NATIVE STARCH EVALUATION AND GENETIC DISTANCE ANALYSIS USING AFLP OF ELITE CASSAVA (*Manihot esculenta* CRANTZ) GENOTYPES FROM MALAWI

By

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DEDICATION

I dedicate this piece of work to the following people:

My late brother Yahaya Benesi who died in the gold mines of South Africa, trying to secure funds to keep me and my young brother in school. Poor Yahaya your dreams have been fulfilled though in your absentia physically, but spiritually we are together. May your soul rest in peace.

My wife (Hawa) has had a tough time in my absentia to manage the family and missed me a lot. My children also missed me a lot in the course of my studies. Your patience, endurance, understanding and encouragement have led to the accomplishment of such an important piece of work.

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CHAPTER 1

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub with an edible root, which grows in tropical and subtropical areas of the world. It belongs to the genus *Manihot* and botanical family *Euphorbiaceae*. It 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 (Onwueme, 1978; Encarta Encyclopedia, 2001; IITA, 2001). Cassava roots can be stored in the ground for up to 24 months, and some varieties for up to 36 months after planting. This enables harvesting to be delayed until marketing, processing or other conditions are favourable (CGIAR Research, 2001; IITA, 2001).

Cassava provides a major source of calories for poor families because of the high starch content and ease of management. Farmers can also dig up the starchy roots of cassava and eat it six months to three years after planting. Hence, cassava cultivation is very flexible and it saves many millions of people under different and difficult circumstances. In Africa, people also eat the leaves of cassava as a green vegetable, which provides a cheap and rich source of protein and vitamins A and B (FAO, 1993; IITA, 2001). In Southeast Asia and Latin America, cassava has also taken on an economic role. Various industries use it as a binding agent, because it is an inexpensive source of starch. Cassava starch is also used in the production of paper, textiles, and as monosodium glutamate (MSG), and as an important flavouring agent in Asian cooking (CGIAR Research, 2001; IITA, 2001). In Africa, cassava is beginning to be used in industries like textile, wood, as binding agent, and as partial substitution for wheat flour. This provides an income to resource-poor farmers and it saves foreign exchange for nationals. Opportunities for product and market diversification are excellent in several countries, such as Nigeria, Uganda, Malawi (CGIAR Research, 2001), and of late also South Africa.

Cassava is the most important root crop in Malawi (FAO, 1993; Anonymous, 1994; Moyo *et al.*, 1998). Cassava 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. It is also a staple food crop in the Shire highlands. Cassava is an important crop for ensuring sustained food security during periods of drought and is also a vital cash crop throughout the country (Moyo *et al.*, 1998). The leaves of cassava are important vegetables for most rural households. These leaves are rich in protein, vitamins and minerals. Cassava leaves are particularly important because they are available throughout the year (Onwueme, 1978; FAO, 1993;). The importance of cassava as a food security crop became more apparent with changes in climatic, physical and socio-economic environments in the early to mid 1990s. Examples of this are persistent droughts and increase of prices of farm inputs largely caused by devaluation of the Malawi Kwacha and the removal of subsidies (Minde *et al.*, 1997). Cassava tolerates drought, poor quality soil, less elaborate management and is widely adapted. These attributes make it the best candidate crop being promoted by the Government of Malawi in crop diversification for achieving food security (FAO, 1993). Of late, cassava is increasingly becoming an important industrial crop in Malawi.

Although there are diverse uses of cassava, as food as well as in the commercial sector, research has concentrated on the development of disease and pest resistant and high yielding cassava varieties. Both International Centres as well as the National Agricultural Research Systems have only put their effort in developing pest and disease resistant, early maturing, drought tolerant and high yielding cassava varieties (CGIAR Research, 2001; IITA, 2001). The notion has been to improve cassava production and increase food security in Africa. Work in post harvest aspects is emphasised in the development and dissemination of processing machines and other tools, which can reduce processing time, labour, as well as production losses.

In May 2001, there was a symposium on the "Promotion of Cassava Commercialisation in Malawi". The commercial sector and other players were brought together to share ideas on possible uses of cassava, the technologies that are available, who has started using cassava in the industries and how much, who is ready to go into the use of cassava in their industries, in what forms and how much, and also to plan the way forward. There was an outcry from the commercial sector that most of them have started using cassava and a lot more would like to go into cassava use in many areas. However, there is a problem in that not much information is available on the qualities of the available varieties for different industrial uses. Since they are

profit oriented they would like to use the best products for quality output, hence more profit. Therefore, there is need to venture and look into specific qualities that are suitable for specific uses in industries. This assessment should be incorporated in the cassava-breeding programme.

There are a number of cassava varieties, which are officially released in Malawi and a number more, locally bred and introduced clones from IITA, are promising. All these cassava genotypes need to be evaluated for specific qualities for industrial uses like starch extraction rates, starch quality and dry matter. Hence the need to initiate this study to show which genotypes are suitable for industrial use, especially the ones good for starch extraction and producing starch of high quality. The quality of cassava starch from Malawian genotypes will be determined. The information obtained in this study, will encourage more industries to use cassava starch since all specifications will be available. The potential investors in starch production in Malawi will have a wider scope of which varieties to use in their prospective plants. The influence of the genotype by environment interaction on the cassava starch will be revealed. Farmers will have an advantage in that they will know which varieties to grow for ready market in the starch processing industry. The fingerprinting will reveal which genotypes are closely related, which will be vital for future planning of the cassava breeding programme.

Hence, the objectives of the study are: (1) To determine the genetic distances/relatedness of Malawi cassava commercially used varieties and to cluster them using AFLP and morphologic descriptors; (2) To evaluate the Malawi cassava commercially used varieties and some promising clones for starch extraction and the quality of the native starch from them; (3) To assess the feasibility of use of native cassava starch in the industrial sector in Malawi; and (4) To evaluate the Malawi cassava commercially used varieties and some promising clones for dry matter content.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical taxonomy of cassava

2.1.1 Classification

2.1.1.1 Taxonomical description of cassava

The word cassava seems to have been derived from the word *casabe*, an Amazonian Indian *Taino (Arawak)* word for cassava bread. Cassava is a name used in the West Indies and most of the English speaking world. In American English and also in the United States it is called *manioc*. In Brazil, cassava in Portuguese is known as *mandioca*, and in Spanish speaking Latin America it is known as *yuca*. In the Pacific Oceanic it is recognised as *tapioca* (Chiwona-Karltun, 2001; Encarta Encyclopedia, 2001; IITA, 2001), and is scientifically known as *Manihot esculenta* Crantz (Onwueme, 1978; CGIAR Research, 2001; Chiwona-Karltun, 2001; Encarta Encyclopedia, 2001; IITA, 2001). Onwueme (1978) repeated that it is synonymous with *Manihot utilissima* Pohl.

Cassava is one of the 100 species of the genus *Manihot*. It includes several rubber producing plants (Chiwona-Karltun, 2001) that may be useful gene sources in cassava improvement (Rogers and Appan, 1973). Cassava is a dicotyledonous plant belonging to the botanical family *Euphorbiaceae* (Onwueme, 1978; Hallack, 2001; IITA, 2001). Of all the *Euphorbiaceae* it is only *Manihot esculenta* that produces tuberous roots that has led to its domestication (Chiwona-Karltun, 2001). Members of the *Euphorbiaceae* family are characterized by vessels composed of sector cells and include several commercially important plants. Some of these plants are rubber trees (*Hevea brasilliensis*), oil plants (*Ricinus comunis*), root crops (*Manihot* species) and ornamental plants (*Euphorbia* species) (Osiru *et al.*, 1996). However, cassava is widely distributed in the tropical and subtropical areas and is the only species from the genus *Manihot* that is widely cultivated. The other *Manihot* species which have had minor uses, especially as alternative sources of latex for rubber production are *Manihot glaziovii* and *Manihot caerulescens* (Franche *et al.*, 1991).

2.1.1.2 Varieties

Numerous cassava varieties exist in each locality where the crop is grown. The cultivars have been distinguished by morphological characteristics such as leaf characteristics, colour and shape, branching habit, plant height, colour of stem and petiole, root characteristics, time of maturity, root yield and cyanogenic glucoside content in the roots (Onwueme, 1978; IITA, 1990; Osiru *et al.*, 1996). The last named characteristic has been used to group cassava cultivars into two major groups: the 'bitter' varieties, in which the cyanogenic glucoside is distributed throughout the tuber and is at a high level, and the 'sweet' varieties, in which the glucoside is confined to the peel and at a low level (Onwueme, 1978). The flesh of 'sweet' varieties is relatively free of glucoside, although it still contains small amounts (Purseglove, 1968). Encarta Encyclopedia (2001) classifies the bitter cassava as *Manihot esculenta* and the 'sweet' cassava as *Manihot duleis.* The other authors classify cassava as *Manihot esculenta* despite of its bitterness. Cassava is further subdivided into 'bitter' or 'sweet' varieties (Sauer, 1963; Onwueme, 1978; CGIAR Research, 2001; Encarta Encyclopedia, 2001; Hallack, 2001; IITA, 2001;). Onwueme (1978) advises that caution should be exercised in using levels of glucoside as a distinguishing characteristic for cassava cultivars since exact level of glucoside in a particular cultivar will vary according to the environmental conditions under which the plant is grown. This observation agrees with the results obtained in Malawi where the same variety had high glucoside in one area and tasted bitter under such conditions, and had very low glucoside content in the other area and tasted very sweet (Benesi *et al.*, 1999). The glucoside content of the cultivar may be high under some conditions and low under others. Hence the use of bitterness in the classification up to a point of even giving different scientific names may not be correct. The bitter type of cassava requires special processing through either grating and pressing or fermentation, followed by heating to make the products safe for consumption (Hallack, 2001).

2.12 Cassava plant and its growth cycle

2.1.2.1 Stems and leaves

The bushy plant of cassava is propagated mainly from stem cuttings (IITA, 1990; Hallack, 2001), however under natural conditions, as well as in the plant breeding process propagation by seed is common. When cuttings are planted in moist soil under favourable conditions they produce sprouts and roots within a week. When propagated by seed, plant establishment is slower since the seeds take 30 to 45 days to germinate (IITA, 1990). In addition the plant itself is weaker and smaller. The seedlings also segregate into different types (Osiru *et al.*, 1996).

Since cassava is mainly propagated through cuttings, the shoot system develops from axillary buds located on the nodes of the cuttings. The number of shoots that develop depends on several factors, which include length of cuttings, size and moisture content of the cutting and genotype (IITA, 1990; Osiru *et al.*, 1996; Zacarias, 1997).

The cassava plant grows as a shrub (Figures 2.1 a and b), with the stem reaching heights of up to four metres in some varieties and environments, or only attaining one metre or so in some of the dwarf varieties (Onwueme, 1978; IITA, 1990). Information provided by Hallack (2001) indicates that cassava grows very tall in Sierra Leone, at times reaching four and a half metres (15 feet) high. Osiru *et al.* (1996) also reported that some cassava varieties reached a height of four metres. In Malawi, cassava normally gains a height of one to two metres but in some areas varieties grow up to three metres high. The colour of the mature stem surface varies between cultivars, but usually ranges from silvery green to dark brown. In older parts of the stem, prominent knob like leaf scars are present, marking the nodal positions where leaves were originally attached. The distance between nodes varies with cultivars and also environmental conditions, being shortest when adverse environmental conditions exist, and longest when growth conditions are favourable (Onwueme, 1978; IITA, 1990; Osiru *et al.*, 1996).

Figure 2.1a: A young cassava plant

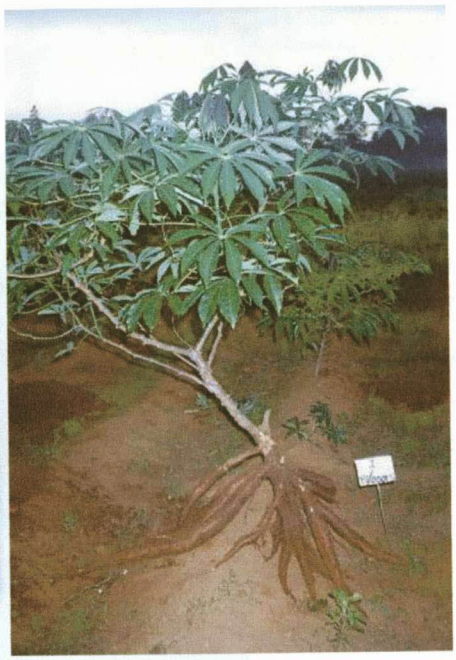

Figure 2.1b: A mature harvested cassava plant

Mature leaves are formed 45-75 days after cuttings are planted. The leaves are arranged spirally on raised nodal portions on the stem. The pyllotaxis is two-fifth spiral (Onwueme, 1978; IITA, 1990). Each leaf is subtended by three to five stipules, each about one centimetre long. The length of the leaf stalk (petiole) varies between five to 30 cm long. The lamina is simple with a smooth margin but deeply palmate or lobed. The number of lamina-lobes varies between three and nine (usually odd numbers). Since the cassava leaves are normally consumed as a vegetable in Africa, large leaves are often harvested singly at intervals. Successional picking of leaves does not significantly reduce the growth of the plant or the alternative food supply, which is the tuberous root (Hallack, 2001). Leaf area approaches its maximum size in four to five months after planting depending on planting time (Williams and Ghazali, 1969; Onwueme, 1978; IITA, 1990).

2.1.2.2 Flowers, fruits and seeds

Cassava is monoecious. Flowering of cassava plants may begin as early as six weeks after planting, although the actual time of flowering depends upon cultivar, time of planting and environment. Flowering is frequent and regular in some cultivars, while in others it is rare or non-existent (Onwueme, 1978; IITA, 1990).

Cassava flowers are borne on terminal panicles, with the axis of the branch being continuous with that of the panicle inflorescence. The male flowers occur near the tip, while the female flowers occur close to the base. Each flower, whether female or male, has five yellowish or reddish perianths. The male flower has 10 stamens arranged in two whorls of five stamens each. The filaments are free and the anthers are small. The female flower has an ovary mounted on a 10 lobed glandular disc. The stigma has three locules and six ridges. The stigma has three lobes which unite to form a single style. The female flower opens first while the male flower opens a week later encouraging cross-pollination. Self-pollination can occur when male and female flowers, located on different branches of the same plant, open at the same time (Onwueme, 1978; IITA, 1990; Osiru *et al.*, 1996).

After pollination and fertilisation, the ovary will develop into a fruit in 70 to 90 days. The mature fruit is a globular capsule (with a diameter of 1 to 1.5 cm), with six narrow longitudinal wings along which it naturally splits explosively to release the seed (Onwueme, 1978; IITA, 1990; Osiru *et al.*, 1996).

The cassava seed is ellipsoidal of 1-1.5 cm long. It has a brittle testa which is grey and mottled with dark blotches. Seeds can be light-grey, brownish or dark-grey, with dark blotches. A large caruncle is located at the micropylar end of the seed (Onwueme, 1978; IITA, 1990; Osiru *et al.*, 1996).

2.1.2.3 Root

When cassava is grown from cuttings, adventitious roots usually arise from the base of the cutting. These roots later develop into a fibrous root system which are the main feeder roots of the plant. The roots may penetrate to a depth of 50-100 cm (Onwueme, 1978). After 30 to 60 days from planting, some of these fibrous roots begin to swell and become tuberous (IITA, 1990). Apparently, all the fibrous roots are initially active in nutrient absorption; but once one of them becomes tuberous, its ability to function in nutrient absorption decreases considerably (Adrian *et al.*, 1969). Only a few, usually less than 10, fibrous roots on each plant become tuberous so that most of the fibrous roots remain thin and continue to function in nutrient absorption (Onwueme, 1978; IITA, 1990). For most of the cultivars, the number of adventitious

roots that develop into tubers is limited, and beyond six to nine months after planting, there will be no further addition to the number of tuberous roots (Beck, 1960). After this time, no other adventitious roots change from fibrous to tuberous condition. The actual number of roots that eventually form tubers depends on several factors, including genotype, assimilate supply, photoperiod and temperature (IITA, 1990; Osiru *et al.*, 1996). A mature cassava tuber (excluding the tail) may range in length from 15-100 cm, and in weight from 0.5–2.0 kg, depending on variety and growing conditions (Onwueme, 1978). Silvestre (1989) reported that each cassava root may weigh 2.0–5.0 kg. The distribution of the roots and tubers in a plant is a varietal characteristic, but can be influenced by the orientation of planting in which the original stem cutting was planted. Onwueme (1978) reported that if a cutting is planted vertically the roots are concentrated at the base of the cutting (Figure 2.2). When the cutting is planted at an angle the roots are loosely arranged and shallower than upright planting (Figure 2.3). Cuttings that are planted horizontally tend to form roots at nearly all the nodes, and the roots are shallow and loosely arranged (Jennings, 1970; Gurnah, 1974).

Figure 2.2: Most roots form at the extreme base of cuttings if cassava is planted upright

Figure 2.3: Roots scattered along the cutting if cassava is planted at an angle or horizontal

2.1.3 Environmental conditions for cassava growth

Cassava is a crop of the lowland tropics. It does best in a warm, moist climate where mean temperatures range from 25–29 °C. It does very poorly in cold climates, and at temperatures below 10 °C, growth of the plant is arrested. It cannot withstand frost at the time of the active growth period, therefore, it can only be profitably grown in

regions which are frost free for at least the duration required for the crop to mature (Onwueme, 1978).

Cassava does best when the rainfall is between 1000–1500 mm per year, and well distributed. However, the crop is well adapted to cultivation under drought conditions, and it can be profitably grown in areas where the annual rainfall is as low as 500 mm (Onwueme, 1978). Zacarias (1997) indicated that cassava is grown from the equator to slightly beyond the tropics of Cancer and Capricorn, with annual rainfall of 500-5000 mm. When moisture availability is low, the cassava plant ceases to grow and sheds some of its older leaves, thereby reducing its total transpiring surface. When moisture is again amply available, the plant quickly resumes growth and produces new leaves (Onwueme, 1978). This behaviour makes cassava a valuable crop in places where, and at times, the rainfall is low, or uncertain, or both. It is only during the first few weeks after planting that the cassava plant is unable to tolerate drought to an appreciable extent.

The best soil for cassava cultivation is a light, sandy loam of medium fertility. Good drainage is also important. On clay or poorly drained soils, root growth is poor so that the shoot-to root ratio is considerably decreased. Moreover, the poor soil aeration conditions cause formation of few tubers. Gravely or stony soils tend to hinder root penetration and are therefore unsuitable. Saline soils are also unsuitable. Cassava can grow and yield reasonably well on soils of low fertility where production of other crops would be uneconomical. Under conditions of very high fertility, cassava tends to produce excessive vegetation at the expense of tuber formation (Onwueme, 1978; Sreekumani *et al.*, 1988). Zacarias (1997) indicated that cassava is grown on soils ranging from slightly acidic to slightly alkaline.

Tuber formation in cassava is under photoperiodic control. Under short day conditions tuberisation occurs readily but when the day length is greater than 10–12 hours, tuberisation is delayed and subsequent yields are lower (Bolhuis, 1966; Mogilner *et al.*, 1967). For this reason, cassava is most productive between latitudes 15°N and 15°S. At the higher latitudes, the best growing season (summer) corresponds to the time of long photoperiods, which do not stimulate tuberisation (Onwueme, 1978).

Zacarias (1997) also pointed out that cassava is grown at altitudes from sea level to 2200 metres above sea level. While Onwueme (1978) says that when cassava is grown at altitudes above 1000 m, it tends to grow slowly and yield poorly, and at present, most of the cassava cultivation is done at lower altitudes.

2.2 Origin, evolution, history and diffusion of cassava

2.2.1 Origin of cassava

It has proven difficult to establish the origin of cassava using traditional evidence. The reason is that cassava growers did not store seed of the plant. The major planting material was non-storable stem cuttings. Since no persistent parts of the plant can be identified in the graves or other archaeological remains, archaeologists have used starch particles left behind on processing and cooking equipment. These have been identified to be of cassava origin and have aided in postulating the most likely origin of cassava (Chiwona-Karltun, 2001). All species of the genus *Manihot* are native to the new world tropics and occur naturally in the Western Hemisphere between the southern USA (33°N) and Argentina (33°S) (Fregene *et al.*, 1994). This genus is important economically because of the species *Manihot esculenta.* This species is believed to have originated in Brazil and Central America (Barnes, 1954; Rogers, 1963; Schwerin, 1970). Purseglove (1968) reported that it was grown as a crop in Peru some 4000 years ago and in Mexico 2000 years ago, and it is now not known in the wild state (Umanah and Hartmann, 1973). Recent studies in Brazil using molecular genetics, have shown that Brazilian *Manihot esculenta* sudsp. *Flabellifolia* species from the Amazon Basin are the most likely source and sites of domestication (Allem, 1994; Haysom *et al.*, 1994; Second *et al.*, 1997; Allem, 1999; Olsen and Schaal, 1999; Schaal, 2001). Cassava is today a widely cultivated tropical and subtropical root crop (Umanah and Hartmann, 1973).

2.2.2 Introduction of cassava into Africa and Malawi

The Portuguese first brought cassava to Africa in the form of flour or 'farinha'. The Tupinamba Indians of Eastern Brazil had taught the Portuguese techniques of *manioc* preparation and production, and they had developed a liking for the various processed

forms (Ross, 1975; Carter *et al.*, 1992). The first mention of cassava cultivation in Africa dates back to 1558 (Mauny, 1953; Pasch, 1980; Silvestre and Arraudeau, 1983). At first, it was cultivated with a sole purpose of provision of chips to slaves, until about 1600. Jones (1959) and Ross (1975) propose that multiple, and more-or-less simultaneous introductions took place at Portuguese trading stations.

Knowledge of the diffusion of cassava in the interior 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).

Cassava spread in Africa through various mechanisms. The most important appear to have been initial contact with the Portuguese-Brazilian culture, through which the crop gained a foothold, by river and possibly over land trade, and by mass migration. In the 19th and 20th century, colonial administrators encouraged its diffusion and increased cultivation (Carter *et al.*, 1992).

Cassava was present on the western shores of Lake Nyasa and Tanganyika in the second half of the 19th century when Europeans first explored the area (Carter *et al.*, 1992). This may not be true since Carter himself and his collegues (1992) pointed out that the information on the diffusion of cassava in East Africa is the most speculative. Cassava was introduced at the Portuguese trading stations in East Africa during the 17th and 18th centuries and therefore it must have reached Malawi earlier than the 19th century, as, during the 16th Century AD, there was a vast trading empire established by the Maravi people. The first European to make contact with the area now known as Malawi may have been the Portuguese explorer Gaspar Bocarro, whose diary published in 1492 made reference to the great inland lake in central Africa.

The slave trade which ravaged most of Africa from the 16th Century to the 19th Century also left its imprints on Malawi's historical development. The Arab slave traders arrived on the shores of Lake Malawi from Zanzibar Island in the Indian Ocean in search of slaves sometime after 1840 and were to continue until the late 19th Century. By the time David Livingstone reached the lake he named "Lake Nyasa" in 1859, there was well established trading (Anonymous, 2002).

In addition there was a lot of migration in Malawi. The Bantu-speaking people arrived around the 14th century, and they soon coalesced into the Maravi kingdom (late 15th–late 18th century), centred in the Shire River valley. In the 18th century, the kingdom conquered portions of modern Zimbabwe and Mozambique. However, shortly thereafter it declined as a result of internal rivalries and incursions by the Yao, who sold their Malawi captives as slaves to Arab and Swahili merchants living on the Indian Ocean coast. In the 1840s the region was thrown into further turmoil by the arrival of the warlike Ngoni from South Africa.

Cassava brown streak disease (CBSD) was first recorded and described by Story in a progress report in 1936 at the foothills of the Usumbara Mountains of Tanganyika (now Tanzania). Nichols (1950) later reported that the disease was endemic in all East African coastal cassava growing areas, from the North-East boarder of Kenya to Mozambique and was widespread at low altitudes in Nyasaland (now Malawi). A survey is normally planned to be carried out where it is commonly found. Thus, CBSD could not be wide spread in an area with no cassava. For the disease to be widespread, it means that cassava has been in the area for some time.

Looking at how rich the knowledge on cassava is along the western shores of Lake Malawi (Lake Nyasa), it means that the crop was introduced much earlier. After independence in 1964, the government encouraged the people to grow maize across the country but it did not succeed in that area since the people were already used to cassava as their staple and it was well established by then in their cropping system.

2.2.3 Current distribution of cassava in Malawi

2.2.3.1.1 Agroecological zones of Malawi

Malawi is a small country of 12.3 million hectares (45,745 square miles) with about 10 million people. However it has a wide range of agroecological zones as shown in Figure 2.4.

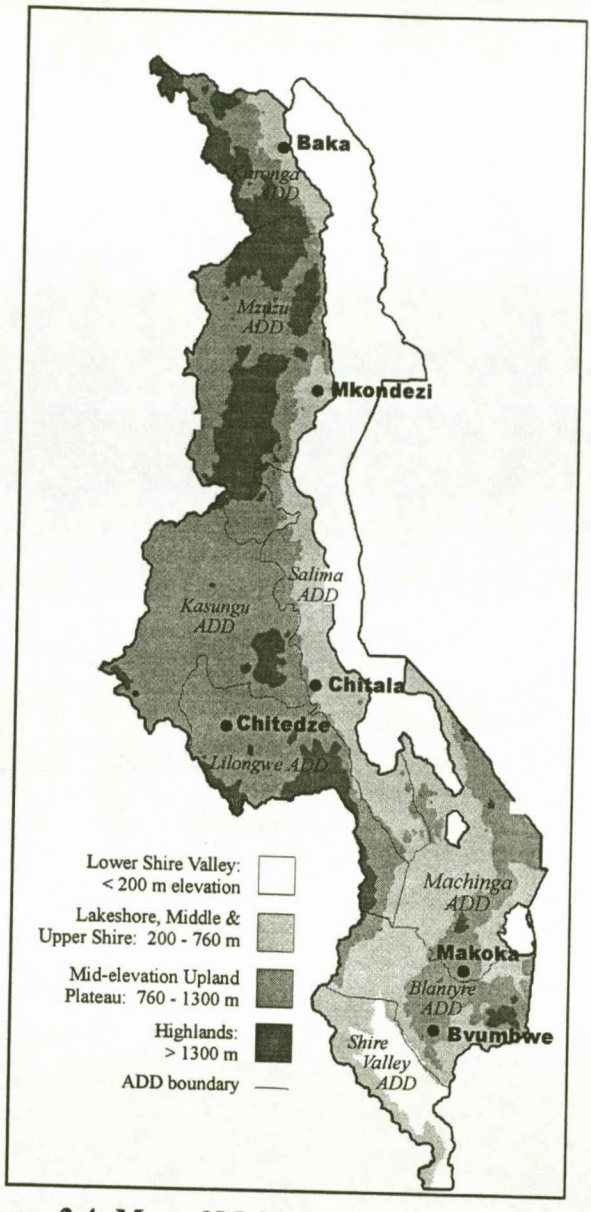

Figure 2.4: Map of Malawi showing Agro-ecological zones and Agricultural Development Divisions (ADD).

Source: GIS office Chitedze Research Station, 1999.

2.2.3.1.2 Types and varieties available

Farmers cultivate a number of varieties, most of them local. From the survey areas of Moyo *et al.* (1998) (one in the North, one in the centre and one in the South) it was found that 26 varieties were grown at Chintheche in Nkhata Bay district in the North, 11 at Lisasadzi in Kasungu district in the Centre and 13 at Mulanje South in Mulanje district in the South. These varieties are mostly low yielding and susceptible to

cassava mosaic disease, cassava green mite and cassava mealybug. The varieties farmers grow depend on use. In Nkhata Bay, where cassava is a major staple crop, farmers prefer bitter varieties to shield them against theft and monkeys. On the other hand in Kasungu where cassava is grown for a snack and sale or in Mulanje where it is grown for a snack, sale and / or processed into chips, sweet types are preferred. Apart from eating qualities, farmers prefer varieties that have big roots, are high yielding, with high dry matter content, are early maturing, and those whose leaves produce a good vegetable (Moyo *et al.*, 1998).

2.2.3.1.3 Cyanoglucocides, taste and distribution in cassava

Several plants which are used as food contain natural toxins. Examples include beans, peas, sorghum, potatoes and cassava. Our ancestors who domesticated these plants solved this problem in either of two ways. The first way was to select varieties with negligible toxin levels. The second way was to devise processing methods that reduced toxins to negligible levels (Nordenskiold, 1924; Chiwona-Karltun, 2001).

A written report attests that in Ecuador 'sweet' or 'bitter' cassava were considered as different crops two centuries ago. This is still the case in several Amerindians or mixed blood communities. In the main area of cultivation of bitter cassava, sweet cassava is regarded as a legume to be grown next to a house (Narvaez *et al.*, 2001). Generally, cassava cultivars are classified into two groups referred to as 'bitter' and 'sweet' by many of the farming communities (Nye, 1991).

The bitter taste in cassava roots has been shown to be positively associated with levels of cyanogenic glucosides (Sinha and Nair, 1968; Sundaresan *et al.*, 1987) but Bokanga (1994) found a strong association. The results of the studies of Chiwona-Karltun *et al.* (2000) found a strong correlation ($r>0.98$) between mean taste and mean danger scores for the 25 most common cultivars grown in the study area (Nkhata Bay district in the North of Malawi). This also supports the recent finding that 'linamarin is a contributor of bitterness in the parenchyma of cassava roots in addition to several other substances that may modify the bitterness of the cortex (King and Bradbury, 1995).

There is documentation of different geographical distribution of 'sweet' and 'bitter' cassava cultivars. It is noted that the 'sweet' cultivars have a wider range of distribution than the bitter ones, that is, there are many areas where only 'sweet' cultivars are grown and eaten. Observations in South America and Africa have shown that where cassava is of major importance as a staple food the 'bitter' cassava comprises a higher percentage of the farming system and the 'sweet' cultivars play a minor role (Chiwona-Karltun, 2001). This is also the case in Malawi.

2.3 Importance of cassava

2.3.1 Adaptability of cassava in relation to other crops

Cassava is a perennial although in agriculture it is usually harvested during the first or second year. Abandoned stands of cassava may continue to grow for several years. The plant encounters quite frequently adverse conditions during its period of growth for one season to another. The most common interseasonal condition in cassava growing areas is drought. During dry spells, between seasons, the cassava plant reduces or ceases its growth, and sheds a considerable proportion of its old leaves. Increase in tuber size is also halted at this time. When the next rainy (growing) season returns, the plant is able to resume vigorous growth and rapid tuber bulking (Onwueme, 1978). When cassava is grown in subtropical areas or at very high altitude, cold periods may also result in cessation of cassava growth.

The successful integration of cassava in African cropping and dietary patterns takes on special importance as Africa is the only region where per capita food production has apparently been declining in the last two decades. During that period, overall cassava production in Africa has nearly doubled, even if according to official statistics, this has not allowed it to keep pace with population increase (De Bruijn and Fresco, 1989).

What makes cassava so special and different from other African staples, such as maize and sorghum, and even other moisture rich starchy staples such as yam and plantain? Its most important feature is the width of its ecological amplitude, that is, its adaptability to a wide variety of ecological and agronomic conditions. In contrast to

other staples, it grows well under marginal conditions since it is also an efficient extractor of soil nutrients even in worn-out soils (Silvestre, 1989), as well as favourable conditions of soil fertility and rainfall. It has no critical growth stage after establishment, during which a short stress period might decrease yield. The implication is not only that the crop is found in a wide range of environments across the continent, but also within the same agroecological zone, it can adapt to microvariations in relief, soils and cropping systems. Apart from this ecological versatility, cassava also displays certain characteristics that makes it adaptable to a variety of socio-economic conditions. Its tolerance to low field labour inputs and variability in planting and harvesting dates makes it much less tightly constrained by seasonality than other staples while it remains a high producer of dry matter (and protein, if aerial parts are included) per unit of land and labour. Although fresh cassava starts to deteriorate within 24 hours and processing is rather labour intensive, its products can be relatively easily stored and transported. Finally, its economically valuable parts are not required for reproduction (Jones, 1959; De Vries, 1978).

In areas with wet rice cultivation in West Africa, cassava may be produced on residual moisture during the dry season, provided that soils are not water-logged (Carter *et al.*, 1992). This system is commonly practiced in Karonga district, in the North of Malawi.

In areas of relatively high soil fertility and high population densities, permanent cropping may have evolved even in precolonial times (Gleave and White, 1969). Subsequent introduction of perennial cash or plantain crops, such as coffee and cocoa, created a need for an 'easy' staple with few demands on labour, high productivity per unit of land area and which could be grown between the young trees as the plantation was establishing (Carter *et al.*, 1992).

