotype over environments (Zobel *et al*., 1988; Crossa, 1990; Crossa *et al*., 1990; Gauch and Zobel, 1996; Purchase, 1997).

High IPCA scores (either negative or positive as it is a relative value) indicate specific adaptation of genotype to certain environments. IPCA scores that approximate to zero (0) indicates that genotypes are more stable over all environments sampled. Based on IPCA scores in this study, the most stable genotypes were 83350, LCN8010, Maunjili and CH92/082. The most unstable genotype was Sauti (Figure 5.3) but was specifically adapted to Makoka. Mkondezi, Silira and

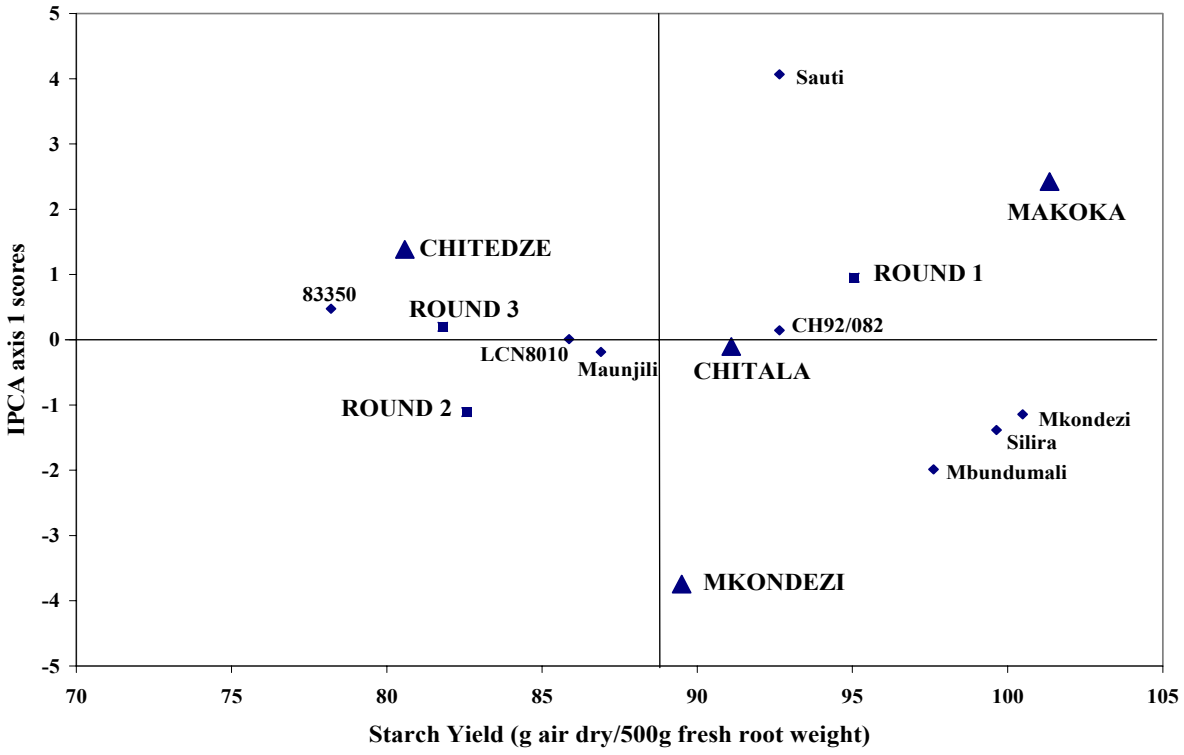


Figure 5.3 Biplot for AMMI IPCA axis 1 scores against means for starch yield for genotype by location and round of starch extraction for cassava genotypes evaluated in Malawi

Mbundumali were stable to some extent, but were most suitable to be grown at Makoka, Chitala and Mkondezi. Results suggested that Sauti, Mkondezi, Silira and Mbundumali would respond to good management. Makoka and Mkondezi were the most unstable sites. These sites have the ability to give high starch yields with proper choice of cassava varieties and by targeting starch extraction at appropriate time (Figure 5.3). The most stable site was Chitala. This site is good for smallholder farmers who cannot shoulder risks.

5.3.3.4 *Contributions of sources of variation for starch extraction rate on fresh root weight basis*

Combined ANOVA results for starch extraction rates on fresh root weight basis over locations and rounds of starch extraction indicated highly significant differences ($p<0.001$; Table 5.7) in the main effects and their interactions (Table 5.7). Locations contributed 12.25%, rounds of starch extraction 8.52%, and genotypes 28.89% of the variation of the total sum of squares. The interaction of genotype by location contributed 10.28%, genotype by round of starch extraction 2.83%, and genotype by location by round of starch extraction 7.57% of the variation of the total sum of squares (Table 5.7). The trend for contributions of sources of variation for starch extraction rate on fresh root weight basis was similar to that of starch yield.

5.3.3.5 *AMMI for starch extraction rate on fresh root weight basis*

AMMI results are given in Figure 5.4. AMMI analysis of cassava starch extraction rate on fresh root weight basis for GxE interaction revealed that IPCA axis 1 explained 86.35% of the GxE interaction. IPCA scores revealed that the most stable genotypes were 83350, LCN8010, Maunjili and CH92/082. The most unstable genotype was Sauti (Figure 5.4) and was most suited at Makoka. Mkondezi, Silira and Mbundumali were stable to some extent, and their most suitable locations to be grown for starch extraction were Makoka, Chitala and Mkondezi. Makoka and Mkondezi were the most unstable sites for growing cassava intended for starch extraction (Figure 5.4). Of the two sites, Makoka had better opportunities of realising better returns (Figure 5.4). Chitala was the most stable site.

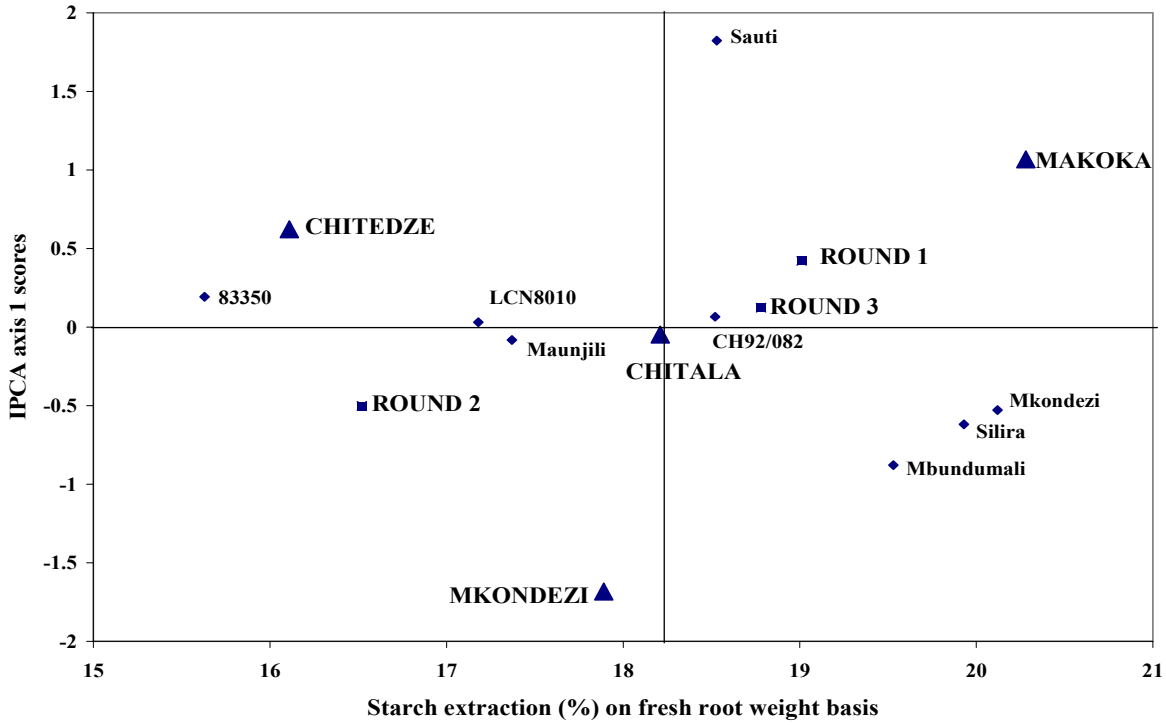


Figure 5.4 Biplot for AMMI IPCA Axis 1 scores against means for starch extraction rate on fresh root weight basis for genotype by location and round of starch extraction for cassava genotypes evaluated in Malawi

5.3.3.6 *Contributions of sources of variation for starch content on fresh root weight basis*

Combined ANOVA results for starch content on fresh root weight basis over locations and round of starch extraction indicated highly significant differences ($p<0.001$; Table 5.7) in the main effects and their interactions. Locations contributed 20.61%, rounds of starch extraction 10.27%, and genotypes 31.51% of the variation of the total sum of squares. The interaction of genotype by location contributed 11.61%, genotype by rounds of starch extraction 2.52%, and genotype by location by round contributed 4.95% of the variation of the total sum of squares (Table 5.7). Genetic make up of the plant and location had more influence on starch content on fresh weight basis than rounds of starch extraction. The interaction of genotype by round made negligible contribution to the total variation of the sum of squares.

5.3.3.7 *AMMI for starch content on fresh root weight basis*

AMMI results are given in Figure 5.5. AMMI analysis of cassava starch content on fresh root weight basis for interactions of genotype by location and round of starch extraction indicated that IPCA axis 1 explained 80.87% of the GxE interaction. IPCA scores showed that the most stable genotypes were 83350, LCN8010, Maunjili, Mbundumali and Silira. The most unstable genotype

was Sauti (Figure 5.5) whose most suitable location was Makoka. Makoka and Mkondezi were the most unstable sites for starch content on fresh root weight basis (Figure 5.5). Of the two sites, Makoka had better opportunities of realising better returns (Figure 5.5).

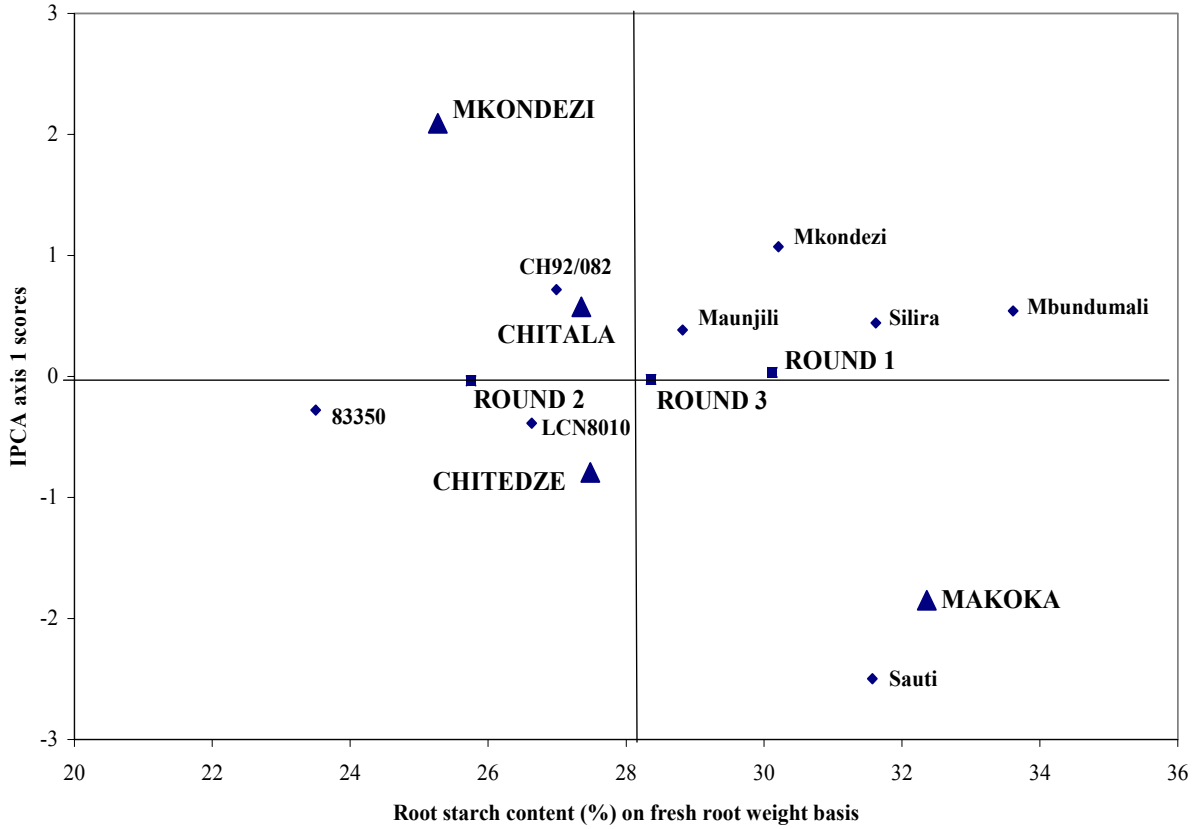


Figure 5.5 Biplot for AMMI IPCA axis 1 scores against means for starch content on fresh root weight basis for genotype by location and round for cassava genotypes evaluated in Malawi

5.3.3.8 *Contributions of sources of variation for root dry matter content*

Combined ANOVA results for root dry matter content over locations and round of extraction indicated highly significant differences ($p<0.001$; Table 5.8) in the main effects and their interactions. Locations contributed 23.09%, round of starch extraction 6.90%, and genotypes 31.83% of the variation of the total sum of squares. The interaction of genotype by location contributed 9.92%, genotype by round of starch extraction 2.84%, and genotype by location by round of starch extraction contributed 4.52% of the variation of the total sum of squares (Table 5.8). The trend of contributions of sources of variation to total variation of sum of squares for root dry matter was similar to that of starch content on fresh root weight basis.

Table 5.8 ANOVA for starch extraction on dry root weight basis, root dry matter and total soluble solutes (TSS) for fresh cassava roots for Malawian cassava genotypes

Sources of Variation	df	Root dry matter content (%)			Starch extraction (%) on DWB			TSS (Brix%)		
		Sum of Squares (SS)		Contribution to total SS (%)	Sum of Squares (SS)		Contribution to total SS (%)	Sum of Squares (SS)		Contribution to total SS (%)
Total	431	13057.92			25732.35			747.67		
Location (L)	3	3015.70	***	23.09	7029.65	***	27.32	52.80	***	7.06
Round (R)	2	901.03	***	6.90	3302.78	***	12.84	243.35	***	32.55
L x R	6	120.51	***	0.92	1306.13	***	5.08	54.36	***	7.27
Rep (L x R)	36	249.95	NS	1.91	2569.95	***	9.99	47.61	***	6.37
Genotype (G)	8	4940.18	***	37.83	1811.44	***	7.04	74.46	***	9.96
G x L	24	1295.11	***	9.92	639.56	NS	2.49	24.75	*	3.31
G x R	16	370.31	***	2.84	669.92	*	2.60	22.36	**	2.99
G x L x R	48	590.20	***	4.52	2348.90	***	9.13	40.10	NS	5.36
Residual	288	1574.92			6054.01			187.89		

*** p≤0.001; ** p≤0.01; * p≤0.05; NS = not significant; G = genotypes; L = locations; R = rounds of starch extraction; DWB = dry root weight basis; df = degrees of freedom

5.3.3.9 *AMMI for root dry matter content*

AMMI results are given in Figure 5.6. AMMI analysis of root dry matter content for interactions of genotype by location and round of starch extraction revealed that IPCA axis 1 explained 75.98% of the GxE interaction. IPCA scores revealed that the stable genotypes were 83350,

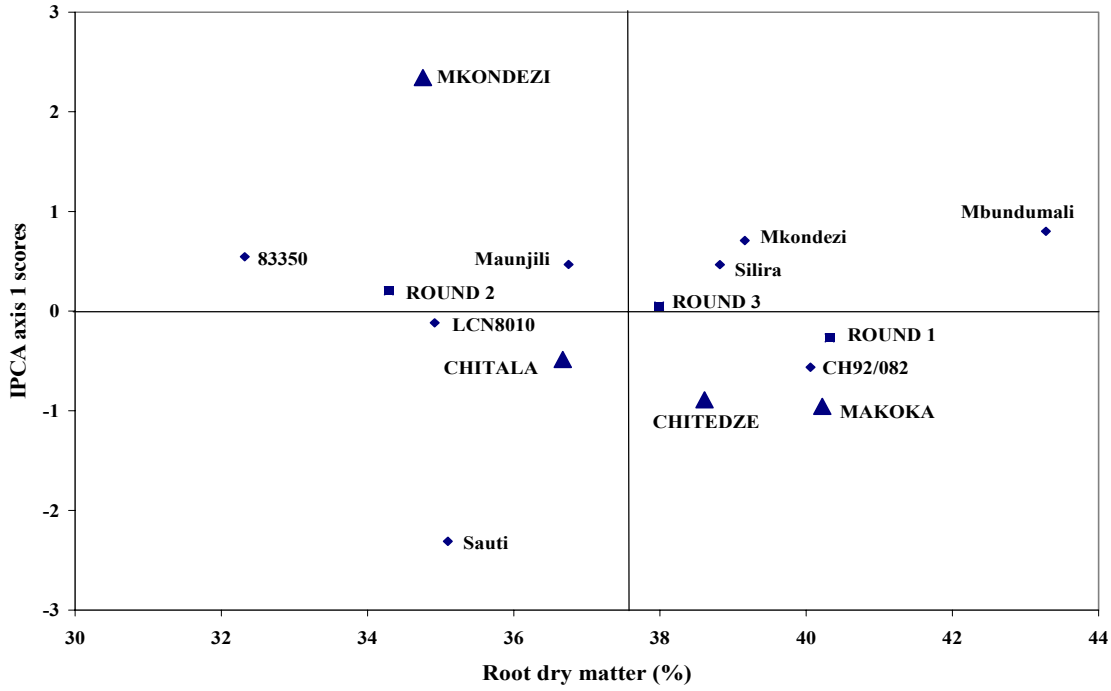


Figure 5.6 Biplot for AMMI IPCA axis 1 scores against means for root dry matter for genotype by location and round of starch extraction for cassava genotypes evaluated in Malawi

LCN8010, Maunjili, and Silira, while the most unstable genotype was Sauti (Figure 5.6). Mkondezi was the most unstable trial site since root dry matter content significantly varied with genotype and season of determination.

5.3.4 Starch extraction rate on dry root weight basis

5.3.4.1 *ANOVA for starch extraction rate on dry root weight basis*

ANOVA results for different trial sites for starch extraction on dry root weight basis indicated highly significant ($p<0.001$; Table 5.4) differences among genotypes at each of the four trial locations. Highly significant ($p<0.001$; Table 5.6) differences among rounds of starch extraction at Chitedze, Makoka and Mkondezi were detected. Cassava genotypes that gave the highest starch extraction rate on dry root weight basis at Chitedze were Silira and Mkondezi, while Mbundumali (the best performer in terms of starch yield, starch content on fresh root weight basis and starch extraction rate on fresh root weight basis) gave the lowest starch extraction rate on dry root weight basis (Table 5.4). Cassava genotypes that gave the highest starch extraction rate on dry root weight basis at Makoka were Silira and Mkondezi, while Mbundumali gave the lowest starch extraction rate on dry root basis, in contrast to results of starch yield and starch extraction rate on fresh root weight basis (Tables 5.3 and 5.4). Cassava genotypes that gave the highest starch extraction rate on dry root weight basis at Chitala were Silira, Mkondezi, 83350 and LCN8010. Mbundumali, CH92/082 (the best performer in terms of starch yield, starch extraction rate on fresh root weight basis and starch content on fresh root weight basis) and Maunjili were the poorest performers in terms of starch extraction rate on dry root weight basis (Tables 5.3 and 5.4). Cassava genotypes that gave the highest starch extraction rate on dry root weight basis at Mkondezi were Mkondezi, Silira and LCN8010. Sauti (which gave the lowest starch yield, starch extraction on fresh root weight basis and starch content on fresh root weight basis,) and Mbundumali (the best performer in terms of starch yield, starch extraction rate on fresh root weight basis and starch content on fresh root weight basis) gave the lowest starch extraction rate on dry root weight basis (Tables 5.3 and 5.4). Round 3 of starch extraction gave the highest starch extraction rates on dry root weight basis followed by round 2 of starch extraction (the poorest performer for starch yield, starch extraction on fresh root weight basis and starch content on fresh root weight basis). Round 1 of starch extraction (the best performer for starch yield, starch extraction on fresh root weight basis and starch content on fresh root weight basis) had the lowest starch extraction rate on dry root weight basis (Tables 5.5 and 5.6). Mkondezi research station with the lowest starch content on fresh root weight basis, gave the

highest starch extraction rate on dry root weight basis (Tables 5.3 and 5.4). Hence, no relationship existed between starch extraction on dry root weight basis and starch yield.

5.3.4.2 *Contributions of sources of variation for starch extraction on dry root weight basis*

Combined ANOVA results for starch extraction on dry root weight basis over locations and round of starch extraction indicated highly significant differences ($p<0.001$; Table 5.8) in the main effects. Locations contributed 27.32%, rounds of starch extraction 12.84%, and genotypes 7.04% of variation of the total sum of squares. The interaction of genotype by location was not significant and contributed 2.46%. The interaction of genotype by round of starch extraction was significant ($p<0.05$; Table 5.8) and contributed 2.60%, and genotype by location by round of starch extraction was highly significant and contributed 9.13% of variation of the total sum of squares (Table 5.8). Location and round of starch extraction had the greatest influence on starch extraction on dry weight basis. This could be due to the fact that location and round of starch extraction and season had an influence on starch yield as well as root dry matter content which are the components in the calculation of starch extraction rate on dry root weight basis.

5.3.4.3 *AMMI for starch extraction rate on dry root weight basis*

AMMI results are given in Figure 5.7. AMMI analysis of starch extraction rate on dry root

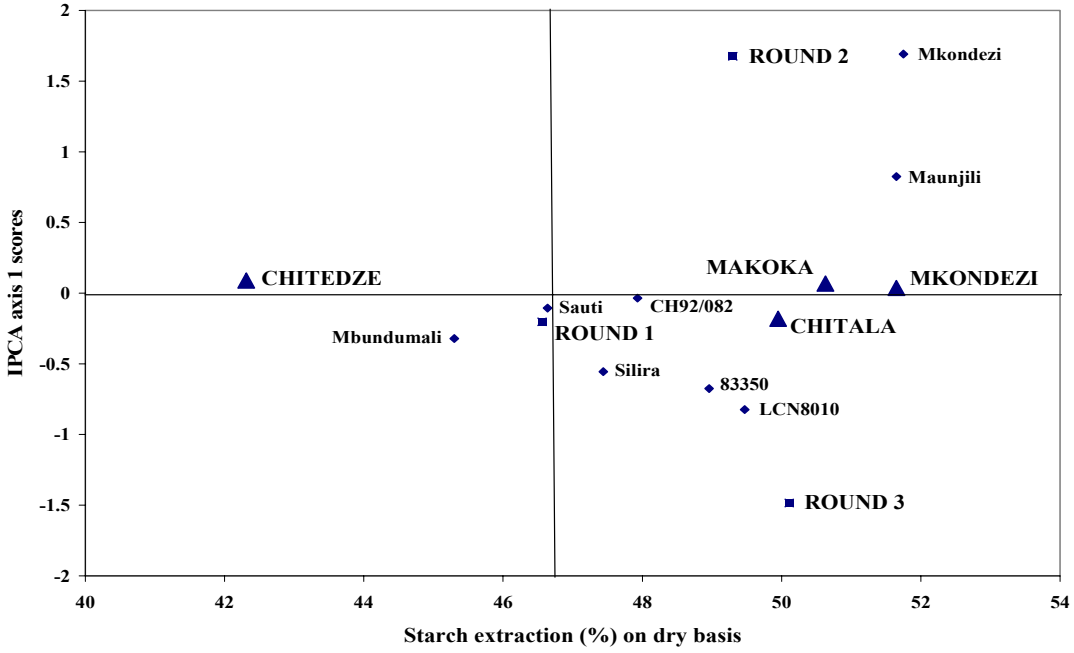


Figure 5.7 Biplot for AMMI IPCA axis 1 scores against means for starch extraction on dry root weight basis for genotype by location and round for cassava genotypes evaluated in Malawi

weight basis for interactions of genotype by location and round of starch extraction indicated that IPCA axis 1 explained 66.55% of the GxE interaction. AMMI analysis revealed that the variety Mkondezi was unstable and gave the highest starch extraction rate on dry root weight basis (Figure 5.7). Round 2 and 3 of starch extractions were unstable for starch extraction rate on dry root weight basis (Figure 5.7). These results indicated that different trial sites had different rankings of round 2 and 3 of starch extractions.

5.3.5 Total soluble solutes (TSS)

5.3.5.1 *Analysis of variance for TSS*

ANOVA results at different trial sites for TSS indicated highly significant ($p<0.001$; Tables 5.4 and 5.8) differences among genotypes and rounds of starch extraction at each of the four trial locations. Cassava cultivars that had the highest TSS values at Chitedze were Mbundumali and Silira, while Mkondezi had the lowest TSS values but was not significantly different from Sauti, 83350 and LCN8010 (Table 5.4). Cassava varieties that had the highest TSS values at Makoka were Mbundumali, LCN8010 and Silira, while 83350 had the lowest TSS values but was not significantly different from Maunjili, Sauti, CH92/082 and Mkondezi (Table 5.4). The cassava genotype that had the highest TSS value at Chitala was Mbundumali but was not significantly different from Silira, while 83350 had the lowest TSS values but was not significantly different from Maunjili, LCN8010, CH92/082, Sauti, and Mkondezi (Table 5.4). The cassava genotype that had the highest TSS value at Mkondezi was Mbundumali but was not significantly different from Silira, 83350 and CH92/082, while Sauti had the lowest TSS value but was not significantly different from Mkondezi, LCN8010 and Maunjili (Table 5.4). Rankings of genotypes varied greatly from one location to the other except for Mbundumali and Silira which had significantly higher TSS values compared to the rest of the varieties at all the four trial sites (Table 5.4).

Differences in TSS values among locations were highly significant ($p<0.001$; Table 5.4). Cassava varieties grown at Chitala had significantly higher TSS values compared to the rest of the trial sites, with Makoka having the lowest TSS values (Table 5.4).

ANOVA results for different rounds of starch extraction for TSS indicated highly significant ($p<0.001$; Table 5.6) differences among genotypes and locations in each of the three rounds of starch extraction. Mbundumali and Silira had the highest TSS values in all three rounds of starch extraction. Genotype 83350 had a high TSS value in round 1 of starch extraction but had the lowest or was among the lowest in round 2 and 3 of starch extractions. Mkondezi was among the genotypes with the highest TSS values in round 2 of starch extraction but was among those with

low TSS values in round 1 and 3 of starch extractions. Sauti was among the cultivars with high TSS values in round 3 of starch extraction but was among those with low TSS values in round 1 and 2 (Table 5.6).

There were highly significant ($p<0.001$; Table 5.6) differences in TSS among the rounds of starch extractions. Round 1 of starch extraction gave the highest TSS values, followed by round 3 of starch extraction (Table 5.6). Combined analysis across locations and rounds of starch extraction indicated that Mbundumali and Silira had the highest TSS values, while 83350 and Sauti had the lowest TSS values, though not significantly different from TSS values for Mkondezi, Maunjili, CH92/082 and LCN8010 (Tables 5.4 and 5.6).

5.3.5.2 *Contributions of sources of variation for TSS*

Combined ANOVA results for TSS over locations and rounds of starch extraction indicated highly significant differences ($p<0.001$; Table 5.8) in the main effects and their interactions. Locations contributed 7.06%, rounds of starch extraction 32.55%, and genotypes 9.96% of the variation of the total sum of squares. The interaction of genotype by location contributed 3.31%, genotype by round 2.99%, and genotype by location by round of starch extraction 5.36% of variation of the total sum of squares (Table 5.8). Rounds of starch extraction had the greatest influence on TSS compared to the rest of sources of variations to total sum of squares.

5.3.5.2 *AMMI for TSS*

AMMI results are given in Figure 5.8. AMMI analysis for TSS for interactions of genotype by

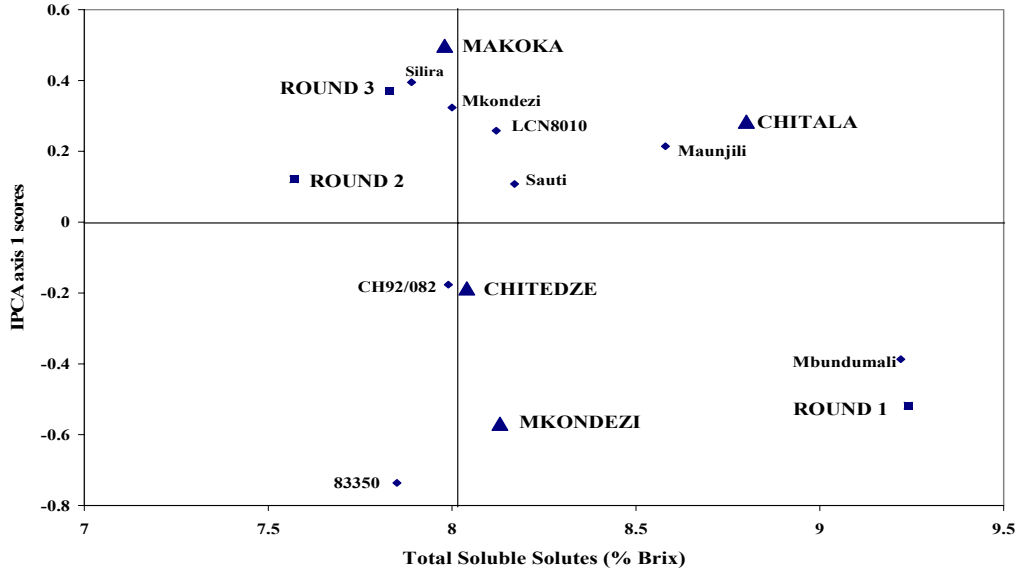


Figure 5.8 Biplot for AMMI IPCA axis 1 scores against means for TSS for genotype by location and round for cassava genotypes evaluated in Malawi

location and round of starch extraction indicated that IPCA axis 1 explained 66.55% of the GxE interaction. AMMI analysis revealed that Silira, Mkondezi, LCN8010, 83350 and Mbundumali were unstable varieties (Figure 5.8). Silira, Mkondezi and LCN8010 had high TSS values at Makoka, Maunjili at Chitala, and 83350 at Mkondezi. Mkondezi and Makoka were the most unstable sites for TSS (Figure 5.8).

5.4 Conclusions and recommendations

This study showed that starch yield levels can efficiently be monitored using starch extraction rate on fresh root weight basis in determining the optimum time to harvest cassava for starch extraction. Starch content on fresh root basis and root dry matter content can be used to determine the optimum time to harvest cassava for starch extraction. It is advisable not to use starch extraction rate on dry root basis and TSS to monitor starch yield levels as they have no direct relationships.

The genotype greatly contributed to the variation of the total sum of squares for starch yield, starch extraction rate on fresh weight basis, starch content on fresh weight basis, and root dry matter content. This suggested that high starch yields could be realised by selection of suitable varieties for starch extraction. Generally, varieties which gave high starch yields were Mkondezi, Silira, Mbundumali and CH92/082. However, some varieties like Sauti could produce high starch yields when grown in its suitable location like Makoka. These results furthermore suggested that starch yield, starch content on fresh root weight basis and root dry matter content can be improved through conventional breeding approaches due to high heritability of these traits.

High contribution of location to the variation of the total sum of squares for starch yield, starch extraction rate on fresh weight basis, starch content on fresh weight basis, and root dry matter content suggested that high starch yields, starch content on fresh root weight basis and root dry matter content can be achieved by growing cassava in appropriate cassava production areas. Makoka and Chitala were the best sites for growing cassava for starch extraction, while Chitedze was the poorest.

Rounds of starch extraction (October-November as round 1, January as round 2 and March-April as round 3) made substantial contribution to the variation of the total sum of squares for starch yield, starch extraction rate on fresh weight basis, root dry matter content and starch content on

fresh weight basis. A proper round of starch extraction which gives the highest starch yield needs to be targeted, which was round 1 and followed by round 3 of starch extractions, in this study. It is therefore advisable to do cassava starch extractions between October and November (Round 1) since the highest starch extraction rates are achieved during this period, and drying of the extracted starch using the open air method is fast and convenient. Harvesting cassava during this time of year furthermore releases the land for cultivation during the following growing season. This underscores the importance of growing of early maturing varieties as highlighted by the farmers. The role of high temperatures as it hasten cassava growth and bulking of tuberous roots is equally important since in conjunction with the use of early maturing varieties can lead to realisation of optimum starch yields when harvested between October and November for starch extraction. Equally high starch extraction rates are achieved between March and April (round 3) but it is too late for the land to be available for other rain-fed crops. In January (round 2), low starch yields are realised, and drying of the starch using the open air method is difficult due to wet weather conditions and high humidity prevailing during that period of the year.

Locations and rounds of starch extraction were the major contributors to the variation of total sum of squares for starch extraction rate on dry weight basis and TSS. This implicates that these traits are polygenic and breeding for these traits using conventional breeding methods can be extremely difficult. Fortunately, these parameters are not related to traits of interest like starch yield.

CHAPTER 6

EFFECT OF GENOTYPE AND PYROCONVERSION ON PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF CASSAVA STARCH

6.1 Introduction

6.1.1 Quality and uses of starch

Native starches from different crops, varieties and species have their own unique properties. These inherent characteristics are exploited by food processors to meet specific needs. Native starches, however, lack the versatility to function adequately in the entire range of food products currently available on the market. The diversity of the modern food industry and enormous variety of food products require that starch be able to tolerate a wide range of processing techniques as well as various distribution, storage, and final preparation conditions. These demands are met by modifying native starches through chemical and physical methods. Starch is used in various industries and each industry needs a particular type of starch which meets its requirements (Thomas and Atwell, 1999). Cassava starch can be modified to provide characteristics that are required for more specialised food and industrial products.

Starches normally have good thickening properties because of the high molecular weight of the polymeric components. For applications that typically utilise high starch (i.e. solid) content such as candies and food coatings, the common practice is to use starches that have been converted. The process results in starch products that contain reduced molecular weight polymers and viscosity. The most common conversion methods used in the starch industry include acid hydrolysis, oxidation, enzyme conversion and pyroconversion (Thomas and Atwell, 1999).

6.1.2 Pyroconversion (dextrinisation) of starch

Pyroconversion of starch is achieved by dry roasting acidified starch. These products are referred to as dextrins or more accurately pyrodextrins. Depending upon the reaction conditions, like pH, moisture, temperature and length of treatment, pyroconversion produces a range of products that vary in viscosity, cold-water solubility, colour, reducing sugar content and stability. Pyrodextrins are classified as white dextrins, yellow dextrins and British gums, depending upon processing

conditions and resultant properties. Depending upon conditions of dextrinisation, both hydrolysis and repolymerisation can occur (Figure 6.1). Because of typically low viscosity, good film forming ability, and high solubility in water, pyrodextrins are used in coating of food and can replace more costly gums in many of these applications. Special high-viscosity dextrins can be used as fat replacers in bakery and dairy products (Thomas and Atwell, 1999).

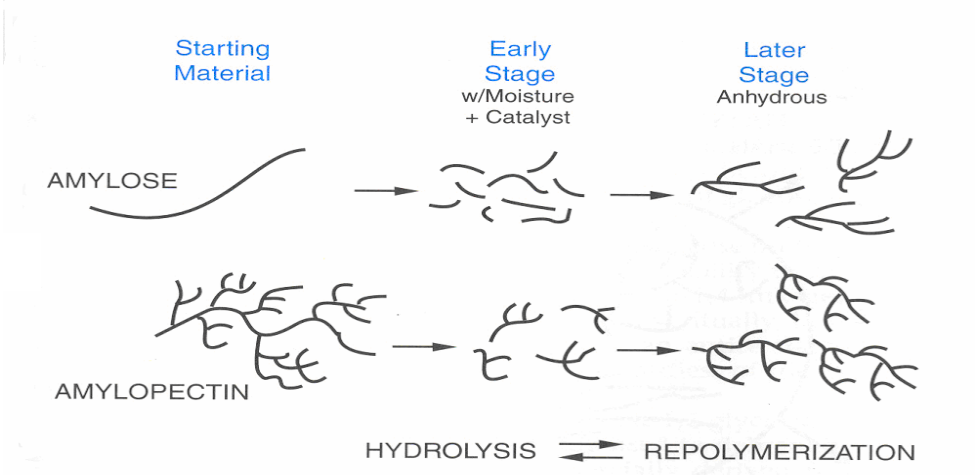


Figure 6.1 Molecular transformation that occur during pyroconversion (Thomas and Atwell, 1999)

Crystallisation of films correlates with the degree of dextrinisation and dextrins of low dextrose content are ideal for starch-based adhesives (Wurzburg, 1986b).

Differential Scanning Calorimetry (DSC) has been used to study thermal properties associated with starch gelatinisation. Gelatinisation is a process through which the internal structure of a starch granule disintegrates, releasing the polysaccharide into the surrounding medium, and is accompanied by a variety of changes. When starch granules are heated in water beyond a critical temperature, the granules absorb a large amount of water and swell to several times their original size. Over a critical temperature range, starch granules undergo an irreversible process, which is marked by crystalline melting, loss of birefringence and starch solubilisation (Singh *et al*., 2005). DSC monitors changes in the physical and chemical properties of starches, offering a thermodynamic approach to the study of starch gelatinisation (Donovan *et al*., 1983; Singh *et al*., 2005). Seetharaman *et al*. (2001) reported several significant relationships between starch thermal properties measured using DSC and pasting properties measured using the rapid visco-analyser (RVA). Singh *et al*. (2005) observed highly positive correlations between onset temperature and peak viscosity, trough and final viscosity ($p<0.001$) for Argentinean maize starches. Zobel (1984) reported that the temperature at which gelatinisation is initiated is in general agreement with values for loss of birefringence.

Cassava dextrin has definite advantages like bland taste, higher stickiness and neutral flavour unlike cereal dextrins which have a lipid flavour. Most re-moisturising gums are made from dextrins (Moorthy, 1994). Knowledge of physical, chemical and functional properties of starch, being produced or worked with, is important. Hence, there exists a need to characterise starch from different sources and varieties.

The objectives of this study were to (1) characterise native starch from 10 elite Malawian cassava genotypes, (2) customise pyroconversion of cassava starch using Malawian cassava genotypes, and (3) delineate the effect of pyroconversion on functional and physicochemical properties of cassava starch.

6.2 Materials and methods

6.2.1 Characterisation of cassava native starch

Protein content, whiteness, pH and ash content of cassava starch for 10 elite cassava genotypes included in this study were adapted and determined according to Benesi (2002). This study therefore focused on moisture content, microscopic examinations and functional properties for native and pyrodextrins for cassava starch.

6.2.1.1 *Moisture content of native starch*

Moisture content was determined using a method described by ISO (1997) as follows: Starch samples (5g; w_1) were dried for 24 hours at 105^{o}C and cooled down in a desiccator. After removal from the desiccator, samples were weighed immediately (w_2). Percentage moisture content (MC %) of each sample (average results of two samples) was calculated as follows:

$$MC\ \% = 100-[(100 * (w_2 / w_1)]$$

6.2.1.2 *Microscopic examination for native starch and dextrins*

Starch granule shape and size was examined using a Confocal/Bino microscope as follows: Two drops of distilled water was put on a clean slide, a small amount of starch (about 2mg) was dispersed into the water while making sure that the starch grains settled down, and thinly spread onto the slide. The slide was examined on a Nikon Eclipse TE2000-E microscope using light

microscopy. Pictures of starch granules with a scale of granule sizes were observed and saved. Twenty granules were randomly sampled for each treatment/variety, and were measured for size.

6.2.2 Standardisation of pyroconversion of cassava starch

6.2.2.1 *Acidification and drying of starch*

Air-dried cassava starch samples (1000g) were acidified with 750ml (0.1M) hydrochloric acid (HCl; Masumbu, 2002). Mixing was done manually and the acidified starch was left to stand for 15min. Acidified starch samples were subsequently sun dried on wooden trays for two days. After drying to a moisture content of about 13%, the acidified starches were pound, sieved through a 0.25mm mesh size using sieve number 60, and kept in closed containers according to the recommendations of Wurzburg (1986b) and Masumbu (2002).

6.2.2.2 *Dextrinisation of cassava starch*

Dextrinisation is affected by time, concentration of the catalyst, temperature and type of starch. The study of Masumbu (2002) reported the most effective concentration of catalyst as 0.1M HCl and dextrinisation temperature of 100^{o}C for cassava starch, but results on the period of dextrinisation was not conclusive. Hence, there was a need to standardise the pyroconversion for dextrinising cassava starch. A composite sample of cassava starch from five genotypes namely TMS60142A, TMS60121, TMS84563, 81/00015 and LCN8010 were acidified as described in section 6.2.2.1. Moisture content of the composite sample was determined, followed by dextrinising for 15, 30, 40, 45, 50, 55, 60, 70, 75, 90, 120, 150, and 180min. The degree of dextrinisation was monitored by cold-water solubility and reducing sugar content analyses (Thomas and Atwell, 1999). Dextrinisation was done by heating 500g of acidified starch placed in a 4l porcelain dish, which was placed in an air oven set and equilibrated at 100°C before adding acidified starches. Stirring was done every 5min to ensure uniform dextrinisation and samples of about 50g were drawn at times as shown above in order to assess the effect of heating time on extent of dextrinisation. Dextrins were immediately neutralised to a pH between 5 and 6 by adding 0.4ml of 25% aqueous ammonia (NH_3). The container was closed and the contents shaken to ensure neutralisation. The dextrinisation process was quenched by placing the container and contents into an ice bath and allowed to cool to room temperature (Wurzburg, 1986b). Finally, the dextrin was quickly sieved through a 0.25mm mesh sieve to remove lumps and was kept in closed containers to stop dextrins from equilibrating to the ambient moisture content (Smith, 1967; Corbishley and Miller, 1984).

