ETHNO-BOTANICAL AND GENETIC VARIATIONS AMONG SWEETPOTATO ACCESSIONS GROWN IN MALAWI

BY

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DECLARATION

I, **FELISTUS PATIENCE NDINGO-CHIPUNGU,** do hereby declare that the work contained in this thesis is my own, unless otherwise stated, and has not been presented for any degree in any university or institution of learning.

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DEDICATION

This piece of work is dedicated to my husband **Amos Allan**, our two sons **Allan Tata** and **Kajiyani Jeremy** and our little daughter **Yankho**, the answer and our **Hope**. You all gave me every help and encouragement throughout the programme despite strained maternal love and guidance because of this academic assignment. I thank my **Lord** for all of you.

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SUMMARY

Sweetpotato (*Ipomoea batatas* L. Lam) is the second important root crop after cassava and one of the most widely grown crops for food and cash in Malawi. Production of the crop is done by small scale farmers in different environments where its lesser need for inputs gives it an important advantage over maize, the staple crop. Farmers grow many sweetpotato cultivars including landraces that are native in various communities but have not been exploited in the building up of a biological system capable of achieving a biological feasibility, consumer demand, and production economics in Malawi. Landraces contain some important traits, a genetic diversity that is useful for effective crop improvement. The location and measure of the extent of diversity in terms of indigenous knowledge, morphological, genetic, stability and nutritional variations among landraces is an important component in sweetpotato research and development. A study, which involved germplasm collection coupled with the compilation of indigenous knowledge was conducted in 2003 in some districts of the Northern (Chitipa, Karonga and Mzimba) and Southern (Nsanje, Chikwawa, Mulanje and Phalombe Districts) regions of Malawi. A total of 268 accessions were collected and planted at Bvumbwe Research Station during the 2004 and 2005 rainfall seasons for morphological, genetic diversity and nutritional analyses.

An analysis of indigenous knowledge revealed that farmers who produce sweetpotato for subsistence grew 8.86 ± 4.34 of cultivars in one field. The cultivars which varied by names, phenotypes and attributes were grown in mixtures to satisfy various requirements as determined by environmental and socio-economic differences. Meanings of cultivar names, which varied between study sites, revealed important insights of sweetpotato attributes which formed the basis for cultivar selection. Farmers and meanings of names characterized cultivars by agronomic (yield, earliness to maturity, root size, root numbers *etc.*), post-harvest (shelf life), gastronomic (taste, texture, aroma) and market (earliness to maturity, shelf life and gastronomic) values and differences.

Use of seven morphological traits and ten SSR primers showed significant morphological and genetic variation ($p < 0.05$) among three eco-geographical populations namely the Northern, South Eastern and the Lower Shire Valley of landraces. The Shire Valley population which gave a Shannon Weaver diversity index of 0.67 on morphological traits and Nei's genetic diversity of 0.37 on SSR markers exhibited highest morphological and genetic diversity implying an eco-geographical area for *in situ* conservation concentration.

Multilocation trials showed larger effects of environment (62.86%) on sweetpotato root yield (t/ha) than the genotypes (14.25%). The elite genotype, LU96/303 was the most stable across six evaluation sites: Bvumbwe, Makoka, Chitedze, Chitala, Lunyangwa and Baka Research stations. The elite genotype LU96/334 was the least stable. The variation in performance of the genotypes in different locations and seasons implies cultivar selection for specific environments. Alternatively, there is need for concerted efforts in the development of stable genotypes that will suit the varying production environments.

Nutritional assessment of 15 genotypes showed significant differences ($p< 0.05$) in concentration of β-carotene, vitamin C, iron, zinc and copper which were affected by storage root age and peeling. While cultivar Zondeni ranked the highest in β-carotene (13.93 mg/100g, unpeeled fresh roots), different genotypes attained different ranks for vitamin C, zinc, iron and copper. Naive and trained panels also ranked the 15 genotypes according to yield potential, texture, taste, aroma and colour suggesting farmers' involvement in variety development.

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LIST OF ACRONYMS AND ABBREVIATIONS

ADD	Agricultural Development Division
Admarc	Agricultural Development and Marketing Cooperation
AFLP	Amplified fragment length polymorphism
AMMI	Additive Main effects and Multiplicative Interaction
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials Standards
AVRDC	Asian Vegetable Research and Development Centre
β	Beta
^{o}C	Degrees Celsius
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Center
CIP	International Potato Center
cm	centimetre
CTAB	Cetyltrimethylammonium bromide
CU	Copper
CV	Coefficient of variation
D	Genetic diversity
DARS	Department of Agricultural Research Services
Df	Degrees of freedom
DM	Dry matter content
DNA	Deoxyribonucleic acid
dNTP	2'-deoxynucleoside 5'-triphosphate
DWB	Dry weight basis
EDTA	Ethylene-diaminetetraacetate
FAO	Food and Agricultural Organisation
Fe	Iron
FEWS	Famine Early Warning System
FWB	Fresh weight basis
g	Gram
G	Genotype
GD	Genetic distance
GDP	Gross domestic product
GoM	Government of Malawi
GEI	Genotype by environment interaction

ha	hectare
HYV	High yielding varieties
H’	Shannon-Weaver Diversity Index
IBPGR	International Board for Plant Genetic Resources
IITA	International Institute of Tropical Agriculture
IK	Indigenous knowledge
IPCA	Interactive principle component analysis
IPICS	International Programme in the Chemical Sciences
ISP	International Science Programme
Kg	Kilogram
L	Litre
L	Location
LRS	Lunyangwa Research Station
LSD	Least significant difference
LU	Lunyangwa Research Station
M	Metre
M	Molar
MoALD	Ministry of Agriculture and Livestock Development
MAP	Months after planting
MAS	Marker-assisted selection
Masl	Metres above sea level
MET	Multi-environment trials
Mg	Milligram
$MgCl_2$	Magnesium chloride
ml	millilitre
mm	millimetres
mM	Millimolar
MoALD	Ministry of Agriculture and Livestock Development
MS	Mean Square
NaCl	Sodium chloride
ng	Nanogram
NGO	Non-Governmental Organisation
nm	Nanometre
NS	Not significant
NSCM41	National Seed Company of Malawi 41
NTSYS	Numerical taxonomy multivariate analysis system
OD	Optical Density

PCA	Principle component analysis
PCR	Polymerase chain reaction
PGR	Plant genetic Resources
PGRFA	Plant genetic Resources for Food and Agriculture
pH	Power of hydrogen
PIC	Polymorphism Index Content
PPM	Parts per million
PPB	Participatory Plant Breeding
PVS	Participatory Variety Selection
R	Replication
RAE	Retinol Activity Equivalent
RAPD	Random amplified polymorphic DNA
RCBD	Randomised Complete Block Design
RDA	Recommended Dietary Allowance
RE	Retinol Equivalent
RSA	Republic of South Africa
SAHN	Sequential, Agglomerative, Hierarchical, and Nested
SARRNET	Southern Africa Root Crops Research Network
SD	Standard deviation
SE	Standard error
SIMINT	Similarity for Interval Data
SPSS	Statistical Package for Social Scientist
SS	Sums of squares
SSR	Simple sequence repeat
µg	Microgram
µl	Microlitre
t	ton
Taq	*Themophilus aquaticus*
TBE	tris-borate EDTA
TE	tris-EDTA
TSS	Total Sum of Squares
µg	microgram
µl	microlitre
UPGMA	Unweighted pair group method of arithmetic averages
UNEP	United Nations Environment Programme
UN-SCN	United Nations Standing Committee on Nutrition
UV/VIS	Ultraviolet and Visible Spectroscopy

VAD	Vitamin A deficiency
v/v	Volume per volume
WHO	World Health Organisation
w/v	Weight per volume

CHAPTER 1

GENERAL INTRODUCTION

1.1 Genetic diversity as a basis for crop improvement

Sweetpotato (*Ipomoea batatas* L. Lam) is one of the world's most important, versatile, and underexploited food crops (Scott and Maldonado, 1998). Worldwide, sweetpotato is a seventh most important staple crop on fresh-weight basis and a fifth crop in developing countries after rice, wheat, maize, and cassava (FAO, 2004). The bulk of the crop (82.7%) is grown in China (FAO, 2004). In Africa, sweetpotato production is concentrated in countries around Lake Victoria, with Uganda having an annual production of 2.6 metric tons (FAO, 2004).

In Malawi, sweetpotato is the second important root crop after cassava and the most widely spread crop grown throughout the country. To emphasize the importance of sweetpotato in Malawi, its production increased from 317,714 metric tones in 1995 to 1,494,112 metric tones in 2006 (FEWS/MoAFS, 1995; 2006). In 1997/98, the contribution of sweetpotato to national food energy was estimated at 17.8% (Akoroda, 1999).

Due to land pressure, cultivation by smallholders is expanding to marginal and less fertile areas leading to severe deforestation, soil erosion and general degradation of the natural resource base. It is estimated that 72 per cent of 1.8-2.0 million smallholder farm families in Malawi cultivate less than 1.0 ha, 41 per cent of these cultivate less than 0.5 ha with an average area of 0.28 ha. About six per cent of the households cultivate more than 2.0 ha (Malawi Government, 1999). Given the fact that much of the existing arable land is decreasing, genetic improvement of crops is considered as the most viable and sustainable approach to increase agricultural productivity (Tanksley and McCouch, 1997).

Effective crop improvement depends on the extent of genetic diversity in the gene pools. Systematic plant breeding over the past century has contributed tremendously to increased crop productivity accompanied by efficient utilisation of agricultural inputs (Warburton *et al.,* 2002). However, increased productivity has often resulted in decreased genetic diversity within gene pools (Fernie *et al.,* 2006) such as landraces which have a diverse genetic base but are rarely integrated into the plant breeding programs due to their low production performance.

1.2 The sweetpotato plant

1.2.1 Origin and distribution of the sweetpotato plant

Sweetpotato originates from Central America from where it spread into Europe and Africa during the big explorations (Purseglove, 1988). The greatest diversity of sweetpotato is found in the countries of Guatemala, Colombia, Ecuador and Peru (Austin, 1983). It was transported to some islands of the Pacific and became an important crop in New Zealand (Yen, 1974). Sweetpotato rapidly disseminated to all parts of the tropics with the establishment of the world commerce (Simmonds, 1979). The Portuguese carried the sweetpotato from the Caribbean and South America to Europe, Africa and Asia in the 17th and 18th and century (Yen, 1974).

1.2.2 Sweetpotato classification

I. batatas is a dicotyledonous perennial plant of the *Convolvulaceae* family (genus *Ipomea*), the morning glory (Austin, 1987) characterised by its succulent, edible tuberous storage roots. Sweetpotato belongs to the series Batatas which has 13 wild species that are considered to be related to it (Austin and Huaman, 1996). The genus *Ipomoea* is thought to contain over 500 species with ploidy levels ranging from 2x to 6x (Ozias-Akins and Jarret, 1994) sweetpotato being the only *Ipomoea* of economic importance as a food crop (Woolfe, 1992) with both 4x and 6x forms (2n=4x=60 or 2n=6x=90). *I. batatas* (L.) Lam probably originated from a cross between the ancestors of *Ipomoea trifida* (Jarret and Austin, 1994; Huang and Sun, 2000) and another wild *Ipomoea spp.*, in Central or northern South America, 5000 years ago, and is among the earliest domesticates of man.

1.3 Sweetpotato in the agricultural system of Malawi

1.3.1 Contribution of root and tuber crops to food security in Malawi

It is envisaged that self food sufficiency in Malawi can be achieved through among other things, more diversification of efficient and drought tolerant crops such as cassava and sweetpotato. Root and tuber crops produce large quantities of dietary energy and have stable yields under conditions in which other crops may fail. They produce remarkable quantities of energy per day in comparison to cereals. In addition, root and tubers are an important source of vitamins, minerals and essential amino acids such as lysine. They adapt well to marginal environments which accounts for a third of the land in Malawi.

Due to bad farming practices and increased pressure on arable land, soil fertility has declined and can only be restored through the application of chemical fertilizers or organic manure. Due to their high cost most rural farmers can not afford fertilizers to improve cereal yields. Furthermore, livestock that can produce manure is very limited in Malawi. Consequently, roots and tuber crops are being promoted because they do not require large volumes of fertilizers like maize and other cereals (Onweme, 1978).

Cassava and sweetpotato gained importance as food and cash crops following the drought years of the early 1990s when maize production was reduced by almost half of the normal production (MoALD, 1995). In 2004/05 season, root and tuber crops contributed 47% maize equivalent of food production (FAO, 2005). Thus while cereal production was 1.34 million metric tons, root and tuber crops production was 1.17 million metric tons of maize equivalent in the country. Similarly, in 2004, root and tuber crops accounted for 38% of total food production in Malawi (FAO, 2004).

1.3.2 Sweetpotato production trends in Malawi

Owing to the recurrent droughts and erratic rainfall pattern that the country has experienced in recent years, there have been consistent increases in hectarage, production and yield per unit area year on year from 1994 and peaked in 2001/002 (Table 1.1). The lesser need of inputs gives the sweetpotato an important advantage over the staple crop maize, which has been adversely affected by fertilizer price increases. The downward production trends from 2003 to 2005 are therefore consequences of fertilizer subsidies by Malawi Government with farmers switching to more maize production suggesting that sweetpotato is important in times of maize failure. Besides being a source of food, the crop is also used for cash generation. A crop budget study by Akoroda and Mwabumba (2000) showed that sweetpotato netted a profit of 102% of the invested cost of production. Apart from labour input, no fertiliser and chemicals are applied to the sweetpotato crop in Malawi, hence the high net profits. The leaves are an important source of vegetable for most rural households in the country. The vines also contribute to livestock feed.

Table 1.1 Sweetpotato production trends in Malawi (FEWS/MoAFS 1995 to 2005)

Year	Area (ha)	Production (tonnes)	Yield (t/ha)
1994/95	60,701	317,714	5.23
1995/96	68,804	596,469	8.66
1996/97	91,884	860,085	9.36
1997/98	136,709	1,447,994	10.59
1998/99	140,904	1,561,345	11.08
1999/20	166,251	1,966,833	11.83
2000/01	190,947	2,534,896	13.27
2001/02	219,766	2,783,307	12.66
2002/03	112,287	1,444,087	13.43
2003/04	147,000	1,573,000	11.00
2004/05	122,000	1,041,790	10.40

1.3.4 Constraints to sweetpotato production in Malawi

Although sweetpotato is adapted to many agro-ecologies (Ewell, 1990), the average yield in Malawi as is the case with the Region, is low (Abubakar, 1992). While sweetpotato yield on farmers fields ranged from 5 to 13t/ha (Table 1.1) over a 10 year period, the yield gap has not improved much when compared with yield on research fields which ranges between 20-30 t/ha (Chipungu *et al.,* 1999). Factors contributing to the low yields include; shortage of planting material, pests (sweetpotato weevil) and diseases, late planting due to shortage of labour force, use of low yielding and late maturing cultivars and poor cultural practices in general (Moyo *et al*., 1998). Sweetpotato is however one of the crops with highest yield potential per unit area among the major starch staple crops (Woolfe, 1992).

1.3.5 Sweetpotato breeding research in Malawi

The sweetpotato breeding program in Malawi has been designed to address some of the production constraints that contribute to low yields. The program focuses on agronomic performance in order to improve food security of the majority of Malawians. The success of the program has mainly been accomplished through the release of superior cultivars (Table 1.2). Introductions, initially from the International Institute of Tropical Agriculture (IITA) and currently, from International Potato Center (CIP) in Lima (Peru) and Nairobi (Kenya), Tanzania, South Africa and Asian Vegetable Research and Development Centre (AVRDC) have been sources of germplasm for yield evaluation. Of the seven officially released and improved sweetpotato varieties only one (Lunyangwa (LRS 407) is a local cross between two introduced varieties; SPN/O (Kemb 10) and Tomato Roma, the rest are introductions (Table 1.2). In terms of adoption, only the cultivar Kenya (Table 1.2) is widely spread; the other released cultivars have however not been successfully adopted probably due to consumer/farmer dissatisfaction with other attributes of the cultivars other than yield (Moyo *et al.,* 1998; 2004). Nweke *et al.,* (1994) indicated that some small farmers still prefer a selection of local landraces to improved cultivars, whereas breeding lines and improved varieties supplied by research do find their way to the farmer slowly.

Table 1.2 Improved and released sweetpotato cultivars in Malawi (Moyo *et al.,* 1998)

Varities	Yield (t/ha)	Origin	Remarks
Kenya (SPN/O, Kemb 10)	20-25	Tanzania	Released in 1988, widely grown.
Lunyangwa	20	Local bred	Released early 1990, low adoption
Kakoma (TIS 3017)	20-25	IITA	Released in 1994, low adoption
Semusa (Cemsa 74-288)	25-30	CIP	Released in 1999, Highest yielding among the released, being adopted
Mugamba (Mogamba)	20-25	CIP	Released in 1999, low adoption
Tainoni (Tainon 57)	20	AVRDC	Released in 1999, low adoption
Salera (CIP1941 121)	20-25	CIP	Released in 2002, low adoption

1.4 Problem statement

Poverty in Malawi as described by Malawi Government (GoM, 2002a) is widespread, deep and severe. An FAO report (2003) showed that 15 to 20 percent of the population was in a destitute situation as they had inadequate food throughout the year. The destitute mostly comprised the handicapped, elderly, child-headed households, and orphan-dominated households. The report also showed that 20 to 25 percent of the population was critically poor for they had inadequate food for about 6 to 9 months. The group had land holding sizes of about 0.5 to 0.8 ha and they grew local maize without fertilizer. Another 20 to 25 percent was moderately poor for they had inadequate food for 3 to 6 months, their landholding size was 0.8 to 1.0 ha and they grew hybrid and local maize varieties. Clearly, one of the key causes to poverty and food insecurity is shortage of land. Literature shows that 55 percent of the smallholder farmers had less than one hectare of cultivable land in 1998 (NSO, 1998). Land shortage is due to increase in population, which in 1998 was estimated at 11 million. As indicated by Rubey (2003), an over-emphasis on maize production simply perpetuates current cycles and does little to help rural smallholders escape from poverty. Even with significant yield increases, farmers with a hectare or less will never devote most of their land to maize for self sufficiency. Diversification of crops rather than increased maize production is the way off this poverty treadmill. Owing to the single rainy season lasting at best 4-5 months between November and March, sweetpotato has the advantage of having a short growth cycle of 4-5 months after planting to supplement/relay food availability in Malawi. As such, the sweetpotato is a suitable crop for poor resource farmers for food and cash income. The advantage is that sweetpotato is already a common crop in the multicropping system for subsistence and cash.

The conventional methodology of testing new varieties on-station, followed by multi-location on-farm trials assumes that researchers are the custodians of knowledge and the source of technical innovations; that extension is a delivery mechanism and that farmers are the ultimate users (Bruin and Meerman, 2001). However, to optimally exploit the crop's potential, there is need to look beyond the conventional research approach. Indigenous knowledge (IK) of traditional varieties of crops is a valuable complement to germplasm collection, maintenance and crop improvement (Cardoso *et al.*, 1998; Chiwona-Karltun *et al.*, 1998). An understanding of IK helps the research scientist to identify attributes of cultivars that are important to farmers, and thus worthy of consideration when releasing cultivars for production. It is envisaged that sweetpotato research and technology development will reduce both yield and knowledge gap between farmers and researchers and therefore exploiting the crops' potential in all aspects to achieve economic contributions in the country.

In Malawi, studies have shown that multiples of cultivars are grown to satisfy varying needs (Moyo *et al.,* 1998; 2004). Since farmers grow multiples of varieties, it is assumed that sweetpotato genetic diversity in the country exist, but probably not evenly distributed geographically due to the various social-economic, cultural, agro-ecological factors among societies. Other than farmers' direct use for food and feed, these landraces in Malawi have not been exploited in the building up of a biological system capable of achieving a biological feasibility, consumer demand, and production economics and therefore not yet effectively utilized in the improvement programs. Landraces are a useful resource in plant breeding as they are highly heterogeneous, having been selected for subsistence agricultural environments where low, but stable yields are important and natural environmental fluctuation requires a broad genetic base (McCouch, 2004).

In a well organised crop improvement program, it is essential to collect and conserve farmers' varieties before disseminating improved ones especially in areas where diversity is high. The reason for a prior collection is that improved varieties tend to replace folk varieties leading to genetic erosion. Gollin and Smale (1999) describe the concerns of high yielding varieties triggering genetic erosion, thereby affecting genetic diversity. In Mzimba, Lilongwe and Mulanje Districts of Malawi, an improved sweetpotato variety Kenya was reported to be grown by 49%, 57% and 76% of farmers respectively (Moyo *et al.,* 2004).

In order to maintain and exploit genetic resources efficiently, an understanding of the location, amount and distribution of genetic variation within and among accessions is an important step in any improvement program. Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including (i) analysis of genetic variability in cultivars (Smith, 1984; Cox *et al*., 1986), (ii) identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell, 1998), and (iii) introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al.,* 1998).

Sweetpotato in Malawi is grown in diverse and mostly in marginal environments by small-scale farmers using different varieties and cultural practices. Although sweetpotato is adapted to many agro-ecologies, the yield depends on management systems, environmental conditions and the variety amongst other factors (Ewell, 2000). Mulindangabo (1987) reported of sweetpotato yields ranging from 1.5 to 30.6 t/ha due to differences in soil fertility and crop management in Rwanda. Other studies on several traits have also shown that sweetpotato is sensitive to environmental variation (Naskar and Singh, 1992; Ngeve, 1993). Farmers select cultivars that perform in their environment. Generation of superior and broadly adapted cultivars for diverse environments has generally been difficult partly due to inadequate definition of selection criteria by breeders who may not be familiar with the requirements of the farmers (Hardon and de Boef, 1993).

Sweetpotato (orange fleshed) is an important crop in the provision of essential nutrients particularly vitamin A. Reports indicate that an estimated 250 million children in the developing countries, Malawi inclusive are vitamin A-deficient, putting them at risk not only for night blindness, but also for other highly infectious diseases (CIP Annual Report, 1998). The extent of vitamin A deficiency in Malawi has been brought to light through the efforts of the Ministry of Health and Population, with support from UNICEF, who distributes vitamin A supplement to children that are at risk in Malawi. However, the distribution of vitamin A capsules to malnourished children and lactating mothers is a short-term, limited and unsustainable solution because of its complete dependence on donor funds. A more viable and sustainable way would be the adoption and consumption of orange-fleshed sweetpotato varieties which are being promoted to alleviate vitamin A deficiency (CIP, 1999). Regular intakes of (100 grams per day) of range-fleshed sweetpotato roots having moderate β-carotene concentrations (3mg/100g) provide the required daily allowances of VA for children under five years of age (Tsou and Hong, 1992). In addition, sweetpotato roots are rich in vitamin C and other mineral salts. Exploiting the genetic variation for micronutrient density in a crop is one of the most powerful tools to change the nutrient balance of a given diet on a large scale. The role of plant breeding in micronutrient malnutrition is the identification and deliverance of nutrient density traits into cultivars of highest yield and organoleptic preference for a crop such as sweetpotato in Malawi. Therefore, efforts have to be done in breeding in order to maintain progress in both yield and nutritional quality concurrently.

Characterization of landraces in an effort to conserve and generate the necessary knowledge gap has not been carried out on sweetpotato in Malawi. This study therefore engaged the application of ethno-botanical, morphological, molecular (DNA-based), adaptational, nutritional and preference analyses to generate information that will facilitate the exploitation of the crops' potential, ultimately achieving economic contributions in the country through improved sweetpotato yields per unit area and time, widespread production and increased consumption of preferred and nutrient dense sweetpotato varieties.

1.5 General objective of the study

The general objective of the study was to develop a framework for sweetpotato genetic improvement in Malawi in order to increase economic returns in terms of yield and quality while conserving the biodiversity that currently prevails in the landraces of the crop. The development of a comprehensive, well documented and accessible germplasm and willingness of investing resources in the long-term programs of germplasm diversification need to be the essential purposes of plant breeding. Conventional sweetpotato improvement programs with the aid of biotechnological tools can improve the low yields of sweetpotato in Malawi. Various studies for the framework development were conducted.

In chapter two, germplasm collection and IK surveys which were conducted in seven districts of Malawi are reported. The aim of the IK study was to determine ethno-botanic variation of sweetpotato cultivars grown by farmers in various areas. IK results revealed farmers' classifications of cultivars. Meanings of names of cultivars cultivar characteristics. The accessions which were planted at Bvumbwe Research Station revealed geographical variation using morphological traits.

Revelations of genetic diversity of sweetpotato accessions in Malawi using SSR are presented in chapter three. Relationships among accessions using genetic distances and clusters were established. Distantly related accessions were delineated for use in the improvement and conservation strategies.

Yield superiority and stability of elite clones in six agro-ecological zones of Malawi are reported in chapter four. Evaluation sites were grouped together for effective evaluation for yield performance.

Genotypic variations in amounts of iron, zinc, copper, beta-carotene and vitamin C as the first step in sweetpotato bio-fortification programs are reported in chapter five. Peeled and unpeeled storage root samples of 15 genotypes were evaluated for these nutrients. Estimates of adequacy of each nutrient for Recommended Daily Allowance (RDA) are also reported for different age groups.

Studies on cultivar preference involving naive and trained panelists on 15 genotypes on organoleptic and agronomic traits are reported in chapter six. Farmers' role in germplasm management and evaluation in Malawi are discussed.

Conclusions and recommendations for further studies are presented in chapter seven.

CHAPTER 2

ETHNO-BOTANICAL AND MORPHOLOGICAL VARIATIONS IN SWEETPOTATO ACCESSIONS

Abstract

This study was conducted in order to better understand the potential of indigenous knowledge (IK) mainly regarding preferences, names, classifications and utilization of farmers' sweetpotato varieties, and thereby subsequently, improve the contribution of agricultural research to farmers' livelihoods. Conducted in 2003, the study which involved a germplasm collection survey, coupled with the compilation of indigenous knowledge covered Chitipa, Karonga, Mzimba, Nsanje, Chikwawa, Mulanje and Phalombe Districts of Malawi. A total of 268 accessions were collected and planted at Bvumbwe Research Station in 2004 and 2005 to determine morphological trait variations. The survey results revealed that farmers who grew sweetpotato for subsitence had 8.86±3.43 cultivars grown in a mixture. The cultivars grown diferred by names, phenotypes and attributes. The naming of cultivars was itself found to be unreliable even within an ethnic group, as some cultivars shared names, while in some cases a cultivar was known by more than one name, indicative of potential duplications in the collection. Nonetheless, it was found that despite these potential difficulties, the meanings given to cultivars entailed varietal characteristics. In terms of attributes, the various cultivars were characterised by agronomic (yield, earliness to maturity, root size, root numbers *etc.*), post-harvest (shelf life), gastronomic (taste, texture, aroma) and market (earliness to maturity, shelf life and gastronomic) values and differences to satisfy growers requirements as determined by environmental and socio-economic differences. Similarities in preferences for cultivar attributes were detectable among the study areas. Morphological analysis using seven traits showed significant variation within and among the accessions which were grouped into three eco-geographical sources of Malawi namely Northern, South Eastern and Lower Shire Valley populations ($p < 0.05$). While some morphological classes within traits occurred at high frequencies, some occurred at low and some were totally missing in other populations. The Shannon Weaver diversity index showed that the Shire Valley population had the highest Shannon Weaver diversity index (0.67) providing information on geographical areas that have high phenotypic indices which can be centers for collection concentration and *in-situ* conservation. Studies of the kind reported in this paper can help foster interaction and complimentarity between formal breeding by scientists and the expertise of farmers, areas which indeed require further exploration.

Key words: sweetpotato, Malawi, germplasm, IK, morphological traits

2.1 Introduction

Plant genetic resources for food and agriculture (PGRFA) which comprise diversity of genetic material contained in landraces, modern cultivars, wild relatives and other wild species are the basis of global food security (Rao, 2004). Genetic diversity provides farmers and plant breeders with options to develop, through selection and breeding, new and more productive crops resistant to virulent pests and diseases and adapted to changing environments (Rao, 2004). International development agencies, believe that the breeding of new crops is important for ensuring food security and developing practices of sustainable agriculture through the development of crops suitable for different environments. One approach for building a germplasm collection is to collect material from diverse geographical origins with a concentration of accessions from proposed centres of diversity. The determination of the extent of phenotypic and genotypic diversity in a germplasm collection provides an indication of redundancies or exclusion and can aid in managing a core collection. In the following literature review, the rationale for studying and conserving the genetic diversity and associated indigenous knowledge (IK) of crops in general, and sweetpotato in particular, and their value to plant breeding programs is highlighted. Various approaches of diversity measurements used in the current research study are highlighted.