Cassava's botanical characteristics confirm its suitability for low external input conditions, hence referred to as a low-risk and low-input crop. Again cassava's botanical properties allows premature and repeated harvesting of minuscule roots (piece meal), and harvesting of growing points as vegetables (Carter *et al.*, 1992).

Hence, 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 (Moyo *et al.*, 1998; Hallack, 2001; IITA, 2001). These features not only explain the successful introduction of cassava in Africa but also gives the understanding of how the crop fits into a great variety of African farming systems, that is why it occupies certain 'niches'. This confirms its importance in Africa.

2.3.1 Uses of cassava

Cassava is the second most important staple food crop in Sub-Saharan Africa providing an average of 285 Calories per person per day (FAO, 2000). Cassava leaf consumption is part of the many cassava farming and food systems (Onwueme, 1978; Chiwona-Karltun, 2001). In a study across Africa, 81% of the included cassava growing communities reported consuming cassava leaves (Nweke *et al.*, 1994). Jones (1957) reported that it was interesting to note that the consumption of cassava leaves is frequent rather than sporadic, and was probably an African invention. Besides protein, cassava leaves contain vitamins such as vitamin C, beta-carotene, vitamin B1 and B2, niacin and minerals including ferric oxide and calcium (Johnson and Raymond, 1968; Lancaster and Brooks, 1983). Many farmers that grow and consume cassava consider it to be a complete crop, since the roots provide the bulky energy and the leaves provide the stew that goes with it (Chiwona-Karltun, 2001).

Cassava is used as food in various ways. The fresh roots of 'sweet' cassava are eaten as a snack, boiled or roasted then consumed (Moyo *et al.*, 1998). The fresh cassava tuber also finds considerable use as feed for livestock. Sheep, goats, cattle, and particularly pigs are often fed on fresh cassava tubers which they find palatable (Onwueme, 1978). Cassava is also processed into various products which can be easily stored and transported. These include flour, chips and starch (Onwueme, 1978). Umanah and Hartmann (1973) also reported that cassava is used as a vegetable and as a source of high quality industrial starch.

Cassava flour is made either from fermented and dried cassava roots or dried chips. The flour is used for human consumption as well as industrial use (Moyo *et al.*, 1998; Benesi *et al.*, 2001).

Cassava chips and pellets are produced mostly for feeding livestock (Onwueme, 1978) while in Malawi cassava chips are produced mainly for human consumption using the 'sweet' cultivars (Moyo *et al.*, 1998). The bitter cassava varieties undergo fermentation before making intermediate products pending milling into flour.

2.4 Cassava starch

2.4.1 Uses of cassava starch and development strategies

Cassava starch is used directly in different ways or as a raw material for further processing (FAO, 2000). It also has various uses including multiple applications in the food industry (Niba *et al.*, 2001). Cassava starch has high potential for growth both for industrial and human uses. The unique properties of cassava starch suggest its use for speciality markets such as: baby foods, non-allergenic products and food for hospitalized persons. Cassava starch can be modified to provide characteristics that are required for more specialized food and industrial products.

A major strategy concern of growth through innovation requires research and development effort. Growth through competition may require improvements throughout the entire cassava production, processing and marketing continuum. Unmodified or native starches retain their identity as cassava-derived (FAO, 2000). Hence, native starches would be more competitive if their inherent special traits set them apart from competing starches like those derived from maize (FAO, 2000). Therefore, it is important to determine and capitalize on the special traits of cassava starch that are not available in competing starches. There is also need for cassava producing countries to develop their domestic market before entering into the export market (FAO, 2000).

Cassava breeding and development should follow even more closely a tailoring approach to suit final uses. Thus cassava bound for fresh food is likely to contain less hydrocyanogenic glucosides than varieties slated for processing. Also, varieties destined for processing for starch should have high levels of starch and have peel and root forms suitable for ease of processing (FAO, 2000).

2.4.2 Cyanogenic potential in cassava starch

Numfor and Walter (1996) reported cyanogenic glucosides potential of 1.94 mg HCN/kg in native starch. This amount is low and safe with respect to the classification of cassava toxicity. This is so since Koch classified cassava roots according to their degree of toxicity based on Boorsman's statement before 1905 that 50-60 mg HCN is the theoretical lethal dose for an adult man weighing 50 kg (Koch, 1933). This led to the publication by Bolhuis (1954) of Koch's arbitrary classification based on the yield hydrocyanogenic acid (HCN) from peeled raw cassava roots (Table 2.1).

Table 2.1: Arbitrary classification of cassava toxicity (Boorsman, 1905; Kochs 1933; Bolhuis, 1954).

Cyanogenic potential	Risk assessment
Less than 50 mg HCN/Kg fresh root	Harmless
50 – 100 mg HCN/Kg fresh root	Moderately poisonous to poisonous
More than 100 mg HCN/Kg fresh root	Very poisonous

The findings of Numfor and Walter (1996) agree with those of Fabiano *et al.* (2001), who found that there was a large reduction (97.50 %) in cyanogenic potential during starch extraction (thus, from 295 mg HCN/kg fresh root to 15.2 mg HCN/kg wet starch) from fresh root to wet starch (Table 2.2). The cyanogenic potential is further reduced in dry starch by 99.7 % (thus, from 295 mg HCN/kg fresh root to just 1.6 mg HCN/kg dry starch) from fresh root to dry starch (Table 2.2). According to the classification of cassava toxicity levels (Koch, 1933; Boorsman, 1905; Bolhuis, 1954), the cassava which was used by Fabiano *et al.* (2001) for starch extraction was highly toxic (295 mg HCN/kg fresh root) in terms of cyanogenic glucoside potential. However, the starch extraction process reduced the cyanogenic glucoside potential to very safe levels of 1.6 mg HCN/kg dry starch. Hence, whether one uses varieties with low or high cyanogenic glucoside potential, or, as farmers refer to them as 'sweet' or 'bitter' the end product which is dry native starch is safe for human consumption.

Table 2.2: Determination of residual cyanide in cassava starch (Fabiano *et al.*, 2001)

Material	Moisture (%)	g Fresh matter	mg HCN /kg	Total mg HCN	%of initial HCN
Fresh roots	65.2	2000	295.0	205.00	100.00
Wet pulp	75.9	626	11.7	1.80	0.90
Dry pulp	11.2	221	0.8	0.16	0.08
Wet starch	44.8	625	15.2	5.20	2.50
Dry starch	11.2	415	1.6	0.60	0.30
Effluent	98.9	8 l	16.9 mg/l	135.00	66.00

2.4.3 Demand of starch and other cassava intermediate products in Malawi

Cassava is becoming one of the most important raw materials in the local industries in the country. SARRNET (2001) studies revealed that cassava is used in the biscuit, plywood and textile industries as well as in bakeries. There are unconfirmed reports that cassava is also used in the breweries of local thick beer named Chibuku. This is due to the fact that cassava has for a long time been considered a poor man's product. During this study, several industries were visited which included: David Whitehead and Sons, Rab Processors, Chibuku brewery, Grain and Milling Company, Trans Globe Produce Export Limited, Universal Industries and Mr K. Kholomana. Results obtained from these discussions are highlighted below.

David Whitehead and Sons has been importing maize starch from Zimbabwe, but because of problems of foreign currency it has turned to using cassava flour. It was indicated that demand for cassava flour was steadily increasing. For example, not long ago, the company was using about 600 kg of flour per day. Currently, it is using about 900 kg per day. This represents a demand of about 30 metric tons per month which represents 480 metric tons per annum. However, it was reported that the capacity of the company requires 60 metric tons per month. In the same study it was reported that these figures should be used with prudence because it has been noticed that each visitor to these company is given a different set of data. The company is interested in small-scale starch processing for national and regional markets (SARRNET, 2001).

Trans Globe Produce Export Limited, used to export cassava chips and flour to Zimbabwe and South Africa a few years ago. However, this was stopped. The

organization has established that there is demand for cassava flour in South Africa and it is currently exploring ways of resuming the exportation to this country. Management considered using more cassava and starch. However, it was indicated that as long as maize prices remain low compared to those of cassava, use of cassava and its processed products such as chips will be very low. The situation has changed since the price of maize increased from MK250 to MK830 per 50kg bag in 2001 due to a maize shortage in the country and the SADC region as a whole (SARRNET, 2001).

Rab Processors Limited is one of the major processors of cassava in the country. Rab Processors has been exporting cassava flour to Zimbabwe but the market has been on the decline since consumers prefer maize flour. The cassava flour that is exported to Zimbabwe is mainly used in the cloth manufacturing factories where it is used as binding material. The demand is not steady and it has been in the range of 200 metric tons per year but three years ago it reached as high as 5000 metric tons per annum. Dry cassava chips are bought both from local farmers as well as from Mozambique. The main problem is that normally because of the high demand on the market for fresh cassava, prices are very high. There are times when some local biscuit manufacturing companies, most particularly, Universal Industries press orders for cassava flour from Rab Processors (SARRNET, 2001).

Universal Industries Company Limited uses small amounts of cassava in most of its products. It incorporates between 10 to 30 % of cassava flour into its products. Currently, the company is operating towards full capacity utilization. In most cases, the company uses 10 to 15 metric tons of cassava flour per month. Besides the local suppliers, the company has bought Njuli Farm in Chiradzulu district where it is growing both cassava and Irish potatoes for its use. The company is also exploring the possibilities of manufacturing its own glucose since this is currently being imported from Zimbabwe (SARRNET, 2001).

2.4.4 Current sources of starch in Malawi

All the starch which is being used in Malawi is imported. The type of starch in use is modified corn starch. Since the starch is imported, the much needed foreign reserves is depleted, and the products become more expensive than if the starch could have been produced locally. It could also be of advantage if cassava starch was being used since it does not require modification and the extraction is relatively easy compared to corn starch.

2.5 Problems of cassava research, production and utilization

2.5.1 Policies towards cassava research and production

Apart from its promotion as an anti-locust crop in east Africa in the 1930s few governments' policies seem to have singled out cassava versus other crops. In contrast, maize has benefited from active government interventions in many countries, in the areas of marketing, prices, supply of fertiliser and improved seed, and agricultural research. Even in the preindependence period, white settlers and colonial authorities favoured maize, and this may have had an effect on its acceptance among small farmers (Carter *et al.*, 1992). This has also been the case in Malawi. Even in the area where people have been growing cassava as staple, after independence, the government was forcing them to grow maize. All these efforts were a failure along the western shores of lake Malawi. As a result their staple has been cassava till now. This might have really affected the attitude of people towards cassava and its products. Due to climatic changes and socio-economic (like removal subsidies on inputs) changes, and decline in soil fertility which has led to mass starvation, the governments have recognised the importance of cassava, and have a tough time to convince the same farmers and donors that cassava is an important crop.

2.5.2 Constraints to cassava production

Cassava yield potential is constrained by shortage of high yielding varieties; high incidence of pests and diseases; shortage of resistant varieties to pests and diseases; use of inappropriate cultural practices; post harvest losses (since cassava roots are highly perishable); limited modes of utilization; and shortage of clean and healthy planting

material (Benesi *et al.*, 1998; 2000; 2001; Moyo *et al.*, 1998). These constraints influenced the Department of Agricultural Research and Technical Services to formulate a research programme to improve the production and utilization of cassava.

2.6 Realisation of importance of cassava

The importance of cassava as a food security crop became more apparent with changes in climatic, physical and socioeconomic environments in the early to mid 1990s: persistent droughts and increases in the prices of farm inputs that was largely caused by the devaluation of the Malawi Kwacha and the removal of subsidies (Minde *et al.*, 1997). Hence, cassava has gained special attention, as a food security crop because of drought and increase in the cost of inputs as required by other crops, in the recent years. Cassava tolerates drought, poor quality soil, less elaborate management and is widely adapted. These attributes make it the best candidate crop being promoted by the Government of Malawi in crop diversification for achieving food security (FAO, 1993). Of late, cassava is increasingly becoming an important industrial crop.

President J.A. Kufuor launched the President's Special Initiative (PSI) on August 16, 2001, in Accra, Ghana, under which the government is going to promote an aggressive export of garments, textiles and cassava starch to earn Ghana about $690 million by the year 2003. The ambitious initiative is going to culminate in an overall export about U$ 4.4 billion over the four-year period. The cassava project is going to create a ready market for 25,000 farmers in 10 selected districts which have comparative advantage in the production of cassava. In addition, about 70,000 jobs would be created in other areas. Cassava was chosen as an industrial crop, for the production of starch because more than 90 % of Ghanaian farmers cultivate the crop either as a main crop or in combination with others. The starch, which is produced from cassava, is used in the paper, textile, food, pharmaceutical, oil drilling and petrol-chemical industries. The two key by-products, pulp and juice, are used as cattle fodder and fertilizer respectively (*Daily Graphic, 2001*).

2.7 Applied research and development

2.7.1 Global strategies in research and uplifting cassava

Although cassava is an important crop, it has largely been ignored as a target for genetic improvement until recently. This was due to its predominant cultivation by subsistence farmers. Now it is considered as a priority tropical crop for improvement as indicated by the formation of the Cassava Biotechnology Network (CBN) and the areas of research include: genetic characterisation; disease and pest resistance; improved nutritional quality and cyanogenesis (Beeching *et al.*, 1993).

2.7.2 African strategies in research and uplifting cassava

The International Institute of Tropical Agriculture (IITA) was founded in 1967 with a mandate for improving food production in the humid tropics and to develop sustainable production systems. Its mission is to enhance the food security, income, and well-being of resource-poor people primarily in the humid and subhumid zones of Sub-Saharan Africa.

IITA conducts research, germplasm conservation, training, and information exchange activities in partnership with regional bodies and national programs including universities, non-governmental organizations (NGOs), and the private sector. The research agenda addresses crop improvement, plant health, and resource and crop management within a food systems framework and targeted at the identified needs. Research focuses on smallholder cropping and post harvest systems on the following food crops: cassava, cowpea, maize, plantain and banana, soybean, and yam. It works in collaboration with other research institutions that are available in various countries.

2.7.3 SADC strategies in research and uplifting cassava

The Southern Africa Root Crops Research Network (SARRNET) was launched in September 1993, as a follow up from an earlier network for Eastern and Southern Africa Root Crops Research Network (ESARRN). The International Institute of Tropical Agriculture (IITA) is executing the project with the responsibility of coordinating the network. Twelve Southern African Development Community

(SADC) countries are part of the network. These are Angola, Botswana, Lesotho, Malawi, Mauritius, Mozambique, Namibia, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe. The Democratic Republic of the Congo, a recent SADC member state is not yet a SARRNET country. SARRNET grouping is working under the umbrella of Southern Africa Centre for Cooperation in Agricultural Research and Natural Resources (SACCAR) Board which ensures compliance to legal requirements on property rights and biosafety in the international exchange of germplasm.

The mandate of SARRNET is basically applied/participatory research and development on cassava and sweetpotato including demand - led processing and utilisation. Aspects such as human resources development (training), information and technology exchange and institutional capacity building within SADC governments are also the focus of SARRNET. Its main applied research objectives are in the fields of the development and /or introduction and evaluation of improved germplasm, managing pests and diseases through an ecologically sustainable plant protection (ESPP) approach, surveying production systems, development and dissemination of post harvest technologies and the establishment of multiplication and distribution systems for improved planting materials alongside marketing issues.

2.7.4 Malawi strategies in research and uplifting cassava

Organised research to address the constraints to cassava production started in 1978. The overall objective was to improve the production and utilization of cassava and sweetpotato. Improved production and utilization would contribute to sustained food security and income for smallholder farmers in Malawi. In addition to the overall objective, programmes had specific objectives, that is, to generate, evaluate and select improved cassava and sweetpotato clones for high yield, tolerance to pests and diseases, consumer/end-user acceptability, early maturity, good in ground storage, quality, and ecological adaptability (Benesi *et al.,* 1998; 2000); to develop efficient and appropriate cultural practices for cassava production; develop appropriate processing, product development and storage methods; develop ecologically sustainable plant protection technologies; establish a rapid and efficient system for multiplication and distribution of cassava planting material; and carry out surveys in order to evaluate adoption rate and impact of new technologies (Benesi *et al.*, 1995; Moyo *et al.*, 1998). Some of these

specific objectives were achieved by carrying out work on breeding (germplasm development); agronomy (cultural practices); plant protection (pest and disease control); post-harvest handling (processing, storage and product development); multiplication and distribution of planting material; and surveys for adoption rate and impact assessment of new technologies (Benesi *et al.*, 1995).

The execution of the applied research programme to address cassava constraints follows a multidisciplinary and multisectoral approach. Personnel from the Department of Agricultural Research and Technical Services and other players manage applied research trials on-stations and on-farm. The team is composed of breeders, pathologists, entomologists and agronomists. These scientists are based at Chitedze, Bvumbwe, Makoka and Lunyangwa research stations as well as Bunda College, Chancellor College, and SARRNET (Malawi office).

Research on cassava is also carried out in collaboration with farmers, Agricultural Development Divisions (ADDs), NGOs, the International Institute for Tropical Agriculture (IITA) and other institutions. These institutions play a key role in the multiplication and distribution of cassava planting material and exchange of germplasm. ADD's identify critical problems farmers face in cassava production. They also supervise on-farm testing of promising technologies. Of late industries are also involved in the evaluation of cassava varieties/clones for specific uses in the industries. Hence, participatory research is employed in technology generation (Benesi *et al.*, 2001). Through these programmes, improved cassava varieties and other technologies have been released, which are currently widely adopted by farmers.

2.7.5 Establishment of cassava networks at global, continental (Africa), regional (SADC), and national levels

It is pleasing to note that cassava is now recognised as an important crop. Efforts are set in the uplifting of the crop at global, continental, regional as well as at national level.

The establishment of the global CBN, efforts to establish CBN Africa, establishment of SARRNET, and Root and Tuber Crops Commodity in Malawi is a positive response to the importance of the crop.

2.7.6 Change of attitude of donors towards funding of cassava research, production, processing, utilization and marketing strategies.

The other encouraging news is the change of attitude of donors towards funding cassava research and development. In the past donors like Rockefeller Foundation and USAID would not even look at a cassava proposal, but rather funded cereals like maize. Now they are quite committed in funding cassava research projects. Some of the donors who have been and are funding cassava research and development programmes are: IDRC, UNDP, FAO, USAID, IFS and of late the Rockefeller Foundation. Some more are showing interest in supporting cassava programmes.

2.7.7 Development of new technology and dissemination

Through collaborative efforts in cassava research and development in Malawi, several cassava varieties have been released for use in the production system by farmers (Benesi *et al.*, 1999). Many more promising clones both sweet and bitter are being evaluated on-farm, and are expected to be released soon (Benesi *et al.,* 2000).

Investigations on land preparation, time of planting, planting methods and patterns, including weeding, time of harvesting, inter-cropping systems and crop hygiene have been carried out. Appropriate technologies have been disseminated to farmers and most of them have been adopted (Anonymous, 1994; Moyo *et al.*, 1998).

Work has also been done on pest and disease control leading to the following recommendations: use of tolerant varieties; biological control (for cassava mealybug and cassava green mite using predators and parastoids) and crop hygiene against cassava mealybug, cassava scales, and cassava mosaic virus (CMD).

Processing equipment such as grating, pressing and chipping machines have been evaluated and are in use by smallholder farmers. These machines have been introduced with the aim of reducing drudgery in cassava processing and also to

improve the quality of the intermediate product. New cassava products (instant baby food, doughnuts, strips, mandazi, cakes, biscuits, chapati, buns, etc.) have been developed or introduced to enhance the diversification of utilization and add value to cassava.

A sustainable system of multiplication and distribution of cassava planting material is being established by having different levels of multiplication sites, thus, primary, secondary and tertiary multiplication sites (Benesi *et al.*, 1995).

Several surveys have been carried out to measure the current status and impact of adoption of new improved technology. These surveys include: the Adoption Rate and Impact Assessment of Accelerated Multiplication and Distribution of Cassava Planting Material as a Drought Recovery Measure in Malawi; the Current Status of Cassava and Sweetpotato Production and Utilization in Malawi; and follow up on on-farm testing of improved technologies.

The users readily adopt technology only when they are involved in the development of the technology. In the execution of the root and tuber crops programmes, participatory approaches are used, at the same time involving many players including the farmers themselves in problem identification and prioritisation and on-farm evaluation. The technology, which has been officially released, are disseminated to the rest of the users through NGOs, religious groups and organizations, in addition to the Department of Extension. The technicians disseminate the technologies to farmers and other end-users through meetings, demonstrations, open days, field days, and exchange visits (Benesi *et al.*, 2000).

Leaflets are also produced and circulated for awareness and wide adoption of the technology. Information is also available in libraries in the form of annual, survey and monitoring reports, and proceedings of workshops. Messages on the new technologies are also passed on through mass media that is, through radio, television, agricultural film shows and newspapers.

Training is a also provided to both technical personnel, farmers and other key players in the dissemination of the technology.

2.7.8 Impact of root and tuber crops programmes in Malawi

The Government of Malawi made a right move in the establishment of the Root and Tuber Crops Improvement Programme. Through its activities in collaboration with other stakeholders including the farmers themselves, in addition to the market opportunities for planting materials, fresh roots, and raw materials for industries a significant impact has been achieved in cassava production.

The vigorous promotion in advising farmers to include root crops like cassava in crop diversification and realisation of the benefits by the farmers in the growing of cassava has led to significant increase in area planted to cassava from 63,965 hectares in 1992 to 201,703 hectares in 2001. The production of cassava has also increased tremendously from 128,827 metric tons in 1992 to 960,315 metric tons in 2001 (Ministry of Agriculture, 1992 to 2001). Now cassava is going commercial in the form of feed formulation, starch production for industrial use, and flour for various uses including exportation.

2.8 Characterisation of cassava

2.8.1 Need for knowledge of genetic diversity in cassava improvement

Beeching *et al.* (1993) pointed out that a prerequisite for any programme of genetic improvement of cassava is knowledge of the extent of genetic variation present between cultivars and genetic distance between cassava and closely related species with which hybrids could be produced. This can be achieved through characterisation of germplasm using either morphological, biochemical or DNA markers.

2.8.1.1 Morphological characterisation: applications, advantages and disadvantages

Although cassava is one of the most important tropical crops, the origin and domestication of the crop and relationship with other species of the genus *Manihot* have not been clearly explained and are still under debate.

Commonly, 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 cultivars and also to study the taxonomic status. Certification of new varieties is based on the genetic purity of a particular crop. However, traditionally those assessments depend on botanical traits. Most of them are ambiguous descriptors, and have limited use for cultivar identification (Stegemann, 1984; Zacarias, 1997). Such characteristics are often controlled by multiple genes, are subject to varying degrees of environmental modifications and interactions. Natural specific hybridisation and introgression occurring in plants can make this problem even more difficult. Many of these traits are also difficult to analyse because they do not have the simple genetic control assumed by many in genetic models (Liu and Fumier, 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. Furthermore, these methods involve a lengthy survey of plant growth that is costly, labour intensive and vulnerable to environmental conditions. In cassava breeding programmes the major emphasis has been on the collection and conservation of genetic pools (CIAT, 1993) and characterisation to remove duplicates.

The morphological traits of plants are grouped as either variable (polygenic) or constant (monogenic) characteristics. The variable characteristics are those associated with large genotype by environment interaction. Constant characteristics are those typifying the species or cultivar, for example petiole colour, root skin colour and stem colour (Onwueme, 1978; Zacarias, 1997). Cassava is a crop, which is grown in several different ecological environments and for this reason it is difficult to describe their morphological characteristics especially the variable traits. The influence of the environment on the genotype is always important.

Characters such as plant height, tuberous root weight and other morphological characters of cassava, have been studied by Mathura *et al.* (1986) over four consecutive years under Tripura agroclimatic conditions. They concluded that the phenotypic variance was higher than genotypic variance in their study. All measured characters in consideration except for plant height, affected the yield.

Cassava cultivars are generally distinguished on the basis of morphological traits. They have a wide variability of botanical characteristics. Such features are not very distinct and sometimes quite variable, but have to be used by local farmers for basic identification of plant material. Numerous cultivars are distinguished by morphological characters like plant height. Size, shape and colour of leaf and root, and position of root can also be of importance. Another obstacle to the identification of cultivars is the existence of considerable linguistic pluralism. As in most parts of the world, the cultivated forms of crops have never been unequivocally named. Each site has its own series of names for different cultivars with specific meanings. Cassava morphological classification has played an important role in rectifying ambiguities of cultivars. Researchers have tried to characterise them using the adapted International Board for Plant Genetic Resources (IBPGR) descriptor. IBPGR has defined a set of relatively stable morphological traits useful for cassava genotype characterisation. They include characterisation of shoot and root parts of cassava with quantitative and qualitative measurements. In some distinct cultivars, the plants' architecture can be very different with the presence or absence of branches as well as levels of branching, colour of unexpanded apical leaf and also the presence or absence of pubescence. Generally the accessions are made up of large numbers, sometimes with lack of definitive identification, and complicated by the influence of the changing environmental condition which makes it difficult to tell which is which (Waycott and Fort, 1994). Picking of cassava leaves for use as a vegetable in Africa also 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 issue of duplication, and genetic diversity.

Methods of germplasm conservation and maintenance vary according to several factors including the species, their geographic distribution, breeding system involved and seed behaviour. However, two main methods of conservation have so far been identified, namely *in situ* and *ex situ* conservation (Pirreno, 1992). *Ex situ* conservation plays a major role in preserving the genetic diversity of cassava (Ocampo *et al.*, 1994). In cassava generally field gene banks are used, where the accessions are conserved in the field, are regenerated every one or two years, and are maintained by vegetative means in the field. New plantings are made from stem cuttings from old fields. In addition to high cost of field maintenance, the valuable

germplasm is often exposed to pest and disease attacks, soil and climatic stress. The main danger of this method of conservation is the loss of accessions due to biotic and abiotic factors. The other method used for cassava germplasm conservation is *in vitro* (Ng, 1992), which is practiced when facilities are available

Manihot species have been extensively classified using their morphology (Rogers and Appan, 1973). Bonierbale *et al.* (1997) assessed the genetic diversity of 105 genotypes by analysing them with morphological and AFLP methods to estimate genetic similarities among taxa and evaluate intra- and inter-specific variability. The results showed that individuals grouped according to prior taxonomic classification using morphological descriptors. A large percentage of duplicates at CIAT were identified using passport, morphological and isozyme characterisation (Ocampo *et al.*, 1994). Such redundancy makes it more expensive to maintain and manage existing collections and slows down the introduction of new germplasm.

Since Mendel, breeders and geneticists have used morphological characteristics such as leaf and flower attributes to follow segregation of genes and hybrids, but most agronomic traits are not associated with easily observed phenotypic markers (Kochert, 1994).

One of the earliest breeding procedures used with cross-pollinated crops was mass selection. The principle advantage of this method in breeding is the simplicity and the ease with which it can be carried out. It is effective for the improvement of characters with high heritability that can be visibly identified. One of the weaknesses of mass selection is that the selection of characters with low heritability is relatively ineffective, since plants which are superior due to genotypic influence cannot be distinguished from those plants which are superior due to environmental influence (Poehlman, 1987). As an alternative, a number of laboratory methods have been successfully developed and applied in the past decades for use in effective characterisation such as isozyme analysis (Bassiri, 1976; Arus *et al.*, 1982; Stegemann, 1984; Ocampo *et al.*, 1994; Nienhuis *et al.*, 1995; Laminski *et al.*, 1997), seed storage electrophoresis (Ladizinsky and Haymowitz, 1979; Gupta and Robbelen, 1986; Vaughan and Denford, 1986; Nienhuis *et al.*, 1995), and high performance liquid chromatography (Burnouf and Bietz, 1987; Buehler *et al.*, 1989).

The main constraint to these techniques is the limited amount of polymorphism they are able to detect among closely related genotypes (Hu and Quiros, 1991). Isozymes often show low levels of polymorphism and problems of reproducibility due to tissue type and conditions (Zacarias, 1997). With the advent of molecular techniques, DNA based procedures have been proposed for cultivar identification. DNA sequences show greater variation than amino acid changes in isozymes. The composition of DNA is also consistent among tissues and are not affected by environmental changes (Beeching *et al.*, 1993). The development of DNA markers provides the opportunity to detect, monitor and manipulate genetic variation (Yamamoto *et al.*, 1994) more precisely than in the case of morphological and biochemical markers. They are potentially unlimited in number and can be organised into a genetic linkage map (Soller and Beckmann, 1983). These techniques include DNA fingerprinting (Nybom *et al.*, 1990).

2.8.1.2 DNA fingerprinting and its application

DNA fingerprinting is a technique which has been widely adopted in order to differentiate among genotypes at species and subspecies level (McClean *et al.*, 1994). Cultivar identification can be achieved more accurately using DNA fingerprinting data, especially in materials characterised by high genetic variation between cultivars and no variation within, such as vegetatively propagated cultivars derived from cross pollinating species. The most closely related cultivars are usually distinguished by DNA fingerprinting methods (Nybom, 1994). Compared to morphological and biochemical characteristics, the DNA genome provides a significantly more powerful source of genetic polymorphism (Beckmann and Soller, 1986). The advantages of DNA fingerprinting over morphological and biochemical markers are: the number of polymorphisms is primarily limited only by genome size; many different single copy clones can be tested, hence, genetic linkage maps can be constructed which contain a very large number of markers at close intervals; provide an opportunity to develop detailed genetic maps from a limited number of crosses; linkages between markers and genes of interest permits one to infer the presence of desirable genes by assaying for DNA markers; they are not environmentally or developmentally influenced, thus, they are phenotypically neutral and independent of allelic and non-allelic interactions.

They also enable the investigator to detect the exact genetic constitution of an individual plant in a segregating population (Phillip *et al.*, 1994).

The application of DNA fingerprinting could be very valuable in the identification of cultivars and species (Kuhnlein *et al.*, 1990), and could help to create more efficient breeding programmes through the detection of genetic linkages between DNA fingerprinting bands and agriculturally important quantitative trait loci (QTL). The high variability of DNA fingerprinting described in humans, animals and plants allows the identification of different individual genotypes and species (Lin *et al.*, 1993)

2.9 DNA marker types, procedures, advantages and disadvantages

2.9.1 Principles for DNA marker techniques

DNA fingerprinting involves the display of a set of DNA fragments from a DNA sample. A variety of DNA fingerprinting techniques are presently available (Vos *et al.*, 1995) most of which use PCR for detection of fragments. The choice of which fingerprinting technique to use, is dependent on application, such as DNA typing, DNA marker mapping, and the organism under investigation, for example, prokaryotes, plants, animals or humans. Ideally, a fingerprinting technique should require no prior investments in terms of sequence analysis, primer synthesis or characterisation of DNA probes (Vos *et al.*, 1995). All the DNA techniques make use of DNA which has been extracted or isolated from either plant or animal organisms.

2.9.2 DNA extraction procedures

Plants can be grown in a variety of environments and in different locations and still provide starting material for DNA isolation (Young, 1994). Any part of a plant can be used to extract DNA. The most common starting material is young leaves. They can be fresh, lyophilised, dried in an oven or in some cases dried at room temperature (Kochert, 1994). Reports of using seeds as source of DNA material for DNA extraction are very scarce.

Several methods for DNA extraction have been developed (Murry and Thompson, 1980; Dellaporta *et al.*, 1983; Tai and Tanksley, 1990; John, 1992). The common methods have the objective of simplicity, speed, and utilisation of a small amount of starting material (Lamalay *et al.*, 1990). Simplicity and speed are absolutely essential for processing large numbers of individuals. Ability to extract DNA from small amounts of starting material is advantageous if large quantities of starting material are hard to obtain (Young, 1994). The plant DNA needs to be pure enough so that it will digest reproducibly with restriction enzymes, and the resultant preparations can be satisfactorily separated by gel electrophoresis.

In most grasses, such as rice, maize and bamboo, very simple isolation procedures can be used. For other plants, including legumes such as groundnuts or alfalfa, special steps must be taken to purify the DNA (Kochert, 1994). Preparation of high quality DNA from polyphenol-containing plants such as field (faba) bean (*Vicia faba*), tomato (*Lycopersion esculentum*), and potato (*Solanum tuberosum*) was difficult (Pich and Schubert, 1993). The greatest problems in those plants are probably due to DNA degradation caused by carbohydrates, glycoproteins and secondary plant products such as phenolic terpenoids and tannins which may bind to DNA after cell lysis (John, 1992; Pich and Schubert, 1993; Kochert, 1994), which tend to tonic purify the plant DNA and prevent proper digestion and electrophoresis (Kochert, 1994). The same problem was also reported by John (1992) in cotton (*Gosspium hirsutum*) where DNA extraction was notoriously difficult. The leaves of grape vine (*Vinis vinfera* L.) have relatively high mineral content and other unknown compounds responsible for low yielding DNA due to inability to precipitate the DNA (Harding and Roubelakis-Angelakis, 1994).