Three periods of dextrinisation (40, 50 and 60min) were applied in the dextrinisation of all acidified starch samples from 10 Malawian cassava genotypes.

6.2.2.3 *Solubility of native starch and dextrins*

Cold-water solubility of starch and dextrins was determined using 10% and 1% (w/v) starch, respectively, in water at 25°C.

Cold-water soluble material in unmodified or slightly modified starch (Smith, 1967; Lyne, 1976)

A starch sample (20g), in triplicate, was placed in a 250ml Erlenmeyer flask and dispersed in 200ml distilled water at 25°C. The container was closed and the slurry agitated continuously at a moderate speed for 30min. The suspension was filtered through a Whatman No.12 filter paper under gravity. The filtrate was collected in a dry receiver, and the first 25ml of filtrate was returned to the suspension. An aliquot of filtrate (100ml) was pipetted into a pre-dried and pre-weighed evaporating dish and the solution evaporated to apparent dryness in a steam bath. The residue was dried to a constant mass in a vacuum oven at 100°C for two hours, cooled in a desiccator and weighed.

%Soluble = (Residue mass in g * 200ml/100ml * Sample mass in g) * 100%

Change in solubility was calculated to asses the rate at which solubility of pyrodextrins changed per unit time (15min) as period of dextrinisation progressed, while cumulative solubility indicated solubility of pyrodextrin after a certain period of dextrinisation. Change in solubility per 15min was calculated as follows:

Change in solubility% = [15 / (time2 in minutes) - time1 in minutes)] * (solubility% at time2 – solubility% at time 1]

Cold-water soluble material in dextrins (Smith, 1967; Lyne, 1976; Osunsami *et al.*, 1989)

A sample of dextrin (2g), in triplicate, was weighed and heated as described in section 6.2.2.3.

%Soluble = (Residue mass in g * 200ml/Aliquot volume * Sample mass in g) * 100%

6.2.2.4 *Reducing sugar value (ferricyanide number)*

Reducing sugar values (ferricyanide number) of dextrins of the composite sample dextrinised at different periods were determined as described by Smith (1967), with the aim of elucidating the relationship between cold-water solubility and reducing sugar values in the customisation of pyroconversion of cassava starch using Malawian cassava genotypes.

Reagents for reducing sugar analysis

Reagents were prepared as follows: Ferricyanide reagent (0.1M) was prepared by dissolving 16.5g of potassium ferricyanide and 22.0g of anhydrous sodium carbonate in distilled water and made up to 1000ml with distilled water. The solution was allowed to stand for four days and any precipitate formed was gravity-filtered. When not in use, the filtrate was stored in brown glass in the dark since it deteriorates when exposed to light. With proper storage, the reagent can be stable for several months. Zinc sulphate-acetic acid solution was prepared by dissolving 70.0g of potassium chloride, 22.0g zinc sulphate heptahydrate and 200ml glacial acetic acid in distilled water and made up to 1000ml. Potassium iodide (20%) and 1% (w/v) starch indicator was prepared by dispersing an aqueous starch mixture in water, followed by boiling with constant stirring till fully gelled and then cooled.

Procedure for determining reducing sugar value

A starch sample was ground and sieved with a 0.25mm sieve prior to analysis. A sample (200mg) whose moisture content and water solubility at 25°C were known, was added to distilled water (25ml) and boiled for 15min while mixing the contents continuously so as to disperse and gelatinise the sample uniformly. A total of 25ml alkaline ferricyanide reagent was added to the hot sample dispersion and the contents of the bottle were mixed. The container was closed with a 1-hole rubber stopper containing a short length of capillary tubing which served as a vent and placed immediately in a vigorously boiling water bath, in a position so that the bottle was immersed to the neck in boiling water. The container was kept in the bath for 15min and the contents swirled frequently to ensure rapid and uniform heat transfer. The container and contents were cooled immediately in an ice-and-water bath. Sixty ml of zinc sulphate-acetic acid solution was added carefully to avoid loss of sample through excessive foaming, and the contents were mixed followed by an addition of 20ml of 20% (w/v) potassium iodide solution. The iodine, liberated by reaction of iodide ions with excess ferricyanide ions, was titrated with standard 0.05M sodium thiosulphate solution.

The starch sample usually served as the indicator for end point detection, but if the product was modified and did not provide the characteristic, intense blue or purple colour starch indicator solution was added. In addition, starch indicator was added to blank titrations that showed no blue colour upon addition of the potassium iodide.

A blank determination was performed on 25ml of the ferricyanide reagent by adding 25ml distilled water, 60ml zinc sulphate-acetic acid reagent, and 20ml 20% (w/v) potassium iodide, as described previously. The reaction mixture was titrated with standard 0.05M thiosulphate solution, starch indicator solution was added, as the end point was approached, as judged by perceptible fading of the yellow ferricyanide colour. The reducing sugar value, or ferricyanide number, expressed as the number of millilitres of 0.1M ferricyanide solution reacting with 1g of dry sample was calculated as follows:

Reducing sugar value = [(Blank titter – Sample titter in ml) * thiosulphate molarity * 10]/Dry sample weight

6.2.2.5 *Characterisation of cassava starch for thermal dynamics using DSC*

Starch and dextrin samples were analysed on a TA Instrument Q100 V9.0 Build275 DSC system with a refrigerated cooling system. Prior to analysis, the Q100 DSC was calibrated with a certified sample of Indium (temperature and enthalpy calibrations), and sapphire for the baseline and cell constant calibration. Samples were weighed into a standard aluminium pan with lid, and sealed with a DSC sample press. A sample of approximately 5mg was taken.

Curves were evaluated using a standard TA Instruments Universal Data Analysis programme. As a basis for comparison, all curves were rescaled to the same X and Y scales, and the peak area/maximum was determined between 10 and 195°C or at the point where a plateau was reached before the 195°C point.

6.2.2.6 *Moisture content of dextrins*

Moisture content for dextrins was analysed using the Dean and Stark direct distillation method (Pearson, 1975). A dextrin sample (5g) was weighed into a 250ml quick-fit round-bottomed flask followed by addition of 150ml toluene and some glass beads to act as boiling aids. The flask was placed on a heating mantle and connected to a Dean and Stark apparatus, which was connected to a condenser with running water. The mixture was refluxed at moderate heating for 30min until

the distilled water in the receiver no longer increased in volume. Any water droplets not mixed with the water in the receiving tube were pushed down the receiving tube using a long wire. After the boiling mixture cooled to room temperature, the water in the receiving tube was drained into a pre-weighed tube and the water determined by mass. A blank determination was done using 150ml of toluene without dextrin samples.

%Moisture = (Mass of water drained into the tube in g/ Mass of dextrin sample used in g) * 100%

6.3 Results and discussion

6.3.1 Protein content, whiteness, pH and ash content for native cassava starch

Earlier work done by Benesi (2002) characterised starch from these genotypes for protein content, whiteness, pH and ash content. All cassava genotypes produced starch in which no protein was detected, and ash contents ranged from 0.11 to 0.20%, which was much lower than the recommended maximum of 0.5% (Thomas and Atwell, 1999; National Starch and Chemical Company, 2002). The pH for native cassava starch ranged from 5.0 to 5.5 at both sites, which was within the recommended range of between 4.5 and 7.0 (National Starch and Chemical Company, 2002). The whiteness of cassava starch ranged from 94.50 to 95.75, using a HunterLab ColorFlex $45^o/0^o$ (Table 6.1). The current study therefore determined the physico-chemical and functional properties of starch from the same genotypes and pyroconversion of starch from cassava.

Table 6.1 Protein content, whiteness, pH and ash content of native cassava starch for 10 Malawian cassava genotypes (Benesi, 2002)

Genotype	Starch Protein content (%)	Whiteness of starch (%)	pH of starch	Starch Ash content (%)
Silira (TMS60142B)	0.00	95.27	5.5	0.12
Sauti (CH92/077)	0.00	95.86	5.2	0.10
CH92/082	0.00	94.55	5.5	0.12
TMS4(2)1425	0.00	95.28	5.2	0.17
LCN8010	0.00	94.17	5.4	0.15
83350	0.00	95.75	5.2	0.20
81/00015	0.00	94.46	5.4	0.12
Mbundumali	0.00	95.57	5.3	0.15
Mkondezi (MK91/478)	0.00	94.50	5.3	0.11
Maunjili (TMS91934)	0.00	95.22	5.0	0.11
Nzeru maize starch (control)	0.66	95.20	4.7	0.14
PIM maize starch (control)	0.09	96.46	5.0	0.15
MPL Amylmaize starch (control)	0.00	97.28	4.1	0.12

6.3.2 Moisture content, solubility, microscopic and DSC analyses

Moisture content range for native cassava starch from the 10 varieties ranged from 10.47 to 12.83% which was below the recommended maximum of 14% (Table 6.2; National Starch and Chemical Company, 2002). Native cassava starch was insoluble in cold water, since solubility of native starch from Malawian cassava genotypes ranged from 0.29 to 0.50% which was lower than for amylmaize starch, which had a solubility of 0.58% (Table 6.2). Therefore, all native

Table 6.2 Moisture content, solubility, granule size, enthalpy of gelatinisation (ΔH_G) and DSC onset and maximum peaks of native cassava starch for 10 Malawian cassava genotypes and controls

Genotype	MC (%)	Sol. (%)	Granule size (µm)				Peak onset (^oC)	Peak Max. (^oC)	ΔH_G (J/g)
			Mean	Mode	Min.	Max.			
Mbundumali	12.23	0.30	14.93	12.9	10.0	22.9	33.40	86.34	348.0
83350	12.67	0.29	13.86	14.3	8.6	20.0	68.99	119.34	321.1
LCN8010	12.91	0.29	15.07	14.3	10.0	20.0	46.67	105.33	310.8
Mkondezi	10.97	0.50	16.18	17.1	11.4	22.9	44.73	105.97	319.5
81/00015	12.70	0.34	14.43	12.9	8.6	20.0	38.01	88.93	350.8
Silira	10.81	0.29	11.79	12.9	8.6	18.6	48.91	110.13	359.2
Sauti	10.47	0.39	14.64	14.3	8.6	24.3	45.77	107.57	331.4
CH92/082	12.22	0.34	13.86	14.3	8.6	18.6	28.56	87.15	333.5
Maunjili	10.75	0.41	12.64	15.7	5.7	18.6	39.20	97.79	372.7
TMS4(2)1425	12.83	0.30	10.14	10.0	4.3	14.3	43.06	102.60	316.4
Amylmaize starch	12.48	0.58	11.79	12.9	4.3	21.4	74.30	124.44	299.6
Maize starch	nd	nd	13.25	12.9	4.3	22.9	46.46	109.19	335.8

MC = moisture content; Sol. = solubility; Min. = minimum; Max. = maximum; J = Joules; nd = not determined

starches were in line with what is reported in literature (Wurzburg, 1986a; Lepoutre and Inoue, 1989; Uniqema Company Limited, 2002). Starch contains aggregates called granules. Size, shape and structure of these granules vary substantially according to varieties and crops from which starch was extracted. The variety Mkondezi had the largest granules as revealed by the mean, mode as well as range of starch granule sizes. Other varieties that had large granules in terms of mean granule size, were Mbundumali, 81/00015 and Sauti. The size distribution of granules within samples in terms of mode, minimum and maximum sizes revealed that apart from Mkondezi, other varieties with mostly large granules were 83350, LCN8010, Sauti, CH92/082 and Maunjili (Table 6.2 and Figures 6.2a and b). Although the mode showed that most of the granules for Maunjili were quite large (15.7µm) the range was from 5.7 to 18.6µm as compared to Mkondezi whose range was from 11.4 to 22.9µm, and Sauti that had a range of 8.6 to 24.3µm (Table 6.2). TMS4(2)1425 had the smallest granules as revealed by the mean, mode as well as range. However, the granule sizes of cassava starch from 10 varieties were

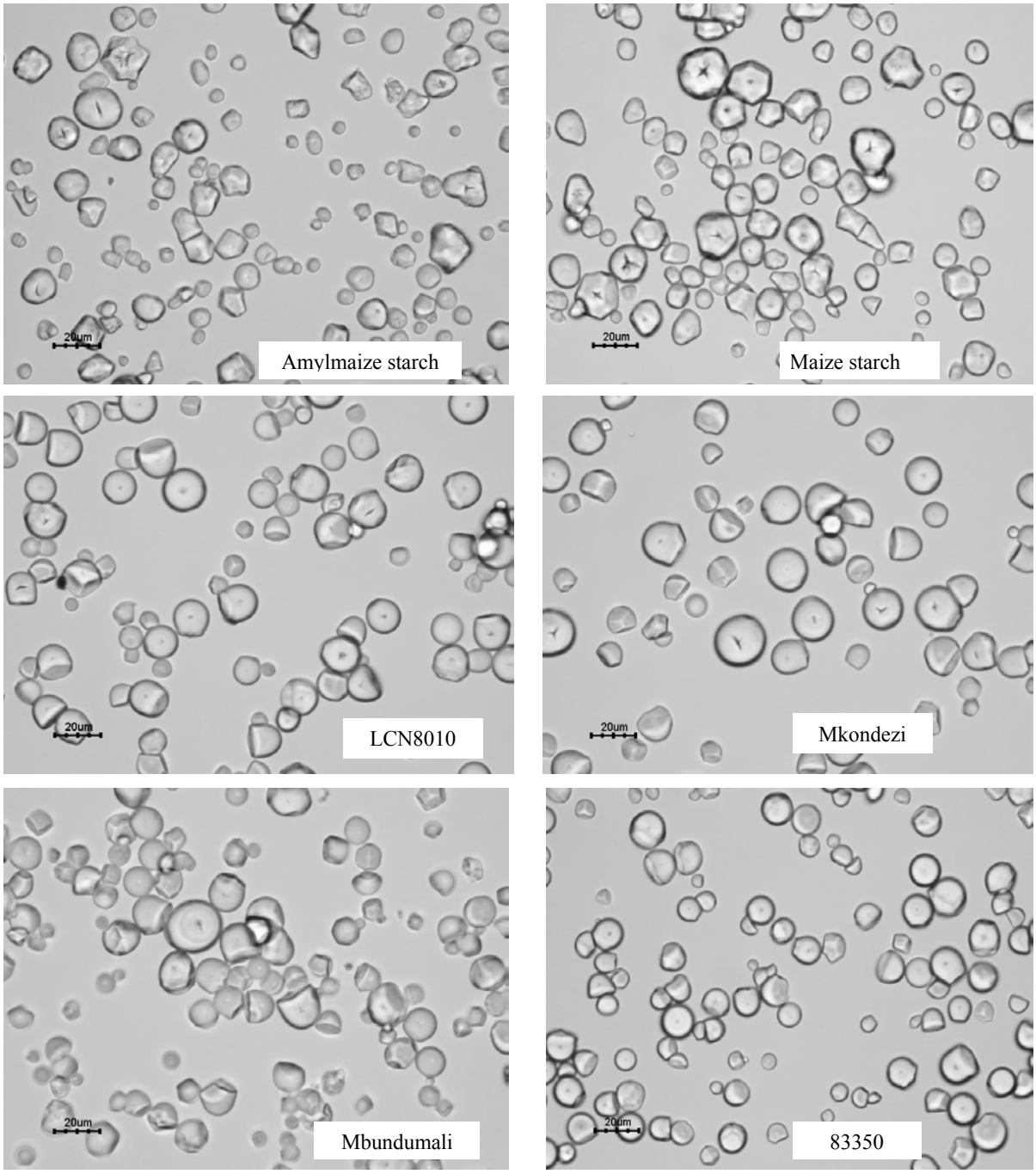


Figure 6.2a Granule shapes and size distribution for cassava starch from four Malawian cassava varieties and two controls (maize starch)

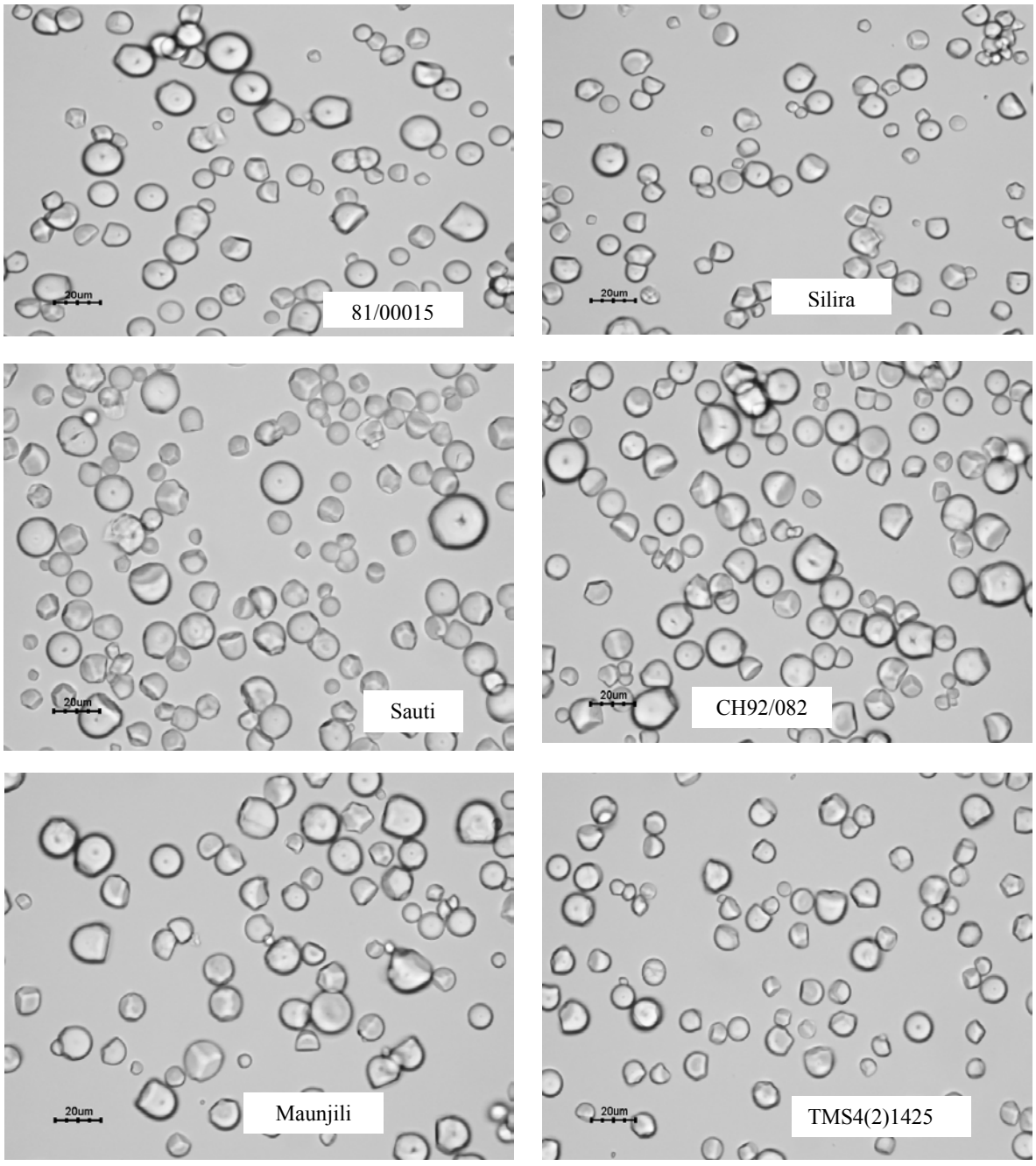


Figure 6.2b Granular shapes and size distribution for cassava starch from six Malawian cassava varieties

generally medium to small (Figures 6.2a and b). It is reported in literature that the diameter for cassava starch granules ranges from 4-35μm (Onwueme, 1978; Moorthy, 1994; Thomas and Atwell, 1999).

Shapes of cassava starch granules from 10 Malawian cassava genotypes are presented in Figures 6.2a and b. Cassava starch granules were mostly round or oval, with a flat surface on one side containing a conical pit which extended to a well. Moorthy (1994) described it as a eccentric hilum, while Thomas and Atwell (1999) described it as truncated or kettledrum. Amylmaize starch from Malawi Pharmacies Limited (MPL) granules were round or irregular, and some were polygonal, while maize starch (from Aldrich) granules were round, irregular but most were polygonal in shape (Figure 6.2a).

Analysis of starch using DSC indicated that there existed wide variability in the thermodynamics of native cassava starch from different varieties. Variety 83350 had the highest peak onset temperature of 68.99^{o}C, which was close to that of amylmaize starch (74.30^{o}C), which is used by MPL, while CH92/082 had the lowest peak onset temperature of 28.56^{o}C (Table 6.2 and Figure 6.3). Temperature for onset of peaks for the other varieties (which ranged from 33.40 to 48.91^{o}C) were close to that of maize starch from Aldrich (46.46^{o}C). Thus, cassava starches generally had lower peak onset temperatures than amylmaize starch except for 83350 (Table 6.2 and Figure 6.3). Results agreed with reports in literature that onset of gelatinisation temperature of cassava starch is much lower (49-70°C) than that of maize starch (62-73°C; Jarowenko, 1977; Wurzburg, 1986a).

The highest peak maximum temperature was measured for amylmaize starch (124.44^{o}C) that was similar to that of maize starch (109.19^{o}C), followed by those of cassava starches from 83350 (119.34^{o}C) and Silira (110.13^{o}C), while Mbundumali, 81/00015 and CH92/082 had the lowest peak maximum temperatures of 86.34, 88.93 and 87.15^{o}C, respectively (Table 6.2 and Figure 6.3). There was no diversity for enthalpies of gelatinisation (ΔH_G) for native cassava starches as well as for maize starch from Aldrich, since all were higher than 310J/g, while the ΔH_G for amylmaize starch was below 300J/g (Table 6.2 and Figure 6.3). Low ΔH_G values, and high peak maximum temperatures of starch are attributed to melting of fatty acid amylose complexes during gelatinisation (Moorthy, 1994; Thomas and Atwell, 1999).

DSC thermograms showed that peak onset and peak maximum temperatures from 83350 and amylmaize starches were similar in functional properties (Figure 6.3). Higher melting forms observed on DSC scans have been confirmed as evidence of formation of V-type structures on

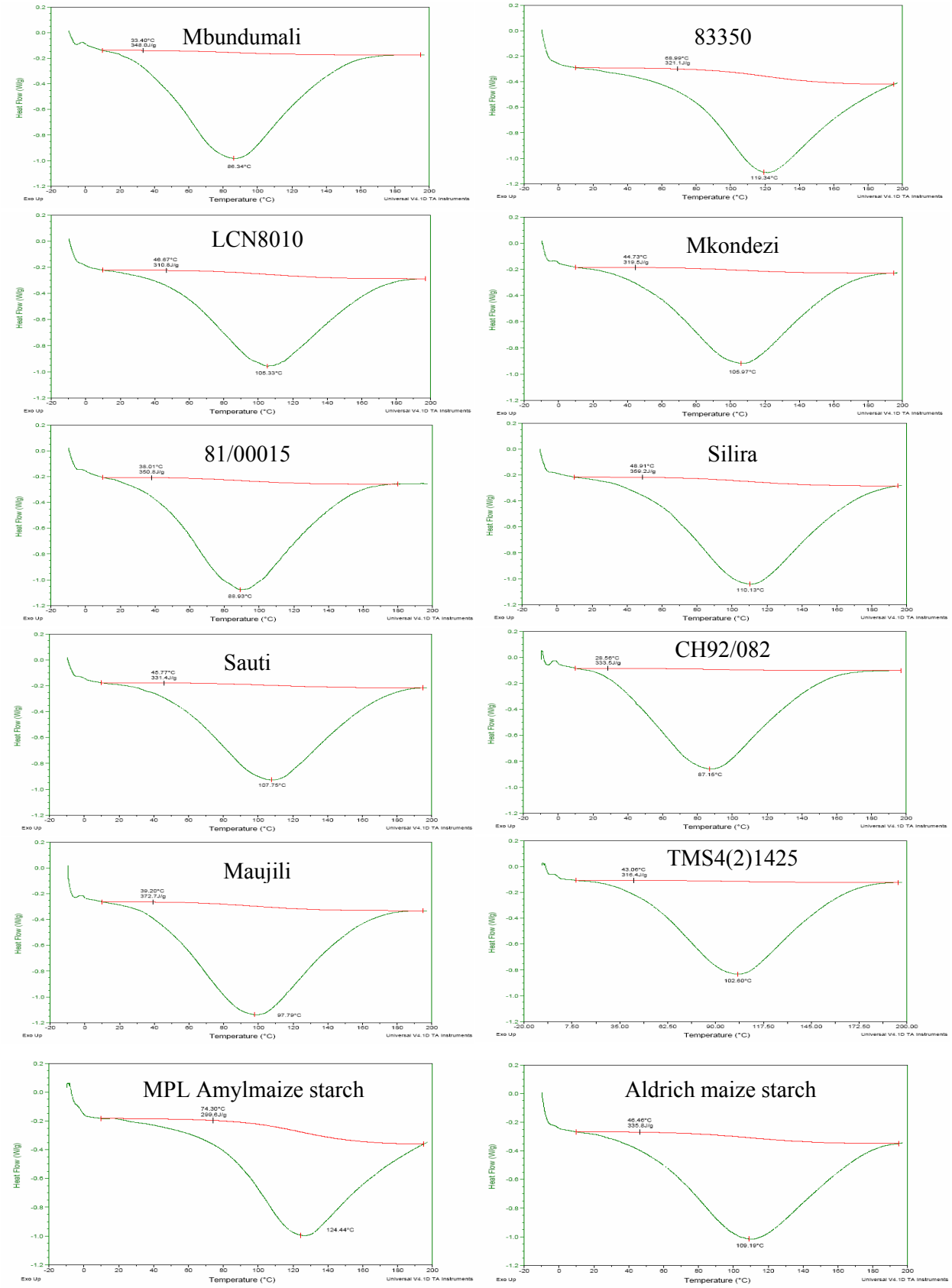


Figure 6.3 DSC thermograms for gelatinisation of native cassava starch from Malawi cassava genotypes and controls

wheat starch gels during gelatinisation using X-ray (Zobel, 1984). DSC tracings revealed major structural changes that occurred beyond birefringence end-point. Hence, DSC scans indicated that the temperature range needed to provide maximum disruption of structure to achieve granule swelling and high viscosities in the hot starch paste (Zobel, 1984). Other studies indicated close correspondence between DSC scans and Brabender Amylgraph viscosity maximum. DSC showed additional changes in starch after completion of viscosity increase (Moorthy, 1994). Moorthy (1994) observed a relationship between gelatinisation temperature and pasting temperature.

6.3.3 Pyroconversion of cassava starch

6.3.3.1 *Determination of the best dextrinisation period for cassava starch*

Solubility of all unmodified starches in cold-water was less than 1% (Table 6.2). Results agreed with literature values (Wurzburg, 1986a; Lepoutre and Inoue, 1989). On the other hand, pyrodextrins are known to have increased solubility due to hydrolysis of starch polymers as dextrinisation progressed (Figure 6.1). As starch dextrinises, there is hydrolysis of the α-1,4 glucosidic links in amylose and amylopectin, resulting in the formation of shorter chain lengths and low molecular weight saccharides with corresponding weakening of hydrogen bonds holding granules together (Wurzburg, 1986a; Thomas and Atwell, 1999). This firstly permits some parts of the granule to be dispersed and later the entire granule becomes soluble in cold water (Wurzburg, 1986a). Dextrinisation increases with temperature (Kennedy and Fischer, 1984; Wurzburg, 1986b). Balagopalan *et al.* (1988) indicated that acidified starches are effectively dextrinised. The study of Masumbu (2002) concluded that 100^{o}C and 0.1M HCl at a rate of 750ml per 1000g starch, was an appropriate combination of temperature, water and acid concentration for pyroconversion of cassava starch.

Determination of the best dextrinisation period for cassava starch, and the relationships between solubility and reducing sugar values were conducted using a composite sample of cassava starch as described in the materials and methods. Time of dextrinisation had a big effect on the pyroconversion of cassava starch (Figure 6.4). Figure 6.4 indicated that the period between one and 60min was the most active period for dextrinisation of cassava starch. The concentration of sampling between 40 and 75min was correct as it captured the critical period of dextrinisation of cassava starch. Results disagreed with the findings of Masumbu (2002) who reported that solubility of dextrins increased to about 100% after three hours at 100^{o}C with starch treated with

0.1M HCl. Figure 6.4 indicated that there existed a close relationship between solubility and reducing sugar values as both revealed the critical period of dextrinisation. The critical period for dextrinisation of cassava was between 40 and 60min, since pyrodextrin from a composite starch sample achieved close to 100% solubility within 60min of dextrinisation (Figure 6.4). Hence, individual starch samples from different cassava genotypes were dextrinised for 40, 50 and 60min. Since both solubility and reducing sugar values corresponded in monitoring the degree of dextrinisation, cold-water solubility is being recommended due to its ease of determination compared to reducing sugar analysis.

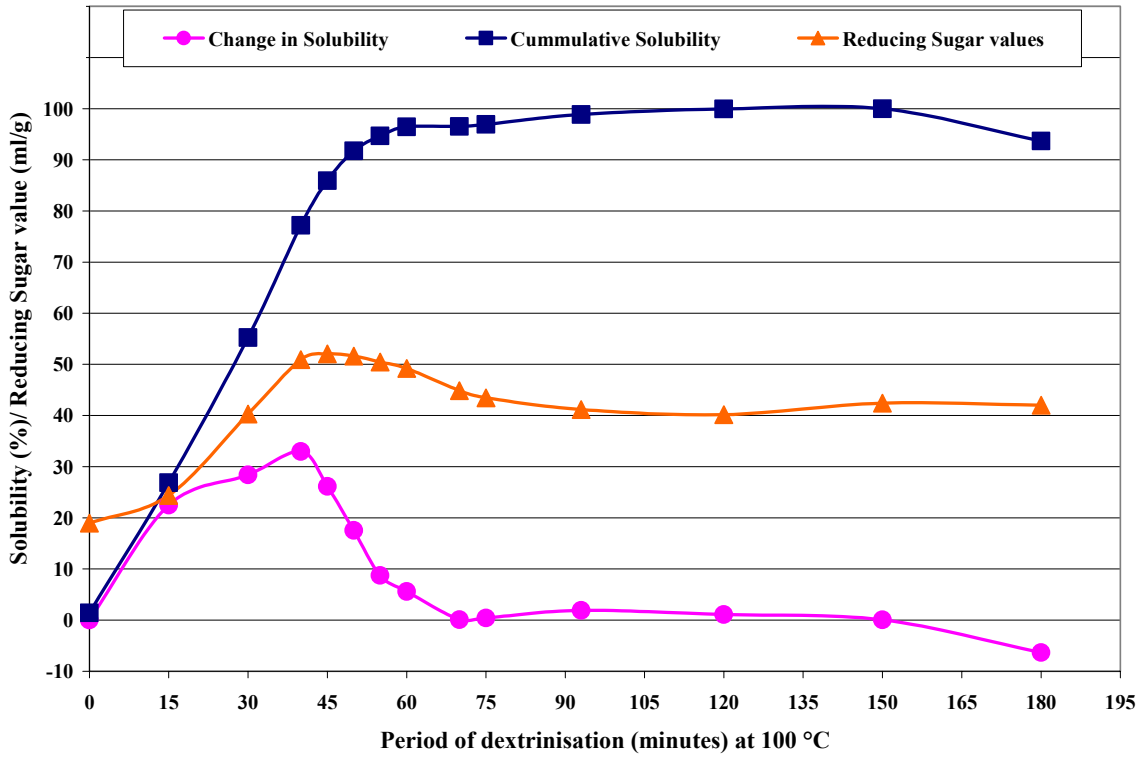


Figure 6.4 Effect of period of dextrinisation on solubility and reducing sugar values in cassava starch

Microscopic examination and DSC analysis of Mkondezi native starch and its pyrodextrins, dextrinised at 40, 50 and 60min showed that dextrinisation had no effect on granule size and shape, but loss of focus was observed with time of dextrinisation (Figure 6.5). Results indicated the extent of hydrolysis of polymers due to pyroconversion that occur within the starch granule with period of dextrinisation without affecting the granule size and shape.

DSC scans indicated that starch from the variety Mkondezi which was dextrinised for 40min at 100^{o}C had the same thermogram, peak onset temperature and peak maximum temperature compared to amylmaize starch (Figure 6.6), which is being used by MPL in the pharmaceutical

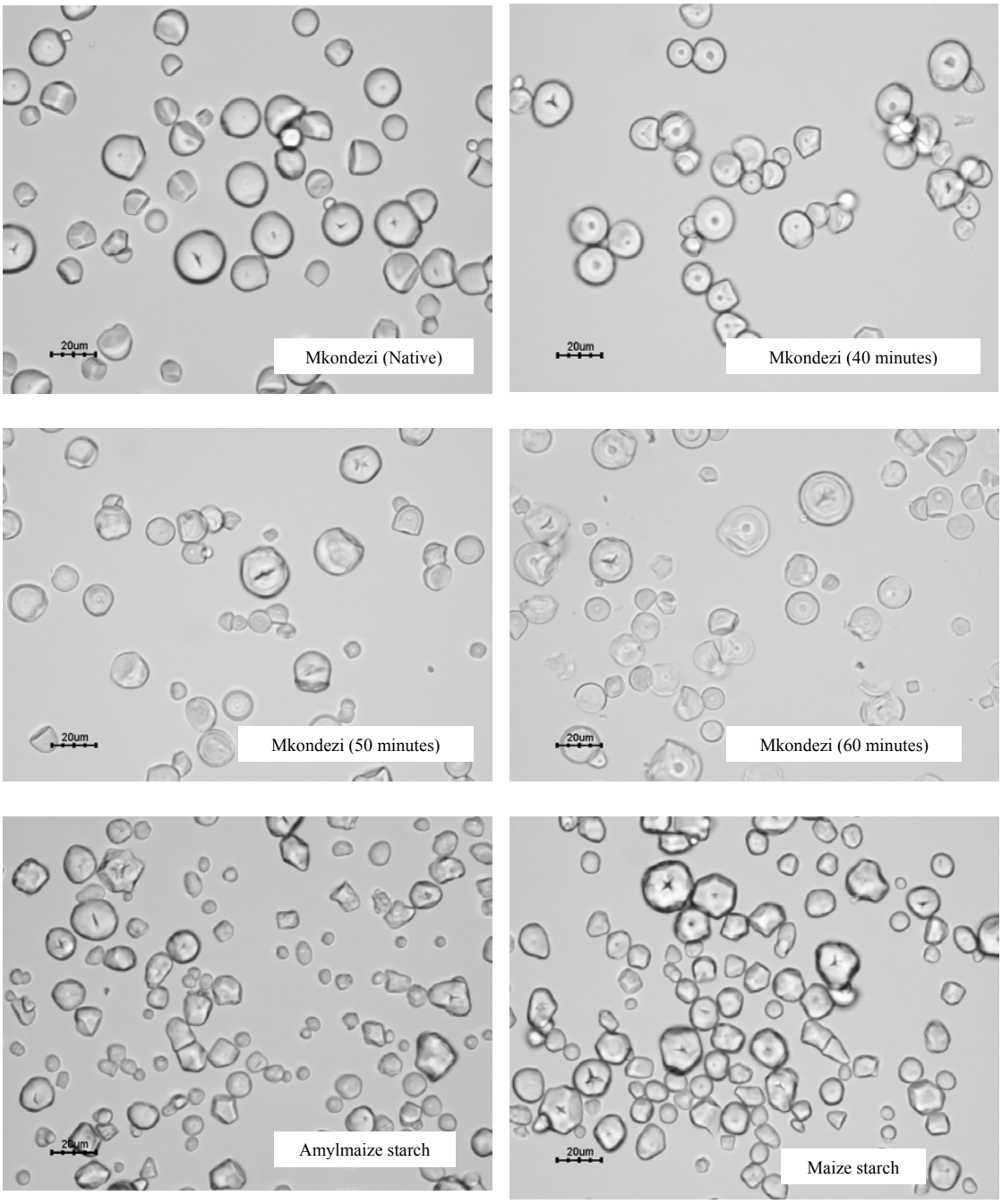


Figure 6.5 Effect of pyroconversion on granular shapes and sizes of cassava starch from the variety Mkondezi and two controls

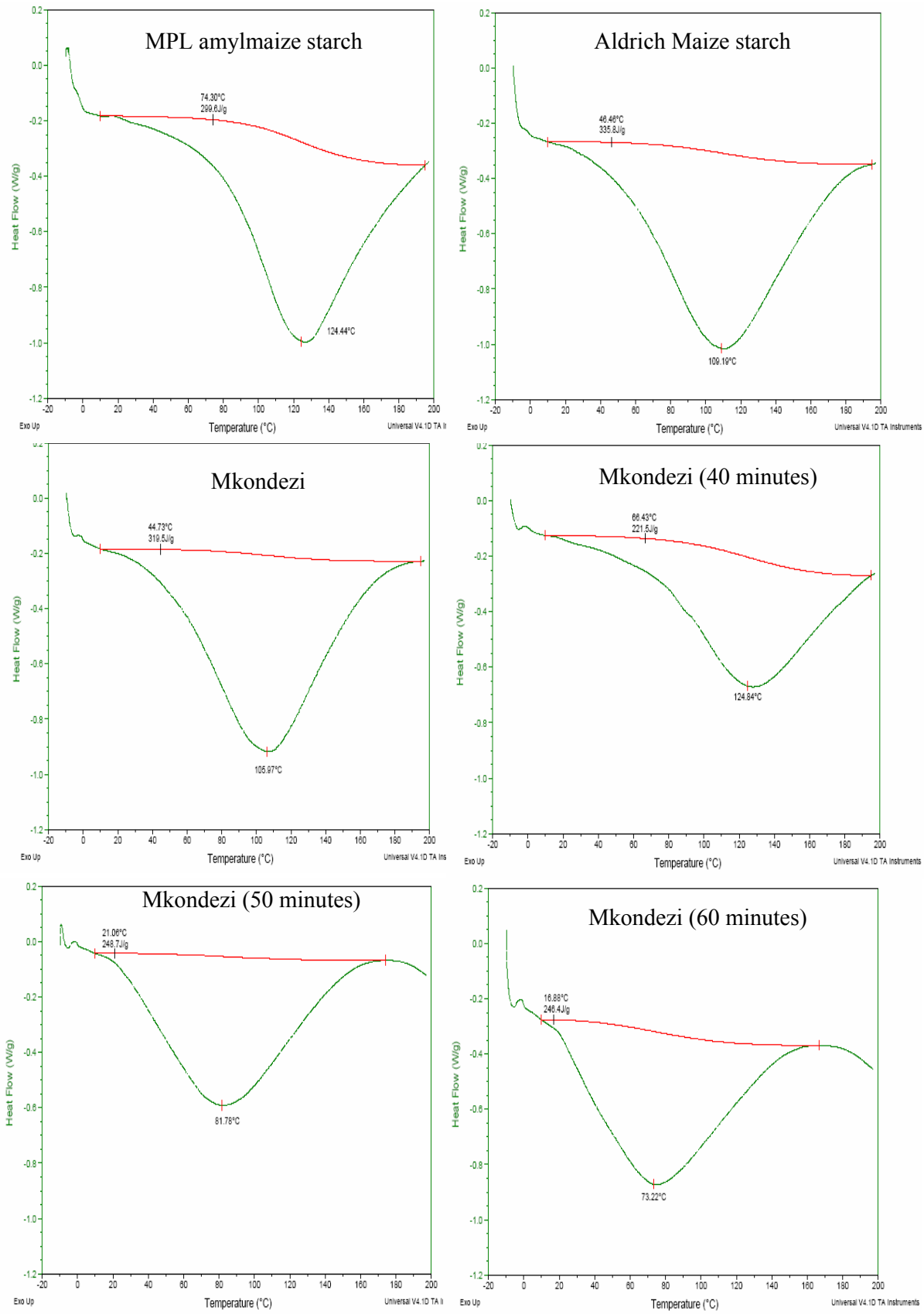


Figure 6.6 DSC thermograms for gelatinisation of native cassava starch of the variety Mkondezi and two controls

industry. Results suggested the possibility of exploiting cassava starch uses in specific industries by varying the dextrinisation period.

5.3.4.2 *Pyroconversion of cassava starch and characterisation of pyrodextrins*

Pyrodextrin from CH92/082 did not show differences in cold water solubility between dextrinised and undextrinised starch results and were excluded for the dextrinisation study, but was included in microscopic and DSC analyses. There exists a need to look closely at this genotype's pyroconversion in order to establish the cause for its behaviour since solubility tests were repeated more than three times but similar results were obtained. Hence, nine genotypes are presented in Figure 6.7. Dextrinisation of cassava starch from nine genotypes for 40, 50 and 60min at 100^{o}C revealed that there existed diversity in terms of resistance of starch to dextrinisation. Starch from 81/00015 and Silira attained about 80% cold-water solubility at 50min of dextrinisation. Starch from 81/00015, Silira, Mbundumali and Sauti attained more than 90% cold-water solubility at 60min of dextrinisation (Figure 6.7). Starch from LCN8010, 83350, Maunjili and TMS4(2)1425 did not attain cold-water solubility of 70 or 80% at 60min of dextrinisation, while starch from Mkondezi variety attained 83% after 60min of dextrinisation. Results suggested that starch from LCN8010, 83350, Maunjili, TMS4(2)1425 and Mkondezi could achieve 90% cold-water solubility after dextrinising for longer than 60min since solubility were still increasing at 60min. However, this hypothesis needs to be established by dextrinising each of the resistant starches for up to 180min. Silira and 81/00015 pyrodextrins almost reached the plateau of solubility within 60min of dextrinisation.