2.2 Literature review

2.2.1 Genetic diversity

Genetic diversity refers to the variety of genes that exist in a particular organism or species (Long *et al.,* 2000). Genetic diversity is the raw material on which agriculture develops crops, utilizing important characteristics for pest resistance, nutritional quality and other factors, to meet both predictable and unforeseen ecological and economic circumstances (Cooper *et al*., 1992). This diversity, that includes landraces, provides unique traits not present in existing cultivars or breeding materials (Plucknett *et al.,* 1987). The unique traits may include security against pests and unfavourable climatic conditions. Such traits ensure provide a wealth of plant material with varied usefulness.

2.2.2 Importance of genetic diversity to plant breeding

In any plant improvement program, crop breeders require germplasm that constitute the genetic variability of traits such as yield and yield components, resistance/tolerance to pests, diseases, droughts or other adverse conditions, maturity periods, quality and others. Genetic uniformity in crops is in most cases undesirable in terms of firstly, vulnerability of the crop to epidemics and environmental disasters (Ko *et al.,* 1994) and secondly, breeders are faced with a limited source of desired traits for improving varietal characteristics. It is well known that a narrow genetic base leads to genetic homogeneity which renders the crop to be vulnerable. Examples of vulnerability due to genetic homogeneity are the outbreaks of severe epidemics such as corn leaf blight in the United States of America (USA) and pearl millet downy mildew in India caused by extensive use of a single source of male sterility that became susceptible to disease (Arunachalam, 2000).

Crop breeding programs which are genetically broad based provide continuous gains under selection (Smith and Duvick, 1989), while the narrowly based one provide a slow response to selection and increases the likelihood of crises triggered by outbreaks of disease and insects. A good example involve a commercial sunflower industry of Australia, China, South Africa, India and the United States which was threatened by new races of rust and have been resolved by transferring resistance genes from ancient sunflower variety cultivated by Indian farmers in South-Western USA (Anonymous, 1993). Some genes have been found to be valuable and economically important in some crops. For instance, a single wild tomato species contributed valuable genes to tomato and added value worth US$ 8 million per annum to US tomato processor (UNEP, 1995).

2.2.3 Value of local varieties to farmers

Landraces also known as traditional, local or primitive varieties have been defined as geographically or ecologically distinctive populations which are conspicuously diverse in their genetic composition both between and within populations (Brown, 1978). The genetic composition of landraces is shaped by household agronomical practices and natural selection pressure over generations of cultivation (Smale *et al.,* 1999). Although high-yielding, modern varieties of staple crops exist in the world today, farmers in cradles of crop genetic diversity continue to plant landraces (Bellon and Brush, 1994; Meng, 1997). Farmers that utilise these unimproved varieties are resource poor who are ecologically and economically marginalised as they live in low- potential areas with less access to markets and are confronted with other developmental restrictions (Wolfe, 1985). Farmers retain these landraces for various reasons which includes; storage, cooking, nutritional and processing qualities, market, resistance to local biotic and abiotic stresses (Bellon, 1991). These local varieties reveal a significant comparative advantage for the ecologically marginalised areas where modern plant breeding has failed to offer more profitable alternatives to farmers' varieties (Ceccarelli, 1993).

2.2.4 Extent of sweetpotato diversity

Sweetpotato give rise to large numbers of landraces grown in Africa, Asia and Oceania (Yen, 1974; Carey,1996) due to its characteristic of being an obligate out crossing and capacity to flower and set seeds (Hernandez and Miller, 1964); unstable and tendency to mutate (Collins and Cannon, 1983). Based on the large numbers of landraces and their adaptation to local conditions Eastern Africa is considered the secondary centre of diversity for sweetpotato (IBPGR, 1981). Presently, many cultivars are grown throughout the world and they vary in many attributes including leaf shape, colour and size, floral morphology, vigour, maturity period (3.5-6 months), storage root shape, colour, size, yield, food value (e.g. carotene content), taste, processing characteristics such as ease of slicing and drying, flour quality, resistance to insect pests and diseases, adaptation to different soils or agro-ecologies and consumer acceptance (Bashaasha *et al.,* 1995). As of 1998, CIP had 5,526 cultivated accessions from 57 countries, 2,589 of them from Latin America (Zhang *et al.,* 1998). This chapter report of 268 accessions collected from seven districts of Malawi which were planted at Bvumbwe Research Station for morphological and molecular characterization.

2.2.5 Rationale for preserving genetic diversity and its associated IK

Genetic diversity is disappearing at unprecedented rates (Ehrlich and Wilson, 1991). Gollin and Smale (1999) and Chang (1984) describe the concerns affecting genetic diversity where high yielding varieties are triggering genetic erosion and genetic uniformity resulting in the increased virulence of pests and pathogens, and the multifaceted depletion of the genetic diversity available to agriculture. It has further been argued (Avery, 1997) that habitat loss is the most important contributing factor to species extinction. This destruction threatens human civilisation which depends in numerous ways on the biological diversity (Ehrlich and Wilson, 1991). The Leipzig Declaration, under which governments committed themselves to implementing the Global Plan of Action, emphasized the need to enhance food security through conserving and sustainably using Plant Genetic Resources (PGR) which constitutes genetic diversity (Cooper *et al.,* 1998). Sustainable use includes incorporation of these resources to their direct use.

However, as efforts to collect landraces are being made, very little cultural information is included in the data collection. As such with time, there shall be the germplasm available but the indigenous knowledge that shaped the cultivar selection will not be available. Knight (1980) called for the systematic documentation of traditional farmers' knowledge into "information bank" from which agronomists, extension workers, and other farmers draw enlightenment.

2.2.6 Importance of Indigenous knowledge

Proper characterisation and evaluation of germplasm collections are important components of effective management of genetic resources (Frankel, 1989; Blakeney, 2002) by eliminating unnecessary duplications (Greene and Pederson, 1996). The main methods of diversity characterisation are IK, morphological and molecular tools.

IK is local knowledge unique to a given culture or society and that is contained in the heads of the farmers and agricultural workers (Kloppenburg, 1991; Warren and McKiernan, 1995). Ethno-botanical methods are used to investigate IK from local people, for instance, on how they characterise and value genetic diversity and how their practices and cultures maintain genetic resources and shape crop evolution. Previously disregarded by scientists, indigenous knowledge is now seen as a valuable resource that complements biological analyses in the identification of where and how variation and genetic diversity in crops is found and valued by farmers. Ethno-botanical research on genetic diversity provides information to research programs in locating, collecting, conserving, characterising and evaluating genetic resources. Ethno-botanical methods of analysis include field observation, in-depth interviews, participant observation, participative technology analysis, surveys, group discussions, village workshop and use of diagrams (Warren and McKiernan, 1995).

2.2.7 Morphological characters

Morphological characterisation is the first step in the description and classification of germplasm (Smith and Smith, 1989). They are markers that plant breeders have relied on before the development of molecular markers. The application of morphological characters has been used as a powerful tool in the classification of lines, to study taxonomic status, identification, determination of genetic variation and correlation of characters with agronomic potential (Millan *et el.,* 1996). In Malawi, phenotypic traits are still important to breeders as a cost effective marker especially where the capacity to use molecular markers in breeding programs is not yet fully developed.

Morphological characterisation is done to identify morphotypes. A morphotype is a group of plants showing morphological similarities, apparently of the same phenotype, but not necessarily of the same genetic constitution (Arbizu *et al.,* 1996). A duplicate identification assessment based on morphological characteristics using a descriptor list has been developed on sweetpotato accessions (Huaman, 1991). The shape of the mature leaves, the pigmentation of abaxial leaf, petiole pigmentation and length, vine internode diameter and length, vine pigmentation, plant type, leaf colour, storage root skin and flesh colour have been used as morphological indicators on sweetpotato (Mok and Schmiediche, 1998). Duplicate accessions of sweetpotato have been routinely identified at CIP where for instance, the Peruvian accessions maintained in the field gene bank held at CIP has so far reduced the size of the collection from 1939 to 909. In other crops, various numerical traits have been successfully used to classify and measure the patterns of phenotypic diversity in the relationships of species and germplasm collections of a variety of crops (Revilla and Tracy, 1995). The advantage of duplicate identification in a collection is the reduction in redundancy that ultimately save management and operating costs of germplasm banks.

2.2.8 The concept of core collection

The concept of core collection was introduced to rationalise the evaluation of a collection and to improve the use of germplasm (Frankel and Brown, 1984). Characterisation and evaluation of large collections is costly and time-consuming (Frankel, 1989). As such, there is need for reducing it by establishing a core collection that is manageable but satisfies the need of the breeders and other users. The core collection consists of a limited set of accessions derived from the existing germplasm collections chosen to represent the genetic spectrum in the whole collection (Brown, 1995). Arbizu *et al.* (1996) reported systematic activities involved in coming up with a core collection (Figure 2.1) using Andean Root and Tuber Crops (ARTC). Thus, after duplicate identification, further studies on genetic diversity and evaluations such as agronomic characters, nutrition, reaction to biotic and abiotic factors *etc.* can be done to establish a core collection with the widest diversity representation in terms of morphology, geographical coverage and genes. Duplicate analysis is mostly based on phenotypic values: frequencies, mean, range, variance, Shannon index *etc.* (Galway, 1995).

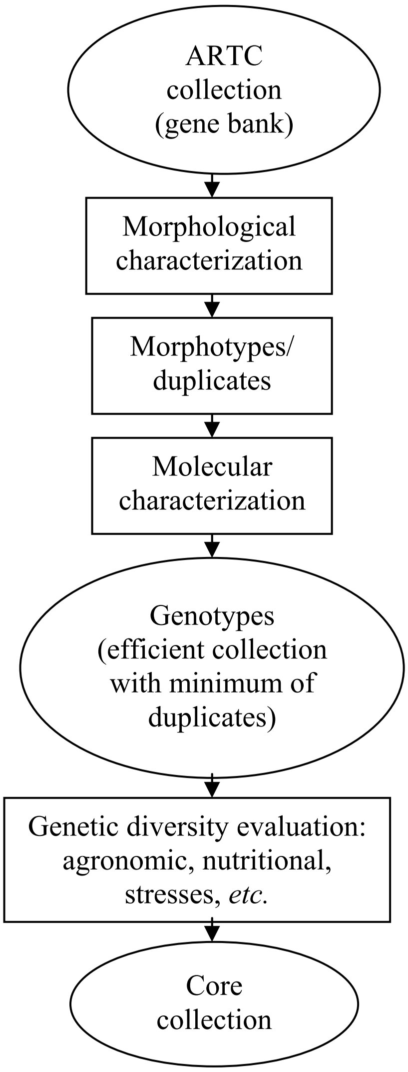

Figure 2.1 Strategy for handling collections of Andean root and tuber crops.

2.3 Justification of the study

There is a worldwide concern over the loss of diversity of plant genetic resources. A particular worry is the substitution of a diverse set of genetically variable crop landraces by a few genetically uniform modern varieties (Brush, 1991). Amidst these concerns, efforts to conserve sweetpotato landrace diversity and associated local knowledge are not yet in place in Malawi. This study was therefore initiated to collect and characterise sweetpotato landraces and other varieties in the country using farmers' knowledge. To determine geographical variations in terms of locality and quantity, morphological analysis was done as the first step in the conservation and maintenance of germplasm.

2.3.1 General objective

The general objective of the study was to characterise sweetpotato germplasm in Malawi using farmers' indigenous knowledge and morphological traits.

2.3.2 Specific objectives

(i) To determine the important traits of varieties grown on a single farm and explore possible complementarities among the traits

(ii) To determine between farm variation in terms of varietal combinations and the effect of geographical distance

(iii) To locate and quantify morphological diversity of sweetpotato accessions within and among geographical sources of origins

2.4 Materials and Methods

2.4.1 Germplasm collection and indigenous knowledge survey

Prior information on areas of high sweetpotato production and varietal diversity areas was obtained from Karonga, Mzuzu, Blantyre and Shire Valley Agricultural Development Divisions (ADDs, Figure 2.2). All the descriptors of the collections were put in passport data according to CIP standard descriptor (Huaman, 1991). Documentation of indigenous knowledge involved interviews of open-ended questions (Chiona-Kartun *et al.,* 1998) on the cultivar names and their meanings, cultivar attributes, preferences and reasons, uses, advantages and disadvantages of various sweetpotato cultivars, cropping systems and field sizes allocated to sweetpotato relative to other crops, gender roles in sweetpotato production, ranking of crops in food and cash importance and cash generated through sales (Appendix I). An open ended format was used to easily gather the scope of knowledge to be incorporated in sweetpotato improvement program as opposed to use of a questionnaire only which may not recognise the value of perspectives (Huntington, 1998).

This method of gathering information has been successfully used in the context of IK documentation (Brooke, 1993; Ferguson and Messier, 1997). The IK survey was conducted in May 2003 in Chitipa, Karonga and Mzimba districts and in July 2003 in Chikwawa, Nsanje, Mulanje and Phalombe districts (Figure 2.2) covering ten ethnic groups (Table 2.1) and 20 farmers in each district. In a study area, farmers were randomly sampled after every ten kilometers. Sweetpotato germplasm was collected from three districts of the Northern Region, two of the South Eastern Region and another two of the far Southern Regions of Malawi referred to as the Lower Shire Valley (Tables 2.1 and 2.2 and Figure 2.2).

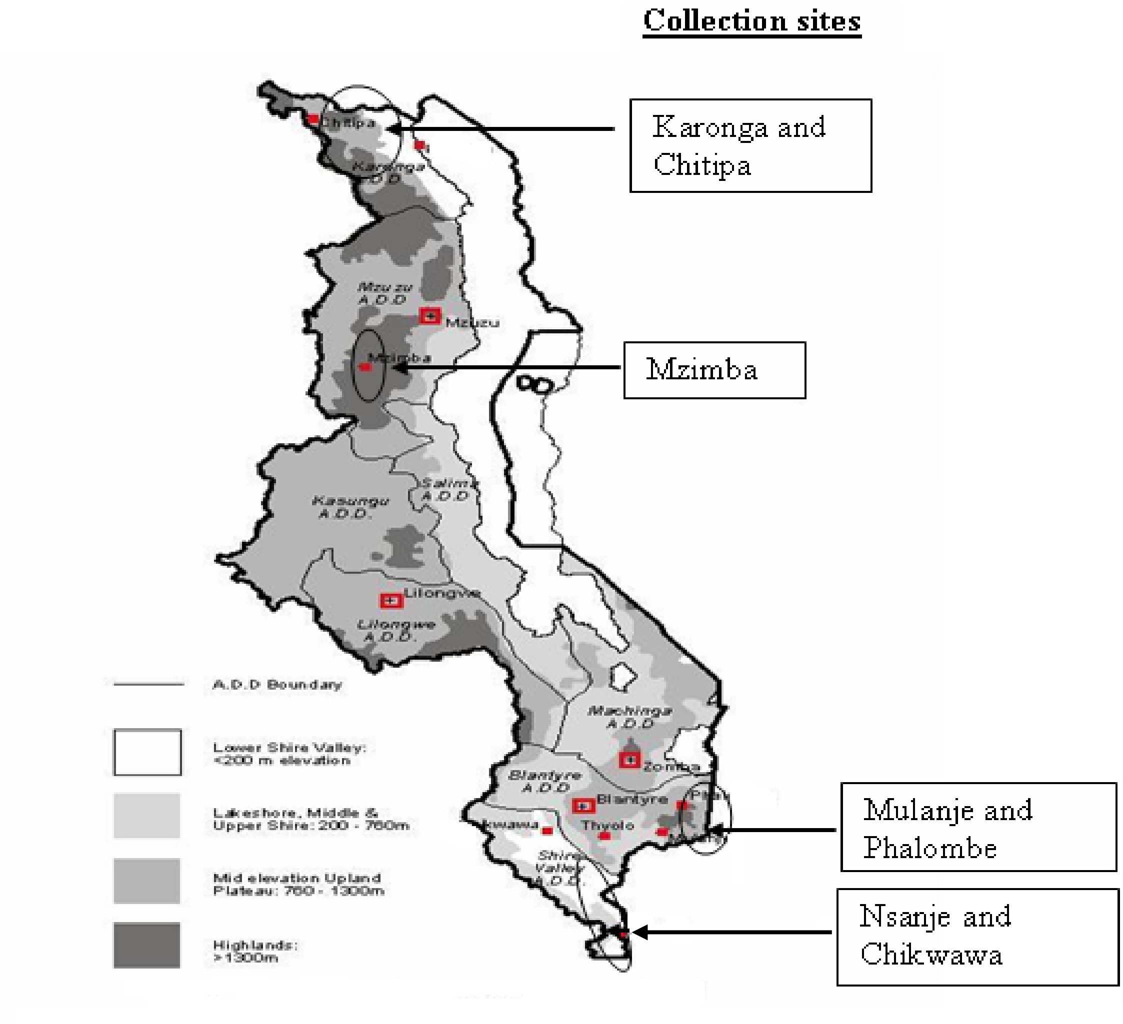

Figure 2.2 Map of Malawi showing elevations of collection sites and Agricultural Development Divisions (ADD) boundaries

Chitipa and Karonga which share boundaries are districts in the far North of Malawi. Chitipa is bordered by Tanzania to the north and Zambia to the west while Karonga, a lakeshore district of Lake Malawi is bordered by Tanzania to the north and the Lake to the east. Mzimba is a district located south wards of the northern region bordered by Zambia to the west. Chitipa and Karonga districts are in the Karonga Agricultural Development Division while Mzimba is in Mzuzu ADD. In the southern Region of Malawi, collection was concentrated along the Shire River Banks in Chikwawa and Nsanje (Shire Valley ADD). Nsanje is the farthest District of southern Malawi with southern, eastern and western sides bordered by Mozambique. The western part of Chikwawa is bordered by Mozambique. Mulanje and Phalombe are districts next to each other to the south east of Blantyre in the southern region which are both partly bordered by Mozambique to the east. Phalombe and Mulanje districts are under Blantyre ADD. The collection districts were characterised by different altitudes, longitudes and latitudes, soils, rainfall amounts and temperatures (Table 2.2). In the Northern Region, the ethnic group in majority was Tumbuka, while the Sena in Chikwawa and Nsanje dominated.

Table 2.1 Ethnic groups in study areas

District	Traditional Authority	Ethnic groups
Chitipa	Mwaulambia, Mwenemisuku	Lambia, Sukwa, Tumbuka,
Karonga	Wasambo, Mwakawoko	Tumbuka, Nkhonde, Nyachusa, Ndali
Mzimba	Mpherembe, Mthwalo	Tumbuka
Chikwawa	Makhwira	Sena, Mang'anja
Nsanje	Mlolo, Ndamera	Sena
Mulanje	Nthiramanja, Mabuka	Lomwe, Yao
Phalombe	Nkhumba	Lomwe

A complementary study by 12 farmers being key informants who grew large numbers of cultivars per field and randomly sampled from survey areas of Chikwawa and Nsanje was done at Bvumbwe Research Station field at harvesting time. These farmers were not the donors of germplasm from Chikwawa and Nsanje Districts. The complementary study was conducted to assess if the accessions collected were a true representation of the cultivars grown in the two Districts by either identifying cultivars in the Bvumbwe field or providing a list of cultivars missing. The Bvumbwe field collection included a good number of accessions which were collected as 'unknown' and duplicates due to naming. Involvement of key informants from the Shire Valley was therefore done to help with the identification of obvious duplicates and the accessions tagged as 'unknowns'. In the process of identification at Bvumbwe, the farmers were first interviewed individually while indoors using a questionnaire (Appendix I) to give names and characteristics of cultivars being grown or known. After indoor interviews, farmers were then let to walk in the field for cultivar identification. The complementary study was also designed to reveal similarities or differences between the Northern and Southern Region collections with the aid of farmers. These farmers were not the donors of germplasm from Chikwawa and Nsanje Districts. The complementary study was conducted to assess if the accessions collected were a true representation of the cultivars grown in the two Districts by either identifying cultivars in the Bvumbwe field or providing a list of cultivars missing. The Bvumbwe field collection included a good number of accessions which were collected as ‘unknown’ and duplicates due to naming.

Table 2.2 Altitude, longitude, latitude, soil type, mean rainfall, minimum and maximum temperatures (^{0}C) of collection sites

District	Altitude (masl)	Longitude	Latitude	Soil type	Mean rainfall (mm)	Mean temperature (1995-2004)	
						Maxi-mum	Mini-mum
Chitipa	700 to 900	32^{0} 56′ to 33^{0} 41′	9^{0} 21′ to 9^{0} 54′	Lithosols	900	26.6	16.4
Karonga	700	33^{0} 41′ to 34^{0} 08′	9^{0} 35′ to 10^{0} 20′	Lithosols	1077	30.3	20.8
Mzimba	1500	33^{0} 12′ to 34^{0} 05′	11^{0} 00′ to 12^{0} 44′	Lithosols	1209	24.9	13.4
Mulanje	700	35^{0} 17′ to 35^{0} 47′	15^{0} 27′to 16^{0} 12′	Ferruginous	1629	28.6	16.6
Phalombe	600 to 700	35^{0} 30′to 35^{0} 49′	15^{0} 27′to 15^{0} 52′	Ferruginous	800	29.3	19.0
Nsanje	100 to 200	34^{0} 53′to 35^{0} 20′	16^{0} 20′to 17^{0} 10′	Alluvial	890	33.3	21.1
Chikwawa	100 to 200	34^{0} 10′ to 35^{0} 05′	15^{0} 45′to 16^{0} 40′	Alluvial	1142	32.1	20.6

* masl = meters above sea level

Involvement of key informants from the Shire Valley was therefore done to help with the identification of obvious duplicates and the accessions tagged as ‘unknowns’. In the process of identification at Bvumbwe, the farmers were first interviewed individually while indoors using a questionnaire (Appendix I) to give names and characteristics of cultivars being grown or known. After indoor interviews, farmers were then let to walk in the field for cultivar identification.

The complimentary study was also designed to reveal similarities or differences between the Northern and Southern Region collections with the aid of farmers. These farmers were not the donors of germplasm from Chikwawa and Nsanje Districts. The complementary study was conducted to assess if the accessions collected were a true representation of the cultivars grown in the two Districts by either identifying cultivars in the Bvumbwe field or providing a list of cultivars missing. The Bvumbwe field collection included a good number of accessions which were collected as ‘unknown’ and duplicates due to naming. Involvement of key informants from the Shire Valley was therefore done to help with the identification of obvious duplicates and the accessions tagged as ‘unknowns’. In the process of identification at Bvumbwe, the farmers were first interviewed individually while indoors using a questionnaire (Appendix I) to give names and characteristics of cultivars being grown or known. After indoor interviews, farmers were then let to walk in the field for cultivar identification. The complementary study was also designed to reveal similarities or differences between the Northern and Southern Region collections with the aid of farmers.

2.4.2 Morphological characterisation

Further, detailed comparisons using morphological traits (Table 2.3) to help define which of those accessions are potential duplicates (Huaman *et al.,* 1991) were carried out at Bvumbwe Research Station (longitude 35^0 07”E and latitude 15^0 53” S and 1146 meters above sea level). The rainfall, temperature and soil characteristics of Bvumbwe Research Station are presented in section 4.4.2.

The experimental field was ploughed by a tractor and ridges constructed at 90 cm apart. Plots were laid in a check plot design consisting of 2 ridges of 3 meters long. Sweetpotato vines (25-30 cm long) were planted along the ridges at 30cm apart and planted on 24th and 28th of January 2004 and 2005 respectively. The fields were weeded using hand hoes. Fertiliser and pesticides were not used in the field. Characterisation of above ground morphology of plants started at 80-100 days after planting (Mok and Schmiediche, 1998). Seven IBPGR descriptor characters for sweetpotato (Huaman, 1988; CIP *et al.,* 1991) were used for the discriminatory assessment. The descriptors used had a total of 47 different character states (classes) from the seven morphological traits. Morphological indicators on roots were done at harvest (5 months after planting). Data was collected from four randomly sampled plants per accession. These descriptors were qualitatively and quantitatively scored (Huaman, 1991).

Table 2.3 Phenotypic classes of morphological traits used for diversity analysis

Character	Abbre-viation	Character states	IBPGR/ CIP code	No. of classes
Vine inter node length	VL	Very short (<3 cm); short (3-5 cm); intermediate 6-9cm; Long (10-12cm); very long (>12 cm)	1, 3, 5, 7, 9	5
Vine inter node diameter	VD	Very thin (< 4mm); Thin (4-6 mm); Intermediate (7–9 mm); Thick (10–12 mm); Very thick (>12)	1, 3, 5, 7, 9	5
Vine tip hairiness	TP	None; Sparse; Moderate; Heavy; Very heavy	0, 3, 5, 7, 9	5
Leaf lobe number	LN	1, 3, 5, 7, 9	1, 3, 5, 7, 9	5
Petiole pigmentation	PP	Green; Green with purple near stem; Green with purple near leaf; Green with purple at both ends; Green with purple stripes; Purple with green near leaf; Some petioles purple others green Totally and Mostly purple	1, 2, 3, 4, 5, 6, 7, 8, 9	9
Storage root shape	RS	Round; round elliptic; elliptic; obovate; ovate; oblong; long oblong; long elliptic; long irregular or curved.	1, 2, 3, 4, 5, 6, 7, 8, 9	9
Predominant flesh colour	FC	White; Cream; Dark cream; Pale yellow; Dark yellow; Pale orange; Intermediate orange; Dark orange; Strongly pigmented with anthocyanins	1, 2, 3, 4, 5, 6, 7, 8, 9	9
Total classes				**47**

2.4.3 Data analysis

2.4.3.1 Indigenous knowledge data analysis

SPSS (SPSS, 1999) software was used to analyse frequencies of the data collected from farmers. Noteworthy excerpts that illustrate the salient themes from the interviews have been quoted in English (Chiona-Kartun *et al.,* 1998).

2.4.3.2 Morphological data analysis

The mean values of all sampled observations for the seven phenotypic traits over three eco-geographical sources and two accession status (Table 2.4) were analysed for their variance and significance using Agrobase^{TM} (Agronomix Sofware Inc., 1999). Accessions were grouped into three populations: Northern, South East and the Lower Shire Valley populations based on agro-ecological zones. The soil types, altitude, production conditions (residual moisture or direct rainfed) and temperature ranges of collection sites are presented in Tables 2.2 and 2.4. The Northern population constituted accessions from Chitipa, Karonga and Mzimba, the South East population from Phalombe and Mulanje and the Lower Shire Valley population from Nsanje and Chikwawa Districts. Accessions were also categorized as either landraces or introductions to compare their morphological differences. The statistical software SPSS (SPSS, 1999) was used to obtain frequencies of the average data over the two years. The χ^2 analysis was carried out to test deviation from the overall mean of all the characters and sources of origin. The variation in frequency in the multi-category attributes was described using the Shannon-Weaver Diversity Index (Shannon, 1948) to measure phenotypic diversity for each trait.

Table 2.4 Eco-geographical characteristics of collection sites

Eco-geographical source	Classifying variables
Northern Region (Chitipa, Karonga, Mzimba)	Mid to high altitude (700 to 1500 m. a. s. l), lithosols soil type, rainfed, low, medium and high temperatures
South Eastern Region (Phalombe, Mulanje)	Mid altitude (600 to 700 m. a. s. l), Ferruginous soil types and rainfed, medium temperatures
Lower Shire Valley (Chikwawa, Nsanje)	Low altitude (100 to 200 m. a. s. l), alluvial soils, grown under residual moisture, high temperatures

The Shannon-Weaver Diversity Index (*H'*), often referred to as the Shannon Diversity Index, provides information about the distribution of observations among categories for multi-category attributes (Peeters and Martinelli, 1989; Grenier *et al.,* 2001; Adugna and Labuschagne, 2002). The index was calculated by the formula:

$$\boldsymbol{H'} = -\sum_{n=1}^{n} pi \, log_e pi \qquad (1)$$

where n is the number of character states (classes) and p_i is the proportion of entries in the *i*th category of an *n*-class (total number of accessions, N) attribute (Pielou, 1966; Jain *et al.,* 1975). If a trait is mainly represented by one category in the database, a low index value will be assigned, but if an attribute is evenly represented among all of the categories, a high index value will be assigned. The additivity of *H'* allows characters to be pooled over groups (Tolbert *et al.,* 1979). The average diversity (Ĥ’) over n traits was estimated as:

$$\boldsymbol{\hat{H}'} = \Sigma \, H'/n \qquad (2)$$

The interpretation is that the greater the number of variants in each phenotypic class of a given character and the more equal their proportions, the greater is the diversity (Pielou, 1966). The minimum value of the index is zero for a uniform population. The value of the index increases with increase within population variability and reaches maximum value when all phenotypic classes have equal frequencies (Yang *et al.,* 1991).