The extraction procedures for plant genomic DNA consist of grinding plant tissue into a powder using either a mortar and pestle in liquid nitrogen or a mechanical grinder (Kochert, 1994). This disrupts the plant cell wall and cell membranes to release the cell constituents into an extraction buffer which contains compounds to protect the DNA from the activity of endogenous nucleases (Yu and Pauls, 1994). Different sorts of extraction buffer have been used, but most of them have to maintain the pH at around 8.0. Salts such as SDS or sarkosyl have to solubilise the plant membranes and provide the means to rapidly inactivate DNase. Since DNase requires magnesium ions

for activity, EDTA is often added to sequester the magnesium ions. Some protein denaturants, such as phenol, chloroform, or urea may also be used. Any added detergents or incubation of extracts at elevated temperature also aid in the inactivation of DNase (Kochert, 1994).

Most proteins are removed by treatment with chloroform, phenol or protease and the DNA is reprecipitated with alcohol and solubilised in buffer. In cases with problems related to plant DNA degradation by secondary products, these bind to DNA upon cell lysis and cannot be removed by conventional extraction procedures (Kochert, 1994). For such plants the common methods for DNA isolation include time consuming and expensive procedures (Pich and Schubert, 1993). For yields of good DNA of high quality, special steps must be taken. This can be accomplished by breaking open the cells of fresh tissue in a blender or Polytron homogeniser and making a crude nuclear preparation of DNA isolation. The supernatant from nuclear preparations contain the bulk of the carbohydrates and glycoproteins. Selective preparation by CTAB of nucleic acids from solutions containing polysaccharides can also be used.

John (1992) developed a DNA extraction method based on a modification of the method of Chirgwin *et al.* (1979) for the extraction of the *Malvaceae, Bombaceae* and *Moraceae* families. The procedure includes high buffering capacity, alkaline pH, and most important polyvinylpirrolidone (PVP), through which hydrogen bonding, complexes with phenolics, are effectively removed from the homogenate. The homogenate is then subjected to ultracentrifugation to isolate the DNA. Pich and Schubert (1993) developed a method based on modified protocols of Dellaporta *et al.* (1983) and John (1992). It combines a complexation of polyphenolic compounds by PVP, followed by cell lysis and selective precipitation and centrifugation for removal of PVP complexes and DNA recovery.

Harding and Roubelakis-Angelakis (1994) have isolated DNA from grape vine using *in vitro* plants, glasshouse grown plants and callus. Using CTAB and phenol methods for both *in vitro* and glasshouse grown plants, these procedures resulted in the formation of coloured complexes. Separation of nucleic compounds from cytoplasmic compounds after ultracentrifugation was essential for the isolation of high molecular weight DNA (30-80 kbp). Results obtained using *in vitro* plants have proved to be

less problematic compared to glasshouse plants, reflecting a reduction in the level of natural products.

CIAT (1990) has used a DNA extraction method based on the protocol of Dellaporta *et al.* (1983) to extract DNA from cassava plants. Proteins and polysaccharides are removed using potassium acetate preparations. Ribonuclease treatment can be used to remove RNA, after isolation of DNA with alcohol (Kochert, 1994).

As long as an extraction provides DNA in sufficient quantity and quality for direct use, for restriction as a template, Southern or polymerase chain reaction (PCR) analysis, the method is probably satisfactory (Pich and Schubert, 1993; Kochert, 1994; Young, 1994). DNA used for genetic mapping through PCR or PCR based techniques does not need to be purified. DNA used for the RFLP technique needs to be pure enough so that it will digest reproducibly with restriction enzymes, and the resultant preparation can be satisfactorily separated by gel electrophoresis. In only the most recalcitrant cases, or the cases where an accurate estimate of DNA quality is needed, it is necessary to purify the DNA with more complex methods such as equilibrium centrifugation on cesium chloride gradients (Kochert, 1994).

2.9.3 DNA marker techniques

2.9.3.1 Restriction Fragment Length Polymorphism (RFLP) technique

The development of RFLP technology represents an important contribution to breeding programmes (Burr *et al.*, 1983; Young *et al.*, 1988). It signified several improvements over the use of isozymes markers. The RFLP technique generated more detectable loci alleles, was not sensitive to environmental factors, and could 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 (Wang and Tanksley, 1989; Song *et al.*, 1990; Kessell *et al.*, 1992), and populations (Bonierbale *et al.*, 1988; Miller and Tanksley, 1990), for identification of cultivars and genotypes (Smith *et al.*, 1990; Melchinger *et al.*, 1991; Livini *et al.*, 1992) for mapping genes that control quantitave as well as qualitative traits (Osborn *et al.*, 1987; Beavis and

Grant, 1991). These procedures are therefore able to disclose unlimited polymorphic markers.

The RFLP method is lengthy, and time consuming, costly and labour intensive (Marsan *et al.*, 1993). It also requires specific probes (Tommerup *et al.*, 1995). The method is difficult in some species with large and complex genomes (Marsan *et al.*, 1993). 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 (5-10 µl per lane), and involves special manipulation requiring high level of expertise and skill (Beeching *et al.*, 1994).

The most important advantage of RFLP markers is that they are co-dominantly inherited, thus they can distinguish between homozygous and heterozygous loci. They can also provide complete genetic information at each RFLP locus (Rafalski and Tingey, 1993). RFLP has been particularly useful in mapping species that display a high level of intraspecific variation. The preliminary genetic map of cassava was based on RFLP (Fregene *et al.*, 1994; 1997). Several maps have also been reported in maize (*Zea mays*) (Burr *et al.*, 1983; Helentjaris *et al.*, 1986; Gardiner *et al.*, 1993). Other species mapped with RFLP include barley (Garmer *et al.*, 1993), sorghum (Xu *et al.*, 1994), sunflower (Berry *et al.*, 1995), alfalfa (Kiss *et al.*, 1993), rice (McCouch *et al.*, 1988) and wheat (Chao *et al.*, 1989; Devos and Gale, 1993).

RFLP has also been used to assess the genetic diversity within cassava and between *Manihot* species (Beeching *et al.*, 1993; 1994). RFLP have also been applied in the studies of analysis for phylogenetic relationships of species within the genus *Manihot* (Haysom *et al.*, 1994; Schaal, 2001).

2.9.3.2 Polymerase Chain Reaction (PCR) technique

Williams *et al.* (1990), and Welsch and McClelland (1990) reported a new technique based on the amplification of random DNA sequences by polymerase chain reaction (PCR) (Saiki *et al.*, 1988). The development of PCR led to the introduction of several

new techniques for genome analysis based on the selective amplification of genomic DNA fragments. The PCR reaction requires: deoxynucleotides to provide both energy and nucleotides for synthesis of DNA; DNA polymerase; primer; template and buffer containing magnesium (Taylor, 1991). The typical PCR amplification utilises oligonucleotide primers that hybridise to opposite strands. The product of DNA synthesis of one primer serves as a template for another primer. The PCR procedure requires repeated cycles of DNA denaturation, annealing and extension by DNA polymerase, resulting in an exponential increase in the number of copies of the region bound by the primer (Saiki *et al.*, 1988). The technique can be applied to detect polymorphism in various plants, animals, bacterial species (Welsch and McClelland, 1990; Williams *et al.*, 1990) and fungi (Manilus *et al.*, 1993).

The introduction of the PCR technique has revolutionised standard molecular techniques and has allowed the development of new tools serving to detect DNA polymorphism (Hu and Quiros, 1991). The electrophoresis pattern of DNA fragments can be used for assaying diversity (Tommerup *et al.*, 1995). Polymorphism between two individuals is generally scored as presence or absence (non-amplification) of a particular DNA fragment. The absence may result from deletion of a priming site, insertion rendering priming sites too distinct of a priming site, insertions that change the size of the DNA segment without preventing its amplification, or simple base changes in either or both primer sites binding the DNA fragment (Williams *et al.*, 1990).

The main advantage of this technique over RFLP analysis is its inherent simplistic analysis and the ability to conduct PCR tests with extremely small quantities of tissue for DNA extraction (Edwards *et al.*, 1991; Welsch *et al.*, 1991). On the other hand, PCR is limited in its usefulness because of the time and expenses required to obtain the DNA sequence information to enable primer design (Samec and Nasinec, 1995).

2.9.3.3 Random Amplified Polymorphic DNA (RAPD) technique

One variation of a specific class of molecular markers is random amplified polymorphic DNA (RAPD). RAPD markers are generated by the use of short (10-mer) synthetic oligonucleotides in a single primer (Williams *et al.*, 1990) using a

modified PCR reaction (Saiki *et al.*, 1988). In this technique decamer primer of arbitrary sequence is allowed to anneal at 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 agarose gel and stained with ethidium bromide (Whitkus *et al.*, 1992). They commonly result in DNA fragment patterns which indicate that they are polymorphic between genotypes (Nybom, 1994), therefore assaying within them can lead to identification of genes of importance (Tommerup *et al.*, 1995).

There are several advantages of RAPDs compared to RFLP and isozymes techniques. The RAPD analysis is relatively simple, rapid, does not involve radioactive isotopes and costs less than RFLPs (Barua *et al.*, 1993; Varghese *et al.*, 1997). It does not require target DNA sequence information, and it can provide markers in the genomic regions not accessible by RFLP analysis (Williams *et al.*, 1990). The technique uses very small amounts of genomic DNA which allows the analysis of single seeds or young seedlings (Hu and Quiros, 1991). Another advantage of the RAPD method is that a universal set of primers can be used for genomic analysis for any organism (Welsch and McClelland, 1990). These advantages make RAPD method suitable for genetic studies.

Short primers have been used to amplify segments of genomic DNA from a wide range of species of various organisms including plants (Williams *et al.*, 1990; Caetano-Annollés *et al.*, 1991; Martin *et al.*, 1991).

In cassava, RAPD technology has been used to explore genetic diversity in a collection (Marmey *et al.*, 1994; Ocampo *et al.*, 1994; Laminski *et al.*, 1997), and relationships between *Manihot* species even at cultivar level (Buso *et al.*, 1994; Nassar 1994; Schaal *et al.*, 1994; Laminski *et al.*, 1997), developed linkage maps (Fregene *et al.*, 1994), and for studies of evolutionary relationships (Carvalho *et al.*, 1995).

However reproducibility of banding patterns can be affected by different concentrations of reaction component compounds and cycling conditions (Weeden *et al.*, 1992). Polymorphism detected by RAPDS are inherited in a dominant fashion

(Williams *et al.*, 1990; Welsch *et al.*, 1991). Buso *et al.* (1994) suggested that the RAPD technique has serious limitations for use in cassava, due to the plants' high heterozygosity.

2.9.3.4 Amplified Fragment Length Polymorphism (AFLP) technique

Marc Zabeau and colleagues at Keygene, Wageningen, Netherlands (Zabeau, 1992; Zabeau and Vos, 1993; Vos *et al.*, 1995), developed the Amplified Fragment Length Polymorphism (AFLP) technique. The AFLP technique is based on the detection of genomic restriction fragments by PCR amplification, and can be used for DNA of any origin or complexity. Fingerprints are produced without prior sequence knowledge using a limited set of generic primers. The number of fragments detected in a single reaction can be tuned by selection of specific primer sets. The technique is the most reproducible. The fingerprints can be used to distinguish even between closely related organisms, including near isogenic lines (NILs). It easily allows scoring a very large number of markers in a given population. It is also robust and reliable because stringent reaction conditions are used for primer annealing. The reliability of the AFLP technique is combined with the power of the PCR technique (Vos *et al.*, 1995; Winter and Kahl, 1995; Powell *et al.*, 1996; Blears *et al.*, 1998).

In addition, AFLP is a highly sensitive method for DNA fingerprinting to be used in a variety of fields, including plant and animal breeding, medical diagnostics, forensic analysis and microbial typing, to name a few. This technology has also been used to generate DNA based markers for cloning genes involved in phototropic responses in higher plants that have only been identified genetically by mutant phenotypes (Liscum and Briggs, 1995). 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 provides support for the contention that AFLP technology can be exploited for gene isolation by positional cloning. The drawback of AFLP is that markers are mostly dominant.

Several researchers have applied AFLP in assessing the genetic diversity of various cassava germplasm collections. Bonierbale *et al.* (1997) assessed the genetic diversity of 105 genotypes by analysing them with AFLP to estimate genetic similarities among taxa and evaluate intra- and inter-specific variability. Results showed that: individuals grouped according to prior taxonomic classification; *M. aesculifolia, M. brachyloba* and *M. carthaginensis* were the most distant taxa to the crop cassava. The results also agree with the proposal that the subspecific taxa of *M. esculenta* are most related to cassava and support 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; and 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* spp. along with classical botany and ecology using AFLP to characterise the genetic structure of cassava in relation to its wild relatives; and the domestication process of cassava. The results showed that the genetic diversity of cassava itself is high, but a diversity nearly equal, although slightly divergent, is found in a single Amazonian field at the level of AFLP. Although domestication appears to have evolved primarily from *M. esculenta* sub-species (ssp.) *flabellifolia* and *Peruvian*, it seems that some other species (spp.) have also contributed. The important genetic recombination suggested to be at the origin of the diversity of cassava gives a favourable perspective for various strategies of genetic mapping and gene tagging since this crop is multiplied vegetatively.

Raji *et al.* (2001) assessed the diversity of 500 African landraces of cassava using RAPD and AFLP. The results showed that the cluster analysis data provided similar genetic relationships of the population; However, the AFLP technique detected a much higher level of polymorphism giving a better diversity structure than RAPD. These findings agree with the results of Wang *et al.* (1998) who found that the AFLP system provides a higher number of loci detected per run than RAPD, and that AFLP revealed more polymorphisms than RFLP based on cassava chloroplast DNA.

Morillo *et al.* (2001) used mapped AFLP and SSR as evidence of introgression in a set of 60 plants. AFLP and SSR bands that appeared in some varieties of cassava and not in *Manihot esculenta* ssp. *flabellifolia*, the presumed ancestor of cassava, were considered introgressed bands. In the case of AFLP, the sequence of one band has so far allowed the design of new primers to amplify part of the same sequence also in *flabellifolia* sp. These findings confirm the introgression of *Manihot glaziovii* in some cultivars.

Cassava Bacterial Blight (CBB) is a disease of economic importance in cassava production. CBB's pathotype was studied between 1995 and 1999 in Colombia using molecular markers (RFLP and AFLP), and virulence assays. The first study from 1995 to 1996 revealed the geographical differentiation of RFLP haplotypes in ecozones. Restrepo *et al.* (1999) pointed out that to develop an appropriate CBB management strategy, the genetic diversity of the pathogen's population must be assessed.

Restrepo *et al.* (1999) characterised the genetic diversity of Colombian *Xanthomonas* isolates by AFLP analysis using primer combinations. Six *Xanthomonas* strains were tested with 64 primer combinations to select the best selective primers. Eight primer combinations were selected according to their reproducibility, number of polymorphic bands, and polymorphism detected between *Xanthomonas* strains. Fourty seven *Xanthomonas* strains, originating from different Colombian ecozones were analysed with the selected combinations. Some primer combinations differentiated *Xanthomonas* strains that were not distinguished by RFLP analysis, thus, AFLP fingerprinting allowed a better definition of genetic relationships among *Xanthomonas* strains.

Sanchez *et al.* (1998) used two primer combinations and two *Xanthomonas* strains on 93 varieties of *Manohot esculenta* Crantz by AFLP analysis to assess genetic diversity and resistance to CBB. The results demonstrate that resistance to CBB is broadly distributed in cassava germplasm, and that AFLP analysis is an effective and efficient means of providing quantitative estimates of genetic similarities among cassava accessions.

The recently constructed molecular genetic map from a F1 cross of non-inbred parents using RFLP, AFLP, EST and SSR markers, mapped resistance of bacterial blight in cassava, based on this map. Nine QTLs located in linkage groups B, D, L, N, and X were found to explain the pathotypic variance of the crop's response to *Xanthomonas* in the greenhouse, while linkage group D is found to be involved in field resistance (Restrepo *et al.*, 2001).

Narvaez *et al.* (2001) used AFLP and SSR markers to study the traditional cassava varieties from various Amerindian communities, thus, in French Guiana (bitter) and Ecuador (sweet) compared to those of 'Caboclo' community in the upper Rio Negro (Brazil) area (mostly bitter), and a collection representative of the core collection in CIAT. The Amerindian cassava varieties from French Guiana and Ecuador were found mostly 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 found intermediate, although more related to Amerindian bitter or sweet varieties, respectively. This pattern of variation expresses a progressive historical merging of sweet and bitter varieties in a continuum. The most recent bred varieties tend to be hybrids derived between sweet and bitter varieties.

CHAPTER 3

EVALUATION OF ROOT DRY MATTER, EXTRACTION AND QUALITY OF STARCH FROM MALAWI ELITE CASSAVA GENOTYPES, AND ITS INDUSTRIAL USE

3.1 Introduction

Cassava is produced mainly for household consumption, as staple diet in most parts of the southern and northern regions of Malawi (especially among low income households) and as a snack and substitute to bread among most households including those in the high income category (Mataya *et al.*, 2001). Over the years, both hectarage and production levels of the crop has significantly increased. This crop is now contributing 25-60 % of the national food balance sheet in some SARRNET countries (SARRNET, 2001). Although demand for cassava products in the processing industries including bakeries, timber and textile industries is steadily rising, they predominantly depend on imports to meet their requirements. This clearly demonstrates that there is an opportunity for small as well as large scale farmers to invest and profit from this commodity (Benesi *et al.*, 2001; Mataya *et al.*, 2001).

Although cassava has become an important crop in most SADC countries such as Malawi, Tanzania, and Zambia, information is lacking on the supply and household demand for fresh and processed products from this crop. It has, therefore, been difficult to develop reliable strategies that address issues on post-harvest utilization and commercialisation of cassava (SARRNET, 2001).

Dry matter content is closely related to starch content in cassava, since 70-90% of cassava dry root matter is starch. This makes dry matter an important trait to cassava producers since it is a crop grown largely for its carbohydrate content. Dry weight of the parenchyma varies very widely in cassava over years and over environments. Some genotypes have variation for dry matter content depending on edaphic-climatic and agronomic production conditions (CIAT, 1995).

Cassava production is constrained by major cassava diseases, low rainfall and low market prices. Land size is also a limitation to increase cassava production.

Generally, there is high labour cost but farmers have no money to hire labourers. As a result most cassava growers resort to food for work. In other areas like Mulanje and Dedza, theft prevents people from growing more of the sweet cassava varieties.

Industries in Malawi have had a negative attitude towards the use of cassava starch. This has been because some industries bought local products in the name of cassava starch which were either not as efficient as the corn starch which is normally used or in some industries led to a total failure (Fungulani and Maseko, 2001; Itaye, 2001). This scenario has been worrisome given the knowledge that cassava starch is being extensively used in many parts of the world. The Malawi main foreign exchange earner (tobacco) is facing the problem of the antismoking lobby. Means need to be identified to protect the little foreign reserves obtained. As industries are importing all the starch they are using, cassava starch is an alternative since the cassava is grown throughout the country and is a low input crop. In addition, the starch extraction from cassava roots is very simple. This situation posed a challenge worthwhile to be investigated. There was also a need to determine if the poor quality starch is associated with cassava genotypes which are grown in Malawi.

In some cases cassava production is increasing, hence there is need to find markets for cassava so that it should not only be taken as a food security crop but also a cash crop. This is because most of the cassava products are multipurpose, thus, used as food and also have commercial value. This can only be achieved if cassava products can be used in various industries especially starch which has various uses in various specialised industries. The objectives of this study therefore were:

1. To evaluate the elite Malawi cassava genotypes for dry matter content;
2. To evaluate the native starch extraction rate for Malawi cassava elite genotypes;
3. To evaluate the elite Malawi cassava genotypes for quality of the native starch extracted from them;
4. To assess the genotype by environment interaction (GxE) of the starch quality traits included in this study; and
5. To assess the feasibility of use of native cassava starch in the industrial sector in Malawi.

3.2 Materials and methods

The recommended varieties (Mbundumali, Gomani, Maunjili, Silira and Mkondezi), the locally bred clones (CH92/108, CH92/077, CH92/082, CH92/105, CH92/112 and MK95/054), and the introduced clones (TMS4(2)1425, TME1, 83350, TMS60142A, 81/00015, I84563, LCN8010, 30786 and TMS60121) were evaluated at the Chitedze and Makoka research stations.

The trials were planted in November 2000 and were harvested in December 2001. The 2000/01 season's rainfall for these stations is presented in Table 3.1. Effective rains started in November at both sites. The general climatic, soil and altitude data for these sites are presented in Table 3.2, while the locations of the research stations (sites) and agro-ecological zones are presented in Figure 2.4 (previous chapter). The detailed weather data during the period of harvesting and starch extraction (December 2001) are presented in Table 3.3.

Table 3.1: Monthly rainfall (mm) for Chitedze and Makoka in 2000/01 season

	2000						2001									
Site	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Chitedze	17.3	148.4	79.8	318.0	237.5	174.4	16.5	0.0	0.0	0.0	0.0	0.0	2.0	60.9	194.3	1249.1
Makoka	30.3	164.9	176.6	130.6	395.7	295.1	3.8	0.8	0.0	0.1	7.0	1.6	6.2	53.9	154.6	1421.2

Table 3.2: The climatic and edaphic description of the test sites (Chitedze and Makoka)

Site	Altitude (m.a.s.l)	Rainfall (mm)	Mean Max. Temp. (°C)	Mean Min. Temp. (°C)	Soil Texture	Soil pH
Makoka	1029	1021	34	10	Sandy loam to sandy clay loam	5.0-6.5
Chitedze	1097	892	34	10	Sandy loam clay to sandy lam	5.5-6.7

Rainfall = Mean annual rainfall; Max.= Maximum; Min. = Minimum; Temp. = Temperature; m.a.s.l = metres above sea level (Benesi *et al.*, 1999).

A randomised complete block design was used with four replicates. The plot sizes were four ridges, each with 12 plants (gross) and a net of two inner ridges each with 10 middle plants. The ridges were 0. 9 m apart and plants were also 0.9 m apart along the ridge. The length of the cassava planting stakes were 25 cm long and were planted in a slanting position at about 60 °. Sprouting rate (up to 1 MAP), dry matter content in

roots, starch extraction rate, pH, moisture content, ash content and colour of the starch were among the data which were recorded.

Table 3.3: Detailed weather data for Chitedze and Makoka during the period of harvesting and starch extraction (December 2001)

Date	Site	Temperature (°C)		Wind	RH (%)	Sun (h)	Rain	Evap.
		Min.	Max.	(km/h)			(mm)	(mm)
3	Makoka	23.7	32.2	2.3	51	6.2	0.0	6.1
4	Makoka	20.5	26.4	1.4	88	0.8	76.8	-
5	Makoka	18.4	29.6	1.5	76	7.8	0.0	5.1
6	Makoka	19.3	30.6	1.6	60	11.7	0.0	6.1
7	Makoka	20.0	30.0	2.0	60	9.3	0.0	6.6
8	Makoka	20.4	30.8	1.9	69	7.6	0.5	4.1
9	Makoka	19.9	28.3	1.3	-	1.1	0.0	3.6
10	Makoka	21.1	31.3	1.2	71	8.9	4.4	4.9
18	Chitedze	18.5	26.9	0.6	79	2.1	3.9	-
19	Chitedze	18.9	29.4	0.6	77	4.8	4.5	-
20	Chitedze	8.5	30.9	0.9	72	8.9	9.1	-
21	Chitedze	18.9	30.9	0.7	68	7.6	8.0	-
22	Chitedze	17.9	27.0	0.5	78	4.0	4.2	-
23	Chitedze	18.3	28.9	0.6	-	8.5	26.9	-
24	Chitedze	18.5	27.2	0.5	79	4.3	0.0	-
25	Chitedze	20.0	27.6	0.7	81	4.5	0.0	-
26	Chitedze	19.3	28.0	0.6	-	4.3	51.2	-
27	Chitedze	17.5	23.5	0.7	88	0.2	3.2	-
28	Chitedze	19.2	26.0	0.5	80	2.9	22.0	-
29	Chitedze	18.1	27.7	0.5	-	2.6	9.1	-
30	Chitedze	19.0	27.9	0.8	83	6.8	0.0	-
31	Chitedze	19.5	28.5	0.9	85	0.3	0.5	-

Min.=Minimum; Max.=Maximum; h=Hours; Evap.=Evaporation

3.2.1 Storage root dry matter content

The cassava storage root dry matter content was determined using the conventional way. Containers (Jumbo bags) were weighed on balance to the nearest 1 mg (w_0). About five undamaged roots were randomly selected. Then the medial sections of the selected roots were shredded into thin slices, mixed thoroughly and a duplicate of 200 g samples for each genotype were weighed to the nearest 1 mg and put in containers (w_1). The samples were placed in an open-top drying container, such as a paper bag, and were dried at 65 °C for 72 hours. The samples were weighed immediately after removal from the oven to the nearest 1 mg (w_2). These steps were carried out within 24 hours after harvest to avoid post harvest changes through physiological deterioration or

moisture loss of the root. Then dry matter content percent (DM %) was calculated as follows: DM % = 100 x (w_2 - w_0) / (w_1 - w_0).

3.2.2 Native starch extraction

The native cassava starch extraction was done using a modification of the method of Numfor and Walter (1996). Fresh roots were washed, peeled and chipped to about 1 cm cubes and were then pulverised in a high speed industrial blender for five minutes. The pulp was suspended in 10x its volume of water, stirred for five minutes and filtered using double cheese (muslin) cloth. The filtrate was allowed to stand for two hours for the starch to sediment and the top liquid was decanted and discarded. The sediment was broken, water added as in the first step, stirred for five minutes and then filtered using double cheese (muslin) cloth. The filtrate was allowed to stand for two hours for the starch to sediment and the top liquid was decanted and discarded. The sediment was washed and dried at 50 °C for 12 hours in an air convection oven.

3.2.3 Ash content

The method for determination of ash content in starch as described by ISO (1997a) was used. Ashing crucibles were carefully cleaned and were heated for half an hour in a furnace at 900 °C. The crucibles were cooled in a desiccator containing a dry dying agent (Silica gel) to room temperature for 30 minutes, and were weighed on a balance to the nearest 0.1 mg (w_0). Starch samples of 5 g were weighed to the nearest 0.1 mg and were put in crucibles (w_1). The samples were incinerated on a Bunsen burner until completely carbonised. Then the incineration was completed in the furnace at 900 °C for five hours. The incinerated samples and their containers were cooled in a desiccator containing dry Silica gel to room temperature for one hour. The samples were weighed immediately after removal from the desiccator to the nearest 0.1 mg (w_2). Ash in percent of sample (average two results) was calculated (ISO, 1997a; ISI, 2002a) as follows: Ash content % = 100 x (w_2 - w_0) / (w_1 - w_0)

3.2.4 pH of starch

The method for pH analysis as described by Anonymous (1999) and ISI (2002c) was used. This method is applicable to native and modified starch, glucose syrups and hydrolysates. Starch samples of 5 g were weighed to the nearest g in a beaker. Twenty millilitres of distilled water was added and stirred for 5 minutes. The starch was allowed to settle and the pH was measured in the water phase.

3.2.5 Moisture content for starch

The method for moisture content determination as described by ISO (1997b) was used. The method is applicable to starch, dextrose - anhydrous and monohydrate - and total sugar. Petri-dishes with lids were washed, air dried overnight and were then further dried in an oven at 105 °C overnight. They were then cooled to room temperature in a desiccator with dry Silica gel for 40 minutes, then were weighed to the nearest 1 mg (w_0). Starch samples of 5 g to the nearest 1 mg were weighed and put in the petri-dishes (w_1). The dishes and samples were dried for 24 hours at 105 °C. Then the dried samples and their containers were cooled down in a desiccator with dry Silica gel for 1 hour and were weighed immediately after removal from the desiccator to the nearest 1 mg (w_2) (ISI, 2002b; ISO, 1997b). Moisture content in percent (MC %) of sample (average results of two samples) was calculated as follows: $MC\ \% = 100-(100 \times (w_2 - w_0) / (w_1 - w_0))$.

3.2.6 Protein content for starch

The starch protein content was analysed using an automatic Protein/Nitrogen Determinator LECO FP-528. Duplicate starch samples of about 3 g were dried in an oven at 105 °C for 72 hours. Then the dried starch was cooled in a desiccator containing dry Silica gel for 1 hour. Samples of 0.30 g were weighed immediately after removal from the desiccator and then were loaded into the protein analyser.

3.2.7 Whiteness of starch

The whiteness of the starch was measured using a HunterLab ColorFlex $45^{o}/0^{o}$ at the Southern African Grain Laboratory, in South Africa. Colour values measured using the ColorFlex are relative to the absolute value of a perfect reflecting diffuser as measured under the same geometric conditions (ASTM Method E 308). The Hunter scale (L) measures lightness and varies from 100 for perfect white to zero for black, approximately as the eye would evaluate it.

3.2.8 Feasibility study on industrial use of native cassava starch in Malawi

The Feasibility study on the industrial use of native cassava starch was included in this study since the industries were hesitant to use cassava. It was important there to find out why it was so and let them also to try and use a real cassava starch produced at Chitedze Research Station.

Industrial use of cassava products was reviewed from the proceedings of the cassava commercialisation symposium (Fungulani and Maseko, 2001; Itaye, 2001; Mataya, 2001; Munthali, 2001). The review also included previous visits to the industries by various stakeholders, and previous studies that were done by different players like the University of Malawi in collaboration with Packaging Industries Malawi Limited (PIM).

The industrial uses of cassava starch was sought from various industries in Malawi by visiting them and holding discussions with the relevant authorities and departments. Trials were also set up with various industries using cassava starch, that is: David Whitehead and Sons in the textile industry, Nzeru radio company in the battery making industry and Malawi Pharmacies Limited (MPL) in the pharmaceutical industry. Cassava starch, which was produced at Chitedze research station, was used for all the trials in the different industries.

3.2.9 Data analysis

A range of statistical analyses were conducted using the Agrobase computer package (Agrobase, 2000) as follows:

3.2.9.1 Analysis of variance

An analysis of variance (ANOVA) was performed on dry matter content, starch extraction rate, protein content, starch moisture content, starch pH and starch ash content of each of the individual trials, using the statistical software computer programme, Agrobase 20 (Agrobase, 2000). Thereafter combined analyses of variance were performed on the pooled data of both trials for Chitedze and Makoka. The genotype by environment interactions for the traits which were significant were managed using different stability methods.

In a conventional cultivar evaluation, trials in which the traits of G genotypes is measured in E locations over R replicates, the classic model to analyse various traits' variation contained in GER observations, is the analysis of variance (Fisher, 1918). After removing the replicate effect when combining the data, the GE observations are partitioned into two sources: (a) additive main effects for genotypes and environments and (b) the non-additive effects due to genotype by environment interaction. The analysis of variance of the combined data expresses the observed (Y_{ij}) mean yield of the i^{th} genotype at the j^{th} environment as:

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

Where μ is the general mean, G_i, E_j and GE_{ij} represent the effect of the genotype, environment and genotype by environment interaction respectively, and e_{ij} is the average of random errors associated with the r^{th} plot that receives the i^{th} genotype in the j^{th} environment. The non-additive interaction (GE_{ij}) implies that an expected value (Y_{ij}) depends not only on the levels of G and E separately, but also on the particular combination of levels G and E (Crossa, 1990).

One of the principal deficiencies of the combined analysis of variance of multilocation trials is that it does not explore the underlying structure within the observed non-additive genotype by environment interaction. Analysis of variance fails to determine the pattern of response of genotypes and environments, in other words the valuable information contained in (G-1)(E-1) degrees of freedom is practically wasted if no further analysis is performed (Crossa, 1990).

A useful aspect of analysis of variance is that variance components related to the different sources of variation, including genotype and GxE interaction, can be estimated. In general, variance component methodology is important in multilocation trials since errors in determining the performance of a genotype arise largely from GxE interaction. Therefore, knowledge of the size of the interaction is required to obtain efficient estimates of genotype effects and determine optimum resource allocations (number of plots and locations to be included in future trials). In a breeding programme, variance component methodology is used to measure genetic variability and to estimate the heritability and predicted gain of the trait under selection. However, the nature and causes of the GxE interaction cannot be established with variance components (Crossa, 1990).

When genotype by environment interactions are present, the effects of genotypes and environments are statistically non-additive, which simply means that differences between genotypes depend on the environment. Existing genotype by environment interactions may, though not necessarily, lead to different rank orders of genotypes in different environments. It is especially crossover or qualitative interactions that are important in agricultural production, in contrast to non-crossover or quantitative interactions (Gail and Simon, 1985; Baker, 1988; Crossa, 1990).