Starch from Mbundumali, LCN8010, Mkondezi and 81/00015 had larger starch granules, while Silira and TMS4(2)1425 had smaller starch granules even after pyroconversion (Table 6.3). Pyroconversion of cassava starch from different cassava varieties did not affect granule size (Table 6.3) and shape (Figure 6.5).

DSC analysis showed that physicochemical and functional properties of native starch from 83350 was similar to amylmaize starch, which is being used by MPL in Malawi (Table 6.2 and Figure 6.8a), except for ΔH_G, which was higher for 83350 native starch than for amylmaize starch. Dextrinisation of 83350 starch for 40min at 100^{o}C led to the reduction of peak onset temperature from 69 to 34^{o}C, and peak maximum temperature from 119 to 98^{o}C, hence were lower than that of amylmaize starch (Figure 6.8a). Enthalpy of gelatinisation for 83350 pyrodextrin (at 40min dextrinisation) was reduced from 321 to 296J/g. The resultant ΔH_G after dextrinisation was almost similar to that of amylmaize starch (299 J/g; Figure 6.8a).

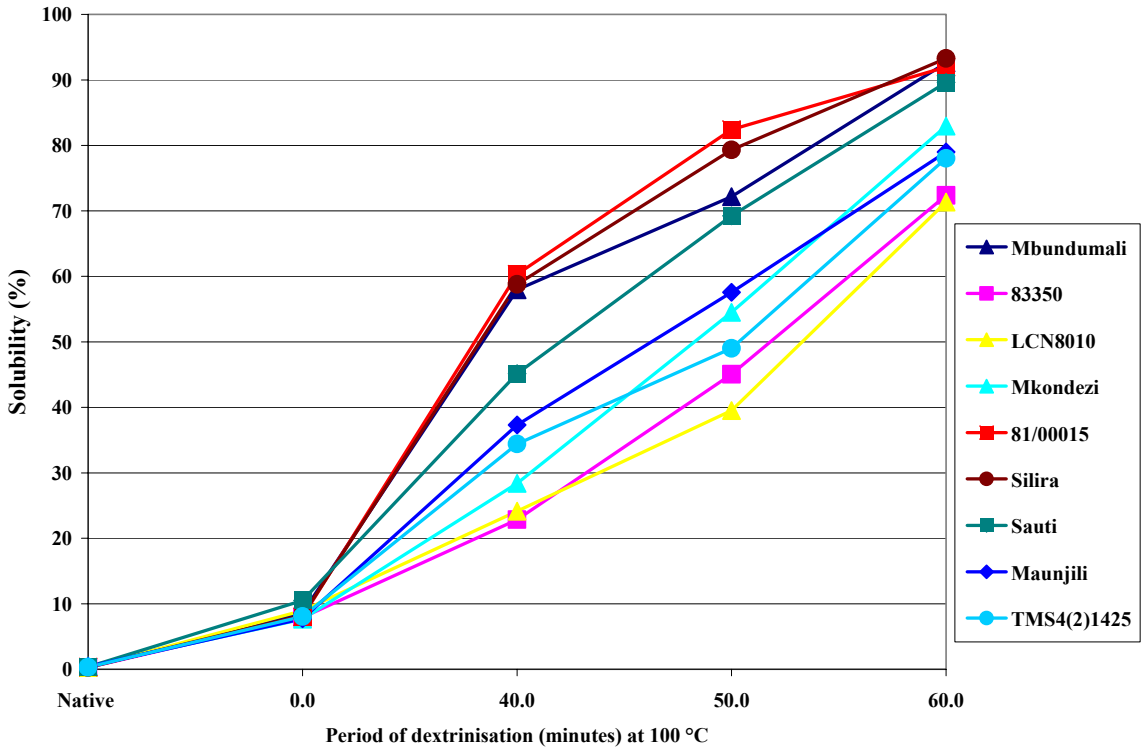


Figure 6.7 Pyroconversion of cassava starch from nine Malawian cassava varieties

Dextrinisation of Mbundumali starch for 40min at 100°C led to a reduction in ΔH_G from 348 to 284J/g. The peak onset and peak maximum temperatures increased from 33 to 45°C, and from 86 to 100°C, respectively (Figure 6.8a), which was closer to amylmaize starch than the native starch.

Dextrinisation of TMS4(2)1425 starch at 100°C for 40min did not have any effect on functional properties. However, solubility results suggested that dextrinising of TMS4(2)1425 starch for 60min could have an effect on its functional properties (Figures 6.7 and 6.8b). While dextrinisation of LCN8010, Silira and Maunjili starches for 40min at 100°C did not affect both peak onset and peak maximum temperatures, ΔH_Gs were reduced from 310 to 236J/g for LCN8010, from 359 to 249J/g for Silira, and from 373 to 286J/g for Maunjili (Figures 6.8a and 6.8b).

Dextrinisation of Mkondezi starch for 40min at 100°C increased both peak onset (from 45 to 66°C) and peak maximum (from 106 to 126°C) temperatures, getting close to those of amylmaize starch. Pyroconversion of Mkondezi starch led to a reduction in ΔH_G from 320 to 222J/g (Figure 6.8b).

Table 6.3 Effect of pyroconversion on granular sizes of cassava starch from Malawian cassava varieties and two controls

Variety	Native					40min					50min					60min				
	Mean	SD	Mode	Min.	Max.	Mean	SD	Mode.	Min.	Max.	Mean	SD	Mode	Min.	Max.	Mean	SD	Mode	Min.	Max.
Mbundumali	14.93	2.949	12.9	10.0	22.9	14.86	2.946	15.7	10.0	20.0	14.14	2.659	15.7	10.0	18.6	15.86	2.853	14.3	11.4	22.9
83350	13.86	2.746	14.3	8.6	20.0	13.71	2.336	12.9	10.0	18.6	13.36	2.138	12.9	8.6	17.1	13.79	2.941	10.0	10.0	18.6
LCN8010	15.07	2.761	14.3	10.0	20.0	15.21	3.084	14.3	8.6	21.4	17.64	5.083	12.9	10.0	30.0	14.71	1.860	15.7	11.4	17.1
Mkondezi	16.18	2.916	17.1	11.4	22.9	13.93	3.930	12.9	7.1	24.3	15.07	2.721	12.9	11.4	20.0	15.57	3.371	14.3	10.0	22.9
81/00015	14.43	3.140	12.9	8.6	20.0	14.71	3.043	14.3	10.0	22.9	16.71	3.825	20.0	8.6	22.9	17.14	3.679	20.0	11.4	22.9
Silira	11.79	2.312	12.9	8.6	18.6	11.36	2.600	12.9	7.1	17.1	12.00	2.597	8.6	8.6	15.7	10.64	1.427	10.0	7.1	12.9
Sauti	14.64	3.819	14.3	8.6	24.3	13.29	2.823	14.3	8.6	20.0	13.79	3.568	14.3	7.1	20.0	13.00	3.036	12.9	8.6	20.0
CH92/082	13.86	3.078	14.3	8.6	18.6	11.57	2.699	10.0	7.1	15.7	12.57	2.726	11.4	8.6	18.6	12.14	3.022	14.3	7.1	17.1
Maunjili	12.64	3.687	15.7	5.7	18.6	13.36	3.383	15.7	7.1	18.6	13.36	2.465	14.3	8.6	18.6	14.21	3.919	12.9	5.7	20.0
TMS4(2)1425	10.14	2.738	10.0	4.3	14.3	11.50	2.559	11.4	5.7	15.7	10.93	2.633	12.9	7.1	17.1	13.07	3.014	11.4	8.6	18.6
Amylmaize starch	11.79	4.747	12.9	4.3	21.4	11.79	11.790	4.7	12.9	4.3	11.790	4.747	12.9	4.3	21.4	11.79	4.747	12.9	4.3	21.4
Maize starch	13.25	4.245	12.9	4.3	22.9	13.25	13.250	4.2	12.9	4.3	13.250	4.245	12.9	4.3	22.9	13.25	4.245	12.9	4.3	22.9

SD = standard deviation; Min. = minimum; Max. = maximum; min = minutes

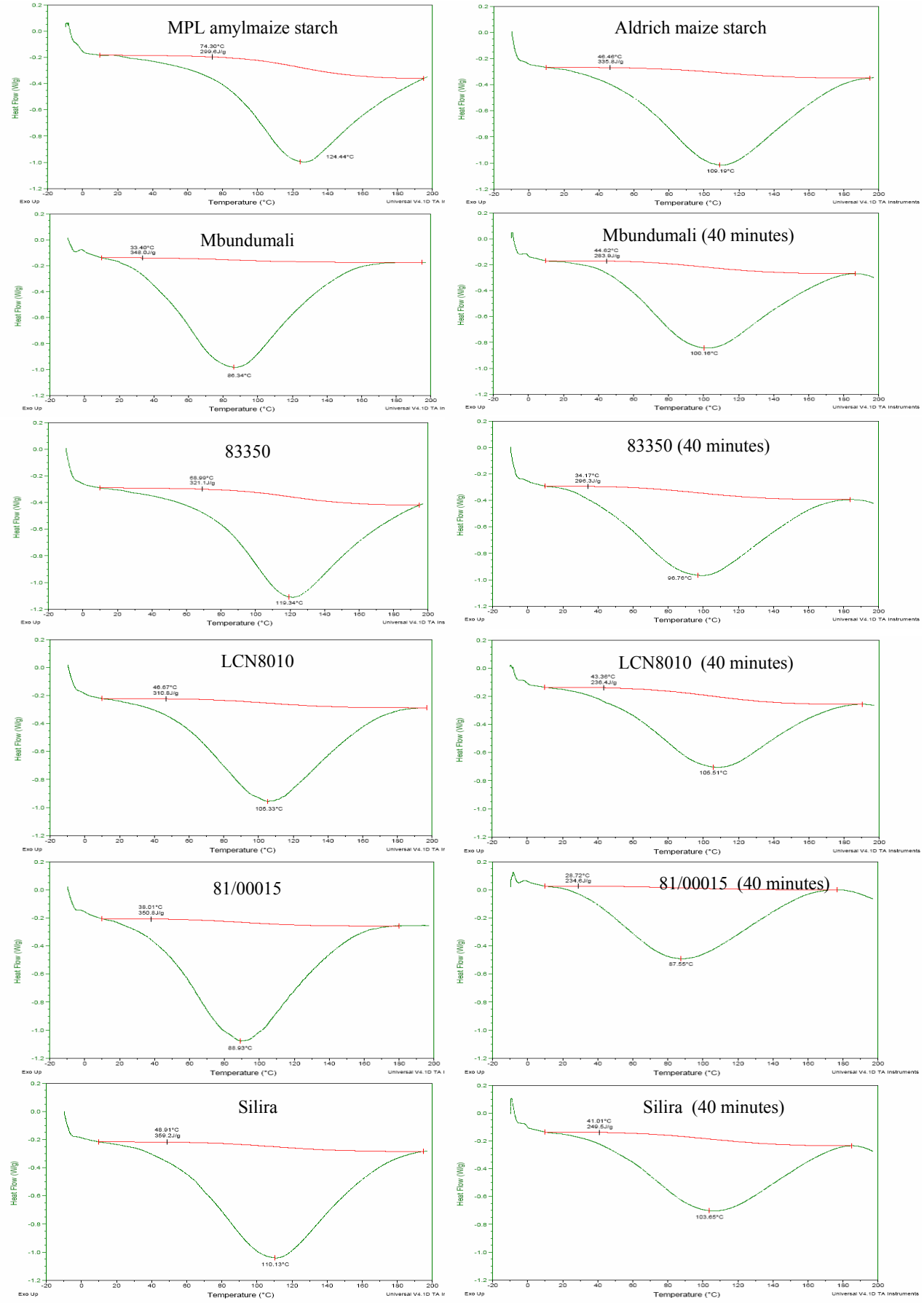


Figure 6.8a DSC thermograms for gelatinisation of native cassava starch from Malawian cassava genotypes and two controls

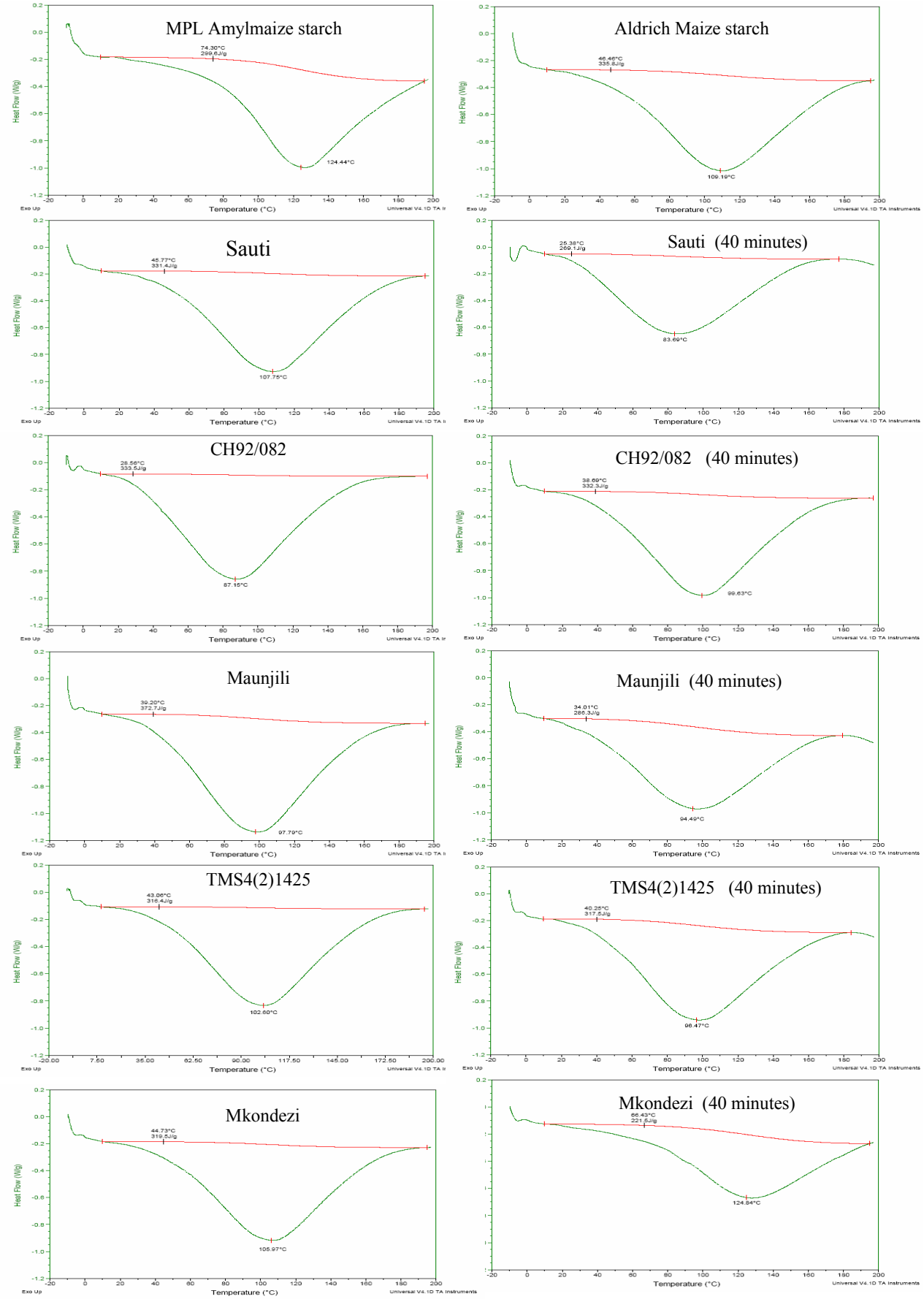


Figure 6.8b DSC thermograms for gelatinisation of native cassava starch from Malawian cassava genotypes and two controls

Dextrinisation of 81/00015 starch for 40min at 100°C led to a reduction in ΔH_G from 331 to 235J/g, and peak onset temperature from 38 to 29°C. The peak maximum temperature was not affected by pyroconversion (Figure 6.8a).

Dextrinisation of Sauti starch for 40min at 100°C led to a reduction in ΔH_G from 331 to 269J/g, peak onset temperature from 46 to 25°C, and peak maximum temperature from 108 to 84°C (Figure 6.8b).

Dextrinisation of CH92/082 starch for 40min at 100°C increased both peak onset (from 29 to 39°C) and peak maximum (from 87 to 100°C) temperatures, but ΔH_G was not affected by pyroconversion (Figure 6.8b).

Results indicated that the tested Malawian cassava genotypes produced starch which differed greatly in functional properties for both native and modified starches. Different cassava genotypes responded differently to pyroconversion. Starch from some varieties were resistant to dextrinisation while others were easily dextrinised, and peak onset and peak maximum temperatures were increased for some genotypes and ΔH_Gs, while it was the opposite for others. Shapes of DSC scans differed greatly among genotypes and pyroconversion. Hence, there exists a need to modify starch to meet diverse needs of various industries. The period of dextrinisation depends on the intended use of the starch or pyrodextrin, and the cassava variety from which the starch is extracted.

6.4 Conclusions and recommendations

Microscopic examination and DSC analysis revealed that Mkondezi, Sauti, 83350, LCN8010, CH92/082 and Maunjili had large starch granules, while Silira and TMS4(2)1425 had the smallest starch granules. Starch granule sizes of cassava starch from the 10 Malawian cassava genotypes were generally medium to small, and were within the values as reported in literature (4-35μm). Pyroconversion of cassava starch from different cassava varieties did not affect granule sizes and shapes, but loss of focus of the starch granules under a light microscope was observed with time of dextrinisation. The lack of change in granule size and shape after pyroconversion is of great importance to industries since the same machine which is used for native starch can be used for pyrodextrins without any problem like clogging.

A close relationship between solubility and reduced sugar values was observed. Results indicated that the critical period for dextrinisation of cassava was between 40 and 60min. Dextrinisation of cassava starch from nine Malawian cassava genotypes for 40, 50 and 60min at 100^{o}C indicated that there existed diversity in terms of resistance of the starch to dextrinisation. Starch from 81/00015, Silira, Mbundumali and Sauti were easily dextrinised, that is, within 60min and maintained their white colour, bland taste and odour. Starch from LCN8010, 83350, Maunjili, TMS4(2)1425 and Mkondezi were resistant to dextrinisation. The appropriate time for dextrinisation of cassava starch at 100^{o}C with the aim of producing white dextrin should be within 70min. Dextrinisation of easily dextrinised starch for longer than 75min at 100^{o}C leads to repolymerisation. Starches resistant to dextrinisation need to be dextrinised for longer than 70 minutes although this resulted in a corresponding change in colour and odour of the pyrodextrin during standardisation of pyrocoversion. Hence, starches resistant to dextrinisation are desired by those who use yellow dextrins and British gum since the long period of dextrinisation leads to change in colour without causing repolymerisation of the pyrodextrin. There exists a need to establish the appropriate time to dextrinise each of the resistant starches and the corresponding changes in colour and odour that occur with period of dextrinisation.

Cassava starches generally had lower gelatinisation temperatures, and higher enthalpies of gelatinisation than for amylmaize starch except for 83350. Starches with low gelatinisation temperatures are preferred for use in hot-setting adhesives in making of corrugated boxes, as it requires the starch granule to gelatinise rapidly and develop the tack needed to hold the board together as it moves through the process. In addition, less heating is required to gelatinise cassava starch which leads to saving of energy.

DSC scans indicated that native starch from 83350, and pyrodextrin from Mkondezi starch, which was dextrinised for 40min at 100^{o}C, had the same thermogram, peak onset temperature and peak maximum temperature as for amylmaize starch. This is important to both the nation and industries since amylmaize starch is imported and the landing cost is very high. The use of native starch from 83350 and pyrodextrin from Mkondezi will save a lot of foreign currency. The locally produced starches which meet the required specifications will be cheaper, which in turn will make the products affordable to consumers thereby improving the wellbeing of Malawians. There exists a need to conduct trials for specialised cassava starches in various specialised industries like pharmaceutical and production of adhesives. DSC monitors changes in the physical and chemical properties of starches, offering a thermodynamic approach to the study of starch gelatinisation. Several positive and significant relationships between starch thermal

properties were measured using DSC and pasting properties. DSC peak onset temperature has been reported to be highly and positively correlated with peak viscosity, trough and final viscosity, and loss of birefringence have been reported. DSC is therefore an important technique to study gelatinisation of starch, since it sums up most of the different starch variables and gives a complete picture of the structural and functional properties of starch.

The tested cassava genotypes produced starch with diverse functional properties from the sampled genotypes. Hence, it is possible to exploit Malawian cassava germplasm to meet a wide range of specialised starch uses with native starches from different varieties, or through conventional breeding, to produce suitable varieties which can produce starch tailored for specific uses. Pyroconversion of cassava starch was shown to increase the diversity of starch functional properties to meet most specialised uses of starch.

There exists a need to characterise starches from accessions of the entire Malawian cassava germplasm collection to determine the extent of variation of starches from the collection which will address the diverse needs of various industries.

CHAPTER 7

COMPARISON AND CONSOLIDATION OF DIVERSITY ANALYSES OF MALAWIAN CASSAVA GERMPLASM

7.1 Introduction

Tropical root and tuber crops are cultivated over large areas in different parts of the world and have been an important staple or subsidiary food especially for the low-income group. Recently, the importance of these crops in the industrial sector has been recognised. They are rich in starch and cassava has the highest starch content, and can form the major source of various intermediate products including flour, starch and dextrins for food, feed, confectionery, wood, pharmaceutical, adhesives, explosives, and other industrial uses. In addition, root and tuber crops' starches, especially cassava, have some special properties not found in cereal starches (Moorthy, 1994). The performance of these products in food, feed and other industries vary according to the crop or variety from which the product was obtained (Benesi *et al*., 2003; Singh *et al*., 2005). Hence, the wider the genetic diversity the higher the chances of obtaining starches and flour with various functional properties that meet the needs of different industries.

Genetic diversity analysis of cassava germplasm is important for conserving, evaluating, and utilising genetic resources, and for proper handling of seed certification programmes (Frankel, 1989). Characterisation allows the study of germplasm diversity as a possible source of genes that can improve the performance of cultivars. In addition, diversity analysis can determine the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes with the purpose of protecting the breeder's intellectual property rights (Franco *et al*., 2001; Subudhi *et al*., 2002). In the past, plant breeders made selections of breeding material based on morphological characteristics that were readily observable and co-inherited with the desired trait. Although these types of morphological characterisation provided useful information to users, they are subjected to environmental influences, time-consuming, must be assessed during a fixed vegetative phase of the crop, and are subjective (Morell *et al*., 1995; Swanepoel, 1999). In addition, morphology is not efficient to discriminate between closely related genotypes (Perkin-Elmer Corporation, 1997). Conversely, DNA characterisation techniques are accurate detectors, and independent on the environment and crop growing cycle (Kumar, 1999). However, they

require specialised knowledge, laboratory equipment and chemical supplies making them more expensive than morphological descriptors.

Analyses of plant genomes using DNA markers allow breeders to rapidly develop crop varieties with enhanced productivity (Altaf-Khan *et al*., 2002). Genome studies provide a number of practical applications, like variety identification through DNA fingerprinting, development of genetic maps that facilitate indirect selection of economically important traits (for example, disease resistance) without cumbersome screening, cloning of important genes, and evolutionary and phylogenetic studies. AFLP is a powerful, reliable, stable and rapid assay with genome mapping applications (Guthridge *et al*., 2001; Altaf-Khan *et al*., 2002). Its analysis offers a rapid and efficient technique for detecting large numbers of DNA markers, and construction of high-density molecular linkage maps of plant genomes. The efficiency of generating AFLP markers appears to be much higher compared to other DNA markers. Most AFLP markers are dominant and show Mendelian inheritance (Maughan *et al*., 1996; Kumar, 1999). AFLP markers have proved to be efficient and reliable in supporting conventional plant breeding programmes. Marker-assisted breeding or selection (MAS) offers the potential of deploying favourable gene combinations and predicting better outcomes (Kumar, 1999; Guthridge *et al*., 2001).

The objectives of this study were to (1) compare the discrimination power of AFLP and morphological markers in cassava genetic diversity analysis, (2) compare the results of analysing genetic diversity for cassava using cluster analysis and principal component analysis (PCA) with the aid of NTSYS computer package, and (3) relate genetic diversity of cassava analyses of Malawian cassava genotypes using AFLP and morphological markers with starch extraction, starch characterisation, and pyroconversion.

7.2 Materials and Methods

7.2.1 Ethnobotany and distribution of cassava pests and diseases in Malawi

Exploration of cassava germplasm, which included assessment of severity and distribution of cassava diseases and pests, were done as described in section 3.2.1. Ethnobotany data was collected as outlined in section 3.2.1.

7.2.2 Morphological markers

Twenty eight cassava accessions described in section 4.2.1 were used. Morphological characterisation and conversion of morphological data into a binary matrix were done as described in section 3.2.2.

7.2.3 DNA analysis

Twenty eight cassava accessions described in section 4.2.1 were used. AFLP analysis methods used for AFLP diversity assessment were the same as described in section 4.2.2.

7.2.4 Starch extraction parameters

Materials and methods described in section 5.2 were applied.

7.2.5 Data analysis

Univariate and multivariate analyses were done using morphological as well as AFLP data. Similarities for morphological and a combination of AFLP and morphological data were calculated using Dice similarity coefficient (Dice, 1945; Nei and Li, 1979) with the aid of NTSYSpc version 2.11c computer package (Rohlf, 2000), and Euclidean distances were obtained using NCSS 2000 (Hintze, 1998). Dendrograms were constructed using UPGMA clustering in the SAHN programme parameters under NTSYSpc computer package (Rohlf, 2000) and NCSS 2000. Principal Component Analysis (PCA) was performed to analyse correlations (Pearson product-moment correlations), followed by generation of Eigenvectors and Eigen values, and PCA projections were constructed using NTSYSpc computer package. Comparison of morphological and AFLP analysis was done by correlating Dice similarity confidents for each, using Agrobase (2000).

Marker techniques were statistically compared by applying linear correlations using Agrobase (2000). ANOVA was performed on root dry matter content, starch yield, starch extraction rate on wet and dry matter basis, starch content, and TSS, of each of the individual trials, using Agrobase (2000). Combined ANOVAs were performed on the pooled data of all starch extraction trials at Chitedze, Chitala, Mkondezi and Makoka research stations across two years and three rounds of starch extractions. Significant genotype by environment interactions for

starch extraction traits were managed using additive main effects and multiplicative interactions (AMMI) performed with Agrobase (2000).

7.3 Results and discussion

7.3.1 Comparison of NTSYS and NCSS computer programmes

NCSS statistical computer package generated a similar dendrogram as NTSYS statistical computer package although some minor differences were observed (Figures 7.1 and 7.2). Although Kachamba broke away from the rest of the accessions in cluster 1, it was still within cluster 1 with NTSYS analysis (Figure 7.1) but it formed its own cluster III with NCSS analysis (Figure 7.2). Correlation of actual and dendrogram genetic distances generated by NCSS showed positive and significant correlation of r=0.645. The difference between actual and dendrogram genetic distances ranged from -48.57 to 21.48% (Appendix 4). The divergence is large which can lead to errors when dendrograms are interpreted using actual genetic distances instead of dendrogram genetic distances. Results implied that the use of actual genetic distances when interpreting dendrograms is not quite correct. NTSYS was able to generate three-dimensional PCA plots, which could be rotated in any direction giving a better definition structure than a two-dimensional PCA plot, which is generated by NCSS, let alone a one-dimensional dendrogram generated by NTSYS or NCSS. Therefore, NTSYS is being recommended for use in PCA analysis of germplasm diversity analysis. Any of the two computer packages can be used for cluster analysis.

7.3.2 Cluster analysis and genetic distances

7.3.2.1 *Morphological cluster analysis using both NTSYS and NCSS*

Morphological data for 28 selected accessions was converted into a binary matrix and used to generate similarity matrix of coefficients (Table 7.1) followed by UPGMA clustering of accessions using NTSYS (Figure 7.1) and NCSS (Figure 7.2). The resulting dendrograms revealed two clusters, I and II. Most of the accessions belonged to cluster I which further formed two major sub-clusters A and B (Figures 7.1 and 7.2).

Cluster I sub-cluster A group i contained four accessions of which three were local cultivars and TMS4(2)1425 was an introduction from IITA (Figures 7.1 and 7.2). Mgwalangwa and Manyokola5 were collected from the south and Mbundumali from the centre of Malawi. The

Table 7.1 Genetic distances for morphological (below diagonal) and a combination of AFLP and morphological (above diagonal) Dice based on Dice similarity coefficients for 28 characterised accessions

Genotypes	Manyokola5	Mwatatu	Gomani2	Beatrice	Nyachikundi	Kachamba	Fyoka	Nyainki	Twentetwente	MasoAzungu	Masangwi	Mgwalangwa	Matuvi	Thipula
Manyokola5		0.878	0.829	0.854	0.827	0.874	0.817	0.842	0.821	0.834	0.839	0.863	0.839	0.851
Mwatatu	0.583		0.869	0.844	0.862	0.859	0.807	0.861	0.825	0.813	0.815	0.825	0.814	0.847
Gomani2	0.417	0.500		0.833	0.868	0.859	0.809	0.857	0.817	0.816	0.803	0.813	0.821	0.851
Beatrice	0.500	0.583	0.250		0.839	0.845	0.809	0.829	0.810	0.827	0.816	0.835	0.813	0.842
Nyachikundi	0.333	0.333	0.500	0.417		0.880	0.819	0.844	0.834	0.848	0.814	0.834	0.826	0.853
Kachamba	0.500	0.333	0.333	0.417	0.333		0.821	0.868	0.844	0.860	0.840	0.849	0.831	0.846
Fyoka	0.417	0.667	0.583	0.333	0.583	0.167		0.821	0.819	0.816	0.806	0.822	0.838	0.809
Nyainki	0.500	0.583	0.667	0.250	0.417	0.250	0.583		0.851	0.843	0.825	0.828	0.842	0.835
Twentetwente	0.583	0.583	0.500	0.500	0.333	0.250	0.583	0.583		0.845	0.824	0.812	0.830	0.824
MasoAzungu	0.174	0.261	0.348	0.174	0.609	0.087	0.435	0.348	0.174		0.820	0.832	0.842	0.816
Masangwi	0.583	0.667	0.583	0.417	0.333	0.250	0.583	0.667	0.917	0.174		0.882	0.855	0.846
Mgwalangwa	0.750	0.417	0.500	0.417	0.333	0.333	0.333	0.500	0.667	0.174	0.583		0.861	0.870
Matuvi	0.500	0.583	0.667	0.333	0.583	0.333	0.583	0.833	0.583	0.435	0.667	0.500		0.850
Thipula	0.500	0.583	0.333	0.333	0.333	0.250	0.500	0.500	0.417	0.348	0.500	0.583	0.500	
Biliwili	0.500	0.417	0.583	0.250	0.500	0.250	0.500	0.750	0.500	0.435	0.500	0.500	0.750	0.250
Depwete	0.500	0.583	0.667	0.333	0.583	0.333	0.583	0.833	0.583	0.435	0.667	0.500	1.000	0.500
Sekelemani	0.667	0.583	0.333	0.833	0.417	0.417	0.333	0.417	0.667	0.174	0.583	0.583	0.500	0.333
Yizaso	0.333	0.250	0.583	0.083	0.417	0.333	0.333	0.500	0.250	0.435	0.333	0.417	0.417	0.417
TMS4(2)1425	0.750	0.500	0.250	0.500	0.500	0.500	0.333	0.333	0.417	0.261	0.417	0.500	0.500	0.500
CH92/082	0.583	0.667	0.583	0.500	0.333	0.500	0.417	0.417	0.583	0.261	0.667	0.417	0.417	0.333
81/00015	0.583	0.500	0.500	0.667	0.417	0.500	0.333	0.333	0.583	0.261	0.500	0.500	0.333	0.250
Gomani1	0.417	0.500	1.000	0.250	0.500	0.333	0.583	0.667	0.500	0.348	0.583	0.500	0.667	0.333
MK95/054	0.417	0.250	0.583	0.333	0.583	0.167	0.417	0.333	0.417	0.522	0.417	0.500	0.250	0.250
83350	0.750	0.833	0.500	0.583	0.333	0.417	0.500	0.583	0.750	0.174	0.833	0.583	0.583	0.500
LCN8010	0.417	0.583	0.750	0.250	0.417	0.417	0.583	0.500	0.500	0.261	0.583	0.333	0.500	0.333
Mbundumali1	1.000	0.609	0.417	0.500	0.333	0.500	0.435	0.500	0.609	0.174	0.609	0.750	0.500	0.500
Sauti	0.480	0.480	0.560	0.320	0.400	0.400	0.320	0.240	0.320	0.250	0.400	0.400	0.400	0.320
Maunjili	0.500	0.417	0.417	0.500	0.417	0.500	0.333	0.167	0.500	0.261	0.417	0.417	0.333	0.167

Table 7.1 Genetic distances for morphological (below diagonal) and a combination of AFLP and morphological (above diagonal) based on Dice similarity coefficients for 28 characterised accessions (continued)

Genotypes	Biliwili	Depwete	Sekelemani	Yizaso	TMS4(2)1425	CH92/082	81/00015	Gomani1	MK95/054	83350	LCN8010	Mbundumali1	Sauti	Maunjili
Manyokola5	0.839	0.843	0.841	0.844	0.835	0.837	0.827	0.811	0.822	0.826	0.823	0.948	0.820	0.834
Mwatatu	0.824	0.833	0.820	0.835	0.842	0.832	0.825	0.843	0.831	0.839	0.823	0.877	0.812	0.823
Gomani2	0.837	0.839	0.808	0.852	0.837	0.832	0.848	0.939	0.837	0.821	0.834	0.828	0.810	0.821
Beatrice	0.822	0.810	0.881	0.844	0.818	0.819	0.820	0.815	0.806	0.819	0.805	0.840	0.817	0.819
Nyachikundi	0.843	0.835	0.825	0.860	0.837	0.847	0.834	0.849	0.825	0.830	0.805	0.838	0.808	0.828
Kachamba	0.834	0.854	0.826	0.859	0.851	0.854	0.855	0.850	0.830	0.823	0.831	0.878	0.817	0.833
Fyoka	0.815	0.830	0.813	0.823	0.805	0.815	0.792	0.805	0.768	0.793	0.788	0.821	0.805	0.806
Nyainki	0.843	0.841	0.821	0.828	0.825	0.815	0.830	0.854	0.820	0.818	0.838	0.855	0.805	0.824
Twentetwente	0.822	0.814	0.823	0.819	0.808	0.820	0.823	0.814	0.796	0.807	0.804	0.825	0.769	0.813
MasoAzungu	0.827	0.837	0.830	0.833	0.833	0.810	0.821	0.809	0.841	0.813	0.813	0.826	0.799	0.818
Masangwi	0.842	0.859	0.856	0.835	0.802	0.831	0.802	0.804	0.786	0.811	0.813	0.832	0.827	0.837
Mgwalangwa	0.872	0.872	0.868	0.840	0.825	0.839	0.821	0.818	0.813	0.824	0.808	0.857	0.831	0.850
Matuvi	0.874	0.862	0.854	0.826	0.829	0.806	0.809	0.821	0.806	0.810	0.817	0.834	0.799	0.818
Thipula	0.840	0.846	0.838	0.843	0.828	0.835	0.826	0.848	0.815	0.840	0.807	0.850	0.821	0.835
Biliwili		0.872	0.852	0.830	0.809	0.818	0.811	0.851	0.804	0.823	0.807	0.846	0.828	0.837
Depwete	0.750		0.872	0.833	0.826	0.831	0.813	0.832	0.800	0.822	0.818	0.852	0.821	0.833
Sekelemani	0.417	0.500		0.827	0.831	0.821	0.824	0.811	0.826	0.829	0.812	0.840	0.826	0.834
Yizaso	0.250	0.417	0.167		0.866	0.844	0.855	0.858	0.839	0.840	0.830	0.847	0.834	0.851
TMS4(2)1425	0.500	0.500	0.667	0.167		0.830	0.875	0.841	0.870	0.837	0.840	0.834	0.816	0.845
CH92/082	0.250	0.417	0.583	0.417	0.417		0.862	0.842	0.820	0.840	0.836	0.839	0.840	0.851
81/00015	0.250	0.333	0.750	0.333	0.500	0.833		0.861	0.866	0.853	0.847	0.830	0.818	0.846
Gomani1	0.583	0.667	0.333	0.583	0.250	0.583	0.500		0.852	0.827	0.818	0.823	0.823	0.831
MK95/054	0.417	0.250	0.417	0.583	0.333	0.500	0.583	0.583		0.851	0.829	0.814	0.804	0.826
83350	0.417	0.583	0.750	0.333	0.583	0.833	0.667	0.500	0.417		0.841	0.829	0.826	0.864
LCN8010	0.333	0.500	0.333	0.583	0.333	0.667	0.500	0.750	0.417	0.583		0.829	0.832	0.853
Mbundumali1	0.500	0.500	0.667	0.333	0.750	0.609	0.583	0.417	0.417	0.783	0.435		0.831	0.832
Sauti	0.320	0.400	0.400	0.480	0.560	0.640	0.480	0.560	0.400	0.560	0.640	0.500		0.876
Maunjili	0.250	0.333	0.583	0.417	0.500	0.667	0.667	0.417	0.417	0.583	0.667	0.522	0.720	

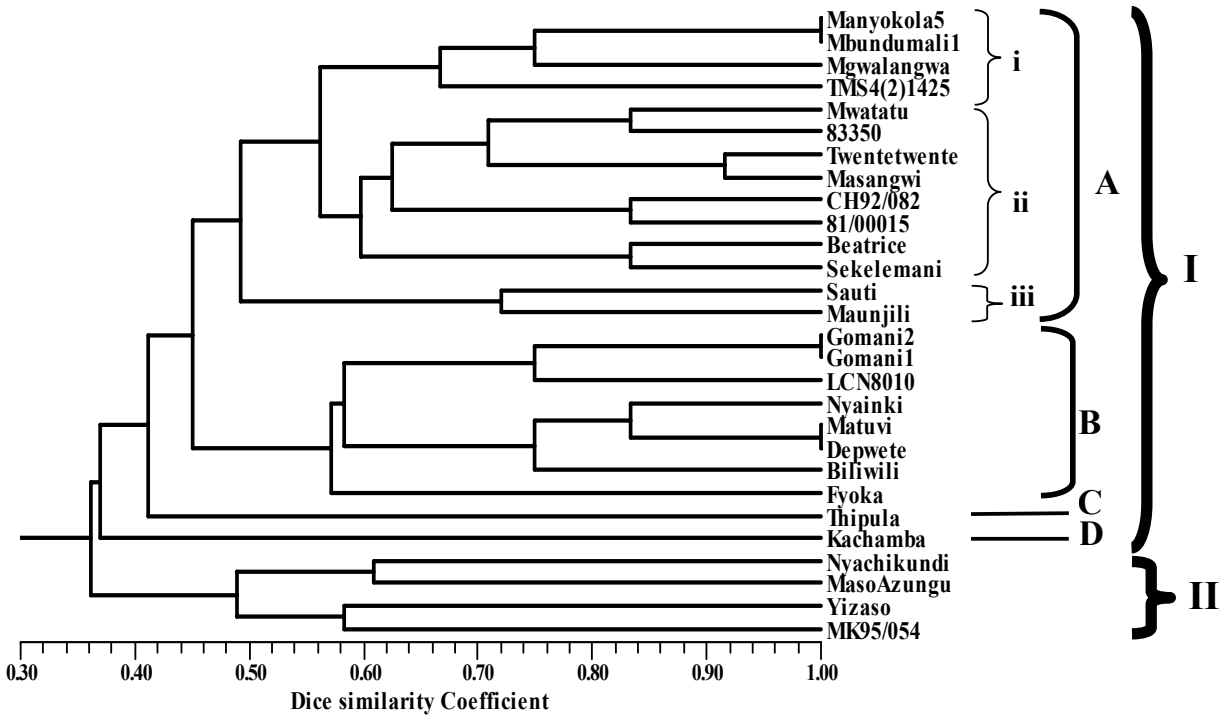


Figure 7.1 Dendrogram for morphological characterisation of 28 analysed accessions using NTSYS computer package, Dice similarity and UPGMA clustering

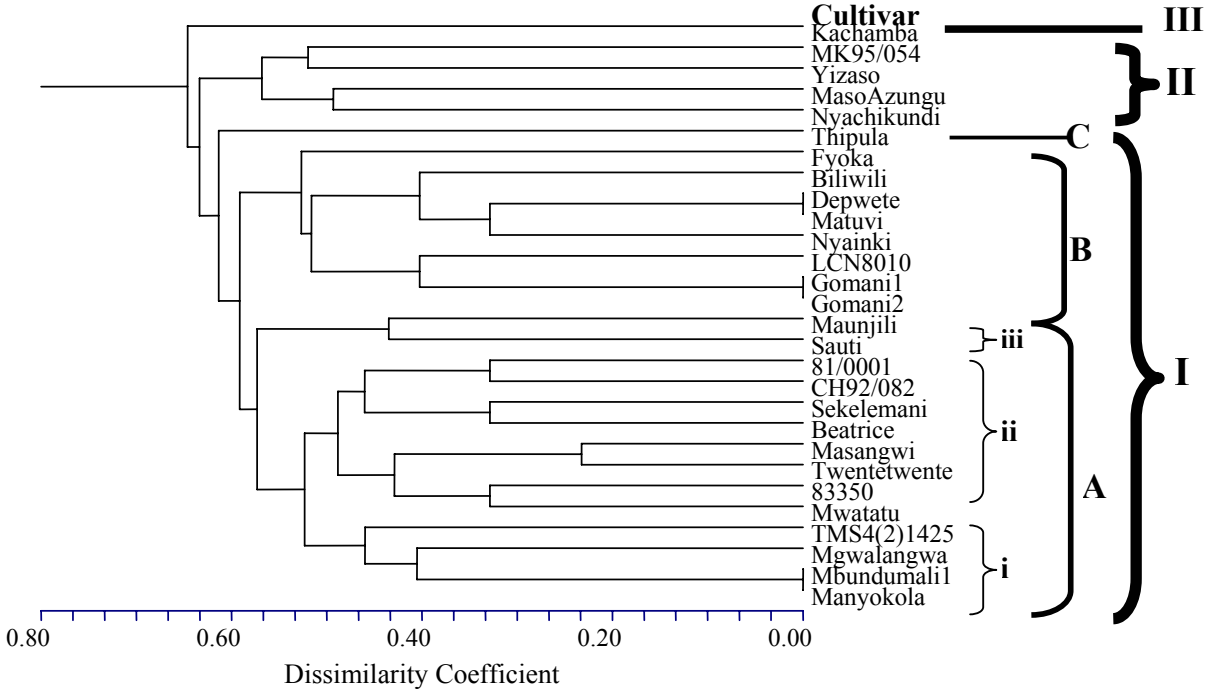


Figure 7.2 Dendrogram for morphological characterisation of 28 analysed accessions with NCSS computer package using Euclidian distances and UPGMA clustering

closest accessions in this cluster were Mbundumali1 and Manyokola5 with a GS of 1.000 (Table 7.1 and Figure 7.1) and a genetic distance (GD) of 0.000 (Figure 7.2 and Appendix 4). Accessions in this cluster were characterised by silvery green mature stems, white root outer and inner skin colours and green shoot tips.