To test for the differences among pairs of Ĥ' values at P< 0.05, a t- statistic according to Jain *et al.* (1975) was used as given by the formula:

$$t = (H'_1 - H'_2)/[\mathrm{var}\ (H'_1) + \mathrm{var}\ (H'_2)]^{1/2} \qquad (3)$$

The degrees of freedom (df) of the t test is:

$$\mathrm{df} = [\mathrm{var}(H'_1) + \mathrm{var}\ (H'_2)]^2/[\mathrm{var}(H'_1)^2/N_1 + \mathrm{var}(H'_2)^2/N_2] \qquad (4)$$

Where N_1 and N_2 are the number of entries used in calculating $\mathrm{var}(H'_1)$ and $\mathrm{var}(H'_2)$ respectively. The variance is provided by the following formula:

$$\mathrm{var}(H') = [\sum_{i=1}^{n} pi\ log_e^2 pi - (\sum_{i=1}^{n} \mathrm{pi}\ \log_e \mathrm{pi})^2/N] + [n-1/2N^2] \qquad (5)$$

2.5 Results and Discussion

2.5.1 Ethno-botanical studies of sweetpotato germplasm

2.5.1.1 Number of cultivars grown per farmer

A total of 268 germplasm accessions (Table 2.5, Appendix II) from ten ethnic groups were collected from the seven districts. Most accessions were from Nsanje District (29%). Chitipa Mzimba and Chikwawa accounted for 22%, 17% and 16% respectively to the total germplasm. Mulanje (1%) contributed the least. The high contribution of accessions by Nsanje associated with the highest number (16) and mean number of cultivars (6.41) obtained in one field (Table 2.5). Chitipa and Mzimba had 12 accessions as the highest number in a field with means of 4.83 and 3.54 respectively. In Mulanje and Phalombe, a maximum number of two and three cultivars respectively were recorded from one field. In each district, the minimum number of accessions per field was one. Generally, 44% of farmers grow 3.85 cultivars in one field.

Table 2.5 Number of accessions per district and farmer

	Accessions per district		Number of Farmers per district			Cultivars per farmer	
District	**Number**	**%**	**Interviewed**	**Donors**	**%**	**Mean**	**Maximum**
Chitipa	58	22	20	12	60	4.83	12
Karonga	29	11	20	8	40	3.62	6
Mzimba	46	17	20	13	65	3.54	12
Chikwawa	42	16	20	11	55	3.81	8
Nsanje	77	29	20	12	60	6.41	16
Phalombe	12	4	20	4	20	3.00	3
Mulanje	4	1	20	2	10	2.00	2
Total	**268**	**100**	**140**	**62**	**44**		
Mean	**38.26**	**14.29**	**20**	**8.86**	**44**	**3.85**	**8.43**
± SD	**25.54**			**4.34**		**1.40**	**5.16**

Key: SD= Standard Deviation

2.5.1.2 Custodians of cultivar diversity

The results on germplasm collection show that not all growers within a community play the same role in maintaining diversity of sweetpotato cultivars (Table 2.5). In Mzimba, 65% of the 20 farmers interviewed were the sources of the accessions while Chitipa and Nsanje were 60% each. Phalombe and Mulanje farmers contributed only 20% and 10 percent respectively. In total, only 44% of 140 farmers interviewed were custodians of cultivar diversity. The rest of farmers (56%) grew one or two of the improved cultivars.

There was a marked difference in the maintenance of a variety of cultivars by gender and age, income group (based on reason for production) and proximity and access to urban centers (Tables 2.6 and 2.7). Table 2.7 presents two main age categories of farmers who emerged as custodians of sweetpotato cultivar diversity during collection (accession donors) based on the life expectancy of Malawi (USAID/Malawi, 2005) which is below 40 years (37 years). It is of prime importance to note that 64.71% of the custodians of sweetpotato cultivar diversity in this study were farmers above 40 years old of which 18.14% and 46.57% were males and females respectively (Table 2.6). These findings therefore suggest that sweetpotato cultivar diversity is at risk of being eroded since its existence is at the mercy of people who have already lived their expected age. Farmers under 40 contributed only 35.29% (4.71% males and 30.57% females) to the total accessions collected. In total, 77.14% (30.57% females under 40 years and 46.57% females above 40 years) of the farmers who were the sources of the accessions were women indicating that sweetpotato cultivation is principally a woman’s job. Of these women, 32.57% were household heads. During the collection expedition, these women demonstrated an in depth knowledge of the various cultivars in terms of organoleptic, agronomic, post-harvest and marketing values. Citing an example, 11 cultivars were collected as ‘unknown’ because the ‘owner’ (wife) was not around, yet the husband was hosting the team. A worse situation was encountered where a ‘husband’ could not identify a cultivar he prefers most as the wife who knows this cultivar was not around.

Table 2.6 Demographic characteristics (%) of germplasm custodians

		Below 40							**Above 40**			**Female Headed Household (%)**
		Males (%)			**Females (%)**							
	n= 62	**Below 30**	**30 to 39**	**Cumulative**	**Below 30**	**30 to 39**	**Cumulative**	**Total**	**Males (%)**	**Females (%)**	**Total**	
Chitipa	12	2	3	**5**	11	13	**24**	**29**	25	46	**71**	**16**
Karonga	8	5	3	**8**	15	26	**41**	**49**	15	36	**51**	**29**
Mzimba	13	5	5	**10**	9	16	**25**	**35**	22	43	**65**	**22**
Chikwawa	11	2	2	**4**	11	13	**24**	**28**	24	48	**72**	**19**
Nsanje	12	3	3	**6**	6	19	**25**	**31**	16	53	**69**	**17**
Phalombe	4	0	0	**0**	0	25	**25**	**25**	25	50	**75**	**25**
Mulanje	2	0	0	**0**	0	50	**50**	**50**	0	50	**50**	**100**
Mean	**8.86**	**2.43**	**2.29**	**4.71**	**7.43**	**23.14**	**30.57**	**35.29**	**18.14**	**46.57**	**64.71**	**32.57**
Standard deviation	**4.34**	**2.07**	**1.80**	**3.77**	**3.77**	**3.26**	**6.82**	**9.95**	**10.64**	**17.16**	**23.34**	**30.08**

The study also revealed that generally where production was for sale, farmers concentrated on 1 or 2 high yielding and early maturing cultivars that had higher/superior market value (Table 2.7). In contrast, farmers who grew for food or for both food and cash grow multiples of cultivars with different attributes to sustain food at household level.

Table 2.7 Reasons for sweetpotato production

District	Number of farmers (%)		
	Cash	**Food**	**Cash and food**
Chitipa	28	52	20
Karonga	18	46	36
Mzimba	36	37	27
Chikwawa	21	43	36
Nsanje	26	48	26
Phalombe	21	19	60
Mulanje	79	4	17
Standard deviation	**21.26**	**17.61**	**14.41**

Based on the reasons for sweetpotato production, three groups of sweetpotato growers were identified in all the study sites. Thus there were farmers who grew sweetpotato for (1) cash, (2) food and (3) both, food and cash (Table 2.7). Mulanje had the highest numbers of farmers who grew sweetpotato for sale (79%) seconded by Mzimba (36%). Farmers from Mulanje and Mzimba are at an advantage in terms of access (good road infrastructure) and proximity (close) to Blantyre and Mzuzu City markets respectively for selling the sweetpotato (Figure 2.2). In contrast, the majority of farmers in most remote areas of Chitipa, Karonga, Mzimba, Nsanje, Chikwawa and Phalombe grew sweetpotato for food or both (cash and food). Phalombe had the highest percentage of farmers growing sweetpotato for both, cash and food (Table 2.8). The findings in general indicate that sweetpotato in 6 of the 7 districts (Table 2.8) is mostly grown for food except in Mulanje where production is mainly for cash.

2.5.1.3 Relative importance of sweetpotato in comparison with other crops

Sweetpotato was grown in association with other crops that vary in importance among the different ethnic groups. The ranking of crops by farmers for food and cash varied in the 7 districts (Table 2.8). Maize was a major crop in almost all study areas with the exception of Karonga and Nsanje, where cassava and sorghum, respectively, are important food crops. The sweetpotato crop ranked third for food in all the seven districts. As a source of cash income, sweetpotato ranked first, third and fourth in Mulanje, Chikwawa and Phalombe Districts respectively while in the other four Districts, the crop ranked second for cash. In a baseline survey in Malawi, Moyo *et al*. (1998) found that sweetpotato was in general, a third source of both food and cash. The ranking of sweetpotato as one of the first 3 important food crops in the current and former studies indicates that the crop satisfies farmers' needs for food and cash in the mixed cropping systems where its main contribution is food security. Production of crops in general for sweetpotato farmers under study was on a small scale and scattered patches of land where the primary concern is meeting the needs of the family rather than maximizing profits.

Table 2.8: Ranking of farmers'major crops for food (F) and cash (C)

District	**CP**		**KA**		**MZ**		**NE**		**CK**		**MJ**		**PE**	
Crop	F	C	F	C	F	C	F	C	F	C	F	C	F	C
Maize	1	4	4	3	1	3	2	4	1	5	1	4	1	1
Cassava	5	5	1	4	2	6	-	-	-	-	2	6	4	5
Sweetpotato	3	2	3	2	3	2	3	2	3	3	3	1	3	4
Beans	4	1	5	5	5	4	-	-	-	-	7	7	2	2
Rice	-	-	2	1	-	-	5	3	4	2	-	-	5	3
Banana	2	3	6	6	7	7	8	6	7	4	6	3	7	7
Groundnuts	6	6	7	7	4	1	7	5		-	-	-	-	-
Sorghum	-	-	-	-	-	-	1	7	2	6	-	-	-	-
Cotton	-	-	-	-	-	-	4	1	5	1	-	-	-	-
Sugarcane	-	-	-	-	-	-	-	-	-	-	5	2	-	-
Pearl millet	-	-	-	-	-	-	6	8	6	7	-	-	-	-
Finger millet	7	7	-	-	6	5	-	-	-	-	-	-	-	-
Pigeon peas	-	-	-	-	-	-	-	-	-	-	4	5	6	6

Key: CP= Chitipa; KA= Karonga; MZ= Mzimba; NE=Nsanje; CK= Chikwawa; MJ=Mulanje; PE=Phalombe; *only 7 crops were considered for the comparison

2.5.1.4 Prevalently grown accessions

Of the 268 accessions collected, six of the seven improved and released cultivars: Kenya (Kemb 10), Kakoma (TIS 3017), Mugamba (Mogamba (CIP 440034), Salera (CIP 1941/121) Semusa (Cemsa 74-228 (CIP 400004) and Lunyangwa (LRS 407) were collected and morphologically identified by the scientists. The rest of the accessions were landraces.

The commonest cultivar found to be grown in all study areas was Kenya which was collected under ten different names (Table 2.9).

Table 2.9 Multiple names and meanings of Kenya in Malawi

Name of cultivar	Status	Meaning
Kenya	Official and common name	Introduced into Malawi through Kenya
Other names and meanings of Kenya		
Name	**Meaning**	
Vision	Introduced into communities by World Vision International (WVI; NGO)	
Admarc	A parastatal for agricultural produce with markets spread in rural areas	
41	Likened to early maturing maize variety (NSCM 41)	
Boma	Variety recommended by Government	
Research	From the Research Department	
Hybrid	An improved cultivar	
Kasungu	A District in Malawi	
Kenya mtuwa kuwalo	Among Kenya cultivars, this one has a whitish skin colour	
Kenya yellow	Among Kenya cultivars, this one has yellow flesh colour	

Bred in Tanzania and introduced through Kenya, the cultivar was officially released in Malawi in 1988 (Moyo *et al.,* 2001). As in the original country, Tanzania (Kapinga *et al.,* 1995), the cultivar Kenya is preferred by farmers, urban traders and consumers in Malawi. Due to its wide adaptability to different agro-ecologies and higher consumer acceptance, this cultivar has been adopted by Tanzania's neighbouring countries of Kenya, Uganda, Zambia, Malawi and Botswana under names: Kemb 10, Tanzania, Chingovwa, Kenya and SPN/O respectively (Kapinga *et al.,* 2000). Another widely sampled cultivar was Zondeni which was also collected under ten different names in four ethnic languages. Zondeni is a preferred local landrace in Malawi, but just like Kenya, Zondeni does not keep long in store.

2.5.1.5 Sources of new cultivars

Sweetpotato has been produced by growers in study areas for as long as their memories could remember. Some old varieties have been dropped, others maintained and new ones adopted for various reasons (Chiwona-Karltun *et al.,* 1998) Sources of new cultivars were relatives and neighbours from within, other districts, neighbouring countries (Zambia in Chitipa, Tanzania in Karonga and Mozambique in Nsanje) and local markets. Improved cultivars (mainly Kenya) were reported to be introduced in an area by Non Governmental Organisations such as World Vision International, Action Aid Malawi, *etc*. In Nsanje and Chikwawa, several Chinese introductions originating from China were collected.

2.5.1.6 Cultivar variability by names

Many variations in phenotypic characteristics such as leaf shape, root flesh and skin colour were observed in the germplasm collection. Farmers were able to recognize the morphological features and used these features to name and identify their varieties. As indicated by Bashaasha *et al.* (1995) varieties are identified on the basis of root yield, quality, colour, size, time to maturity, sweetness (Taste), place of origin and the person who introduced the variety *etc*. Similarly these characteristics were found to be the basis for cultivar naming in the current study (Table 2.9). However, the naming of cultivars in all study areas appeared to be imprecise and confusing even within an ethnic group. For example, two cultivars Babache and Mfumu, prevailing cultivar names and commonly grown in Nsanje and Chikwawa had multiple names that were found to be shared between them. Babache had eight while Mfumu had nine names which were also shared with other cultivar names apart from these two. Both, Babache and Mfumu have skins that are purplish in colour. Generally, cultivars had more than one name which is in agreement with the results by Chiona-Kartun *et al.* (1998) who reported that the less commonly grown cultivars of cassava seemed to have different names in different parts of study districts. The implication of the confusions in naming is that apart from obvious duplicates of prevalently grown and known cultivars, it is difficult to screen the accessions for the formulation of a core collection based on the names in Malawi. Conventional methods of cultivar identification such as morphological and molecular markers which were also applied in the current study are therefore being strongly recommended.

2.5.1.7 Accession identification and duplicate assessment by farmers

In the complementary identification studies at Bvumbwe Research Station, farmers from Nsanje and Chikwawa competently identified cultivars grown in their districts including three from Phalombe which were obvious duplicates of accessions from their areas. Below ground and above ground phenotypic features were used for identification. In total, with the aid of the farmers, 75 phenotypic and obvious duplicates were identified and tagged for elimination. However, using the same features, farmers could not identify cultivars from Mzimba, Chitipa and Karonga except two: tsambalimodzi from Mzimba which was also collected as ‘unknown 13’ from Karonga, called supuni/namasupuni in Nsanje, Chikwawa and Phalombe; and Kadidimi which was identified as Thinda of Chikwawa and Nsanje which also share names with other cultivars Babache and Mfumu. The failure of twelve farmers from Nsanje and Chikwawa to identify the cultivars from the northern districts and relate them with their cultivars is a strong indication that the accessions from the northern districts were phenotypically different from the collections of the southern districts.

Although ethnic preference on phenotypic traits may not totally be overruled, growers in all study areas indicated no preference for phenotypic traits as a selection criterion for a cultivar (except that cultivars that have lobed leaves were preferred for vegetables than unlobed leaves). However, no farmer indicated leaves as a selection criterion for a sweetpotato cultivar. Phenotypic traits were found to be very important but only for use in cultivar differenciation. The failure of farmers to identify accessions from the northern region could therefore be due to phenotypic differences of the germplasm between the two sources which may be attributed to climatic and soil differences (Table 2.2) other than ethnic preferences. As indicated by Zimmerer and Douches (1991), farmers define local cultivars by their agro-morphological characteristics. Based on preferred agro-morphological criteria, farmers decide on cultivars to select and maintain. For instance, yield responses of different cultivars in the different environments might have influenced cultivar selection over years to suit different sites manifesting into the current phenotypic differences. A clear example would be Kenya, a cultivar not commonly grown in Nsanje and Chikwawa due to its vegetative and luxurious growth at the expense of root yield in the alluvial soils grown under residual moisture in the warm to hot conditions of this area. The few farmers who had grown and or/ tasted Kenya indicated high organoleptic preference of the cultivar but poor agronomic performance. While the cultivar Kenya was common in the Northern and South Eastern districts the cultivar was found to be grown by one and two farmers only in Chikwawa and Nsanje respectively although many other farmers had grown the cultivar before.

It is of prime importance to note that although farmers in the complementary study managed to identify most of their accessions, not all were identified. Thus, although the involvement of farmers was a powerful tool in the discrimination between the Northern and Southern populations, the findings of Chiona-Kartun *et al.* (1998) who reported that cassava farmers sometimes classified plants from the same cultivar into subgroups, based on slight differences in morphology should be taken into consideration. Farmers failed to identify obvious duplicates contained in the northern population suggesting that prolonged interaction with their cultivars was more important in the identification process at Bvumbwe. Farmers may ably identify cultivars after long and close interaction, but not necessarily duplicates of new cultivars.

2.5.1.8 Variability in cultivars by attributes

Although cultivar naming has been shown to be imprecise and confusing among and within ethnic groups as discussed in Section 2.5.1.6, the meanings of the names were however important as they provided a powerful tool in cultivar identification and classification. Consequently, important information on agronomic and end-user preference on sweetpotato cultivars in general were delineated. Examples are provided in Tables 2.9 and 2.10 which present names and meanings of some cultivars grown in different study areas. As opposed to Table 2.9 which presents place (Kasungu), organization (WVI), phenotypic traits (Kenya mtuwa kuwalo) and accession status (hybrid, research) of the cultivar for naming, Table 2.10 presents agro-morphologic (Nkhodole dee, Bangakhala *etc.*), organoleptic (Suga ntape, phala la mwana *etc.*) and market (41) values for naming. While names by phenotype were important in visual identification of cultivars, the names in Table 2.10 provide descriptions of individual cultivars by attributes and the role it plays to food security. The process of naming of cultivars was found to be similar in all ethinic groups. This similarity in naming could be expected since sweetpotato growers were found to be small scale farmers where crop production was mainly for subsistence and food security. Food security attributes of cultivars were therefore highlighted and found to be the basis for such names as those presented in Table 2.10.

Attribute based names entailed cultivar classification, thus cultivars with phenonotypic based names could be classified into similar or different character classes of attributes. For example, the cultivar ‘Kenya mtuwa kuwalo’ (Table 2.9) can be classified as ‘early or late maturing’ in the attribute ‘time to maturity’ as presented in Table 2.11. This classification of cultivars was found to be the basis for cultivar selection in all study sites. Similarities in the classification of cultivars were also noted among the ethinic groups as farmers in different areas highlighted the same attributes to be important. Based on known meanings and farmer classification, the following variables were found to determine the value placed on the different cultivars: diversity in growth period (early and late maturity), texture (soft and mealy), taste (sweet and flat), storability (based on time frame), marketability (taste texture, maturity, high yields), yield (low and high yields) and pest resistance (field weevil tolerance).

Table 2.10 Some of the ethnic names describing attributes used in cultivar selection

Attribute	Name /cultivar	Meaning
Early maturity	Ndabwera ndi nthumbo-yanga kwathu	A pregnant bride
	Nkhodole dee	Stick the vine in the soil, and before getting home, if you turn around, the sweetpotato is ready for lifting
Late maturity	Bangakhala	‘stay there’ (an insult due to lateness)
High yielding	Sakana nthaka	Does well regardless of soil type
	Chitsiru pa mchenga	Does well even on the sandy soil (an insult)
	Chisungabalanda	Nurtures the believed
	Chisungabapina	Nurtures the less privileged
Shelf life	Chisungabalanda	Nurtures the believed
	Chisungabapina	Nurtures the less privileged
	Ndakwata banja ndi nzeru zanga	Married wisely (good choice)
Taste/texture	Njipe nunkhe	Nice but smells (smells like herbs)
	Phala la mwana	Baby’s porridge (too moist)
	Mzungu	Good looking, sweet, but moist
	Njipe yekha	The only tasty one
	Suga ntape	Lots of sugar
Market / Saleability	Forty one (41)	Likened to early maturing maize variety (NSCM 41)

During collection, farmers demonstrated knowledge of traits of each of the cultivars being grown. Relative comparisons of cultivars using the traits showed that farmers pay attention to their cultivars. The distribution of cultivars based on traits as provided by farmers in the various districts is summarised in Table 2.11. The classification of cultivars for yield, maturity and organoleptic taste, is on relative comparisons of the cultivars grown by the farmer. However on storage, the cultivars with high shelf life are the ones which can keep for a minimum of one month after harvest while cultivars expressing high weevil resistance/tolerance can keep in ground for a month after maturity and before harvest. The results in Table 2.11 indicate that while percentages are high in cultivars with some desired attributes, they are low in others.

High percentages of cultivars with low tolerance to weevil attack (69.14%) and short shelf life (69.29%) were observed denoting lack of diversity for these attributes at farmers' disposal for selection. Table 2.11 however indicates that in most study areas, there has been selection for good organoleptic traits which constitute mealy texture, good sweetness, low or no fibres and good flavour. The percentages with an average of 65.71% for the cultivars within organoleptic attribute were high followed by high yield (50.14%) and early maturity (49.57%). Such a selection has a high likelihood for selecting against other desirable attributes which occurred in low percentages such as high tolerance to weevil (30.86%) and long shelf life (32.14%).

As presented in Table 2.11, along the desirable attributes are also undesirable attributes present. These undesirable attributes are low yielding, late-maturing, short shelf life, susceptibility to sweetpotato weevil and poor gastronomic attributes (taste, texture, fibre content and aroma). Cultivars with undesirable traits are present in the farming communities, on account of associated desirable attributes, as no farmer in any of the study areas actually selected cultivars on the basis of these 'bad' attribtes *per se*. For instance, late maturing cultivars take a longer period in the field which is considered a bad attribute. However, 22% of the late-maturing cultivars in Table 2.11 were resistant/ tolerant to sweetpotato weevil attack, (owing to low yield as small roots were not exposed and therefore not easily accessed by weevils, and some were deep rooting), 37 % of them had long shelf-life after harvest, 49% of them had high dry matter content and 8% of them were considered to be high-yielding. In the present study, it was found that it is the association of desirable and undesirable attributes that is responsible for the cultivar mixtures in an effort to tap and make use of important traits that are contained in different cultivars.

Table 2.11 Distribution of accessions (%) for various trait classifications in districts

		Yield levels		Maturity period		Organoleptic taste		Tolerance to weevil		Post-harvest shelf life	
District	**N**	**High**	**Low**	**early**	**late**	**good**	**Bad**	**high**	**low**	**long**	**short**
Chitipa	58	49	51	48	52	67	33	48	52	26	74
Karonga	29	58	42	37	63	64	36	39	61	36	64
Mzimba	46	53	47	66	34	71	29	25	75	22	78
Chikwawa	42	36	64	33	67	69	31	31	69	32	68
Nsanje	77	48	52	45	55	73	27	26	74	41	69
Phalombe	12	57	31	68	42	66	34	22	78	18	82
Mulanje	4	50	50	50	50	50	50	25	75	50	50
Mean	**38.29**	**50.14**	**48.14**	**49.57**	**51.86**	**65.71**	**34.29**	**30.86**	**69.14**	**32.14**	**69.29**
± SD	**25.54**	**10.09**	**10.09**	**23.28**	**22.77**	**12.35**	**12.35**	**15.05**	**15.05**	**18.77**	**18.05**

2.5.1.9 Basis for cultivar mixtures

Wolfe (1985) defined cultivar mixture as 'mixtures of cultivars that vary for many characters including disease resistance, but have sufficient similarity to be grown together'. Cultivar mixture provides a range of desired traits to meet varying needs at household level. Sweetpotato cultivars in the current study were planted either in a total random within and between rows, or - in many cases - in separate patches or ridges within a field. Sweetpotato cultivars were classified by the farmers according to their role in food security which was one of the major reasons noted in the study for cultivar diversity and production of a mixture of cultivars.

In Malawi, sweetpotato has the advantage of a short growth cycle of just three to four months after planting, which enables it to supplement/ relay food availability before the next maize harvest. A baseline survey results (Moyo *et al.,* 2004) showed that as high as 84% of respondents prefer early maturity as an important characteristic of sweetpotato which they considered for selection. Early-maturing cultivars are planted for harvest and use within the shortest time possible (three to four months after planting) when previous maize stocks have been exhausted and farmers are waiting for the next maize crop to be harvested. In normal years, the months most affected by food deficits in Malawi are between January and March. A farmer Agripa Kaonga from Karonga expressed that *'it is a disgrace to the family to start using maize from the stalk (field), as it is a sign of perpetual hunger in the seasons to come; the maize has to dry completely and be harvested before use, and in the meantime we survive on early-maturing sweetpotato cultivars.*' In Nsanje, four different varieties referred to as *Nkhodoledee* (Table 2.10) were found to be commonly grown by seven farmers as early-maturing cultivars. The literal meaning of the name is that after planting, even before you get home, if you look back, the sweetpotato is ready for harvest. Additionally, farmers who produce the crop for sale would want to be the first at the markets in order to f*etc.*h premium prices before the market becomes saturated. The importance of early maturity was emphasized by farmers in both rainfed conditions and under residual moisture. In the rainy season, sweetpotato is usually planted late due to labour bottlenecks, as such cultivars that utilize the available water efficiently are required, while under residual moisture, sweetpotato may be planted late on account of late drying-off of excess rain water and harvest has to be carried out before the next floods.

It was found that farmers grow multiples of varieties which differ in terms of yield within and between localities. Instances were encountered where a variety could be ranked high yielding by one farmer but low yielding by the next farmer within study localities. A farmer, Dora Mzumara of Karonga (personal communication, 2003), asserted that sweetpotato yields vary from season to season, and more especially from site to site (referring to different field patches). In some households, planting is carried out by (a) specific family member (s), believed to have ‘good hands for root yield’ (root size). Thus the varying performance of varieties from farmer to farmer, site to site and season to season, justifies the existence of diversified production environments. In marginal environments where variability between production sites is high and climatic conditions are erratic within and between seasons, farmers often respond by enhancing diversity, both of crops and of varieties of crops to allow versatility and thus greater household food security (Jiggins, 1990).

In Malawi, the rainy season lasts about four months. As such, due to the prevailing dry conditions, piecemeal harvesting which is practiced in other countries such as Uganda (Smit, 1997) is not possible. Yet the preferred form of sweetpotato utilisation is in the form of fresh roots. A cultivar that has a longer shelf life in local storage facilities is important to both growers and traders. The cultivars that have a short shelf (less than a month in storage) are used up at harvest, marketed or processed (rarely) into dried chips. The roots with long shelf life are stored for future use or sale. Depending on the variety, roots can store for a period of up to six months depending on the variety. Sweetpotato roots are stored in pits in cool to warm areas of Chitipa, Mzimba and Karonga. In hot areas of Chikwawa and Nsanje roots are stored on the open and raised shelves, called *nsanja.* In some cases, roots are piled on sand on the floor in a well-ventilated hut where water is sprinkled daily to prevent them from shrivelling. However, open storage brings an additional problem as some roots sprout. The sprouts have to be removed as they appear.

Tolerance/resistance to weevil attack was also highlighted as an important attribute in all study areas. Although farmers may not know (apart from root size and exposure) the physical traits that have been shown to be linked to weevil resistance, such as rooting depth, arrangement and root shape (Kabi *et al.,* 2001), they however know the varieties that are resistant to weevil attack. Varieties were classified as early and late to weevil while in the field. Roots damaged by weevil have an off-flavour, do not keep in store after lifting, and cannot be marketed. In Uganda, up to 73% crop loss on account of sweetpotato weevil, and plant diseases that follow weevil attack, has been reported (Smit, 1997). The combination of texture (mealy), taste and aroma are of major importance and influence cultivar selection.