3.2.9.2 Shukla's procedure of stability variance

Shukla's stability variance for each genotype across environments was determined by Agrobase 20 statistical programme (Agrobase, 2000). Shukla (1972) defined the stability variance (σ^2_i) of genotype *i* as its variance across environments after the main effects of environmental means had been removed. Since the genotype main effect is constant, the stability variance is based on the residual ($GE_{ij} + e_{ij}$) matrix. The stability variance (σ^2_i) is estimated as follows:

$$\sigma^{2i} = \frac{1}{(G-1)(G-)(E-1)}[G(G-1)\Sigma_j(X_{ij} - \overline{X}_{i\cdot} - \overline{X}_{\cdot j} + \overline{X}_{\cdot\cdot})^2 - \Sigma_i\Sigma_j(X_{ij} - \overline{X}_{i\cdot} - \overline{X}_{\cdot j} + \overline{X}_{\cdot\cdot})^2]$$

3.2.9.3 Lin and Binn's cultivar superiority measure (P_i)

The data set was analysed according to the procedure recommended by Lin and Binn (1988) where the values estimated are the squares of the differences between an entry (genotype) mean and the maximum genotype's mean at a location, summed and divided by twice the number of locations. The computations were performed with the aid of Agrobase 20 statistical programme (Agrobase, 2000).

3.2.9.4 Wricke's ecovalence

Wricke (1962) defined the concept of ecovalence as the contribution of each genotype to the genotype by environment interaction sum of squares (SS). The ecovalence (Wi) or stability of the i^{th} genotype is its interaction with environments, squared and summed across environments, and expressed as: $W_i = \sum_j (Y_{ij} - \overline{Y}_{i\cdot} - \overline{Y}_{\cdot j} + \overline{Y}_{\cdot\cdot})^2$ where $\overline{Y}_{ij}$ is the mean performance of genotype *i* in the j^{th} environment and $\overline{Y}_i$ and $\overline{Y}_j$ are the genotype and environment mean deviations respectively, and $\overline{Y}_{\cdot\cdot}$ is the overall mean. Accordingly, genotypes with low ecovalence have smaller fluctuations from the mean across different environments and are therefore more stable.

3.2.9.5 Additive Main Effects and Multiplicative Interaction Method (AMMI)

The additive main effects and multiplicative interaction method, widely known as the AMMI model, combines analysis of variance for genotype and environment main effects with principal components analysis of the genotype by environment (GxE) interaction into a unified approach (Gauch, 1988; Zobel *et al.*, 1988), and is especially useful in analysing multi-location trials (Gauch and Zobel, 1988). The AMMI analysis first fits the additive main effects of genotypes and environments by the usual analysis of variance and then describes the non-additive part, the genotype by environment interaction, by principal component analysis (PCA). The results can be graphically represented in an easily interpretable and informative biplot which shows both main effects and GxE interaction. The AMMI model has been used extensively and with success over the past few years to analyse and understand various crop genotype by environment interactions by Smith and Smith (1992); Yau (1995); Steyn

et al. (1993), Crossa *et al.* (1990), and by many others. The AMMI analysis was performed using Agrobase 20 (Agrobase, 2000).

The AMMI model equation is expressed as follows:

$$Y_{ij} = \mu + G_i + E_j + (\sum_{n-i}^{h} k_n V_{ni} S_{nj}) + e_{ij}$$

Where k_n is the singular value for interaction principal component of the n^{th} axis, V_{ni} is the eigenvector of the i^{th} genotype for the n^{th} axis, S_{nj} is eigenvector of the j^{th} environment for the

n^{th} axis and $\sum_{n-1}^{h} V_{ni} = \sum_{n-1} S_{nj} = 1$ (Gauch, 1988; Zobel *et al.*, 1988).

The AMMI model is particularly useful in understanding GxE interactions and summarising patterns and relationships of genotypes and environments (Crossa, 1990; Crossa *et al.*, 1990; Zobel, 1990). In the initial analysis of variance (ANOVA) the total variation is partioned into three orthogonal sources, visably genotypes (G), environments (E) and GxE interactions (Purchase, 1997).

Several authors, including Westcott (1986); Becker and Léon (1988); and Hohls (1995), have questioned whether significant IPCA axes are interpretable in terms of known properties of the genotypes and environments. Gauch and Zobel (1996) state that not only has extensive experience indicated the interpretability of a relatively large IPCA 1, but that from a statistical perspective, significant model parameters indicate that identifiable physical or biological causes are at work. By various means the pattern in AMMI parameters or biplots can usually be interpreted clearly in terms of evident environmental or genetic factors. AMMI results may also illuminate plant physiological processes that cause genotypes to interact with environments, for example the growth period of a variety (Smit and De Beer, 1991).

Another primary use of the AMMI model is to improve the accuracy of data estimates. Gains in accuracy of data estimates are equivalent to increasing the number of replicates by a factor of two to five (Zobel *et al.*, 1988; Crossa *et al.*,

1990), so that AMMI analysis offers a remarkably cost-effective means for improving research efficiency and increasing returns on investment (Gauch and Zobel, 1996).

3.2.9.6 Combined comparison of stability analysis procedures

To statistically compare the four stability analysis procedures used in this study, it was decided to use Spearman's coefficient of rank correlation (Steel and Torrie, 1980). Spearman's coefficient of rank correlation applies to data in the form of ranks. All the cultivars evaluated at both locations were assigned stability values according to the procedure and definition used, which were then ranked in order to determine Spearman's rank correlation coefficient between different procedures (Purchase, 1997).

Assume n genotypes are arranged in the same following order according to two stability parameters, and X_i indicates the ranking order (or ranking number) of the i^{th} genotype for the first parameter, while Y_i indicates the ranking number for the i^{th} genotype of the second parameter, then $d_i = X_i - Y_i$ ($i = 1,2, ..., n$) and Spearman's rank correlation coefficient (r_s) can be described as $r_s = 1 - \frac{6\sum d_i^2}{n(n^2 - 1)}$

Ranking numbers are whole numbers and when two or more equal ranking numbers occur, the average of the ranking numbers that they otherwise would have received, are ascribed to each genotype (Purchase, 1997).

The significance of r_s can be tested by means of Student's *t* test, where

$$t = \frac{r_s\sqrt{n-2}}{\sqrt{1-r_s^2}}$$

with n-2 degrees of freedom.

3.3 Results and discussions

3.3.1 Dry matter content in cassava roots

The analysis of variance (ANOVA) results (Table 3.4) for root dry matter content at each location show that there were statistically significant differences among the genotypes that were evaluated at Makoka (p=0.0144). The genotypes that had the

highest root dry matter content at Makoka were 30786, Silira, Mbundumali and Mkondezi (Table 3.4), and their dry matter was significantly higher than for CH92/112 and 83350. At Chitedze, there were no significant differences in root dry matter among the genotypes. Mbundumali, Silira and TMS60142A had the highest dry matter content and the ones with the least dry matter were CH92/112 and Mk95/054. It has been observed here that Mbundumali and Silira had the highest dry matter at both locations while CH92/112 had the lowest dry matter at both locations (Table 3.4).

The combined analysis of variance results for root dry matter over locations show that there were significant differences in the main effects for root dry matter content at $p=0.0053$ for locations, and $p=0.0296$ for the genotypes, but there was no significant interaction between the genotypes and the locations (Tables 3.4 and 3.5).

Although the locations gave highly significant differences in root dry matter content in cassava ($p<0.01$) its contribution to the variation of the total sum of squares (SS) was only 7.94 % (Table 3.5) as compared to the genotypes which contributed up to 36 % of the total variation. The low contribution of variation of the locations to the total SS, coupled with insignificant interaction of the genotype by the environments, and the main effects for the replicates (blocks) (Table 3.5) suggests that dry matter content is not as much influenced by environment as by genetic differences. These results agree with the suggestion of Pérez *et al.* (2001) that dry matter content in roots of cassava is likely to be controlled by one or a few major genes. However, the results differ with the suggestions of CIAT that the performance of genotypes on root dry matter content strongly depend on edaphic-climatic and agronomic conditions (CIAT, 1995).

The results of the combined ANOVA show that the treatments' main effects for location at Makoka influenced the genotypes to have higher root dry matter content than Chitedze (Table 3.5). The treatments' main effects for genotypes showed that Mbundumali, Silira and TMS60142A gave significantly higher root dry matter content compared to CH92/112 and 83350 (Table 3.5).

The results also suggest that Malawi's cassava genotypes have high root dry matter since the Malawi average dry matter was 42.57 % which was much higher than the conventional 30 % root dry matter which is universally agreed upon as an average root dry matter for cassava.

3.3.2 Starch extraction from cassava roots

The analysis of variance (ANOVA) results (Table 3.4) for starch extraction at each location show that there were no significant differences in starch extraction among the genotypes at both locations.

The combined analysis of variance results for starch extraction over locations show that there were no significant differences in starch extraction among the genotypes, but there were highly significant ($p=0.0001$) differences between the locations. Makoka gave a higher starch extraction rate than Chitedze. These results agree with observations which were made that the cassava roots at Makoka were very mealy as compared to the same genotypes at Chitedze. These differences could be attributed to the differences in the distribution of the rainfall at these two locations (Table 3.1). It is observed that at Makoka the only month when there was no rain was in June but throughout the season in each month there was some rain. Rain at Chitedze stopped in April. There was no rain from May to September. This extended dry weather might have forced the plants at Chitedze to use their food reserves by breaking down some of the starch into sugars for plant use and survival during the dry season.

The locations made the largest contribution to the variation of the total sum of squares (SS) up to 40 % (Table 3.5). These results suggest that when and where cassava is grown and harvested for starch extraction matters if one wants to maximise the starch yield from the tubers. It has been observed from this study that rainfall distribution is one of the major factors that affect starch extraction in cassava roots. If the rainfall is distributed evenly throughout the season, then harvesting of the cassava roots for starch extraction can be done any time during the season. If there is a long dry season then it is suggested that harvesting needs to be done soon after the rainy season or to wait until deep into the next rainy season for the plants to redeposit the starch into the

tuberous roots. As to exactly when the harvesting for starch extraction must be done in areas with an extended dry season is yet to be established.

Table 3.4: Mean root dry matter and starch extraction for 20 Malawi cassava genotypes evaluated at Chitedze and Makoka in 2000/01

	Root dry matter			Starch extraction rate		
Genotype	**Chitedze**	**Makoka**	**Mean**	**Chitedze**	**Makoka**	**Mean**
Silira	46.10	46.85	46.48	30.68	42.88	36.78
CH92/077	39.05	41.25	40.15	43.43	42.49	42.96
CH92/082	42.75	41.53	42.14	39.04	47.35	43.19
TMS4(2)1425	39.23	43.03	41.13	41.27	45.68	43.47
CH92/112	37.63	38.85	38.24	27.35	41.33	34.34
CH92/105	39.75	42.58	41.16	34.87	36.24	35.56
LCN8010	40.40	43.60	42.00	34.01	46.68	40.35
30786	40.55	47.68	44.11	32.54	44.29	38.42
83350	40.95	38.15	39.55	34.72	52.05	43.38
TME1	41.30	45.35	43.33	27.62	43.82	35.72
81/00015	40.05	43.75	41.90	39.33	47.30	43.31
CH92/108	39.83	44.73	42.28	34.06	33.18	33.62
MK95/054	39.00	44.90	41.95	29.26	43.72	36.49
Mbundumali	46.68	46.48	46.58	33.36	40.95	37.16
Gomani	43.10	43.05	43.08	34.11	45.86	39.99
Mkondezi	39.65	46.28	42.96	26.25	41.69	33.97
TMS60121	43.58	43.15	43.36	33.29	45.40	39.34
TMS84563	42.88	41.80	42.34	29.85	45.43	37.64
Maunjili	44.65	43.68	44.16	37.71	40.11	38.91
TMS60142A	44.68	45.40	45.54	38.39	42.24	40.31
Mean	**41.44**	**43.69**	**42.57**	**34.06**	**43.43**	**38.74**
CV (%)	8.73	4.83	6.97	15.16	10.51	12.58
SED for G (±)	3.634	2.108	2.101	5.164	4.565	3.446
SED for L (±)	-	-	0.664	-	-	1.090
LSD for G	NS	7.54	6.97	NS	NS	NS
LSD for L	-	-	2.21	-	-	3.62
Sign. for G	NS	**	*	NS	NS	NS
Sign. for L	-	-	**	-	-	***
Sign. for GxL	-	-	NS	-	-	NS
R-squared	91.86	97.29	94.58	66.83	66.56	79.81
Heritability	84.60	94.60	89.60	40.90	37.90	21.90

***, **, * and NS = Significant at P≤0.001, P≤0.01, P≤0.05 and not significant, respectively; CV=coefficient of variation; SED=standard error deviation; Sign.=probability level of significance; G=genotype; L=location

There was no significant interaction between genotypes and locations in starch extraction (Tables 3.4 and 3.5). This means that the performance of the cassava genotypes for starch extraction did not depend on the locality.

Table 3.5: Analysis of variance (ANOVA) for root dry matter and starch extraction rate

Source of Variation	df	Dry matter (%)		Starch extraction rate (%)	
		Sum of Squares (SS)	Contribution to SS (%)	Sum of Squares (SS)	Contribution to SS (%)
Total	79	970	-	4470	-
Location (L)	1	77 **	7.94	1759 ***	39.35
Rep (location)	2	56	5.77	314	7.02
Genotype (G)	19	344 *	35.46	839	18.77
G x L	19	158	16.29	656	14.68
Error	38	335	34.54	902	20.18

3.3.3 Protein content in cassava starch

The analysis of variance (ANOVA) results (Table 3.6) for starch protein content at each location show that there were highly significant (p=0.0001) differences in starch protein content among the genotypes at both locations. All the cassava genotypes produced starch without any protein just like the corn starch that was obtained from Malawi Pharmacies Limited (MPL) which is being used in the pharmaceutical industry. The highest protein content was recorded in corn flour followed by corn starch which was obtained from Nzeru radio company, then there followed the corn starch which was obtained from Packaging Industries Malawi Limited (PIM) (Table 3.6). The trend was similar at both locations.

The combined analysis of variance results for starch protein content over locations show that there were highly significant differences in the main effects for protein content among the genotypes at p=0.0001, but there were no significant differences in protein content between the locations, and the interaction of the genotype by location (Tables 3.6 and 3.7).

The locations and the interaction of genotype by location contributed nothing to the variation of the total SS while the genotypes contributed 99.99 % of the variation of the total SS. This indicates that it did not matter where the crop was grown; all the cassava genotypes produced native starch without any protein. The corn starch which was obtained from Nzeru radio company gave the highest mean protein content of 0.66 %, followed by the corn starch which was obtained from PIM with a mean protein content of 0.09 %. The corn starch which was obtained from MPL had no

protein, just like the starch from cassava. The corn flour gave the highest mean protein content of 6.21 % (Table 3.6).

The lower the protein content in the starch, the higher the quality of that starch. The industries do not like starch with a high protein content, so much so that different industries have set different maximum protein content in starch but all are lower than 1.0 %. Thus, National Starch and Chemical Company, a member of the ICI group, and Nzeru radio company, recommends a maximum of 0.50 %, while African products Pty Limited and MPL recommends a maximum of 0.35 %. The results confirm that maize flour is not suitable for industrial use based on its protein content although its look and fineness was very similar to starch. The results also suggest that native cassava starch gave high quality starch in terms of protein content irrespective of which cassava genotype was used. The native cassava starch was comparable to corn starch which is used in the pharmaceutical industry.

3.3.4 Whiteness of starch

The results of the ANOVA (Table 3.6) for the white colour of starch at each location show that there were statistically significant differences among the genotypes at p=0.0156 for Chitedze and p=0.0005 for Makoka. The genotypes that produced extra white native starch at Chitedze were Gomani, Mbundumali, 83350, CH92/077, TMS60142A and Maunjili but there were no significant differences in whiteness with the rest of the genotypes except for CH92/112 (Table 3.6). The colour of the corn starch which was obtained from Nzeru radio company was statistically similar to the starch produced from any cassava genotype. The starch which was obtained from PIM was statistically different from only starch produced from CH92/112. The corn starch from MPL was the whitest, however, its whiteness was only statistically different from the native cassava starch from CH92/082, 81/00015, TME1, Mk95/054, Mkondezi, Silira, CH92/92/105, LCN8010, and CH92/112, while the rest of the cassava genotypes were statistically similar to corn starch from MPL (Table 3.6).

The genotypes that produced extra white native cassava starch at Makoka were Gomani, Silira and CH92/077 and they were significantly different from LCN8010, TMS84563, and 30786, but there were no significant differences in the whiteness of

native starch produced from the rest of the cassava genotypes (Table 3.6). The colour of corn starch from Nzeru radio company was comparable to the native cassava starch despite the genotype from which it was produced. The corn starch from PIM was whiter than the cassava starch from 30786. The corn starch from MPL was again the whitest, but it was significantly different from the starch from most of the cassava genotypes, except CH92/082, 81/00015, MK95/054, LCN8010, TMS84563 and 30786 (Table 3.6).

The combined ANOVA for white colour of starch over locations show that there were significant differences in the main effects ($p=0.0381$) between the locations, and among the genotypes ($p=0.0001$, Tables 3.6 and 3.8), but the interaction of genotype by location was not significant. The genotypes contributed the greatest amount of variation to the total SS (72.40 %) as compared to locations which contributed only 2.6 %, and the GxE interaction contributed 12.50 %. Although the genotype by location (GxE) interaction was not significant, it still contributed a much higher amount of variation to the total SS than locations (Table 3.7).

The combined ANOVA over locations shows that the most extra white starch was the corn starch from MPL, but there were no significant differences with the starch from Gomani and CH92/077 (Table 3.6). Corn starch from Nzeru radio company was not different from the native cassava starch extracted from the rest of the cultivars except from the ones extracted from Gomani and CH92/077 which were significantly superior to the corn starch from Nzeru radio company in terms of whiteness (Table 3.6). Generally, Chitedze produced whiter starch than Makoka (Table 3.6). Whiteness is normally a function of handling during the starch extraction process. The colour of the cassava root pulp and the presence or absence of root defects like root rots and/ or cassava brown streak disease (CBSD) affect cassava tuberous roots.

Table 3.6: Protein content and whiteness for starch from 20 Malawi cassava genotypes

	Starch protein content			Whiteness of the starch		
Genotype	**Chitedze**	**Makoka**	**Mean**	**Chitedze**	**Makoka**	**Mean**
Silira	0.00	0.00	0.00	94.75	95.80	95.27
CH92/077	0.00	0.00	0.00	96.02	95.71	95.86
CH92/082	0.00	0.00	0.00	94.94	94.17	94.55
TMS4(2)1425	0.00	0.00	0.00	95.36	95.20	95.28
CH92/112	0.00	0.00	0.00	93.49	94.40	93.94
CH92/105	0.00	0.00	0.00	94.56	95.14	94.85
LCN8010	0.00	0.00	0.00	94.63	93.72	94.17
30786	0.00	0.00	0.00	94.94	93.20	94.07
83350	0.00	0.00	0.00	96.11	95.40	95.75
TME1	0.00	0.00	0.00	94.51	94.46	94.49
81/00015	0.00	0.00	0.00	94.84	94.08	94.46
CH92/108	0.00	0.00	0.00	95.42	95.45	95.43
MK95/054	0.00	0.00	0.00	93.77	93.81	93.79
Mbundumali	0.00	0.00	0.00	96.28	94.87	95.57
Gomani	0.00	0.00	0.00	96.33	96.82	96.57
Mkondezi	0.00	0.00	0.00	94.81	94.18	94.50
TMS60121	0.00	0.00	0.00	95.38	95.20	95.29
TMS84563	0.00	0.00	0.00	95.22	93.45	94.33
Maunjili	0.00	0.00	0.00	95.48	94.95	95.22
TMS60142A	0.00	0.00	0.00	95.64	94.65	95.14
Nzeru corn starch	0.67	0.65	0.66	95.20	95.20	95.20
PIM corn starch	0.09	0.10	0.09	96.46	96.46	96.46
MPL corn starch	0.00	0.00	0.00	97.28	97.28	97.28
Corn flour	6.22	6.20	6.21	94.20	94.21	94.20
Mean	**0.29**	**0.28**	**0.29**	**95.33**	**94.91**	**95.12**
CV (%)	5.63	2.76	4.99	0.83	0.74	0.79
SED for G (±)	0.016	0.008	0.009	0.788	0.706	0.529
SED for L (±)	-	-	0.003	-	-	0.153
LSD for G	0.04	0.02	0.02	1.97	1.76	1.28
LSD for L	-	-	NS	-	-	0.37
Sign. for G	***	***	***	*	***	***
Sign. for L	-	-	NS	-	-	*
Sign. for G x L	-	-	NS	-	-	NS
R-squared	99.99	100.00	99.99	74.00	81.86	78.66
Heritability	100.00	100.00	100.00	60.00	76.60	82.80

***, * and NS = Significant at P≤0.001, P≤0.05 and not significant, respectively; CV=coefficient of variation; SED=standard error deviation; Sign.=probability level of significance; G=genotype; L=location; MPL=Malawi pharmacies limited; PIM= Packaging industries Malawi limited; Nzeru=Nzeru radio company.

Table 3.7: Analysis of variance (ANOVA) for starch protein content and whiteness

Source of Variation	df	Protein content (%)		Whiteness (L)	
		Sum of Squares (SS)	Contribution to SS (%)	Sum of Squares (SS)	Contribution to SS (%)
Total	95	147.848	-	120.7	-
Location (L)	1	0.000	0.00	2.6 *	2.15
Rep (location)	2	0.000	0.00	7.5	6.21
Genotype (G)	23	147.839 ***	99.99	72.4 ***	58.88
G x L	23	0.001	0.00	12.5	10.36
Error	46	0.008	0.01	25.8	21.30

In general, the starches which were tested for white colour were very white with only minor differences. This is so because the standard method which is being used to differentiate the colour of wheat flour (Maartin colour grader) could not pick up the differences in the whiteness of the starch. Hence, a more sensitive method was applied called HunterLab ColorFlex by using a HunterLab ColorFlex $45^{o}/0^{o}$ which managed to identify the minute differences in starch whiteness which have been presented and discussed above. Thus, the colour of all the starches which were evaluated were acceptable in terms of whiteness, and that is why the Maartin colour grader could not differentiate between them.

The colour of starch is very important when it comes to some industrial uses like pharmaceutical and textile industries where the colour of the final product matters. Hence, there is a need to use white starch so that the final product has no discolouration or spots of off colour, as it affects the quality of the product for consumers. PIM and Nzeru radio company indicated that the colour of the starch does not matter since the quality of their final product does not depend on the original colour of the starch but the physical/chemical properties of the starch. This study therefore showed that cassava can be grown anywhere and any genotype can be used to produce white starch acceptable to all industries.

3.3.5 pH of starch

The analysis of variance results (Table 3.8) for pH of starch at each location show that there were highly significant (p=0.0001) differences among the genotypes at both locations. The cassava genotypes that produced starch with the highest pH at Chitedze

were CH92/108, Silira, CH92/082 and TME1, while the lowest pH was detected in corn starch from Nzeru radio company and MPL (Table 3.8). The cassava genotypes that produced starch with the highest pH at Makoka were CH92/108, Silira Gomani and MK95/054, while the corn starch from Nzeru radio company and MPL gave the lowest pH (Table 3.8).

The pH for native starch ranged from 4.7 to 5.8 at both sites. This range was within the recommended range of between 4.5 and 7.0 for National starch and chemical company, and between 4.5 and 6.0 for Nzeru radio company limited. What was surprising was that the pH for corn starch from MPL have the lowest. MPL starch was leading in terms of whiteness and protein content compared to other corn starch from Nzeru radio company and PIM. However the MPL starch caused no problem in the pharmaceutical uses (Table 3.8).

The combined analysis of variance results for pH of starch over locations show that there were highly significant differences ($p=0.0001$) in the main effects (locations and genotypes) and their interaction (Tables 3.8 and 3.9). The locations contributed 13.51 % while genotypes contributed the bulk of the variation of the total SS by 74.82 %, while the interaction of the GxE (genotype by location) contributed 11.66 % (Table 3.9).

The combined analysis over locations show that the cassava genotypes which gave high mean pH values were CH92/108, Silira MK95/054, Gomani and CH92/082 (Table 3.8). The starch which gave the lowest pH values was corn starch from MPL. The pH value for MPL starch was 4.1 which was lower than the minimum recommended value of 4.5 (Table 3.8).

Makoka gave a higher mean pH value of 5.4 for starch than Chitedze which had a mean pH of 5.0 (Table 3.8). Although the difference of these sites were statistically significant from each other, both sites gave starch pH values which were within the recommended values of between 4.5 and 7.0 (African products limited, 2001; National starch and chemicals company, 2002).

Since the interaction between genotypes and locations were highly significant, it was difficult to recommend which genotypes were suitable at which location using main effect means. Therefore it was necessary to conduct stability analyses to determine which genotypes were stable. If genotypes were unstable, then it was wise to determine under which environments they were suitable in terms of pH for their starch. The summary of the four stability parameters for pH of starch are given in Table 3.10.

3.3.5.1 Lin and Binn's cultivar superiority measure (P_i) on pH of starch

According to the Lin and Binn's (1988) cultivar performance measure (P_i) as a stability statistic the genotypes 83350, CH92/108, TMS4(2)1425, LCN8010, TME1 and CH92/112 were the most stable clones while CH92/077, CH92/082 and Mkondezi were unstable (Table 3.10).

3.3.5.2 Wricke's ecovalence (Wi) on pH of starch

Wricke's ecovalence value of stability for each of the 20 cassava genotypes evaluated over the two locations were generated and the ranks were given accordingly as presented in Table 3.10. According to Wi-ecovalence, the most stable genotypes in terms of pH of starch extracted from those genotypes were Silira, CH92/112, CH92/105, Maunjili, Mkondezi, TMS84563 and TMS60142A. The most unstable genotypes were Gomani, Mbundumali, 83350, CH92/082, TMS4(2)1425, 30786, TME1 and TMS60121 (Table 3.10).

3.3.5.3 Shukla's procedure of stability variance – no covariate for pH of starch

In Table 3.10, Shukla's (1972) stability variance (σ^2_i) values, as well as the ranking order of the genotypes' stability are given. According to the stability variance – no covariate, the cassava genotypes which were stable were Mkondezi, Maunjili Silira, CH92/112, CH92/105 TMS60121, TMS60142A and CH92/077, while Gomani, Mbundumali and 83350 were the most unstable genotypes (Table 3.10).

Table 3.8: pH, moisture content and ash content for starch from 20 Malawi cassava genotypes evaluated at Chitedze and Makoka in 2000/01 season

	pH for the starch			Starch moisture content			Starch ash content		
Genotype	Chitedze	Makoka	Mean	Chitedze	Makoka	Mean	Chitedze	Makoka	Mean
Silira	5.5	5.5	5.5	13.10	14.20	13.65	0.13	0.11	0.12
CH92/077	5.2	5.1	5.2	12.60	13.70	13.15	0.09	0.11	0.10
CH92/082	5.4	5.5	5.5	12.20	14.30	13.25	0.09	0.15	0.12
TMS4(2)1425	5.0	5.3	5.2	11.20	14.20	12.70	0.22	0.13	0.17
CH92/112	5.1	5.5	5.3	10.30	13.70	12.00	0.15	0.13	0.14
CH92/105	4.8	5.5	5.2	11.40	12.30	11.85	0.13	0.13	0.13
LCN8010	5.1	5.7	5.4	11.80	13.40	12.60	0.20	0.14	0.15
30786	5.2	5.6	5.4	11.80	13.50	12.45	0.10	0.16	0.13
83350	5.2	5.1	5.2	12.50	12.90	12.70	0.25	0.15	0.20
TME1	5.3	5.3	5.3	11.60	13.70	12.65	0.20	0.11	0.15
81/00015	5.1	5.6	5.4	11.80	14.00	12.90	0.13	0.11	0.12
CH92/108	5.5	6.2	5.9	12.20	14.00	13.10	0.22	0.13	0.17
MK95/054	5.0	6.0	5.5	12.70	12.90	12.80	0.11	0.16	0.13
Mbundumali	4.8	5.7	5.3	12.60	13.10	12.85	0.12	0.19	0.15
Gomani	5.1	5.9	5.5	12.80	12.60	12.70	0.09	0.19	0.14
Mkondezi	4.9	5.7	5.3	12.60	13.20	12.90	0.12	0.09	0.11
TMS60121	5.0	5.8	5.4	12.00	13.70	12.85	0.18	0.09	0.13
TMS84563	4.7	5.8	5.3	12.10	12.00	12.05	0.12	0.13	0.12
Maunjili	4.9	5.1	5.0	12.80	13.90	13.35	0.13	0.10	0.11
TMS60142A	4.9	5.7	5.3	14.00	12.70	13.35	0.14	0.09	0.11
Nzeru corn starch	4.7	4.7	4.7	11.20	11.20	11.20	0.15	0.14	0.14
PIM corn starch	5.1	5.0	5.0	11.80	11.80	11.80	0.18	0.13	0.15
MPL corn starch	4.1	4.1	4.1	9.90	9.90	9.90	0.11	0.14	0.12
Corn flour	3.4	3.4	3.4	10.60	10.60	10.60	0.86	0.70	0.78
Mean	**5.0**	**5.4**	**5.2**	**11.97**	**12.98**	**12.47**	**0.17**	**0.15**	**0.16**
CV (%)	0.19	0.15	0.17	5.96	4.54	5.25	11.40	9.36	12.04
SED for G (±)	0.010	0.008	0.006	0.714	0.589	0.463	0.020	0.014	0.014
SED for L (±)	-	-	0.002	-	-	0.134	-	-	0.004
LSD for G	0.02	0.02	0.02	1.78	1.47	1.12	0.05	0.04	0.03
LSD for L	-	-	0.01	-	-	0.32	-	-	0.01
Sign. for G	***	***	***	**	***	***	***	***	***
Sign. for L	-	-	***	-	-	***	-	-	***
Sign. for GxL	-	-	***	-	-	***	-	-	***
R-squared	99.98	99.99	99.99	77.27	88.87	86.68	99.17	99.30	99.00
Heritability	100.00	100.00	84.40	70.60	87.40	59.95	99.20	99.30	94.30

***, **, * and NS = Significant at P≤0.001, P≤0.01, P≤0.05 and not significant, respectively; CV=coefficient of variation; SED=standard error deviation; Sign.=probability level of significance; G=genotype; L=location; MPL=Malawi pharmacies limited; PIM= Packaging industries Malawi limited; Nzeru=Nzeru radio company.

Table 3.9: Analysis of variance (ANOVA) for pH, moisture and ash content for 20 Malawi cassava genotypes evaluated at Chitedze and Makoka in 2000/01 season

Source of Variation	df	pH for starch		Moisture content (%)		Ash content (%)	
		Sum of Squares (SS)	Contribution to SS (%)	Sum of Squares (SS)	Contribution to SS (%)	Sum of Squares (SS)	Contribution to SS (%)
Total	95	30.227	-	147.85	-	1.752	-
Location (L)	1	4.084 ***	13.51	24.60 ***	16.64	0.011 ***	0.61
Rep (location)	2	0.001	0.00	0.52	0.35	0.014	0.79
Genotype (G)	23	22.615 ***	74.82	73.32 ***	49.59	1.617 ***	92.29
G x L	23	3.523 ***	11.66	29.71 ***	20.10	0.093 ***	5.30
Error	46	0.004	0.01	19.70	13.32	0.018	1.01

3.3.5.4 Additive main effects and multiplicative interaction (AMMI) for pH of starch

The AMMI analysis for cassava starch pH revealed that IPCA 1 explained 99.99 % of the GxE interaction. The IPCA scores of a genotype by environment interaction in the AMMI analysis are an indication of the stability of a genotype over environments. The greater the IPCA scores, either negative or positive as it is a relative value, the more specifically adapted a genotype is to certain environments. The more the IPCA scores approximate to zero (0), the more stable the genotype is over all environments sampled. Thus, using AMMI IPCA scores, the most stable genotypes were 81/00015, 30786, CH92/112, LCN6010 and CH92/108 (Table 3.10) while the most unstable genotypes were TMS84563, CH92/077, 83350 and MK95/054.

3.3.5.5 Overall ranking of genotypes for pH of starch

The overall ranking of the genotypes for stability using the four stability parameters with equal weighting show that the overall most stable genotypes for pH of starch were CH92/112, 81/00015, CH92/077, CH92/082, MK95/054, Gomani and Mbundumali (Table 3.10).