Cluster I sub-cluster A group ii contained eight accessions of which five were local cultivars (Figures 7.1 and 7.2). Twentetwente and Sekelemani were collected from the north, Mwatatu and Beatrice from the centre and Masangwi from the south of Malawi. CH92/082 was locally screened. Accessions 81/00015 and 83350 were introductions from IITA. The closest accessions in this cluster were Masangwi and Twentetwente with a GS of 0.917 (Table 7.1 and Figure 7.1) and a GD of 0.232 (Figure 7.2 and Appendix 4). Accessions in this cluster were characterised by hairless apical unexpanded leaves and green shoot tips.

Cluster I sub-cluster A group iii contained Sauti and Maunjili (Figures 7.1 and 7.2). Seed for Sauti (CH92/077) and plantlets for Maunjili (TMS91934) came from IITA and might have been developed from a common gene pool. These accessions were characterised by green mature leaves, elliptic shaped central lobes, mainly green but with some pale red sllading petioles, light brown to orange mature stems, medium height of first branches, dark brown outer root skin colour, white inner root skin colour and green shoot tips.

Cluster I sub-cluster B contained eight accessions of which seven were local cultivars (Figures 7.1 and 7.2). Fyoka, Biliwili, Nyainki, and Depwete were collected from the north, Gomani1 and 2 from the centre and Matuvi from the south of Malawi. LCN8010 was an introduction from IITA. The closest accessions in this cluster were Depwete and Matuvi, and Gomani1 and 2, which were morphologically similar either using NTSYS or NCSS with a GS of 1.000 (Table 7.1 and Figure 7.1) and a GD of 0.000 (Figure 7.2 and Appendix 4). Accessions in this cluster were characterised by purple apical unexpanded leaves, dark green mature leaves and white root inner skin.

Cluster I sub-cluster C contained only Thipula, which broke away from other accessions in cluster I (Figures 7.1 and 7.2). Cluster I sub-cluster D contained Kachamba in NTSYS dendrogram (Figure 7.1) while it formed its own cluster III in the NCSS dendrogram (Figure 7.2). Morphological characterisation indicated that these two accessions were genetically unique since they did not associate with any heterotic group and are therefore the best candidates as parents in a breeding programme.

Cluster II contained four accessions, of which MasoAzungu and Nyachikundi were collected from the north of Malawi, while Yizaso and MK95/054 were locally screened but their seeds came from IITA (Figures 7.1 and 7.2). Accessions in this cluster were grouped according to geographic origin. The wide diversity in this cluster gives opportunity for use in a breeding programme by direct selection or crosses. It is therefore not surprising that Yizaso was selected and officially released in Malawi. Clustering according to geographic origin and the formation of clear heterotic groups is of importance in establishing a core collection with the aim of reducing the number of accessions to be conserved in a field gene bank while maintaining the wide genetic diversity since management of field gene bank is expensive.

7.3.2.2 *AFLP cluster analysis using NTSYS*

AFLP cluster analysis using NTSYS was discussed in chapter 4 in terms of clustering patterns (Table 4.5 and Figure 7.3).

7.3.3 Comparison of morphological, AFLP and a combination of AFLP and morphological markers analysed using NTSYS

7.3.3.1 *Dendrograms and genetic distances*

Morphological versus AFLP dendrograms and Dice similarity matrices

Morphological and AFLP dendrograms and similarity matrices were similar for many clusters and genetic distances. Gomani1 and 2, and Mbundumali1 and Manyokola5 clustered together in both dendrograms and were the most similar accessions based both on morphological and AFLP markers. However, Matuvi and Depwete that were 100% similar based on morphological data (Figure 7.1) and Dice similarity matrix (Table 7.1), clustered separately based on AFLP data (Figure 7.3) and Dice similarity matrix (Table 4.5) but still within clusters IB. Matuvi, Depwete and Biliwili, as well as Maunjili and Sauti clustered together in both dendrograms. These accessions are morphologically and genetically similar. Therefore, AFLP analysis confirmed morphological characterisation.

Accessions in group III of the AFLP dendrogram grouped together in cluster IA of the morphological dendrogram, except for LCN8010 and MK95/054. Most of the accessions in clusters IB and IIA of the morphological dendrogram (Figure 7.1) clustered together in clusters I and II of the AFLP dendrogram, except for LCN8010, MK95/054 and Fyoka.

Some differences were observed between morphological and AFLP dendrograms and similarity matrices for some clusters and genetic distances. Accessions in group III of the AFLP dendrogram (Figure 7.3) were randomly clustered in the morphological dendrogram (Figure 7.1). Fyoka clustered separately from other accessions in the AFLP dendrogram, but within group IB of morphological dendrogram. Accessions in cluster IIA of the morphological dendrogram clustered separately from other accessions but randomly clustered within the AFLP dendrogram.

Morphological characterisation showed a high range of genetic similarity (GS) values of 0.087-1.000 compared to AFLP with a GS range of 0.778-0.946. The narrow GS range agreed with previous AFLP studies by Fregene *et al*. (2000). Fregene *et al*. (2000) reported a GS range of 0.83-1.00 on cassava and Herselman (2003) a GS range of 0.977-0.997 for groundnut, which were narrower than the GS range of 0.778-0.946 obtained in the current study. Similar results were obtained when other DNA techniques were used by Gepts (1991) on common beans, Miller and Tanksley (1990) on tomato, Shattuk-Eidens *et al*. (1990) on melons, and Keim *et al*. (1990) on soybean. These results could be due to the fact that morphological variations are controlled by a few major genes and may be caused by changes in a few loci (Halward *et al*., 1992) that might be subjected to intense selection pressure. As a result, morphological variation is likely to increase during domestication while molecular markers which are not subjected to direct selection, often decrease (Gepts, 1991). However, these results disagreed with the findings of cassava genetic diversity studies of Roa *et al*. (1997) and Wong *et al*. (1999) who found a wide GS range in cassava using AFLP analysis.

Morphological versus AFLP versus a combination of AFLP and morphological dendrograms and Dice similarity matrices

Sub-clusters for a combination of AFLP and morphological data were more related to the ones for AFLP (Figure 7.3) than morphological analysis (Figures 7.1). However, the major clusters for AFLP and a combination of AFLP and morphological analyses differed in the sense that cluster III of the AFLP dendrogram (Figure 7.3) was included within cluster I in group IB of a combination of AFLP and morphological data (Figure 7.4). Cluster IB of the AFLP dendrogram was included in cluster II of a combination of AFLP and morphological dendrogram. Beatrice and Sekelemani were found to be closely related using morphological analysis with a GS of 0.833 while AFLP analysis allocated Beatrice to cluster IA and Sekelemani to cluster IB (Figures 7.1 and 7.3). A combination of AFLP and morphological analysis clustered Beatrice and Sekelemani together, which supported morphological analysis (Figures 7.1, 7.3 and 7.4). Fyoka's

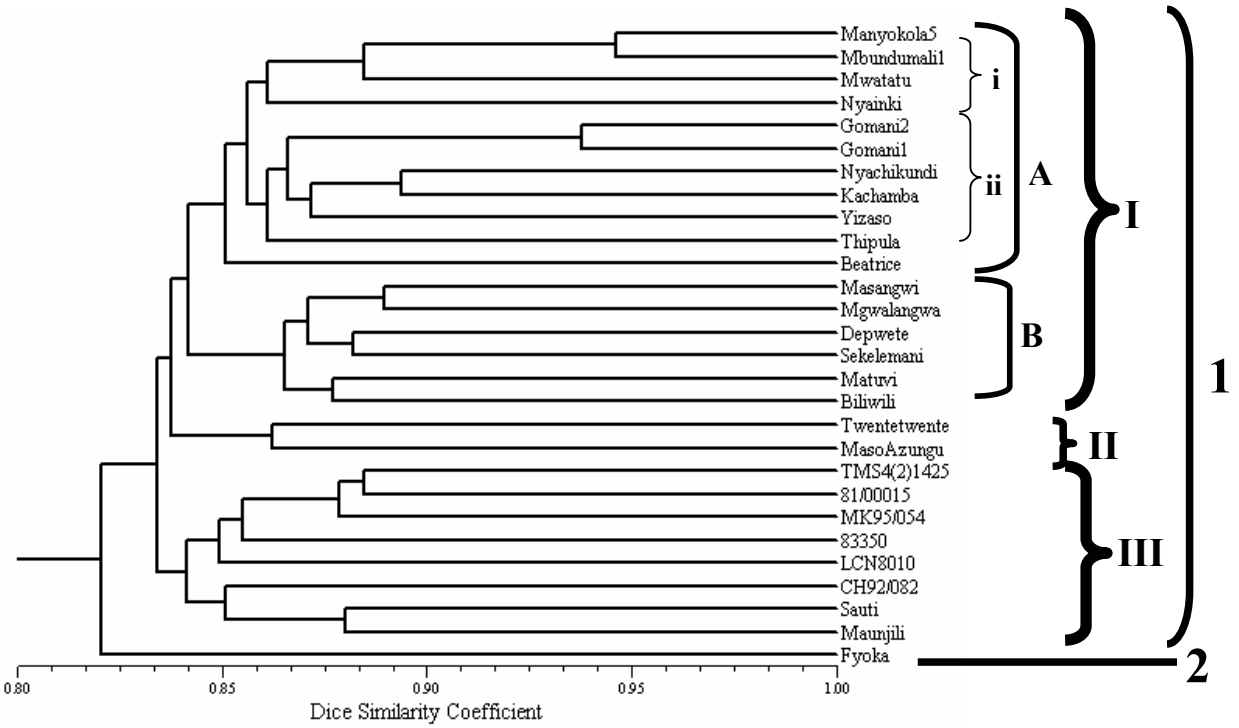


Figure 7.3 Dendrogram for characterisation of 28 analysed cassava accessions using six AFLP primer pairs with the aid of NTSYS computer package, Dice similarity coefficient and UPGMA clustering

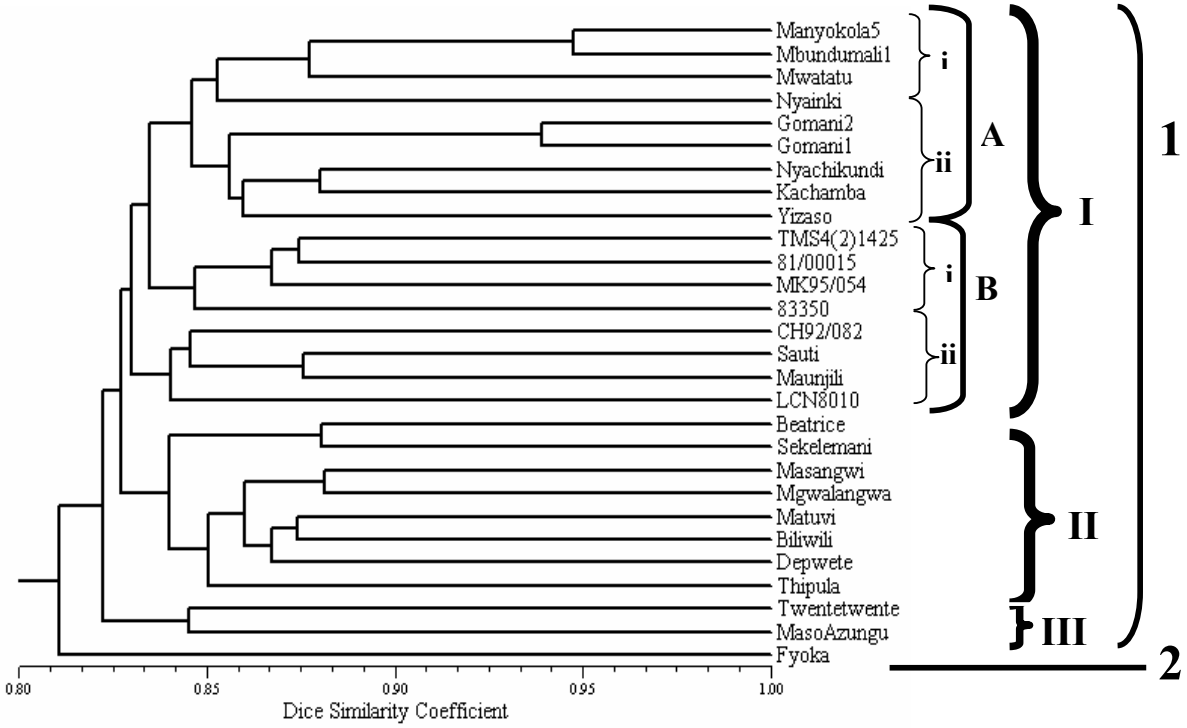


Figure 7.4 Dendrogram for characterisation of 28 analysed cassava accessions using a combination of AFLP and morphological markers with the aid of NTSYS computer package, Dice similarity coefficient and UPGMA clustering

clustering did not change, and MasoAzungu and Twentetwente were clustered together by AFLP and a combination of AFLP and morphological analyses although the cluster moved in the dendrogram when clustering was done using AFLP and a combination of AFLP and morphological markers. These accessions were allocated to different clusters using morphological analysis (Figures 7.1, 7.3 and 7.4). Yizaso and MK95/054 clustered together in the morphological dendrogram (Figure 7.1) but belonged to different clusters with AFLP and a combination of AFLP and morphological analyses (Figures 7.3 and 7.4). Hence, in the combined dendrogram, some accessions rather clustered according to morphological than AFLP data. The cluster with Manyokola5 and Yizaso was identical in both AFLP and a combination of AFLP and morphological dendrograms. Accessions in cluster IB (AFLP) clustered together in a combination of AFLP and morphological dendrogram in cluster II. Thipula's clustering was different in the two dendrograms. Genetic distance were in the same range for both AFLP and combined dendrograms.

The clustering based on a combination of AFLP and morphological analysis largely resembled that of AFLP analysis, probably due to the large number of data points obtained from AFLP analysis of 623 markers compared to 41 markers for the morphological matrix. As a result, a greater degree of discrimination was obtained with AFLP than morphological analysis alone, as suggested by Nemara (2003). Swanepoel (1999) and Roldan-Ruiz *et al*. (2001) suggested that the combination of morphological and molecular markers could serve as a major source of information in separating closely related accessions in different crops. Hence, germplasm characterisation using a combination of AFLP and morphological data is better because more data points are achieved and both AFLP and morphological markers are incorporated which results in a more accurate diversity analysis than using only one of them.

7.3.3.2 *Principal component analysis versus cluster analysis*

Grouping of accessions using cluster analysis and PCA were similar but not completely congruent (Figures 7.1, 7.3-7.7). Dendrograms as well as PCAs revealed similar major clusters. PCA gave a better definition structure than the dendrogram since PCA used three dimensions, which compared to only one for a dendrogram. The better presentation using PCA was observed by the difference in relatedness of some accessions. For example, when morphological data was used, Kachamba and Fyoka were distant in the dendrogram (GS of 0.167; Table 7.1) but a PCA plot revealed that they were not as distant as the dendrogram portrayed (Figures 7.1 and 7.5). Gomani1, Gomani2 and LCN8010 formed a cluster (IB) with Matuvi, Depwete, Nyainki,

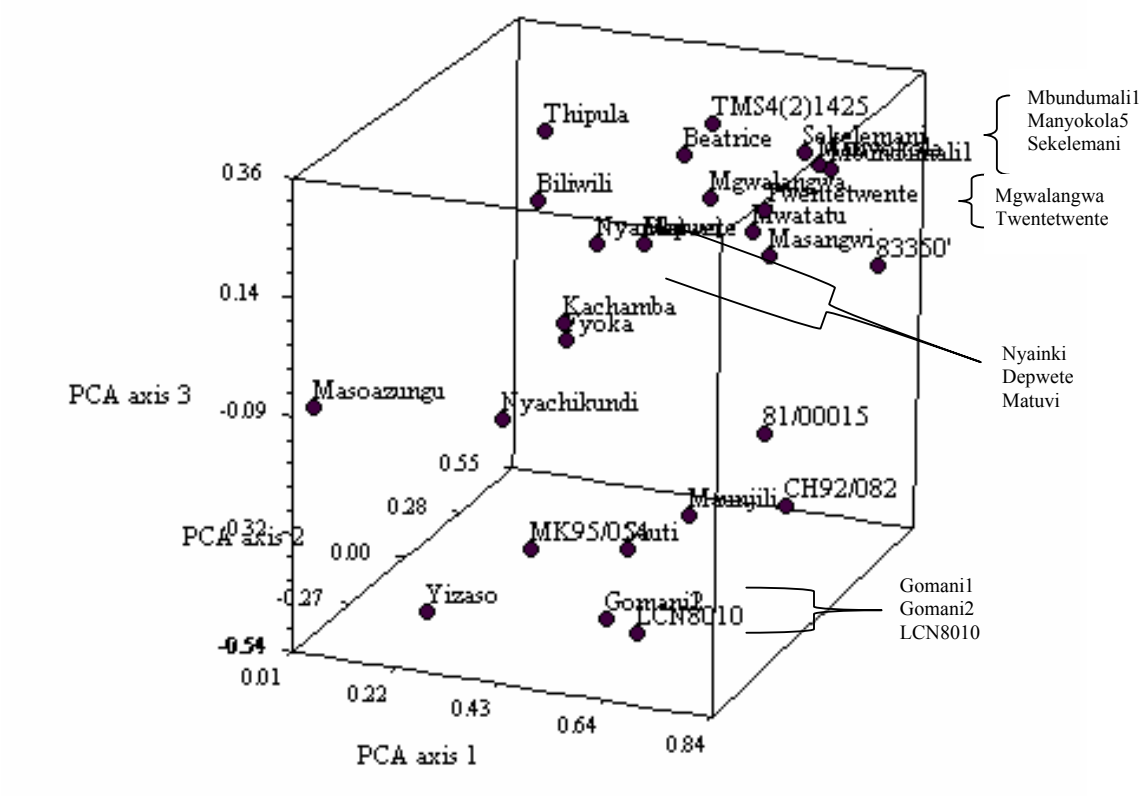


Figure 7.5 PCA plot for characterisation of 28 analysed cassava accessions using morphological markers with the aid of NTSYS computer package

Biliwili and Fyoka in the dendrogram but clustered separately from those accessions in the PCA plot (Figures 7.1 and 7.5, and Table 7.1).

The same trend was observed with AFLP analysis where the major clusters were similar for cluster and PCA analyses although the degree of definition varied with some accessions. For example, locally screened and introduced clones were grouped together in cluster III in Figure 7.3 and in the PCA plot (Figure 7.6). Mbundumali1 and Manyokola5 were closely related in both the dendrograms as well as PCA plots but Gomani1 and 2 were closer in the dendrogram compared to the PCA plot (Figures 7.3 and 7.6). Twentetwente and MasoAzungu clustered closer to group IB than group IA in the dendrogram but clustered with the accessions of group IA in the PCA plot. Fyoka that clustered separately from all accessions in the dendrogram clustered between accessions of groups IA and IB in the PCA plot. Similar trends were observed when the combined dendrogram and PCA plot were compared (Figures 7.4 and 7.7).

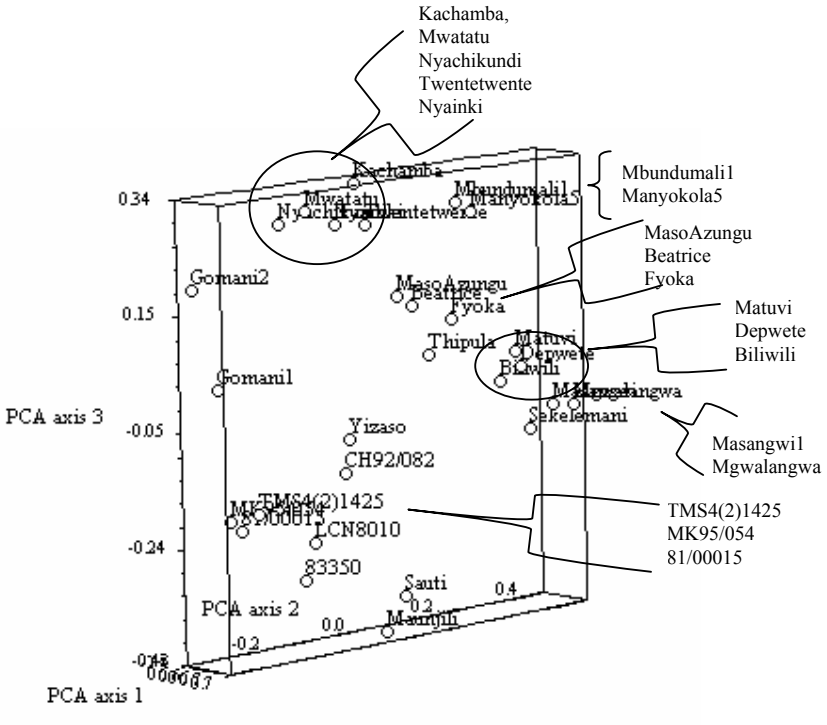


Figure 7.6 PCA plot for characterisation of 28 analysed cassava accessions using all six AFLP primer pairs

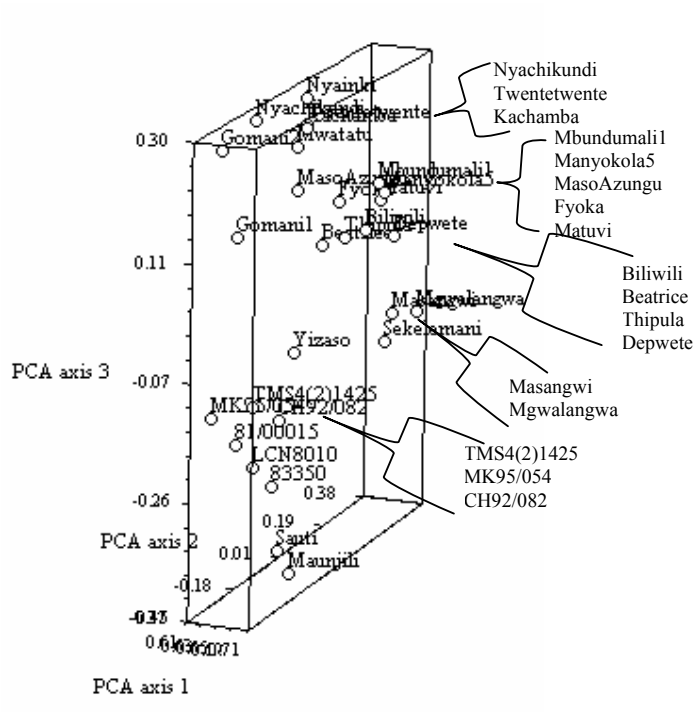


Figure 7.7 PCA plot for characterisation of 28 analysed cassava accessions using a combination of AFLP and morphological markers

7.3.3.3 *Correlations and congruencies of marker techniques*

Correlations between genetic distance matrices based on AFLP, morphological and a combination of AFLP and morphological traits were significant (Table 7.2). The correspondence might be because clustering of Manyokola5 and Mbundumali1, Matuvi, Depwete, and Biliwili, Gomani1 and 2, and Sauti and Maunjili, and TMS4(2)1425, 81/00015, CH92/082, Sauti and Maunjili clustered together in the main groups of all three dendrograms and PCA plots (Figures 7.1, 7.3-7.7), which was confirmed by significant correlation of the two marker techniques. In addition, morphological as well as AFLP diversity analyses indicated that Thipula and Fyoka were distantly related from the other accessions (Figures 7.1-7.4). These results showed that a high degree of relationship existed between AFLP and morphological genetic diversity analyses methods. In similar studies, Benesi (2002) reported a strong correlation between AFLP and morphological analyses on cassava. Nemara (2003) reported a significant correlation between AFLP and morphological genetic diversity analyses on sorghum. Tatineni *et al*. (1996) reported a high correlation between RAPD and morphological characters on cotton.

Table 7.2 Correlation matrix for AFLP and morphological genetic diversity analyses

	AFLP markers	Morphological traits
Morphological traits	0.562 ***	
Combination of AFLP and Morphological traits	0.981 ***	0.554 ***

*** $p \leq 0.001$

These results disagreed with findings of similar studies by Adugna (2002) on linseed, Ben-Har *et al*. (1995) and Swanepoel (1999) on maize inbred line collections, and Roldan-Ruiz *et al*. (2001) on perennial ryegrass. Their results indicated that these two methods were different and highly variable and might be due to the inclusion of more polygenic compared to salient traits found in descriptors. Adugna (2002) reported that the constituents of the clusters in his study were different mainly due to the major effects of environment on the 12 quantitative traits used for morphological descriptors, which suggested a shift from morphological to agronomical characterisation of linseed. Quantitative traits controlled by a number of genes with small effects are tremendously influenced by the environment (Poehlman, 1987). The correspondence between morphological and AFLP analyses found in the current study might be due to the fact that mainly salient traits, as given in the descriptors which was developed by IPGRI, were used as recommended by Berthaud (1997) and Elias *et al*. (2001) since they are less affected by the environment and developmental stage of the plant. AFLP uses the same principle of revealing salient fragments, which typify individuals or populations. Hence, one cannot expect to obtain

significant correlation when polygenic traits are compared with AFLP analysis. Salient traits should be compared with AFLP analysis (Elias *et al*., 2001) while polygenic traits should be compared with quantitative trait loci (QTLs). The other reason why other studies found no correspondence between AFLP and morphological characterisation could be due to the problem of conversion of morphological data into a binary matrix. Most researchers do not use salient traits like colour since they do not know how to covert them into a binary matrix and most statistical computer packages dealing with genetic distance analyses use binary data. Sometimes even quantitative traits are not converted correctly into a binary matrix. Results of such analyses cannot be related to those of AFLP analysis. Hence, it is not surprising that Ben-Har *et al*. (1995), Swanepoel (1999), Roldan-Ruiz *et al*. (2001) and Adugna (2002) emphasised that results of their studies should not be regarded as indications of weaknesses or limitations of these two systems. These authors indicated that the characterisation using morphological and AFLP analyses independently provided the overall interrelationship of the studied accessions.

7.3.4 Comparison of genetic diversity analysis with evaluated characteristics

7.3.4.1 *Ethnobotany*

Farmers reported diverse characters which assisted them in the identification and selection of preferred cultivars and for economic gains. These characters ranged from sweet/bitter, maturity period, food quality, processing amenability and suitability in intercropping with various crops and cropping systems. Cassava accessions were diverse in each of these characters. The entire range of cultivars were available in the farmers fields. For example, cultivars ranged from very sweet to very bitter, from early maturing to late maturing, from susceptible to resistant to cassava diseases and pests prevalent in Malawi, low and high yielding cultivars, unstable and stable. Ethnobotany recorded high levels of phenotypic variation of cultivars in farmers fields. This correlated with high levels of genetic diversity detected using morphological analysis (GS of 0.083-1.000), in contrast with low levels of diversity detected using AFLP analysis (GS of 0.778-0.946). This could be due to the fact that farmers do selection based on morphological characters which are also used in morphological characterisation.

7.3.4.2 *Starch extraction potential*

The diversity of Malawian cassava germplasm was reflected in starch extraction, starch characterisation and pyroconversion studies. Cassava starch extraction from 10 elite genotypes which represented a wide genetic diversity within a working collection, showed wide variation in

starch extraction potential. AMMI analysis showed a diversity within 10 genotypes in terms of stability of genotypes. Both stable and unstable genotypes in terms of starch extraction potential were shown. Mbundumali1, Mkondezi, CH92/082 and TMS4(2)1425 gave high starch extraction rates at all four sites. Genotype 83350 gave the lowest starch extraction rate. However, it was observed that when harvesting for starch extraction, 83350 and LCN8010 had more and bigger roots per plant than the other genotypes. Sometimes 83350 could give four times the yield of other genotypes like Mbundumali and Silira (data not shown). Based on cluster analysis, Mbundumali1, CH92/082 and TMS4(2)1425 belonged to cluster IA (Figures 7.1 and 7.2) for morphological analysis, while AFLP analysis allocated Mbundumali1 to cluster IA, and CH92/082 to cluster II (Figure 7.3). Genotype 83350, which had the lowest starch extraction rate belonged to cluster IA for morphological analysis together with Mbundumali1, CH92/082 and TMS4(2)1425 (Figures 7.1 and 7.2). The same trend was observed when AFLP analysis clustered 83350 together with CH92/082 and TMS4(2)1425 (Figure 7.4). A combination of AFLP and morphological analysis clustered 83350 and TMS4(2)1425 together in cluster IB (Figure 7.4). These results implied that neither morphological nor AFLP markers were related to cassava starch extraction rate on fresh root weight basis. Thus, morphological and AFLP characterisation for genetic diversity can not elucidate cassava starch extraction potential.

7.3.4.3 *Cassava starch*

Characterisation of native cassava starch showed a wide diversity in terms of functional properties but no variation in terms of granule size and shape for 10 elite genotypes. The diversity in functional properties for native cassava starch was in terms of peak onset and maximum temperatures, ΔH_G (Table 5.2) and DSC thermograms (Figure 5.3). For example, DSC scan for 83350 native starch was similar to that of amylmaize starch (Figure 5.3). Characterisation of starch had no relationship with morphological nor AFLP characterisation for genetic diversity.

7.3.4.4 *Pyroconversion of starch*

Nine genotypes which were included in the pyroconversion of starches showed that 81/00015, Silira and Mbundumali1 were amenable to dextrinisation while LCN8010 and 83350 were tolerant to dextrinisation. Results showed no correspondence between dextrinisation period and genetic diversity analysis using either morphological or AFLP analyses since 81/00015 and

83350 clustered together using morphological, AFLP and a combination of AFLP and morphological characterisation (Figures 7.1, 7.3 and 7.4).

7.4 Conclusions and recommendations

Although AFLP and morphological analyses were in correspondence, they were not perfectly congruent as confirmed by the correlation coefficient of 0.554 (Table 7.2). The 44.6% difference might be due to differences in clustering of some accessions. These results suggested that the use a combination of both methods should be employed.

There existed a strong relationship between morphological and AFLP analyses. However, morphological analysis revealed more diversity than AFLP, while AFLP analysis uniquely distinguished all accessions even morphologically identical ones. Hence, results supported the idea of combining both AFLP and morphological characterisation on cassava germplasm for more accurate results. Each method showed its own unique and important features.

Characterisation of cassava accessions at DNA level can help to identify genetically representative, non-redundant sets of germplasm for cassava breeding and conservation purposes. Significant correlation coefficients obtained between genetic distance values from morphological and AFLP marker techniques indicated a comparable genetic diversity level. AFLP markers were more powerful than morphology in distinguishing accessions. DNA-based markers clearly suggested that conventional methods have been effective in selecting unique collections, and that diversity assessed by molecular markers may efficiently represent the genetic diversity in morphological traits. Morphological and AFLP characterisations confirmed the wide diversity of Malawian cassava germplasm reported by farmers in the field.

Morphological markers failed to uniquely distinguish all 28 analysed accessions but revealed a high degree of GS ranging between 0.083-1.000 compared to the AFLP GS range of 0.778-0.946. PCA revealed that coordinate 1 (PCA axis 1) for AFLP characterisation displayed narrow genetic diversity while PCA axes 2 and 3 displayed wide genetic diversity. AFLP analysis of cassava germplasm grouped accessions according to geographical origin. Results suggested that AFLP analysis was more powerful in distinguishing accessions which morphological analysis failed to distinguish and that PCA provided a better diversity structure than cluster analysis. Genetic diversity presented by PCA (especially three-dimensional PCA plots) gave a clearer

picture of germplasm diversity compared to dendrograms generated through cluster analysis. Therefore, the use of PCA in diversity studies is being recommended.

No relationships were observed between genetic diversity using morphological or AFLP markers and cassava starch characterisation, cassava starch extraction potential, starch physical and functional properties, and pyroconversion of cassava starch. Hence, genetic diversity studies using either of these methods can not elucidate the performance of genotypes for starch yield, and starch functional properties. This study found that LCN8010, and Maunjili were stable for starch extraction but gave low rates of starch extraction. CH92/082 was the most stable genotype which gave above average extraction rate. Mkondezi, Mbundumali and Silira were stable to some extent and gave the highest starch extraction rates. Since GxE was significant for starch extraction rate on fresh root weight basis, stable varieties with high starch extraction rates are being recommended to be grown across the country but unstable varieties like Sauti need to be grown in specific environments to optimise starch extraction. There exists need to execute GxE trials for Malawian cassava germplasm to establish their performance for starch extraction and starch characteristics in various agroecologies.

CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Commercialisation of cassava coupled with biotic and abiotic stresses on cassava lead to genetic erosion and there exists a need to explore cassava germplasm. Information on the levels and patterns of genetic diversity is valuable for efficient management of germplasm and for effective utilisation of materials in breeding programmes to meet the ever-changing needs of growers and consumers in the face of changing and unpredictable environmental challenges. Therefore, cassava germplasm was collected and characterised using morphological and AFLP markers. Previous screening of AFLP primer combinations for use in cassava genetic diversity was fragmented and no consensus conclusions were reached. Hence, AFLP primer pairs and combination of primer pairs were screened.

Exploration of cassava germplasm was carried out. Ethnobotany and prevalence data for major diseases and pests of cassava were gathered during germplasm exploration in Malawi. Results indicated that CMD, CBSD, CGM and CM were pandemic in Malawi. The combination of germplasm collection, gathering of ethnobotany data and surveying for diseases and pests in exploration of cassava germplasm is an innovation. Different pests and diseases have different optimum times of expression hence, surveys for pests and diseases should be carried out in hot-spot areas during appropriate time frames for each pest and disease. A survey for CBSD should include assessment of root symptoms, hence special arrangements need to be made with farmers to give room for destructive sampling.

Preferences of sweet and bitter cassava varieties by farmers in Malawi are diverse according to areas and uses. Farmers who use cassava as a staple crop prefer bitter cassava and those who grow cassava for fresh market or snack prefer sweet cassava. Cassava breeding programmes need to be strategised to address specific needs of farmers and consumers. Introduction of bitter cassava in predominantly sweet cassava growing and consuming areas need to be accompanied with information regarding dangers of consuming unprocessed bitter cassava, detection methods to separate those which need processing from the directly consumed varieties, and processing methods for bitter cassava to avoid exposure of those communities to the effects of cyanogens.

Implementation of strict crop hygiene in a field gene bank and establishment of a field gene bank in a low pressure area for the major cassava pests and diseases was a new initiative and helped to minimise re-infection of the conserved germplasm. There exists a need to study the effect of establishing the cassava field gene bank in cool or cold areas with the aim of prolonging the

period of cassava growing before rejuvenating by replanting. This would minimise costs for managing of field gene banks.

Use of a combination of M-CAA/E-ACA, M-CAA/E-AAC, M-CTT/E-ACT and M-CTT/E-ACC AFLP primers when assessing genetic diversity of cassava gave accurate results which were comparable to use of six primer pairs which included M-CAT/E-ACA and M-CAT/E-AAC, in addition to the above mentioned primer pairs. Use of single primer pair in analysing cassava genetic diversity is being discouraged due to low resolution compared to a combination of the efficient six primer pairs. A single primer pair should only be used on special occasions like critical financial constraints but will be at the expense of accuracy. Use of correlations of single and combinations of primer pairs as a measure of accuracy and relationships among primer pairs during screening was a new initiative which added accuracy to selection of primer pairs in conjunction with the previously used parameters like number of amplified fragments, percentage polymorphism, and ability to differentiate analysed accessions.

AFLP characterisation using cluster analysis showed narrow genetic diversity but uniquely differentiated all analysed accessions while an AFLP PCA plot revealed wider genetic diversity. Morphological cluster analysis and PCA indicated wide genetic diversity but could not uniquely differentiate all characterised accessions. A combination of morphological and AFLP markers is proposed. However, it has to be borne in mind that AFLP analysis is expensive, requires expertise, specialised equipment and chemicals compared to morphological characterisation which is relatively cheap. Since AFLP analysis confirmed morphological characterisation, the combination of AFLP and morphological methods should be planned in such a way that morphological characterisation is conducted first to reduce the bulk of the germplasm, AFLP should only be used for morphologically similar accessions and samples from different morphological heterotic groups and representatives of introductions.

Beatrice was outstanding in farmers' fields in terms of yield and resistance to pests and diseases as reported by farmers and observed by explorers. There exists a need to evaluate this cultivar for official recommendation/release for a wider community to benefit from it. Fyoka, Thipula and Kachamba were distantly related to other accessions, hence were best candidates as parents in a breeding programme. Representatives of heterotic groups should be used as parents to hasten genetic gains in the development of improved varieties.

Cassava starch has a lot of advantages compared to maize starch. Due to the diversity of uses of starch in various industries, there existed a need to asses diversity of physical and functional properties in starch and pyrodextrins from different Malawian cassava varieties. There existed a need to investigate the effect of genotype and season on starch extraction. Effects of season at time

of harvest on starch extraction and reporting of starch extraction rate on fresh root weight basis is a new study area. Previous studies investigated the effects of season on starch content and root dry matter content in other areas, hence this is the first time Malawian cassava varieties have been considered in such a study.

Starch should be extracted between October and early December using early maturing varieties. During that period high starch extraction rates are achieved, convenient for open-air drying of starch, and land is released for growing other crops in the following season. Further studies need to be conducted on the effect of irrigation on starch extraction and the economics of growing one cassava crop for 1.5 to two years compared to growing a new crop in each year for starch extraction.

Mkondezi, Silira, Mbundumali and CH92/082 are recommended to be grown across the country for starch extraction while Sauti is suited to specific areas like Makoka. The establishment of a cassava starch plant needs feasibility studies for best possible site since the agroecology of cassava growing influences starch extraction potential.

Cassava starch and dextrins are diverse in functional properties. Therefore, starch from different varieties can address needs of different industries. Pyroconversion of cassava starch gives a wider opportunity of meeting more specialised needs like production of cold-setting adhesives. Use of locally produced starch and dextrins will create job opportunities and save the much needed foreign exchange.

This study elucidated some of the important questions regarding cassava production in Malawi. Data from this study can be applied to breeding programmes and the cassava industry in order to add an improved crop and produce for the industry which will benefit farmers, industry and the country.

SUMMARY

Key words: cassava, characterisation, germplasm, starch, ethnobotany, morphological, AFLP, pyroconversion, modification, solubility

Cassava has been neglected and considered as a primitive crop, food for the poor and a crop with poor nutritional value for a long time. Cassava provides more dietary energy per unit area than any staple crop. Population increase, multiplicity of cassava uses, unusual climatic variations and prospects of global warming highlight advantages of possible uses of this hardy drought tolerant crop. Policy makers realised the importance of cassava and are encouraging its promotion through research, production and utilisation. Since the crop has lagged behind for a long time, there exist knowledge gaps in many areas.

Commercialisation of cassava, and biotic and abiotic factors encourage genetic erosion. Management and use of germplasm in production and breeding programmes need knowledge of genetic diversity. Hence, there existed a need to collect, characterise, evaluate, conserve and use Malawian cassava germplasm.

Indigenous knowledge revealed wide genetic diversity because farmers reported a wide range of quantitative and quality attributes, which they preferred or disliked in existing Malawian cassava germplasm. Morphological markers failed to uniquely distinguish all analysed accessions. Manyokola5 and Mbundumali1, Gomani1 and 2, and Matuvi and Depwete were morphologically identical. However, morphological markers revealed high levels of genetic diversity with Dice genetic similarity (GS) ranging between 0.083-1.000. The most distant accessions were Beatrice (local cultivar) and Yizaso (CH92/112; locally screened). AFLP characterisation using cluster analysis indicated narrow genetic diversity with a GS range between 0.778-0.946 and uniquely differentiated all analysed accessions. The closest accessions were Mabundumali1 and Manyokola5 (GS=0.946), and Gomani1 and 2 (GS=0.938). PCA analysis for AFLP markers revealed that PCA axis 1 displayed narrow genetic diversity but PCA axes 2 and 3 displayed wide genetic diversity. AFLP analysis of cassava germplasm grouped accessions according to pedigree and geographical origins. Morphological and AFLP markers were not similar but significantly correlated, indicating the need to use both markers in characterisation of cassava germplasm.