Characteristics of 'bad' roots included high fibre content, low dry matter content, insufficiently sweetness or too sweet taste, and unpleasant flavour.

Different studies have shown areas of high crop genetic diversity; as in places where production is particularly difficult such as desert margins and high altitude areas, where the environment is extremely variable and access to resources and markets is difficult (Brush, 1991; Brush and Meng, 1998). Substantial evidence is accumulating on the way high levels of crop genetic diversity of farmers' traditional cultivars meet the needs of resource poor farmers. Farmers in subsistence farming communities grow several cultivars in a field or adjacent field as a strategy to cope with heterogeneous and uncertain ecological and socio-economic conditions (Bellon, 1996). Other advantage for high genetic diversity include: risk avoidance or management with respect to climatic uncertainties and pest and disease problems (Zimmerer and Douches, 1991; Sawadogo *et al.,* 2001); food security and nutritional well being (Johns, 2002); income generation (Smale *et al.,* 1999; Gauchan *et al.,* 2003). Other than high yields Finckh *et al.,* 2000), sweetpotato cultivar mixtures in Malawi are used as a resource management strategy amid unfavourable environmental conditions and limited labour resources to ensure food security. A farmer uses varieties with different known characteristics to manipulate labour and environmental resources to achieve food security.

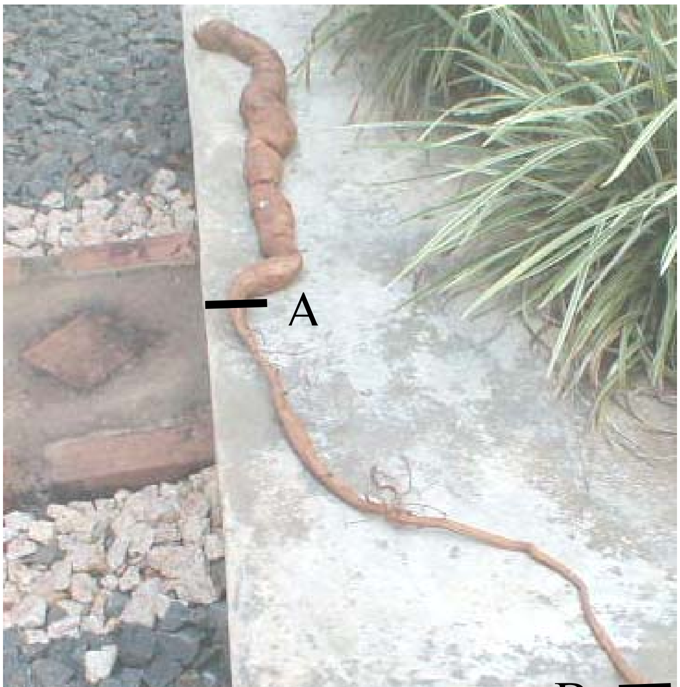

Figure 2.3 Long sweetpotato root stalk (1.36 meters from A to B)

Farmers revealed that harvesting and utilization of storage roots starts with the early-maturing cultivars which were susceptible to weevil attack. According to the farmers' classification, some cultivars susceptible to weevil attack were also early-maturing and high-yielding with large roots that become exposed above the ground (shallow-rooted). This local knowledge has scientific backing in the findings of Alcazar *et al.* (1997) who showed that shallow rooting varieties were four times more infested than varieties that rooted 8 cm below the soil surface. As depicted in Figure 2.3, the larvae of the weevil which is responsible for root damage has problems to get to the root as it is far from the plant on which the weevil lay its eggs. These cultivars may be low yielding, late maturing and yet deep rooting (unexposed roots), an attribute that contribute to tolerance to weevil attack. Although early maturity was considered to be very important than late maturity, further discussions with farmers revealed that late maturing cultivars equally play important roles to food security. Utilization of early maturing varieties was relayed with late-maturing cultivars that were available in times of abundance (when maize was in use for food).

Figure 2.4 Harvestable roots with smooth and regular root shape

Many situations were encountered where less-preferred cultivars were planted on a large scale due to their abundance in terms of planting material from source (previous field). Such an attribute is among those neglected by researchers in cultivar development and evaluation. For instance, it is assumed that Semusa, the highest yielding among the seven released cultivars (Chipungu *et al.,* 1999) and which has been heavily distributed into the farming community by Non Governmental Organizations is weak in sprouting, as observed by the failure to establish very well in the farming system. The cultivar gives large and smooth roots that are all harvestable leaving non in the ground to sprout (Figure 2.4). It is important to note that improved cultivars may not easily compete with landraces in sprouting, as most of their roots have regular root shapes that are easy to harvest (no breakages) and big enough to be easily harvested (Figure 2.4), while among the local varieties, some have many small unusable roots that are left in the soil. Some local varieties have long and irregular roots (Figure 2.5) that break during harvesting and those are the ones which provide sprouts later.

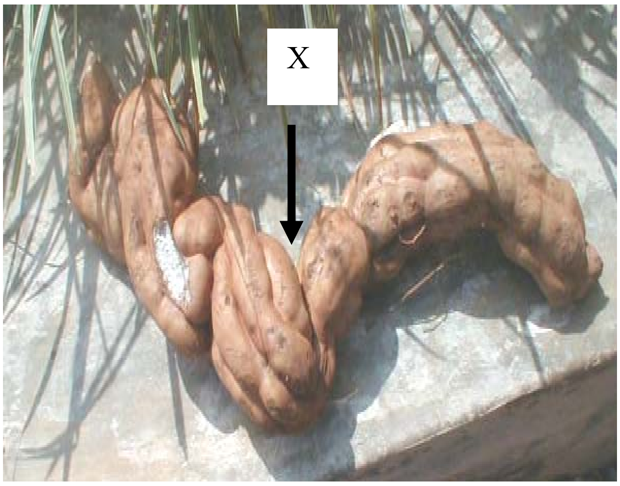

Figure 2.5 Irregular root shape, broken at point X during harvesting.

Relying on sprouts for planting material therefore may lead to the natural elimination of some cultivars. Technological information on maintaining vines of improved cultivars during the dry period needs to be transmitted to the farmers. Unfortunately, sweetpotato is given the least priority among crops, as regards extension aimed at helping farmers to improve production practices.

2.5.1.11 Sweetpotato ethno-botanical variability and its implications to cultivar improvement

The knowledge of cultivars in relation to their respective classes of attributes provided by farmers to researchers is an important activity for the formulation of breeding objectives that are directed at meeting the needs of the end users. Findings on cultivar identification by classes suggest that while farmers are important in the provision of the necessary cultivars defined by their classes of attributes, breeders should effectively discriminate these cultivars using tools and methods for further improvement in order to meet the needs of these farmers better. The process of selection and maintenance of varieties exist since farmers' concerns in various agro-ecologies are varied, and that a good performance with respect to certain concerns often implies poorer performance with respect to others, therefore, several varieties are maintained to satisfy the various concerns. The biggest challenge for promoting sustainable management strategies by which farmers may ensure food security is therefore the initiation of opportunities and prospects for diversifying the ethno-botanical base that farmers use for their own selection efforts. Since farmers often manage a combination of cultivars and multiply or eliminate varieties as they evaluate their performance over time Brush *et al.,* (1981) researchers can easy their work by combining attributes into different cultivars with the knowledge base that farmers grow and manage multiples of cultivars.

2.5.2 Sweetpotato morphological diversity analysis

The total number of accessions under morphological evaluation in 2004 season was 268 landraces and 35 introductions. In 2005 the total number of landraces evaluated was 193 (Appendix II) since 75 obvious duplicates were removed.

2.5.2.1 Existence of morphological variability in sources of origin

An analysis of variance on the seven traits was used to differentiate the populations understudy.

Table 2.12 Mean squares of ANOVA on sources of origins

Source	df	VL	VD	TP	LN	PP	RS	FC
Entry	227	4.87**	3.66**	3.92**	4.29**	3.21**	4.09**	3.45**
Years	1	1.63 ns	0.65 ns	0.48 ns	0.97ns	1.72 ns	1.36 ns	1.23ns
Eco-geographical populations	2	1.83*	2.77*	1.93*	3.49**	2.19*	3.42**	2.22*
Status	1	3.78**	2.63*	3.11**	2.93*	1.81*	2.34*	2.31*

df= degrees of freedom; *, **= Significant at $p<0.05$ and 0.01 respectively

The mean squares for analysis of variance over the two years, three eco-geographical populations and two populations based on status as landrace or introduction are presented in Table 2.12. Variability among the accessions (entry) and populations was highly significant ($p<0.01$) for the seven traits, indicative of the existence of a wide range of morphological diversity within and among sources of origin for these traits. Accession variability contributed a larger proportion of the variance (Table 2.12) than the year and populations. Since seasonal variability (years) was absent in the seven traits, means of the two years were used for analysis.

2.5.2.2 Distribution of traits over sources of origin

A χ2 test (Table 2.13) was used to compare the distributions of character states in the different sources of origin. Phenotypic percent frequencies of the character states in their respective classes are presented in Table 2.14. The chi-square test for homogeneity of frequencies of character classes of a trait showed that traits varied significantly ($P < 0.05$ and 0.01) among the three eco-geographical and two status populations. The total proportion of trait exceeded the anticipated indicating highly significant inter-population differences in character frequencies.

Table 2.13 Chi-square for sources of origin on seven morphological traits

	VL	**VD**	**TP**	**PP**	**FC**	**RS**	**LLN**
Source	χ^2	χ^2	χ^2	χ^2	χ^2	χ^2	χ^2
Eco-geographical populations	12.72*	28.92**	13.87	17.98*	6.33*	28.88*	7.36*
Status populations	16.41**	17.22*	13.81	14.93*	16.12*	23.02*	7.23*

*, **= Significant at p<0.05 and 0.01 respectively

The performance of accessions under field conditions at Bvumbwe showed wide ranges of variability exhibited by frequencies in the various character states and sources of origins indicating prevalence of diversity among the accessions. Internode diameter is a very important characteristic in cultivar release as it affects vine sprouting after planting in the field. Very thin vines easily dry up when planted due to excessive heat and moisture stress. Cultivar survival under farmers' selection and natural forces (drought, chilly temperatures, sun heat) may therefore be very low if vines are very thin. Very few accessions were observed in the very thin category in all the sources of origin. Selection against the very thin vine accessions may have contributed to low frequency due to failure of vines to survive under harsh field conditions. Accessions in the intermediate thickness prevailed among the populations with South East population having the highest accessions (54%) of the intermediate thickness. While the frequencies for very thick vines were low across sources of origins, the introduced population has the highest frequency (14%) and the landrace had 7% (Table 2.13). The introductions had 2% very thin, 17% thin, 46% intermediate, 21% thick and 14% very thick. The introductions are therefore a good source of the very thick vines which is an important attribute in harsh field conditions.

As in the vine internode diameter, vine internode length (>12cm) also affect sprouting as only a few nodes (at most 2 to 3) are available for sprouting on a 25 to 30 cm long planting vine. It is recommended that at planting, three to four nodes should be inserted underground (GoM, 1996). Vine sprouting is high when more nodes are buried in the soil at planting as opposed to one or two nodes which may easily dry up in soils with limited moisture content. The majority of accessions across the geographical sources (Table 2.13) had short and intermediate internode length. The SE population had the highest accessions in the intermediate category (63%) followed by Shire Valley population (50%).

The Northern population had a high number of accessions in thick category (54%). The very short and very long internode lengths were very few although present in all populations with the Shire Valley having the highest number of the very short internodes (11%). The landraces had very few accessions that were very short (6%), long (8%) and very long (1%) internode lengths which entailed that there has been selection against these traits. The introduction registered non for long to very long but were a good source for very short (11%), short (60%) and the intermediate (29%) lengths. A released variety Tainoni, had a combination of very thin vines and long internode lengths. The cultivar has problems with sprouting, hence one of the reasons of failure to establish in the farming communities.

Most of the accessions in the study were in the categories of no hair, sparsely present and moderately present. While the South East and Northern populations did not register accessions in the heavy to very heavy hairiness categories, the Shire Valley population had heavy (2%) to very heavy hairiness (3%) accessions (Table 2.13). The high percentage of heavy hairly accessions in the low altitude areas which are also characterised by high temperatures is attributed to selection against the heavy insect attacks. Reference accessions were Nyamajoya ya Malawi (the hairly one from Malawi) and Nyamajoya ya Mozambique (the hairly one from Mozambique) which were said to be less preferred by elegant grasshoppers which are leaf eaters and problem insects in the area.

Table 2.14 Distribution (%) of morphological character classes by sources of origin on N (number) accessions

		Internode diameter					Inter node length					Tip hairiness					Petiole pigmentation								
Population	**N**	**1**	**3**	**5**	**7**	**9**	**1**	**3**	**5**	**7**	**9**	**0**	**3**	**5**	**7**	**9**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
NR	101	2	32	45	18	13	4	31	54	9	2	32	49	18	1	0	34	6	11	18	6	3	0	11	11
SE	12	0	15	54	29	3	3	63	19	15	0	29	50	21	0	0	71	0	9	0	0	0	0	8	13
SV	80	1	11	52	33	5	11	50	39	1	1	40	42	14	2	3	28	18	10	18	6	0	0	6	17
Landraces	193	1	19	50	27	7	6	48	37	8	1	34	47	18	1	1	44	8	10	12	4	1	0	8	14
Introductions	35	2	17	46	21	14	11	60	29	0	0	23	54	14	7	9	40	0	14	14	0	0	3	20	9

		Predominant root flesh colour								Root shape									Leaf lobe number				
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**1**	**3**	**5**	**7**	**9**
NR	101	31	47	10	7	1	1	1	3	11	6	16	3	8	0	4	20	32	13	26	43	9	10
SE	12	44	23	13	17	0	1	0	3	2	15	0	8	0	4	2	33	38	38	19	25	13	6
SV	80	20	62	7	10	1	1	0	1	2	7	7	7	2	2	7	33	35	8	14	13	28	37
Landraces	193	32	44	6	11	1	1	1	3	5	9	8	6	3	2	4	29	35	20	20	27	17	18
Introductions	35	49	10	13	0	3	6	3	17	26	29	13	6	11	9	6	0	0	1	22	66	0	11

The results also showed that the green colour of petiole pigmentation for the South East population was the highest (71%). Not all character states of petiole pigmentation were represented in the nine classes in all sources of origin where the green coloured petioles constituted 44% of the landraces (Table 2.13). Accessions in the ‘purple with green near leaf’ are totally missing among the landraces. The introductions constituted 40% of the green petioles but have no representation in green with purple near stem, green with purple at both ends and green with purple stripes which were supplemented by the landraces in the germplasm (Table 2.13).

While the majority of the accessions had the white, cream and yellow root flesh colour, the distribution of the accessions among the character states of the root flesh colour was absent in other states in different geographical areas. The landraces had a total of 44% of the cream accessions and 32% of the white ones while the introductions had only 10% of the cream and 49% of the white. The introductions are a source of the dark orange (17%) which were only 3% in the landrace.

Among the local geographical areas, the majority of the accessions had elliptic and long irregular root shapes with landrace averaging 29% and 35% respectively. Such root shapes are generally a problem when processing (peeling) and marketing. The Shire Valley population had however a higher frequency of the round roots 11%. When compared with the landraces, the introductions had mostly the round (26%) and round elliptic (29%) roots which have good marketing values. Number of lobes is a trait which is represented in all character states ranging from 18% to 22% among the character traits in the landraces. Most of the introductions however had five lobes (66%) and none had seven lobes.

2.5.2.3 Shannon Weaver Diversity Index on morphological traits

Estimates of phenotypic diversity with respect to geographical origin are a prerequisite for a sound genetic conservation strategy. Diversity analysis of world collections of crop germplasm and wild relatives used Shannon and Weaver index (H’) to reveal variability of characters. Several authors have used this index extensively to estimate the phenotypic diversity in crop germplasm collections including Yoon *et al.* (2000); Kebebew *et al.* (2002); Upadhyaya *et al.* (2002).

In the current study, the Shannon Weaver Index (H’) was calculated to compare phenotypic diversity of characters between populations. The Shannon Weaver index was tested for significant difference within and between populations using t-test. It is observed in Table 2.15 that the different traits had different source areas of highest diversity which were significantly different at $p \leq 0.05$. It has therefore been shown that among sources of origin, there is low diversity for some characters and high diversity for others. For instance, vine internode length (VL) highest diversity in the Northern population (0.85) while the number of lobes (LN=0.84) and root flesh colour (FC=0.82) had highest diversity in the Shire Valley population. These results where different traits had different areas of highest diversity suggest that some sources have the potential to offer highest diversity for a particular trait(s) than others (Tables 2.13 and 2.15). The introductions however are the highest sources of tip hairiness (0.84).

Table 2.15 Estimates of H' and Ĥ of populations using morphological traits

		H'								
Source	**N**	**VL**	**VD**	**TP**	**LN**	**PP**	**RS**	**FC**	**Ĥ**	**± SE**
NR	101	0.85	0.68	0.42	0.60	0.55	0.39	0.67	**0.59**	**0.03**
SE	12	0.67	0.62	0.38	0.33	0.39	0.17	0.58	**0.45**	**0.01**
SV	80	0.71	0.65	0.40	0.84	0.60	0.65	0.82	**0.67**	**0.02**
± SE		**0.01**	**0.03**	**0.02**	**0.01**	**0.03**	**0.02**	**0.02**	**0.01**	
Landraces	193	0.72	0.64	0.40	0.61	0.53	0.45	0.69	**0.58**	**0.02**
Introductions	35	0.57	0.64	0.84	0.53	0.70	0.69	0.57	**0.65**	**0.02**
± SE		**0.04**	**0.01**	**0.03**	**0.02**	**0.03**	**0.01**	**0.04**	**0.02**	

Based on the average diversity calculated for the different sources of origins and using the t test, the Shire Valley had the highest diversity (0.67) which was significantly different from the other two populations at $p \leq 0.05$. The differences in diversity within a character class could be attributed to diversity in weather conditions, soil type and a wide range of altitude (Table 2.4). In addition, natural selection (such as drought) as well as artificial selection by man may be assumed to have been designed to suit prevailing conditions that are different from site to site. Thus, although there is no preference for morphological traits by farmers in the ethno-botanical studies (Section 2.5.1.7), morphological diversity may be attributed to selection process for the cultivars with desired agronomic and post-harvest attributes (Section 2.5.1.8) in combination with natural selection in the various agro-ecological areas. Similarly, the introductions in Malawi had nothing to do with phenotypic attributes, apart from flesh colour (deliberate introduction for orange fleshed), root shape and agronomic traits (yield and maturity period), consequently diversifying phenotypic traits as well.

2.5.2.4 Implications of morphological analysis to conservation

The results of the character distribution pattern as given by percent frequencies and the phenotypic diversity analyses permit some broad generalisation about collection and conservation of sweetpotato germplasm in Malawi. The current study on morphological characterisation has revealed that high phenotypic diversity exist among and within populations of different the three geographical origins for some traits and not others. The study indicates that the amount of variability is not uniformly distributed in all geographic regions for morphological traits under study. From a phenotypic point of view, the Lower Shire Valley population appears to be a suitable site for exploration and *in situ* conservation. Future collections should focus on these areas that have relatively large variation. However, the collection expedition should also take into account the due cause for genetic erosion, drought and environmental degradation in Mzimba around Ekwendeni, Mulanje and Phalombe Districts which have been affected by commercialization.

2.5 Conclusions

An understanding of farmers' cultivars and present diversification strategies is of fundamental importance to researchers who need to consider farmer preferences carefully when developing new cultivars. A study aimed at locating and analysing the existence of cultivar diversity was conducted using ethno-botanical and morphological methods. The study has revealed that cultivar names from the various districts differed owing to differences in ethnic groups. An an nalysis of farmers' preferences based on the meanings of the names and farmers' classification of the different cultivars, yielded some similarities between the geographical origins of accessions and interesting insights whose implications require further exploration in terms of research and development and maintenance of genetic diversity. It has also been revealed that depending on purpose for production, farmers grow one or many cultivars of sweetpotato together. The survey results revealed that farmers who grew sweetpotato for subsitence had 8.86±3.43 cultivars grown in a mixture. Cultivar diversity and mixture in terms of maturity period, shelf life after harvest and resistance to sweetpotato weevil were noted to be important in ensuring household food security over longer periods of time. However, cultivars with undesirable attributes such as late maturity, low yields and low dry matter content were also present, and were grown along with those that had desirable cultivars on account of associated good attributes complimenting each other. It was therefore established that based on purpose for production, there exist a high level of cultivar variation in farmers fields for agronomic, gastronomic, post harvest and economic value. The knowledge held by local people can help foster interaction and complimentarity between scientists and farmers and therefore calls for further exploration.

On morphological traits, frequency analysis of classes within traits gave indications of prevalent classes for each trait in the country. As some classes within traits occurred at high frequencies, some occurred at low frequency and some were totally missing in the local germplasm. The Shannon Weaver diversity index showed that the Shire Valley population had the highest Shannon Weaver diversity index (0.67). The Shannon Weaver Diversity Index provided information on geographical areas that have high phenotypic indices which can be centers for collection concentration and *in-situ* conservation.

CHAPTER 3

ECO-GEOGRAPHICAL GENETIC DIVERSITY AMONG SWEETPOTATO ACCESSIONS USING SIMPLE SEQUENCE REPEATS

Abstract

Genetic diversity among sweetpotato accessions in Malawi has not been examined using molecular markers. Fifty-nine sweetpotato accessions from three eco-geographical populations of the Northern and South Eastern Regions and the Lower Shire Valley of the country were examined using ten Simple Sequence Repeats (SSR) primers. The results generated 2-5 alleles per primer and a polymorphism Index Content (PIC) of 0.0 to 0.77 among and within primers and population indicative of diversity among accessions within and between populations. Only five primers discriminated 60% of the genetic variation entailing their importance in sweetpotato SSR analysis. The Nei's genetic diversity was highest in the Shire Valley population (0.37) indicating an area of most diversity. The accessions showed mean morphologic and SSR distances of 0.40 and 0.63 respectively suggesting more genetic than morphologic divergence among accessions. Further, cluster analysis exhibited a tendency of some accessions to group according to eco-geographical origins in both morphological and SSR UPGMA dendrograms. The collection seem to contain a high genetic diversity which is important for the development of better performing cultivars in Malawi.

Key words: SSR, sweetpotato accessions, genetic diversity, Malawi

3.1 Introduction

Identification and release of sweetpotato cultivars in Malawi is mainly based on morphological and agronomical characteristics (Chipungu *et al.,* 1999). In Chapter 2 of this thesis, farmers' classifed sweetpotato cultivars using attributes that were used for cultivar selection. Further, morphological analysis of accessions aided diversity measurement of phenotypic variability and its geographical location. Even though morphological analyses are useful, they are limited because they may be affected by environmental factors, are time-consuming and they need to be assessed at a specific vegetative phase of the crop (Swanepoel, 1999). DNA markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects, and allow cultivar identification early in plant development (Tanksley *et al.,* 1989; Melchinger *et al.,* 1994). DNA markers are increasingly employed to examine genetic diversity for germplasm conservation, or genetic enhancement.

3.2 Literature review

3.2.1 Utilization of molecular markers in the analysis of genetic diversity and germplasm improvement

The ultimate goal of plant breeding is to generate improved varieties with a combination of traits reflecting preference of end users, adaptation to a particular cropping system and environmental conditions targeted by the breeding program (Poehlman and Sleper, 1995). Superior cultivars with high productivity can be selected by identifying quantitative traits loci (QTL) manifested with DNA markers. The development of distinct, uniform and stable varieties (Cooke, 1995) has been facilitated by practicing marker-assisted selection and by estimating the similarity and difference among plant individuals (Stuber *et al.,* 1999). Selection of genotypes can be performed through targeted molecular markers rather than for the trait itself (Karp and Edwards, 1997). Since marker technology became available for detecting polymorphisms at the DNA level, they have been used in many cases to survey genetic diversity within germplasm collections (Karp *et al.,* 1997), providing information about crop evolution (Fahima *et al.,* 1999), identification of geographic regions with high diversity (Semagn *et al.,* 2000) and definition of genetically similar groups of varieties or accessions (Dudley *et al.,* 1991). DNA markers have also been widely used to assess precisely genomic diversity in crops, such as wheat (Koebner, 2003), rice (Mackill *et al.,* 1999), barley (Donini *et al.,* 2001) and maize (Gauthier *et al.,* 2002). A variety of DNA-based markers have been developed for measuring genetic diversity in crop species (Schut *et al.,* 1997). In Sweetpotato DNA markers have been used to finger print varieties using Polymerase Chain Reaction (PCR) based methods using arbitrary primers like Random Amplified Polymorphic DNA (RAPD; Williams *et al.,* 1990), Amplified Fragment Length Polymorphism (AFLP; Connolly *et al.,* 1994; Villordon and LaBonte, 1995; Vos *et al.,* 1995; Zhang *et al.,* 1998a; Huamán *et al.,* 1999).

3.2.2 Simple Sequence Repeats

Generally, an ideal genetic marker should be stable, polymorphic, easy to detect or observe, heritable in a simple manner, co-dominant, reproducible within and among different laboratories and detectable with methodology applicable to many distinct species. Different types of molecular markers have been used to assess genetic diversity in crop species, but no single technique is universally ideal. Molecular markers can be classified into three categories based on their detection method: (1) hybridization-based such as restriction fragment length polymorphisms (RFLPs); (2) polymerase chain reaction (PCR)-based such as random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs), and (3) DNA chip and/or sequence-based such as single nucleotide polymorphisms (SNPs) (Gupta *et al.,* 1999; Collard *et al.,* 2005). In the current study, a PCR based method SSR, was used for diversity analysis of sweetpotato accessions.

Microsatellites, also called SSR, are important molecular markers in plant breeding. SSR markers are stretches of 1-6 nucleotide units repeated in tandem and randomly spread in eukaryotic genomes (Litt and Lutty, 1989). They were first referred to as microsatellites by Litt and Lutty (1989) and later, as simple sequence repeats (SSRs) by Jacob *et al.* (1991). The fragment polymorphism is due to variation in the total sequence length as determined by the number of repeat units (Morgante and Olivieri, 1993). The use of SSR markers is primarily deduced from their abundant distribution and hyper-variability in the whole genome (Morgante and Olivieri, 1993). Due to the existence of these hyper-variable regions, SSR markers exhibit a high power in distinguishing between closely related genotypes. Such differences are detected on polyacrylamide or agarose gels, where repeat lengths migrate different distances according to sizes (Robinson and Harris, 1999).

In plant genome analyses, microsatellites are frequently used to fingerprint genotypes (Plaschke *et al.,* 1995; Bryan *et al.,* 1997). Primers designed to flank simple sequence repeats (SSRs) loci were developed (Jarret and Bowen, 1994) and used to genotype sweetpotato cultivars. The SSR has been used by Zhang *et al.* (2000) and Buteler *et al.* (1999) to assess the genetic identity and diversity in sweetpotato. Dominant molecular markers are routinely being used to eliminate redundancy (Zhang *et al.,* 2001) in addition to genetic diversity assessment (Zhang *et al.,* 1998b) at CIP. SSRs are also employed as genetic markers for linkage mapping (Bell and Ecker, 1994; Cregan, *et al.,* 1994) or marker assisted selection (Yu *et al.,* 1994). In the current study, genetic diversity assessment on sweetpotato using SSR was the readily available molecular method of analysis in terms of both equipment and technical expertise.

3.3 Justification of the study

Proper characterisation and evaluation of germplasm collections are important components of effective management of genetic resources (Frankel, 1989; Blakeney, 2002) by eliminating unnecessary duplications (Greene and Pederson, 1996). In order to maintain and exploit sweetpotato genetic resources efficiently, an understanding of the amount and distribution of genetic variation within and among accessions is very important. Generations of new and improved cultivars can be enhanced by new sources of genetic variation and therefore criteria for parental stock selection need to be considered not only by agronomic value, but also from the point of view of their genetic dissimilarity. Parental genotypes that are genetically diverse produce highly variable progenies by heterosis effect (Messmer *et al.,* 1993). Although morphological and agronomical traits have been very important in diversity estimates, their use however has been limited as they are perceived to be lengthy, largely affected by environment and development stage of the plant (Kumar, 1999). Molecular markers and SSR in particular have provided a powerful tool for breeders to search for new sources of variation and to investigate genetic factors controlling quantitatively inherited traits. SSR markers are becoming the preferred molecular markers in crop breeding because of their properties of genetic co-dominance, high reproducibility, multiallelic variation and most practical markers for genomic mapping, variety identification and marker-assisted selection. Further, to develop a practical and cost-effective approach for sweetpotato diversity analysis a few and well chosen primers in SSR amplification are required as resources are a constraint.