Table 3.10: Summary of stability statistics for pH in starch from 20 Malawi cassava genotypes tested at Chitedze and Makoka in 2000/01 season

Genotype	Cultivar superiority		Wi-Ecovalence		Stability variance-no covariate.		AMMI		Overall Rank
	Pi GxE Stat.	Rank	Wi GxE Stat.	Rank	δ^2_i GxE Stat.	Rank	IPCA scores	Rank	
Silira	0.0051	9	0.0000	1	-0.0001	2	-0.3806	13	5
CH92/077	0.0082	17	0.0013	6	0.0008	5	-0.4566	16	13
CH92/082	0.0068	16	0.0058	10	0.0042	9	-0.3162	11	14
TMS(2)1425	0.0011	2	0.0058	10	0.0042	9	-0.1601	6	6
CH92/112	0.0035	5	0.0000	1	-0.0001	2	-0.0821	3	1
CH92/105	0.0044	8	0.0003	3	0.0001	2	0.1617	7	3
LCN8010	0.0013	3	0.0019	7	0.0013	6	0.0954	4	3
30786	0.0058	13	0.0058	10	0.0042	9	-0.0743	2	9
83350	0.0004	1	0.0070	11	0.0051	10	-0.4508	15	11
TME1	0.0022	4	0.0058	10	0.0042	9	-0.3806	13	10
81/00015	0.0052	10	0.0000	1	-0.0001	2	0.0096	1	2
CH92/108	0.0011	2	0.0051	9	0.0036	8	0.1461	5	4
MK95/054	0.0053	11	0.0047	8	0.0034	7	0.3938	14	12
Mbundumali	0.0042	7	0.0070	11	0.0051	10	0.3216	12	12
Gomani	0.0064	14	0.0129	12	0.0094	11	0.2300	9	14
Mkondezi	0.0066	15	0.0002	2	0.0000	1	0.2378	10	7
TMS60121	0.0037	6	0.0058	10	0.0042	9	0.2300	9	9
TMS84563	0.0052	10	0.0008	4	0.0005	3	0.4660	17	9
Maunjili	0.0058	13	0.0002	2	0.0000	1	-0.2284	8	4
TMS60142A	0.0055	12	0.0010	5	0.0006	4	0.2378	10	8

3.3.5.6 Comparison of analyses

Since the ranking of the genotypes varied between stability parameters, it was difficult to choose which stability parameter to consider. Spearman's coefficient of rank correlation was then determined for each of the possible pairwise comparisons of the different stability analysis procedures for the respective traits with significant GxE interaction like pH of starch (Table 3.10). Subsequently Student's *t* test was performed to determine the significance of Spearman's rank correlation coefficients and the results are indicated in Table 13. The results show that Wi-ecovalence was significantly (p=0.0001) and positively correlated to stability variance – no covariate. The other stability parameters were not correlated hence each one stood on its own (Table 3.11), and could not displace the other.

The overall rank was not correlated to cultivar superiority measure, but there were significant correlations with Wi-ecovalence, stability variance – no covariate and AMMI. The stability parameter which was closest to the overall rank was Wi-ecovalence, followed by AMMI and the stability variance – no covariate, came third (Table 3.11).

Table 3.11 Spearman's coefficients of rank correlation for four GxE stability analysis procedures conducted for pH of starch from 20 cassava genotypes evaluated over two locations in Malawi

Stability Parameter	Cultivar superiority measure (Pi)	Wi-Ecovalence	Stability variance – no covariate	AMMI
	-0.2275			
Wi-Ecovalence	0.3639			
Stability variance	-0.2700	0.9866		
– no covariate	0.2785	0.0000 ***		
	0.1975	0.1640	0.1149	
AMMI	0.4321	0.5155	0.6499	
	0.3990	0.6795	0.6469	0.6600
Overall Rank	0.1010	0.0019 **	0.0039 *	0.0029 *

The genotypes which were stable suggest that they were universal, thus their pH were not dependent on environment while those which were unstable means that they were specifically adapted to certain environments. Hence there was need to associate the unstable genotypes with their suitable environments. This was only achieved using AMMI. The AMMI biplot (Figure 3.1) shows that TMS84563, CH92/077, 83350 and MK95/054 were the unstable genotypes which needed to be fitted into their appropriate environments. The results presented in Figure 3.1 also show that MK95/054 and TMS84563 were best suited to Makoka while CH92/077 and 83350 were best suited to Chitedze.

The results show that in terms of pH for starch any one of the three methods, thus Wi-ecovalence, stability variance or AMMI can be used for stability analysis. The selection of the parameter depends on ease of calculation, application and diversity of its use. The application of AMMI is recommended since it provides additional

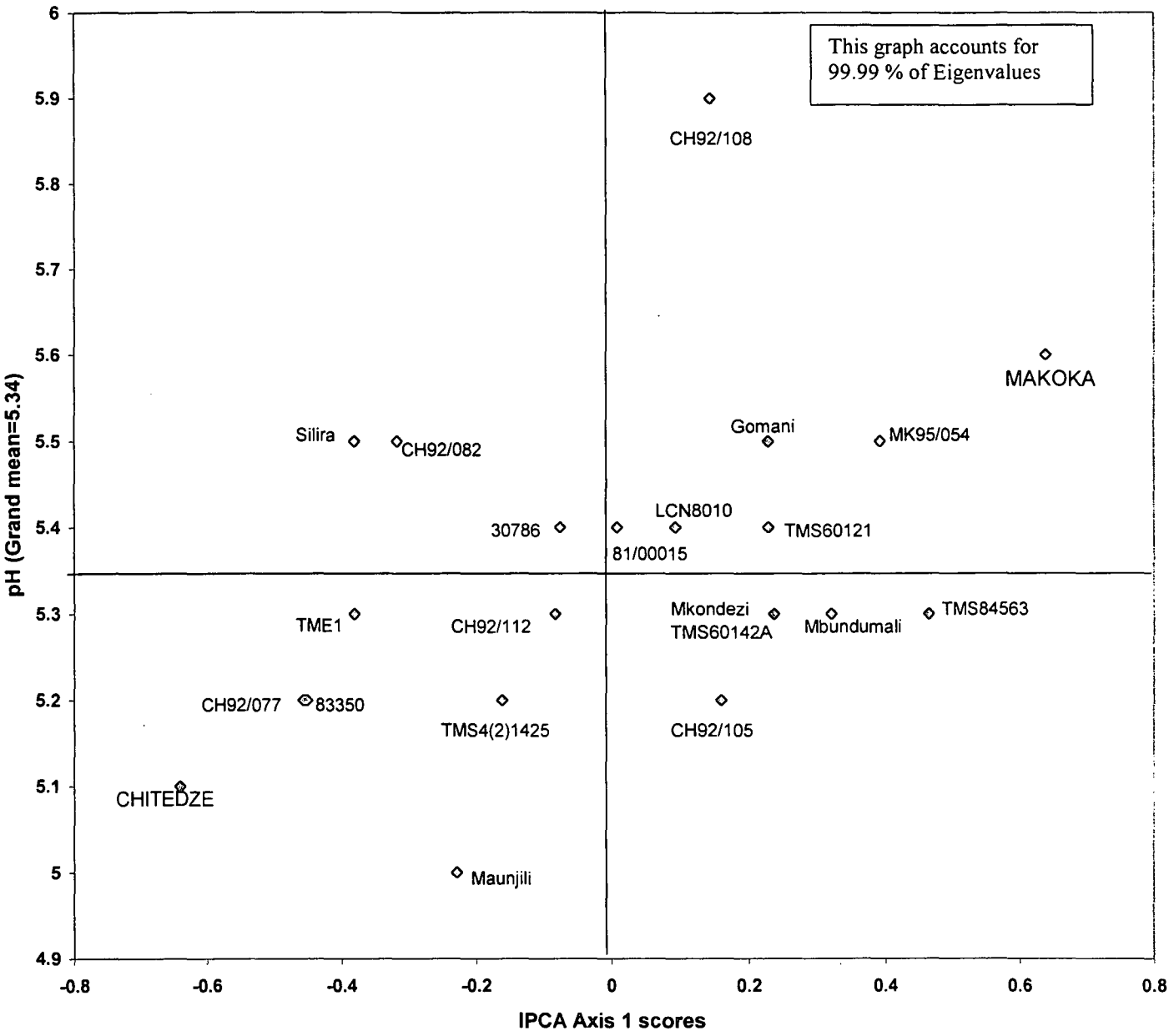

Figure 3.1: Biplot for AMMI IPCA 1 scores against mean pH for 20 Malawi cassava genotypes evaluated at Chitedze and Makoka in 2000/01 season

3.3.6 Moisture content in starch

The analysis of variance results (Table 3.8) for moisture content of starch at each location show that there were highly significant differences among the genotypes at p=0.0024 for Chitedze, and p=0.0001 for Makoka. The cassava genotypes that produced starch with the highest starch moisture content at Chitedze were TMS60142A, Gomani, and Maunjili, while the lowest starch moisture content was detected in corn starch from MPL and cassava starch from CH92/112 (Table 3.8). The cassava genotypes that produced starch with the highest moisture content at Makoka

were CH92/082, Silira, TMS4(2)1425, CH92/108, 81/00015 and Maunjili. Again, MPL corn starch gave the lowest moisture content (Table 3.8).

The range of moisture content for native cassava starch at both sites ranged from 10.3 to 14.3 % which was below the recommended maximum of 15 % for MPL, and only CH92/082 surpassed by the recommended maximum of 14 % for National starch and chemical company, and Nzeru radio company limited by only 0.3 % (Table 3.8).

The combined analysis of variance results for moisture content of starch over locations show that there were highly significant differences (p=0.0001) in the main effects (locations and genotypes) and for their interaction (p=0.0007) (Tables 3.8 and 3.9). The locations contributed 16.64 %, the genotypes contributed the bulk of the variation of the total SS by 49.59 %, while the interaction of the GxE (genotype by location) contributed 20.10 % (Table 3.9).

The combined analysis over locations shows that the cassava genotypes which gave high mean moisture content were Silira, Maunjili, TMS60142A, CH92/082, CH92/077 and CH92/108 (Table 3.8). The starch which gave the lowest moisture content was corn starch from MPL (Table 3.8). The mean moisture content for starch were all below the recommended maximum of 15 or 14 % depending on the industry.

Makoka gave a higher mean moisture content of 12.47 % for starch than Chitedze which had a mean moisture content of 11.97 % (Table 3.8). Although these sites were significantly different from each other, both sites gave a starch moisture content which was below the recommended maximum values of 14 or 15 % (National starch and chemicals company, 2002).

Since the interaction between genotypes and locations were highly significant, it was difficult to recommend which genotypes were suitable at which location using only the main effect means. Therefore, it was necessary to conduct stability analyses to determine which genotypes were stable and which ones were unstable. For those genotypes which were unstable it was wise to determine under which environments they were suitable in terms of moisture content for their starch. The summary of the four stability parameters for moisture content of starch is given in Table 3.12.

3.3.6.1 Lin and Binn's cultivar superiority measure (P_i) on moisture content in starch

According to the Lin and Binn's (1988) cultivar performance measure (P_i) as a stability statistic, the genotypes Silira, Maunjili, CH92/077, TMS60142A and Mkondezi were the most stable clones, while CH92/112, CH92/105, TMS84563 and TMS4(2)1425 were unstable (Table 3.12).

3.3.6.2 Wricke's ecovalence (Wi) on moisture content in starch

Wricke's ecovalence value of stability for each of the 20 cassava genotypes evaluated over the two locations were generated, and the ranks were given accordingly as outlined in Table 3.12. According to Wi-ecovalence, the most stable genotypes in terms of moisture content of starch extracted from those genotypes were Silira, CH92/0772, CH92/105, LCN8010, TMS60121 and TMS60142A. The most unstable genotypes were Gomani, TMS84563, TMS4(2)1425, and MK95/054 (Table 3.12).

3.3.6.3 Shukla's procedure of stability variance for moisture content in starch

In Table 3.12, Shukla's (1972) stability variance (σ^2_i) values, as well as the ranking order of the genotypes' stabilities are given. According to the stability variance – no covariate, the cassava genotypes which were stable were Maunjili Silira, CH92/105, LCN8010, TMS60121, CH92/108 and CH92/077, while TMS60142A, CH92/112, TMS4(2)1425 and Gomani were the most unstable genotypes (Table 3.12).

3.3.6.4 Additive main effects and multiplicative interaction (AMMI) for moisture content in starch

The AMMI analysis for cassava starch moisture content revealed that IPCA 1 explained 99.99 % of the GxE interaction. The IPCA scores of a genotype by environment interaction in the AMMI analysis are an indication of the stability of a genotype over environments. Using AMMI IPCA scores, the most stable genotypes were Silira, CH92/077, Maunjili, CH92/105, LCN6010, TMS60121 and CH92/108 (Table 3.12), while the most unstable genotypes were TMS60142A, CH92/112, TMS4(2)1425 and Gomani.

3.3.6.5 Overall ranking of genotypes for moisture content in starch

The overall ranking of the genotypes for stability using the four stability parameters with equal weighting showed that the overall most stable genotypes for moisture content of starch were Silira, Maunjili, CH92/077, LCN8010, CH92/105 and TMS60121 (Table 3.12), while the most unstable genotypes were TMS4(2)1425, CH92/112 and TMS84563.

3.3.6.6 Comparison of analyses

Spearman's coefficient of rank correlation was determined for each of the possible pairwise comparisons of the different stability analysis procedures for traits with significant GxE interaction (Table 3.12). Subsequently Student's *t* test was performed to determine the significance of Spearman's rank correlation coefficients. The results for moisture content of starch are given in Table 3.13. The results show that Wi-ecovalence was significantly (p=0.0001) and positively correlated to stability variance – no covariate, and AMMI. AMMI was also significantly and positively correlated (p=0.0001) with stability variance – no covariate. Cultivar superiority measure was only correlated to Wi-ecovalence at 5 % alpha level (Table 3.13).

The overall rank was highly significantly correlated to all the stability parameters hence, any one of them could be used to test the stability of the genotypes for moisture content (Table 3.13).

The genotypes which were stable suggest that they were universal, thus their moisture content was not dependent on environment while those which were unstable means that they were specifically adapted to certain environments. The unstable genotypes were associated with their suitable environments using AMMI. The AMMI biplot (Figure 3.2) shows that TMS60142A, CH92/112, TMS4(2)1425, TMS84563 and Gomani were the unstable genotypes which needed to be fitted into their appropriate environments. The results presented in Figure 3.2 also show that TMS4(2)1425 and CH92/112 were best suited to Makoka, while TMS60142A, TMS84563 and Gomani were best suited to Chitedze.

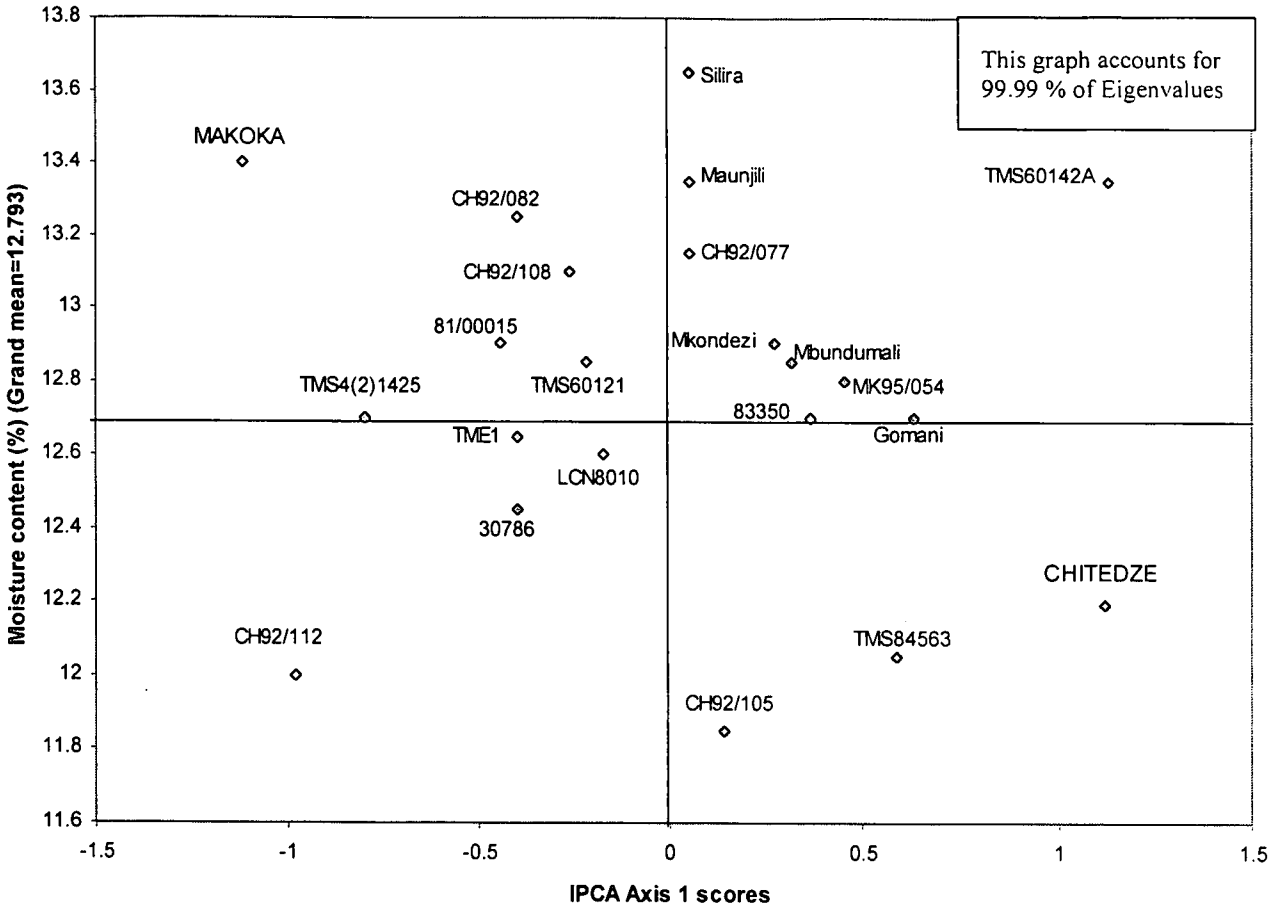

Figure 3.2: Biplot for AMMI IPCA 1 scores against mean moisture content for 20 Malawi cassava genotypes evaluated at to locations in 2000/01 season

The results show that in terms of moisture content for starch any one of the four stability parameters can be used for stability analysis for moisture content in starch for the cassava genotypes. AMMI is generally used since it provides additional information on the allocation of unstable genotypes into their appropriate environments.

Table 3.12: Summary of stability statistics for moisture content of starch from 20 Malawi cassava genotypes, tested at Chitedze and Makoka in 2000/01 season

Genotype	Cultivar superiority		Wi-Ecovalence		Stability variance-no Covariate		AMMI		Overall Rank
	Pi GxE Stat.	Rank	Wi GxE Stat.	Rank	δ^2_i GxE Stat.	Rank	IPCA scores	Rank	
Silira	0.2050	1	0.0066	1	-0.0385	2	0.0515	1	1
CH92/077	0.5800	3	0.0066	1	-0.0385	2	0.0515	1	3
CH92/082	0.8100	6	0.3916	11	0.5319	9	-0.3966	9	8
TMS4(2)1425	1.9625	16	1.5931	16	2.3119	14	-0.8000	14	16
CH92/112	3.5125	19	2.3871	10	3.4881	15	-0.9793	15	15
CH92/105	2.6900	18	0.0496	2	0.0252	1	0.1412	2	5
LCN8010	1.4125	13	0.0741	3	0.0615	3	-0.1725	3	4
30786	1.8500	15	0.3916	11	0.5319	9	-0.3966	9	12
83350	1.0525	10	0.3321	9	0.4437	8	0.3653	8	9
TME1	1.5300	14	0.3916	11	0.5319	9	-0.3966	9	11
81/00015	1.2325	12	0.4851	12	0.6704	10	-0.4415	10	12
CH92/108	0.8325	7	0.1711	6	0.2052	5	-0.2622	5	5
MK95/054	0.9125	9	0.5151	13	0.7148	11	0.4549	11	12
Mbundumali	0.8500	8	0.2556	8	0.3304	7	0.3204	7	7
Gomani	1.0825	10	1.0011	15	1.4348	13	0.6342	13	13
Mkondezi	0.7925	5	0.1891	7	0.2319	6	0.2756	6	6
TMS60121	1.0900	11	0.1176	4	0.1259	4	-0.2174	4	5
TMS84563	2.2250	17	0.8646	14	1.2326	12	0.5894	12	14
Maunjili	0.4000	2	0.0066	1	-0.0385	2	0.0515	1	2
TMS60142A	0.6400	4	3.1626	5	4.6370	16	1.1272	16	10

Table 3.13 Spearman's coefficients of rank correlation for four GxE stability analysis procedures conducted for moisture content for starch from 20 cassava genotypes evaluated over two sites in Malawi

Stability Parameters	Cultivar superiority measure (Pi)	Wi-Ecovalence	Stability variance – no covariate	AMMI
	0.4999			
Wi-Ecovalence	0.0346 *			
Stability variance – no covariate	0.3586 0.1439	0.7892 0.0001 ***		
	0.4118	0.7995	0.9960	
AMMI	0.0895	0.0001 ***	0.0000 ***	
	0.6477	0.9101	0.9054	0.9176
Overall Rank	0.0037 **	0.0000 ***	0.0000 ***	0.0000 ***

3.3.7 Ash content in starch

The analysis of variance results (Table 3.8) for ash content of starch at each location show that there were highly significant (p=0.0001) differences among the genotypes at both locations. The cassava genotypes that produced starch with the highest ash content at Chitedze were 83350, TMS4(2)1425, CH92/108, TME1, LCN 8010 and TMS60121, while the lowest ash content was found for Gomani, CH92/082 and CH92/077 (Table 3.8). The cassava genotypes that produced starch with the highest ash content at Makoka were Gomani, Mbundumali, MK95/054, 30786, CH92/082 and 83350, while TMS60142A, Mkondezi and TMS60121 gave the lowest ash content (Table 3.8).

The range of ash content for native cassava starch at both sites ranged from 0.09 to 0.25 % which was much lower than the recommended maximum of 0.5 % for National starch and chemical company, and Nzeru Radio Company Limited. Thus, there were significant differences in ash content at each location (Table 3.8).

The combined analysis of variance results for ash content of starch over locations show that there were highly significant differences (p=0.0001) in the main effects (locations and genotypes) and their interaction (Tables 3.8 and 3.9). The locations contributed only 0.61 % while genotypes contributed the bulk of the variation of the total SS by 92.29 %, while the interaction of the GxE (genotype by location) contributed 5.30 % (Table 3.9).

The combined analysis over locations show that the cassava genotypes which gave high mean ash content were 83350, CH92/108, TMS4(2)1425, TME1 and Mbundumali (Table 3.8). The starch which gave the lowest ash content were from MPL, 81/00015, TMS60142A, Maunjili, Mkondezi and CH92/077. The ash content of starch from cassava genotypes were not different from the corn starch obtained from various industries (Table 3.8).

Chitedze gave higher mean ash content of 0.17 % for starch than Makoka which had a mean ash content of 0.15 % (Table 3.8). Although statistically these sites were significantly different from each other, both sites gave starch ash content which was

lower than the recommended maximum of 0.5 % (National starch and chemicals company, 2002).

Stability analyses were conducted to determine which genotypes were stable since the GxE interaction was highly significant. The unstable genotypes associated with their suitable environments. The summary of the four stability parameters for ash content of starch is given in Table 3.14.

3.3.7.1 Lin and Binn's cultivar performance measure (P_i) on ash content in starch

According to the Lin and Binn's (1988) cultivar performance measure (P_i) as a stability statistic the genotypes CH92/108, Gomani, MK95/054, LCN8010 and TMS60121 were the most stable ones, while CH92/077, Maunjili, 83350, TMS4(2)1425 and CH92/105 were unstable (Table 3.14).

3.3.7.2 Wricke's ecovalence (Wi) on ash content in starch

Wricke's ecovalence value of stability for each of the 20 cassava genotypes evaluated over the two locations were generated and the ranks were given as shown in Table 3.9. According to Wi-ecovalence, the most stable genotypes in terms of ash content of starch extracted from those genotypes were 81/00015, CH92/112, 30786, LCN8010 and CH92/108. The most unstable genotypes were CH92/077, TMS84563, 83350 and MK95/054 (Table 3.14).

3.3.7.3 Shukla's procedure of stability variance for ash content in starch

In Table 3.9, Shukla's (1972) stability variance (σ^2_i) values, as well as the ranking order of the genotypes' stability are given. According to the stability variance – no covariate the cassava genotypes which were stable were 30786, CH92/112, 81/00015, LCN8010 and CH92/108, while TMS84563, 83350, CH92/077 and MK95/054 were the most unstable genotypes (Table 3.14).

3.3.7.4 Additive main effects and multiplicative interaction (AMMI) for ash content in starch

The AMMI analysis for cassava starch ash content revealed that IPCA 1 explained 99.99 % of the GxE interaction. Using AMMI IPCA scores, the most stable genotypes were Silira, 81/00015, CH92/112, Mkondezi, Maunjili and CH92/105 (Table 3.14), with the most unstable genotypes being Gomani and Mbundumali.

3.3.7.5 Overall ranking of genotypes for ash content in starch

The overall ranking of the genotypes for stability using the four stability parameters with equal weighting show that the overall most stable genotypes for ash content of starch were 81/00015, CH92/112, LCN8010, 30786, CH92/108, Silira, Gomani and Mkondezi (Table 3.14). The most unstable genotypes were CH92/077, TME1 and TMS84563.

3.3.7.6 Comparison of analyses

Spearman's coefficient of rank correlation was conducted for each of the possible pair-wise comparisons of the different stability analysis procedures for the respective traits with significant GxE interaction like ash content of starch (Table 3.14). This was done since the ranking of the genotypes varied from one stability parameter to the other and it was difficult to choose which stability parameter to consider. Subsequently Student's *t* test was performed to determine the significance of Spearman's rank correlation coefficients and the results for ash content of starch are indicated in Table 3.15. The results show that Wi-ecovalence was significantly ($p=0.0001$) and positively correlated to stability variance – no covariate. The other stability parameters were not correlated hence each one stood on its own (Table 3.15) and could not displace the other.

The overall rank was not correlated to cultivar superiority measure at 5 % alpha level of significance, but were significantly correlated at 99.99 % with Wi-ecovalence and stability variance – no covariate. With respect to ash content, AMMI was not correlated to the overall rank stability. The stability parameter that was closest to the

overall rank was Wi-ecovalence, followed by stability variance – covariate (Table 3.15).

The genotypes which were stable suggest that they were universal, thus their starch pH were not dependent on environment while those which were unstable means that they are specifically adapted to certain environments. Hence, the need to associate the unstable genotypes with their suitable environments. This was achieved only using AMMI. The AMMI biplot (Figure 3.3) shows that Gomani, Mbundumali CH92/082, MK95/054, 83350, CH92/108, TME1, TMS4(2)1425 and TMS60121 were the unstable genotypes which needed to be fitted into their appropriate environments. The results presented in Figure 3.3 show that Gomani, Mbundumali, 30786, CH92/082 and MK95/054 were best suited to Makoka while 83350, CH92/108, TME1, TMS4(2)1425 and TMS60121 were best suited to Chitedze.

The results show that in terms of ash content for starch, either Wi-ecovalence or stability variance – no covariate can be used for stability analysis. The selection of the parameter depends on ease of calculation, application and diversity of its use.

Table 3.14: Summary of stability statistics for ash content in starch from 20 Malawi cassava genotypes, which were tested at Chitedze and Makoka in 2000/01 season

Genotype	Cultivar superiority		Wi-Ecovalence		Stability variance-no covariate		AMMI		Overall Rank
	Pi GxE Stat.	Rank	Wi GxE Stat.	Rank	δ^2_i GxE Stat.	Rank	IPCA scores	Rank	
Silira	0.1200	7	0.2379	13	0.1710	14	0.0072	1	5
CH92/077	0.3222	19	0.3425	16	0.2485	16	-0.0701	8	16
CH92/082	0.1254	8	0.1642	11	0.1164	12	-0.1474	12	11
TMS4(2)1425	0.2616	17	0.0421	6	0.0260	7	0.1474	12	10
CH92/112	0.1599	12	0.0111	3	0.0030	2	0.0073	2	2
CH92/105	0.2451	16	0.0430	7	0.0266	8	-0.0314	5	6
LCN8010	0.1018	4	0.0150	4	0.0058	4	0.0846	9	3
30786	0.1152	6	0.0091	2	0.0015	1	-0.1474	12	3
83350	0.3182	18	0.3338	15	0.2420	6	0.1619	13	13
TME1	0.2110	15	0.2379	13	0.1710	14	0.1474	12	15
81/00015	0.1313	9	0.0002	1	-0.0051	3	0.0121	3	1
CH92/108	0.0000	1	0.0351	5	0.0207	5	0.1377	11	4
MK95/054	0.0770	3	0.2548	14	0.1835	15	-0.1329	10	10
Mbundumali	0.1893	13	0.1700	12	0.1206	13	-0.1619	13	12
Gomani	0.0640	2	0.0869	9	0.0591	10	-0.2199	14	5
Mkondezi	0.1537	10	0.0929	10	0.0635	11	0.0266	4	5
TMS60121	0.1044	5	0.0869	9	0.0591	10	0.1474	12	6
TMS84563	0.2040	14	0.3567	16	0.2590	17	-0.0556	6	14
Maunjili	0.3901	20	0.0857	8	0.0582	6	0.0266	4	7
TMS60142A	0.1578	11	0.0929	10	0.0635	11	0.0604	7	8

Table 3.15: Spearman's coefficients of rank correlation for four G x E stability analysis procedures conducted for ash content for starch from 20 cassava genotypes evaluated over two sites in Malawi

Stability Parameter	Cultivar superiority measure (Pi)	Wi-Ecovalence	Stability variance – no covariate	AMMI
	0.3125			
Wi-Ecovalence	0.2068			
Stability variance	0.1313	0.8804		
– no covariate	0.6035	0.0000 ***		
	-0.2004	0.1603	0.0664	
AMMI	0.4254	0.5251	0.7935	
	0.5495	0.8396	0.7214	0.3825
Overall Rank	0.0182 *	0.0000 ***	0.0007 ***	0.1172

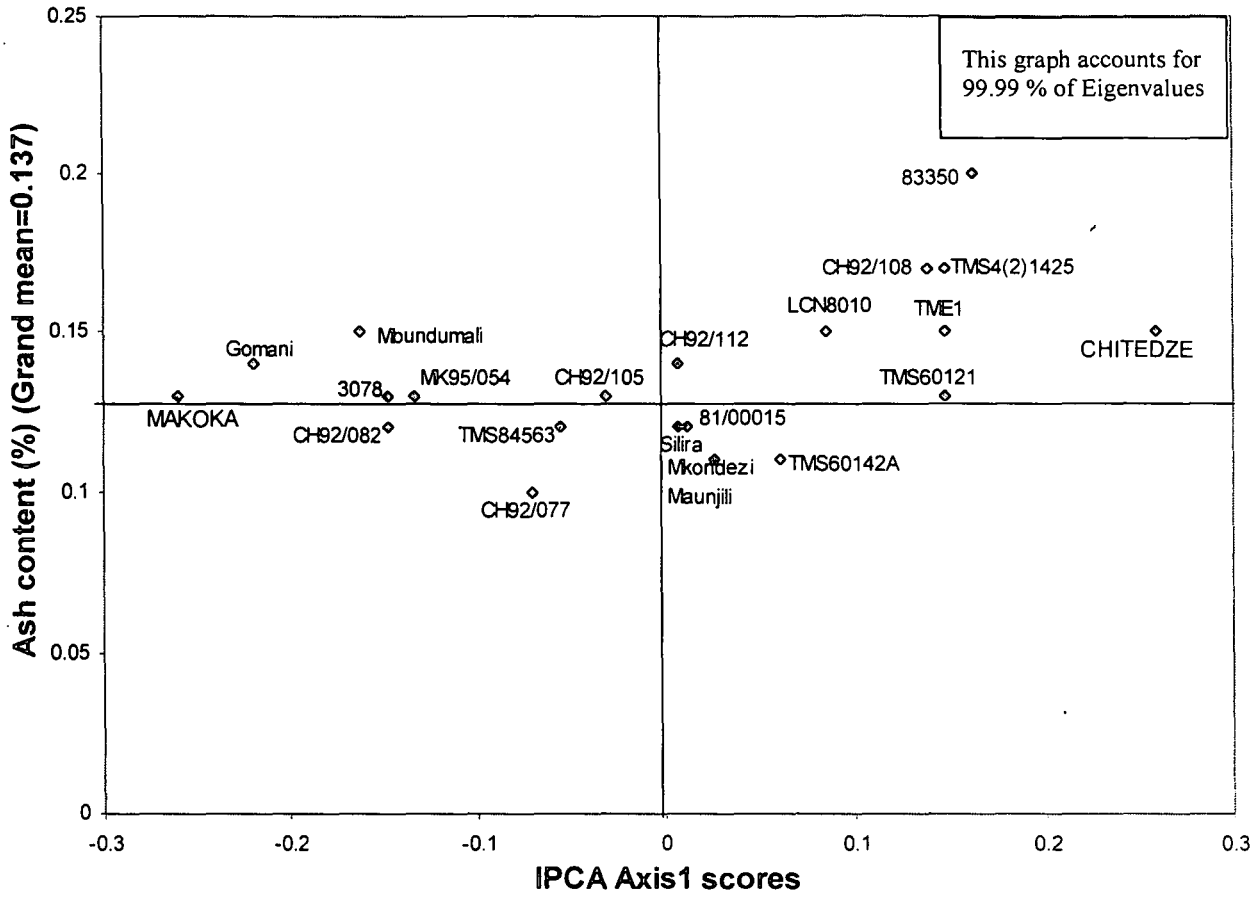

Figure 3.3: Biplot for AMMI IPCA 1 scores against mean ash content for 20 Malawi cassava genotypes evaluated at Chitedze and Makoka in 2000/01 season

3.3.8 Industrial use of native cassava starch in Malawi

3.3.8.1 David Whitehead and Sons Limited

David Whitehead and Sons Limited, which is a textile manufacturing company, revealed that they normally use maize starch. The maize starch is imported, but most of the time they face a problem of foreign exchange, as a result they started looking for alternatives. The company switched to a powder of so called cassava starch obtained from local traders. However, operating at 430 metric tons per annum, the company is facing problems with the quality of the product. The low quality product which is not cassava starch, gives under-sized warp yarns and also causes clogging on the machines when weaving the cloth (Fungulani and Maseko, 2001). After thorough discussion with the company and seeing the product it was discovered that it was

cassava flour and not cassava starch as the traders claimed. Then a study was initiated and 50 kg of cassava starch, which was produced at Chitedze research station was given to them to try in cloth manufacturing. They ran a batch and the results were very good (Fungulani and Maseko, 2001) in the sense that it produced the right size of warp yarns, no clogging was experienced on the machine, and the cloth was of high quality (it was smooth and pure white).