Screening of AFLP primers revealed that M-CAA/E-ACA, M-CAA/E-AAC, M-CAT/E-ACA, M-CAT/E-AAC, M-CTT/E-ACT and M-CTT/E-ACC were convenient and efficient in analysing cassava. Further analysis indicated that a combination of four primer pairs was more cost and time efficient and gave similar resolution compared to a combination of six primer pairs, the best being M-CAA/E-ACA+M-CAA/E-AAC+M-CTT/E-ACT+M-CTT/E-ACC.

Evaluation of genotypes for starch extraction was done just before or at onset of the rainy season (October-November), within the rainy season (January) and towards the end of the rainy season (March-April). Results indicated that the best genotypes were Mkondezi, Silira, Mbundumali and CH92/082. Starch extraction done just before or at onset of the rainy season gave the highest starch extraction rates compared to the middle of the rainy season. Apart from high recovery of starch in October-November, it is convenient for drying starch using open-air method and releases land for other crops in the following rainy season. Makoka was the best site for growing cassava for starch extraction. Starch extraction rate on fresh root weight basis had a direct relationship with starch yield as opposed to starch extraction on dry root weight basis.

Cassava starch from different genotypes were characterised for physical and functional properties. Results indicated that Silira, 81/00015, Mbundumali and Sauti were easily dextrinised and 80% solubility was achieved within 60min of dextrinisation at 100^{o}C after acidification with 0.1M HCl. Pyrodextrin from Mkondezi variety after dextrinisation for 40min turned out to be similar in functional properties to amylmaize starch.

Cassava starches had lower gelatinisation temperatures desired for hot-setting adhesives since less heating is required to gelatinise cassava starch, which leads to energy saving. Functional properties of 83350 native starch was different from the rest of the genotypes but was very close to amylmaize starch as revealed by DSC.

OPSOMMING

Sleutelwoorde: cassava, karakterisering, kiemplasma, stysel, etnobotanie, morfologie, AFLP, piro-omskakeling, modifikasie, oplosbaarheid

Cassava is in die verlede verwaarloos en beskou as ‘n primitiewe gewas, kos vir arm mense en ‘n gewas met swak voedingswaarde. Cassava verskaf meer voedingsenergie per eenheid area as enige ander stapelgewas. Populasie toename, veelvoudige gebruike, ongewone klimaatswisseling, en die moontlikheid van globale verwarming beklemtoon die voordele van hierdie geharde, droogte tolerante gewas. Beleidmakers het die belangrikheid van cassava besef en moedig nou navorsing, produksie en gebruik van cassava aan. Omdat navorsing op die gewas vir lank agterweë gebly het, is daar kennisgapings wat gevul moet word om dit effektief te produseer, prosesseer en gebruik.

Kommersialisering van cassava, en biotiese en abiotiese faktore bevorder genetiese erosie. Bestuur en gebruik rakende kiemplasma in produksie en teelprogramme vereis kennis van genetiese diversiteit. Daarom bestaan daar ‘n behoefte aan versameling, karakterisering, evaluering, bewaring en gebruik van Malawiese kiemplasma.

Inheemse kennis het wye genetiese diversiteit aangetoon, omdat boere ‘n wye spektrum in bestaande kwantitatiewe en kwalitatiewe eienskappe aangetoon het wat hulle in bestaande Malawiese cassava kiemplasma verkies of afkeur. Morfologiese merkers kon nie tussen al die inskrywings onderskei nie. Manyokola5 en Mbundumali1, Gomani1 en 2, en Matuvi en Depwete was morfologies identies. Tog het morfologiese merkers wye genetiese diversiteit aangetoon met die Dice genetiese similariteits (GS) spektrum van 0.083-1.000. Die genotipes met die grootste genetiese afstand was Beatrice (plaaslike cultivar) en Yizaso (CH92/112; plaaslik geëvalueer). AFLP karakterisering met groeperings analise het baie nou genetiese diversiteit aangedui met ‘n GS spektrum van 0.778-0.946, maar alle inskrywings kon gedifferensieer word. Die mees verwante inskrywings was Mabundumali1 en Manyokola5 (GS=0.946), en Gomani1 en 2 (GS=0.938), wat met die morfologiese karakterisering as duplikate geïdentifiseer is. PCA analise vir AFLP merkers het getoon dat die PCA as 1 min genetiese diversiteit wys, maar PCA asse 2 en 3 het wye genetiese diversiteit getoon. AFLP analise van cassava kiemplasma het die inskrywings volgens geografiese oorsprong gegroepeer, wat verwant was aan die stamboom van die inskrywings. Morfologiese en AFLP merkers was nie dieselfde nie, maar was tog

betekenisvol gekorreleer. Dit is dus goed om beide merkersisteme karakterisering van cassava kiemplasma te gebruik in.

Evaluering van AFLP voorvoerders het getoon dat M-CAA/E-ACA, M-CAA/E-AAC, M-CAT/E-ACA, M-CAT/E-AAC, M-CTT/E-ACT en M-CTT/E-ACC cassava effektief analiseer. Verdere analise het getoon dat ‘n kombinasie van vier voorvoerders net so goed was as ‘n kombinasie van ses voorvoerders. Die beste voorvoerders was M-CAA/E-ACA+M-CAA/E-AAC+M-CTT/E-ACT+M-CTT/E-ACC.

Evaluasie van genotipes vir stysel ekstraksie is net voor die begin van die reënseisoen (Oktober-November), in die reënseisoen (Januarie) en naby die einde van die reënseisoen (Maart-April) gedoen. Resultate het getoon dat die beste genotipes Mkondezi, Silira, Mbundumali en CH92/082 was. Styselektraksies wat net voor die begin van die reënseisoen gedoen is het hoër ekstraksie persentasies gegee as in die middel van die reënseisoen. Behalwe dat die ekstraksies so hoog was tydens Oktober-November, is dit ook ‘n baie geskikte tyd om die stysel te droog met die opelug metode, en dit maak die lande ook beskikbaar vir ander gewasse in die opvolgende reënseisoen. Makoka was die beste area om cassava te verbou vir stysel ekstraksie.

Cassava stysel van verskillende genotipes is vir fisiese en funksionele eienskappe gekarakteriseer. Resultate het gewys dat Silira, 81/00015, Mbundumali en Sauti maklik gedektriniseer is en 80% oplosbaarheid is binne 60 min van dekstrinisering gekry by 100^{o}C na asidifisering met 0.1M HCl. Pirodekstrinering van Mkondezi vir 40min het baie dieselfde funksionele eienskappe gewys as die van amylmaize stysel.

Gelvorming vind by laer temperature plaas in cassava stysel as ander stysel, wat voordelig is vir hitte geaktiveerde plakmiddels, omdat minder energie nodig is om gelvorming te induseer in die cassava stysel, wat tot energiebesparing lei. Funksionele eienskappe van 83350 natuurlike stysel was verskillend van die van die ander genotipes, maar dit was baie dieselfde as amylmaize stysel soos gesien met die DSC.

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REFERENCES

Adugna, W.G., 2002. *Genetic diversity analysis of linseed (Linum usitatissimum L.) in different environments.* 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.

Agboola, S.O., J.O. Akingbala and G.B. Oguntimein, 1990. Processing of cassava starch for adhesive production. *Starch* **42**: 12-15.

Agrobase, 2000. *Agrobase 20 for windows.* Copyright 1990 – 1998, 1999 by Agromix Software, Inc. Winnipeg, Manitoba, Canada.

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.

Akinlosotu, T.A., 1982. Seasonal trends of green spider mite *Mononychellus tanajoa* population on cassava *Manihot esculenta* and its relationship with weather factors at Moor Plantation. *Insect Science Application* **3**: 251-254.

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

Allard, R.W., 1988. Genetic changes associated with the evolution of adaptness in cultivated plants and their wild progenitors. *Journal of Heredity* **79**: 255-238.

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

Allem, A.C., 2002. The origins and taxonomy of cassava (*Manihot esculenta* Crantz subspecies *esculenta*). 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., R.A. Mendes, A.N. Salomao and M.L. Burle, 2001. The primary gene pool of cassava (*Manihot esculenta* Crantz subspecies *esculenta* Euphorbiaceae). *Euphytica* **120**: 127-132.

Altaf-Khan, M., G.O. Myers and J.M. Stewart, 2002. Molecular markers, genomics and cotton improvement. In: M.S. Kang (ed.). *Crop improvement challenges in the twenty first century.* Food Products Press, New York. pp.253-284.

Applied Biosystems, 2001. *AFLP Plant Mapping Kit*: Product Insert. USA.

Ashoka, P.V., S.V. Nair and T.M. Kurian, 1984. Influence of stages of harvest on yield and quality of cassava, *Manihot esculenta* Crantz. *Madraos Agricultural Journal* **71**: 447-449.

Bachem, C.W.B., R.S. Van de Hoeven, S.M. Brujin, D. Vreugdenhill, M. Zabeau and R.G.F. Visser, 1996. Visualization of differential gene expression using a novel method of RNA fingerprinting using AFLP: Analysis of gene expression during potato tuber development. *Plant Journal* **9**: 745-753.

Balagopalan, C. B.G. Padmaja, S.K. Nanda and S.N. Moorthy, 1988. *Cassava in Food, Feed and Industry*, CRC Press, Boca Raton, USA, pp.177.

Barua, U.M., K.J. Charmers, C.A. Hackett, W.T.B. Thomas, W. Powel and R. Waugh, 1993. Identification of RAPD markers linked to a *Rhynchosporium secalis* resistance locus in barley using near isogenic lines and bulked segregant analysis. *Heredity* **71**: 177-184.

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.

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.

Becker, J. and M. Heun, 1995. Barley microsatellites: allele variation and mapping. *Plant and Molecular Biology* **27**: 835-845.

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

Benesi, I.R.M., C.C. Moyo, J. Mkumbira, F.P. Chipungu, A.P. Mtukuso and N.M. Mahungu, 2001a. Cassava research and production to enhance commercialisation in Malawi. In: Mahungu, N.M., J.W. Banda and C. Mataya (eds.). *Cassava commercialisation for economic development in Malawi. Proceedings of the Symposium held at Kwacha International Conference Centre*, Blantyre, Malawi, 21–23 May 2001. pp78-86.

Benesi, I.R.M., C.C. Moyo, J. Mkumbira, F.P. Chipungu, A.P. Mtukuso and N.M. Mahungu, 2001b. Cassava: Becoming a giant crop in Malawi-Current status. 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 files-S1-02.pdf.

Benesi, I.R.M., C.C. Moyo, J. Mkumbira, F.P. Chipungu, N.M. Mahungu and V.S. Sandifolo, 2003. The release of three new improved cassava varieties in Malawi. In: Akoroda, M.O. (ed.). *Root crops: The small processor and development of local food industries for market economy. Proceedings of the eighth Triennial Symposium of ISTRC-AB.* Held at IITA, Ibadan, Nigeria, 12-16 November 2001. pp.405-411.

Benesi, I.R.M, C.C. Moyo, J. Mkumbira, N.M. Mahungu and R.F.N. Sauti, 1999. A proposal for the release of three promising cassava clones in Malawi. Presented to the Malawi Agricultural Technology Clearing Committee, 18 May 1999, Natural Resources College, Lilongwe, Malawi.

Benesi, I.R.M., M.T. Labuschagne, A.G.O. Dixon and N.M. Mahungu, 2004. Genotype x Environment interaction effects on native cassava starch quality and potential for starch in the commercial sector. *African Crop Science Journal* **12**: 205-216.

Ben-Har, A., A. Charcrosset, M. Bourgoin and J. Guiard, 1995. Relationships between genetic markers and morphological traits in a maize inbred lines collection. *Euphytica* **84**: 145-154.

Berg, T., 1993. The Science of Plant Breeding: Support or alternative to traditional process? In: De Boef, W., K. Amador, K. Wellard and A. Beddington (eds.). *Cultivation knowledge: Genetic diversity, farmer experimentation and crop research*. Intermediate Technology Publications. pp. 72-77.

Berry, S.T., A.J. Leon, C.C. Hanfrey, P. Challis, A. Burkholza, S.R. Barnes, G.K. Rufener, M. Lee and P.D.S. Caligary, 1995. Molecular markers analysis and construction of linkage map of cultivated sunflower (*Helianthus annuus* L.) using RFLP. *Theoretical and Applied Genetics* **83**: 691-694.

Berthaud, J., 1997. Strategies for conservation of genetic resources in relation with their utilisation. *Euphytica* **96**: 1-12.

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.

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

Bolhuis, G.G., 1966. Influence of the illumination period on root formation in cassava. The Netherlands Journal of Agricultural Sciences 14: 151-262.

Bonierbale, M.W., A.C. Roa, M.M. Maya, M.C. Duque and J. Thome, 1997. Assessment of genetic diversity in *Manihot* species with AFLPs. *African Journal of Root and Tuber Crops* **2:** 139.

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. Sholnick and R.W. Davis, 1980. Construction of genetic linkage map using RFLP. *American Journal of Human Genetics* **32:** 314-331**.**

Boventius, H. and J.I. Weller, 1994. Mapping and analysis of diary cattle quantitative trait loci by maximum likelihood methodology using milk protein genes as genetic markers. *Genetics* **137:** 267-280.

Bradshaw, J.E., C.A. Hackett, R.C. Meyer, D. Milbourne, J.W. McNichol, M.S. Philips and R Waugh, 1998. Identification of AFLP and SSR marker associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection. *Theoretical and Applied Genetics* **97**: 202-210.

Brautlecht, C.A., 1953. *Starch: Its sources, production and uses*. Reinhold Publishing Corporation, New York, USA.

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.

Brush, S., 1995. *In situ* conservation of landraces in centres of crop diversity. *Crop Science* **35**: 346-354.

Brush, S.B., 2000. *Genes in the field: On-farm conservation of crop diversity*. IPGRI, Rome, Italy, and IDRC, Otawa, Canada and Lewis Publishers Roca Raton, London, New York and Washington D.C. pp.288.

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 Thro, A.M. (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.

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

Carter, S.E., L.O. Fresco, P.G. Jones and J.N. Fairbairn, 1992. *An atlas of cassava in Africa*: Historical, agroecological and demographic aspects of crop distribution. CIAT, Cali, Colombia. pp.85.

Central Science Laboratory, 2002. Carbohydrate crops. Available at URL: http://www.csl.gov.uk/groups/acb/crops/carbohydrate.cfm

CGIAR Research, 2001. Cassava (*Manihot esculenta* Crantz). Areas of research. Available at URL: http://www.cgiar.org/areas/cassava.htm

Chao, S., P.J. Sharp, A.J. Worland, E.J. Warham, R.M.D. Koebner and M.D. Gale, 1989. RFLP-based genetic maps of wheat homologous group seven chromosome. *Theoretical and Applied Genetics* **78**: 495-504.

Charrier, A. and F. Lefevre, 1987. The genetic variability of cassava origin, evaluation and utilisation. In: Fauquent, C. and D. Fargette (eds.). *Proceedings of an International Seminar on African cassava mosaic disease and its control*, Cote d'Ivoire, Yamoussoukro. CTA/FAOORSTOM/IITA/IITA/IAPC. pp.77-91.

Chase, M.R., C. Moller, R. Kesseli and K.S. Bawa, 1996. Distant gene flow in tropical trees. *Nature* **383**: 398-399.

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

Chiwona-Karltun, L., 2001. *A Reason to Be Bitter: Cassava Classification From the Farmers' Perspective.* Doctoral Thesis. Division of International Health (IHCAR), Department of Public Health Sciences, Karolinsha Institute, Sweden. Printed by IHCAR, Karolinska Institute, Stockholm, Sweden.

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

Chiwona-Karltun, L., T. Tylleskar, J. Mkumbira, M. Gebre-Medhin and H. Rosling, 2000. Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi. *International Journal of Food Science and Nutrition* **51**: 33-43.

Ciacco, C. F. and B. L. D'Applonia, 1977. Characterisation of starches from various tubers and their uses in bread baking. *Cereal Chemistry* **54**: 1096-1107.

CIAT, 1993. Biotechnology Research Unit. *Annual Report*, Cali, Colombia.

Cock, J. H., 1985. *Cassava: new potential for a neglected crop*. Westview Press, Boulder, Colorado.

Cooke, D. and M.J. Gidley, 1992. Loss of crystalline and molecular order during starch gelatinization and origin of the enthalpic transition. *Carbohydrate Research* **227**: 103–112.

Corbishley, D. A. and W. Miller, 1984. Tapioca, arrowroot, and sago starches: production. In: Whistler, R.L., J.N. BeMiller and E.F. Paschal (eds.). *Starch: Chemistry and Technology*. Second Edition. Academic Press, Inc, New York, USA. pp.469-478.

Crossa, J., 1990. Statistical analyses of multilocation trials. *Advances in Agronomy* **44:** 55-86.

Crossa, J., H.G. Gauch and R.W. Zobel, 1990. Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. *Crop Science* **30**: 493-500.

Danish EU-directorate, 1996. Determination of starch content under water weight of 5050 g tuber. Instruction no. 2 Mkt. 4410-4-96 of July 1996 issued by Ministry of Agriculture and Fishery enforced on 1 July 1996 and revised on 31 December 1997. Available at URL: http://www.starch.dk/isi/methods/13starch.htm.

Dayanandan, S., O.P. Rajora and K.S. Bawa, 1998. Isolation and characterisation of microsatellites in trembling aspen (*Populus tremuloides*). *Theoretical and Applied Genetics* **96:** 950-956.

Demeke, T., D.R. Lynch, L.M. Kawehuk, G.C. Kozub and J.D. Armstrong, 1996. Genetic diversity of potato determined by RAPD analysis. *Plant Cell Reports* **15**: 662-667.

De Boer, W.R., 1975. The archiological evidence for manioc cultivation: a cautionary note. *American Antiquity* **40**: 419-433.

De Verno, L.L. and A. Mosseler, 1997. Genetic variation in red pine (*Pinus resinosa*) revealed by RAPD and RAPD-RFLP analysis. *Canadian Journal of Forest Research* **27**: 1336-1320.

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

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

Dice, L.R., 1945. Measures of amount of ecologic association between species. *Ecology* **26**: 297-302.

Dixon, A.G.O., R. Asiedu and S. K. Hahn, 1992. Cassava germplasm enhancement at IITA. In: Akoroda, M.O. and O.B. Arene (eds.). *Proceedings of the fourth ISTRC-AB Symposium*. ISTRC-AB/IDRC/CTA/IITA. pp.83-87.

Dixon, A.G.O., R. Brandyop and R. Ortiz, 2003. Cassava from poor farmers crop to Pacesctters of African rural development. *Chronica Horticulturae* **43**: 3-69.

Donnan Landscape Services, 2005. Soil pH. *Landscaping information and more*. Available at URL: http://www.donnan.com/soilph.htm

Donovan, J.W. K. Lorenz and K. Kulp, 1983. Differential scanning calorimetry of heat-moisture treated wheat and potato starches. *Cereal Chemistry* **60**: 381–387.

Eckert, D. and J. Thomas Sims, 1995. Recommended soil pH and lime requirement tests. In: Thomas Sims, J. and A. Wolf (eds.). Recommended soil testing procedures for the north–eastern United States of America. *North-east Regional Bulletin* **93**: 11-16.

Edwards, K., C. Johnstone and C. Thompson, 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research* **19**: 1349.

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

Ericksson, G., G. Namkoong and J.H. Roberts, 1993. Dynamic gene conservation for uncertain future. *Ecology and Management* **62**: 15-37.

Essers, A.J.A., A. Abén and P. Rosling, 1992. Insufficient processing of cassava induced acute intoxications and the paralytic disease konzo in a rural area of Mozambique. *Ecology of Food and Nutrition* **27**: 17-27.

Evers, A.D., 1971. Scanning electron microscopy of wheat starch, III: Granule development in the endosperm. *Starch* **23**: 157-162.

Fabiano, E., W.R.L. Masamba and F.F. Masumbu, 2001. Processing of starch from cassava: modification and utilisation of cassava starch in Malawi. In: Mahungu, N.M., J.W. Banda and C. Mataya (eds.). *Cassava commercialisation for economic development in Malawi*. Proceedings of the Symposium held at Kwacha International Conference Centre, Blantyre, Malawi, 21–23 May 2001. p.121.

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

FAO, 2001. *A Global Cassava Development Strategy and Implementation Plan*. Volume 1. Plucknett, D.L. and R.B. Kagbo (eds.). Proceedings of the Validation Forum on the Global Cassava Strategy. Rome, 26-28 April 2000. pp.70.

Franco, J., J. Crossa, J.M. Ribaut, J. Betran, M.L. Warburton and M. Khairallah, 2001. A method for combining molecular markers and phenotypic attributes for classifying plant genotypes. *Theoretical and Applied Genetics* **103**: 944-952.

Frankel, O.H., 1989. Practical considerations relevant to effective evaluation. In: A.H.D. Brown, O.H. Frankel, D.R. Marshall and J.T. Williams (Eds.). *The use of plant genetic resources.* Cambridge University Press, Cambridge, pp.235-260.

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

Fregene, M.A., J. Vargas, J. Ikea, F. Angel and J. Tohme, 1994. Variability of chloroplast DNA and molecular ribosomal DNA in cassava (*Manihot esculenta* Crantz) and its wild relatives. *Theoretical and Applied Genetics* **89**: 719-727.

Fregene, M., F. Angel, R. Gomez, F. Rodriguez, P. Chavarriaga-Aguirre, M. Bonierbale, W. Roca and J. Tohme, 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). A research summary. *African Journal of Root and Tuber Crops* **2**: 150-151.

Fregene, M., M. Suarez, J. Mkumbira, H. Kulembeka, E. Ndedya, A. Kulaya, S. Mitchel, U. Gullberg, H. Rosling, A. Dixon and S. Kresovich, 2001. 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.

Fresco, L.O., 1986. Cassava in shifting cultivation. A systems approach to agricultural technology development in Africa. Amsterdam: Royal Tropical Institute. pp.240.

Fungulani, T. and E. Maseko, 2001. Utilization of cassava starch in textile industry. In: Mahungu, N.M., J.W. Banda and C. Mataya (eds.). *Cassava commercialisation for economic development in Malawi. Proceedings of the Symposium held at Kwacha International Conference Centre*, Blantyre, Malawi, 21–23 May 2001. pp.98-99.

Gardiner, J.M., E.H. Coe, S. Melia-Hanconck, D.A. Hoisington and S. Chao, 1993. Development of a core RFLP map in maize using an immortalised F2 population. *Genetics* **134**: 917-930.

Garmer, H.R., B. Armstrong and D.M. Lininger, 1993. High throughput PCR. *Bio-Techniques* **14**: 112-115.

Gauch, H.G. and R.W. Zobel, 1996. AMMI analysis of yield trials. In: Kang, M.S. and H.G. Gauch (eds.). *Genotype by environment interaction*. CRC Press, Boca Raton, New York. pp.85-122.

Gee, G.W. and J.W. Bauder, 1986. Particle size analysis. In Klute, A. (ed.). Methods of soil analysis. Part 1. Physical and mineralogy methods. *Agronomy Monograph number 9 (Second edition). American Society of Agronomy* **9**: 383-411.

Gepts, P., 1991. Biotechnology sheds light on bean domestication in Latin America. *Diversity* **7**: 49-50.

Geraci, A., I. Divaret, F.M. Raimondo and A.M. Chevre, 2001. Genetic relationship between Sicilian wild populations of Brassica analysed with RAPD markers. *Plant Breeding* **120**: 193-196.

Gianfranceschi, L., N. Seglias, R. Tarchini, M. Komjanc and C. Gessler, 1998. Simple sequence repeats for the genetic analysis of apple. *Theoretical and Applied Genetics* **96:** 1069-1076.

Gibbons, A., 1990. New view of early Amazonia. *Science* **248**: 1488-1490.

Guilford, P., S. Prakash, J.M. Zhu, E. Rikkerink, S. Gardiner, H. Basset and R. Forster, 1997. Microsatellites in *Malus domestica* (apple): abundance polymorphism and cultivar identification. *Theoretical and Applied Genetics* **94:** 249-254.

Guthridge, K.M., M.P. Dupal, R. Kolliker, E.S. Jones, K.F. Smith and J.W. Forster, 2001. AFLP analysis of genetic diversity within and between populations of perennial ryegrass (*Lolium perenne* L.). *Euphytica* **122**: 191-201.

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

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

Halward, T., T. Stalker, E. LaRue and G. Kochert, 1992. Use of single primer DNA amplification in genetic studies in peanut (*Arachis hypogaea* L.). *Plant Molecular Biology* **18**: 315-325.

Hamada, H., M. Petrino and T. Kalunaga, 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. *Proceedings of National Academy of Sciences* **79**: 6465-6469.

Hardon, J.J. and W.S. De Boef, 1993. Linking farmers and breeders in local crop development. In: De Boef, W., K. Amador, K. Wellard and A. Beddington (eds.). *Cultivation knowledge: Genetic diversity, farmer experimentation and crop research*. Intermediate Technology Publications. pp.64-71.

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.

Herselman, L., 2003. Genetic variation among South African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. *Euphytica* **133**: 319-327.

Hershey, C.H., 1987. Cassava germplasm resources. In: Hershey, C.H. (ed.). *Cassava breeding a multidisciplinary review. Proceedings of a workshop held in Philippines*, 4-7 March, 1985. pp.1-24.

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

Hintze, J.L., 1998. NCSS 2000:. Statistical System for Windows. Number Cruncher Statistical Systems, Kaysville, Utah 84037, Canada.

Hokanson, S.C., A.K. Szewc-McFadden, W.F. Lamboy and J.R. McFerson, 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus x domestica* Borkh. core subset collection. *Theoretical and Applied Genetics* **97**: 671-683.

Hosaka, K., M. More and K. Ogawa, 1994. Genetic relationships of Japanese potato cultivars assessed by RAPD analysis. *American Potato* **171**: 535-546.

Hussein, A., W. Bushiuk, H. Ramirez and W. Roca, 1987. Identification of cassava (*Manihot esculenta* Crantz) cultivars by electrophoretic patterns of esterase isozyme. *Seed Science and Technology* **15:** 19-22.

Iglesias, C.A. and C.H. Hershey, 1994. Cassava breeding at CIAT: Heritability estimates and genetic progress in 1980s. *Acta Horticulture* **380:** 149-163.

IITA, 1990. *Cassava in Tropical Africa*. A reference Manual. Balding and Mansell International, Wesbech, UK. pp.176.

IITA, 1985. *Common African Pests and diseases of cassava, yam, sweetpotato and cocoyam*. IITA, Ibadan, Nigeria. pp.108.

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

Iltis, H.H., 1974. Freezing the genetic landscape: The preservation of diversity in cultivated plants as an urgent social responsibility of plant geneticist and plant taxonomist. *Maize Genetics Cooperation News letter* **48**: 199-200.

IPGRI, 1993. Diversity for development. The strategy of the International Plant Genetic Resources Institute. IPGRI. Rome. pp.62.

ISI, 1999-2001. Tapioca (Cassava) starch. Available at URL: http://home3.inet.tele.dk/starch/isi/

ISO, 1997. International standard: ISO 1741.

Itaye, S., 2001. The use of starch in the production of corrugated cartons. In: Mahungu, N.M., J.W. Banda and C. Mataya (eds.). *Cassava commercialisation for economic development in Malawi*. *Proceedings of the Symposium held at Kwacha International Conference Centre,* Blantyre, Malawi, 21–23 May 2001. pp.105-107.

Jarowenko, W., 1977. Starch based adhesives: In: Irving, S. (ed.). *Handbook of Adhesives.* Second Edition, Van Nostrand Reinhold Co., New York, USA. pp.192-211.

Jarvis, D. and T. Hodgkin, 2000. Farmer decision making and genetic diversity: linking multidisciplinary research to implementation on-farm. Chapter 11. In: Brush, S.B. (ed.). *Genes in the field: On-farm conservation of crop diversity*. IPGRI, Rome, Italy, and IDRC, Otawa, Canada and Lewis Publishers Roca Raton, London, New York and Washington D.C. pp.261-278.

Jennings, D.L. and C. Hershey, 1985. Cassava breeding: An article of progress from International programmes. In: Russel, G.E. (ed.). *Progress in Plant Breeding*. I. Butterworths, London. pp. 89-116.

Jones, W.O., 1957. Manioc: An example of innovation in African economies. *Economic Development and Cultural Change* 5: 97-117.

Jones, W.O., 1957. *Manioc in Africa*. Stanford University Press. Stanford, CA. pp.315.

Kapinga, R.N., N. Mlingi and H. Rosling, 1997. Reason for use of bitter cassava in Southern Tanzania. *African Journal of Tropical Root Crops* **2**:81-84.

Karp, A., S. Kresovich, K.V. Bhat, W.G. Ayad and T. Hodgkin, 1997. *Molecular tools in plant genetic resources conservation: a guide to the technologies.* IPGRI technical bulletins, International Plant Genetic Resources Institute, Rome, Italy. Available at URL http://198.93.227.125/publicat/techbull/TB2.pdf.

Kawawo, K., 1980. Cassava: In: Fehr, W.R. and H.H. Hadley (eds.). *Hybridisation of Crop Plants*. ASA, CSSA, Madison, Wisconsin. pp. 225-233.

Keim, P., B.W. Dier, T.C. Olson and R.C. Shoemaker, 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. *Genetics* **16**: 735-742.

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

Kennedy, H. M., 1989. Starch- and dextrin-based adhesives: In: Hemingway, R.W., A.H. Conner and S.J. Branham (eds.). *Adhesives from Renewable Resources.* American Chemical Society, Washington DC, USA. pp.326-336.

Kennedy, H. M. and A.C. Fischer (Jr), 1984. Starch and dextrins in prepared adhesives: In: Whistler, R.L., J.N. BeMiller and E.F. Paschal (eds.). *Starch: Chemistry and Technology*. Second Edition. Academic Press, Inc, New York, USA. pp.563-610.

Koch, L., 1933. *Cassaveselectie (cassava selection)*. Wageningen Agricultural University, Wageningen. pp.86.

Kochert, G., 1994. RFLP technology. In: Phillips, R.L. and I.K. Vasil (eds.). *DNA-based markers in plants.* Kluwer Academic Publishers, London, United Kingdom. pp.8-38.

Krauss, S.L. and R. Peakall, 1998. An evaluation of the AFLP fingerprinting technique for the analysis of paternity in natural populations of *Persoonia mollis* (Proteaceae). *Australian Journal of Botany* **46**: 533-546.

Kumar, L.R., 1999. DNA markers in plant improvement: An overview. *Biotechnology Advances* **17**: 143-182.

Lanner-Herrera, C., T. Bryngelsson and M. Gustafsson, 1997. Relationships of wild *Brassica* species in chromosome number 2n=18, based on comparison on RFLPs. *Genome* **40:** 302-308.

Lazaro, A. and I. Aguinagalde, 1998. Genetic diversity in *Brassica oleracea* L. (Cruciferae) and wild relatives (2n=18) using isozymes. *Annals of Botany* **82**: 821-828.

Lefevre, F. and A. Charrier, 1993. Heredity of seventeen isozyme loci in cassava (*Manihot esculenta* Crantz). *Euphytica* **66:** 171-178.

Lepoutre, P. and M. Inoue, 1989. Glueability at the corrugator. *Tappical Journal* **72**: 113-119.

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

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.

Lozano, J.C., D. Byme and A. Bellotti, 1980. Cassava and ecosystem relationships and their influence on breeding strategy. *Tropical Pest Management* **26**: 180-187.

Lynam, J., 1993. Potential impact of biotechnology on cassava production in the third world. In: Roca, W.M. and A.M. Thro (eds). *Proceedings of the first International Scientific Meeting of the Cassava Biotechnology Network,* 25-28 August 1992. Cartegena, Colombia. pp.22-30.

Lyne, F.A., 1976. Chemical analysis of raw and modified starches: In Radley, J.A. (ed.). *Examination and Analysis of Starch and Starch Products*. Applied Science Publishers limited, London, United Kingdom. pp.133-165.

Magoon, M.L., R. Krishnam and K.V. Bai, 1969. Cytogenetics in flowering plants. *Journal of Heredity* **63**: 11-14.

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

Mahungu, N.M., M. Bodiaka, H. Tata, S. Lukombo and S. N'luta, 2003. Cassava brown streak disease-like symptoms in Democratic Republic of Congo. *Newsletter for SARRNET and Eastern Africa Root Crops Research Network (EARRNET). Roots* **8**: 8-10.

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

Malawi Bureau of Standards, 2004. Cassava and maize starch for textile industry: Specification. Malawi Standards Board, Blantyre, Malawi. ICS67.180.20. MS704:2004

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

Masamba, W. R. L., F. F. F. Masumbu and E. Fabiano, 2001. Advantages of cassava starch over maize starch in a hot setting adhesive formulation. *Malawi Journal of Science and Technology* **6:** 91-97.

Masumbu, F.F.F., 2002. *Production of cold-setting adhesives using starch and dextrins from cassava* (*Manihot esculenta* Crantz). MSc thesis in Applied Chemistry, Department of Chemistry, University of Malawi, Zomba, Malawi.

Mataya, C., 2001. A policy review of cassava processing, marketing and distribution in Malawi. In: Mahungu, N.M., J.W. Banda and C. Mataya (eds.). *Cassava commercialisation for economic development in Malawi. Proceedings of the Symposium held at Kwacha International Conference Centre*, Blantyre, Malawi, 21–23 May 2001. pp.15-23.

Mathura, R., D.G. Dhander and S.P. Varma, 1986. Variability studies of cassava varieties on growth and yield under Tripura-conditions. *Journal of Root Crops* **12**: 25-28.

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.

Mauny, R. **1953**. Notes historiques autour des principales plantes cultivées d'Afrique occidentale. *Bulletin IFAN B* **15**: 684-731.

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

McCouch, S.R., G. Kochert, Z.H. Yu, Z.Y. Wang, G.S. Khush, W.R. Coffman and S.D. Tanksley, 1988. Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics* **76:** 815-829.

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.

Mehlich, A., 1984. Mehlich 3 Soil Test Extractant: A modification of Mehlich 2 Extractant. *Communications in Soil Science and Plant Analysis* **15:** 1409-1416.

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.

Milbourne, D., R. Meyer, J.E. Bradshaw, E. Baird, N. Bonar, J. Provan, W. Powell and R. Waugh, 1997. Comparison of PCR marker based marker systems for analysis of genetic relationships in cultivated potato. *Molecular Breeding* **3**: 127-136.

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 materials 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,* 10–14 March 1997, Harare, Zimbabwe. pp.162–167.

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

Mkumbira, J., L. Chiwona-Karltun, U. 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.

MoALD, 1995. *Agricultural Research Master Plan*.

Moore, C.O., J.V. Tuschhoff, C.W. Hastings and R.V. Schanefelt, 1984. Applications of starches in foods. In: Whistler, R.L., J.N. BeMiller and E.F. Paschal (eds.). *Starch: Chemistry and Technology*. Second Edition. Academic Press, Inc, New York, USA. pp. 575-591.

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

Moorthy, S.N., 2001. Novel Biotechnological Methods of Cassava Starch Modification for Value Added Products. 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 Archive-S3-09.

Moorthy, S.N. and T. Ramanujam, 1986. Variation in properties of starch in cassava varieties in relation to age of crop. *Starch* **38**: 58-61.

Morell, M.K., R. Peakall, R. Appels, L.R. Preston and H.L. Lloyd, 1995. DNA profiling techniques for plant variety identification. *Australian Journal of Experimental Agriculture* **35**: 807-819.

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. carthaginensis* 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.

Moyo, C.C., I.R.M. Benesi, E.A.N. Gondwe and M.O. Mchinanguwo, 1999. Improved cassava varieties are beneficial to farmers. *Newsletter for SARRNET and Eastern Africa Root Crops Research Network (EARRNET). Roots* **6**: 4-5.

Moyo, C.C., I.R.M. Benesi and V.S. Sandifolo, 1998. Current status of cassava and sweetpotato production and utilisation in Malawi. The Department of Agricultural research and Technical Services report submitted to the Ministry of Agriculture and Irrigation Development. pp.41.

Mueller, U.G. and L. Wolfenbarger, 1999. AFLP genotyping and fingerprinting. *Tree* **14**: 389-394.

Munthali, H. M., 2001. The use of starch in the manufacturing of batteries. In: Mahungu, N.M., J.W. Banda and C. Mataya (eds.). *Cassava commercialisation for economic development in Malawi. Proceedings of the Symposium held at Kwacha International Conference Centre*, Blantyre, Malawi, 21–23 May 2001. p.122.

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.M.A., 1978. Genetic resources of cassava: Chromosome behaviour in some Manihot species. *International Journal of Genetics* **38**: 135-137.

Nassar, N.M.A., 1986. Genetic variation of wild Manihot species native to Brazil and its potential for cassava improvement. *Field Crops Research* **13:** 177-184.

Nassar, N.M.A., 1994. Selection and development of apomitic cassava clones. *Ciéncia e Cultura* **1:** 168-171.

Nassar, N.M.A., 2000. The transference of Apomixis genes from *Manihot neusana* Nassar to cassava (*Manihot esculenta* Crantz*). Hereditas* **132**: 167-170.

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 C.P. Costa, 1976. Tuber formation and protein content in some wild cassava (mandioca) species native to central Brazil. *Experientia* **33**: 1304-1306.

Nassar, N.M.A., N.M. Nassar Hala, C. Vieira and A. Carvalho, 1996. Induction of productive aneuploid in cassava (*Manihot esculenta* Crantz). *Brazilian Journal of Genetics* **19:** 123-125.

National Starch and Chemical Company, 2002. *100 Years of Food Starch History*. Available at URL http://www.foodstarch.com/about/abo_fhistory.asp

National Statistics Office (NSO), 1994-1999. *Bill of Entry on Starches, Dextrins and Other Modified Starches.* Zomba, Malawi.

Nei, M. and W. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences* **76**: 5269-5273.

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.

Nemara, G.S., 2003. *Morpho-agronomical and molecular markers based genetic diversity analyses and quality evaluation of sorghum [sorghum bicolor (l.) Moench] genotypes*. 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.

Ngendahayo, M. and A.G.D.O. Dixon, 2001. Effect of harvest on tuber yield, dry matter, starch and harvest index of cassava in two ecological zones in Nigeria. In: Akoroda, M.O. and J.M. Ngeve (eds.). *Root Crops in the Twentyfirst Century. Proceedings of the seventh Triannual Symposium of the International society for Tropical Root Crops-Africa Branch (ISTRC-AB)* held at Centre International des Conférences, Cotonou, Bénin 11-17 October 1998. pp.661-667.

Niba, L.L., M. M. Bokanga and F.L. Jackson, 2001. Clonal Variations in Pasting Profiles and Granular Characteristics of Cassava (*Manihot esculenta*) Starch. 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 Archive-S3-11.

Nichols, R.F., 1950. The brown streak disease of cassava. Distribution, climatic effects and diagnostic symptoms. *East African Agricultural Journal* **15**: 154-160.

Noda, T., Y. Takahata, T. Sato, I. Suda, T. Morishita, K. Ishiguro and O. Yamakawa, 1998. Relationships between chain length distribution of amylopectin and gelatinization properties within the same botanical origin of sweet potato and buckwheat. *Carbohydrate Polymers* **37:** 153–158.

Nordenskiold, E., 1924. The ethnography of South America seen from Mojos in Bolivia. *Journal of Economic Perspective* **9**: 103-115.

Nthonyiwa, A., A. Mhone, H. Kazembe, C. Moyo, V. Sandifolo, S. Jumbo, D. Siyeni, M. Mahungu, V. Rweyendela and J. White, 2005. Cassava starch pilot factory, the first of its kind in Malawi. *Newsletter for SARRNET) and Eastern Africa Root Crops Research Network (EARRNET. Roots* **9**: 20-22.

Numfor, F.A. and W.M. Walter (Jr.), 1996. Cohesiveness of native cassava starch pastes: effect of fermentation. *African Journal of Root and Tuber Crops* **1:** 29-32.

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

Nweke, F.I. and M. Bokanga, 1994. Importance of cassava processing for production in Sub-Saharan Africa. *Acta Horticulture* **375**: 401-411.

Nweke, F.I., A.G.O. Dixon, R. Asiedu and S.A. Folayan, 1994. *Cassava varietal needs of farmers and potential production growth in Africa.* COSCA working paper No. 10. Collaborative study of cassava in Africa. IITA, Ibadan, Nigeria.

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.

Ocampo. C., F. Angel, A. Jimenez, G. Jarramillo, C. Hershey, E. Granados and C. Iglesias, 1995. DNA fingerprinting to confirm possible genetic duplicates 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**: 145-151.

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

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

Osunsami, A.T., J.O. Akingbala and G. B. Oguntimein, 1989. Effect of storage on starch content and modification of cassava starch. *Starch/Starke* **41**: 54-57.

Pearson, D., 1975. *General methods-basic constituents*: Laboratory Techniques In Food Analysis. Butterworth and Company Limited, England, United Kingdom. pp.77.

Pérez, J.C., N. Morante, J. López, J.I. Lenis, G. Jaramillo, H. Ceballos and F. Calle, 2001. Advantages of the New Cassava Breeding Scheme at CIAT. 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 files-S6-22.pdf.

Perkin-Elmer Corporation, 1997. GenScan reference guide: Chemistry reference for the ABI PRISM 320 Genetic Analyser. PE Applied Biosystems.

Phibbs, H., 1997. Paper-based supports. *Journal of American Institute for Conservation* **36**: 263-267.

Phiri, M.A.R., 2001. Applying a sub-sector analysis in studying the marketing of cassava in Malawi: Summary of qualitative assessment of the Sub-Sector. In: Mahungu, N.M., J. W. Banda and C. Mataya (eds.). *Cassava commercialisation for economic development in Malawi. Proceedings of the Symposium held at Kwacha International Conference Centre*, Blantyre, Malawi, 21–23 May 2001. pp.24-36.