3.3.1 General objective

The general objective of the study was to assess the genetic diversity of sweetpotato accessions collected from different geographical areas of Malawi using SSR markers.

3.3.2 Specific objectives

(i) To develop a rational basis for the choice of informative SSR primers for sweetpotato characterisation in Malawi

(ii) To investigate the level of genetic diversity and validate the degree of relatedness of the morphologically divergent sweetpotato accessions from different geographical sources using SSR diversity estimates

(iii) To explore potential associations between similarity patterns using morphological and molecular data

3.4. Materials and Methods

3.4.1 Genomic DNA isolation for SSR markers

A total of 59 accessions (Appendix III) that showed wide morphological distances within and among geographical populations namely the Northern, South East, the Lower Shire Valley and introductions were sampled for further analysis using SSR markers. Bogyo *et al.* (1990) stated that sampling germplasm collections across diverse environments based on morphological variation is considered to be the most effective way of capturing genetic diversity. Sample leaves for DNA extraction were obtained from the Bvumbwe field which was used for morphological characterisation (section 2.4.2). Total genomic DNA from plant tissues of freshly harvested leaves were extracted using a modified CTAB procedure (Doyle and Doyle, 1990; Edwards *et al.,* 1991). Four leaf discs were collected in a 2 ml microfuge tube by punching the discs directly into the tube, using the lid as a punch. To aid grinding, a pinch of carborundum was added and thorough grinding was done using a glass micropestle attached to an industro power tool grinder. After grinding, 500µl of preheated (60^{0}C) CTAB DNA extraction buffer was added, mixed gently and incubated at 60^{0}c (water bath) for 30 minutes to one hour with gentle mixing every 10 minutes. An equal volume (500 µl) of chloroform: isoamylalcohol (24:1) was added and mixed well for 5 minutes on a shaker (not vortexing) to remove contaminating substances. The mixture was centrifuged at 15000g for 15 minutes in a Tomy high speed microcentrifuge. Using a pipette, 450µl of supernatant was transferred to a clean 1.5 ml microfuge. To this was added 100µl of 20% SDS and mixed gently by inverting and incubated at 65^{0}C for 10 minutes in a water bath. Added to the mixture was 500µl of Potassium acetate (Sodium acetate) 5M and mixed gently by inverting and followed by incubation in fridge at 4^{0}C for 20 minutes and centrifuge at 15 000g for 10 minutes. Genomic DNA was precipitated by adding 700µl cold isopropanol to a supernatant transferred in a clean 2ml micro tube, gently mixed and left at -20^{0}C for 1 to 2 hours. After centrifugation at 15 000g for 15 minutes, the alcohol was decanted, and the DNA pellet was rinsed with 70% ethanol and centrifuged again for 5 minutes. The DNA pellet was dried by inverting the tube on tissue paper. The air dried pellets were resuspended in 50µl TE buffer (pH 8.0) and stored at 4°C. Due to the presence of secondary metabolites such as polyphenols, tannins, and polysaccharides which inhibit enzyme action and unamplification in the polymerase chain reaction (PCR) by inhibiting Taq polymerase activity the crude DNA extract was further purified by repeated extraction with phenol-chloroform and chloroform:isoamyl alcohol. Dried DNA was resuspended in 50µl TE buffer.

3.4.2 DNA quantity and quality estimation

Five percent of the extracted DNA concentration in 95% TE buffer was quantified to get optical density (OD) readings using ultraviolet light. The concentration was measured at an absorbance reading of on a 260nm using a UV-1601 spectrophotometer (Shimadzu Cooperation of Kyoto, Japan) to come up with a dilution factor for each sample to get the desired DNA concentration of 25ng/µl for use in amplification reactions. The formula used to get the concentrations was:

$$[DNA = OD_{260} * \text{dilution factor} * \text{constant } (50\mu g/ml)] \quad (1)$$

3.4.3 Preparation of PCR reaction mixture

A PCR master mix was prepared for each primer pair (Table 3.1). An aliquot of the master mix (10 µl) was added to 2µl of a genomic DNA template into a 6 mm PCR tubes.

Table 3.1 Reagents and volumes used for preparing a PCR master mix

Reagent	Stock concentration	Volume in 1µl For 1 reaction	Final concentration	Volume in 1µl For N reaction
PCR grade water	-	5.7	-	N*5.7
dNTP mix	5mM	1.0	0.1mM	N*1.0
10 X PCR buffer	10 X	1.25	1 X	N*1.25
$MgCl_2$	15mM	1.6	1.0mM	N*1.6
Primer-forward	10µM	0.75	0.2µM	N*0.75
Primer-reverse	10µM	0.75	0.2µM	N*0.75
Taq polymerase	5µ/µl	0.06	1µ	N*0.06

3.4.4 Primer optimisation and selection

Ten sweetpotato primers (Table 3.2) were used in the study. Selection of primers was based on the presence of strong bands and polymorphism after amplification.

Table 3.2 Primers and annealing temperatures used for SSR studies

Primer	5'-nucleotide sequence (forward)	5'-nucleotide sequence (Reverse)	Annealing Temp ^{0}C
IB-S09	GCTGCTCAATCCCTCTCCTT	GGAACTCGATACAGCGTGGT	60
IB-S10	CTACGATCTCTCGGTGACG	CAGCTTCTCCACTCCCTAC	60
IB-R13	GTACCGAGCCAGACAGGATG	CCTTTGGGATTGGAACACAC	60
IB-R16	GACTTCCTTGGTGTAGTTGC	AGGGTTAAGCGGGAGACT	60
IB-R19	GGCTAGTGGAGAAGGTCAA	AGAAGTAGAACTCCGTCACC	60
IB-CIP-5R	CCTCAACGAATTTGACCTC	GATGACGGTGTGTCTGAAG	65
IB-242	GCGGAACGGACGAGAAAA	ATGGCAGAGTGAAAATGGAACA	58
IB-286	AGCCACTCCAACAGCACATA	GGTTTCCCAATCAGCAATTC	57
IB-297	GCAATTTCACACACAAACACG	CCCTTCTTCCACCACTTTCA	58
IB-324	TTTGGCATGGGCCTGTATT	GTTCTTCTGCACTGCCTGATTC	56

3.4.5 DNA amplification

Amplification of PCR reaction was done using a minicycler model PTC-150 (MJ Research Inc, Watertown, USA) for 10 amplification cycles. Each cycle consisted of a 30-second denaturing step at 94^{0}C, 15-second annealing step at primer specific temperature (Table 3.2), a 30-second denaturing step at 89^{0}C for 30 seconds, annealing step at primer specific temperature (Table 3.2) for 15 seconds and extension step at 72^{0}C for 15 seconds. In total, there were 35 cycles after which the samples were held at 65^{0}C for 20 minutes. Amplified samples were stored at -20^{0}C.

3.4.6 Detection of microsatellites

Amplified products were detected using silver staining polyacrylamide gels for DNA electrophoresis as outlined in Silver Sequence^{TM} DNA Sequencing System Technical Manual (Promega, 2000). Rain water X was used as a repellent on the long glass plate.

3.4.7 Allele scoring

The banding pattern for each primer pair was scored by visual observation using a gel viewer. Each SSR variant was treated as an allele. The co-run sequence ladder was used to determine the size of the alleles.

3.4.8 Data analysis

3.4.8.1 Statistical analysis for loci variability

In order to investigate the genetic variation among sweetpotato accessions in the study, the 59 accessions were assigned to five population groups (one to five) as described in Table 2.4 namely: Northern, South East and Shire Valley for populations 1, 2 and 3 respectively. The fourth and fifth (4 and 5) populations were the landrace and introduced populations respectively. Genotypic variation was estimated by the number of accessions assayed per locus and the total number of alleles amplified per loci and accession. Graphical presentation of distribution of allele frequency for each locus on the five populations was performed to distinguish groups with less diversity from those that were more diverse. A polymorphism Index Content (PIC), a measure of variability was estimated for each locus (Anderson *et al.,* 1993) using the 59 accessions and on five populations using the formula:

$$PIC = 1-\sum_{i}^{n} P_i^2 \qquad (2)$$

where p_i is the frequency of the ith allele and n is the sample size

PIC is the calculation of allele frequencies for each geographical group. The PIC values provide an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus, but also the relative frequencies of those alleles in the accessions under study. The locus with a large number of alleles occurring at equal frequencies will have the highest PIC values.

Each band was considered as a binary character and was scored as 1 (present) or 0 (absent) for each sample and assembled in a data matrix. The POPGENE freeware version 1.31 (Yeh *et al.,* 1997) was used to estimate two measures of genetic diversity viz the Nei (h) (Nei, 1978) and Shannon's information index (i) of geographical sources.

Pearson's Correlation coefficient was calculated to estimate the degree of association among indices. The significance of the coefficients was calculated at $P< 0.05$ using the t- statistic as given by:

$$t = \frac{r}{\sqrt{[1-r^2)/(n-2)]}} \qquad (3)$$

where r is the coefficient, n is the sample size, in this case the number of accessions (Sokal and Rholf, 1969).

3.4.8.2 Genetic distance (GD) and cluster analysis comparison using SSR and morphological markers

The data on morphological traits (Chapter 2) and SSR products (alleles) of the 59 accessions were transformed into a binary data matrix. The presence of an amplification product (allele) at a particular locus for SSR and a character state in a particular class for morphological traits was recorded as 1 and 0 for present and absent respectively. Based on the presence/absence, dissimilarity coefficients were generated using the SIMINT module (NTSYS pc 2.11c software (Rolhf, 2001). The default parameter DIST (average genetic distance) was used to generate the binary data matrix. A dendrogram was generated from the Sequential, Agglomerative, Hierarchical, and Nested (SAHN) clustering method using the unweighted pair-group method and arithmetic average (UPGMA) (Sneath and Sokal, 1973; Rohlf, 2001) using NTSYSpc. Correlations between similarity matrices from morphological and SSR coefficients were calculated by Pearson product-moment. The significance of the correlation was tested by Mantel's test (Mantel, 1976) using the NTSYS program (MXCOMP option).

3.5 Results and Discussion

3.5.1 Variation of SSR markers

3.5.1.1 Number of alleles and size range

The total number of alleles scored at each locus and five populations are presented in Table 3.3. The total number of alleles scored varied among loci and populations. The highest number scored with reference to all populations was at locus IB-S09 (292 alleles) and in their decreasing order IB-297 (256) IB-242 (253), IB-CIP-5R (230) IB-324 (212), IB-286 (207), IB-S10 and IB-R19 (172each), IB-R16 (143 each) and IB-R13 (126). Among the three eco-geographical populations, the Northern population had the highest number of alleles (67) at locus IB-S09 followed by the Shire Valley (55) generated at locus IB-286. A comparison between the introductions and the landrace populations, the highest number of alleles (135) was generated by the landraces at locus IB-S09 followed by locus IB-297 (119) and locus IB-242 (116). The highest number of alleles in the introduction population was 23 recorded on locus IB-286 (Table 3.3).

Table 3.3 Number of alleles (A) and size ranges (SR)

Population	**Locus IB-R16**		**Locus IB-324**		**Locus IB-297**		**Locus IB-242**		**Locus IB-286**	
	A	SR	A	SR	A	SR	A	SR	A	SR
Shire Valley	27	218-240	38	126-134	47	126-138	46	124-132	55	100-106
South East	7	218	11	126-134	18	126-138	16	124-132	18	100-106
North	32	218-240	50	126-134	54	126-138	54	124-132	19	100-106
Landraces	66	218-240	99	126-134	119	126-138	116	124-132	92	100-106
Introductions	11	218-240	14	126-134	18	126-138	21	124-132	23	100-106
All populations	**143**		**212**		**256**		**253**		**207**	

Population	**Locus IB-R19**		**Locus IB-R13**		**Locus IB CIP-5R**		**Locus IB-SO9**		**Locus IB-S10**	
	A	SR	A	SR	A	SR	A	SR	A	SR
Shire Valley	29	218-222	25	222-226	41	120-128	51	46-51	30	350-396
South East	10	218-222	6	222-226	14	120-128	17	46-51	11	350-396
North	40	218-222	28	222-226	52	120-128	67	46-51	39	350-396
Landraces	79	218-222	59	222-226	107	120-128	135	46-51	80	350-396
Introductions	14	218-222	8	222-226	16	120-128	22	46-51	12	350-396
All populations	**172**		**126**		**230**		**292**		**172**	

Table 3.4 Number and common alleles per locus

SSR locus	Number of alleles per locus	Common alleles per locus
IB-R16	2	1
IB-324	3	0
IB-297	5	0
IB-242	4	0
IB-286	4	0
IB-R19	2	1
IB-R13	2	0
IBCIP-5R	3	0
IB-SO9	3	1
IB-S10	2	1
Total alleles	**30**	
Mean	**3**	

The total number of alleles generated by the ten primers was 30, and among the loci, number of alleles generated varied from two to five alleles (Table 3.4) indicating a greater magnitude of diversity among the sweetpotato accessions. Gichuru *et al.* (2006) also generated two to five polymorphic fragments in 57 sweetpotato landraces from Kenya, Uganda and Tanzania using four SSR primers. A high number of fragments were however reported by Zhang *et al.* (2000) who reported 70 SSR variants from six loci in 113 accessions from 3 geographic origins, averaging 11.67 variants per loci. The large number of variants generated and type of loci in the later study could be attributed to the large number of accessions and the wide geographical sampling range (Zhang *at el*., 2000). Genetic diversity of the studied materials is the most important factor limiting average number of alleles identified per microsatellite locus during screening. However, factors such as number of SSR loci and repeat types and methodologies employed for detection of polymorphic markers influence allelic differences (Legesse *et al.,* 2007).

The allele sizes varied from 46 (IB-S09) to 396 base pairs (locus IB-S10) among the accessions (Table 3.3). Most of the SSR loci exhibited di- (40%) and tri- nucleotide (30%) repeats. Only 20% and 10% contained tetra- and penta-nucleotide repeats respectively (Table 3.3). Seventy five percent and 33.33% of the di- and tri-nucleotide repeats respectively had common alleles (alleles present in all accessions).

3.5.1.2 Allele frequency distribution

Allele distribution on locus specific size among population designated as groups 1 to 5 representing Shire Valley (group 1), South East (group 2), Northern (group 3), landraces (group 4) and introductions (group 5) are presented in Figures 3.1 to 3.10. The plots of allele frequency for locus IB-R16 on specific base pairs are presented in Figure 3.1.

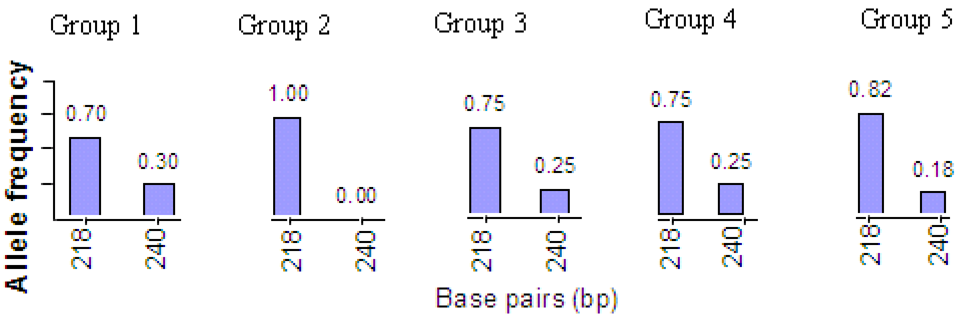

Figure 3.1 Allele frequency at locus IB-R16 on the five population groups

The common allele was 218 bp on all population groups ranging from 70% in group 1 to 100% in group 2. The larger size allele 240 bp was less common, the highest frequency being 30% and totally missing in group 2.

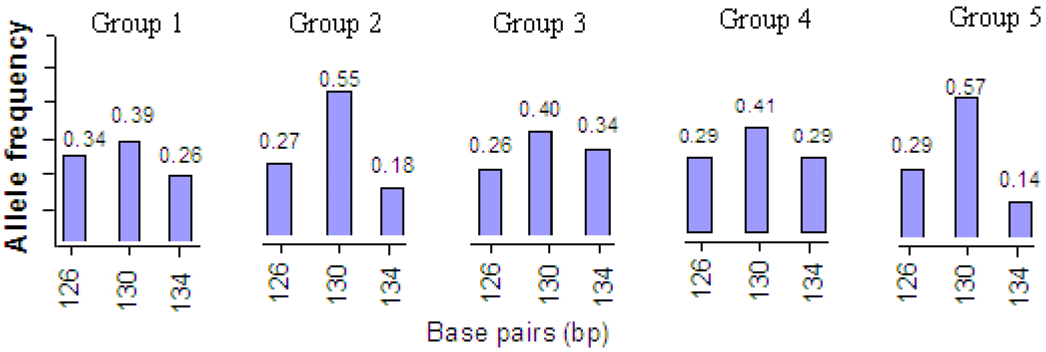

Figure 3.2 Allele frequency at locus IB-324 on the five population groups

Allele frequency distributions at locus IB-324 are presented in Figure 3.2. The common allele in all populations was 130 bp. Group 5 had the highest frequency (57%) of allele 130 bp, and in their decreasing order group 2 (55%), group 4 (41%), group 3 (40%) and group 1 (39%). The different population groups had different allele sizes in lowest frequency; the lowest in group 1, 2, 4 and 5 is 134 bp while group 3 and 4 had 126 bp as the lowest in frequency.

Plots of allele frequency distribution at locus IB-297 are presented in Figure 3.3. This locus showed the most polymorphism of all the ten loci. While the highest frequent allele in group

1 was 134 bp (32%), the most frequent allele in groups 2 to 5 was allele 126 bp with frequencies ranging from 31% (groups 3 and 4) to 33% (groups 2 and 5. In all population groups, allele 138 bp, the largest size was the least frequent allele, its highest being 11% in group 3 and lowest frequency is 6% in groups 1, 2, 4 and 5. In groups 3 (22%) and 5 (28%), allele 130 bp and 134 bp occurred in the same frequencies while 136 and 138 in group 5 (6%) occurred in the same frequency as well.

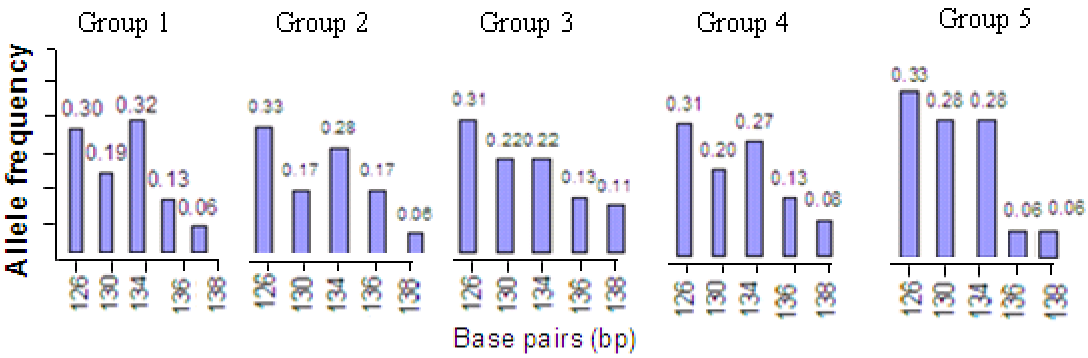

Figure 3.3 Allele frequency at locus IB-297 on the five population groups

Allele frequency plots for locus IB-242 are presented in Figure 3.4. Four alleles were scored in all groups, the smallest allele (124 bp) had the highest frequency range of 37% (group 1) to 44% (group 2). The biggest allele (132 bp) was the next highest in frequency across the populations, ranging from 0.29 (group 5) to 0.33 (groups 1, 3 and 4). The allele found in lowest frequency was different among the different groups. In groups 1 (13%), 2 (6%) and 3 (13%), the lowest allele was 128 bp while in groups 3 (11) and 5 (10%), the lowest allele frequency was 130 bp.

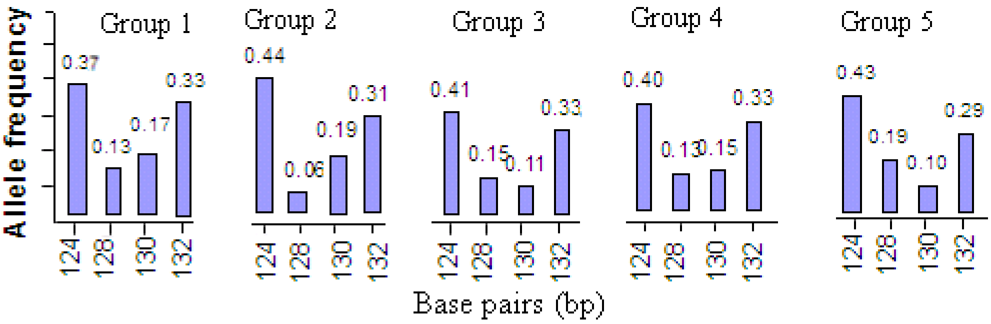

Figure 3.4 Allele frequency at locus IB-242 on five groups

The distribution plot of allele frequency for locus IB-286 is presented in Figure 3.5. The locus had four fragments of size range from 100 to 106 bp. The different groups had different highest and lowest alleles. In group 1 (31%), the allele found in highest frequency was the biggest, 106 bp. In group 2, 3 alleles (100, 102 and 106 bp) occurred in equal frequencies (33) while allele 104 was absent. In group 3, the highest allele in frequency was the smallest (28%) while fragments 102bp and 106 bp occurred in equal frequencies (26%).

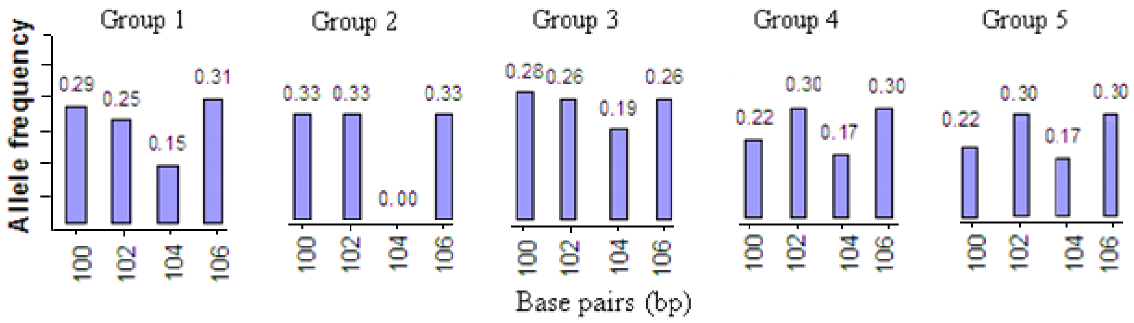

Figure 3.5 Allele frequency at locus IB-286 on the five population groups

Groups 4 and 5 had their fragments occurring in equal frequencies, thus 102 and 106 bp had equal and highest frequency (30%) in both groups while 100 bp (22% each group) was the next highest and 104 bp was the least frequent (17% each group).

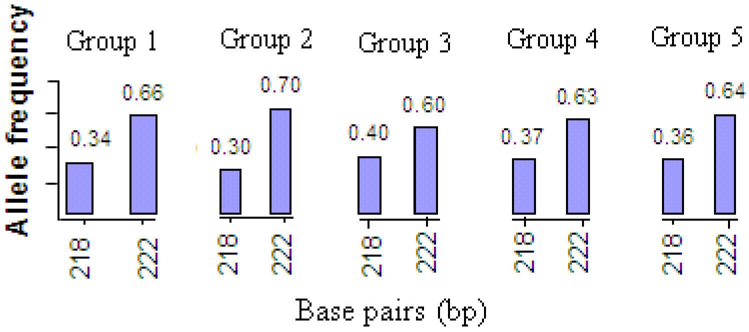

Figure 3.6 Allele frequency at locus IB-R19 on the five population groups

Figure 3.6 is a presentation of allele frequency plots on locus IB-R19. Two fragments were scored in each of the population groups which however displayed different frequencies. The highest allele frequency (70%) was registered in group 2 on the largest fragment (222 bp) and the lowest frequency on same fragment was 60% in group 3. The fragment 218 bp occurred in lower frequencies ranging from 30% (group 2) to 37% (group 4).

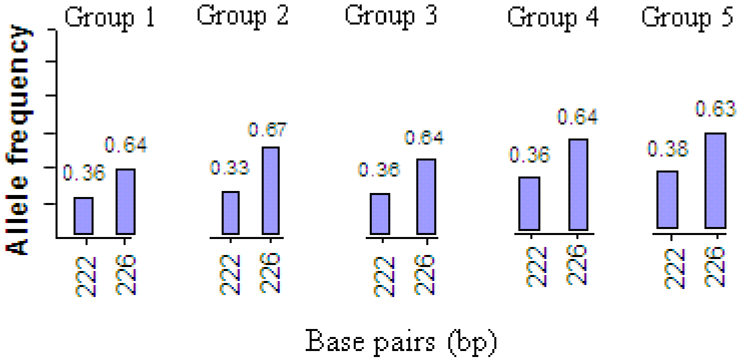

Figure 3.7 Allele frequency at locus IB-R13 on five groups

The distribution plot of allele frequency for locus IB-R13 is presented in Figure 3.7. Only two alleles of size 222 and 226 base pairs were scored in all populations. The bigger fragment 226 bp occurred in highest frequency in all groups ranging from 63% (group 5) to 67% (group 2).

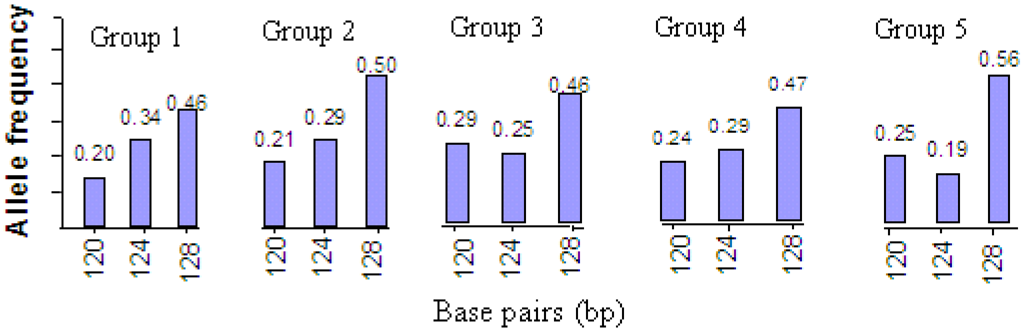

Figure 3.8 Allele frequency at locus IB-CIP-5R on the five population groups

The frequency distribution of three fragments of 120, 124 and 128 base pairs scored at locus IBCIP-5R is presented in Figure 3.8. In all groups, the biggest allele occurred in highest frequency ranging from 46% (groups 1 and 2) to 56% (group 5). However, there was variation in the groups on alleles that occurred in lowest frequencies. Groups 1, 2 and 4 had a similar trend where frequency was highest in the biggest fragment and lowest in the smallest fragment while in groups 3 and 5, the smallest fragment (120 bp) had the second highest frequency after the biggest fragment.

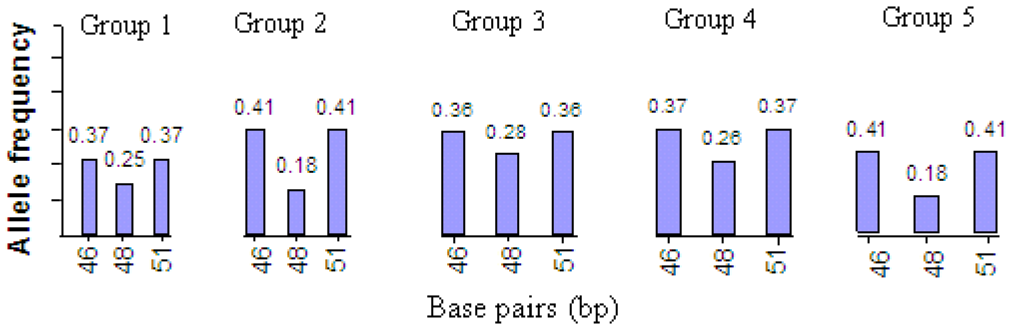

Figure 3.9 Allele frequency at locus IB-S09 on the five population groups

Plots of allele frequency distribution at locus IB-S09 are presented in Figure 3.9. At this locus, three fragments were scored of sizes 46 48 and 51 bp. In each group, the smallest and biggest fragments occurred in the same frequencies, thus 41% in groups 2 and 5; 37% in groups 1 and 4 and 36% in group 3. The allele 48 bp was the least common in all groups with a frequency range of 18% (groups 2 and 5) to 28% (group 3).