Some of the traders who were delivering cassava flour to David Whitehead and Sons Limited in the name of cassava starch were consulted and trained on how to extract starch from cassava roots. As to whether they will start producing real cassava starch is something that needs follow up and monitoring.

3.3.8.2 Packaging Industries (Malawi) Limited (PIM)

Packaging Industries Malawi Limited (PIM) is one of the listed companies on the Malawi stock exchange. It produces a range of corrugated cartons used for packaging of different products for local distribution and exports. Major users of the cartons are soap, oil, confectionery, textile and garment manufacturing industries, and tobacco processing firms (Itaye, 2001).

In the production process, starch is mixed with other chemicals to produce glue for binding lines and fluting. The company uses about 500 metric tons of starch per annum of which all is imported. Although currently only maize starch is used, cassava has in the past been used. PIM, however, discontinued the use of cassava starch in its manufacturing process in 1966 due to the fact that local producers of this type of starch were not able to consistently supply the company in accordance with its required quality standards (Itaye, 2001).

Due to high landed cost of the imported starch, and that the company needs to hold huge stocks of starch to ensure availability of the product, it makes the whole exercise expensive. PIM has been looking for alternative sources of supply. It is for this reason that PIM has since 1997 collaborated with the University of Malawi to assess the possibility of use of cassava starch. When the properly extracted cassava starch was used the results were successful (Itaye, 2001; Peter Chingo and Yoweli Kawiya,

personal communication). It is also observed here that properly extracted cassava starch works in packaging industries but the issue is monitoring of the quality of cassava starch being delivered to industries. Hence, the involvement of Malawi Bureau of Standards (MBS) in the cassava commercialisation especially in the starch production is evident.

3.3.8.3 Nzeru Radio Company Limited

Starch is an important component in the manufacturing of batteries (dry cells) amongst the other components that are used. Starch helps in the retention of water which facilitates electrical flow in the battery, prevents leakage of fluid components, and ensures portability of the battery (Munthali, 2001).

Nzeru Radio Company was visited and discussions were held with relevant authorities. It was pointed out that corn starch is imported and used. The requirement is about 18 metric tons per annum. It was also indicated that the starch which is required in the battery making industry needs to be: low in iron; have a maximum protein content of 0.50 %; have a maximum ash content of 0.50 %; maximum moisture content of 14 %; maximum acid insolubles of 0.10 %; and should have a pH within the range of between 4.5 and 6.0. It was also agreed that 5.0 kg of cassava starch extracted at Chitedze research station be delivered for trial in battery making. The cassava starch was delivered and was tested in their laboratory. It was reported that the cassava starch passed all the tests they conducted on it. The paste made using cassava starch passed the tests for pH, viscosity and specific gravity. That is why they proceeded with the make of the batteries. The batteries were successfully made on 30th January 2002. No problem was experienced in the course of making the batteries, and the paste was just like corn starch in terms of fluidity and viscosity. The batteries were tested after one month and they met the requirements. The minimum period of observation is up to six months after production. If starch of poor quality is used, the Zinc cane gets corroded within a short period of time and/ or cause leaking of batteries. After a period of 10 months, the cells that were made using cassava starch were still fine and the Zinc canes (the negative part of the battery) were intact, that is, not eaten away or corroded.

However, it is required to try to make the batteries on the machine to ascertain that no clogging occurs on the machine. Clogging is not expected because the quality of the paste was similar to the one which is being used in the machine. In the further studies, 25 kg of cassava starch will be needed to run a batch on the machine.

3.3.8.4 Malawi Pharmacies Limited (MPL)

Malawi Pharmacies Limited (MPL) were visited and discussions were held with the relevant authorities. It was indicated that good starch in the pharmaceutical industry should especially be free of pathogenic bacteria like *E. coli,* salmonella, heavy metals, and foreign particles. The particle size of the starch should be 23 μm or less and the moisture content should be less than 15 %.

Maize starch is being used which is imported and normally 1.0 metric ton of starch is used per annum. The high landing cost of starch makes it necessary to look for alternatives. The suggestion of use of cassava starch was welcomed but trials had to be conducted. It was agreed that cassava starch amounting to 5.0 kg be brought for testing. The cassava starch passed all the tests hence they proceeded to make the tablets. The compression for making the tablets was similar to the ones made from corn flour. The disintegration of the tablets was also tested. Normally, tablets need to disintegrate within 4-15 minutes up to six months after production. The ones made from cassava starch passed initial disintegration tests but after one month the tablets did not disintegrate. The quality assurance manager (Mr. Manjolo) made the tablets without consulting the specialist (Mr. A. Chigombe). When discussing the results, the specialist indicated that he was satisfied with the results since lack of disintegration of the tablets after one month was not a problem. Starch works as a binder as well as a disintegrator. If the starch is wet it works as a binder but when dried it works as a disintegrator. The quality assurance manager accepted that the starch which he used for coating the tablets was not further dried. The specialist emphasised that this happens to any type of starch if the starch to be used as a disintegrator is not properly dried. However, further trials were recommended to coat the tablets with properly dried cassava starch. The tablets also passed the friability test, where 10 tablets are put in a machine and the machine is run for five minutes. The loss in weight should not

exceed 2 %. From the discussion it was concluded that use of cassava starch in tablet making was possible.

3.3.8.5 Malawi Bureau of Standards (MBS)

The policing body for quality control of products and services in Malawi by the name of Malawi bureau of standards (MBS) was also visited and discussions were held with the relevant authorities. It was found that MBS had neither the international nor national standards for starch. It was agreed that they were going to source international standards and try to come up with national standards for starch. MBS was finalising on the national standards for cassava flour after which national standards for starch were going to be developed. With that effect, a sample of cassava starch amounting to 5.0 kg, which was produced at Chitedze research station was delivered to assist in the development of standards.

MBS needs to be committed in the quality control of cassava starch to be taken up by industries for use in their industries without hesitation.

3.4 Conclusions and recommendations

The genotypic influence on root dry matter is much greater than for the environment. This agrees with the hypothesis that one or a few major genes control root dry matter in cassava.

Rainfall distribution affected the starch extraction from cassava roots. The more evenly the rain is distributed throughout the season, the wider the harvesting time for roots to achieve high starch extraction rate. However, work needs to be done to establish when exactly harvesting for starch should be done to maximise starch extraction.

All the cassava genotypes produced starch which had no protein, and their ash and moisture contents were much lower than the recommended maximums. Hence, cassava starch was of high quality in terms of protein, ash and moisture content. The whiteness and pH of cassava starch was within the recommended range.

The four stability parameters which were applied for starch moisture and ash content, and pH were compared using Spearman's coefficient of rank correlation.

The results showed that in terms of pH, Wi-ecovalence was significantly correlated to stability variance – no covariate. The overall rank was not correlated to cultivar superiority measure, but there were significant correlations with Wi-ecovalence, stability variance – no covariate and AMMI. The stability parameter which was closest to the overall rank was Wi-ecovalence, followed by AMMI and lastly stability variance – no covariate. The application of AMMI is recommended since it provides additional information on the allocation of unstable genotypes into their appropriate environments. The results show that MK95/054 and TMS84563 were best suited to Makoka, while CH92/077 and 83350 were best suited to Chitedze.

The results showed that in terms of moisture content, Wi-ecovalence was correlated to stability variance – no covariate, and AMMI. AMMI was also correlated with stability variance – no covariate. Cultivar superiority measure was correlated with Wi-ecovalence. The overall rank was highly significantly correlated to all the stability parameters hence, any one of them could be used to test the stability of the genotypes for moisture content. AMMI is generally used since it provides additional information on the allocation of unstable genotypes into their appropriate environments. The results show that in terms of moisture content, TMS4(2)1425 and CH92/112 were best suited to Makoka, while TMS60142A, TMS84563 and Gomani were best suited to Chitedze.

The results showed that in terms of ash content, Wi-ecovalence was correlated to stability variance – no covariate. The other stability parameters were not correlated hence each one stood on its own and could not displace the other. The overall rank was not correlated to cultivar superiority measure, but were correlated to Wi-ecovalence and stability variance – no covariate. With respect to ash content AMMI was not correlated with the overall rank stability. The stability parameter that was closest to the overall rank was Wi-ecovalence, followed by stability variance – no covariate. AMMI was used to allocate the unstable genotypes to their appropriate environments. The results show that Gomani, Mbundumali, 30786, CH92/082 and

MK95/054 were best suited to Makoka, while 83350, CH92/108, TME1, TMS4(2)1425 and TMS60121 were best suited to Chitedze.

It has been established that the use of cassava starch in packaging, pharmaceutical and textile industries, and in battery manufacturing were successful. Industries are now looking forward to reputable local suppliers of cassava starch. This study has therefore dispelled fears that cassava starch is of low quality and that it does not work in some industries. Hence, there is need for strict quality control for cassava starch so that industries receive what they pay for. The fact that cassava starch found in Africa is of low quality is not due to genotypic attributes as revealed by this study but due to poor processing of cassava in Africa.

MBS needs to move forward with speed to develop national standards for starch. This will help them to enforce the quality control for any starch produced as per requirements of the Malawi industries.

CHAPTER 4

GENETIC DISTANCE ANALYSIS OF ELITE CASSAVA (*Manihot esculenta* CRANTZ) GENOTYPES FROM MALAWI

4.1 Introduction

The starchy roots of cassava have become the most important source of dietary energy in Sub-Saharan Africa (FAO, 2000; Scott *et al.*, 2000). This is due to its high and stable yield (Akoroda, 1995), especially in areas with arable land shortage and declining soil fertility (Romanoff and Lynam, 1992). Other advantages of cassava is its flexibility in planting and harvesting time, its suitability in intercropping, and that it is vegetatively propagated. Most small scale farmers grow a number of cultivars, each with locally preferred characteristics such as "good" taste, early maturing or "good" processing characteristics (Salick *et al.*, 1997; Chiwona-Karltun *et al.*, 2000; Mkumbira *et al.*, 2001). Cassava is grown in almost all areas, and it is the most important root crop in Malawi. It is grown for food, as cash crop and for industrial uses.

Ethnobotanists have suggested that the diversity of cassava is very high, particularly in the Amazon basin and it is classified by the Indians according to different varietal names (Second *et al.*, 1997). For example, Chernela (1987) found that the Tukano use 137 different names and Kerr (1987) reported the use of 40 varietal names in Desana. They have also suggested that dynamic management of cassava diversity involves the exchange or gifts of cuttings among Indian tribes, over different geographic areas, as well as the integration of varieties, vegetatively propagated from seed. Emperaire *et al.* (1997) observed that a single "roca" (traditional field) included 26 groups of plants with different varietal names (Second *et al.*, 1997). Chiwona-Karltun *et al.* (1998) also reported that African small scale farmers mainly acquire new cultivars from their neighbours or by collecting seedlings volunteers from sexually propagated cassava found in fields left fallow for several years. Sauti *et al.* (1998) also reported that about 400 cultivars of cassava are available in Malawi. A study in one area of northern Malawi showed that farmers have descriptive names for up to 50 cultivars just in one village (Chiwona-Karltun *et al.*, 2000). The number of plants of each cultivar differs considerably and changes over time. Local cultivar names denote phenotypic

attributes, place of origin, the person that introduced it or that it originates from a volunteer seedling (Chiwona-Karltun *et al.,* 1998). The performance of a cultivar within a local environment and farming system determines whether it will be adopted and continue to be cultivated. There is evidence from several parts of Africa that very few cassava cultivars actually originate from formal breeding programmes (Nweke *et al.*, 1994; Spencer, 1994; Chiwona-Karltun *et al.*, 1998).

Breeding research and the management of comprehensive germplasm collections for cassava in Africa is internationally co-ordinated by the International Institute for Tropical Agriculture (IITA). This institute has developed improved clones, which are high yielding, of high quality, and resistant to pests and diseases. The Malawi National Root Crops Program, in collaboration with SARRNET (Southern Africa Root Crops Research Network), carries out the development and distribution of improved lines in Malawi. Breeding and selection trials using seedling and clonal lines from local and introduced material are aimed at improving productivity and root characteristics, resistance to pests and diseases, and adaptability to environmental stresses in various ecological zones. Participatory approaches with reference to variety selection of cassava and identification of research targets are being used in Malawi (Thro *et al.,* 1996; Benesi *et al.,* 1999; 2000).

Relying exclusively only on a few improved cultivars would necessarily narrow the genetic basis and result in genetic erosion of cassava. This was reported in West and Central Africa where two clones (TMS30572 and TMS4(2)1425), had been successfully adopted (Mignouna and Dixon, 1997). It has been observed that farmers in Malawi continually replace and update varieties. This was evident during a participatory approach in on-farm trials especially in the Karonga district, in the north of Malawi. When farmers come across an ideal clone, it quickly replaces the local cultivars (Benesi *et al.,* 1999; Moyo *et al.*, 1999). Hence, in addition to *in vitro* conservation facilities like germplasm collections, on-farm conservation by small-scale farmers plays a crucial role in maintaining a broad genetic variability of cassava (Brush, 1995).

The estimation of the diversity of plant genetic resources 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) (Zacarias, 1997).

A prerequisite for the genetic improvement of cassava, is a knowledge of the extent of genetic variation (Beeching *et al.*, 1993). The starting point for genetic improvement on cassava in Malawi, is the working collection, which includes the recommended local varieties, locally bred and introduced varieties, as well as local elite and introduced clones. Hence, the objectives of this study are: (1) To determine the genetic diversity of the elite cassava genotypes in the Malawi working collection; and (2) To compare the use of morphological and molecular data in characterising cassava genotypes.

4.2 Materials and methods

The recommended varieties (Mbundumali, Gomani, Maunjili, Silira and Mkondezi), locally bred clones (CH92/112, CH92/105, CH92/082, and CH92/077, and introduced clones (TMS4(2)1425, TME1, 30786, TMS60121, 83350, LCN8010 and TMS60142A) were evaluated at Chitedze and Makoka research stations.

Trials were planted in November 2000 and were harvested in December 2001. The 2000/01 season's rainfall for these stations is presented in Table 3.1. The general climatic, soil and altitude for these sites are presented in Table 3.2, while the locations of the research stations (sites) and agro-ecological zones are presented in Figure 2.4.

A randomised complete block design was used with four replicates. The plot sizes were four ridges, each with 12 plants (gross) and a net of two inner ridges each with 10 middle plants. The ridges were 0. 9 m apart and the plants were also 0.9 m apart along the ridge. The length of the cassava planting stakes were 25 cm long and were planted in a slanting position at approximately 60°.

4.2.1 Morphological characterisation

Morphological characterisation was conducted at harvesting using the modified IBPGR descriptors (Nweke *et al.*, 1994; Mahungu and Kanju, 1997; IITA, 1998) (Appendix 1). The morphological data was converted into a binary matrix. Different ways were employed to code the data into binary form.

Traits with only two categories of description: number of leaf lobes, root constrictions and root texture.

The traits that had only two categories of description were scored normally in the binary matrix. The number of leaf lobes were scored as (0) for 5 and (1) for 7. Root constriction is classified as absent (0) or present (1). Root texture was scored as smooth (1) and rough (0) (Appendix 2).

Traits with more than two categories of description concerning colour, shape and position

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

Hairiness of apical unexpanded leaves

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

Branching levels

Branching level is classified as 1=(non-branching) (for those genotypes which do not branch), 2=(few branches) (for those genotypes with branches between one and three) and 3=(profuse branching) (for those genotypes with four branches and more). The mean for the number of branching levels over 20 plants in each plot, and across replicates and sites were calculated, and fitted into their respective groups as described above. If a genotype's mean number of branching levels was two, it was scored as one against a column for few branches, and zero for none and zero for profuse (Appendix 2).

Branching and plant heights

Branching height and plant height followed a similar pattern of coding for binary matrix but had different classes. Branching height is classified as low (for those plants whose first branches were 50 cm high and below), medium (for those plants whose first branches were between 50 cm and 100 cm high), and high (for those plants whose heights of first branches were over 100 cm). The mean for branching height over 20 plants in each plot, and across replicates and sites was calculated, and then fitted into their respective groups as low, medium or high. If a genotype's mean branching height was 60 cm, then it was scored as one against a column for medium, and zero for low and zero for high (Appendix 2).

Plant height is classified as short (for those plant whose mean height was 100 cm and below), medium (for those plant whose mean height was between 100 cm and 200 cm), and tall (for those plants whose mean height was over 200 cm). The mean for branching height over 20 plants in each plot, and across replicates and sites was calculated, and fitted into their respective groups as short, medium or tall. If a genotype's mean branching height was 60 cm, then it was scored as one against a column for medium, and zero for short and tall (Appendix 2).

All the traits recorded according to the descriptor list (Appendix 1) were converted to binary data to give a true description of that genotype, whether the traits were variable (quantitative) or constant (quantitative).

The columns which contained monomorphic data was disregarded and only columns with polymorphic data was used in the cluster analysis and dendrogramme construction. Genetic distance between individual genotypes was calculated with the Euclidean distance using the formula: $E^2_{ij} = \sum_k (x_{ki} - x_{kj})^2$, where k is the number of bands and x_{ki} and x_{kj} are the frequencies of occurrence of 0 and 1 of the k^{th} band in an individual i and j, respectively. The morphological data was transformed into a binary matrix and analysed using the Number Cruncher Statistical System (NCSS 2000) (Hintze, 1998). Dendrogrammes were constructed using the Unweighted Pair Group Method of Arithmetic Averages (UPGMA) in NCSS 2000.

4.2.2 Plant material

DNA was extracted from fresh leaves of clean cassava cuttings that were morphologically described at Makoka and Chitedze, and collected and planted in an isolated field at Chitedze.

4.2.3 DNA extraction and quantification

A method of Edwards *et al.* (1991) was used in the DNA extraction from cassava with minor modifications. Fresh young leaves were collected and kept on ice. A well-chilled mortar and pestle was used to grind the plant material to a fine powder in the presence of liquid Nitrogen. The ground powder was transferred into a 50 ml centrifuge tube and 10 ml of extraction-buffer, preheated to 65°C added, as well as 1 ml of CTAB-buffer and 2 ml 5M NaCl. The homogenate was vortexed and incubated in a water bath at 65°C for 1 hour, inverting it every 10 to 20 minutes. Ten millilitres of chloroform-isoamylalcohol (24:1) was added to the homogenate and mixed, followed by centrifugation for 15 minutes at 10000 rpm at room temperature. Chloroform extractions were repeated until the interface was visibly clear. Absolute cold ethanol in a 1:2 ratio was added, and left overnight at 4 °C to precipitate the DNA. The DNA was spooled with a glass Pasteur-pipette, washed in 70 % ethanol three times and dissolved in 250 µl sterile distilled water.

DNA concentration and purity determination, as well as the rest of the molecular genetic analysis was done at the University of the Free State, South Africa. Purity and concentrations of DNA was determined as follows:

$$\text{Purity} = \frac{\text{260nm reading}}{\text{280nm reading}}$$

DNA concentration = optical density x dilution x constant = µg/ml]

4.2.4 AFLP

Visualisation of genomic DNA on a 1 % agarose gel was used to confirm the integrity of the genomic DNA in TAE buffer for 90 minutes at 80 volts. DNA was visualised using ethidium bromide under UV light.

AFLP was performed according to Vos *et al.* (1995) using EcoRI and MseI restriction enzymes (GIBCO BRL products). Genomic DNA (250 ng) was digested in 25 µl containing 5 µl reaction containing: 5x reaction buffer, 23 µl sterile distilled water and 2 µl of EcoRI/MseI (2 units) enzyme. The reaction mixture was incubated at 37 °C for two hours, followed by incubation at 70 °C for 15 minutes to inactivate the restriction endonucleases.

Ligation of adaptors

Adaptor ligation buffer, (24 µl) and 1 µl T4 DNA ligase (1 unit/µl) was added to the digested DNA and incubated at 20 °C for two hours. After incubation, the ligation mixture was diluted 1:10 in TE buffer (10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA).

Pre-Selective amplification reactions

Pre-selective amplification reactions were performed in 25 µl reactions containing: 5 µl of diluted (1:10) DNA for each genotype, 40 µl of pre-amp primer mix, 5 µl of 10x PCR buffer with Mg^{+2} (200 mM Tris-HCL (pH 8.4), 15 mM MgCl, 500 mM KCl) and 0.2 µl (1 unit/µl) Taq DNA polymerase. PCR was performed for 20 cycles at 94 °C for 30 seconds, 56 °C for 60 seconds and 72 °C for 2 minutes. After the PCR reaction, 12 µl of the pre-selective amplification was visualised using a 1 % agarose gel run at 85 volts for 90 minutes. The DNA was visualised using ethidium bromide under UV light. The

pre-selective amplified DNA was diluted 1:50 in TE buffer (10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA).

Selective amplification reactions

Primers (MseI-CAA, MseI-CAT, MseI-CTG, MseI-CTA, MseI-CAG, MseI-CTC, EcoRI-ACA, EcoRI-AAC, EcoRI-ACT and EcoRI-ACC) were screened in all possible combinations.

PCR reactions were performed in 20 µl reactions containing: 7.4 µl sterile distilled water, 2 µl 10x PCR buffer [2] (200 mM Tris-HCL (pH 8.4), 15 mM MgCl, 500 mM KCl), 0.1 µl (1 unit/µl) Taq polymerase, 1 µl Eco primer (67 ng/µl) (labelled with either FAM or NED), 4.5 µl Mse primer with dNTP mixture, and 5 µl of diluted (1:50) pre-selective amplification product.

The selective PCR parameters were as follows: 94 °C for five minutes, 60 °C for 30 seconds and 72 °C for two minutes, followed by 12 cycles of 94 °C for 30 seconds, 65 °C for 30 seconds with a temperature reduction of 0.7 °C per cycle, and 72 °C for two minutes. This was followed by 23 cycles at 94 °C for 30 seconds, 56 °C for 30 seconds and 72 °C for two minutes.

After amplification, 5 µl of each selective amplification reaction was added to 24 µl formamide and 1 µl Rox 500, denatured at 95 °C for 10 minutes, quick cooled on ice and resolved on a IBI Prism 310 Automated Capillary Sequencer (PI Biosystems).

4.2.5 Genetic distance and cluster analysis

DNA fingerprint analysis was done on a Macintosh (IMAC) computer using GeneScan 3.1 (Perkin-Elmer Corporation, 1997). Fragments larger than 70 bp with a peak height of 40 or more were scored into a binary matrix as present (1) or absent (0).

Genetic distances between individual genotypes was calculated with the Euclidean distance using the formula: $E^2_{ij} = \sum_k (x_{ki} - x_{kj})^2$, where k is the number of bands and x_{ki}

and x_{kj} are the frequencies of occurrence of 0 and 1 of the k^{th} band in an individual i and j, respectively. The molecular data was analysed using the Number Cruncher Statistical System (NCSS 2000) (Hintze, 1998). Dendrogrammes were constructed using the Unweighted Pair Group Method of Arithmetic Averages (UPGMA) in NCSS 2000.

GeneScan version 3.1 (Applied Biosystems) was used to score the bands. The primers were chosen after screening 12 different primer combinations. Bands were scored as present or absent, and the NCSS software computer package was used for the statistical analysis using the Simple Matching or Jaccard's similarity indices and UPGMA or Principle Coordinate analysis of the matrices of similarities which was represented in the form of dendrograms.

4.3 Results and discussions

A dissimilarity matrix based on Euclidean distance was computed by unweighted pair-wise comparison of all 21 genotypes. Genetic distances (GD) ranged from 0.25 (for CH92/108 and 81/00015) to a maximum of 0.80 (for CH92/112 and TMS4(2)1425) (Figure 4.1) (Appendix 3).

Genetic distance data was used to cluster the taxa using the UPGMA algorithm, after the dissimilarity matrix had been transformed into a cophenetic matrix. The goodness-of-fit between the two matrices was calculated as 84.01 %. The resulting phenetic dendrogram revealed three major (I, II and III) clusters at 62 % dissimilarity level (Figure 4.1).

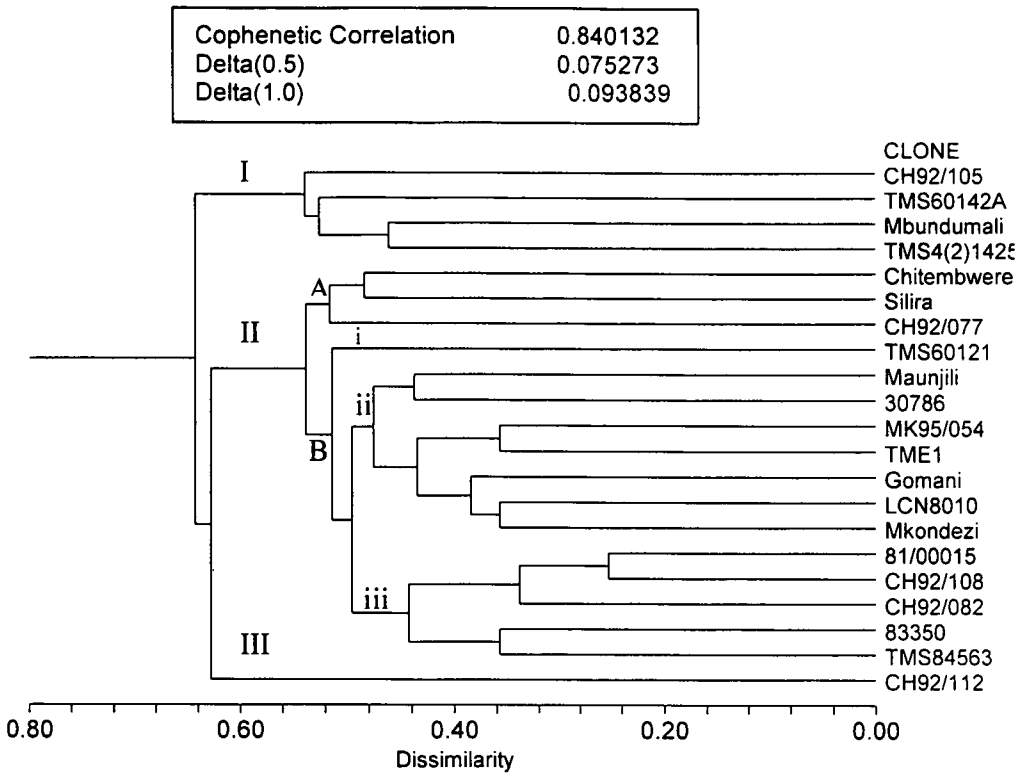

Figure 4.1. **Phenetic dendrogram generated using morphological data of 21 elite Malawi cassava genotypes based on UPGMA from pair-wise comparisons employing Euclidean's coefficients of genetic distance.**

Cluster I consisted of Mbundumali (a local cultivar), CH92/105 (a locally bred clone), and TMS4(2)1425 and TMS60142A (introductions from IITA). The closest genotypes in this cluster were Mbundumali and TMS4(2)1425 with a dissimilarity of 46.13 %. The dissimilarity between Mbundumali and TMS40142A was 50.53 %, while the dissimilarity between Mbundumali and CH92/105 was 52.59 % (Figure 4.1) (Appendix 3). All the genotypes in cluster I were characterised by having smooth roots with white outer skin colour. In addition, all the genotypes in this cluster had silvery green mature stems (Appendix 2).

Cluster II consisted of the other genotypes (Figure 4.1). Within the second cluster, there were different groups and subgroups at 50 % dissimilarity level (Figure 4.1). Cluster II, contained two main groups (A and B), with sub-clusters (i, ii and ii) (Figure 4.1).

Cluster II group A contained three genotypes including Chitembwere (a recommended local variety), Silira (introduction from IITA), and CH92/077 (a locally bred clone) (Figure 4.1). Among these three genotypes, the closest pair was Chitembwere and Silira with a genetic distance of 0.48. The genetic distance between Chitembwere and CH92/077 was 0.51, while between Silira and CH92/077 the genetic distance was 0.53 (Figure 4.1) (Appendix 3). The genotypes are tall, (over 200 cm high), the roots of these genotypes are conical or cylindrical in shape, and their unexpanded apical leaves have little hairs. Furthermore, the genotypes have roots with white outer skin colour and smooth, and stems are silvery green (Figure 4.1) (Appendix 2).

Cluster II, group B sub-cluster i contained only TMS60121, which is an introduction from IITA (Figure 4.1). The closest genotypes to TMS60121 were Gomani and LCN8010 with a genetic distance of 0.44. The most distant genotype to TMS60121 was CH92/105 with a genetic distance of 0.71 (Appendix 3).

Cluster II, group B, sub-cluster ii, contained Gomani (a recommended local variety), Mkondezi (a locally bred variety), Maunjili (introduction from IITA), MK95/054 (locally bred clone) as well as 30786, TME1 and LCN8010 (introduced clones from IITA) (Figure 4.1). The closest genotypes in this sub-grouping are MK95/054 and TME1, and, LCN8010 and Mkondezi with a dissimilarity of 35.73 for both pairs (Appendix 3). MK95/054 is a locally bred clone and is similar to TME1an introduced clone. Mkondezi is locally bred and similar to LCN8010, which is also an IITA clone.

Cluster II, group B, sub-group iii, contained only locally bred clones and introductions from IITA and there were no local cultivars in this group. The introduced clones in this group were 81/00015, 83350 and TMS84563, while the locally bred clones were CH92/108 and CH92/082 (Figure 4.1). The most closely related pair of genotypes among all the characterised genotypes belongs to this group. The closest genotypes were 81/00015 and CH92/108 with a dissimilarity level of 25.27 % (Figure 4.1). The closest pair of genotypes was between a local clone and an introduced genotype (Figure 4.1).

Cluster III contained only one locally bred clone CH92/112 (Figure 4.1). The closest genotype to CH92/112 from another cluster was Silira with a dissimilarity of 54.58 %, while the most distant genotype was TMS4(2)1425 with a dissimilarity of 79.89 % (Appendix 3).

Of the 21 genotypes used in the morphological characterisation, only 16 were included in the AFLP characterisation due to DNA problems in purity and concentration. Only the genotypes present in both characterisations were included in the comparison of the results.