Poehlman, J.M., 1987. Breeding Field Crops. Third Edition, AVI Publishing Company, Inc., Westport, Connecticut, New York. pp.45. Available at URL: http://www.hec.gov.pk.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski, 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* **2**: 225-238.

Purchase, J.L., 1997. *Parametric analysis to describe genotype by environment interaction and yield stability in winter wheat*. Doctoral thesis. University of the Free State, Bloemfontein, South Africa.

Purseglove, J.W., 1968. *Tropical crops. Dicotyledons I*. Longmans, London. pp.332.

Radley, J. A., 1976. *Industrial uses of starch and its derivatives*. Applied Science Publishers, London, UK.

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

Raji, A.A., A.G.O. Dixon and T.A.O. Ladeinde, 2001a. Genetic diversity among Nigerian cassava landraces resistant to cassava mosaic disease and cassava green mite. In: Akoroda, M.O. and J.M. Ngeve (eds.). *Root Crops in the Twentyfirst Century. Proceedings of the seventh Triennial Symposium of the International Society for Tropical Root Crops-Africa Branch (ISTRC-AB*). 11-17 October 1998. Centre International des Conférences, Cotonou, Bénin. pp.443-454.

Raji, A.A., A.G.O. Dixon, I. Fawole and M. Gedil, 2001b. 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.

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.

Restrepo, S., M. Duque, J. Tohme and V. Verdier, 1998. AFLP fingerprinting: an efficient technique for detecting genetic variation of *Xanthomonas axonopodis* pv. *manihotis*. *Microbiology* **145**: 107-114.

Restrepo, S., M. Santaella, C. Lopez, C. Gonzalez, M. Chavez, E. Suarez, G. Mosquera, S. Ojeda, P. Zuluaga, C. Velez, V. Jorge, J. Tohme and V. Verdier, 2001. Cassava Bacterial Blight: Recent Advances in the Understanding and Control of the Disease. 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. CD4, CBN-V Video Archive-S8-18.

Richards, P., 1985. *Indigenous agricultural revolution*. Hutchinson, London, UK. pp.192.

Richardson, P.H., R. Jeffcoat and Y.C. Shi, 2000. High amylose starches: From biosynthesis to their use as food ingredients. *MRS Bulletin* pp.20–24.

Rickard, J. E., M. Asaoka and J. M. V. Blanshard, 1991. The physico-chemical properties of cassava starch. *Tropical Science* **31:** 189-207.

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

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/

Rockefeller Foundation, 1999. Food Security. *Annual Reports Archives*. Available at URL http://www.rockfound.org/

Rockefeller Foundation, 2002. Food Security. *Annual Reports Archives*. Available at URL http://www.rockfound.org/

Rogers, D.J. and S.G. Appan, 1973. *Manihot and manihotoides* (*Euphorbiaceae*): a computer assisted study. Organisation for Flora Neotropica. Monograph 13. Hafner, New York. pp.278.

Rohlf, F. J., 2000. NTSYS-pc: *Numerical taxonomy and multivariate analysis system. Applied Biostatistics*, Exerter Publishing Ltd., New York.

Roldan-Ruiz, I., F.A. Van Eeuwijk, T.J. Gilliland, P. Dubreuil, C. Dillmann, J. Lallemand, M. De Lose and C.P. Baril, 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. *Theoretical and Applied Genetics* **103**: 1138-1150.

Ross, H.B., 1975. *The diffusion of the manioc plant from South America to Africa. An essay in ethnobotanical culture history*. Doctoral thesis.. Faculty of Political science, Colombia University, New York.

Rossetto, M., R.W. Slade, P.R. Baverstock, R.J. Henry and L.S. Lee, 1999. Microsatellite variation and assessment of genetic structure in teatree (*Melaleuca alternifolia* - Myrtaceae). *Molecular Ecology* **8**: 633-643.

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

Salick, J., N. Cellinese and S. Knapp, 1997. Indigenous diversity of cassava: generation, maintenance, use and loss among the Amuesha, Peruvian Upper Amazon. *Economic Botany* **51**: 6-17.

Sandhu, K.S., N. Singh and M. Kaur, 2004. Characteristics of the different corn types and their grain fractions: physicochemical, thermal, morphological, and rheological properties of starches. *Journal of Food Engineering* **64**: 119-127.

Sanni, M.O. and A.O. Olubamiwa, 2003. Post harvest and seasonal changes in four cassava varieties: processing implications. In: Akoroda, M.O. (ed.). *Root crops: The small processor and development of local food industries for market economy. Proceedings of the eighth Triennial Symposium of ISTRC-AB*. Held at IITA, Ibadan, Nigeria, 12-16 November 2001. pp.185-189.

Sarmento, S.B.S., L.J.C.B. Carvalho, M.P. Cereda, M.V.C. Penteado and S.M.S. Piedade, 2000. Growth rate, starch and fibre content in cassava storage roots as an indication of harvesting time. In: Carvalho, L.J.C.B., A.M. Thro and A.D. Vilarinhos (eds.). *Cassava Biotechnology. Proceedings of the fourth International Scientific Meeting of Cassava Biotechnology Network*, held at EMBRAPA, Brazil, 03-07 November 1998. pp.357-366.

Sauti, R.F.N., 1981. Problems of cassava production in Malawi. *Tropical Root Crops. Research strategies for the 1980s*. IDRC, Otawa, Canada. pp.104-106.

Sauti, R.F.N., E.M.H. Khonje, G. Thaulo, M. Chibambo and G.M. Bulla, 1994. The importance of cassava and production constraints in Malawi. In: Akoroda, M.O. (ed.). *Root crops for food security in Africa. Proceedings of the fifth Triennial Symposium of the International Society for Tropical Root Crops-Africa Branch (ISTRC-AB)*. 22-28 November, 1992, Kampala, Uganda. pp.93-96.

Schlötterer, C. and J. Pemberton, 1994 The use of microsatellites for genetic analysis of natural populations. In: Schierwater B., B, Streit, G.P. Wagner and R. DeSalle (eds.). *Molecular Ecology and Evolution: Approaches and Applications*, Birkhäuser Verlag Basel, Switzerland, pp.203-214.

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

Scott, G.J., M.W. Rosegrant and C. Ringler, 2000. Global projections of root and tuber crops to the year 2020. *Food Policy* **25**: 561-597.

Second, G., A.C. Allem, R.A. Mendes, L.J.C.B. Carvalho, L. Emperaire, C. Ingram and C. Colombo, 1997. Molecular marker (AFLP)-based *Manihot* and cassava numerical taxonomy and genetic structure analysis in progress: Implications for their dynamic conservation and genetic mapping. *African Journal of Root and Tuber Crops* **2**: 140-147.

Seetharaman, K., A. Tziotis, F. Borras, P.J. White, M. Ferrer and J. Robutti, 2001. Thermal and functional characterization of starch from Argentinean corn. *Cereal Chemistry* **78:** 379–386.

Sevilla, R., W. Salhuana, J. Rubio, G. Avila, O. Paratori, F. Cardenas, L.H. Zhu, L. Pollak, A. Bejarano, M.X. Santos, C. Diaz, M. Fuentes, H. Sanchez, M. Ferrier and G. Vivo, 1994. Latin American maize project: a comparative genetic resources evaluation project. In: Balfourier, F. and M.R. Perretant (eds.). *Evaluation and exploration of genetic resources, pre-breeding. Proceedings of the genetic section meeting of Eucarpia*, 15-18 March 1994. pp.289-290.

Shattuck-Eidens, D.M., R.N. Bell, S.L. Neuhausen and T. Helentijaris, 1990. DNA sequence variation within maize and melon: observation from polymerase chain reaction amplification and direct sequencing. *Genetics* **126**: 207-217.

Shore, K., 2002. Decades of cassava research bear fruit. *Gene Conserve* **1**: 1-4.

Silvestre, P. and M. Arraudeau, 1983. *Le manioc. Techniques Agricoles et productions Tropicales Collection*. Maisonneuve & Larose Agence de Cooperation Culturalle et Technique, Paris. pp.262.

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

Singh, N., J. Singh, L. Kaur, N.S. Sodhi and B.S. Gill, 2003. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry* **81:** 219–231.

Singh, N., K. S. Sandhu and M. Kaur, 2005. Physicochemical Properties Including Granular Morphology, Amylose Content, Swelling and Solubility, Thermal and Pasting properties of Starches from Normal, Waxy, High Amylose and Sugary Corn. *Progress in Food Biopolymer Research* **1**: 43-50.

Smith, R.J., 1967. Characterisation and analysis of starches. In: Whistler, R.L. and E.F. Paschal (eds.), *Starch: Chemistry and Technology*, Academic Press, New York, USA. pp.569-635.

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.

Snyder, E.M., 1984. Industrial microscopy of starches. In: Whistler, R.L., J.N. BeMiller and E.F. Paschal (eds.). *Starch: Chemistry and Technology*. Second Edition. Academic Press, Inc. pp. 661-689.

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**.**

Sosinski, B. and D.S. Douches, 1996. Using PCR based DNA amplification to fingerprint North American potato cultivars. *Horticulture Science* **31**: 130-133.

Spencer, D.S.C., 1994. Infrastructure and technology constraints to agricultural development in the humid and sub-humid tropics of Africa. IFPRIEPTD discussion paper No. 3.

Sriroth, K., K. Piyachomkwan, S. Chotineeranat, R. Chollakup, V. Santisopasri and C.G. Oates, 2000. Impact of drought during early growth on cassava starch quality. In: Carvalho, L.J.C.B., A.M. Thro and A.D. Vilarinhos. *Cassava Biotechnology. Proceedings*: IV International Scientific Meeting of Cassava Biotechnology Network, held at EMBRAPA, Brazil, 03-07 November 1998. pp 367-376.

Stegemann, H., 1984. Retrospect on 25 years of cultivar identification by protein patterns and prospects for the future. *Seed Science and Technology* **15:** 20-31.

Stevens, D.J. and G.A.H. Elton, 1971. Thermal properties of starch/water system. Measurement of heat of gelatinization by differential scanning calorimeter. *Starch* **23**: 8–11.

Stojsin, D., L.W. Kannenberg, J. Rajnpreht, P.K. Pauls and R. Stojsin, 1996. Genetic relationships among commercial corn hybrids and parents based on RAPD analysis of pericarp and embryo DNA. *Genetics* **28**: 137-150.

Storey, H.H., 1936. Virus diseases of East African plants: VI. A progress report on studies of the diseases of cassava. *East African Agricultural Journal* **2**: 34-39.

Suárez, M.C., A. Bernal, J. Gutiérrez, J. Tohme and M. Fregene, 2000. Developing expressed sequence tags (ESTs) from polymorphic transcript-derived fragments (TDFs) in cassava (*Manihot esculenta* Crantz). *Genome* **43**: 62-67.

Subudhi, P.K., H.T. Nguyen, M.L. Gilbert and D.T. Rosenow, 2002. Sorghum improvement: Past achievements and future prospects. In: Kang, M.S. (ed.). *Crop improvement: Challenges in the Twenty-First Century*. The Haworth Press, Binghamton, NY. pp.109-159.

Sun, G.L., M. William, J. Liu, K.J. Kasha and K.P. Pauls, 2001. Microsatellite and RAPD polymorphism in Ontario corn hybrids are related to the commercial sources and maturity ratings. *Molecular Breeding* **7**: 13-24.

SUNY-ESF, 2005. Soil pH: What it means. Environmental information series. Available at URL: http://www.esf.edu/PUBPROG/brochure

Suzuki, A., Y. Takeda and S. Hizukuri, 1985. Relationship between the molecular structures and retrogradation properties of tapioca, potato and kuzu starches. *Journal of the Japanese Society for starch Science* **32**: 205-212.

Swanepoel, E., 1999. *Identification of genetic distances and heterotic groups of inbred maize (Zea mays) lines using DNA fingerprinting*. M. Sc. Thesis, Department of Plant Breeding, University of the Free State, Bloemfontein, South Africa.

Takeda, Y., K. Shirasaka and A. Suzuki, 1984. Examination of the purity and structure of amylose by gel-permeation chromatography. *Carbohydrate Research* **132**: 83-92.

Tanksley, S.D., N.D. Young, A.H. Paterson and M.W. Bonierbale, 1989. RFLP mapping in plant breeding, new tools for old science. *Bio/Technology* **7:** 257-262.

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

Tatineni, V., R.G. Cantrell and D.D. Davis, 1996. Genetic diversity in elite cotton germplasm determined by morphological characters and RAPDs. *Crop Science* **36**: 186-192.

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

Thomas, C.M., P. Vos, M. Zabeau, D.A. Jones, K.A. Norcott, B.P. Chadwick and J.D.G. Jones, 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato Cf-9 gene for resistance to *Claosporium fulvum*. *Plant Journal* **8:** 785-794.

Thomas, D.J. and W.A. Atwell, 1999. *Starches: Practical guides for the food industry*. Eagan Press Hand Book Series. Eagan Press, St Paul, Minnesota, USA. pp.94.

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.

Towle,M.A., 1961. *The ethnobotany of pre-Colombian Peru*. Aldine Publishing Company, Chicago. pp.180.

Umanah, E.E. and R.W. Hartman, 1973. Chromosome Numbers and Karyotypes of some *Manihot* Species. *Journal of American Society for Horticultural Science* **98**: 272-274.

Uniqema Company Limited, 2002. *Starch: The search for new functionality*. Available at URL http://www.uniqema.com/cpa/lit/cp9

Varghese, Y.A., K. Knaak, M.R. Sethuraj and J. Ecke, 1997. Evaluation of random amplified polymorphic DNA (RAPD) markers in *Hevea braziliensis*. *Plant Breeding* **116:** 47-52.

Veltkamp, H.J, 1986. *Physiological causes of yield variation in cassava* (*Manihot esculenta* Crantz). Agricultural University Wageningen Papers 85-6(1985). Agricultural University, Wageningen, the Netherlands. pp103.

Vos, P. and M. Kuiper, 1998. AFLP analysis. In: Caetano-Annolés, G. and P.M. Gresshoff (eds.). *DNA markers: Protocols, Applications and Overview*. Willey-VCH, New York. pp.115-131.

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.

Wang, M.L., N.A. Barkley, J.K. Yu, R.E. Dean, M.L. Newman, M.E. Sorrells and G.A. Pederson, 2005. Transfer of SSR markers from major cereal crops to minor grass species for germplasm characterisation and evaluation. *Plant Genetic Resources: characterisation and evaluation* **3**: 45-57.

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

Waycott, W. and S.B. Fort, 1994. A differentiation of nearly identical germplasm accessions by combination of molecular morphologic analysis. *Genome* **37:** 577-583.

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.

Welsch, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18:** 7213-7218.

Welsch, J., R.J. Honeycutt, M. McClelland and B.W.S. Sobral, 1991. Parentage determination in maize hybrids using the arbitrary primed polymerase chain reaction (AP-PCR). *Theoretical and Applied Genetics* **82:** 473-476.

Whistler, R.L., 1984. History and future expectation of starch use. In: Whistler, R.L., J.N. BeMiller and E.F. Paschal (eds.). *Starch: Chemistry and Technology*. Second Edition. Academic Press, Inc. pp.1-9.

White, G. and W. Powell, 1997. Isolation and characterisation of microsatellite loci in *Swietenia humulis* (*Meliaceae*): an endangered tropical hardwood species. *Molecular Ecology* **6**: 851-860.

Whitkus, R., J. Doebley and M. Lee, 1992. Comparative genome mapping of sorghum and maize. *Genetics* **132:** 1119-1130.

Williams, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalskiand and S.V. Tingey, 1990. DNA polymorphism 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.

Wong, H.L., H.H. Yeoh and S.H. Lim, 1999. Customisation of AFLP analysis for cassava varietal identification. *Phytochemistry* **50**: 919-924.

Wurzburg, O.B., 1986a. *Modified Starches: Properties and Uses.* CRC Press, Boca Raton, USA. pp. 3-16.

Wurzburg, O.B., 1986b. Converted starches: In: Wurzburg, O. B. (ed.). *Modified Starches: Properties and Uses.* CRC Press, Boca Raton, USA. pp 18-40.

Wyman, A.R. and R. White, 1980. A highly polymorphic locus in human DNA. *Proceedings of the National Academy of Sciences* **77**: 6754-6758.

Xu, G.W., C.W. Magill, K.F. Schertz and G.E. Hart, 1994. AFLP map of *Sorghum bicolor* (L.) Moench. *Theoretical and Applied Genetics* **89:** 139-145.

Xu, M.L., A.E. Melchinger, X.C. Xia and T. Lübberstedt, 1999. High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR and AFLP markers. *Molecular and General Genetics* **261**: 574-581.

Yamamoto, T., A. Nishikawa and K. Oeda, 1994. DNA polymorphism in *Oryza sativa* L. amplified by arbitrary primer PCR. *Euphytica* **78:** 143-148.

Yamin, F.F., M. Lee, L.M. Pollak and P.L. White, 1999. Thermal properties of starch in corn variants isolated after chemical mutagenesis of inbred line B73. *Cereal Chemistry* **76**: 175–181.

Young, A.H., 1984. Fractionation of starch: In: Whistler R.L., J.N. BeMiller and E.F. Paschal (eds.). *Starch: Chemistry and Technology*. Second Edition. Academic Press Inc., New York, USA, pp.249-283.

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.

Yuan, R.C., D.B. Thompson and C.D. Boyer, 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behaviour of maize starches from three wx-containing genotypes in two inbred lines. *Cereal Chemistry* **70**: 81–89.

Zabeau, M., 1992. *European Patent Application*. Publication number EP 0534858

Zabeau, M. and P. Vos, 1993. *European Patent Application*. Publication number EP 0534858.

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.

Zhou, M., K. Robards, M. Glennie-Holmes and S. Helliwell, 1998. Structure and pasting properties of oat starch. *Cereal Chemistry* **75**: 276–281.

Zobel, H.F., 1984. Gelatinisation of starch and mechanical properties of starch pastes. In: Whistler, R.L., J.N. BeMiller and E.F. Paschal (eds.). *Starch: Chemistry and Technology*. Second Edition. Academic Press, Inc, New York, USA. pp 285-305.

Zobel, R.W., M.J. Wright and H.G. Gauch, 1988. Statistical analysis of a yield trial. *Agronomy Journal* **80:** 388-393.

APPENDICES

Appendix 1 Passport data for the Malawian cassava germplasm which was included in this study

Acc. code	Name of cultivar	Name of farmer	Village	District	Region of Malawi	Place of origin	Latitude (^{o})	Longitude (^{o})	Altitude (m)
GNSM 1	Balaka1	Mr Gunda	Makupiza	Mulanje	South	Malawi	S 16.08778	E 035.48160	575
GNSM 2	Makhwekhwele	Mr Gunda	Makupiza	Mulanje	South	Malawi	S 16.08778	E 035.48160	575
GNSM 4	Chithekere1	Mr Gunda	Makupiza	Mulanje	South	Malawi	S 16.08778	E 035.48160	575
GNSM 5	Masangwi	Mr Gunda	Makupiza	Mulanje	South	Malawi	S 16.08778	E 035.48160	575
GNSM 7	Chithekere2	Mr Mbendeza	Makupiza	Mulanje	South	Malawi	S 16.10956	E 035.49201	606
GNSM 8	Kaligonje	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 16.10956	E 035.49201	606
GNSM 9	Mgwalangwa	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 16.10101	E 035.49139	612
GNSM 10	Chithekere3	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 16.10101	E 035.49139	612
GNSM 11	Matuvi	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 16.10101	E 035.49139	612
GNSM 12	Chitedze	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 16.10101	E 035.49139	612
GNSM 13	Manyokola5	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 16.10101	E 035.49139	612
GNSM 14	Balaka	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 16.10101	E 035.49139	612
GNSM 15	Mzonda	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 16.10101	E 035.49139	612
GNSM 16	Bele la dona	Mr S. Nangwande	Mose	Mulanje	South	Malawi	S 15.67991	E 035.63711	767
GNSM 17	Six months1	Mr Byson Timba	Nyezelera	Phalombe	South	Malawi	S 15.67991	E 035.63711	767
GNSM 18	Chilikhano1	Mr Byson Timba	Nyezelera	Phalombe	South	Malawi	S 15.67991	E 035.63711	767
GNSM 19	Mwandilawa	Mr Byson Timba	Nyezelera	Phalombe	South	Malawi	S 15.67991	E 035.63711	767
GNSM 20	Six months2	Mr Benson Likwata	Ndungunya	Phalombe	South	Malawi	S 15.68321	E 035.54404	721
GNSM 21	Chilikhano2	Mr Benson Likwata	Ndungunya	Phalombe	South	Malawi	S 15.68321	E 035.54404	721
GNSM 22	Kadekomu	Mr Benson Likwata	Ndungunya	Phalombe	South	Malawi	S 15.68321	E 035.54404	721
GNSM 24	Azungu	Mrs Kostas Chimwaza	Chongole	Nkhotakota	Centre	Malawi	S 13.13199	E 034.33111	486
GNSM 25	Aluphwana	Mr Issa Mpholozi	Chongole	Nkhotakota	Centre	Malawi	S 13.12991	E 034.33046	485
GNSM 26	Mwatatu	Mr John Chenjerani	Chongole	Nkhotakota	Centre	Malawi	S 13.12986	E 034.32990	501
GNSM 27	Gomani2	Mr John Chenjerani	Chongole	Nkhotakota	Centre	Malawi	S 13.12986	E 034.32990	501
GNSM 28	Beatrice	Miss Akuzike Kile	Chongole	Nkhotakota	Centre	Malawi	S 13.12980	E 034.32965	486
GNSM 29	Nyankhwazi	Mr John Chenjerani	Chongole	Nkhotakota	Centre	Malawi	S 13.12986	E 034.32990	501
GNSM 30	Mbawala	Mr Maginas Zembani	Chongole	Nkhotakota	Centre	Malawi	S 13.13173	E 034.33067	496
GNSM 31	Mwaya2	Mr Christopher Thom	Chamba 2	Nkhotakota	Centre	Malawo	S 13.02877	E 034.29456	587
GNSM 32	Kasungwi	Mr John Mapapa	Chamba 2	Nkhotakota	Centre	Malawi	S 13.02877	E 034.29456	587

Appendix 1 Passport data for the Malawian cassava germplasm which was included in this study (Continued)

Acc. code	Name of cultivar	Name of farmer	Village	District	Region of Malawi	Place of origin	Latitude (^{o})	Longitude (^{o})	Altitude (m)
GNSM 33	Guguza	Mrs Loice Mapapa	Chamba 2	Nkhotakota	Centre	Malawi	S 13.02877	E 034.29456	588
GNSM 34	Thipula	Mrs Loice Mapapa	Chamba 2	Nkhotakota	Centre	Malawi	S 13.02877	E 034.29456	587
GNSM 35	Popa	Mrs Joice Banda	Chamba 2	Nkhotakota	Centre	Malawi	S 13.02770	E 034.29590	586
GNSM 37	Kawalika	Mr S. Mdelemani	Munguwo	Nkhotakota	Centre	Malawi	S 12.37759	E 034.05972	577
GNSM 38	Nyaharawa	Mr S. Mdelemani	Munguwo	Nkhotakota	Centre	Malawi	S 12.37759	E 034.05972	577
GNSM 39	Chimphuno	Mr Kingsley Phiri	Munguwo	Nkhotakota	Centre	Malawi	S 12.377581	E 034.06004	541
GNSM 40	Simalilandi	Mrs Irine Zimba	Kamphandira	Nkhotakota	Centre	Malawi	S 12.30623	E 034.02362	500
GNSM 41	Kolowêka1	Mrs Leni Kasakula	Kamphandira	Nkhotakota	Centre	Malawi	S 12.30623	E 034.02362	500
GNSM 42	Chimudini	Mrs Agnes Kaunda	Kamphandira	Nkhotakota	Centre	Malawi	S 12.30585	E 034.02345	502
GNSM 44	Unknown1	Mrs Irine Zimba	Kamphandira	Nkhotakota	Centre	Malawi	S 12.30623	E 034.02362	500
GNSM 45	Biliwili	Miloni Banda	Zinyoni	Nkhata Bay	North	Malawi	S 12.21660	E 033.99935	507
GNSM 46	Ng'wenyani	Shadric V. Banda	Mazinga	Nkhata Bay	North	Malawi	S 11.86871	E 034.15340	562
GNSM 47	Nyamalonje	Shadric V. Banda	Mazinga	Nkhata Bay	North	Malawi	S 11.86871	E 034.15341	562
GNSM 49	Kalomo	Miss Eliza Banda	Mazinga	Nkhata Bay	North	Malawi	S 11.86964	E 034.15773	536
GNSM 50	Depwete	Miss L. Nyalongwe	Gamute	Nkhata Bay	North	Malawi	S 11.73985	E 034.23220	571
GNSM 51	Mpuma	Miss L. Nyalongwe	Gamute	Nkhata Bay	North	Malawi	S 11.73985	E 034.23220	571
GNSM 52	Mgodi	Miss Zudi Thundu	Chilera-Wana	Nkhata Bay	North	Malawi	S 11.64674	E 034.26177	607
GNSM 53	Kanonono	Miss Zudi Thundu	Chilera-Wana	Nkhata Bay	North	Malawi	S 11.64674	E 034.26178	607
GNSM 54	Gomani mfipa	Mrs Jenet Banda	Chilera-Wana	Nkhata Bay	North	Malawi	S 11.64955	E 034.26235	604
GNSM 56	Loresi	Miss Cicilia Manda	Chilera-Wana	Nkhata Bay	North	Malawi	S 11.64951	E 034.26239	609
GNSM 57	Nyachikundi	Miss Cicilia Manda	Chilera-Wana	Nkhata Bay	North	Malawi	S 11.64951	E 034.26239	609
GNSM 58	Gomani mtuŵa	Miss Cicilia Manda	Chilera-Wana	Nkhata Bay	North	Malawi	S 11.64950	E 034.26240	609
GNSM 59	Kachamba	Mrs Oleni Manda	Timbiri	Nkhata Bay	North	Malawi	S 11.54596	E 034.19965	564
GNSM 60	Fyoka	Mrs Mary Mhango	Chamawoya	Nkhata Bay	North	Malawi	S 11.53818	E 034.09536	1005
GNSM 61	Mbundumale	Mrs Mary Mhango	Chamawoya	Nkhata Bay	North	Malawi	S 11.53818	E 034.09537	1005
GNSM 62	Nyainki	Mrs Kerise Boyole	Chamawoya	Nkhata Bay	North	Malawi	S 11.34486	E 034.08986	901
GNSM 64	Munyakayuni	Mrs Mori Msenga	Kaliwo	Rumphi	North	Malawi	S 10.74827	E 034.13468	879
GNSM 67	20:20	Mrs Morini Nyirenda	Dimba	Rumphi	North	Malawi	S 10.70407	E 034.18604	505
GNSM 71	Chitembwere kapanthi	Mrs Trivo Nyirenda	Dimba	Rumphi	North	Malawi	S 10.70605	E 034.18592	505
GNSM 72	Chiswanthema	Mrs Trivo Nyirenda	Dimba	Rumphi	North	Malawi	S 10.70605	E 034.18592	505
GNSM 73	Masoanyoza	Mrs Irine Nyirenda	Dimba	Rumphi	North	Malawi	S 10.70496	E 034.18683	497
GNSM 76	Mangochi	Wilton Mayuni	Muyeleka	Karonga	North	Malawi	S 10.23374	E 034.10123	521
GNSM 78	Sekelemani	Kasaza Ndovi	Kayuni	Karonga	North	Malawi	S 10.07052	E 033.97368	517

Appendix 1 Passport data for the Malawian cassava germplasm which was included in this study (Continued)

Acc. code	Name of cultivar	Name of farmer	Village	District	Region of Malawi	Place of origin	Latitude (^{o})	Longitude (^{o})	Altitude (m)
GNSM 79	Yaboma	Kasaza Ndovi	Kayuni	Karonga	North	Malawi	S 10.07052	E 033.97368	517
GNSM 81	UnknownSelemani	K.K Chirwa	Mwambuli	Karonga	North	Malawi	S 09.97595	E 033.92090	511
GNSM 82	Kasantha1	Ayison Simbeye	Mwankenja	Karonga	North	Malawi	S 09.94210	E 033.76433	572
GNSM 83	Kamphunobii	Ayison Simbeye	Mwankenja	Karonga	North	Malawi	S 09.94210	E 033.76433	572
GNSM 84	Bwanali	Joel Mwenewungu	Timothy	Karonga	North	Malawi	S 09.61423	E 033.79979	524
GNSM 85	Kasantha2	Mrs Mary Kuwokwa	Mwangulukulu	Karonga	North	Malawi	S 09.68833	E 033.84341	524
GNSM 86	Kolowêka2	Charles Mvula	Chawinga	Karonga	North	Malawi	S 09.73244	E 033.86948	515
GNSM 87	Agriculture	Shadric V. Banda	Mazinga	Nkhata Bay	North	Malawi	S 11.86870	E 033.15339	562
BMN1	Manyokola1	A. Misomali	Fikani	Mulanje	South	Malawi	S 16.11077	E 035.53111	636
BMN25	Manyokola2	Mussa Yusufu	Mbamba	Mangochi	South	Malawi	S 14.40856	E 035.40619	940
BMN26	Kabuthu	Mussa Yusufu	Mbamba	Mangochi	South	Malawi	S 14.40856	E 035.40619	940
BMN27	Nakalasi	Mussa Yusufu	Mbamba	Mangochi	South	Malawi	S 14.40856	E 035.40619	940
BMN54	Mbundumali2	Mrs.M.J.Kalikumbi		Karonga	North	Malawi	S 09.61193	E 033.79873	526
BMN57	Mwaya1	W. Nyasulu	Mkombola	Nkhotakota	Centre	Malawi	S 12.64693	E 034.17617	514
BMN58	Manyokola3	Majidu John	Kamelo	Dedza	Centre	Malawi	S 14.10915	E 034.24172	1376
BMN59	Manyokola4	Abasi Aluba	LIkonde	Dedza	Centre	Malawi	S 14.15235	E 034.33425	1343
BMN60	Mkhalatsonga	Aliyele Kalonde	Chambwe	Lilongwe	Centre	Malawi	S 14.09868	E 033.84234	1095
BMN61	Yizaso	Chitedze R. S.	Chitedze R. S.	Lilongwe	Chitala R. S.	IITA*, Malawi	S 13.97765	E 033.64259	1178
BMN63	TMS4(2)1425	Chitedze R. S.	Chitedze R. S.	Lilongwe	IITA	IITA+, Nigeria	S 13.97765	E 033.64259	1178
BMN64	Mkondezi	Chitedze R. S.	Chitedze R. S.	Lilongwe	Mkondezi R. S.	IITA*, Malawi	S 13.97765	E 033.64259	1178
BMN68	CH92/082	Chitedze R. S.	Chitedze R. S.	Lilongwe	Chitala	IITA*, Malawi	S 13.97765	E 033.64259	1178
BMN71	81/00015	Chitedze R. S.	Chitedze R. S.	Lilongwe	IITA	IITA*, Nigeria	S 13.97765	E 033.64259	1178
BMN72	Gomani1	Chitedze R. S.	Chitedze R. S.	Lilongwe	IITA	IITA+, Nigeria	S 13.97765	E 033.64259	1178
BMN73	MK95/054	Chitedze R. S.	Chitedze R. S.	Lilongwe	Mkondezi R. S.	IITA*, Malawi	S 13.97765	E 033.64259	1178
BMN74	83350	Chitedze R. S.	Chitedze R. S.	Lilongwe	IITA	IITA, Nigeria	S 13.97765	E 033.64259	1178
BMN75	LCN8010	Chitedze R. S.	Chitedze R. S.	Lilongwe	IITA	IITA, Nigeria	S 13.97765	E 033.64259	1178
BMN77	Mbundumali1	Chitedze R. S.	Chitedze R. S.	Lilongwe	Centre	Malawi	S 13.97765	E 033.64259	1178
BMN78	Sauti	Chitedze R. S.	Chitedze R. S.	Lilongwe	Chitala R. S.	IITA*, Malawi	S 13.97765	E 033.64259	1178
BMN79	Maunjili	Chitedze R. S.	Chitedze R. S	Lilongwe	IITA	IITA+, Nigeria	S 13.97765	E 033.64259	1178
BMN80	Silira	Chitedze R. S.	Chitedze R. S..	Lilongwe	IITA	IITA+, Nigeria	S 13.97765	E 033.64259	1178

R. S. = Research station; IITA+, Nigeria = the clone was introduced from International Institute of Tropical Agriculture (IITA) in tissue culture plantlet form; IITA*, Malawi = Seeds came from IITA but germination and screening was done in Malawi

Appendix 2 Morphological descriptor for cassava

DESCRIPTION	CATEGORIES	IPGRI CODE
1. Colour of unexpanded apical leaves (CUAL)	1-Light green 2-Dark green 3-Green with red/purple tinge 4-Light purple 5-Purple	4.1.1
2. Hairiness of unexpanded leaves (HUL)	1-None 2-Little 3-Moderate 4-High	4.1.2
3. Mature leaf colour (MLC)	1-Light Green 2-Green 3-Dark green 4-Green purple 5-Purple	4.1.3
4. Leaf central lobe shape (LCLS)	1-Oblanceolate 2-Lincar 3-Elliptic 4-Pandurate 5-Lanceolate	4.1.5
5. Petiole colour (PC)	1-Whole petiole green 2-Mainly green but with some pale red sllading 3-Mainly red to dark 4-Whole petiole red or red/purple	4.1.7
6. Mature stem colour (MSC)	1-Silvery green 2-Light brown/orange 3-Dark brown 4-Reddish-red	4.1.8
7. Height of first branch	In cm	4.1.10
8 Colour of root surface (CRS)	1-White to cream 2-Light brown 3-Dark brown	4.1.12
9. Root inner skin colour (ISC)	1-White 2-Yellow 3-Pink	4.1.13
10. Colour of root pulp (CRP)	1-White or cream 2-Yellow	4.1.14
11. Colour of tip shoots	1-Red 2-Redish 3-Purple 4-Green	
12. Taste of tuberous root	1-Very sweet 2-Sweet 3-Bitter 4-Very bitter	

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions

	Balaka1	Makhwekhwere	Chithekere1	Masangwi	Chithekere2	Kaligonje	Mgwalangwa
Balaka1	1.0000						
Makhwekhwere	0.7500	1.0000					
Chithekere1	0.8333	0.6667	1.0000				
Masangwi	0.6667	0.7500	0.5833	1.0000			
Chithekere2	0.8333	0.6667	1.0000	0.5833	1.0000		
Kaligonje	0.5833	0.5000	0.6667	0.4167	0.6667	1.0000	
Mgwalangwa	0.6667	0.5000	0.5833	0.5833	0.5833	0.5833	1.0000
Chithekere3	0.4167	0.4167	0.5000	0.3333	0.5000	0.6667	0.2500
Matuvi	0.5833	0.6667	0.5833	0.6667	0.5833	0.5833	0.5000
Chitedze	0.5000	0.3333	0.5000	0.5000	0.5000	0.4167	0.3333
Manyokola5	0.9167	0.6667	0.7500	0.5833	0.7500	0.5833	0.7500
Balaka2	1.0000	0.7500	0.8333	0.6667	0.8333	0.5833	0.6667
Mzonda	0.5833	0.6667	0.5000	0.6667	0.5000	0.5000	0.5000
BeleLaDona	0.7500	0.7500	0.7500	0.5833	0.7500	0.5833	0.6667
SixMonths1	0.7500	0.7500	0.6667	0.5833	0.6667	0.5833	0.5833
Chilikhano1	0.4167	0.3333	0.4167	0.3333	0.4167	0.4167	0.2500
Mwandilawa	0.4167	0.4167	0.4167	0.3333	0.4167	0.4167	0.3333
SixMonths2	0.7500	0.7500	0.6667	0.5833	0.6667	0.5833	0.5833
Chilikhano2	0.4167	0.3333	0.4167	0.3333	0.4167	0.4167	0.2500
Kadekomu	0.9167	0.7500	0.7500	0.6667	0.7500	0.5000	0.5833
Azungu	0.5833	0.6667	0.5833	0.5833	0.5833	0.4167	0.3333
Aluphwana	0.7500	0.5833	0.6667	0.7500	0.6667	0.4167	0.6667
Mwatatu	0.6667	0.7500	0.6667	0.6667	0.6667	0.4167	0.4167
Gomani2	0.5000	0.5833	0.5000	0.5833	0.5000	0.3333	0.5000
Beatrice	0.5833	0.4167	0.5000	0.4167	0.5000	0.3333	0.4167
Nyankhwazi	0.5000	0.6667	0.5000	0.6667	0.5000	0.5000	0.4167
Mbawala	0.6667	0.4167	0.5833	0.5833	0.5833	0.5000	0.5833
Mwaya2	0.8333	0.6667	0.7500	0.5000	0.7500	0.6667	0.6667
Kasungwi	0.2500	0.4167	0.1667	0.2500	0.1667	0.2500	0.1667
Guguza	0.5833	0.5000	0.6667	0.4167	0.6667	0.5833	0.4167
Thipula	0.4167	0.4167	0.5000	0.5000	0.5000	0.4167	0.5833
Popa	0.4167	0.4167	0.3333	0.3333	0.3333	0.5000	0.4167
Kawalika	0.5833	0.6667	0.5833	0.6667	0.5833	0.3333	0.5000
Nyaharawa	0.5833	0.5000	0.5833	0.4167	0.5833	0.4167	0.5000
Chimphuno	0.7500	0.6667	0.7500	0.5833	0.7500	0.5833	0.5000
Simalilande	0.3333	0.5000	0.3333	0.4167	0.3333	0.3333	0.5000
Koloweka1	0.5833	0.7500	0.5833	0.6667	0.5833	0.5833	0.4167
Chimdini	0.6667	0.6667	0.6667	0.5833	0.6667	0.5000	0.5000
Unknown1	0.5833	0.3333	0.5000	0.5833	0.5000	0.2500	0.4167
Biliwili	0.5833	0.6667	0.5000	0.5000	0.5000	0.5000	0.5000
Ng'wenyani	0.7500	0.8333	0.7500	0.7500	0.7500	0.5000	0.5000
Nyamalonje	0.5000	0.7500	0.5000	0.6667	0.5000	0.5000	0.3333
Kalomo	0.5833	0.8333	0.5833	0.8333	0.5833	0.4167	0.5000
Depwete	0.5833	0.6667	0.5833	0.6667	0.5833	0.5833	0.5000
Mpuma	0.6667	0.9167	0.6667	0.8333	0.6667	0.5000	0.5000
Mgodi	0.3333	0.4167	0.3333	0.4167	0.3333	0.3333	0.3333
Kanonono	0.3333	0.5000	0.3333	0.4167	0.3333	0.2500	0.4167
GomaniMfipa	0.5833	0.5000	0.6667	0.3333	0.6667	0.4167	0.3333
Loresi	0.5833	0.6667	0.6667	0.5000	0.6667	0.5000	0.3333
Nyachikundi	0.4167	0.5000	0.3333	0.3333	0.3333	0.4167	0.3333
GomaniMtuwa	0.6667	0.6667	0.6667	0.6667	0.6667	0.5833	0.7500
Kachamba	0.5000	0.3333	0.6667	0.2500	0.6667	0.5833	0.3333
Fyoka	0.5000	0.7500	0.4167	0.5833	0.4167	0.2500	0.3333
Mbundumali3	1.0000	0.7500	0.8333	0.6667	0.8333	0.5833	0.6667
Nyainki	0.5833	0.6667	0.5833	0.6667	0.5833	0.4167	0.5000
Munyakayuni	0.4348	0.6957	0.4348	0.6957	0.4348	0.4348	0.4348

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Balaka1	Makhwekhwere	Chithekere1	Masangwi	Chithekere2	Kaligonje	Mgwalangwa
Twentetwente	0.6667	0.7500	0.5833	0.9167	0.5833	0.4167	0.6667
ChitembwereKa.	0.5000	0.6667	0.5000	0.6667	0.5000	0.5833	0.5000
Chiswanthema	0.5833	0.5000	0.4167	0.5000	0.4167	0.3333	0.4167
MasoAzungu	0.3333	0.4167	0.2500	0.2500	0.2500	0.3333	0.2500
Mangochi	0.4167	0.4167	0.5000	0.3333	0.5000	0.5000	0.1667
Sekelemani	0.7500	0.5833	0.6667	0.5833	0.6667	0.5000	0.5833
Yaboma	0.6667	0.4167	0.6667	0.4167	0.6667	0.5000	0.4167
UnknownSe.	0.5833	0.7500	0.5833	0.5833	0.5833	0.5000	0.5000
Kasantha1	0.7500	0.7500	0.7500	0.5833	0.7500	0.5000	0.5833
Kamphunobii	0.7826	0.5217	0.7826	0.6087	0.7826	0.5217	0.6087
Bwanali	0.5000	0.4167	0.5000	0.5000	0.5000	0.4167	0.3333
Kasantha2	0.6667	0.5833	0.6667	0.5833	0.6667	0.5833	0.5000
Koloweka2	0.5217	0.5217	0.4348	0.6957	0.4348	0.3478	0.5217
Agriculture	0.4167	0.5000	0.4167	0.5000	0.4167	0.5000	0.4167
Unknown2	0.3478	0.4348	0.4348	0.5217	0.4348	0.3478	0.3478
Yizaso	0.3333	0.2500	0.3333	0.3333	0.3333	0.5000	0.4167
TMS4(2)1425	0.6667	0.5833	0.6667	0.4167	0.6667	0.5833	0.5000
Mkondezi	0.5833	0.6667	0.5833	0.5833	0.5833	0.3333	0.3333
CH92/082	0.6667	0.5833	0.5833	0.6667	0.5833	0.3333	0.4167
81/00015	0.6667	0.5000	0.5833	0.5000	0.5833	0.3333	0.5000
Gomani1	0.5000	0.5833	0.5000	0.5833	0.5000	0.3333	0.5000
MK95/054	0.5000	0.4167	0.3333	0.4167	0.3333	0.2500	0.5000
83350	0.8333	0.7500	0.7500	0.8333	0.7500	0.5000	0.5833
LCN8010	0.4167	0.6667	0.4167	0.5833	0.4167	0.4167	0.3333
Mbundumali1	0.9167	0.6957	0.7500	0.6087	0.7500	0.5833	0.7500
Sauti	0.4000	0.4000	0.4000	0.4000	0.4000	0.4800	0.4000
Maunjili	0.5000	0.4167	0.4167	0.4167	0.4167	0.5000	0.4167
Silira	0.4167	0.5833	0.4167	0.5000	0.4167	0.3333	0.5000
Manyokola1	0.7500	0.7500	0.6667	0.5833	0.6667	0.5833	0.5833
Manyokola2	0.7500	0.7500	0.6667	0.5833	0.6667	0.5833	0.5833
Kabuthu	0.7500	0.7500	0.6667	0.5833	0.6667	0.5833	0.5833
Nakalasi	0.7500	0.7500	0.6667	0.5833	0.6667	0.5833	0.5833
Mbundumali2	0.9167	0.6667	0.7500	0.5833	0.7500	0.5833	0.7500
Mwaya1	0.8333	0.6667	0.7500	0.5000	0.7500	0.6667	0.6667
Manyokola3	0.9167	0.8333	0.7500	0.7500	0.7500	0.5000	0.5833
Manyokola4	0.9167	0.8333	0.7500	0.7500	0.7500	0.5000	0.5833
Mkhalatsonga	0.9167	0.6667	0.7500	0.5833	0.7500	0.6667	0.6667