The frequency distribution of two fragments; 350 and 396 base pairs scored at locus IB-S10 is presented in Figure 3.10. Of all the loci under studied, locus IB-S10 had the largest fragment sizes. In all groups, the smaller allele, 350 bp had the highest frequency ranging from 75% (group 5) to 62% (group 3). Lowest frequencies were scored in the larger allele and ranged from 25% (group 5) to 38% (group 4).

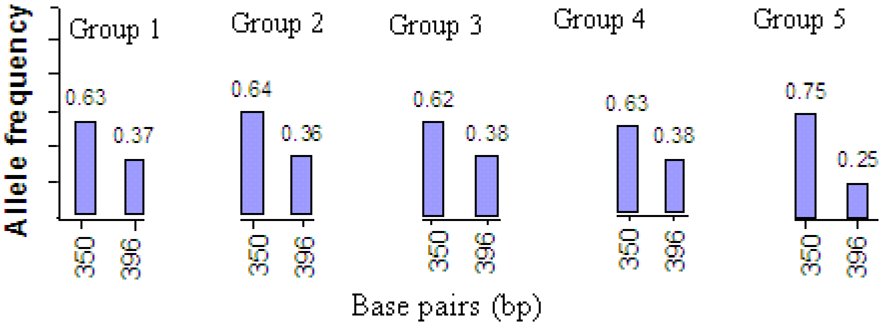

Figure 3.10 Allele frequency at locus IB-S10 on five groups

Different populations can be compared for the frequencies of alleles at all marker loci so that distinctive alleles, allele combinations, and allele frequency patterns can be identified (Brown and Kresovich, 1996). The rationale for this analysis is to distinguish population groups with less diversity from more diverse groups. In rice for example, Xu *et al.* (2004) found that alleles at six SSR loci were represented at frequencies of 17.1% to 33.6% in the world collection, but had completely been lost in the United States cultivars. In the present study at locus IB-R13 and IB-286, alleles 240 and 104 base pairs were totally missing in the South East population most likely, due to genetic erosion. However, these allele sizes are found up to 30% in group 1 for locus IB-R13 and 19% in group 3 for IB-286. Considering the allele frequency profiles across all the populations under the current study an idea is provided on the sampling strategy for conservation and management of accessions.

3.5.1.3 Polymorphism information content (PIC)

To measure the information content of the ten set of primers, PIC values (Table 3.5) were generated for the primer pairs and geographical sources of origins of the accessions. Differences among primer pairs and within and among populations were significant for PIC values at $p < 0.05$. The different loci and populations had different highest and lowest PIC values (Table 3.5).

Table 3.5 PIC values, Nei's (h) diversity measure and Shannon information index (i) over eco-geographical origins

		Primer PIC values													
Population	**N**	**IBR-16**	**IB-324**	**IB-297**	**IB-242**	**IB-286**	**IB-R19**	**IB-R13**	**IB-CIPR5**	**IB-S09**	**IB-S10**	**Mean PIC**	**± SE**	**h**	**i**
Shire Valley	19	0.42	0.66	0.75	0.71	0.46	0.45	0.46	0.63	0.66	0.46	**0.57**	**0.02**	0.37	0.49
South East Region	7	0.00	0.60	0.75	0.67	0.67	0.42	0.44	0.62	0.63	0.46	**0.53**	**0.04**	0.25	0.36
Northern Region	24	0.38	0.66	0.77	0.69	0.39	0.48	0.46	0.64	0.66	0.47	**0.56**	**0.03**	0.32	0.47
Introductions	9	0.30	0.57	0.73	0.69	0.42	0.46	0.47	0.59	0.63	0.38	**0.52**	**0.01**	0.25	0.38
Landraces	50	0.37	0.66	0.77	0.70	0.46	0.46	0.46	0.64	0.66	0.47	**0.57**	**0.04**	0.32	0.47
Mean	**59**	**0.29**	**0.63**	**0.75**	**0.69**	**0.48**	**0.45**	**0.46**	**0.62**	**0.65**	**0.45**	**0.55**		**0.30**	**0.43**
± SE		**0.01**	**0.04**	**0.05**	**0.03**	**0.06**	**0.04**	**0.03**	**0.02**	**0.02**	**0.03**	**0.04**		**0.05**	**0.06**

SE=standard error

Mean primer PIC values ranged from 0.29 (IB-R16) to 0.75 (IB-297) while mean population values ranged from 0.52 (introductions) to 0.57 (landraces and Shire Valley). Thus while two allele types were absent in the South East population (fragment 240 bp at locus IB-R13 and fragment 104 bp at IB-286), some alleles were common in all accessions and others were not evenly distributed as some were found in high frequencies than others (Table 3.5).

PIC values among SSR loci and populations ranged from 0.00 (missing alleles) to 0.77 (highly discriminative with many alleles in equal frequencies). The high mean PIC ranges among loci (0.29 to 0.75) and within and among populations (0.00 to 0.77) reveals high genetic variability (Table 3.5). The mean PIC value estimated across all SSR loci was 0.55 which is higher than 0.46, 0.28 and 0.39 reported for sorghum (Geleta *et al.,* 2006), cucumber (Danin-Poleg *et al.,* 2001) and potato (Ashkenazi *et al.,* 2001) respectively. The current study therefore suggests that the sweetpotato accessions harbour high genetic divergence.

An objective evaluation of genetic diversity among germplasm collections needs to consider, both, the number of alleles per locus and their respective PIC values in combination as suggested by Hao *et al.* (2006). The PIC values per locus in the current study showed a significant and positive correlation with the number of alleles per locus ($r = 0.81$, $P < 0.05$). The results are consistent with those of Yu *et al.* (2003) and Jain *et al.* (2004) in rice ($r = 0.62$, 0.72, respectively) and by Vaz Patto *et al.* (2004) in maize ($r = 0.85$).

The PIC value of markers indicates the usefulness of DNA markers for gene mapping, molecular breeding and germplasm evaluation (Peng and Lapitan, 2005). Based on the level of PIC values given by individual primers, five most important primers were identified: primer IB-297 had the highest PIC level (0.75) detecting the highest level of polymorphism followed by primer IB-242 (0.69), IB-S09 (0.65), IB-324 (0.63) and IB-CIPR 5 (0.62). These five SSR primers can be considered useful in the revelation of polymorphism level and therefore high discrimination capacity in the characterisation of sweetpotato accessions in Malawi. These primers amplified a total of 18 fragments which is 60% of the total 30 fragment types generated (Table 3.3). PIC values higher than 0.5 indicate high polymorphism highly and are informative for genetic studies as indicated by DeWoody *et al.* (1995) and Ram *et al.* (2007).

3.5.2 Genetic diversity among geographical populations

For a better understanding of genetic variation within the germplasm, genetic diversity was investigated among geographical populations where moderate diversity indices were observed. Differences among populations for h and i indices were significant at $p < 0.05$. The results in Table 3.5 shows that the Shire Valley population (h= 0.37) had the highest diversity index while the introductions and the South East populations had the lowest values (h= 0.25 each). The mean genetic diversity, h for all accessions was estimated at 0.30. The Shannon indices were similarly moderate, ranging from 0.36 (South East) to 0.49 (Shire Valley). The mean Shannon index in the current study was 0.43. A high and significant correlation (r=0.84) between the h and i values was observed. Genetic diversity indices indicated that the Shire Valley population had the highest genetic variability (h= 0.37; i= 0.49).Values of mean h (0.2045) and i (0.3193) were reported as the highest in mulberry population studies (Guo Zhao *et al.,* 2006) while Juan *et al.* (2004) reported h= 0.1463 and i= 0.228 as highest in *Medicago citrina* populations which are low values than reported in the current study.

3.5.3 Genetic relationship among accessions using morphological and SSR analyses

Knowledge of the genetic relationship among genotypes is useful in plant breeding programs because it permits the organization of germplasm and provides information for more efficient parental selection. A breeder can use genetic distance information to make informed decisions regarding the choice of genotypes to cross for the development of divergent progenies or to facilitate the identification of diverse parents to cross in hybrid combinations in order to maximize the expression of heterosis (Smith *et al.,* 1990). Further more, fingerprinting of genotypes offers the opportunity for the removal of duplicate accessions. In the Malawi collection, genetic similarities and dissimilarities among accessions is currently lacking to aid breeders in parent selection and core collection formulation. Therefore, the morphological and SSR diversity analysis is a valuable initiative in the provision of genetic information to breeders. A comparison of morphological and SSR markers was necessitated to evaluate the usefulness of the two methods for future reference in the improvement programs of sweetpotato.

3.5.3.1 Genetic distances

Genetic distance is the difference between two entities that can be described by allelic variation (Nei, 1973). The genetic similarity distances between pairs for morphological and SSR analyses for the 59 accessions were estimated. Genetic distance was determined for all 1711 pair wise comparisons of the 59 accessions.

The accessions that showed maximum morphological difference were Chinese and Molele (0.57) while accessions Nasambo and Mwakyusa (0.22) showed the least morphological difference. The accession Chinese is a collection from the Shire Valley and an introduction from China by the Chinese irrigation technical staff while Molele is a collection from the South East population. Mwakyusa and Nasambo revealed morphological closeness and both were collections from Chitipa in the Northern Region. On the molecular studies, the most SSR genetic difference (0.87) was recorded between Mwambande and Khomeni collections from lithosols and alluvial populations respectively. The least SSR genetic difference (0.35) was recorded between Dembuka and Nyamajoya ya Mozambique, both collections from Nsanje district (alluvial population). The mean morphological distance was 0.40 while the SSR mean genetic difference was 0.63. The maximum and minimum morphological and genetic differences in morphological and SSR analyses were recorded on local accessions indicating their dissimilarity and similarity respectively. However, Chinese and Nyamajoya ya Mozambique although regarded as local accessions, are introductions as confirmed by their names.

The frequencies of genetic distance coefficients of the 1711 pair wise comparisons of the 59 accessions for morphological and SSR data showed normal distributions (Figure 3.11). Most of morphological genetic distance coefficients ranged from 0.37 to 0.47 accounting for a total frequency of 81% of pair-wise comparisons among accessions in this range while the SSR genetic distance coefficients range was wider (0.45 to 0.75) with a frequency of 85%. In both analyses, accessions that are distantly and closely related were few. The genetic distances in the two methods were quite different in terms of values and rankings. Thus accessions which showed maximum morphological genetic distance were different from those in the SSR method.

The Mantel (1967) matrix correspondence test demonstrates that there is low correspondence between the distance matrices generated from SSR and morphological traits (r = 0.1095). Morphological traits are known to be less polymorphic, dominant, late in expression, and susceptible to environmental and developmental variations (Gottlieb, 1984; Bretting and Widrlechner, 1995). The implication is that the patterns of relationship obtained from the microsatellite data are therefore more reliable and accurate in the current study than those obtained from the morphological traits in determining genetic diversity.

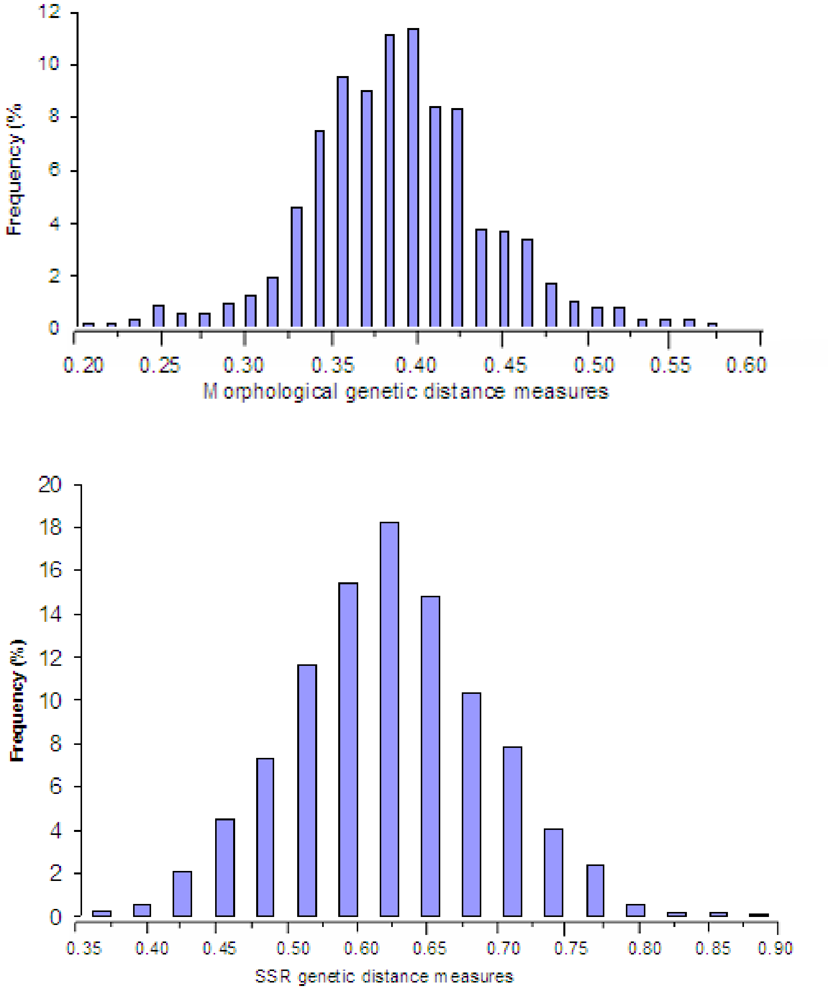

Figure 3.11 Frequency distribution (%) of genetic distance for pairs of 59 accessions for morphological and SSR data

3.5.3.2 Clusters of accessions

UPGMA-based cluster analyses on binary data of seven morphological traits and 59 sweetpotato accessions (Figure 3.12) was used to explore the pattern of morphological diversity. The morphological clustering grouped the accessions into three main clusters A, B and C consisting of a singleton accession in cluster B, 27 in cluster A and 31 accessions in cluster C. The clusters A and C of the morphological dendrogram (Figure 3.12) comprised of accessions from all sources under study namely Northern, South East and Shire Valley and introductions while the singleton cluster contains accession Tchubatchuba from the Northern population. The clusters A and C were further sub grouped to establish any possibilities of the accessions to cluster according to sources of origin.

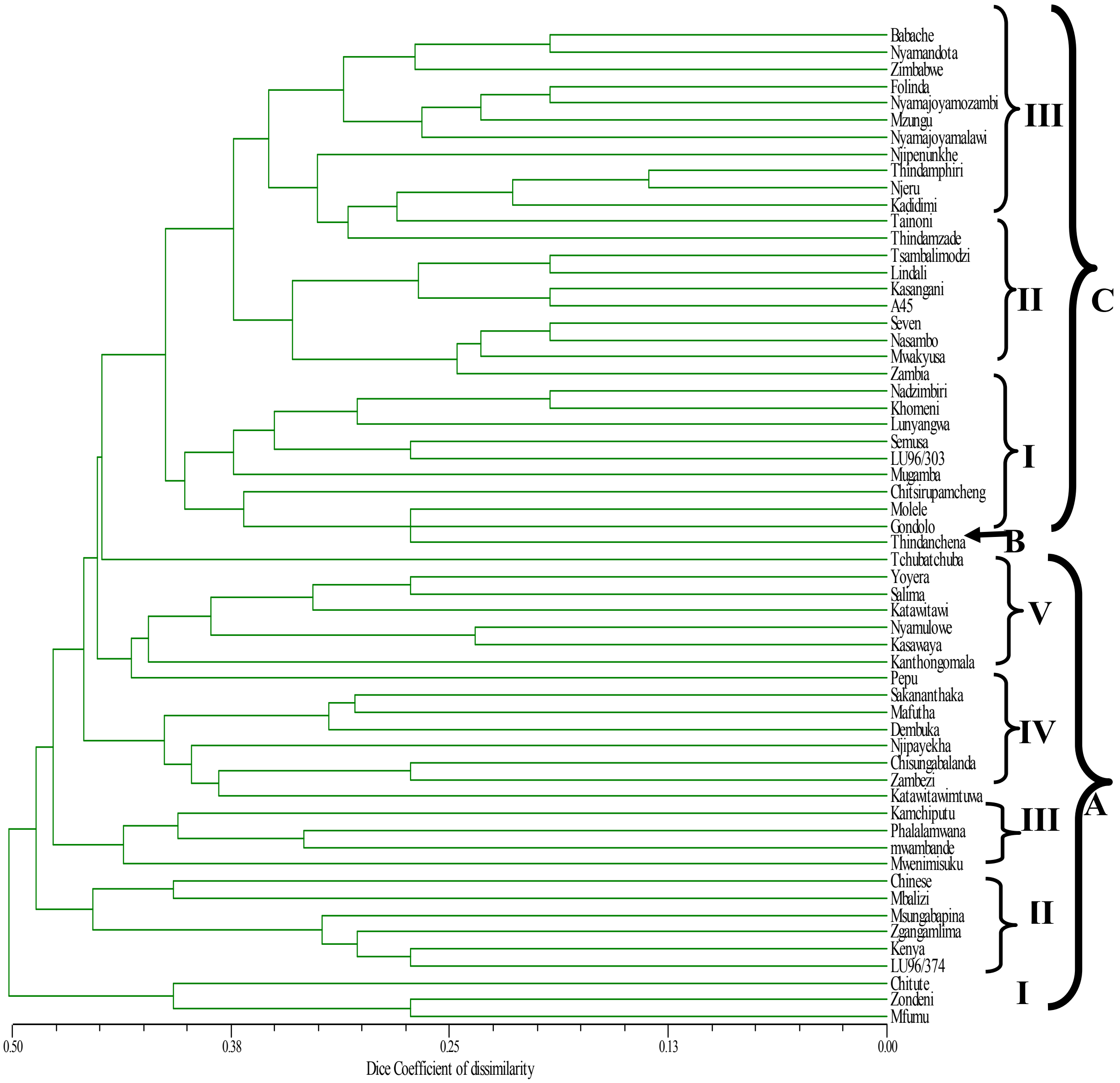

Figure 3.12 Clustering of 59 sweetpotato accessions on seven morphological traits

The composition of sub clusters I, II, III and IV of main cluster A contained accessions from all sources of origins while sub cluster V contained accessions from the Northern population and included Yoyera which was also sampled in the Shire Valley. While sub cluster I of main cluster C contained accessions from all sources of origins, sub cluster II contained accessions from the North including Tsambalimodzi which was also sampled from the Shire Valley and an introduction A45, which originates from the Republic of South Africa.

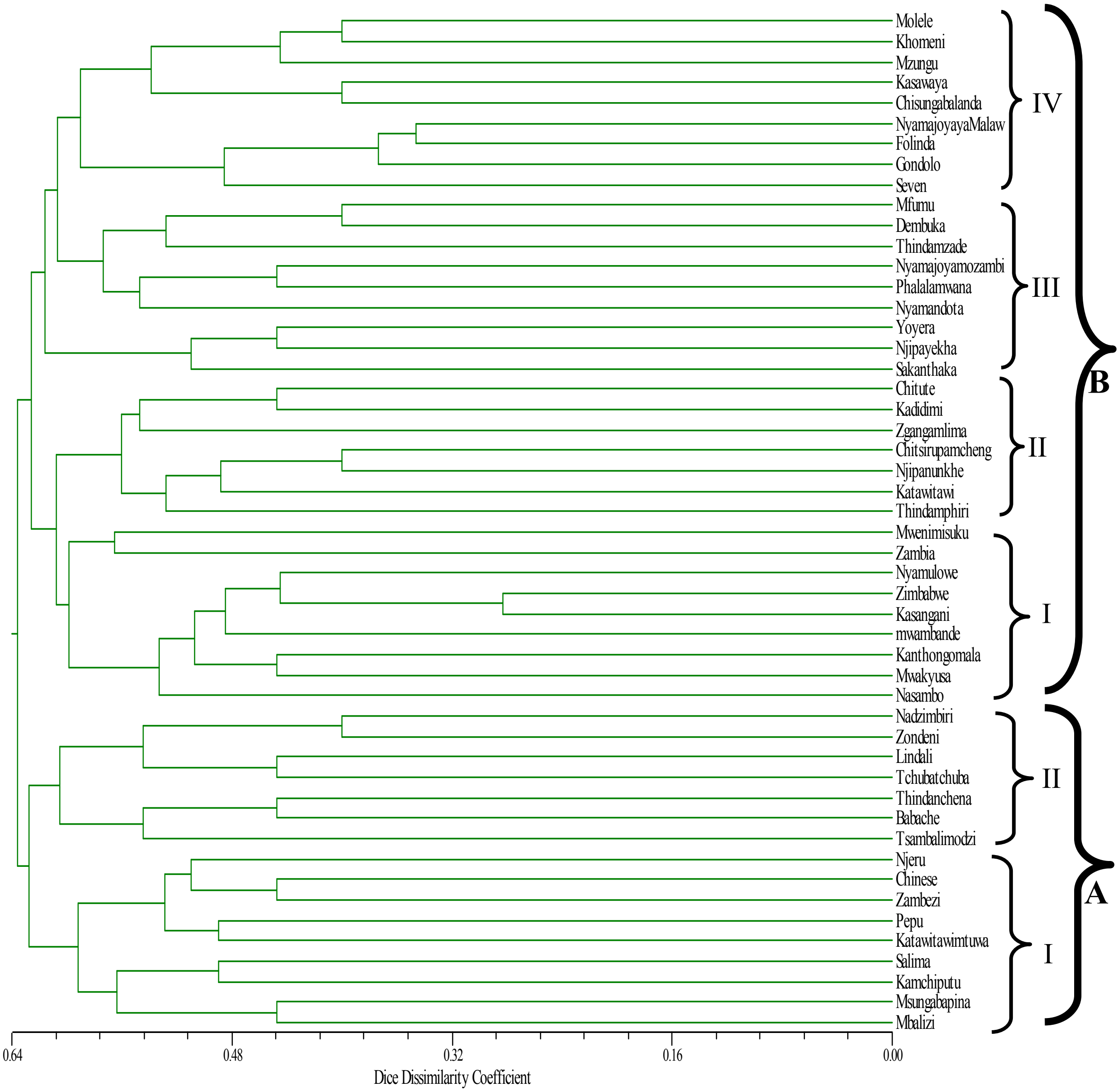

Figure 3.13 SSR clustering of 50 landrace sweetpotato accessions using ten loci

All the accessions in sub cluster III of C originated from the Shire Valley. Phenotypically, significant geographical variations in the accessions collected as discussed section 2.5.2 were observed using seven character states. Further, IK from farmers provided the basis to explain for the high level of morphological variation within a geographical population where in section 2.5.1.7 farmers from the Shire Valley could not identify accessions from the Northern Region an indication of morphological dissimilarity. In SSR analysis, two dendrograms for landraces and introductions that contained 50 and 9 accessions respectively were generated. SSR clustering grouped the 50 landrace accessions into two main groups A and B that composed of 16 and 34 accessions respectively (Figure 3.13). Groups A and B generated sub clusters I to II and I to IV respectively. The accessions in group A and its sub clusters I and II did not show the tendency to cluster according to the three eco-geographical sources. However, sub clusters I and III of main cluster B grouped according to eco-geographical origins. Sub cluster I contained accessions from the North while sub cluster III contained accessions from the Shire Valley.

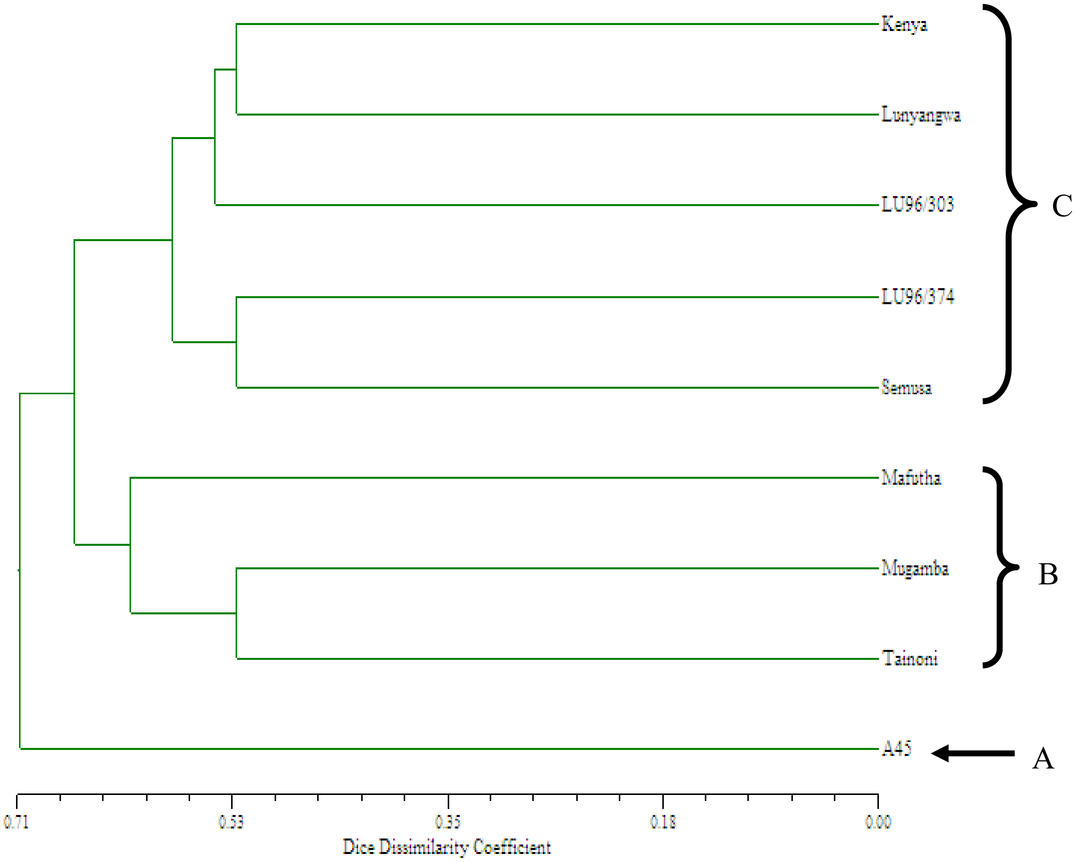

Figure 3.14 SSR clustering of nine introduced sweetpotato accessions using ten loci

A cluster analysis on introductions (Figure 3.14) was performed separately from landraces to establish the extent of relationship among two open pollinated clones; LU96/374 and LU96/303 that were developed from a crossing block where Lunyangwa, Kenya and Semusa were among the parents. Included in Figure 3.14 were other introductions from RSA (A45 and Mafutha), Mugamba from CIP, Kenya and Tainon from AVRDC.

Three clusters were generated (Figure 3.14) where A45 was a singleton in A. Cluster B contained accessions from AVRDC, CIP (Kenya) and RSA. Confirming their similarities, Kenya grouped together with Lunyangwa (Cluster C). Kenya and Tomato roma were the parents of Lunyangwa the only locally bred and released sweetpotato cultivar in Malawi.

However, the clustering pattern show that Kenya and Lunyangwa are more dissimilar (0.53) as presented in Figure 3.14 entailing a similarity of 0.47 only. The open pollinated clones, LU96/374 and LU96/303 in Figure 3.14 clustered together with Lunyangwa, Kenya and Semusa although their coefficients indicate more of dissimilarity between the clones and the parents than similarity. The high level of dissimilarity among accessions of similar parentage is expected since sweetpotato is a hexaploid and an outbreeding species, and therefore the progenies generated could be highly heterozygous (Zhang *et al.,* 2000).

In both morphological and introduced SSR dendrograms, singleton accessions were observed. The individual clustering pattern of these accessions is indicative of being more divergent from the others as they formed single-genotyped clusters. Singletons could be used in breeding programs to develop more productive cultivars.

Generally, morphological clustering of sweetpotato was different from SSR clustering in the present studies as different clusters contained different accessions. However, in both clusters (Figures 3.12 and 3.13), accessions exhibited some degree of clustering according to eco-geographical associations. Thus while some accessions clustered according to source, suggesting a genetic distinction, some accessions grouped together in the same heterotic clusters. The work by Chiguru *et al.* (2006) showed that while morphological clustering was irrespective of geographical origin, an SSR analysis tended to cluster Tanzanian landraces together from the Kenyan and Ugandan accessions.