Although a total of 12 different primer combinations were tested, only four gave usable results as shown in Figure 4.2 a and Figure 4.2 b. Primer combinations MseI-CAT and EcoRI-ACT, MseI-CAT and EcoRI-ACC, MseI-CAG and EcoRI-ACA, and, MseI-CAG and EcoRI-AAC (Figures 4.2 a and b) are the ones which gave usable results and were used to fingerprint all cassava genotypes

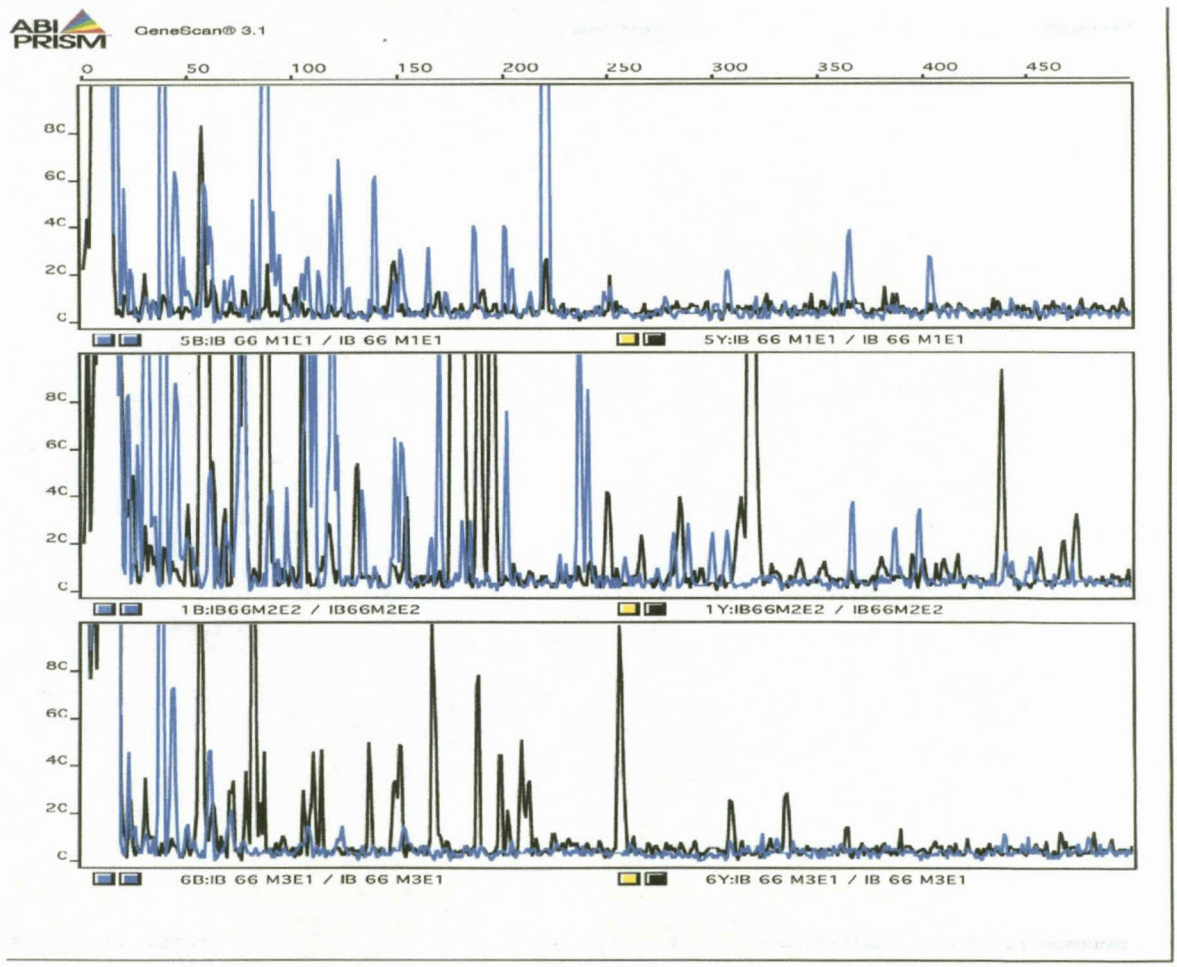

Figure 4.2 a: AFLP electropherograms for different primer combinations in cassava

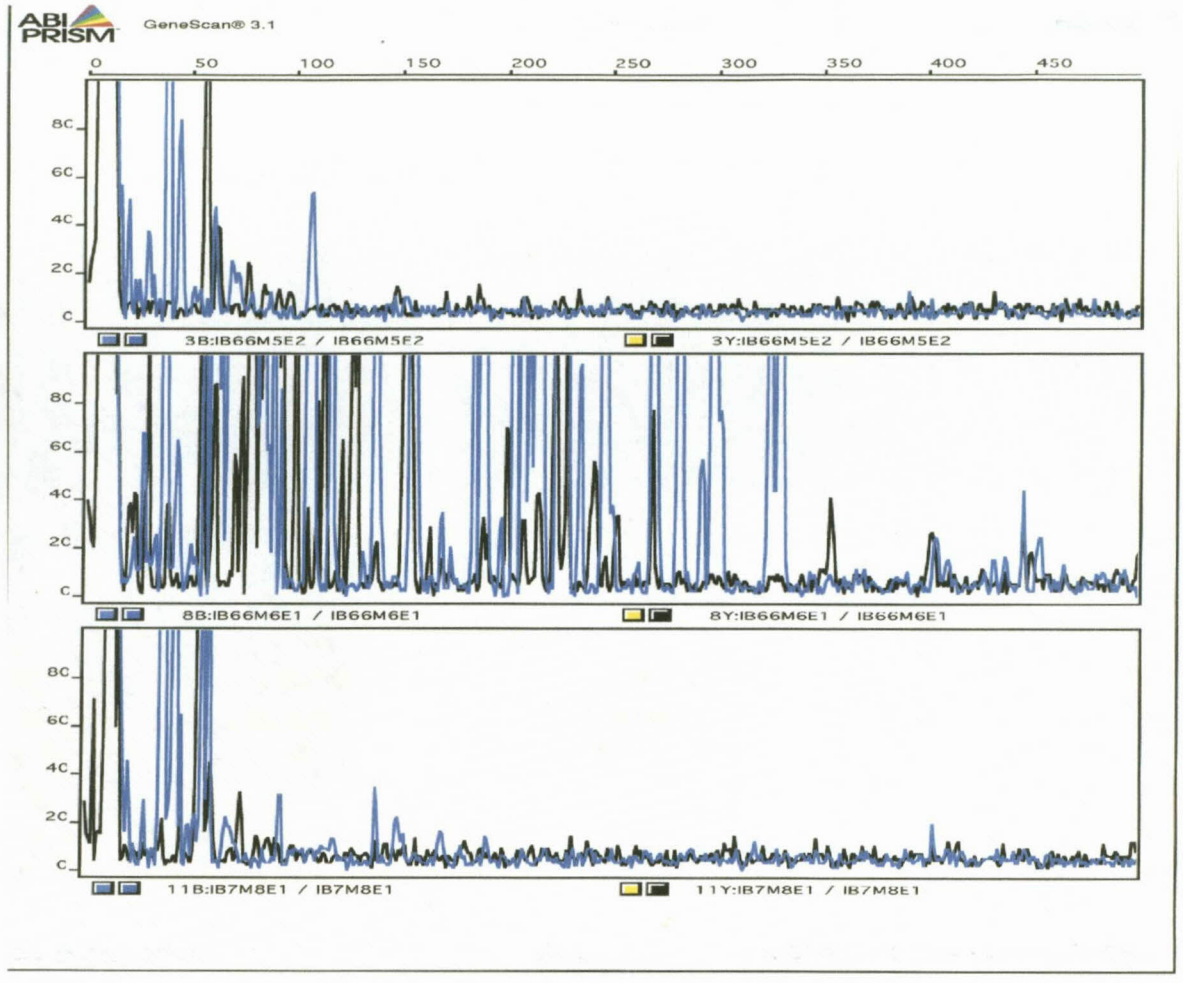

Figure 4.2 b: AFLP electropherograms for different primer combinations in cassava

The genetic distance data were clustered using the UPGMA algorithm. After the dissimilarity matrix had been transformed into a cophenetic matrix, the goodness-of-fit between the two matrices was 81.99 %. The resulting phenetic dendrogram revealed two major (I and II) clusters at 62 % dissimilarity level (Figure 4.3). Genetic distances ranged from 0.39 for Mkondezi and TME1 to a maximum of 0.68 for 30786 and 83350 (Figure 4.3) (Appendix 4).

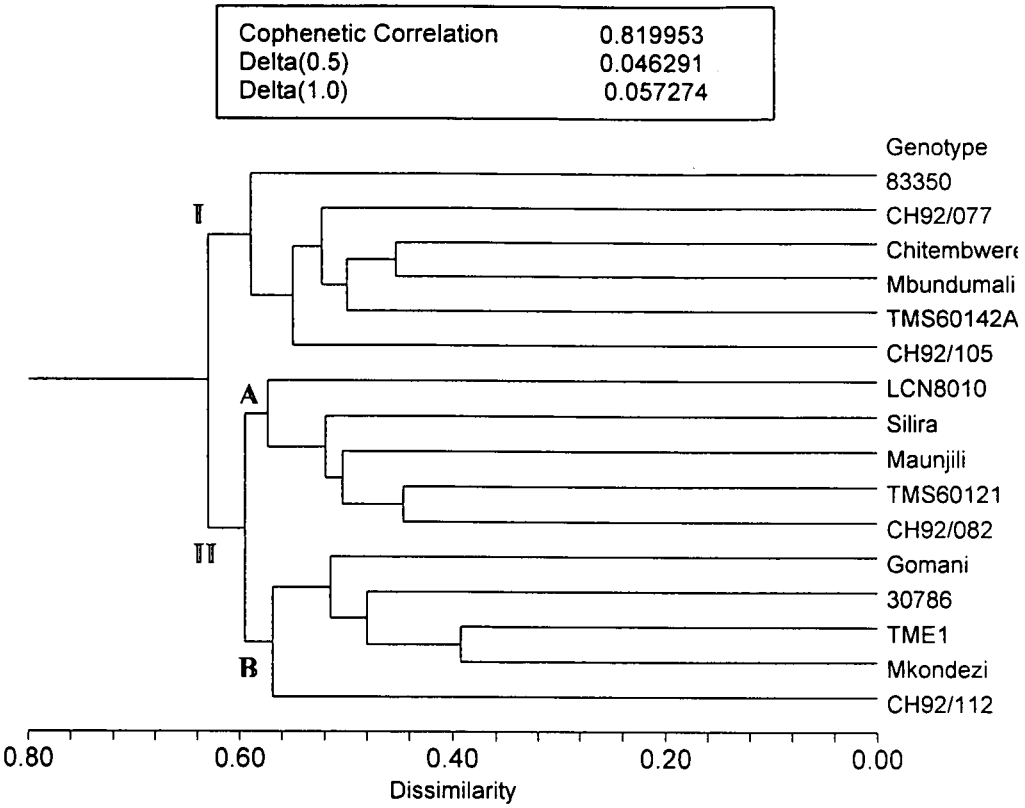

Figure 4.3: **Phenetic dendrogram generated using AFLP data of 16 elite Malawi cassava genotypes based on UPGMA from pair-wise comparisons employing Euclidean's coefficients of genetic distance.**

The genotypes in cluster I include two locally recommended cultivars (Mbundumali and Chitembwere), two locally bred clones (CH92/105 and CH92/077) and two introductions from IITA (83350 and TMS60142A). The most closely related genotypes in this cluster are Mbundumali, Chitembwere and TMS60142A with a dissimilarity of 45.15 % between Mbundumali and Chitembwere, and 52.94 % between Mbundumali and TMS40142A (Figure 4.3) (Appendix 4). These genotypes are characterised by being sweet (low in cyanide). All the genotypes in this cluster have silvery green mature stems.

Cluster II containing the rest of the genotypes can be divided into two sub-clusters (A and B) at 59 % dissimilarity level (Figure 4.3). Five genotypes belong to cluster II group A. This cluster comprises of introductions from IITA, except for CH92/082, which is a locally bred clone. The closest pair was TMS60121 and CH92/082 with a genetic distance of 0.44, while the genetic distance among the genotypes in this

cluster ranged from 0.44 to 0.55. The genotypes in this group are all characterised by profuse branching (Figure 4.3) (Appendix 4).

Cluster II, group B consists of Gomani (a recommended local variety), Mkondezi (a locally bred and released as a variety), CH92/112 (locally bred clone), and 30786 and TME1 (introduced clones from IITA) (Figure 4.3). The most closely related genotypes in this sub-group were Mkondezi and TME1 with a dissimilarity of 39.10 % (Appendix 4). The genetic distance among the genotypes in this cluster ranged from 0.39 to 0.54. The genotypes in this group are characterised by having no hairs on the apical unexpanded leaves.

The clustering between morphological and AFLP was similar although not the same. Genotypes in clusters I and II B ii (morphology) and I and II B (AFLP) are similar. The only difference is that in cluster I genotypes Chitembwere and CH92/077 have been added in cluster I of the AFLP dendrogramme. The only change in cluster II B for AFLP is that LCN8010 has been moved to cluster II A (Figures 4.1 and 4.3). The clustering using AFLP data stresses the importance of branching habit, hairiness and plant height in the characterisation of cassava germplasm in addition to all other traits. These characteristics are therefore more important in genotypic description than was previously thought.

Previous studies have shown weak to no relationships between morphological and molecular genetic distances and clustering (Zacarias, 1997; Sanchez *et al.,* 1998; Carvalho, *et al.,* 2000; Raji *et al.,* 2001). However, the results of this study conflict previous findings with a correlation of r=0.98. This indicates that if constant traits are included in morphological characterisation, and that the data is properly coded in a binary matrix for cluster analysis, the results are as accurate as if molecular data was used. However, AFLP fingerprints give a more descriptive structure relationship than morphological characterisation, and hence can be used to remove duplicates in germplasm collections.

4.4 Conclusions and recommendations

Morphological characterisation identified CH92/112, CH92/105, TMS60142A, TMS4(2)1425 and Mbundumali as the most genetically distant of all the genotypes used in this study. Therefore, these genotypes would maximise the recombination potential if incorporated into breeding programmes.

There was no clear division between genotypes according to their origin and clustering of genotypes was distributed across the local cultivars, locally bred clones, as well as introduced genotypes.

AFLP characterisation identified the most distant genotypes as 83350 and 30786 (Figure 4.3). Most of the genotype groupings contained at least one local cultivar except for group II A that was dominated by introduced clones. However, CH92/112, CH92/108, TMS60142A and TMS4(2)1425 were identified as the most distant genotypes by morphological characterisation.

There was a strong correlation between the morphological and AFLP genetic distances ($r=0.98$). All the genotypes with silvery green stems, profuse branching and with little or no hairs in the apical unexpanded leaves were grouped together by both characterisation methods. This indicates that if constant traits are included in morphological characterisation, and the data interpreted in a binary matrix for cluster analysis, the results are comparable. However, difficulties still exist between phenotypic and genotypic clustering. Therefore a combination of both should be used to determine a true relation of genetic diversity.

AFLP fingerprints gave a more descriptive structure than morphological characterisation and can be used to remove duplicates in germplasm collections.

CHAPTER 5

SUMMARY

The starchy tuberous roots of cassava (*Manihot esculenta* Crantz) provide more than half of the calories consumed by more than 800 million people in the South America, Asia and Africa. Cassava is a staple for more than 30 % of the Malawi population, while in the rest of the country it is grown for food security, as a snack, and a cash crop. A prerequisite for any genetic improvement programme of cassava is knowledge of the extent of genetic variation present between cultivars and genetic distances between them, and between cassava and closely related species with which hybrids could be produced. This can be achieved through characterisation of germplasm either using morphological, biochemical or DNA markers. Industries that use starch in Malawi have not been willing to use cassava starch because the powder sold by some suppliers as 'cassava starch' was inferior. This study was therefore initiated with the following objectives: (1) To determine the genetic distances/relatedness of commercial Malawi cassava varieties and promising clones using AFLP and morphology descriptors; (2) To evaluate the Malawi commercial cassava varieties and promising clones for starch extraction and quality; (3) To assess the feasibility of using native cassava starch in the industrial sector in Malawi; and (4) To evaluate the commercial cassava varieties in Malawi and promising clones for dry matter content.

Trials with 20 cassava genotypes were planted at Chitedze and Makoka in Malawi in November 2000, and were harvested in December 2001. Morphological characterisation was done during plant growth and at harvest. The quality parameters included: protein, moisture and ash content, pH and whiteness. Feasibility studies in the use of cassava starch in the pharmaceutical, textile, battery making and packaging industries started in December 2001.

The results show that all the cassava genotypes produced starch containing no detectable protein, similar to the starch used in the pharmaceutical industry. The moisture content ranged from 11.85 to 13.65 %, which is lower than the recommended maximum of 14 %. The recommended maximum of 0.5 % for ash was much higher than the values of cassava starch, which ranged from 0.10 to 0.20 %. The

recommended pH for starch is between 4.5 and 7.0, and cassava starch was within these limits, ranging from 5.0 to 5.9. The cassava starch was as white as the corn starch currently being used. Trials have shown that cassava starch can successfully be used in the making of tablets, batteries, packaging material and textile manufacture. Thus, native cassava starch is suitable for use in various industries. This study has also dispelled fears that cassava starch is of low quality and that it is not effective in some industries. Hence, the ideal quality of starch was confirmed in this study. What is required is strict quality control for cassava starch so that industry receives what they pay for. MBS needs to develop national standards for starch, which will help in the enforcement of the quality control for any starch produced in Malawi. This task will be facilitated by the recommendations made in this thesis.

Additive main effects and multiplicative interaction (AMMI) was strongly correlated with other stability parameters like Wi-ecovalence, stability variance – no covariate and cultivar superiority measure. AMMI is therefore recommended for use in the stability analysis of starch quality parameters since it provides additional information on the allocation of unstable genotypes into their appropriate environments.

This study has demonstrated that genotype has a greater influence on root dry matter than the environment. This agrees with the hypothesis that one or a few major genes control root dry matter in cassava.

This study has also revealed that the genetic distances for the local cultivars, locally bred clones and introduced genotypes are equally divergent. This is supported by the distribution of the genotypes in different clusters despite their origin Genetic distances determined by morphological characterisation correlates to similar values using AFLP fingerprinting ($r=0.98$). It is however, a prerequisite that morphological characterisation be based on constant traits, and the conversion of the morphologic data into binary characters needs careful consideration to meaningful results.

OPSOMMING

Die styselagtige tuberagtige wortels van cassava (*Manihot esculenta* Crantz) voorsien meer as helfte van die kalorië wat verbruik word deur meer as 800 miljoen mense in Soid Amerika, Asië en Afrika. Cassava is die stapevoedsel van meer as 30% van die Malawi populasie, terwyl dit in die res van die land verbou word vir voedsel sekuriteit, as 'n snoephappie en as 'n kontant gewas. 'n Voorwaarde vir enige program van genetiese verbetering van cassava is kennis van die hoeveelheid genetiese variasie wat teenwoordig is tussen cultivars en die genetiese afstande tussen hulle en met naby verwante spesies waarmee basters gemaak kan word. Dit kan bereik word deur die karakterisering van kiemplasma deur morfologiese, biochemiese of DNA merkers. Industrië wat stysel gebruik in Malawi is onwillig om cassava stysel te gebruik, omdat poeier wat deur handelaars in die naam van cassava stysel aan hulle voorsien is, misluk het. Hierdie studie is dus geïnisieer met die volgende doelwitte: (1) Om die genetiese afstande / verwantskappe van kommersiële cultivars en belowende klone van Malawi te bepaal met die gebruik van AFLP en morfologiese beskrywers; (2) Om die Malawi kommersiële cassava cultivars en belowende klone te evalueer vir hulle stysel ekstraksie en die kwaliteit van ongemodifiseerde stysel; (3) Om die bruikbaarheid van ongemodifiseerde stysel in die industriële sektor van Malawi te ondersoek; (4) Om die Malawi kommersiële cassava cultivars en belowende klone te evalueer vir droë material inhoud.

Proewe met 20 cassava genotipes is geplant by Chitedze en Makoka in Malawi in November 2000, en is geoes in Desember 2001. Morfologiese karakterisering is gedoen tydens die groeiseisoen en met oestyd. Die kwaliteits parameters wat oorweeg is, was: proteïen, vog, as inhoud, pH en witheid. Lewensvatbaarheids studies vir die gebruik van cassava stysel in die farmaseutiese, tekstiel, battery en verpakkings industrië is begin in Desember 2001.

Die resultate het getoon dat al die cassava genotipes stysel geproduseer het wat geen proteïen het nie, soortgelyk aan stysel wat gebruik word in die farmaseutiese bedryf. Die vog inhoud het gewissel van 11.85% tot 13.65% wat laer is as die voorgestelde maksimum van 14%. Die voorgestelde maksimum van 0.5% vir as was baie hoër as die waardes van 0.10% tot 0.2% wat gekry is vir die stysel. Die voorgestelde pH vir

stysel is tussen 4.5 en 7.0, en die cassava stysel se waarde was binne hierdie perke, en het gewissel tussen 5.0 en 5.9. Die cassava stysel was net so wit soos mielie stysel wat tans gebruik word. Proewe het getoon dat cassava stysel suskesvol gebruik kan word vir die maak van tablette, die verdunning van medisyne, batterye en verpakkings materiaal. Dus is ongemodifiseerde stysel bruikbaar in verskeie industrië. Hierdie studie het vrese besweer dat cassava stysel van swak kwaliteit is en dat dit nie effektief in sekere industrië is nie. Daarom is die ideale eienskappe van cassava stysel bevestig in hierdie studie. Streng kontrole is nodig sodat industrië kry waarvoor hulle betaal. MBS moet nasionale standaarde vir stysel ontwikkel wat sal help met die toepassing van kwaliteits kontrole van enige stysel wat geproduseer word in Malawi. Dit behoort maklik te wees omdat hierdie studie die stysel standaarde hersien het.

AMMI (additive main effects and multiplicative interaction) was sterk gekorreleer met ander stabiliteits parameters soos Wi-ekovalensie, stabiliteits variansie – geen kovariaat en cultivar superioriteits analise. AMMI word daarom aanbeveel vir die gebruik in stabiliteits analise van stysel kwaliteits parameters omdat dit addisionele informasie gee oor plasing van onstabiele genotipes in hulle regte omgewings.

Daar is gedemonstreer in hierdie studie dat genotipiese invloed op wortel droë materiaal baie groter is as die omgewings invloed. Dit bevestig die hipotese dat een of 'n paar hoof gene droë materiaal in wortels beheer.

Die studie het aangetoon dat genetiese diversiteit vir plaaslike cultivars, plaaslik geteelde klone en genotipes van buite ewe wyd is. Dit is aangetoon deur die verspreiding van genotipes in verskillende groeperings, ten spyte van hulle afkoms en deur werklike genetiese afstande. Die morfologiese karakterisering van die genotipes was sterk gekorreleer met die van die AFLP vingerafdrukke (r=0.9978) omdat morfologiese beskrywers gebasseer is op konstante (monogeniese) eienskappe. Dit is daarom belangrik dat morfologiese karakterisering gebasseer word op konstante eienskappe, en dat die omskakeling van morfologiese data na 'n binêre matriks effektief gedoen word soos beskryf in Hoofstuk 4, om sinvolle resultate te kry.

CHAPTER 6

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APPENDICES

Appendix 1: Morphological descriptor for cassava

DESCRIPTION	CATEGORIES	IPGRI CODE
1. Colour of unexpended apical leaves (CAUL)	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	4.1.6
3. Mature leaf colour (MLC)	1-Light Green 2-Green 3-Dark green 4-Green purple 5-Purple	4.1.2
4. Mature leaf vein colour (Veins on the lower surface of the first fully expanded Leaf) (MLVS)	1-Light green 2-Dark green 3-Green purple 4-Purple	6.1.6
5. Leaf central lobe shape (LCLS)	1-Oblanceolate 2-Lincar 3-Elliptic 4-Pandurate 5-Lanceolate	4.1.3
6. Number of leaf lobes	Most common number	6.1.3
7. Petiole colour (PC)	1-Whole petiole green 2-Mainly green but and some pale red sllading 3-Mainly red to dark 4-Whole petiole red or red/purple	4.1.5
8. Mature stem colour (MSC)	1-Silvery green 2-Light brown/orange 3-Dark brown 4-Reddish-red	4.1.7

Appendix 1: Morphological descriptor for cassava (continues)

DESCRIPTION	CATEGORIES	IPGRI CODE
9. Number of branching levels	1-Actual number of levels	4.1.8
10. Height of first branch	In cm	4.1.10
11. Height of mature plant	In cm (top canopy)	4.1.11
12 Colour of root surface (CRS)	1-White to cream 2-Light brown 3-Dark brown	4.1.12
13. Root inner skin colour (ISC)	1-White 2-Yellow 3-Pink	6.1.28
14. Colour of root pulp (CRP)	1-White or cream 2-Yellow	4.1.13
15. Root peduncle (RP)	1-Absent 2-Short (less than 5 cm) 3-Intermediate (between 5-10 cm) 4-Long (above 10 cm)	6.1.17
16. Root shape (RS)	1-Conical 2-Conical/cylindrical 3-Cylindrical 4-Fusiform 5-Irregular	6.1.18
17. Root constrictions	1-Absent 2- Present	6.1.19
18. Root position (PR)	1-Tending towards vertical 2-Tending toward horizontal 3-Horizontal 4-Irregular	6.1.20
19. Root texture	1-Smooth 2-Rough	6.1.21

Appendix 2: Conversion of morphological data into binary matrix

CLONE	Colour of unexpanded apical leaves (CUAL)					Hairness of unexpanded apical leaves (HUL)				Mature leaf colour (MLC)				
	CUAL1	CUAL2	CUAL3	CUAL4	CUAL5	HUL1	HUL2	HUL3	HUL4	MLC1	MLC2	MLC3	MLC4	MLC5
CH92/112	0	0	1	0	0	0	1	0	0	1	0	0	0	0
TMS84563	0	0	0	1	0	1	0	0	0	0	1	0	0	0
TMS4(2)1425	1	0	0	0	0	0	0	1	0	0	1	0	0	0
Mkondezi	0	0	0	0	1	1	0	0	0	0	1	0	0	0
TME1	0	0	1	0	0	0	1	0	0	1	0	0	0	0
CH92/105	0	0	1	0	0	0	1	0	0	0	1	0	0	0
30786	0	0	0	0	1	0	1	0	0	0	1	0	0	0
CH92/082	0	0	0	1	0	1	0	0	0	0	1	0	0	0
TMS60121	0	0	0	0	1	1	0	0	0	0	0	1	0	0
CH92/108	0	0	0	1	0	0	1	0	0	0	1	0	0	0
81/00015	0	0	0	1	0	1	0	0	0	0	1	0	0	0
Gomani	0	0	0	0	1	1	0	0	0	0	0	1	0	0
MK95/054	0	0	0	1	0	1	0	0	0	1	0	0	0	0
83350	0	0	0	1	0	1	0	0	0	0	1	0	0	0
LCN8010	0	0	0	0	1	0	1	0	0	0	0	1	0	0
TMS60142A	0	0	1	0	0	0	0	1	0	1	0	0	0	0
Mbundumali	0	0	1	0	0	0	0	1	0	0	1	0	0	0
CH92/077	0	0	1	0	0	0	0	1	0	0	1	0	0	0
Maunjili	0	0	0	1	0	0	1	0	0	0	1	0	0	0
Silira	0	0	0	0	1	1	0	0	0	0	1	0	0	0
Chitembwere	0	0	1	0	0	1	0	0	0	0	1	0	0	0

Appendix 2: Conversion of morphological data into binary matrix (continues)

CLONE	Mature leaf vein colour (MLVC)				Leaf central lobe shape (LCLS)					No. of leaf lobes	Petiole colour			
	MLVC1	MLVC2	MLVC3	MLVC4	LCLS1	LCLS2	LCLS3	LCLS4	LCLS5		PC1	PC2	PC3	PC4
CH92/112	0	0	0	1	0	0	0	0	1	1	0	0	0	1
TMS84563	1	0	0	0	0	0	0	0	1	1	0	1	0	0
TMS4(2)1425	1	0	0	0	0	0	1	0	0	1	0	1	0	0
Mkondezi	0	0	1	0	0	0	0	0	1	0	0	1	0	0
TME1	0	0	1	0	0	0	0	0	1	1	0	0	0	1
CH92/105	1	0	0	0	0	0	0	0	1	0	1	0	0	0
30786	0	0	1	0	0	0	0	0	1	1	0	0	1	0
CH92/082	0	0	1	0	0	0	0	0	1	1	0	1	0	0
TMS60121	0	0	1	0	0	0	0	0	1	0	0	1	0	0
CH92/108	0	0	1	0	0	0	0	0	1	0	0	1	0	0
81/00015	0	0	1	0	0	0	0	0	1	1	0	1	0	0
Gomani	0	0	1	0	0	0	0	0	1	1	0	1	0	0
MK95/054	0	0	1	0	0	0	0	0	1	1	0	0	1	0
83350	1	0	0	0	0	0	0	0	1	1	0	1	0	0
LCN8010	0	0	1	0	0	0	0	0	1	1	0	1	0	0
TMS60142A	0	0	1	0	0	0	0	0	1	1	0	0	1	0
Mbundumali	1	0	0	0	0	0	0	0	1	1	0	1	0	0
CH92/077	0	0	1	0	0	0	1	0	0	0	0	1	0	0
Maunjili	0	0	1	0	0	0	1	0	0	0	0	1	0	0
Silira	0	0	0	1	0	0	0	0	1	1	0	0	0	1
Chitembwere	0	0	1	0	0	0	0	0	1	0	0	0	0	1

Appendix 2: Conversion of morphological data into binary matrix (continues)

CLONE	Mature stem colour (MSC)				Branching habit			Branching height			Plant height		
	MSC1	MSC2	MSC3	MSC4	NONE	FEW	PROFUSE	LOW	MEDIUM	HIGH	SHORT	MEDIUM	TALL
CH92/112	0	1	0	0	0	0	1	0	0	1	0	0	1
TMS84563	0	1	0	0	0	1	0	1	0	0	1	0	0
TMS4(2)1425	1	0	0	0	0	1	0	1	0	0	0	1	0
Mkondezi	0	1	0	0	0	1	0	0	1	0	0	1	0
TME1	0	1	0	0	0	1	0	0	1	0	0	1	0
CH92/105	1	0	0	0	0	1	0	0	1	0	0	1	0
30786	0	1	0	0	0	1	0	1	0	0	0	1	0
CH92/082	0	0	1	0	0	0	1	0	1	0	0	1	0
TMS60121	0	1	0	0	0	1	0	1	0	0	0	1	0
CH92/108	0	0	1	0	0	1	0	1	0	0	0	1	0
81/00015	0	0	1	0	0	1	0	1	0	0	0	1	0
Gomani	0	1	0	0	1	0	0	0	0	1	0	1	0
MK95054	0	1	0	0	0	1	0	0	1	0	0	1	0
83350	1	0	0	0	0	1	0	1	0	0	0	1	0
LCN8010	0	1	0	0	0	1	0	0	1	0	0	1	0
TMS60142A	1	0	0	0	0	1	0	0	1	0	0	1	0
Mbundumali	1	0	0	0	0	1	0	0	1	0	0	0	1
CH92/077	0	1	0	0	0	1	0	0	0	1	0	0	1
Maunjili	0	1	0	0	0	1	0	0	1	0	0	1	0
Silira	0	1	0	0	0	1	0	1	0	0	0	0	1
Chitembwere	0	1	0	0	0	1	0	0	1	0	0	0	1

Appendix 2: Conversion of morphological data into binary matrix (continues)

CLONE	Colour of root surface (CRS)			Root inner skin colour (ISC)			Colour of root pulp (CRP)		Root peduncle (RP)			
	CRS1	CRS2	CRS3	ISC1	ISC2	ISC3	CRP1	CRP2	RP1	RP2	RP3	RP4
CH92/112	0	0	1	1	0	0	1	0	0	0	1	0
TMS84563	0	1	0	1	0	0	1	0	0	1	0	0
TMS4(2)1425	1	0	0	1	0	0	1	0	0	1	0	0
Mkondezi	0	0	1	1	0	0	1	0	0	1	0	0
TME1	0	0	1	1	0	0	1	0	0	1	0	0
CH92/105	1	0	0	1	0	0	1	0	0	0	0	1
30786	0	0	1	1	0	0	1	0	0	1	0	0
CH92/082	0	0	1	1	0	0	1	0	0	1	0	0
TMS60121	0	0	1	1	0	0	1	0	0	0	1	0
CH92/108	0	0	1	1	0	0	1	0	0	1	0	0
81/00015	0	0	1	1	0	0	1	0	0	1	0	0
Gomani	0	0	1	1	0	0	1	0	0	1	0	0
MK95/054	0	0	1	1	0	0	1	0	0	1	0	0
83350	1	0	0	1	0	0	1	0	0	1	0	0
LCN8010	0	0	1	1	0	0	1	0	0	1	0	0
TMS60142A	1	0	0	1	0	0	1	0	0	1	0	0
Mbundumali	1	0	0	1	0	0	1	0	0	1	0	0
CH92/077	0	0	1	1	0	0	1	0	0	1	0	0
Maunjili	0	0	1	1	0	0	1	0	0	1	0	0
Silira	0	0	1	1	0	0	1	0	0	1	0	0
Chitembwere	0	0	1	0	0	1	1	0	0	0	1	0

Appendix 2: Conversion of morphological data into binary matrix (continues)