	Chithekere3	Matuvi	Chitedze	Manyokola5	Balaka2	Mzonda	BeleLaDona
Chithekere3	1.0000						
Matuvi	0.3333	1.0000					
Chitedze	0.5000	0.4167	1.0000				
Manyokola5	0.4167	0.5000	0.4167	1.0000			
Balaka2	0.4167	0.5833	0.5000	0.9167	1.0000		
Mzonda	0.3333	0.4167	0.3333	0.5000	0.5833	1.0000	
BeleLaDona	0.3333	0.5833	0.2500	0.8333	0.7500	0.5000	1.0000
SixMonths1	0.5833	0.4167	0.3333	0.8333	0.7500	0.6667	0.7500
Chilikhano1	0.6667	0.3333	0.5833	0.4167	0.4167	0.3333	0.4167
Mwandilawa	0.7500	0.3333	0.4167	0.4167	0.4167	0.3333	0.4167
SixMonths2	0.5833	0.4167	0.3333	0.8333	0.7500	0.6667	0.7500
Chilikhano2	0.6667	0.3333	0.5833	0.4167	0.4167	0.3333	0.4167
Kadekomu	0.4167	0.5000	0.5833	0.8333	0.9167	0.5833	0.6667
Azungu	0.4167	0.5833	0.3333	0.5000	0.5833	0.5833	0.4167
Aluphwana	0.3333	0.5833	0.5833	0.6667	0.7500	0.5000	0.5000
Mwatatu	0.4167	0.5833	0.3333	0.5833	0.6667	0.6667	0.5000

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Chithekere3	Matuvi	Chitedze	Manyokola5	Balaka2	Mzonda	BeleLaDona
Gomani2	0.3333	0.6667	0.3333	0.4167	0.5000	0.3333	0.5000
Beatrice	0.3333	0.3333	0.5000	0.5000	0.5833	0.4167	0.3333
Nyankhwazi	0.3333	0.9167	0.5000	0.4167	0.5000	0.4167	0.5000
Mbawala	0.3333	0.5833	0.5833	0.5833	0.6667	0.5833	0.4167
Mwaya2	0.5000	0.5000	0.3333	0.9167	0.8333	0.5833	0.8333
Kasungwi	0.3333	0.4167	0.4167	0.1667	0.2500	0.4167	0.1667
Guguza	0.4167	0.7500	0.5000	0.5000	0.5833	0.4167	0.4167
Thipula	0.1667	0.5000	0.1667	0.5000	0.4167	0.5833	0.5833
Popa	0.3333	0.4167	0.3333	0.3333	0.4167	0.6667	0.3333
Kawalika	0.3333	0.5000	0.4167	0.5000	0.5833	0.7500	0.4167
Nyaharawa	0.3333	0.6667	0.4167	0.5000	0.5833	0.3333	0.4167
Chimphuno	0.4167	0.7500	0.4167	0.6667	0.7500	0.4167	0.5833
Simalilande	0.1667	0.4167	0.2500	0.2500	0.3333	0.5833	0.2500
Koloweka1	0.4167	0.8333	0.5000	0.5000	0.5833	0.4167	0.5833
Chimdini	0.5833	0.4167	0.4167	0.5833	0.6667	0.5833	0.5000
Unknown1	0.3333	0.4167	0.4167	0.5000	0.5833	0.4167	0.3333
Biliwili	0.2500	0.7500	0.4167	0.5000	0.5833	0.4167	0.5833
Ng'wenyani	0.5000	0.6667	0.4167	0.6667	0.7500	0.5833	0.5833
Nyamalonje	0.4167	0.7500	0.3333	0.4167	0.5000	0.5833	0.5000
Kalomo	0.3333	0.8333	0.3333	0.5000	0.5833	0.5833	0.5833
Depwete	0.3333	1.0000	0.4167	0.5000	0.5833	0.4167	0.5833
Mpuma	0.4167	0.7500	0.3333	0.5833	0.6667	0.5833	0.6667
Mgodi	0.2500	0.6667	0.3333	0.2500	0.3333	0.1667	0.3333
Kanonono	0.2500	0.4167	0.2500	0.2500	0.3333	0.4167	0.2500
GomaniMfipa	0.5000	0.4167	0.4167	0.5000	0.5833	0.3333	0.4167
Loresi	0.5000	0.5000	0.4167	0.5000	0.5833	0.5833	0.4167
Nyachikundi	0.3333	0.5833	0.4167	0.3333	0.4167	0.4167	0.3333
GomaniMtuwa	0.2500	0.7500	0.3333	0.5833	0.6667	0.5833	0.5833
Kachamba	0.6667	0.3333	0.3333	0.5000	0.5000	0.1667	0.5000
Fyoka	0.2500	0.5833	0.1667	0.4167	0.5000	0.6667	0.5000
Mbundumali3	0.4167	0.5833	0.5000	0.9167	1.0000	0.5833	0.7500
Nyainki	0.1667	0.8333	0.3333	0.5000	0.5833	0.4167	0.5833
Munyakayuni	0.3478	0.7826	0.3478	0.3478	0.4348	0.5217	0.4348
Twentetwente	0.3333	0.5833	0.5000	0.5833	0.6667	0.6667	0.5833
ChitembwereKa.	0.4167	0.8333	0.4167	0.4167	0.5000	0.5833	0.4167
Chiswanthema	0.5833	0.4167	0.5833	0.5000	0.5833	0.4167	0.3333
MasoAzungu	0.2500	0.5000	0.5000	0.2500	0.3333	0.4167	0.2500
Mangochi	0.7500	0.3333	0.5000	0.3333	0.4167	0.4167	0.2500
Sekelemani	0.4167	0.5000	0.5833	0.6667	0.7500	0.4167	0.5000
Yaboma	0.4167	0.4167	0.5000	0.5833	0.6667	0.4167	0.4167
UnknownSe.	0.3333	0.6667	0.3333	0.5000	0.5833	0.4167	0.5833
Kasantha1	0.4167	0.5833	0.4167	0.6667	0.7500	0.5000	0.5833
Kamphunobii	0.4348	0.6087	0.6087	0.6957	0.7826	0.4348	0.5217
Bwanali	0.3333	0.6667	0.5000	0.4167	0.5000	0.5000	0.3333
Kasantha2	0.4167	0.9167	0.5000	0.5833	0.6667	0.4167	0.5000
Koloweka2	0.2609	0.6087	0.4348	0.4348	0.5217	0.3478	0.4348
Agriculture	0.2500	0.7500	0.3333	0.3333	0.4167	0.5000	0.3333
Unknown2	0.3478	0.5217	0.3478	0.3478	0.3478	0.2609	0.5217
Yizaso	0.4167	0.4167	0.3333	0.3333	0.3333	0.3333	0.3333
TMS4(2)1425	0.5000	0.5000	0.5000	0.7500	0.6667	0.4167	0.7500
Mkondezi	0.5833	0.5000	0.4167	0.5000	0.5833	0.4167	0.4167
CH92/082	0.6667	0.4167	0.5000	0.5833	0.6667	0.4167	0.4167
81/00015	0.5833	0.3333	0.5833	0.5833	0.6667	0.3333	0.4167
Gomani1	0.3333	0.6667	0.3333	0.4167	0.5000	0.3333	0.5000
MK95/054	0.2500	0.2500	0.5833	0.4167	0.5000	0.4167	0.3333

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Chithekere3	Matuvi	Chitedze	Manyokola5	Balaka2	Mzonda	BeleLaDona
83350	0.5000	0.5833	0.5000	0.7500	0.8333	0.5833	0.5833
LCN8010	0.5833	0.5000	0.2500	0.4167	0.4167	0.3333	0.5000
Mbundumali1	0.4348	0.5000	0.4167	1.0000	0.9167	0.5217	0.8333
Sauti	0.7200	0.4000	0.4000	0.4800	0.4000	0.3200	0.4000
Maunjili	0.7500	0.3333	0.5000	0.5000	0.5000	0.2500	0.3333
Silira	0.4167	0.5000	0.3333	0.3333	0.4167	0.5000	0.3333
Manyokola1	0.5833	0.4167	0.3333	0.8333	0.7500	0.6667	0.7500
Manyokola2	0.5833	0.4167	0.3333	0.8333	0.7500	0.6667	0.7500
Kabuthu	0.5833	0.4167	0.3333	0.8333	0.7500	0.6667	0.7500
Nakalasi	0.5833	0.4167	0.3333	0.8333	0.7500	0.6667	0.7500
Mbundumali2	0.4167	0.5000	0.4167	1.0000	0.9167	0.5000	0.8333
Mwaya1	0.5000	0.5000	0.3333	0.9167	0.8333	0.5833	0.8333
Manyokola3	0.5000	0.5000	0.5000	0.8333	0.9167	0.6667	0.6667
Manyokola4	0.5000	0.5000	0.5000	0.8333	0.9167	0.6667	0.6667
Mkhalatsonga	0.5000	0.5000	0.4167	0.9167	0.9167	0.5000	0.7500

	SixMonths1	Chilikhano1	Mwandilawa	SixMonths2	Chilikhano2	Kadekom	Azungu
SixMonths1	1.0000						
Chilikhano1	0.4167	1.0000					
Mwandilawa	0.5000	0.8333	1.0000				
SixMonths2	1.0000	0.4167	0.5000	1.0000			
Chilikhano2	0.4167	1.0000	0.8333	0.4167	1.0000		
Kadekomu	0.7500	0.5000	0.4167	0.7500	0.5000	1.0000	
Azungu	0.5833	0.2500	0.3333	0.5833	0.2500	0.5833	1.0000
Aluphwana	0.5833	0.4167	0.4167	0.5833	0.4167	0.8333	0.5833
Mwatatu	0.6667	0.2500	0.3333	0.6667	0.2500	0.6667	0.9167
Gomani2	0.3333	0.3333	0.4167	0.3333	0.3333	0.4167	0.4167
Beatrice	0.4167	0.3333	0.2500	0.4167	0.3333	0.6667	0.5833
Nyankhwazi	0.4167	0.4167	0.3333	0.4167	0.4167	0.5833	0.5833
Mbawala	0.4167	0.4167	0.3333	0.4167	0.4167	0.5833	0.5833
Mwaya2	0.9167	0.4167	0.4167	0.9167	0.4167	0.7500	0.5000
Kasungwi	0.2500	0.3333	0.4167	0.2500	0.3333	0.2500	0.3333
Guguza	0.4167	0.3333	0.3333	0.4167	0.3333	0.5000	0.5833
Thipula	0.5000	0.2500	0.2500	0.5000	0.2500	0.4167	0.5000
Popa	0.4167	0.3333	0.4167	0.4167	0.3333	0.4167	0.4167
Kawalika	0.5833	0.3333	0.3333	0.5833	0.3333	0.5833	0.7500
Nyaharawa	0.4167	0.3333	0.3333	0.4167	0.3333	0.5000	0.5833
Chimphuno	0.5833	0.3333	0.3333	0.5833	0.3333	0.6667	0.8333
Simalilande	0.3333	0.0833	0.2500	0.3333	0.0833	0.3333	0.5833
Koloweka1	0.5000	0.4167	0.3333	0.5000	0.4167	0.6667	0.6667
Chimdini	0.7500	0.3333	0.4167	0.7500	0.3333	0.6667	0.5833
Unknown1	0.3333	0.4167	0.3333	0.3333	0.4167	0.5000	0.4167
Biliwili	0.4167	0.3333	0.4167	0.4167	0.3333	0.5000	0.4167
Ng'wenyani	0.7500	0.3333	0.4167	0.7500	0.3333	0.7500	0.8333
Nyamalonje	0.5000	0.2500	0.3333	0.5000	0.2500	0.5000	0.8333
Kalomo	0.5833	0.2500	0.3333	0.5833	0.2500	0.5833	0.6667
Depwete	0.4167	0.3333	0.3333	0.4167	0.3333	0.5000	0.5833
Mpuma	0.6667	0.2500	0.3333	0.6667	0.2500	0.6667	0.7500
Mgodi	0.1667	0.2500	0.3333	0.1667	0.2500	0.2500	0.3333
Kanonono	0.3333	0.1667	0.2500	0.3333	0.1667	0.3333	0.3333
GomaniMfipa	0.4167	0.3333	0.3333	0.4167	0.3333	0.5000	0.5000
Loresi	0.5833	0.2500	0.3333	0.5833	0.2500	0.5833	0.9167
Nyachikundi	0.3333	0.5000	0.4167	0.3333	0.5000	0.5000	0.3333
GomaniMtuwa	0.5000	0.2500	0.3333	0.5000	0.2500	0.5833	0.5833
Kachamba	0.4167	0.5833	0.5833	0.4167	0.5833	0.4167	0.3333

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	SixMonths1	Chilikhano1	Mwandilawa	SixMonths2	Chilikhano2	Kadekom	Azungu
Fyoka	0.5000	0.2500	0.3333	0.5000	0.2500	0.5000	0.5833
Mbundumali3	0.7500	0.4167	0.4167	0.7500	0.4167	0.9167	0.5833
Nyainki	0.4167	0.1667	0.1667	0.4167	0.1667	0.5000	0.5000
Munyakayuni	0.4348	0.2609	0.4348	0.4348	0.2609	0.4348	0.6957
Twentetwente	0.5833	0.3333	0.3333	0.5833	0.3333	0.6667	0.5000
ChitembwereKa.	0.5000	0.3333	0.4167	0.5000	0.3333	0.5000	0.6667
Chiswanthema	0.5000	0.5833	0.7500	0.5000	0.5833	0.5833	0.4167
MasoAzungu	0.2500	0.3333	0.3333	0.2500	0.3333	0.3333	0.3333
Mangochi	0.5000	0.4167	0.5000	0.5000	0.4167	0.4167	0.5000
Sekelemani	0.5833	0.4167	0.3333	0.5833	0.4167	0.8333	0.5833
Yaboma	0.4167	0.2500	0.2500	0.4167	0.2500	0.5833	0.5833
UnknownSe.	0.5000	0.2500	0.2500	0.5000	0.2500	0.5833	0.5833
Kasantha1	0.6667	0.3333	0.3333	0.6667	0.3333	0.7500	0.6667
Kamphunobii	0.5217	0.3478	0.3478	0.5217	0.3478	0.6957	0.4348
Bwanali	0.3333	0.4167	0.3333	0.3333	0.4167	0.4167	0.6667
Kasantha2	0.5000	0.4167	0.4167	0.5000	0.4167	0.5833	0.6667
Koloweka2	0.3478	0.2609	0.3478	0.3478	0.2609	0.5217	0.4348
Agriculture	0.3333	0.2500	0.2500	0.3333	0.2500	0.4167	0.5833
Unknown2	0.3478	0.4348	0.4348	0.3478	0.4348	0.3478	0.2609
Yizaso	0.3333	0.3333	0.3333	0.3333	0.3333	0.2500	0.1667
TMS4(2)1425	0.7500	0.6667	0.5833	0.7500	0.6667	0.7500	0.5000
Mkondezi	0.5833	0.4167	0.5000	0.5833	0.4167	0.5833	0.6667
CH92/082	0.5833	0.5000	0.5833	0.5833	0.5000	0.6667	0.5833
81/00015	0.5000	0.5833	0.5000	0.5000	0.5833	0.7500	0.4167
Gomani1	0.3333	0.3333	0.4167	0.3333	0.3333	0.4167	0.4167
MK95/054	0.3333	0.4167	0.4167	0.3333	0.4167	0.5833	0.1667
83350	0.7500	0.3333	0.4167	0.7500	0.3333	0.8333	0.7500
LCN8010	0.5000	0.4167	0.5000	0.5000	0.4167	0.4167	0.5000
Mbundumali1	0.8696	0.4167	0.4348	0.8696	0.4167	0.8333	0.5217
Sauti	0.6400	0.4800	0.6400	0.6400	0.4800	0.4000	0.4800
Maunjili	0.5000	0.5833	0.6667	0.5000	0.5833	0.5000	0.4167
Silira	0.4167	0.3333	0.4167	0.4167	0.3333	0.4167	0.4167
Manyokola1	1.0000	0.4167	0.5000	1.0000	0.4167	0.7500	0.5833
Manyokola2	1.0000	0.4167	0.5000	1.0000	0.4167	0.7500	0.5833
Kabuthu	1.0000	0.4167	0.5000	1.0000	0.4167	0.7500	0.5833
Nakalasi	1.0000	0.4167	0.5000	1.0000	0.4167	0.7500	0.5833
Mbundumali2	0.8333	0.4167	0.4167	0.8333	0.4167	0.8333	0.5000
Mwaya1	0.9167	0.4167	0.4167	0.9167	0.4167	0.7500	0.5000
Manyokola3	0.8333	0.4167	0.5000	0.8333	0.4167	0.9167	0.6667
Manyokola4	0.8333	0.4167	0.5000	0.8333	0.4167	0.9167	0.6667
Mkhalatsonga	0.7500	0.5000	0.5000	0.7500	0.5000	0.8333	0.5000

	Aluphwana	Mwatatu	Gomani2	Beatrice	Nyankhwazi	Mbawala	Mwaya2
Aluphwana	1.0000						
Mwatatu	0.6667	1.0000					
Gomani2	0.5833	0.5000	1.0000				
Beatrice	0.5833	0.5833	0.2500	1.0000			
Nyankhwazi	0.6667	0.5833	0.5833	0.4167	1.0000		
Mbawala	0.5833	0.5833	0.3333	0.6667	0.5000	1.0000	
Mwaya2	0.5833	0.5833	0.4167	0.4167	0.4167	0.5000	1.0000
Kasungwi	0.1667	0.3333	0.4167	0.1667	0.4167	0.2500	0.1667
Guguza	0.5000	0.5833	0.4167	0.4167	0.6667	0.6667	0.5000
Thipula	0.5000	0.5833	0.3333	0.3333	0.5000	0.4167	0.5000
Popa	0.4167	0.4167	0.3333	0.5000	0.4167	0.5000	0.4167

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Aluphwana	Mwatatu	Gomani2	Beatrice	Nyankhwazi	Mbawala	Mwaya2
Kawalika	0.5833	0.8333	0.4167	0.5833	0.5000	0.7500	0.5000
Nyaharawa	0.5000	0.5000	0.5000	0.5833	0.5833	0.5833	0.5000
Chimphuno	0.6667	0.7500	0.5833	0.5000	0.6667	0.5833	0.6667
Simalilande	0.4167	0.5833	0.3333	0.4167	0.4167	0.5000	0.2500
Koloweka1	0.6667	0.6667	0.5833	0.5000	0.9167	0.5000	0.5000
Chimdini	0.5833	0.6667	0.4167	0.6667	0.4167	0.5000	0.6667
Unknown1	0.5833	0.5000	0.5000	0.5000	0.3333	0.5833	0.4167
Biliwili	0.5000	0.4167	0.5833	0.2500	0.6667	0.5000	0.5000
Ng'wenyani	0.7500	0.9167	0.5833	0.5000	0.6667	0.5000	0.6667
Nyamalonje	0.5000	0.8333	0.5000	0.5000	0.7500	0.5833	0.4167
Kalomo	0.6667	0.7500	0.6667	0.3333	0.8333	0.4167	0.5000
Depwete	0.5833	0.5833	0.6667	0.3333	0.9167	0.5833	0.5000
Mpuma	0.6667	0.8333	0.6667	0.4167	0.7500	0.4167	0.5833
Mgodi	0.4167	0.3333	0.7500	0.2500	0.5833	0.3333	0.2500
Kanonono	0.3333	0.4167	0.5000	0.3333	0.4167	0.2500	0.2500
GomaniMfipa	0.4167	0.5833	0.6667	0.4167	0.3333	0.4167	0.5000
Loresi	0.5000	0.8333	0.3333	0.5833	0.5000	0.5833	0.5000
Nyachikundi	0.4167	0.3333	0.5000	0.4167	0.6667	0.3333	0.3333
GomaniMtuwa	0.7500	0.6667	0.7500	0.3333	0.6667	0.5000	0.5833
Kachamba	0.3333	0.3333	0.3333	0.4167	0.2500	0.3333	0.5000
Fyoka	0.4167	0.6667	0.5833	0.3333	0.5833	0.4167	0.4167
Mbundumali3	0.7500	0.6667	0.5000	0.5833	0.5000	0.6667	0.8333
Nyainki	0.5833	0.5833	0.6667	0.2500	0.7500	0.4167	0.5000
Munyakayuni	0.6087	0.6957	0.6087	0.3478	0.7826	0.5217	0.3478
Twentetwente	0.6667	0.5833	0.5000	0.5000	0.5833	0.6667	0.5000
ChitembwereKa.	0.5833	0.6667	0.5000	0.3333	0.8333	0.5000	0.4167
Chiswanthema	0.5833	0.4167	0.5833	0.4167	0.4167	0.5000	0.4167
MasoAzungu	0.2500	0.3333	0.3333	0.2500	0.5000	0.3333	0.2500
Mangochi	0.3333	0.5000	0.4167	0.4167	0.3333	0.4167	0.4167
Sekelemani	0.7500	0.5833	0.3333	0.8333	0.5833	0.6667	0.5833
Yaboma	0.5000	0.5833	0.3333	0.8333	0.3333	0.6667	0.5000
UnknownSe.	0.5000	0.5833	0.5000	0.5000	0.6667	0.5000	0.5000
Kasantha1	0.6667	0.7500	0.5000	0.5833	0.5833	0.5833	0.6667
Kamphunobii	0.6957	0.5217	0.5217	0.5217	0.5217	0.6087	0.6087
Bwanali	0.5000	0.6667	0.5000	0.5833	0.5833	0.7500	0.4167
Kasantha2	0.6667	0.6667	0.5833	0.4167	0.8333	0.6667	0.5833
Koloweka2	0.6957	0.4348	0.6087	0.5217	0.6087	0.4348	0.3478
Agriculture	0.5000	0.5833	0.4167	0.3333	0.7500	0.5000	0.3333
Unknown2	0.4348	0.3478	0.5217	0.1739	0.5217	0.1739	0.3478
Yizaso	0.3333	0.2500	0.5833	0.0833	0.3333	0.1667	0.4167
TMS4(2)1425	0.5833	0.5000	0.2500	0.5000	0.5833	0.5000	0.7500
Mkondezi	0.5833	0.7500	0.7500	0.4167	0.5000	0.3333	0.5000
CH92/082	0.6667	0.6667	0.5833	0.5000	0.4167	0.4167	0.5000
81/00015	0.6667	0.5000	0.5000	0.6667	0.4167	0.5000	0.5000
Gomani1	0.5833	0.5000	1.0000	0.2500	0.5833	0.3333	0.4167
MK95/054	0.5833	0.2500	0.5833	0.3333	0.3333	0.2500	0.3333
83350	0.8333	0.8333	0.5000	0.5833	0.5833	0.5833	0.6667
LCN8010	0.4167	0.5833	0.7500	0.2500	0.5000	0.1667	0.4167
Mbundumali1	0.6667	0.6087	0.4167	0.5000	0.4167	0.5833	0.9167
Sauti	0.4800	0.4800	0.5600	0.3200	0.4000	0.3200	0.5600
Maunjili	0.4167	0.4167	0.4167	0.5000	0.3333	0.5000	0.4167
Silira	0.4167	0.5000	0.6667	0.3333	0.5000	0.3333	0.3333
Manyokola1	0.5833	0.6667	0.3333	0.4167	0.4167	0.4167	0.9167
Manyokola2	0.5833	0.6667	0.3333	0.4167	0.4167	0.4167	0.9167
Kabuthu	0.5833	0.6667	0.3333	0.4167	0.4167	0.4167	0.9167

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Aluphwana	Mwatatu	Gomani2	Beatrice	Nyankhwazi	Mbawala	Mwaya2
Nakalasi	0.5833	0.6667	0.3333	0.4167	0.4167	0.4167	0.9167
Mbundumali2	0.6667	0.5833	0.4167	0.5000	0.4167	0.5833	0.9167
Mwaya1	0.5833	0.5833	0.4167	0.4167	0.4167	0.5000	1.0000
Manyokola3	0.7500	0.7500	0.4167	0.5833	0.5000	0.5833	0.7500
Manyokola4	0.7500	0.7500	0.4167	0.5833	0.5000	0.5833	0.7500
Mkhalatsonga	0.6667	0.5833	0.4167	0.5000	0.4167	0.5833	0.8333

	Kasungwi	Guguza	Thipula	Popa	Kawalika	Nyaharawa	Chimphuno
Kasungwi	1.0000						
Guguza	0.5000	1.0000					
Thipula	0.2500	0.4167	1.0000				
Popa	0.4167	0.4167	0.5000	1.0000			
Kawalika	0.3333	0.5833	0.5000	0.4167	1.0000		
Nyaharawa	0.3333	0.6667	0.3333	0.4167	0.5833	1.0000	
Chimphuno	0.2500	0.6667	0.4167	0.3333	0.5833	0.7500	1.0000
Simalilande	0.4167	0.4167	0.4167	0.5000	0.6667	0.4167	0.4167
Koloweka1	0.3333	0.5833	0.4167	0.4167	0.5000	0.5000	0.7500
Chimdini	0.2500	0.4167	0.3333	0.5000	0.6667	0.6667	0.5833
Unknown1	0.2500	0.5000	0.3333	0.3333	0.5000	0.5000	0.5000
Biliwili	0.4167	0.5833	0.2500	0.5000	0.4167	0.5000	0.5833
Ng'wenyani	0.3333	0.5833	0.5000	0.3333	0.7500	0.5833	0.8333
Nyamalonje	0.4167	0.5833	0.5000	0.5000	0.6667	0.4167	0.6667
Kalomo	0.4167	0.5833	0.5833	0.3333	0.6667	0.5833	0.6667
Depwete	0.4167	0.7500	0.5000	0.4167	0.5000	0.6667	0.7500
Mpuma	0.3333	0.5000	0.5000	0.3333	0.6667	0.5000	0.7500
Mgodi	0.4167	0.4167	0.1667	0.4167	0.2500	0.5000	0.5000
Kanonono	0.5833	0.3333	0.3333	0.3333	0.5000	0.5833	0.3333
GomaniMfipa	0.5000	0.6667	0.2500	0.2500	0.5000	0.5000	0.5833
Loresi	0.3333	0.6667	0.4167	0.4167	0.7500	0.5833	0.7500
Nyachikundi	0.6667	0.5000	0.3333	0.5833	0.3333	0.5833	0.4167
GomaniMtuwa	0.3333	0.5833	0.5833	0.5000	0.5833	0.5833	0.7500
Kachamba	0.0833	0.4167	0.2500	0.2500	0.2500	0.5000	0.5000
Fyoka	0.5833	0.5000	0.5000	0.4167	0.6667	0.4167	0.4167
Mbundumali3	0.2500	0.5833	0.4167	0.4167	0.5833	0.5833	0.7500
Nyainki	0.3333	0.5833	0.5000	0.2500	0.5000	0.5833	0.6667
Munyakayuni	0.4348	0.6087	0.4348	0.5217	0.6087	0.4348	0.6087
Twentetwente	0.2500	0.4167	0.4167	0.3333	0.7500	0.5000	0.5000
ChitembwereKa.	0.5833	0.6667	0.5833	0.5000	0.5833	0.5833	0.6667
Chiswanthema	0.5833	0.4167	0.1667	0.5000	0.4167	0.4167	0.4167
MasoAzungu	0.8333	0.5833	0.4167	0.5833	0.3333	0.4167	0.3333
Mangochi	0.5833	0.5833	0.1667	0.4167	0.4167	0.3333	0.4167
Sekelemani	0.1667	0.5000	0.3333	0.3333	0.5833	0.5833	0.6667
Yaboma	0.1667	0.5833	0.3333	0.5000	0.5000	0.5833	0.5833
UnknownSe.	0.2500	0.5000	0.4167	0.4167	0.5833	0.5833	0.6667
Kasantha1	0.2500	0.5833	0.5000	0.4167	0.7500	0.6667	0.7500
Kamphunobii	0.2609	0.6957	0.4348	0.3478	0.5217	0.6957	0.6087
Bwanali	0.3333	0.6667	0.4167	0.5833	0.6667	0.6667	0.6667
Kasantha2	0.4167	0.8333	0.5000	0.4167	0.5833	0.7500	0.8333
Koloweka2	0.1739	0.3478	0.4348	0.5217	0.3478	0.4348	0.5217
Agriculture	0.5833	0.7500	0.6667	0.5833	0.5000	0.5000	0.5833
Unknown2	0.2609	0.2609	0.6087	0.2609	0.2609	0.3478	0.3478
Yizaso	0.5000	0.2500	0.4167	0.2500	0.1667	0.3333	0.3333
TMS4(2)1425	0.2500	0.5000	0.5000	0.4167	0.4167	0.4167	0.5833
Mkondezi	0.5000	0.4167	0.3333	0.2500	0.5833	0.5000	0.6667
CH92/082	0.3333	0.3333	0.3333	0.2500	0.5000	0.4167	0.5833

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Kasungwi	Guguza	Thipula	Popa	Kawalika	Nyaharawa	Chimphuno
81/00015	0.2500	0.3333	0.2500	0.2500	0.5000	0.5000	0.5000
Gomani1	0.4167	0.4167	0.3333	0.3333	0.4167	0.5000	0.5833
MK95/054	0.5833	0.1667	0.2500	0.4167	0.2500	0.2500	0.2500
83350	0.2500	0.5000	0.5000	0.3333	0.6667	0.5000	0.7500
LCN8010	0.4167	0.2500	0.3333	0.1667	0.4167	0.3333	0.5000
Mbundumali1	0.1739	0.5000	0.5000	0.3333	0.5217	0.5000	0.6667
Sauti	0.4000	0.3200	0.3200	0.4000	0.3200	0.3200	0.4800
Maunjili	0.4167	0.3333	0.1667	0.2500	0.4167	0.4167	0.4167
Silira	0.6667	0.4167	0.4167	0.3333	0.5833	0.5833	0.4167
Manyokola1	0.2500	0.4167	0.5000	0.4167	0.5833	0.4167	0.5833
Manyokola2	0.2500	0.4167	0.5000	0.4167	0.5833	0.4167	0.5833
Kabuthu	0.2500	0.4167	0.5000	0.4167	0.5833	0.4167	0.5833
Nakalasi	0.2500	0.4167	0.5000	0.4167	0.5833	0.4167	0.5833
Mbundumali2	0.1667	0.5000	0.5000	0.3333	0.5000	0.5000	0.6667
Mwaya1	0.1667	0.5000	0.5000	0.4167	0.5000	0.5000	0.6667
Manyokola3	0.3333	0.5000	0.4167	0.4167	0.6667	0.5000	0.6667
Manyokola4	0.3333	0.5000	0.4167	0.4167	0.6667	0.5000	0.6667
Mkhalatsonga	0.1667	0.5000	0.4167	0.3333	0.5000	0.5000	0.6667

	Simalilande	Koloweka1	Chimdini	Unknown1	Biliwili	Ng'wenyani	Nyamalonje
Simalilande	1.0000						
Koloweka1	0.4167	1.0000					
Chimdini	0.4167	0.5000	1.0000				
Unknown1	0.1667	0.3333	0.5000	1.0000			
Biliwili	0.5000	0.6667	0.3333	0.2500	1.0000		
Ng'wenyani	0.5000	0.7500	0.7500	0.5000	0.5000	1.0000	
Nyamalonje	0.5833	0.8333	0.5000	0.3333	0.5833	0.7500	1.0000
Kalomo	0.5000	0.7500	0.5833	0.4167	0.5833	0.8333	0.7500
Depwete	0.4167	0.8333	0.4167	0.4167	0.7500	0.6667	0.7500
Mpuma	0.5000	0.8333	0.6667	0.4167	0.5833	0.9167	0.8333
Mgodi	0.4167	0.5833	0.3333	0.4167	0.6667	0.4167	0.5000
Kanonono	0.5000	0.3333	0.5833	0.3333	0.4167	0.5000	0.3333
GomaniMfipa	0.2500	0.4167	0.5000	0.5833	0.3333	0.5833	0.4167
Loresi	0.5833	0.5833	0.5833	0.3333	0.4167	0.7500	0.7500
Nyachikundi	0.3333	0.5833	0.4167	0.3333	0.5000	0.4167	0.4167
GomaniMtuwa	0.5833	0.6667	0.5000	0.5000	0.6667	0.7500	0.5833
Kachamba	0.1667	0.3333	0.5000	0.5000	0.2500	0.4167	0.2500
Fyoka	0.5000	0.5000	0.4167	0.3333	0.5000	0.5833	0.6667
Mbundumali3	0.3333	0.5833	0.6667	0.5833	0.5833	0.7500	0.5000
Nyainki	0.4167	0.6667	0.4167	0.4167	0.7500	0.6667	0.5833
Munyakayuni	0.6087	0.7826	0.4348	0.3478	0.6957	0.6957	0.8696
Twentetwente	0.5000	0.5833	0.6667	0.5000	0.5000	0.6667	0.5833
ChitembwereKa.	0.5833	0.7500	0.5000	0.3333	0.5833	0.7500	0.7500
Chiswanthema	0.3333	0.4167	0.5000	0.4167	0.5000	0.5000	0.4167
MasoAzungu	0.4167	0.4167	0.2500	0.2500	0.5000	0.3333	0.4167
Mangochi	0.2500	0.4167	0.5833	0.4167	0.2500	0.5000	0.5000
Sekelemani	0.4167	0.6667	0.6667	0.4167	0.4167	0.6667	0.5000
Yaboma	0.3333	0.4167	0.5833	0.5833	0.3333	0.5000	0.5000
UnknownSe.	0.5833	0.7500	0.5833	0.2500	0.5833	0.6667	0.6667
Kasantha1	0.5000	0.6667	0.7500	0.4167	0.5000	0.8333	0.5833
Kamphunobii	0.2609	0.4348	0.6087	0.6087	0.4348	0.6087	0.3478
Bwanali	0.4167	0.5833	0.5000	0.6667	0.5000	0.5833	0.6667
Kasantha2	0.4167	0.7500	0.5000	0.5000	0.6667	0.7500	0.6667
Koloweka2	0.3478	0.6087	0.4348	0.4348	0.5217	0.5217	0.5217
Agriculture	0.5000	0.6667	0.3333	0.4167	0.5000	0.5833	0.6667

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Simalilande	Koloweka1	Chimdini	Unknown1	Biliwili	Ng'wenyani	Nyamalonje
Unknown2	0.1739	0.4348	0.3478	0.2609	0.3478	0.4348	0.3478
Yizaso	0.1667	0.2500	0.3333	0.3333	0.2500	0.3333	0.1667
TMS4(2)1425	0.2500	0.6667	0.5000	0.2500	0.5000	0.5833	0.5000
Mkondezi	0.3333	0.5833	0.6667	0.5000	0.3333	0.8333	0.5833
CH92/082	0.2500	0.5000	0.6667	0.6667	0.2500	0.7500	0.5000
81/00015	0.2500	0.5000	0.6667	0.5833	0.2500	0.5833	0.3333
Gomani1	0.3333	0.5833	0.4167	0.5000	0.5833	0.5833	0.5000
MK95/054	0.3333	0.3333	0.3333	0.3333	0.4167	0.3333	0.1667
83350	0.4167	0.6667	0.7500	0.5833	0.4167	0.9167	0.6667
LCN8010	0.2500	0.5833	0.5000	0.3333	0.3333	0.6667	0.5833
Mbundumali1	0.2609	0.5000	0.6087	0.5000	0.5000	0.6957	0.4348
Sauti	0.2400	0.4800	0.5600	0.3200	0.3200	0.5600	0.4800
Maunjili	0.2500	0.4167	0.5833	0.3333	0.2500	0.5000	0.4167
Silira	0.5000	0.4167	0.5833	0.3333	0.3333	0.5833	0.4167
Manyokola1	0.3333	0.5000	0.7500	0.3333	0.4167	0.7500	0.5000
Manyokola2	0.3333	0.5000	0.7500	0.3333	0.4167	0.7500	0.5000
Kabuthu	0.3333	0.5000	0.7500	0.3333	0.4167	0.7500	0.5000
Nakalasi	0.3333	0.5000	0.7500	0.3333	0.4167	0.7500	0.5000
Mbundumali2	0.2500	0.5000	0.5833	0.5000	0.5000	0.6667	0.4167
Mwaya1	0.2500	0.5000	0.6667	0.4167	0.5000	0.6667	0.4167
Manyokola3	0.4167	0.5833	0.7500	0.5000	0.5000	0.8333	0.5833
Manyokola4	0.4167	0.5833	0.7500	0.5000	0.5000	0.8333	0.5833
Mkhalatsonga	0.2500	0.5000	0.5833	0.5000	0.5000	0.6667	0.4167

	Kalomo	Depwete	Mpuma	Mgodi	Kanonono	GomaniMf.	Loresi
Kalomo	1.0000						
Depwete	0.8333	1.0000					
Mpuma	0.9167	0.7500	1.0000				
Mgodi	0.5000	0.6667	0.5000	1.0000			
Kanonono	0.5833	0.4167	0.5000	0.5000	1.0000		
GomaniMfipa	0.4167	0.4167	0.5000	0.4167	0.4167	1.0000	
Loresi	0.5833	0.5000	0.6667	0.2500	0.3333	0.5833	1.0000
Nyachikundi	0.5000	0.5833	0.4167	0.5833	0.6667	0.4167	0.3333
GomaniMtuwa	0.7500	0.7500	0.7500	0.5833	0.5000	0.5000	0.5000
Kachamba	0.2500	0.3333	0.3333	0.3333	0.2500	0.5000	0.4167
Fyoka	0.7500	0.5833	0.6667	0.4167	0.5833	0.5000	0.5833
Mbundumali3	0.5833	0.5833	0.6667	0.3333	0.3333	0.5833	0.5833
Nyainki	0.8333	0.8333	0.7500	0.5833	0.5833	0.4167	0.4167
Munyakayuni	0.7826	0.7826	0.7826	0.6087	0.3478	0.3478	0.6087
Twentetwente	0.7500	0.5833	0.7500	0.3333	0.5000	0.3333	0.5000
ChitembwereKa.	0.8333	0.8333	0.7500	0.5000	0.5833	0.3333	0.5833
Chiswanthema	0.4167	0.4167	0.4167	0.5000	0.4167	0.5000	0.4167
MasoAzungu	0.4167	0.5000	0.3333	0.3333	0.4167	0.4167	0.3333
Mangochi	0.3333	0.3333	0.4167	0.3333	0.3333	0.7500	0.5833
Sekelemani	0.5000	0.5000	0.5833	0.2500	0.3333	0.4167	0.5833
Yaboma	0.3333	0.4167	0.4167	0.3333	0.2500	0.5833	0.6667
UnknownSe.	0.6667	0.6667	0.7500	0.4167	0.4167	0.4167	0.5833
Kasantha1	0.6667	0.5833	0.7500	0.3333	0.5000	0.5833	0.6667
Kamphunobii	0.6087	0.6087	0.5217	0.3478	0.4348	0.6087	0.5217
Bwanali	0.5000	0.6667	0.5000	0.5833	0.3333	0.5000	0.5833
Kasantha2	0.7500	0.9167	0.6667	0.5833	0.4167	0.5000	0.5833
Koloweka2	0.6087	0.6087	0.6087	0.5217	0.2609	0.2609	0.3478
Agriculture	0.6667	0.7500	0.5833	0.4167	0.4167	0.4167	0.5000
Unknown2	0.6087	0.5217	0.5217	0.3478	0.3478	0.2609	0.1739
Yizaso	0.4167	0.4167	0.3333	0.4167	0.5000	0.4167	0.0833
TMS4(2)1425	0.4167	0.5000	0.5000	0.2500	0.1667	0.3333	0.5000