The tendency of sweetpotato to cluster according to geographical source was also reported using other molecular methods such as random amplified polymorphic DNA, RAPD (Gichuki *et al.,* 2007), Amplification fragment length polymorphism, AFLP (Zhang *et al.,* 1998) and Selective Amplification of Microsatellite Polymorphic loci, SAMPL (Tseng *et al.,* 2002). The pattern of some accessions to cluster irrespective of eco-geographical origin suggests some similarity among them. In section 2.5.18 farmers revealed the tendency to select for agronomic and gastronomic attributes in all study sites, which entails similarities in some genotypes under production in different areas.

Introductions showed morphological similarity in their clustering pattern (Figure 3.12) with landraces as they did not exhibit their own cluster. It is possible that some of the landraces had a good percentage of introduced germplasm which spread through the process of 'seed' sharing among farmers as highlighted in section 2.5.1.5. Research stations are proposed to be sources for some of these introductions to farmers. A typical example is accession 'seven' whose yield and roots were highly appreciated by workers who picked some of its planting vines at harvesting at Lunyangwa Research Station. The accession was recognized by a technician years later during the collection in Mzimba which had maintained its plot number in the experimental field (plot number seven) as its name. Another example is TBI 11 (CIP440057), an orange fleshed genotype which was screened at research stations in the late 1990s due to very low dry matter content but was collected from Mzimba under the name Zambezi. In addition, introductions by Chinese volunteers in irrigation schemes in the country are also a source of genetic variation. The main point to be delineated from this paragraph is that in each geographical origin, some reasonable proportion of exotic accessions is assumed to exist which may genetically not (necessarily) differ from the designated introductions under the current study, resulting in the observed clustering pattern among the geographical origins.

Several comparisons between morphological and molecular-based studies have indicated that these two methods are different and highly variable (Ben-Har *et al.,* 1995; Burstin and Charcosset, 1997; Roldan-Ruiz *et al.,* 2001). However, the latter authors emphasized that such observation should not be regarded as an indication of weakness or limitation of these two systems. Genotypes that display high phenotypic similarity need not be genetically similar, as environment plays a significant role in phenotypic expression. If similarity between molecular and morphological relationships were found, it might have indicated little effect of environment or a very restricted commercial gene pool (Roldan-Ruiz *et al.,* 2001). Consistency should therefore be expected if the environment has no major impact and if the accessions performed identically across the environments, which was not necessarily the case in this study. However, the characterisation with morphological and SSR analyses independently provided the overall interrelationship of the studied accessions. Morphological traits are expected to provide the general representation of the accession relationships according to their environment, while the SSR provides a more accurate and reliable accession relationships as it deals with their basic DNA.

3.6 Conclusions

Knowledge of the genetic diversity and relationship among genotypes is useful in understanding germplasm organization for more efficient selection of desired cultivars (Roldan-Ruiz *et al.,* 2001). Molecular markers reveal differences at DNA level and thus provide direct, efficient and reliable tools for germplasm conservation and management. In the current study, a genetic diversity analysis was performed on 59 sweetpotato accessions using ten SSR primers. Two to five alleles were generated and a PIC value range from 0.0 to 0.77 among and within primers and populations was revealed indicative of diversity among accessions within and between geographical settings. Distribution of allele frequency presented on graphs showed missing and common alleles among and within populations. The allele distribution profiles distinguished groups with less diversity from those of more diverse providing an idea on sampling strategies for conservation and management of accessions. Among the ten primers, five; IB-297, IB-242, IB-S09, IB-324 and IB-CIPR 5 were identified as most important in SSR analysis of sweetpotato accessions. These five primers discriminated 60% of the genetic variation and are therefore useful in the characterisation of sweetpotato accessions in Malawi.

The Nei's genetic diversity was highest in the Shire Valley population (0.37) suggesting an area of most diversity. The pair of accessions that showed maximum morphological difference (0.57) in traits were both from the Shire Valley population while while a Northern pair showed the least morphological difference (0.22). The most significant SSR genetic difference (0.87) was obtained between accessions from the Shire Valley and Northern populations; the least SSR genetic difference (0.35) was recorded between Shire Valley populations. The mean morphological genetic distance was 0.40 while the SSR mean genetic difference was 0.63. The level of variation for SSR was higher than that of morphological traits implying that the accessions in this study were more genetically divergent. This means that it is possible to exploit distant relatives to broaden the gene pool base of sweetpotato.

A morphological cluster analysis on 59 accessions and SSR cluster analysis on 50 landraces exhibited a tendency of some accessions to cluster according to geographical origins. A cluster analysis on introductions showed genetic related accessions to cluster together. A comparison of morphological and SSR characterisation displayed different amounts and pattern of variability. Both morphological and SSR have their own advantages and disadvantages and therefore both should used. Thus while SSR provides more accurate and reliable information, it is expensive and should be used after preliminary morphological analysis to remove obvious duplicates. However, morphological characterisation is affected by the environment and should only complement the SSR.

CHAPTER 4

ADAPTATION OF SWEETPOTATO GENOTYPES IN VARIOUS AGRO-ECOLOGICAL ZONES OF MALAWI

Abstract

Sweetpotato is grown by small holder farmers across a wide range of environments in Malawi. A multi-location trial at Bvumbwe, Makoka, Chitedze, Chitala, Lunyangwa and Baka Research Stations representative of sweetpotato growing areas of Malawi was undertaken to determine the extent of genotype x environment interactions (GEI) and examine the stability of storage root yield for sweetpotato. Six genotypes were tested and compared with checks, Semusa and Kenya over the 2004, 2005 and 2006 cropping seasons. The GEI analysis was conducted with additive main effects and multiplicative interaction (AMMI). An ANOVA showed highly significant GEI effects ($p \leq 0.001$) than the genetic variation of yield traits. While genotypes only accounted for 14.25%, most of the variance (62.86%) was attributed to environmental effects. The improved check genotype, Semusa was superior for storage root yield performance (28.33t/ha) while AMMI stability analysis revealed that LU96/303 (24.16t/ha) was the most stable across sites. Lunyangwa was the site which provided the lowest yields while Chitedze provided the highest yields. It is therefore suggested that preliminary yield evaluation which has a large number of testing entries should be undertaken at Chitedze in order to capture maximum yielding potential of the genotypes. Lunyangwa should be used to assess the potential of worst performance of genotypes under unfavourable environmental conditions. Identification of low and high yielding environments should help breeders to make a rational decision on sites to be used for yield evaluations that are designed for low input sweetpotato production.

Key words: AMMI, genotype x environment interactions, root yield, sweetpotato, Malawi

4.1 Introduction

In improvement programs of crop varieties, a better understanding of the potential genotypes and their environments is necessary. Bilbro and Ray (1976) indicated that any successful breeding program should focus on genotypic yield level (average yield compared to standards), adaptation (what environment does the genotype best perform in), and stability (how consistent does the genotype yield compared to others). Eberhart and Russell (1966) defined stability as the ability of a genotype to show a minimum interaction with the environment. Thus, a variety or genotype is considered to be more adaptive and stable if it has a high mean yield but a low degree of fluctuation in diverse environments.

In section 2.5.1.9 it was found that farmers selected and grew varieties which differ in terms of yield from one farmer to another within and between localities. For example, the cultivar Kenya commonly grown in Malawi was found to be uncommon in the Shire Valley due to its vegetative and luxurious growth at the expense of root yield when grown in the alluvial soils and residual moisture in the warm to hot conditions of this area (section 2.5.1.7). While ethno-botanical studies revealed similar agro-morphological preferences among farmers (section 2.5.1.8), morphological (section 2.5.2) and molecular (section 3.5.2) studies revealed variations among and within sweetpotato populations. The existence of yield differences among cultivars grown by farmers who have similar agro-morphological preferences such as high yields, early maturity, *etc.* is attributed to differential response of cultivars resulting from different production environments that might have influenced cultivar selection over years in the different sites. The development of varieties, which can be adapted to a wide range of diversified environments, is therefore the ultimate goal of plant breeders in a crop improvement program. Breeding for such genotype stability is accomplished with repetitive field testing, trait evaluation, and selection of genotypes that rank at or near the top of a series of field trials conducted across a range of environments and years. Superior cultivars/genotypes are thus evaluated in multi-environmental trials (MET) to ensure that the selected genotypes have acceptable performance under variable environments of the target region (Yan and Rajcan, 2002). The genotype evaluation process entails selecting the appropriate field trial locations that best represent the target environments for the breeding program. Gauch and Zobel (1997) contend that subdividing growing regions into mega-environments implies higher heritabilities and faster progress for plant breeders, potentially stronger competitiveness for seed producers and higher yields for growers.

4.2 Literature review

This literature study reviews the concept of genotype by environment interaction (GEI) and some of the statistical methods that have been developed for detecting the presence of GEI, its quantification and identification of cultivar superiority and stability.

4.2.1 Concept of Genotype by Environment interaction (GEI)

Sweetpotato is grown under diverse of environments around the world. In crop improvement, a successful new variety must show high performance for yield and other essential agronomic traits. In addition, this superiority should be reliable over a wide range of environmental conditions. However, when conducting multi-environment trials, plant breeders usually observe that genotypes change in their relative performance across testing environments. The different expression of a genotype under different environments is referred to as Genotype x Environment interaction (Dixon *et al.* (1994). Genotypes refer to a set of genes possessed by individuals that are important for the expression of traits under investigation. The environment is usually defined as all non- genetic factors that influence the expression of traits and may include all biophysical factors like water, nutrition, temperature, disease *etc.* which influence the growth and development of the individual plant thereby influencing the expression of the traits (Basford and Cooper, 1998). Dhillon (2001) indicated that various environments may be expected to contribute differently to GEI and to the masking of genotypes. According to Becker & Léon (1988) GEI can be partly understood as the result of differential reactions to environmental stresses, such as drought, diseases and other factors.

Means across environments indicate better performance of genotypes in multilocation trials with non-significant GEI. The presence of GEI is however a major concern since large interactions can reduce gains expected from selection and complicate identification of superior cultivars (Rea and Vieira, 2002). According to Romagosa *et al.* (1993), GEI reduces association between phenotypic and genotypic values, and may cause selections from one environment to perform poorly in another, forcing plant breeders to examine genotypic adaptation. Deciding on what genotype to be selected becomes difficult for a breeder due to changes in ranking of genotypes (Nguyen *et al.,* 1980). As the relative performance of different genotypes differ from one environment to the other for quantitative traits such as yield, ranking of the different genotypes also changes from one environment to the other (Rea and Vieira, 2002). Thus, interactions can complicate the identification of superior genotypes and reduce gain from selection. Plant breeders therefore have to conduct selection over a number of environments. Multi-environment selection experiments require large resources and must be made efficient as much as possible. Knowledge of GEI can help reduce the cost of extensive genotype evaluation by eliminating unnecessary testing sites and by fine tuning the breeding programmes (Shafil *et al.,* 1992; Kang and Magari, 1996). The presence of a large GEI may necessitate establishment of additional testing sites which has cost implications to variety development.

GEI is considered to be quantitative (Baker, 1988) if the ranking of genotypes does not change from one environment to another (thus non-crossover). Qualitative interactions (crossover) complicate selection and identification of superior cultivars. For variety trials which are tested in the same locations (L) and genotypes (g) over years (Y), Genotype by Environment (G x E) analysis of variance may be partitioned into components due to G x L, G x Y and G x L x Y. If G x L is the dominant portion of the G x E, then the specific adaptation is exploitable by grouping regions into homogenous sites that minimise G x E within regions (Mkumbira *et al.,* 2003).

Data collected in G x E trials are intrinsically complex and consist of three major elements: (a) structural patterns, (b) non-structural noise, and (c) relationships among genotypes, environments, and genotypes and environments considered jointly. Pattern implies that a number of genotypes respond to certain environments in a systematic, significant and interpretable manner, whereas noise suggests that the responses are unpredictable. The roles of experimental design and statistical analyses in multilocation trials are to eliminate and discard as much of this unexplainable noise as possible (Crossa, 1990).

4.2.2 The concept of stability

Genetic improvement of sweetpotato root yield is concerned with two parameters: genetic potential and stability over environment. GEI and its effect on the prediction of future performance of a genotype is the essence of the concept of trait stability (Piepho, 1996; Sneller *et al.,* 1997; 1999). Preferred genotypes generally show GEI variances, above average response to environmental yield potential and lower deviations from the expected response within a target production region (Kang, 2002). Further, a genotype that has stable trait expression across environments contributes little to GEI variance and its performance should be more predictable from the main effects of genotypes and environments than the performance of an unstable cultivar (Sneller *et al.,* 1997). Evaluating stability of performance and range of adaptation has become increasingly important for breeding programs. A number of statistical procedures have been developed to facilitate the understanding of genotype by environment interaction, stability of genotypes and their relationships. The joint regression analysis of either phenotypic values or interactions on environment indices, was first discussed by Yates and Cochran (1938) and was later modified and used by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). Part of the genotype stability is expressed in terms of three empirical parameters: the mean performance, the slope of regression line (b_i), and the sum of squares deviation from regression (S^2d_i) (Crossa 1990, Flores *et al.* 1998). A two-stability parameter method similar to that of Eberhart and Russell (1966) was also proposed by Tai (1971). In this method, environmental effects (α_i) and deviation from the linear response (λ_i) can be regarded as special form of the regression parameters (b_i) and (S^2d_i), when the environmental index is assumed to be random (Lin *et al.,* 1986). Wricke (1962) suggested using genotype environment interactions (GEI) for each genotype as a stability measure, which he termed as ecovalance (W_i^2). Shukla (1972) developed an unbiased estimate using stability variance ($\sigma^2{}_i$) of genotypes and a method to test the significance of the ($\sigma^2{}_i$) for determining stability of a genotype. Francis and Kannenberg (1978), used the environmental variance ($S^2{}_i$) and the coefficient of variation (CV_i) and Pinthus (1973), used coefficients of determination (R_i^2) of each genotype as stability parameter.

4.2.3 Additive main effects and multiplicative interaction (AMMI) stability analysis

The additive main effect and multiplicative interaction (AMMI) method integrates analysis of variance and principal components analysis into a unified approach (Gauch, 1988). According to Zobel *et al.* (1988) and Crossa *et al.* (1990), it can be used to analyse multilocation trials. Zobel *et al.* (1988) pointed the flaws of other methods such as (1) analysis of variance (ANOVA) which fails to detect a significant interaction component, (2) principal component analysis (PCA) which fails to identify and separate the significant genotype and environment main effects, and (3), linear regression models which account for only a small portion of the interaction sum of squares. The AMMI method is used for three main purposes. The first is model diagnoses, AMMI is more appropriate in the initial statistical analysis of yield trials, because it provides an analytical tool of diagnosing other models as sub cases when these are better for particular data sets (Gauch, 1988). Secondly, AMMI clarifies the GEI. AMMI summarizes patterns and relationships of genotypes and environments (Zobel *et al.,* 1988; Crossa *et al.,* 1990). The third use is to improve the accuracy of yield estimates. Gains have been obtained in the accuracy of yield estimates that are equivalent to increasing the number of replicates by a factor of two to five (Zobel *et al.,* 1988; Crossa, 1990). Such gains may be used to reduce testing cost by reducing the number of replications, to include more treatments in the experiments, or to improve efficiency in selecting the best genotypes. The AMMI model combines the analysis of variance for the genotype and environment main effects with principal components analysis of the genotype- environment interaction. It has proven useful for understanding complex GEI. The results can be graphed in a useful biplot that shows both main and interaction effects for both the genotypes and environments. AMMI combines analysis of variance (ANOVA) into a single model with additive and multiplicative parameters.

The AMMI model equation is expressed as follows:

$$Y_{ij} = \mu + G_i + E_j + (\sum_{k-1}^{n} \lambda_k \, \alpha_{ik} \, \gamma_{jk}) + e_{ij}$$

Where Y_i is the yield of the ${}_i$th genotype in the ${}_j$th environment; μ is the grand mean; $G_i + E_j$ are the genotype and environment deviation from the grand mean respectively; λ_k is the eigienvalue for interaction principal component of k^{th} axis; α_{ik} and γ_{jk} the genotype and environment principal component scores for axis k; n is the number of principal components retained in the model; and e_{ij} is the error term (Gauch, 1988; Zobel *et al.,* 1988). The combination of ANOVA and principal components analysis in the AMMI model, along with prediction assessment, is a valuable approach for understanding GEI and obtaining better yield estimates. The interaction is explained in the form of a biplot display where, PCA scores are plotted against each other and it provides visual inspection and interpretation of the GEI components. Integrating biplot display and genotypic ability statistics enable genotypes to be grouped based on similarity of performance across diverse environments.

4.3 Justification of the study

Knowledge about the magnitude of GEI is important in order to develop cultivars that combine high yield and stable performance over a wide range of environmental conditions. In Malawi, sweetpotato root yield (t/ha) and yield stability are some of the primary breeding objectives in addition to eating quality (flavor, taste and texture), earliness to maturity (due to the recurrent droughts and dry spells), insect (sweetpotato weevil) and disease tolerance/resistance and post-harvest shelf life (Chipungu *et al.,* 1999; 2001). Multilocation evaluation for yield over seasons are conducted to assess elite genotypes for wide and specific adaptation and stability as the crop has been shown to be very sensitive to environmental changes (Carpena *et al.,* 1982; Janssens, 1984). However, if the Genotype by Year (GxY) component is larger, then multiple year tests are needed as opposed to when the GxY is small. Testing over years is costly and slows the release of varieties although MET is usually undertaken during the advanced stages of testing and on a few potential genotypes. Selecting testing sites within targeted areas needs to be done to maximize the information required as an addition of a second location that has a similar cultivar performance is only an added cost. It is therefore imperative to ascertain if locations are similar or different through the evaluation of a set of genotypes at many possible testing locations. Based on the results, sites that are clearly different are selected as testing sites. Thus, locations are clustered in groups of relative similarity or dissimilarity based on the genotype performance, which is the basis of the current study on sweetpotato yield evaluation. The information gained from the study aims at facilitating the design of a cost effective testing strategy in Malawi to assist in sweetpotato yield evaluations in designated environments.

4.3.1 General objective

To assess sweetpotato elite genotypes for wide and specific adaptation and stability

4.3.2 Specific objectives

(i) To determine the presence and magnitude of GEI for sweetpotato root yield
(ii) To identify superior cultivars in terms of yield potential and stability
(iii) To identify and recommend testing environments for sweetpotato root yield

4.4 Materials and Methods

4.4.1 Plant material

Table 4.1 presents the sweetpotato study genotypes. Materials included selections from open pollinated seeds in a crossing block that was established at Lunyangwa Research Station in 1995. Scarified (using sulphuric acid in 99 parts of water) true seeds were initially planted in a seedling nursery in 1996 where clonal selections were made, hence the coding of LU96. LU96 clones were combined with introductions from CIP and evaluated together in preliminary, advanced and uniform (multilocation) yield trials.

Table 4.1 Sweetpotato genotypes used for GEI study

Clone/Genotype	Source/Origin
LU96/220	Lunyangwa 1996 selections
LU96/274	Lunyangwa 1996 selections
LU96/374	Lunyangwa 1996 selections
LU96/303	Lunyangwa 1996 selections
LU96/334	Lunyangwa 1996 selections
Mugande	CIP, Nairobi
Kenya (SPN/O)	Tanzania bred, came through Kenya
Semusa (Cemsa 74-288)	CIP, Nairobi

Inferior clones in terms of pests and diseases, root yield, root shape, dry matter content, palatability, fibre content *etc.* were being dropped as others advanced with seasons. In the final entry, 6 genotypes were evaluated against Kenya and Semusa (Table 4.1) at six sites.

4.4.2 Evaluation sites and seasons

The multilocation trial was conducted during the 2002/03, 2003/04 and 2004/05 seasons at Bvumbwe, Makoka, Chitedze, Chitala, Lunyangwa and Baka Research Stations (Table 4.2).

Table 4.2 Altitude and soil characteristics of the study sites (MoALD, 1995)

Location	Altitude (masl)	Soil texture	Soil pH
Bvumbwe	1164	Sandy clay loam	4.5-6.0
Makoka	1026	Sandy clay loam	5.2-5.7
Chitedze	1097	Sandy clay loam	5.5-6.7
Chitala	600	Sandy clay	6.1
Lunyangwa	1342	Clay to sandy clay	4.4-5.6
Baka	460	Sandy clay to sandy clay loam	6.0

The testing sites represent sweetpotato agro-ecological zones in Malawi. The sites differ by altitudes, soil texture, pH and climatic characteristics (rainfall and temperatures) as presented in Table 4.2 and Figures 4.1 and 4.2

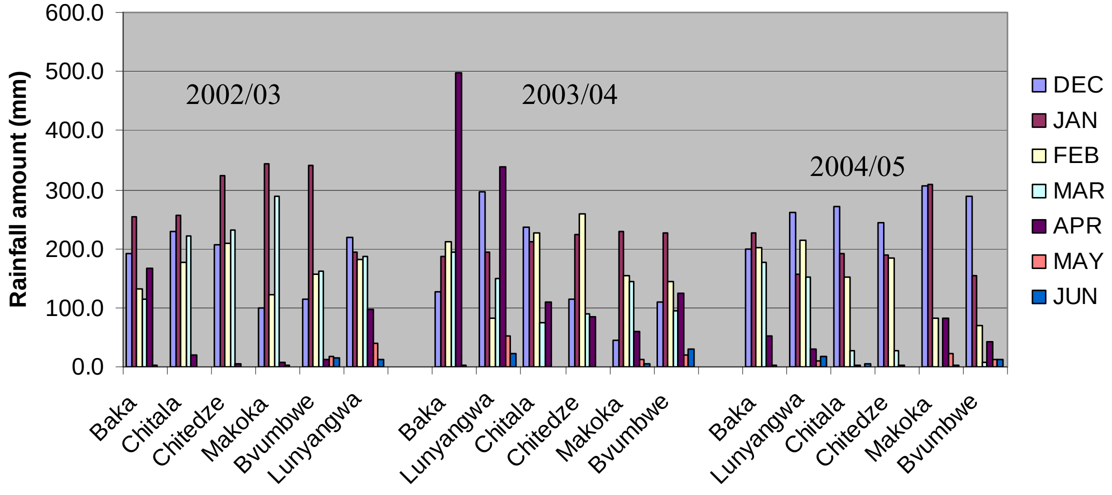

Figure 4.1 Rainfall amounts (mm) for testing sites over the study seasons

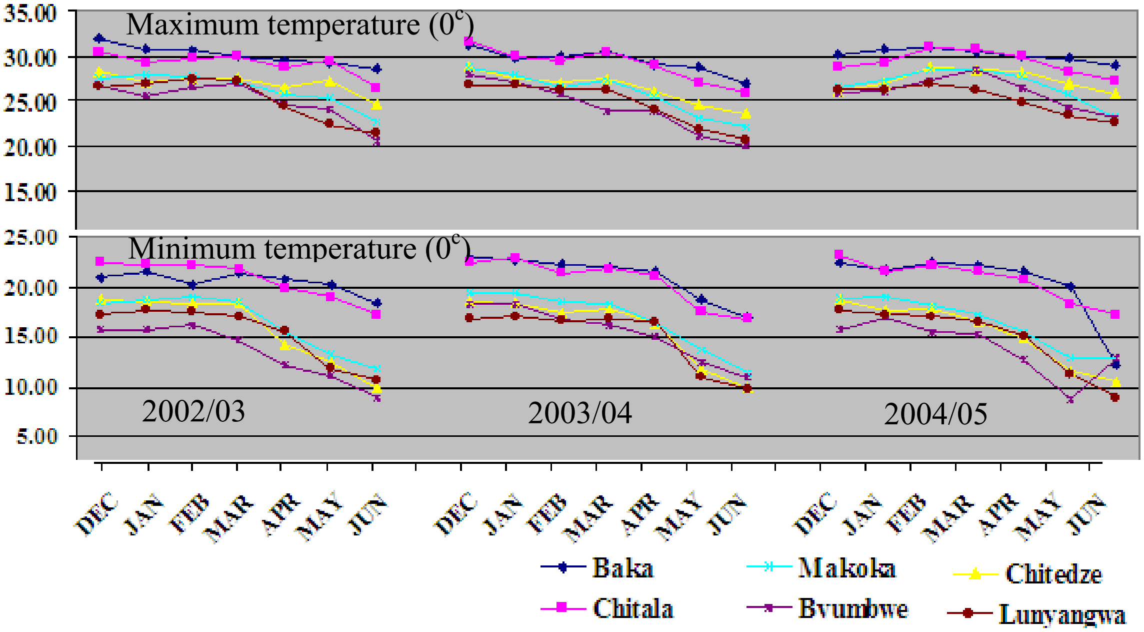

Figure 4.2 Maximum and minimum temperatures for six study sites and three seasons

4.4.3 Trial design and field lay out

The clones were laid out in a Randomized Complete Block Design (RCBD) with 3 replicates at the six locations. The gross plot was 4.8 x 0.9m in size with 4 ridges, whereas the net plot measured 3.6 x 0.9m with 2 ridges. The sweetpotato cuttings were planted at 30 cm apart within ridges. No fertilizers or herbicides were applied to experimental plots.

4.4.4 Collection of data

Harvesting was done five months after planting at all sites. At five months after planting, data on final stand count was recorded and represented final surviving plants in the net plot. Using a weighing scale, storage root yield (kg) per plot was recorded. Only marketable roots were considered for analysis in this study.

4.4.5 Statistical analyses

4.4.5.1 Analysis of variance

The effects of the genotype, location and season as well as their first and second order interactions were determined using ANOVA analysis. The effects of genotypes were assumed to be fixed, while those of seasons and location effects were considered to be random.

Table 4.3 Form of variance analysis and MS expectation for GEI

Source	DF	MS
Seasons (S)	(S-1)	
Locations (L)	(L-1)	
S x L	(S-1)(L-1)	
Reps (L and S)	LS(R-1)	
Genotypes (G)	(G-1)	$\sigma^2 e+r\sigma^2_{gls}+rl\sigma^2_{gs}+rs\sigma^2_{gl}+ rs\sigma^2_{g}$
G x L	(G-1)(L-1)	$\sigma^2 e+r\sigma^2_{gls}+rs\sigma^2_{gl}$
G x S	(G-1)(S-1)	$\sigma^2 e+r\sigma^2_{gls}+rl\sigma^2_{gs}$
G x L x S	(G-1)(L-1)(S-1)	$\sigma^2 e+r\sigma^2_{gls}$
Error	LS(G-1)(R-1)	$\sigma^2 e$

Table 4.3 shows the model of the ANOVA. The ANOVA for estimating variance components are based on the model provided by Allard (1960), which was developed by Comstock and Moll (1963) for the determination of interaction variance components. In the model, S, L, G and R are the number of seasons, locations, genotypes and replications, respectively. The σ^2_e and σ^2_g are components of variance of error and genotypes respectively. Combinations of the subscript identify the components, for the interactions.

4.4.5.2 AMMI stability analysis on root yield

Similarities among test environments based on environmental main and GEI effects were evaluated using AMMI analyses (Agrobase$^{\text{TM}}$, 1999). The method uses a combination of ANOVA and PCA. While ANOVA partitioned the variance into three components: genotype, environment and G×E deviations from the grand mean, the PCA partitioned the G×E deviations into different interaction principal component axes (IPCA). These were tested for statistical significance through ANOVA.

4.5 Results and Discussion

4.5.1 Genotypic variability for root yield

The analysis of variance (Table 4.4) indicated highly significant differences in storage root yield (t/ha) among genotypes at $p \leq 0.001$ within and between locations and seasons. The difference in performance among the genotypes in a given environment is in part due to genetic variability which accounts for 14.25% to the total sum of squares (Table 4.4).

Table 4.4 Combined ANOVA for storage root yield (t/ha) over 3 seasons

Source	Degrees of freedom	Sum of Squares	Mean Squares	% contribution to total Sum of Squares
Location (L)	5	17476.84	3495.37***	27.60
Seasons (S)	2	8523.728	4261.86***	13.46
L*S	10	13803.89	1380.39***	21.80
Genotype (G)	7	9020.829	1288.69***	14.25
G*L	35	5232.823	149.51***	8.26
G*S	14	1100.643	78.62***	1.74
G*L*S	70	3204.916	45.79***	5.06
REP (L*S)	36	964.553	26.79*	1.52
Error	252	3994.911	15.85	6.31
Total	**431**	**63323.13**		
		r^2=0.94	**% CV=19.19**	

Key: *=$p \leq 0.05$; * * *p=≤ 0.001; r^2=% repeatability

The mean storage root yields of the genotypes (Table 4.5) ranged from 11.19t/ha (LU96/334) to 28.33t/ha (Semusa). The coefficient of variance (CV) was 19.91% which was acceptable and reflects to reliability of the study results under field and rain fed conditions. The r^2 value =0.94 for root yield was closer to one, suggesting 94% repeatability of the trial for this trait (thus, getting similar results when repeated). The relative yields of the eight genotypes (Table 4.5) show that Semusa, the highest yielding reference was the most superior (28.33t/ha) in all the three seasons. The rest of the genotypes had different ranks in different seasons.