CLONE	Root shape (RS)					Root constrictions	Root position (PR)				Root texture
	RS1	RS2	RS3	RS4	RS5		PR1	PR2	PR3	PR4	
CH92/112	0	0	0	1	0	0	0	1	0	0	0
TMS84563	0	1	0	0	0	0	0	1	0	0	0
TMS4(2)1425	0	1	0	0	0	0	1	0	0	0	1
Mkondezi	0	1	0	0	0	0	1	0	0	0	0
TME1	0	1	0	0	0	0	0	1	0	0	0
CH92/105	1	0	0	0	0	0	1	0	0	0	1
30786	1	0	0	0	0	0	0	1	0	0	0
CH92/082	0	1	0	0	0	0	0	1	0	0	0
TMS60121	0	0	0	1	0	0	0	1	0	0	0
CH92/108	0	1	0	0	0	0	0	1	0	0	0
81/00015	0	1	0	0	0	0	0	1	0	0	0
Gomani	0	1	0	0	0	0	0	1	0	0	0
MK95/054	0	1	0	0	0	0	0	1	0	0	0
83350	0	1	0	0	0	0	0	1	0	0	0
LCN8010	0	1	0	0	0	0	0	1	0	0	0
TMS60142A	0	0	0	1	0	0	1	0	0	0	1
Mbundumali	0	1	0	0	0	0	0	1	0	0	1
CH92/077	0	1	0	0	0	0	0	1	0	0	0
Maunjili	1	0	0	0	0	0	0	1	0	0	0
Silira	0	1	0	0	0	01	0	1	0	0	0
Chitembwere	0	1	0	0	0	0	0	1	0	0	0

Appendix 3: Hierarchical clustering report for comparison of genetic distances between pairs for the 21 elite Malawi cassava genotypes characterised using morphological characteristics

First Row	Second Row	Actual Distance	Dendrogram Distance	Actual Difference	Percent Difference
1=CH92/112	2=TMS84563	0.684167	0.628159	0.056009	8.19
1=CH92/112	3=TMS4(2)1425	0.798935	0.643114	0.155822	19.50
1=CH92/112	4=Mkondezi	0.684167	0.628159	0.056009	8.19
1=CH92/112	5=TME1	0.505291	0.628159	-0.122868	-24.32
1=CH92/112	6= CH92/105	0.729325	0.643114	0.086211	11.82
1=CH92/112	7=30786	0.618853	0.628159	-0.009306	-1.50
1=CH92/112	8=CH92/082	0.652328	0.628159	0.024169	3.71
1=CH92/112	9=TMS60121	0.601417	0.628159	-0.026742	-4.45
1=CH92/112	10=CH92/108	0.668437	0.628159	0.040279	6.03
1=CH92/112	11=81/00015	0.684167	0.628159	0.056009	8.19
1=CH92/112	12=Gomani	0.618853	0.628159	-0.009306	-1.50
1=CH92/112	13=MK95/054	0.618853	0.628159	-0.009306	-1.50
1=CH92/112	14=83350	0.714590	0.628159	0.086431	12.10
1=CH92/112	15=LCN8010	0.618853	0.628159	-0.009306	-1.50
1=CH92/112	16=TMS60142A	0.684167	0.643114	0.041054	6.00
1=CH92/112	17=Mbundumali	0.684167	0.643114	0.041054	6.00
1=CH92/112	18=CH92/077	0.601417	0.628159	-0.026742	-4.45
1=CH92/112	19=Maunjili	0.668437	0.628159	0.040279	6.03
1=CH92/112	20=Silira	0.545777	0.628159	-0.082382	-15.09
1=CH92/112	21=Chitembwere	0.564933	0.628159	-0.063226	-11.19
2=TMS84563	3=TMS4(2)1425	0.583460	0.643114	-0.059654	-10.22
2=TMS84563	4=Mkondezi	0.505291	0.495376	0.009915	1.96
2=TMS84563	5=TME1	0.583460	0.495376	0.088084	15.10
2=TMS84563	6= CH92/105	0.699544	0.643114	0.056430	8.07
2=TMS84563	7=30786	0.545777	0.495376	0.050401	9.23
2=TMS84563	8=CH92/082	0.505291	0.442966	0.062325	12.33
2=TMS84563	9=TMS60121	0.564933	0.513604	0.051328	9.09
2=TMS84563	10=CH92/108	0.483779	0.442966	0.040814	8.44
2=TMS84563	11=81/00015	0.412568	0.442966	-0.030397	-7.37
2=TMS84563	12=Gomani	0.545777	0.495376	0.050401	9.23
2=TMS84563	13=MK95/054	0.505291	0.495376	0.009915	1.96
2=TMS84563	14=83350	0.357295	0.357295	0.000000	0.00
2=TMS84563	15=LCN8010	0.545777	0.495376	0.050401	9.23
2=TMS84563	16=TMS60142A	0.714590	0.643114	0.071476	10.00
2=TMS84563	17=Mbundumali	0.545777	0.643114	-0.097337	-17.83
2=TMS84563	18=CH92/077	0.564933	0.538321	0.026612	4.71
2=TMS84563	19=Maunjili	0.564933	0.495376	0.069557	12.31
2=TMS84563	20=Silira	0.461266	0.538321	-0.077056	-16.71
2=TMS84563	21=Chitembwere	0.601417	0.538321	0.063096	10.49
3=TMS4(2)1425	4=Mkondezi	0.583460	0.643114	-0.059654	-10.22
3=TMS4(2)1425	5=TME1	0.684167	0.643114	0.041054	6.00
3=TMS4(2)1425	6= CH92/105	0.564933	0.538927	0.026006	4.60
3=TMS4(2)1425	7=30786	0.652328	0.643114	0.009215	1.41
3=TMS4(2)1425	8=CH92/082	0.652328	0.643114	0.009215	1.41
3=TMS4(2)1425	9=TMS60121	0.699544	0.643114	0.056430	8.07

Clustering Method = Group Average (Unweighted Pair-Group); Distance Type = Euclidean; Scale Type = Standard Deviation

Appendix 3: Hierarchical clustering report for comparison of genetic distances between pairs for the 21 elite Malawi cassava genotypes characterised using morphological characteristics (continues)

First Row	Second Row	Actual Distance	Dendrogram Distance	Actual Difference	Percent Difference
3=TMS4(2)1425	10=CH92/108	0.601417	0.643114	-0.041697	-6.93
3=TMS4(2)1425	11=81/00015	0.583460	0.643114	-0.059654	-10.22
3=TMS4(2)1425	12=Gomani	0.684167	0.643114	0.041054	6.00
3=TMS4(2)1425	13=MK95/054	0.684167	0.643114	0.041054	6.00
3=TMS4(2)1425	14=83350	0.461266	0.643114	-0.181848	-39.42
3=TMS4(2)1425	15=LCN8010	0.652328	0.643114	0.009215	1.41
3=TMS4(2)1425	16=TMS60142A	0.545777	0.525534	0.020243	3.71
3=TMS4(2)1425	17=Mbundumali	0.461266	0.461266	0.000000	0.00
3=TMS4(2)1425	18=CH92/077	0.601417	0.643114	-0.041697	-6.93
3=TMS4(2)1425	19=Maunjili	0.635811	0.643114	-0.007303	-1.15
3=TMS4(2)1425	20=Silira	0.652328	0.643114	0.009215	1.41
3=TMS4(2)1425	21=Chitembwere	0.757937	0.643114	0.114823	15.15
4=Mkondezi	5=TME1	0.461266	0.435042	0.026223	5.69
4=Mkondezi	6= CH92/105	0.635811	0.643114	-0.007303	-1.15
4=Mkondezi	7=30786	0.461266	0.475552	-0.014287	-3.10
4=Mkondezi	8=CH92/082	0.412568	0.495376	-0.082807	-20.07
4=Mkondezi	9=TMS60121	0.483779	0.513604	-0.029825	-6.16
4=Mkondezi	10=CH92/108	0.483779	0.495376	-0.011596	-2.40
4=Mkondezi	11=81/00015	0.412568	0.495376	-0.082807	-20.07
4=Mkondezi	12=Gomani	0.412568	0.384932	0.027637	6.70
4=Mkondezi	13=MK95/054	0.412568	0.435042	-0.022474	-5.45
4=Mkondezi	14=83350	0.505291	0.495376	0.009915	1.96
4=Mkondezi	15=LCN8010	0.357295	0.357295	0.000000	0.00
4=Mkondezi	16=TMS60142A	0.583460	0.643114	-0.059654	-10.22
4=Mkondezi	17=Mbundumali	0.583460	0.643114	-0.059654	-10.22
4=Mkondezi	18=CH92/077	0.525924	0.538321	-0.012397	-2.36
4=Mkondezi	19=Maunjili	0.483779	0.475552	0.008227	1.70
4=Mkondezi	20=Silira	0.461266	0.538321	-0.077056	-16.71
4=Mkondezi	21=Chitembwere	0.525924	0.538321	-0.012397	-2.36
5=TME1	6= CH92/105	0.635811	0.643114	-0.007303	-1.15
5=TME1	7=30786	0.461266	0.475552	-0.014287	-3.10
5=TME1	8=CH92/082	0.505291	0.495376	0.009915	1.96
5=TME1	9=TMS60121	0.564933	0.513604	0.051328	9.09
5=TME1	10=CH92/108	0.483779	0.495376	-0.011596	-2.40
5=TME1	11=81/00015	0.505291	0.495376	0.009915	1.96
5=TME1	12=Gomani	0.505291	0.435042	0.070249	13.90
5=TME1	13=MK95/054	0.357295	0.357295	0.000000	0.00
5=TME1	14=83350	0.583460	0.495376	0.088084	15.10
5=TME1	15=LCN8010	0.357295	0.435042	-0.077748	-21.76
5=TME1	16=TMS60142A	0.545777	0.643114	-0.097337	-17.83
5=TME1	17=Mbundumali	0.583460	0.643114	-0.059654	-10.22
5=TME1	18=CH92/077	0.525924	0.538321	-0.012397	-2.36
5=TME1	19=Maunjili	0.483779	0.475552	0.008227	1.70
5=TME1	20=Silira	0.505291	0.538321	-0.033030	-6.54
5=TME1	21=Chitembwere	0.483779	0.538321	-0.054542	-11.27

Clustering Method = Group Average (Unweighted Pair-Group); Distance Type = Euclidean; Scale Type = Standard Deviation

Appendix 3: Hierarchical clustering report for comparison of genetic distances between pairs for the 21 elite Malawi cassava genotypes characterised using morphological characteristics (continues)

First Row	Second Row	Actual Distance	Dendrogram Distance	Actual Difference	Percent difference
6= CH92/105	7=30786	0.635811	0.643114	-0.007303	-1.15
6= CH92/105	8=CH92/082	0.699544	0.643114	0.056430	8.07
6= CH92/105	9=TMS60121	0.714590	0.643114	0.071476	10.00
6= CH92/105	10=CH92/108	0.652328	0.643114	0.009215	1.41
6= CH92/105	11=81/00015	0.699544	0.643114	0.056430	8.07
6= CH92/105	12=Gomani	0.757937	0.643114	0.114823	15.15
6= CH92/105	13=MK95/054	0.699544	0.643114	0.056430	8.07
6= CH92/105	14=83350	0.601417	0.643114	-0.041697	-6.93
6= CH92/105	15=LCN8010	0.668437	0.643114	0.025324	3.79
6= CH92/105	16=TMS60142A	0.525924	0.538927	-0.013003	-2.47
6= CH92/105	17=Mbundumali	0.525924	0.538927	-0.013003	-2.47
6= CH92/105	18=CH92/077	0.714590	0.643114	0.071476	10.00
6= CH92/105	19=Maunjili	0.618853	0.643114	-0.024261	-3.92
6= CH92/105	20=Silira	0.729325	0.643114	0.086211	11.82
6= CH92/105	21=Chitembwere	0.684167	0.643114	0.041054	6.00
7=30786	8=CH92/082	0.545777	0.495376	0.050401	9.23
7=30786	9=TMS60121	0.483779	0.513604	-0.029825	-6.16
7=30786	10=CH92/108	0.437595	0.495376	-0.057781	-13.20
7=30786	11=81/00015	0.461266	0.495376	-0.034110	-7.39
7=30786	12=Gomani	0.505291	0.475552	0.029739	5.89
7=30786	13=MK95/054	0.461266	0.475552	-0.014287	-3.10
7=30786	14=83350	0.545777	0.495376	0.050401	9.23
7=30786	15=LCN8010	0.412568	0.475552	-0.062984	-15.27
7=30786	16=TMS60142A	0.618853	0.643114	-0.024261	-3.92
7=30786	17=Mbundumali	0.652328	0.643114	0.009215	1.41
7=30786	18=CH92/077	0.564933	0.538321	0.026612	4.71
7=30786	19=Maunjili	0.437595	0.437595	0.000000	0.00
7=30786	20=Silira	0.461266	0.538321	-0.077056	-16.71
7=30786	21=Chitembwere	0.601417	0.538321	0.063096	10.49
8=CH92/082	9=TMS60121	0.564933	0.513604	0.051328	9.09
8=CH92/082	10=CH92/108	0.385922	0.338826	0.047096	12.20
8=CH92/082	11=81/00015	0.291730	0.338826	-0.047096	-16.14
8=CH92/082	12=Gomani	0.461266	0.495376	-0.034110	-7.39
8=CH92/082	13=MK95/054	0.412568	0.495376	-0.082807	-20.07
8=CH92/082	14=83350	0.461266	0.442966	0.018300	3.97
8=CH92/082	15=LCN8010	0.461266	0.495376	-0.034110	-7.39
8=CH92/082	16=TMS60142A	0.652328	0.643114	0.009215	1.41
8=CH92/082	17=Mbundumali	0.583460	0.643114	-0.059654	-10.22
8=CH92/082	18=CH92/077	0.564933	0.538321	0.026612	4.71
8=CH92/082	19=Maunjili	0.483779	0.495376	-0.011596	-2.40
8=CH92/082	20=Silira	0.545777	0.538321	0.007456	1.37
8=CH92/082	21=Chitembwere	0.564933	0.538321	0.026612	4.71
9=TMS60121	10=CH92/108	0.505291	0.513604	-0.008313	-1.65
9=TMS60121	11=81/00015	0.483779	0.513604	-0.029825	-6.16

Clustering Method = Group Average (Unweighted Pair-Group); Distance Type = Euclidean; Scale Type = Standard Deviation

Appendix 3: Hierarchical clustering report for comparison of genetic distances between pairs for the 21 elite Malawi cassava genotypes characterised using morphological characteristics (continues)

First Row	Second Row	Actual Distance	Dendrogram Distance	Actual Difference	Percent Difference
9=TMS60121	12=Gomani	0.437595	0.513604	-0.076009	-17.37
9=TMS60121	13=MK95/054	0.525924	0.513604	0.012319	2.34
9=TMS60121	14=83350	0.564933	0.513604	0.051328	9.09
9=TMS60121	15=LCN8010	0.437595	0.513604	-0.076009	-17.37
9=TMS60121	16=TMS60142A	0.668437	0.643114	0.025324	3.79
9=TMS60121	17=Mbundumali	0.699544	0.643114	0.056430	8.07
9=TMS60121	18=CH92/077	0.583460	0.538321	0.045139	7.74
9=TMS60121	19=Maunjili	0.545777	0.513604	0.032173	5.89
9=TMS60121	20=Silira	0.525924	0.538321	-0.012397	-2.36
9=TMS60121	21=Chitembwere	0.545777	0.538321	0.007456	1.37
10=CH92/108	11=81/00015	0.252646	0.252646	0.000000	0.00
10=CH92/108	12=Gomani	0.525924	0.495376	0.030548	5.81
10=CH92/108	13=MK95/054	0.483779	0.495376	-0.011596	-2.40
10=CH92/108	14=83350	0.437595	0.442966	-0.005371	-1.23
10=CH92/108	15=LCN8010	0.437595	0.495376	-0.057781	-13.20
10=CH92/108	16=TMS60142A	0.668437	0.643114	0.025324	3.79
10=CH92/108	17=Mbundumali	0.601417	0.643114	-0.041697	-6.93
10=CH92/108	18=CH92/077	0.505291	0.538321	-0.033030	-6.54
10=CH92/108	19=Maunjili	0.412568	0.495376	-0.082807	-20.07
10=CH92/108	20=Silira	0.525924	0.538321	-0.012397	-2.36
10=CH92/108	21=Chitembwere	0.583460	0.538321	0.045139	7.74
11=81/00015	12=Gomani	0.461266	0.495376	-0.034110	-7.39
11=81/00015	13=MK95/054	0.412568	0.495376	-0.082807	-20.07
11=81/00015	14=83350	0.357295	0.442966	-0.085671	-23.98
11=81/00015	15=LCN8010	0.461266	0.495376	-0.034110	-7.39
11=81/00015	16=TMS60142A	0.652328	0.643114	0.009215	1.41
11=81/00015	17=Mbundumali	0.583460	0.643114	-0.059654	-10.22
11=81/00015	18=CH92/077	0.525924	0.538321	-0.012397	-2.36
11=81/00015	19=Maunjili	0.483779	0.495376	-0.011596	-2.40
11=81/00015	20=Silira	0.461266	0.538321	-0.077056	-16.71
11=81/00015	21=Chitembwere	0.564933	0.538321	0.026612	4.71
12=Gomani	13=MK95/054	0.461266	0.435042	0.026223	5.69
12=Gomani	14=83350	0.545777	0.495376	0.050401	9.23
12=Gomani	15=LCN8010	0.357295	0.384932	-0.027637	-7.74
12=Gomani	16=TMS60142A	0.684167	0.643114	0.041054	6.00
12=Gomani	17=Mbundumali	0.652328	0.643114	0.009215	1.41
12=Gomani	18=CH92/077	0.525924	0.538321	-0.012397	-2.36
12=Gomani	19=Maunjili	0.564933	0.475552	0.089380	15.82
12=Gomani	20=Silira	0.505291	0.538321	-0.033030	-6.54
12=Gomani	21=Chitembwere	0.601417	0.538321	0.063096	10.49
13=MK95/054	14=83350	0.505291	0.495376	0.009915	1.96
13=MK95/054	15=LCN8010	0.412568	0.435042	-0.022474	-5.45
13=MK95/054	16=TMS60142A	0.545777	0.643114	-0.097337	-17.83

Clustering Method = Group Average (Unweighted Pair-Group); Distance Type = Euclidean; Scale Type = Standard Deviation

Appendix 3: Hierarchical clustering report for comparison of genetic distances between pairs for the 21 elite Malawi cassava genotypes characterised using morphological characteristics (continues)

First Row	Second Row	Actual Distance	Dendrogram Distance	Actual Difference	Percent Difference
13=MK95/054	17=Mbundumali	0.618853	0.643114	-0.024261	-3.92
13=MK95/054	18=CH92/077	0.564933	0.538321	0.026612	4.71
13=MK95/054	19=Maunjili	0.483779	0.475552	0.008227	1.70
13=MK95/054	20=Silira	0.505291	0.538321	-0.033030	-6.54
13=MK95/054	21=Chitembwere	0.525924	0.538321	-0.012397	-2.36
14=83350	15=LCN8010	0.545777	0.495376	0.050401	9.23
14=83350	16=TMS60142A	0.618853	0.643114	-0.024261	-3.92
14=83350	17=Mbundumali	0.461266	0.643114	-0.181848	-39.42
14=83350	18=CH92/077	0.601417	0.538321	0.063096	10.49
14=83350	19=Maunjili	0.564933	0.495376	0.069557	12.31
14=83350	20=Silira	0.505291	0.538321	-0.033030	-6.54
14=83350	21=Chitembwere	0.635811	0.538321	0.097490	15.33
15=LCN8010	16=TMS60142A	0.618853	0.643114	-0.024261	-3.92
15=LCN8010	17=Mbundumali	0.583460	0.643114	-0.059654	-10.22
15=LCN8010	18=CH92/077	0.525924	0.538321	-0.012397	-2.36
15=LCN8010	19=Maunjili	0.437595	0.475552	-0.037957	-8.67
15=LCN8010	20=Silira	0.505291	0.538321	-0.033030	-6.54
15=LCN8010	21=Chitembwere	0.564933	0.538321	0.026612	4.71
16=TMS60142A	17=Mbundumali	0.505291	0.525534	-0.020243	-4.01
16=TMS60142A	18=CH92/077	0.668437	0.643114	0.025324	3.79
16=TMS60142A	19=Maunjili	0.668437	0.643114	0.025324	3.79
16=TMS60142A	20=Silira	0.714590	0.643114	0.071476	10.00
16=TMS60142A	21=Chitembwere	0.699544	0.643114	0.056430	8.07
17=Mbundumali	18=CH92/077	0.525924	0.643114	-0.117190	-22.28
17=Mbundumali	19=Maunjili	0.635811	0.643114	-0.007303	-1.15
17=Mbundumali	20=Silira	0.583460	0.643114	-0.059654	-10.22
17=Mbundumali	21=Chitembwere	0.601417	0.643114	-0.041697	-6.93
18=CH92/077	19=Maunjili	0.461266	0.538321	-0.077056	-16.71
18=CH92/077	20=Silira	0.525924	0.515607	0.010316	1.96
18=CH92/077	21=Chitembwere	0.505291	0.515607	-0.010316	-2.04
19=Maunjili	20=Silira	0.601417	0.538321	0.063096	10.49
19=Maunjili	21=Chitembwere	0.583460	0.538321	0.045139	7.74
21=Chitembwere	21=Chitembwere	0.483779	0.483779	0.000000	0.00

Clustering Method = Group Average (Unweighted Pair-Group); Distance Type = Euclidean; Scale Type = Standard Deviation

Appendix 4: **Hierarchical clustering report for comparison of genetic distances between pairs for the 16 elite Malawi cassava genotypes characterised using AFLP fingerprinting**

First Row	Second Row	Actual Distance	Dendrogram Distance	Actual Difference	Percent Difference
1= CH92/112	2=Mkondezi	0.541289	0.566985	-0.025696	-4.75
1= CH92/112	3=TME1	0.517219	0.566985	-0.049765	-9.62
1= CH92/112	4=CH92/105	0.677199	0.628867	0.048332	7.14
1= CH92/112	5=30786	0.581016	0.566985	0.014031	2.41
1= CH92/112	6=CH92/082	0.613022	0.592908	0.020114	3.28
1= CH92/112	7=TMS60121	0.628414	0.592908	0.035506	5.65
1= CH92/112	8=Gomani	0.628414	0.566985	0.061430	9.78
1= CH92/112	9=83350	0.575509	0.628867	-0.053359	-9.27
1= CH92/112	10=LCN8010	0.613022	0.592908	0.020114	3.28
1= CH92/112	11=TMS60142A	0.633462	0.628867	0.004594	0.73
1= CH92/112	12=Mbundumali	0.643438	0.628867	0.014571	2.26
1= CH92/112	13=CH92/077	0.681886	0.628867	0.053018	7.78
1= CH92/112	14=Maunjili	0.628414	0.592908	0.035506	5.65
1= CH92/112	15=Silira	0.633462	0.592908	0.040554	6.40
1= CH92/112	16=Chitembwere	0.653262	0.628867	0.024395	3.73
2=Mkondezi	3 =TME1	0.390981	0.390981	0.000000	0.00
2=Mkondezi	4=CH92/105	0.575509	0.628867	-0.053359	-9.27
2=Mkondezi	5=30786	0.498405	0.478436	0.019970	4.01
2=Mkondezi	6=CH92/082	0.558661	0.592908	-0.034248	-6.13
2=Mkondezi	7=TMS60121	0.618195	0.592908	0.025287	4.09
2=Mkondezi	8=Gomani	0.478852	0.512907	-0.034055	-7.11
2=Mkondezi	9=83350	0.607805	0.628867	-0.021063	-3.47
2=Mkondezi	10=LCN8010	0.591877	0.592908	-0.001031	-0.17
2=Mkondezi	11=TMS60142A	0.602542	0.628867	-0.026325	-4.37
2=Mkondezi	12=Mbundumali	0.602542	0.628867	-0.026325	-4.37
2=Mkondezi	13=CH92/077	0.602542	0.628867	-0.026325	-4.37
2=Mkondezi	14=Maunjili	0.575509	0.592908	-0.017400	-3.02
2=Mkondezi	15=Silira	0.623326	0.592908	0.030417	4.88
2=Mkondezi	16=Chitembwere	0.591877	0.628867	-0.036990	-6.25
3=TME1	4=CH92/105	0.575509	0.628867	-0.053359	-9.27
3=TME1	5=30786	0.458466	0.478436	-0.019970	-4.36
3=TME1	6=CH92/082	0.535373	0.592908	-0.057535	-10.75
3=TME1	7=TMS60121	0.586472	0.592908	-0.006437	-1.10
3=TME1	8=Gomani	0.478852	0.512907	-0.034055	-7.11
3=TME1	9=83350	0.607805	0.628867	-0.021063	-3.47
3=TME1	10=LCN8010	0.581016	0.592908	-0.011892	-2.05
3=TME1	11=TMS60142A	0.623326	0.628867	-0.005542	-0.89
3=TME1	12=Mbundumali	0.643438	0.628867	0.014571	2.26
3=TME1	13=CH92/077	0.643438	0.628867	0.014571	2.26
3=TME1	14=Maunjili	0.564333	0.592908	-0.028576	-5.06
3=TME1	15=Silira	0.613022	0.592908	0.020114	3.28
3=TME1	16=Chitembwere	0.602542	0.628867	-0.026325	-4.37

Clustering Method = Group Average (Unweighted Pair-Group); Distance Type = Euclidean; Scale Type = Standard Deviation

Appendix 4: **Hierarchical clustering report for comparison of genetic distances between pairs for the 16 elite Malawi cassava genotypes characterised using AFLP fingerprinting (continues)**

First Row	Second Row	Actual Distance	Dendrogram Distance	Actual Difference	Percent Difference
4=CH92/105	5=30786	0.643438	0.628867	0.014571	2.26
4=CH92/105	6=CH92/082	0.633462	0.628867	0.004594	0.73
4=CH92/105	7=TMS60121	0.658119	0.628867	0.029252	4.44
4=CH92/105	8=Gomani	0.541289	0.628867	-0.087579	-16.18
4=CH92/105	9=83350	0.618195	0.592716	0.025480	4.12
4=CH92/105	10=LCN8010	0.569948	0.628867	-0.058919	-10.34
4=CH92/105	11=TMS60142A	0.569948	0.548267	0.021681	3.80
4=CH92/105	12=Mbundumali	0.569948	0.548267	0.021681	3.80
4=CH92/105	13=CH92/077	0.581016	0.548267	0.032749	5.64
4=CH92/105	14=Maunjili	0.638470	0.628867	0.009602	1.50
4=CH92/105	15=Silira	0.662941	0.628867	0.034073	5.14
4=CH92/105	16=Chitembwere	0.472155	0.548267	-0.076112	-16.12
5=30786	6=CH92/082	0.504754	0.592908	-0.088154	-17.46
5=30786	7=TMS60121	0.523341	0.592908	-0.069568	-13.29
5=30786	8=Gomani	0.581016	0.512907	0.068109	11.72
5=30786	9=83350	0.681886	0.628867	0.053018	7.78
5=30786	10=LCN8010	0.564333	0.592908	-0.028576	-5.06
5=30786	11=TMS60142A	0.628414	0.628867	-0.000453	-0.07
5=30786	12=Mbundumali	0.677199	0.628867	0.048332	7.14
5=30786	13=CH92/077	0.638470	0.628867	0.009602	1.50
5=30786	14=Maunjili	0.569948	0.592908	-0.022960	-4.03
5=30786	15=Silira	0.552931	0.592908	-0.039978	-7.23
5=30786	16=Chitembwere	0.648369	0.628867	0.019501	3.01
6=CH92/082	7=TMS60121	0.444356	0.444356	0.000000	0.00
6=CH92/082	8=Gomani	0.591877	0.592908	-0.001031	-0.17
6=CH92/082	9=83350	0.662941	0.628867	0.034073	5.14
6=CH92/082	10=LCN8010	0.552931	0.571632	-0.018701	-3.38
6=CH92/082	11=TMS60142A	0.628414	0.628867	-0.000453	-0.07
6=CH92/082	12=Mbundumali	0.648369	0.628867	0.019501	3.01
6=CH92/082	13=CH92/077	0.618195	0.628867	-0.010672	-1.73
6=CH92/082	14=Maunjili	0.511025	0.501499	0.009526	1.86
6=CH92/082	15=Silira	0.517219	0.517195	0.000024	0.00
6=CH92/082	16=Chitembwere	0.628414	0.628867	-0.000453	-0.07
7=TMS60121	8=Gomani	0.658119	0.592908	0.065211	9.91
7=TMS60121	9=83350	0.638470	0.628867	0.009602	1.50
7=TMS60121	10=LCN8010	0.523341	0.571632	-0.048291	-9.23
7=TMS60121	11=TMS60142A	0.613022	0.628867	-0.015845	-2.58
7=TMS60121	12=Mbundumali	0.653262	0.628867	0.024395	3.73
7=TMS60121	13=CH92/077	0.633462	0.628867	0.004594	0.73
7=TMS60121	14=Maunjili	0.491974	0.501499	-0.009526	-1.94
7=TMS60121	15=Silira	0.511025	0.517195	-0.006170	-1.21
7=TMS60121	16=Chitembwere	0.672480	0.628867	0.043613	6.49

Clustering Method = Group Average (Unweighted Pair-Group); Distance Type = Euclidean;
Scale Type = Standard Deviation

Appendix 4: **Hierarchical clustering report for comparison of genetic distances between pairs for the 16 elite Malawi cassava genotypes characterised using AFLP fingerprinting (continues)**

First Row	Second Row	Actual Distance	Dendrogram Distance	Actual Difference	Percent Difference
8=Gomani	9=83350	0.667727	0.628867	0.038860	5.82
8=Gomani	10=LCN8010	0.591877	0.592908	-0.001031	-0.17
8=Gomani	11=TMS60142A	0.591877	0.628867	-0.036990	-6.25
8=Gomani	12=Mbundumali	0.581016	0.628867	-0.047851	-8.24
8=Gomani	13=CH92/077	0.591877	0.628867	-0.036990	-6.25
8=Gomani	14=Maunjili	0.638470	0.592908	0.045561	7.14
8=Gomani	15=Silira	0.662941	0.592908	0.070032	10.56
8= Gomani	16=Chitembwere	0.547141	0.628867	-0.081727	-14.94
9=83350	10=LCN8010	0.623326	0.628867	-0.005542	-0.89
9=83350	11=TMS60142A	0.602542	0.592716	0.009827	1.63
9=83350	12=Mbundumali	0.569948	0.592716	-0.022768	-3.99
9=83350	13=CH92/077	0.591877	0.592716	-0.000839	-0.14
9=83350	14=Maunjili	0.667727	0.628867	0.038860	5.82
9=83350	15=Silira	0.662941	0.628867	0.034073	5.14
9=83350	16=Chitembwere	0.581016	0.592716	-0.011700	-2.01
10=LCN8010	11=TMS60142A	0.597234	0.628867	-0.031634	-5.30
10=LCN8010	12=Mbundumali	0.618195	0.628867	-0.010672	-1.73
10=LCN8010	13=CH92/077	0.597234	0.628867	-0.031634	-5.30
10=LCN8010	14=Maunjili	0.613022	0.571632	0.041390	6.75
10=LCN8010	15=Silira	0.597234	0.571632	0.025602	4.29
10=LCN8010	16=Chitembwere	0.618195	0.628867	-0.010672	-1.73
11=TMS60142A	12=Mbundumali	0.529391	0.497376	0.032015	6.05
11=TMS60142A	13=CH92/077	0.541289	0.521088	0.020201	3.73
11=TMS60142A	14=Maunjili	0.653262	0.628867	0.024395	3.73
11=TMS60142A	15=Silira	0.638470	0.628867	0.009602	1.50
11=TMS60142A	16=Chitembwere	0.465361	0.497376	-0.032015	-6.88
12=Mbundumali	13=CH92/077	0.504754	0.521088	-0.016333	-3.24
12=Mbundumali	14=Maunjili	0.653262	0.628867	0.024395	3.73
12=Mbundumali	15=Silira	0.648369	0.628867	0.019501	3.01
12=Mbundumali	16=Chitembwere	0.451466	0.451466	0.000000	0.00
13=CH92/077	14=Maunjili	0.672480	0.628867	0.043613	6.49
13=CH92/077	15=Silira	0.618195	0.628867	-0.010672	-1.73
13=CH92/077	16=Chitembwere	0.517219	0.521088	-0.003868	-0.75
14=Maunjili	15=Silira	0.523341	0.517195	0.006146	1.17
14=Maunjili	16=Chitembwere	0.672480	0.628867	0.043613	6.49
15=Silira	16=Chitembwere	0.648369	0.628867	0.019501	3.01

Clustering Method = Group Average (Unweighted Pair-Group); Distance Type = Euclidean; Scale Type = Standard Deviation