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Kalomo	Depwete	Mpuma	Mgodi	Kanonono	GomaniM	Loresi
Mkondezi	0.6667	0.5000	0.7500	0.5000	0.5833	0.7500	0.5833
CH92/082	0.5833	0.4167	0.6667	0.3333	0.4167	0.5833	0.5000
81/00015	0.4167	0.3333	0.5000	0.2500	0.4167	0.5833	0.4167
Gomani1	0.6667	0.6667	0.6667	0.7500	0.5000	0.6667	0.3333
MK95/054	0.3333	0.2500	0.3333	0.4167	0.5000	0.4167	0.1667
83350	0.7500	0.5833	0.8333	0.3333	0.4167	0.5000	0.6667
LCN8010	0.6667	0.5000	0.7500	0.5000	0.5000	0.5833	0.4167
Mbundumali1	0.5217	0.5000	0.6087	0.2500	0.2609	0.5000	0.5217
Sauti	0.4000	0.4000	0.4800	0.4800	0.3200	0.4800	0.4000
Maunjili	0.3333	0.3333	0.4167	0.3333	0.4167	0.5000	0.4167
Silira	0.6667	0.5000	0.5833	0.4167	0.8333	0.5833	0.4167
Manyokola1	0.5833	0.4167	0.6667	0.1667	0.3333	0.4167	0.5833
Manyokola2	0.5833	0.4167	0.6667	0.1667	0.3333	0.4167	0.5833
Kabuthu	0.5833	0.4167	0.6667	0.1667	0.3333	0.4167	0.5833
Nakalasi	0.5833	0.4167	0.6667	0.1667	0.3333	0.4167	0.5833
Mbundumali2	0.5000	0.5000	0.5833	0.2500	0.2500	0.5000	0.5000
Mwaya1	0.5000	0.5000	0.5833	0.2500	0.2500	0.5000	0.5000
Manyokola3	0.6667	0.5000	0.7500	0.2500	0.4167	0.5000	0.6667
Manyokola4	0.6667	0.5000	0.7500	0.2500	0.4167	0.5000	0.6667
Mkhalatsonga	0.5000	0.5000	0.5833	0.2500	0.2500	0.5000	0.5000

	Nyachikundi	GomaniMtuwa	Kachamba	Fyoka	Mbundumali3	Nyainki	Munyakayuni
Nyachikundi	1.0000						
GomaniMtuwa	0.5000	1.0000					
Kachamba	0.3333	0.3333	1.0000				
Fyoka	0.5833	0.5000	0.1667	1.0000			
Mbundumali3	0.4167	0.6667	0.5000	0.5000	1.0000		
Nyainki	0.4167	0.7500	0.2500	0.5833	0.5833	1.0000	
Munyakayuni	0.4348	0.6957	0.1739	0.6087	0.4348	0.6087	1.0000
Twentetwente	0.3333	0.5833	0.2500	0.5833	0.6667	0.5833	0.6087
ChitembwereKa.	0.6667	0.7500	0.2500	0.5833	0.5000	0.6667	0.7826
Chiswanthema	0.5833	0.4167	0.3333	0.5000	0.5833	0.2500	0.5217
MasoAzungu	0.5833	0.4167	0.0833	0.4167	0.3333	0.4167	0.4348
Mangochi	0.4167	0.2500	0.4167	0.4167	0.4167	0.1667	0.4348
Sekelemani	0.4167	0.5000	0.4167	0.3333	0.7500	0.4167	0.4348
Yaboma	0.3333	0.4167	0.5833	0.3333	0.6667	0.3333	0.3478
UnknownSe.	0.4167	0.5833	0.4167	0.5000	0.5833	0.5833	0.6087
Kasantha1	0.4167	0.6667	0.4167	0.5000	0.7500	0.5833	0.5217
Kamphunobii	0.4348	0.6087	0.5217	0.4348	0.7826	0.6087	0.3636
Bwanali	0.5000	0.5833	0.4167	0.4167	0.5000	0.5000	0.6087
Kasantha2	0.5833	0.7500	0.4167	0.5000	0.6667	0.7500	0.6957
Koloweka2	0.3478	0.6087	0.2609	0.3478	0.5217	0.5217	0.6364
Agriculture	0.5833	0.6667	0.1667	0.5000	0.4167	0.5833	0.6957
Unknown2	0.2609	0.4348	0.3478	0.3478	0.3478	0.6087	0.3636
Yizaso	0.4167	0.5000	0.3333	0.3333	0.3333	0.5000	0.1739
TMS4(2)1425	0.5000	0.4167	0.5000	0.3333	0.6667	0.3333	0.4348
Mkondezi	0.5000	0.5833	0.4167	0.5833	0.5833	0.5000	0.5217
CH92/082	0.3333	0.5000	0.5000	0.4167	0.6667	0.4167	0.4348
81/00015	0.4167	0.4167	0.5000	0.3333	0.6667	0.3333	0.2609
Gomani1	0.5000	0.7500	0.3333	0.5833	0.5000	0.6667	0.6087
MK95/054	0.5833	0.5000	0.1667	0.4167	0.5000	0.3333	0.2609
83350	0.3333	0.6667	0.4167	0.5000	0.8333	0.5833	0.6087
LCN8010	0.4167	0.5000	0.4167	0.5833	0.4167	0.5000	0.5217
Mbundumali1	0.3333	0.5833	0.5000	0.4348	0.9167	0.5000	0.3636
Sauti	0.4000	0.4000	0.4000	0.3200	0.4000	0.2400	0.5000

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	Nyachikundi	GomaniMtuwa	Kachamba	Fyoka	Mbundumali3	Nyainki	Munyakayuni
Maunjili	0.4167	0.2500	0.5000	0.3333	0.5000	0.1667	0.3478
Silira	0.6667	0.5833	0.2500	0.6667	0.4167	0.5000	0.4348
Manyokola1	0.3333	0.5000	0.4167	0.5000	0.7500	0.4167	0.4348
Manyokola2	0.3333	0.5000	0.4167	0.5000	0.7500	0.4167	0.4348
Kabuthu	0.3333	0.5000	0.4167	0.5000	0.7500	0.4167	0.4348
Nakalasi	0.3333	0.5000	0.4167	0.5000	0.7500	0.4167	0.4348
Mbundumali2	0.3333	0.5833	0.5000	0.4167	0.9167	0.5000	0.3478
Mwaya1	0.3333	0.5833	0.5000	0.4167	0.8333	0.5000	0.3478
Manyokola3	0.4167	0.5833	0.4167	0.5833	0.9167	0.5000	0.5217
Manyokola4	0.4167	0.5833	0.4167	0.5833	0.9167	0.5000	0.5217
Mkhalatsonga	0.3333	0.5833	0.5833	0.4167	0.9167	0.5000	0.3478

	Twentetwente	ChitembwereKa.	Chiswanthema	MasoAzungu	Mangochi	Sekelemi	Yaboma
Twentetwente	1.0000						
ChitembwereKa.	0.5833	1.0000					
Chiswanthema	0.5000	0.5000	1.0000				
MasoAzungu	0.2500	0.5833	0.4167	1.0000			
Mangochi	0.3333	0.4167	0.6667	0.4167	1.0000		
Sekelemani	0.6667	0.5000	0.5000	0.2500	0.4167	1.0000	
Yaboma	0.4167	0.3333	0.4167	0.2500	0.5000	0.6667	1.0000
UnknownSe.	0.6667	0.5833	0.3333	0.4167	0.3333	0.6667	0.4167
Kasantha1	0.6667	0.5833	0.4167	0.4167	0.4167	0.7500	0.5000
Kamphunobii	0.6087	0.5217	0.5217	0.3478	0.4348	0.6087	0.6957
Bwanali	0.4167	0.5833	0.4167	0.4167	0.4167	0.4167	0.6667
Kasantha2	0.5000	0.8333	0.5000	0.5000	0.4167	0.5833	0.5000
Koloweka2	0.6087	0.5217	0.5217	0.3478	0.2609	0.6087	0.5217
Agriculture	0.4167	0.8333	0.3333	0.7500	0.4167	0.4167	0.3333
Unknown2	0.4348	0.4348	0.2609	0.4348	0.1739	0.2609	0.1739
Yizaso	0.2500	0.4167	0.3333	0.4167	0.3333	0.1667	0.1667
TMS4(2)1425	0.4167	0.5000	0.5000	0.3333	0.4167	0.6667	0.4167
Mkondezi	0.5000	0.5833	0.6667	0.3333	0.6667	0.5000	0.4167
CH92/082	0.5833	0.5000	0.6667	0.2500	0.5833	0.5833	0.5000
81/00015	0.5833	0.3333	0.5833	0.2500	0.5000	0.7500	0.5000
Gomani1	0.5000	0.5000	0.5833	0.3333	0.4167	0.3333	0.3333
MK95/054	0.4167	0.3333	0.6667	0.5000	0.3333	0.4167	0.2500
83350	0.7500	0.6667	0.5833	0.2500	0.5000	0.7500	0.5833
LCN8010	0.5000	0.5000	0.5000	0.2500	0.5000	0.3333	0.2500
Mbundumali1	0.6087	0.4348	0.5217	0.2500	0.3478	0.6667	0.5833
Sauti	0.3200	0.4800	0.7200	0.2400	0.7200	0.4000	0.3200
Maunjili	0.5000	0.4167	0.7500	0.2500	0.6667	0.5833	0.4167
Silira	0.5833	0.6667	0.5833	0.5000	0.5000	0.4167	0.2500
Manyokola1	0.5833	0.5000	0.5000	0.2500	0.5000	0.5833	0.4167
Manyokola2	0.5833	0.5000	0.5000	0.2500	0.5000	0.5833	0.4167
Kabuthu	0.5833	0.5000	0.5000	0.2500	0.5000	0.5833	0.4167
Nakalasi	0.5833	0.5000	0.5000	0.2500	0.5000	0.5833	0.4167
Mbundumali2	0.5833	0.4167	0.5000	0.2500	0.3333	0.6667	0.5833
Mwaya1	0.5000	0.4167	0.4167	0.2500	0.4167	0.5833	0.5000
Manyokola3	0.7500	0.5833	0.6667	0.3333	0.5000	0.7500	0.5833
Manyokola4	0.7500	0.5833	0.6667	0.3333	0.5000	0.7500	0.5833
Mkhalatsonga	0.5833	0.4167	0.5000	0.2500	0.3333	0.6667	0.5833

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	UnknownS	Kasantha1	Kamphunobii	Bwanali	Kasantha2	Koloweka2	Agriculture
UnknownSe.	1.0000						
Kasantha1	0.8333	1.0000					
Kamphunobii	0.4348	0.6087	1.0000				
Bwanali	0.4167	0.5000	0.5217	1.0000			
Kasantha2	0.5833	0.6667	0.6957	0.7500	1.0000		
Koloweka2	0.6087	0.5217	0.5455	0.4348	0.5217	1.0000	
Agriculture	0.5833	0.5833	0.4348	0.5833	0.7500	0.5217	1.0000
Unknown2	0.4348	0.4348	0.4545	0.2609	0.4348	0.5455	0.4348
Yizaso	0.1667	0.2500	0.4348	0.2500	0.4167	0.2609	0.3333
TMS4(2)1425	0.5000	0.5833	0.4348	0.4167	0.5833	0.3478	0.4167
Mkondezi	0.5000	0.6667	0.5217	0.5000	0.5833	0.4348	0.4167
CH92/082	0.4167	0.5833	0.6087	0.4167	0.5000	0.5217	0.3333
81/00015	0.5000	0.6667	0.6087	0.3333	0.4167	0.4348	0.2500
Gomani1	0.5000	0.5000	0.5217	0.5000	0.5833	0.6087	0.4167
MK95/054	0.2500	0.3333	0.4348	0.1667	0.2500	0.4348	0.2500
83350	0.5833	0.7500	0.6957	0.5000	0.6667	0.6087	0.5000
LCN8010	0.5000	0.5000	0.3478	0.3333	0.4167	0.4348	0.3333
Mbundumali1	0.5000	0.6667	0.6957	0.4167	0.5833	0.4348	0.3333
Sauti	0.3200	0.4000	0.3333	0.4000	0.4800	0.4167	0.3200
Maunjili	0.4167	0.5000	0.4348	0.3333	0.4167	0.3478	0.2500
Silira	0.5000	0.5833	0.5217	0.3333	0.5000	0.3478	0.5000
Manyokola1	0.5000	0.6667	0.5217	0.3333	0.5000	0.3478	0.3333
Manyokola2	0.5000	0.6667	0.5217	0.3333	0.5000	0.3478	0.3333
Kabuthu	0.5000	0.6667	0.5217	0.3333	0.5000	0.3478	0.3333
Nakalasi	0.5000	0.6667	0.5217	0.3333	0.5000	0.3478	0.3333
Mbundumali2	0.5000	0.6667	0.6957	0.4167	0.5833	0.4348	0.3333
Mwaya1	0.5000	0.6667	0.6087	0.4167	0.5833	0.3478	0.3333
Manyokola3	0.5833	0.7500	0.6957	0.4167	0.5833	0.5217	0.4167
Manyokola4	0.5833	0.7500	0.6957	0.4167	0.5833	0.5217	0.4167
Mkhalatsonga	0.5000	0.6667	0.6957	0.4167	0.5833	0.4348	0.3333

	Unknown2	Yizaso	TMS4(2)	Mkondez	CH92/08	81/0001	Gomani1
Unknown2	1.0000						
Yizaso	0.6087	1.0000					
TMS4(2)1425	0.3478	0.1667	1.0000				
Mkondezi	0.4348	0.5000	0.4167	1.0000			
CH92/082	0.4348	0.4167	0.4167	0.8333	1.0000		
81/00015	0.3478	0.3333	0.5000	0.6667	0.8333	1.0000	
Gomani1	0.5217	0.5833	0.2500	0.7500	0.5833	0.5000	1.0000
MK95/054	0.3478	0.5833	0.3333	0.5000	0.5000	0.5833	0.5833
83350	0.4348	0.3333	0.5833	0.7500	0.8333	0.6667	0.5000
LCN8010	0.6087	0.5833	0.3333	0.8333	0.6667	0.5000	0.7500
Mbundumali1	0.3478	0.3333	0.7500	0.5217	0.6087	0.5833	0.4167
Sauti	0.3333	0.4800	0.5600	0.7200	0.6400	0.4800	0.5600
Maunjili	0.3478	0.4167	0.5000	0.6667	0.6667	0.6667	0.4167
Silira	0.4348	0.5833	0.2500	0.7500	0.5833	0.5833	0.6667
Manyokola1	0.3478	0.3333	0.7500	0.5833	0.5833	0.5000	0.3333
Manyokola2	0.3478	0.3333	0.7500	0.5833	0.5833	0.5000	0.3333
Kabuthu	0.3478	0.3333	0.7500	0.5833	0.5833	0.5000	0.3333
Nakalasi	0.3478	0.3333	0.7500	0.5833	0.5833	0.5000	0.3333
Mbundumali2	0.3478	0.3333	0.7500	0.5000	0.5833	0.5833	0.4167
Mwaya1	0.3478	0.4167	0.7500	0.5000	0.5000	0.5000	0.4167
Manyokola3	0.3478	0.2500	0.6667	0.6667	0.7500	0.6667	0.4167
Manyokola4	0.3478	0.2500	0.6667	0.6667	0.7500	0.6667	0.4167
Mkhalatsonga	0.4348	0.4167	0.6667	0.5000	0.5833	0.5833	0.4167

Appendix 3 Genetic distances for morphological analysis on 93 analysed accessions (Continued)

	MK95/054	83350	LCN8010	Mbunduma	Sauti	Maunjil	Silira
MK95/054	1.0000						
83350	0.4167	1.0000					
LCN8010	0.4167	0.5833	1.0000				
Mbundumali1	0.4167	0.7826	0.4348	1.0000			
Sauti	0.4000	0.5600	0.6400	0.5000	1.0000		
Maunjili	0.4167	0.5833	0.6667	0.5217	0.7200	1.0000	
Silira	0.5833	0.5000	0.6667	0.3478	0.4800	0.5833	1.0000
Manyokola1	0.3333	0.7500	0.5000	0.8696	0.6400	0.5000	0.4167
Manyokola2	0.3333	0.7500	0.5000	0.8696	0.6400	0.5000	0.4167
Kabuthu	0.3333	0.7500	0.5000	0.8696	0.6400	0.5000	0.4167
Nakalasi	0.3333	0.7500	0.5000	0.8696	0.6400	0.5000	0.4167
Mbundumali2	0.4167	0.7500	0.4167	1.0000	0.4800	0.5000	0.3333
Mwaya1	0.3333	0.6667	0.4167	0.9167	0.5600	0.4167	0.3333
Manyokola3	0.5000	0.9167	0.5000	0.8696	0.4800	0.5833	0.5000
Manyokola4	0.5000	0.9167	0.5000	0.8696	0.4800	0.5833	0.5000
Mkhalatsonga	0.4167	0.7500	0.5000	0.9167	0.4000	0.5833	0.3333

	Manyokola1	Manyokola2	Kabuthu	Nakalasi	Mbundumali2	Mwaya1	Manyokola3	Manyokola4
Manyokola1	1.0000							
Manyokola2	1.0000	1.0000						
Kabuthu	1.0000	1.0000	1.0000					
Nakalasi	1.0000	1.0000	1.0000	1.0000				
Mbundumali2	0.8333	0.8333	0.8333	0.8333	1.0000			
Mwaya1	0.9167	0.9167	0.9167	0.9167	0.9167	1.0000		
Manyokola3	0.8333	0.8333	0.8333	0.8333	0.8333	0.7500	1.0000	
Manyokola4	0.8333	0.8333	0.8333	0.8333	0.8333	0.7500	1.0000	1.0000
Mkhalatsonga	0.7500	0.7500	0.7500	0.7500	0.9167	0.8333	0.8333	0.8333

ChitembwereKa. = ChitembwereKapanthi; UnknownSe. = UnkhnownSekelemani; GomaniMf. = GomaniMfipa

Appendix 4 Actual and dendrogram genetic distances for morphological analysis on 28 analysed accessions using NCSS

First Row	Second Row	Actual Dist.	Dendr. Dist.	Diff. %	First Row	Second Row	Actual Dist.	Dendr. Dist.	Diff. %
Manyokola5	Mwatatu	0.520	0.523	-0.69	Mwatatu	Mbundumali1	0.500	0.523	-4.69
Manyokola5	Gomani2	0.615	0.592	3.82	Mwatatu	Sauti	0.593	0.573	3.29
Manyokola5	Beatrice	0.545	0.523	4.00	Mwatatu	Maunjili	0.615	0.573	6.81
Manyokola5	Nyachikundi	0.658	0.634	3.61	Gomani2	Beatrice	0.678	0.592	12.72
Manyokola5	Kachamba	0.569	0.646	-13.48	Gomani2	Nyachikundi	0.569	0.634	-11.30
Manyokola5	Fyoka	0.615	0.592	3.82	Gomani2	Kachamba	0.658	0.646	1.72
Manyokola5	Nyainki	0.569	0.592	-3.88	Gomani2	Fyoka	0.520	0.527	-1.36
Manyokola5	Twentetwente	0.520	0.523	-0.69	Gomani2	Nyainki	0.465	0.516	-11.04
Manyokola5	Masoazungu	0.717	0.634	11.55	Gomani2	Twentetwente	0.569	0.592	-3.88
Manyokola5	Masangwi	0.520	0.523	-0.69	Gomani2	Masoazungu	0.637	0.634	0.45
Manyokola5	Mgwalangwa	0.403	0.405	-0.69	Gomani2	Masangwi	0.520	0.592	-13.80
Manyokola5	Matuvi	0.569	0.592	-3.88	Gomani2	Mgwalangwa	0.569	0.592	-3.88
Manyokola5	Thipula	0.569	0.614	-7.75	Gomani2	Matuvi	0.465	0.516	-11.04
Manyokola5	Biliwili	0.569	0.592	-3.88	Gomani2	Thipula	0.658	0.614	6.68
Manyokola5	Depwete	0.569	0.592	-3.88	Gomani2	Biliwili	0.520	0.516	0.68
Manyokola5	Sekelemani	0.435	0.523	-20.35	Gomani2	Depwete	0.465	0.516	-11.04
Manyokola5	Yizaso	0.658	0.634	3.61	Gomani2	Sekelemani	0.637	0.592	7.08
Manyokola5	TMS4(2)1425	0.403	0.460	-14.27	Gomani2	Yizaso	0.520	0.634	-21.92
Manyokola5	CH92/082	0.520	0.523	-0.69	Gomani2	TMS4(2)1425	0.697	0.592	15.18
Manyokola5	81/00015	0.520	0.523	-0.69	Gomani2	CH92/082	0.520	0.592	-13.80
Manyokola5	Gomani1	0.615	0.592	3.82	Gomani2	81/00015	0.569	0.592	-3.88
Manyokola5	MK95/054	0.615	0.634	-3.04	Gomani2	Gomani1	0.000	0.000	0.00
Manyokola5	83350'	0.403	0.523	-29.99	Gomani2	MK95/054	0.520	0.634	-21.92
Manyokola5	LCN8010	0.615	0.592	3.82	Gomani2	83350'	0.569	0.592	-3.88
Manyokola5	Mbundumali1	0.000	0.000	0.00	Gomani2	LCN8010	0.403	0.403	0.00
Manyokola5	Sauti	0.593	0.573	3.29	Gomani2	Mbundumali1	0.624	0.592	5.13
Manyokola5	Maunjili	0.569	0.573	-0.66	Gomani2	Sauti	0.545	0.592	-8.50
Mwatatu	Gomani2	0.569	0.592	-3.88	Gomani2	Maunjili	0.615	0.592	3.82
Mwatatu	Beatrice	0.493	0.488	0.95	Beatrice	Nyachikundi	0.593	0.634	-6.93
Mwatatu	Nyachikundi	0.658	0.634	3.61	Beatrice	Kachamba	0.593	0.646	-9.03
Mwatatu	Kachamba	0.658	0.646	1.72	Beatrice	Fyoka	0.637	0.592	7.08
Mwatatu	Fyoka	0.465	0.592	-27.23	Beatrice	Nyainki	0.678	0.592	12.72
Mwatatu	Nyainki	0.520	0.592	-13.80	Beatrice	Twentetwente	0.545	0.488	10.41
Mwatatu	Twentetwente	0.520	0.429	17.46	Beatrice	Masoazungu	0.697	0.634	9.12
Mwatatu	Masoazungu	0.678	0.634	6.49	Beatrice	Masangwi	0.593	0.488	17.59
Mwatatu	Masangwi	0.465	0.429	7.72	Beatrice	Mgwalangwa	0.593	0.523	11.69
Mwatatu	Mgwalangwa	0.615	0.523	14.90	Beatrice	Matuvi	0.637	0.592	7.08
Mwatatu	Matuvi	0.520	0.592	-13.80	Beatrice	Thipula	0.637	0.614	3.62
Mwatatu	Thipula	0.520	0.614	-18.04	Beatrice	Biliwili	0.678	0.592	12.72
Mwatatu	Biliwili	0.615	0.592	3.82	Beatrice	Depwete	0.637	0.592	7.08
Mwatatu	Depwete	0.520	0.592	-13.80	Beatrice	Sekelemani	0.329	0.329	0.00
Mwatatu	Sekelemani	0.493	0.488	0.95	Beatrice	Yizaso	0.753	0.634	15.87
Mwatatu	Yizaso	0.697	0.634	9.12	Beatrice	TMS4(2)1425	0.545	0.523	4.00
Mwatatu	TMS4(2)1425	0.569	0.523	8.08	Beatrice	CH92/082	0.545	0.460	15.59
Mwatatu	CH92/082	0.465	0.488	-5.05	Beatrice	81/00015	0.435	0.460	-5.82
Mwatatu	81/00015	0.569	0.488	14.22	Beatrice	Gomani1	0.678	0.592	12.72
Mwatatu	Gomani1	0.569	0.592	-3.88	Beatrice	MK95/054	0.637	0.634	0.45
Mwatatu	MK95/054	0.697	0.634	9.12	Beatrice	83350'	0.493	0.488	0.95
Mwatatu	83350'	0.329	0.329	0.00	Beatrice	LCN8010	0.678	0.592	12.72
Mwatatu	LCN8010	0.520	0.592	-13.80	Beatrice	Mbundumali1	0.553	0.523	5.30

Dist. = distance; Dendr. = dendrogram; Diff. = difference

Appendix 4 Actual and dendrogram genetic distances for morphological analysis on 28 analysed accessions using NCSS (continued**)**

First Row	Second Row	Actual Dist.	Dendr. Dist.	Diff. %	First Row	Second Row	Actual Dist.	Dendr. Dist.	Diff. %
Beatrice	Sauti	0.658	0.573	12.83	Fyoka	Masangwi	0.520	0.592	-13.80
Beatrice	Maunjili	0.545	0.573	-5.14	Fyoka	Mgwalangwa	0.658	0.592	10.04
Nyachikundi	Kachamba	0.658	0.646	1.72	Fyoka	Matuvi	0.520	0.527	-1.36
Nyachikundi	Fyoka	0.520	0.634	-21.92	Fyoka	Thipula	0.569	0.614	-7.75
Nyachikundi	Nyainki	0.615	0.634	-3.04	Fyoka	Biliwili	0.569	0.527	7.47
Nyachikundi	Twentetwente	0.658	0.634	3.61	Fyoka	Depwete	0.520	0.527	-1.36
Nyachikundi	Masoazungu	0.493	0.493	0.00	Fyoka	Sekelemani	0.637	0.592	7.08
Nyachikundi	Masangwi	0.658	0.634	3.61	Fyoka	Yizaso	0.658	0.634	3.61
Nyachikundi	Mgwalangwa	0.658	0.634	3.61	Fyoka	TMS4(2)1425	0.658	0.592	10.04
Nyachikundi	Matuvi	0.520	0.634	-21.92	Fyoka	CH92/082	0.615	0.592	3.82
Nyachikundi	Thipula	0.658	0.634	3.61	Fyoka	81/00015	0.658	0.592	10.04
Nyachikundi	Biliwili	0.569	0.634	-11.30	Fyoka	Gomani1	0.520	0.527	-1.36
Nyachikundi	Depwete	0.520	0.634	-21.92	Fyoka	MK95/054	0.615	0.634	-3.04
Nyachikundi	Sekelemani	0.593	0.634	-6.93	Fyoka	83350'	0.569	0.592	-3.88
Nyachikundi	Yizaso	0.615	0.568	7.62	Fyoka	LCN8010	0.520	0.527	-1.36
Nyachikundi	TMS4(2)1425	0.569	0.634	-11.30	Fyoka	Mbundumali1	0.601	0.592	1.55
Nyachikundi	CH92/082	0.658	0.634	3.61	Fyoka	Sauti	0.678	0.592	12.72
Nyachikundi	81/00015	0.615	0.634	-3.04	Fyoka	Maunjili	0.658	0.592	10.04
Nyachikundi	Gomani1	0.569	0.634	-11.30	Nyainki	Twentetwente	0.520	0.592	-13.80
Nyachikundi	MK95/054	0.520	0.568	-9.31	Nyainki	Masoazungu	0.637	0.634	0.45
Nyachikundi	83350'	0.658	0.634	3.61	Nyainki	Masangwi	0.465	0.592	-27.23
Nyachikundi	LCN8010	0.615	0.634	-3.04	Nyainki	Mgwalangwa	0.569	0.592	-3.88
Nyachikundi	Mbundumali1	0.667	0.634	4.92	Nyainki	Matuvi	0.329	0.329	0.00
Nyachikundi	Sauti	0.637	0.634	0.45	Nyainki	Thipula	0.569	0.614	-7.75
Nyachikundi	Maunjili	0.615	0.634	-3.04	Nyainki	Biliwili	0.403	0.403	0.00
Kachamba	Fyoka	0.735	0.646	12.10	Nyainki	Depwete	0.329	0.329	0.00
Kachamba	Nyainki	0.697	0.646	7.34	Nyainki	Sekelemani	0.593	0.592	0.19
Kachamba	Twentetwente	0.697	0.646	7.34	Nyainki	Yizaso	0.569	0.634	-11.30
Kachamba	Masoazungu	0.753	0.646	14.22	Nyainki	TMS4(2)1425	0.658	0.592	10.04
Kachamba	Masangwi	0.697	0.646	7.34	Nyainki	CH92/082	0.615	0.592	3.82
Kachamba	Mgwalangwa	0.658	0.646	1.72	Nyainki	81/00015	0.658	0.592	10.04
Kachamba	Matuvi	0.658	0.646	1.72	Nyainki	Gomani1	0.465	0.516	-11.04
Kachamba	Thipula	0.697	0.646	7.34	Nyainki	MK95/054	0.658	0.634	3.61
Kachamba	Biliwili	0.697	0.646	7.34	Nyainki	83350'	0.520	0.592	-13.80
Kachamba	Depwete	0.658	0.646	1.72	Nyainki	LCN8010	0.569	0.516	9.34
Kachamba	Sekelemani	0.593	0.646	-9.03	Nyainki	Mbundumali1	0.577	0.592	-2.47
Kachamba	Yizaso	0.658	0.646	1.72	Nyainki	Sauti	0.717	0.592	17.44
Kachamba	TMS4(2)1425	0.569	0.646	-13.48	Nyainki	Maunjili	0.735	0.592	19.53
Kachamba	CH92/082	0.569	0.646	-13.48	Twentetwente	Masoazungu	0.717	0.634	11.55
Kachamba	81/00015	0.569	0.646	-13.48	Twentetwente	Masangwi	0.232	0.232	0.00
Kachamba	Gomani1	0.658	0.646	1.72	Twentetwente	Mgwalangwa	0.465	0.523	-12.57
Kachamba	MK95/054	0.735	0.646	12.10	Twentetwente	Matuvi	0.520	0.592	-13.80
Kachamba	83350'	0.615	0.646	-5.06	Twentetwente	Thipula	0.615	0.614	0.24
Kachamba	LCN8010	0.615	0.646	-5.06	Twentetwente	Biliwili	0.569	0.592	-3.88
Kachamba	Mbundumali1	0.577	0.646	-11.94	Twentetwente	Depwete	0.520	0.592	-13.80
Kachamba	Sauti	0.637	0.646	-1.50	Twentetwente	Sekelemani	0.435	0.488	-12.31
Kachamba	Maunjili	0.569	0.646	-13.48	Twentetwente	Yizaso	0.697	0.634	9.12
Fyoka	Nyainki	0.520	0.527	-1.36	Twentetwente	TMS4(2)1425	0.615	0.523	14.90
Fyoka	Twentetwente	0.520	0.592	-13.80	Twentetwente	CH92/082	0.520	0.488	6.04
Fyoka	Masoazungu	0.593	0.634	-6.93	Twentetwente	81/00015	0.520	0.488	6.04

Appendix 4 Actual and dendrogram genetic distances for morphological analysis on 28 analysed accessions using NCSS (continued**)**

First Row	Second Row	Actual Dist.	Dendr. Dist.	Diff. %	First Row	Second Row	Actual Dist.	Dendr. Dist.	Diff. %
Twentetwente	Gomani1	0.569	0.592	-3.88	Mgwalangwa	81/00015	0.569	0.523	8.08
Twentetwente	MK95/054	0.615	0.634	-3.04	Mgwalangwa	Gomani1	0.569	0.592	-3.88
Twentetwente	83350'	0.403	0.429	-6.55	Mgwalangwa	MK95/054	0.569	0.634	-11.30
Twentetwente	LCN8010	0.569	0.592	-3.88	Mgwalangwa	83350'	0.520	0.523	-0.69
Twentetwente	Mbundumali1	0.500	0.523	-4.69	Mgwalangwa	LCN8010	0.658	0.592	10.04
Twentetwente	Sauti	0.678	0.573	15.43	Mgwalangwa	Mbundumali1	0.408	0.405	0.68
Twentetwente	Maunjili	0.569	0.573	-0.66	Mgwalangwa	Sauti	0.637	0.573	9.97
Masoazungu	Masangwi	0.717	0.634	11.55	Mgwalangwa	Maunjili	0.615	0.573	6.81
Masoazungu	Mgwalangwa	0.717	0.634	11.55	Matuvi	Thipula	0.569	0.614	-7.75
Masoazungu	Matuvi	0.593	0.634	-6.93	Matuvi	Biliwili	0.403	0.403	0.00
Masoazungu	Thipula	0.637	0.634	0.45	Matuvi	Depwete	0.000	0.000	0.00
Masoazungu	Biliwili	0.593	0.634	-6.93	Matuvi	Sekelemani	0.545	0.592	-8.50
Masoazungu	Depwete	0.593	0.634	-6.93	Matuvi	Yizaso	0.615	0.634	-3.04
Masoazungu	Sekelemani	0.697	0.634	9.12	Matuvi	TMS4(2)1425	0.569	0.592	-3.88
Masoazungu	Yizaso	0.593	0.568	4.13	Matuvi	CH92/082	0.615	0.592	3.82
Masoazungu	TMS4(2)1425	0.678	0.634	6.49	Matuvi	81/00015	0.658	0.592	10.04
Masoazungu	CH92/082	0.678	0.634	6.49	Matuvi	Gomani1	0.465	0.516	-11.04
Masoazungu	81/00015	0.678	0.634	6.49	Matuvi	MK95/054	0.697	0.634	9.12
Masoazungu	Gomani1	0.637	0.634	0.45	Matuvi	83350'	0.520	0.592	-13.80
Masoazungu	MK95/054	0.545	0.568	-4.22	Matuvi	LCN8010	0.569	0.516	9.34
Masoazungu	83350'	0.717	0.634	11.55	Matuvi	Mbundumali1	0.577	0.592	-2.47
Masoazungu	LCN8010	0.678	0.634	6.49	Matuvi	Sauti	0.637	0.592	7.08
Masoazungu	Mbundumali1	0.726	0.634	12.75	Matuvi	Maunjili	0.658	0.592	10.04
Masoazungu	Sauti	0.697	0.634	9.12	Thipula	Biliwili	0.697	0.614	12.02
Masoazungu	Maunjili	0.678	0.634	6.49	Thipula	Depwete	0.569	0.614	-7.75
Masangwi	Mgwalangwa	0.520	0.523	-0.69	Thipula	Sekelemani	0.637	0.614	3.62
Masangwi	Matuvi	0.465	0.592	-27.23	Thipula	Yizaso	0.615	0.634	-3.04
Masangwi	Thipula	0.569	0.614	-7.75	Thipula	TMS4(2)1425	0.569	0.614	-7.75
Masangwi	Biliwili	0.569	0.592	-3.88	Thipula	CH92/082	0.658	0.614	6.68
Masangwi	Depwete	0.465	0.592	-27.23	Thipula	81/00015	0.697	0.614	12.02
Masangwi	Sekelemani	0.493	0.488	0.95	Thipula	Gomani1	0.658	0.614	6.68
Masangwi	Yizaso	0.658	0.634	3.61	Thipula	MK95/054	0.697	0.634	9.12
Masangwi	TMS4(2)1425	0.615	0.523	14.90	Thipula	83350'	0.569	0.614	-7.75
Masangwi	CH92/082	0.465	0.488	-5.05	Thipula	LCN8010	0.658	0.614	6.68
Masangwi	81/00015	0.569	0.488	14.22	Thipula	Mbundumali1	0.577	0.614	-6.29
Masangwi	Gomani1	0.520	0.592	-13.80	Thipula	Sauti	0.678	0.614	9.47
Masangwi	MK95/054	0.615	0.634	-3.04	Thipula	Maunjili	0.735	0.614	16.54
Masangwi	83350'	0.329	0.429	-30.50	Biliwili	Depwete	0.403	0.403	0.00
Masangwi	LCN8010	0.520	0.592	-13.80	Biliwili	Sekelemani	0.593	0.592	0.19
Masangwi	Mbundumali1	0.500	0.523	-4.69	Biliwili	Yizaso	0.697	0.634	9.12
Masangwi	Sauti	0.637	0.573	9.97	Biliwili	TMS4(2)1425	0.569	0.592	-3.88
Masangwi	Maunjili	0.615	0.573	6.81	Biliwili	CH92/082	0.697	0.592	15.18
Mgwalangwa	Matuvi	0.569	0.592	-3.88	Biliwili	81/00015	0.697	0.592	15.18
Mgwalangwa	Thipula	0.520	0.614	-18.04	Biliwili	Gomani1	0.520	0.516	0.68
Mgwalangwa	Biliwili	0.569	0.592	-3.88	Biliwili	MK95/054	0.615	0.634	-3.04
Mgwalangwa	Depwete	0.569	0.592	-3.88	Biliwili	83350'	0.615	0.592	3.82
Mgwalangwa	Sekelemani	0.493	0.523	-6.14	Biliwili	LCN8010	0.658	0.516	21.48
Mgwalangwa	Yizaso	0.615	0.634	-3.04	Biliwili	Mbundumali1	0.577	0.592	-2.47
Mgwalangwa	TMS4(2)1425	0.569	0.460	19.20	Biliwili	Sauti	0.678	0.592	12.72
Mgwalangwa	CH92/082	0.615	0.523	14.90	Biliwili	Maunjili	0.697	0.592	15.18

Appendix 4 Actual and dendrogram genetic distances for morphological analysis on 28 analysed accessions using NCSS (continued**)**

First Row	Second Row	Actual Dist.	Dendr. Dist.	Diff. %	First Row	Second Row	Actual Dist.	Dendr. Dist.	Diff. %
Depwete	Sekelemani	0.545	0.592	-8.50	TMS4(2)1425	Mbundumali1	0.408	0.460	-12.71
Depwete	Yizaso	0.615	0.634	-3.04	TMS4(2)1425	Sauti	0.545	0.573	-5.14
Depwete	TMS4(2)1425	0.569	0.592	-3.88	TMS4(2)1425	Maunjili	0.569	0.573	-0.66
Depwete	CH92/082	0.615	0.592	3.82	CH92/082	81/00015	0.329	0.329	0.00
Depwete	81/00015	0.658	0.592	10.04	CH92/082	Gomani1	0.520	0.592	-13.80
Depwete	Gomani1	0.465	0.516	-11.04	CH92/082	MK95/054	0.569	0.634	-11.30
Depwete	MK95/054	0.697	0.634	9.12	CH92/082	83350'	0.329	0.488	-48.57
Depwete	83350'	0.520	0.592	-13.80	CH92/082	LCN8010	0.465	0.592	-27.23
Depwete	LCN8010	0.569	0.516	9.34	CH92/082	Mbundumali1	0.500	0.523	-4.69
Depwete	Mbundumali1	0.577	0.592	-2.47	CH92/082	Sauti	0.493	0.573	-16.23
Depwete	Sauti	0.637	0.592	7.08	CH92/082	Maunjili	0.465	0.573	-23.28
Depwete	Maunjili	0.658	0.592	10.04	81/00015	Gomani1	0.569	0.592	-3.88
Sekelemani	Yizaso	0.717	0.634	11.55	81/00015	MK95/054	0.520	0.634	-21.92
Sekelemani	TMS4(2)1425	0.435	0.523	-20.35	81/00015	83350'	0.465	0.488	-5.05
Sekelemani	CH92/082	0.493	0.460	6.68	81/00015	LCN8010	0.569	0.592	-3.88
Sekelemani	81/00015	0.368	0.460	-25.20	81/00015	Mbundumali1	0.527	0.523	0.68
Sekelemani	Gomani1	0.637	0.592	7.08	81/00015	Sauti	0.593	0.573	3.29
Sekelemani	MK95/054	0.593	0.634	-6.93	81/00015	Maunjili	0.465	0.573	-23.28
Sekelemani	83350'	0.368	0.488	-32.88	Gomani1	MK95/054	0.520	0.634	-21.92
Sekelemani	LCN8010	0.637	0.592	7.08	Gomani1	83350'	0.569	0.592	-3.88
Sekelemani	Mbundumali1	0.441	0.523	-18.71	Gomani1	LCN8010	0.403	0.403	0.00
Sekelemani	Sauti	0.615	0.573	6.81	Gomani1	Mbundumali1	0.624	0.592	5.13
Sekelemani	Maunjili	0.493	0.573	-16.23	Gomani1	Sauti	0.545	0.592	-8.50
Yizaso	TMS4(2)1425	0.735	0.634	13.79	Gomani1	Maunjili	0.615	0.592	3.82
Yizaso	CH92/082	0.615	0.634	-3.04	MK95/054	83350'	0.615	0.634	-3.04
Yizaso	81/00015	0.658	0.634	3.61	MK95/054	LCN8010	0.615	0.634	-3.04
Yizaso	Gomani1	0.520	0.634	-21.92	MK95/054	Mbundumali1	0.624	0.634	-1.64
Yizaso	MK95/054	0.520	0.520	0.00	MK95/054	Sauti	0.637	0.634	0.45
Yizaso	83350'	0.658	0.634	3.61	MK95/054	Maunjili	0.615	0.634	-3.04
Yizaso	LCN8010	0.520	0.634	-21.92	83350'	LCN8010	0.520	0.592	-13.80
Yizaso	Mbundumali1	0.667	0.634	4.92	83350'	Mbundumali1	0.373	0.523	-40.46
Yizaso	Sauti	0.593	0.634	-6.93	83350'	Sauti	0.545	0.573	-5.14
Yizaso	Maunjili	0.615	0.634	-3.04	83350'	Maunjili	0.520	0.573	-10.27
TMS4(2)1425	CH92/082	0.615	0.523	14.90	LCN8010	Mbundumali1	0.601	0.592	1.55
TMS4(2)1425	81/00015	0.569	0.523	8.08	LCN8010	Sauti	0.493	0.592	-19.95
TMS4(2)1425	Gomani1	0.697	0.592	15.18	LCN8010	Maunjili	0.465	0.592	-27.23
TMS4(2)1425	MK95/054	0.658	0.634	3.61	Mbundumali1	Sauti	0.577	0.573	0.71
TMS4(2)1425	83350'	0.520	0.523	-0.69	Mbundumali1	Maunjili	0.553	0.573	-3.71
TMS4(2)1425	LCN8010	0.658	0.592	10.04	Sauti	Maunjili	0.435	0.435	0.00