Table 4.5 Across locations genotypic means for storage root yield (t/ha)

Genotype	2003	2004	2005	Mean
Semusa	30.94	34.39	19.65	**28.33**
Lu96/303	26.17	28.33	17.98	**24.16**
Lu96/220	26.20	24.33	16.85	**22.46**
Lu96/374	22.50	26.11	13.80	**20.80**
Mugande	21.83	23.72	15.01	**20.19**
Kenya	21.11	24.28	13.10	**19.50**
Lu96/274	22.72	24.56	10.90	**19.39**
Lu96/334	10.56	13.56	9.47	**11.19**
Mean	**22.75**	**24.91**	**14.60**	**20.75±2.36**
LSD (genotypes)	**2.34**	**2.77**	**1.79**	
LSD (Location *Season)				**1.89**
Sig. (Location *Season)				** * * ***

Key: * * *p=≤0.001; Sig. =significant; L=Location; S=season; LSD=least significance difference

The selection criterion for early maturing and high yielding cultivars in Malawi is based on mean root yields of 20t/ha at 5 months after planting (Chipungu *et al.,*1999). The weakness of this selection criterion is that a cultivar may perform so well in one season but not others and still achieve a mean tonnage of 20t/ha over seasons. Such a selection criterion however, overlooks the issue of stability in yield as confirmed by the performance of cultivars in 2005 which was below 20t/ha. Considering the LSD, the root yield means were over 20t/ha and above the lower yielding check, Kenya except for LU96/334 (Table 4.5). Five of the six testing genotypes are therefore strong candidates for release in the national program assuming their performance is convincing in other traits such as tolerance/resistance to pests and diseases, palatability (dry matter content, colour, taste, texture, fiber content *etc.*), root sizes and numbers and adaptability to a wide environments.

4.5.2 Presence and magnitude of GEI on root yield

ANOVA (Table 4.4) showed significant effects of genotypes, locations, seasons and their interactions ($P \leq 0.001$) for root yield. Location which is a predictable environment had a variability of 27.60% of the total variation of yield and was attributed to the differences in soil type and pH and altitude (Table 4.2). The variability among seasons which is the unpredictable environment (13.46% contribution to total SS) may be attributed to rain fall and temperatures

(Figures 4.1 and 4.2) more especially to the rainfall amount which varied more than temperature ranges (Figures 4.1 and 4.2) in the seasons. When GEI is due to variation in predictable environmental factors, sweetpotato breeders can have the alternatives of either developing specific varieties for different environments (locations, soil types, management systems, *etc*.), or broadly adapted varieties that can perform well under variable conditions. However, when GEI results from variation in unpredictable environmental factors, such as year to year variation in rainfall distribution, as is the case in this study, the breeder needs to develop stable varieties that can perform reasonably well under a range of conditions. Such breeding strategies can assist the farmers in risk avoidance. Ceccarelli (1994) and Piepho (1998) indicated that farmers perceive yield stability as the most important factor that can contribute to minimize crop failure, especially in marginal environments. In general, the environment accounted for 62.86% (27.60 + 13.46% + 21.80%) of the total variation. The results (Table 4.5) were indicative of a high influence of the environment on root yield. Significant GEI suggests the need for evaluation of candidate genotypes over multiple sites and seasons for accurate inference (Sial *et al.,* 2001). Further exploration of the response of root to environment, the presence of GEI altered genotypic rankings in different sites and seasons (Table 4.6). The best yield was produced by Semusa in 2003/04 and 2002/03 seasons (34.39 and 30.94t/ha respectively) while in 2004/05, LU96/303 was the best cultivar (19.65t/ha). The most productive season was 2003/04 (24.91t/ha) while 2004/05 (14.60t/ha) was the worst season (Table 4.6). The seasonal difference is attributed to uneven distribution of rainfall amounts in 2004/05 season (Figure 4.1). The season had recurrent dry spells between January and March although the monthly amount in some sites is more or less similar to other seasons. The seasons 2002/03 and 2003/04 were similar in terms of rainfall (Figure 4.1) amounts and distribution. All the three seasons were similar in terms of minimum and maximum temperatures (Figure 4.2).

The best site in terms of performance was Chitedze in 2003/04 (45.00t/ha) and 2002/03 (39.54t/ha). In 2004/05, Baka ranked first for yield (22.29t/ha). For each location, Semusa ranked first in five sites in 2003/04, four sites in 2004/05 and three sites in 2002/03. LU96/303 ranked first in each season at Makoka while LU96/334 ranked the last in each location of 2002/03 and 2003/04 seasons and in three sites in 2004/05 season. The rest of the testing clones varied a lot from position 2 to 8 across sites over the seasons, yet successful new cultivars must show high performance for yield and other essential agronomic traits over a wide range of environments (Becker and Leon, 1988).

Table 4.6 Yield (t/ha) and performance rank (R) over 3 seasons and 6 sites

Genotype	BK	R	MK	R	CH	R	BV	R	CZ	R	LU	R	O M	R
2002/03 season														
Semusa	42.67	1	21.67	3	28.67	1	16.00	6	63.33	1	13.33	5	**30.94**	**1**
Mugande	32.33	3	13.00	7	14.67	7	16.33	5	39.67	5	15.00	2	**26.20**	**2**
LU96/220	38.21	2	21.00	4	24.67	2	19.00	4	42.33	4	12.00	6	**26.17**	**3**
Kenya	21.67	7	20.33	6	19.33	5	21.67	1	33.00	7	10.67	7	**22.72**	**4**
LU96/303	30.00	4	24.67	1	20.33	4	20.00	3	45.00	2	17.00	1	**22.50**	**5**
LU96/274	22.67	5	20.33	5	15.67	6	20.67	2	43.00	3	14.00	3	**21.83**	**6**
LU96/374	22.33	6	23.00	2	24.33	3	13.33	7	38.33	6	13.67	4	**21.11**	**7**
LU96/334	14.67	8	11.00	8	8.33	8	7.67	8	11.67	8	10.00	8	**10.56**	**8**
Mean	**28.07**		**19.38**		**19.50**		**16.83**		**39.54**		**13.21**		**22.75**	
2003/04 season														
Semusa	39.33	1	18.33	4	25.67	1	34.67	1	62.33	1	25.67	1	**34.39**	**1**
Mugande	19.33	7	17.33	6	24.67	2	20.00	7	43.33	7	24.67	2	**24.56**	**4**
LU96/220	25.67	3	17.00	7	21.67	4	21.67	6	46.00	5	21.67	4	**24.33**	**5**
Kenya	20.33	5	18.33	5	17.67	7	27.33	3	46.33	4	17.67	7	**23.72**	**7**
LU96/303	32.00	2	22.00	1	24.33	3	23.00	4	44.67	6	24.33	3	**28.33**	**2**
LU96/274	19.67	6	20.00	3	19.67	5	22.33	5	48.00	3	19.67	5	**24.28**	**6**
LU96/374	22.00	4	20.33	2	18.00	6	33.33	2	49.67	2	18.00	6	**26.11**	**3**
LU96/334	10.67	8	11.33	8	16.67	8	13.33	8	19.67	8	16.67	8	**13.56**	**8**
Mean	**23.63**		**18.08**		**21.04**		**24.46**		**45.00**		**21.04**		**24.91**	
2004/05 season														
Semusa	35.7	2	27.00	4	12.04	1	19.00	1	16.98	1	7.20	2	**17.98**	**2**
Mugande	19.44	4	24.67	7	8.38	3	17.67	3	12.69	3	7.20	1	**13.80**	**4**
Lu96/220	33.95	3	25.00	6	6.58	4	12.67	7	16.72	2	6.17	4	**16.85**	**3**
Kenya	14.61	7	26.67	5	5.25	6	16.67	4	10.91	5	4.53	8	**10.90**	**7**
LU96/303	36.01	1	28.33	1	9.77	2	17.67	2	10.65	6	5.45	6	**19.65**	**1**
Lu96/274	7.51	8	27.00	3	2.57	7	15.67	5	7.20	8	5.45	5	**9.47**	**8**
Lu96/374	16.46	5	28.33	2	5.97	5	14.67	6	12.34	4	5.04	7	**15.01**	**5**
Lu96/334	14.61	6	15.00	8	1.95	8	10.33	8	8.23	7	6.69	3	**13.10**	**6**
Mean	**22.29**		**25.25**		**6.56**		**15.54**		**11.97**		**5.97**		**14.60**	

Key: BK=Baka; MK=Makoka; CH=Chitala; BV=Bvumbwe; CZ=Chitedze; LU=Lunyangwa; OM = overall mean

4.5.2.1 Root yield AMMI for the genotypes across environments

The AMMI analysis of variance of root yield (kg/ha) of 8 genotypes in 18 environments showed that 62.86% of the total SS was attributable to environmental effects, only 14.25% to genotypic effects, and 15.06% to GEI effects (Table 4.4). A large SS for environments suggests that the environments were diverse, with the large differences among environmental means causing most of the variation in root yield.

Table 4.7 AMMI analysis of variance and % GEI explained for root yield

Source	df	SS	MS	% explained	% cumulative
Environment (E)	17	39804.55	2341.44* * *	62.86	62.86
Genotype (G)	7	9020.78	1288.68* * *	14.25	77.11
Rep (GxE)	36	964.56	26.79* * *	1.52	78.63
GxE	119	9538.29	80.15* * *	15.06	93.69
Error	252	3994.91	15.85* * *	6.31	100.00
Total	**431**	**63323.09**			
IPCA 1	23	4919.65	4919.65* * *	51.58	51.58
IPCA 2	21	2545.50	2545.50* * *	26.69	78.27
IPCA 3	19	866.67	866.67* * *	9.09	87.35
IPCA 4	17	588.76	588.76* * *	6.17	93.52
IPCA 5	15	414.98	414.9*	4.35	97.87
IPCA 6	13	130.42	130.42ns	1.37	99.24
IPCA 2	11	72.31	72.31	0.76	100

Key: * and * * *p=≤ 0.05, and 0.001; ns = not significant

The magnitude of the GEI (15.06%) sum of squares was above the one for genotypes (14.25%), indicating that there were differences in genotypic response across environments. Results of the AMMI analysis (Table 4.7) also showed that the first principal component axis (PCA 1) captured 51.58% of the interaction sum of squares. Similarly, the second principal component axis (PCA 2) explained a further 26.69% of the GEI sum of squares. The mean squares for the PCA 1 and PCA 2 were significant at P≤0.001 and cumulatively contributed to 78.27% of the total GEI. An F-test at P≤0.01 suggested that two principal component axes of the interaction were significant for the model with 44 degrees of freedom. The prediction assessment indicated that AMMI with only two IPCA axes was the best model (Zobel *et al.,* 1988). Further interaction principal component axes captured mostly noise and therefore did not help to predict validation observations. Thus, the interaction of the 8 genotypes with 18 environments was best predicted by the first two principal components. In similar studies on linseed yield by Adugna and Labuschagne (2002) the two IPCAs accounted for 69.5% of the total interaction and the remaining 30.5% was the residual or noise not interpretable and was therefore discarded.

To further investigate main effects and interactions across location environments, a biplot (Figure 4.3) was used where both the location environments and genotypes were plotted on the same graph to show associations between genotypes and location environments. Genotype and location environment that appear almost on horizontal line have a similar interaction pattern for the IPCA 2 and those that fall along the perpendicular line or close to it have similar interaction pattern along IPCA 1. The sign (plus or minus) of the interaction is associated with the location of the quadrant. The bottom left quadrant is associated with a negative interaction along the axes and the top right of the quadrant with a positive interaction. The other two quadrants have different signs of interactions for both axes. The IPCA scores of a genotype 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 IPCA scores approximate to zero (0), the more stable the genotype is over all environments sampled. Figure 4.3 is a biplot generated using genotypic and environmental scores of the first two AMMI components as suggested by Vargas and Crossa (2000). Genotype LU96/334 is best for Lunyangwa while Kenya, LU96/274 and LU96/374 are best for Bvumbwe and Makoka and LU96/303 for Chitala. The genotype LU96/303 is near Chitala and therefore best suited for this site. However, the genotype LU96/303 is the only one located near the plot origins of both IPCA1 and 2 and therefore less responsive than the vertex genotypes, thus points near the origin have little interaction. The closer the IPCA scores are to zero, the more stable the genotypes are across their testing environments (Yau, 1995; Purchase, 1997). Such a stable performance is a desirable attribute of cultivars, particularly in Malawi where environmental variations especially the rainfall pattern are very high and unpredictable as exhibited by Figure 4.2. Breeding efforts for such environments should give more emphasis to developing widely adapted varieties.

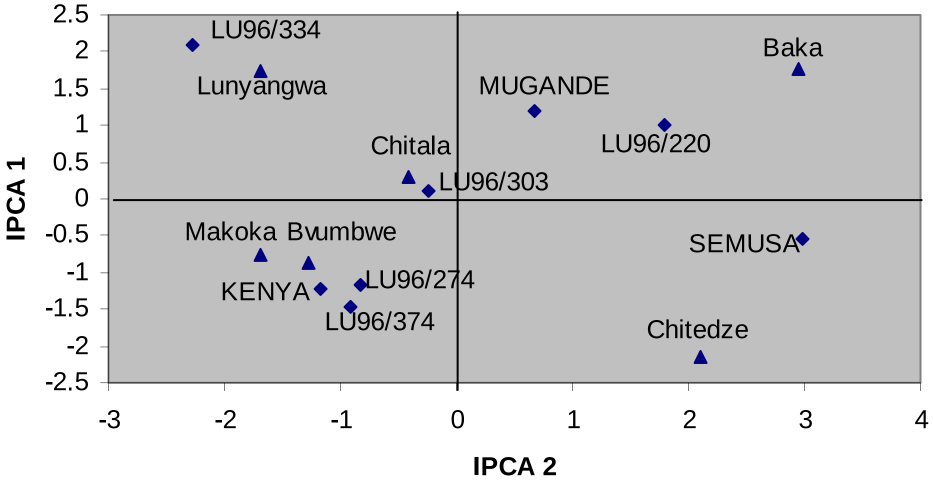

Figure 4.3 Biplot of 8 genotypes and six locations for IPCA scores 1 and 2

The position of cultivar Kenya (mid way for both IPCA 1 and 2) confirms that the cultivar is unstable as reported by survey findings in section 2.5.1.4 of chapter two where in some areas, its yield performance was very low. Since Kenya was ranked as a high yielding cultivar by some farmers during ethno-botanical studies, breeding for specific localities need to be encouraged using these existing evaluation sites. Semusa was unstable (Figure 4.4) over the sites when variation was explained by IPCA 1 (51.58%) but stabilised (Figure 4.3) when IPCA 2 was included where it became closer to zero. In contrast, LU96/334 is a non-adapted genotype as indicated by its large IPCA 1 score and large IPCA 2 score, thus relatively unstable. Mugande and LU96/220 are testing genotypes of interest (high yield) which are moderately stable and are not associated with any site. Other genotypes of interest are LU96/274 and LU96/374 which are stable when explained by IPCA 2. Points that are near each other as Makoka and Bvumbwe for environments and Kenya, LU96/274 and LU96/374 for genotypes denote similarity in terms of interaction pattern. Conversely, points distant from each other as Semusa and LU96/334 genotypes and Lunyangwa and Chitedze for sites are dissimilar with completely different values of interaction.

Figure 4.4 is a biplot that shows not only the average yield of a genotype but also how it is achieved (PCA 1 effects). That is, the biplot also shows the yield of a genotype at individual sites. Genotype Semusa had the highest average yield because it yielded the highest at sites Chitedze and Baka, and yielded above average at all other sites.

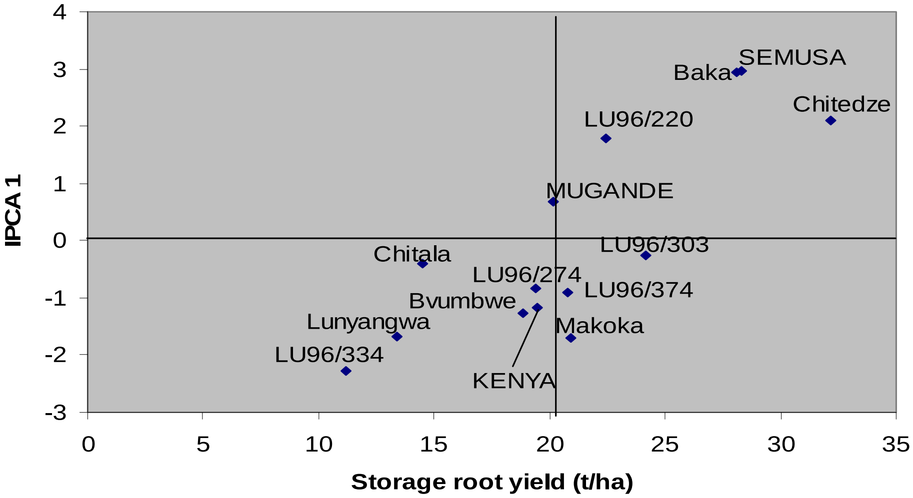

Figure 4.4 Biplot of 8 genotypes and six locations for root yield and IPCA 1 scores

On the other hand, the average yield of genotype LU96/334 was the least while the yield of Mugande was average. LU96/220, LU96/303 and LU96/374 yielded above average. Piepho (1996) indicated that if cultivars are being selected for a large group of environments, stability and mean yield across all environments are more important than yield for specific environments. In this study therefore, LU96/303 is considered the most stable genotype (Figures 4.3 and 4.4) suitable for the variable production conditions under small holder farmers in the country. In addition, the cropping seasons have been observed to be variable as shown by the erratic rains in 2005 seasons which resulted in low root yields across sites for most of the genotypes. Chitedze and Baka were conducive to high yields (above average). Although Bvumbwe and Makoka were close to each other, thus relatively similar (Figure 4.4), Makoka yields were however above average (Figure 4.5) and therefore a better site of the two.

Knowledge of GEI helps plant breeders to reduce the cost of extensive genotype evaluation by eliminating unnecessary testing sites (Shafii *et al.,* 1992; Kang and Magari, 1996). Conversely, the presence of a large GEI may necessitate the establishment of additional testing sites.

4.6 Conclusions

The combined analysis of variance across locations and seasons on the sweetpotato root yield (t/ha) showed highly significant ($p<0.001$) differences in the performance of genotypes. While genotypes only accounted for 14.25% most of the variance (62.86%) was attributed to the environment. These results were indicative of the presence of environmental effects that necessitated stability analysis to identify stable genotypes. ANOVA on storage root yield identified the genotype Semusa as the highest yielding (28.33t/ha). However, AMMI stability analysis revealed that LU96/303 (24.16t/ha) was the most stable. While Lunyangwa was a low yielding site, Chitedze was the highest. The economic implication for preliminary yield evaluation which has a large number of testing entries is that Chitedze should be used for screening to capture maximum yield potential. In times of limited resources, Baka and Bvumbwe sites are not necessary for yield evaluation as they are similar to Chitedze and Makoka respectively. However, AMMI analyses for other traits such as disease resistance/tolerance may require the inclusion of these sites for those other variables.

CHAPTER 5

THE NUTRITIONAL VALUE OF 15 SWEETPOTATO GENOTYPES

Abstract

Sweetpotato is a major food security crop in Malawi and Sub-Saharan Africa where micronutrient deficiency, especially iron and zinc in humans is an important health problem. High nutrient dense sweetpotato genotypes suits subsistence communities in alleviating the deficiencies. Using 15 sweetpotato genotypes grown at Bvumbwe Research Station in 2005 season, a study was undertaken to determine the extent of genotypic variability for β-carotene, ascorbic acid, iron, zinc and copper concentrations as influenced by storage root age and peeling. Analysis of variance (ANOVA) on β-carotene content identified Zondeni as an excellent source of the nutrient (13.93 mg/100g) in unpeeled storage roots of five months old. Zondeni maintained its superiority as the age of storage roots at harvest varied from 4 to 7 months old. Results also showed that the genotypes can reliably be differentiated based on ascorbic acid, iron, zinc and copper at different storage root ages and peeling. The existence for genetic variation for these traits entails the potential to breed for higher levels of these nutrients.

Key words: sweetpotato, genotypes, β-carotene, ascorbic acid, iron, zinc, copper, peeling, root age

5.1 Introduction

Food security exists when people at all times have both physical and economic access to enough safe and nutritious food which meets their dietary needs (FAO, 1996). At the dawn of the new millennium (2000-2002) more than 850 million people worldwide were undernourished (FAO, 2004). This indicates that of the 6 billion people in the world, one out of seven suffer from hunger. However, a much larger population of people suffer from a different, stealthier form of hunger than the simple lack of sufficient quantities of foodstuffs. Micronutrient malnutrition, or "hidden hunger" is caused by a lack of food of sufficient dietary quality (Kennedy *et al.,* 2003). Monotonous diets based on cereals and other starchy staple foods frequently fail to deliver sufficient quantities of essential minerals and vitamins like iodine, iron, zinc and vitamin A (Demment *et al.,* 2003). Generally, the dietary intakes of economically and socially deprived communities in developing countries largely consist of plant-based staple foods, while fruits, vegetables and animal products are seldom consumed. These communities are thus predisposed to micronutrient deficiencies resulting into malnutrition. This is now a massive and rapidly growing public health problem among most poor people in many developing nations. In order to address the prevalent micronutrient deficiencies directly, the conventional approach is supplementation (supplying micronutrients such as Vitamin A mega doses), food fortification, dietary diversification and nutrition education. Additional measures include public health like control of parasites (through deworming), sanitation improvement and personal hygiene.

The most effective and long term solution to addressing micronutrient deficiencies appears to be bio-fortification where plants are bred to fortify themselves (Bouis *et al.,* 2000). Focusing research and development on bio-fortification approach on staple food crops could increase the micronutrient intake of the poor, who are most at risk of suffering from vitamin and mineral deficiencies. Bio-fortification which is self-targeting (Bouis, 2002; Welch and Graham, 2002) provides food-based strategies which empowers individuals and households to take ultimate responsibility over the quality of their diet through their own production of nutrient-rich foods and informed consumption choices. These strategies are said to be "the ideal long-term goal towards which society strives - provision of assurance of access to a nutritionally adequate diet achieved through diversity of food availability, wise consumer selection, proper preparation, and adequate feeding" (Howson *et al.,* 1998). These food-based strategies are also appealing because they can address multiple nutrients simultaneously, including dietary energy, proteins and various micronutrients, without the risk of antagonistic nutrient interactions or overload.

This chapter concerns expanding knowledge through the identification of mineral and vitamin rich sweetpotato cultivars in Malawi that may assist in addressing five different micronutrient deficiencies (iron, zinc, copper, vitamins A and C).

5.2 Literature review

The role and sources of five micronutrients investigated in the study for better human well being are discussed. The contribution of agriculture and plant breeding as strategies to micronutrient reduction are also highlighted.

5.2.1 Micronutrients, sources and deficiency symptoms in humans

Micronutrients which are the essential vitamins and minerals are required by human beings to stimulate cellular growth and metabolism (Combs, 1998). Human beings require various trace elements and vitamins for growth and health. The important elements are: arsenic, boron, chromium, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, vanadium and zinc. Vitamins occur in two major groups namely water soluble vitamins; ascorbic acid, biotin, cobalamin, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin and fat-soluble vitamins; retinoic acid, calciferol, tocopherol, phylloquinone and menoquinone (Combs, 1998). A deficiency in one or more of these nutrients may result in stunted growth (Allen, 1994), increased morbidity (Tomkins, 1993) or delayed cognitive function. Micronutrient deficiencies usually occur when the habitual diet lacks diversity or is overly dependent on a single staple food such as monotonous cereal- or tuber-based diets (FAO/WHO, 2002). Deficiencies of iron, iodine and vitamin A are the most widespread micronutrient malnutrition forms with public health consequences (Mason and Garcia, 1993).

5.2.1.1 Sources and roles of Vitamin A

The fat soluble vitamin A is required by all body tissues for normal growth and tissue repair. The visual and immune systems are particularly dependent upon this vitamin for normal functioning. World Health Organisation (WHO) defines vitamin A deficiency (VAD) as tissue concentration of vitamin A low enough to have adverse health consequences even if there is no evidence of clinical xerophthalmia (WHO, 1996). Vitamin A in the form of retinol is present in a variety of foods including eggs, milk and fish, or in its precursor form as carotene in yellow fruits and vegetables, green leafy vegetables and red palm oil. Retinol forms of vitamin A are more readily absorbed by the body than carotene, although the bioavailability of carotene can be enhanced by consuming dietary sources of fat at the same time. Since there are losses during the absorption of carotene and its conversion into retinol, it is convenient to describe the vitamin A activity of foods in terms of 'retinol equivalents.' Normally, 1µg retinol equivalent is taken as corresponding to 6 µg beta-carotene, although there are strong indications that this factor generally underestimates the retinol equivalents of most diets. In the most recent edition of the Dietary Reference Intakes (Food and Nutrition Board, 2001), the vitamin A equivalency factors for provitamin A carotenoids from foods were increased from 6:1 to 12:1 for ß-carotene [12 µg ß-carotene = 1 µg retinol = 1 retinol activity equivalent (RAE)]. The equivalency factors were increased because recent evidence indicates that the efficiency of absorption of ß-carotene from foods is lower than previously estimated which is 16% compared with 33% (Van het Hof *et al.,* 1999; Food and Nutrition Board, 2001). The latest RDA (Table 5.1) revised by the Food and Nutrition Board of the in 2001 is based on the amount needed to ensure adequate stores of vitamin A in the body to support normal reproductive and immune function and vision.

Table 5.1 RDA for Vitamin A (Retinol; Food and Nutrition Board, 2001)

Life Stage	**Age**	**Males (µg /day)**	**Females (µg /day)**
Infants	0-6 months	400	400
Infants	7-12 months	500	500
Children	1-3 years	300	300
Children	4-8 years	400	400
Children	9-13 years	600	600
Adolescents	14-18 years	900	700
Adults	19 years and older	900	700
Pregnancy	19-years and older	-	770
Breastfeeding	19-years and older	-	1,300

5.2.1.2 Sources and roles of Vitamin C

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. Unlike most mammals, humans do not have the ability to make their own vitamin C. Therefore vitamin C must be obtained through the diet. Food sources for vitamin C include guavas, citrus fruits, pawpaw, red sweet pepper and vegetables. Vitamin C is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments, bone and neurotransmitter (norepinephrine) which is critical for brain function. In addition, vitamin C is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat to cellular organelles called mitochondria, for conversion to energy (Carr and Frei, 1999). Severe vitamin C deficiency has been known for many centuries as the potentially fatal disease, scurvy. Symptoms of scurvy include bleeding and bruising easily, hair and tooth loss, joint pain and swelling. Such symptoms appear to be related to the weakening of blood vessels, connective tissue, and bone, which contain collagen. Early symptoms of scurvy such as fatigue may result from diminished levels of carnitine, needed to derive energy from fat or decreased synthesis of the neurotransmitter, norepinephrine. Scurvy is rare in developed countries because it can be prevented by as little as 10 mg of vitamin C daily (Simon and Hudes, 2000).

Table 5.2 RDA for Vitamin C (Food and Nutrition Board, 2001)

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	40	40
Infants	7-12 months	50	50
Children	1-3 years	15	15
Children	4-8 years	25	25
Children	9-13 years	45	45
Adolescents	14-18 years	75	65
Adults	19 years and older	90	75
Smokers	19 years and older	125	110
Pregnancy	19-years and older	-	85
Breastfeeding	19 years and older	-	120

However, recent cases have occurred in children and the elderly on very restricted diets (Weinstein *et al.,* 2001). Table 5.2 presents the recommended dietary allowance for vitamin C (Food and Nutrition Board, 2000). The recommended intake for smokers is 35 mg/day higher than for nonsmokers due to increased oxidative stress from the toxins in cigarette smoke and generally have lower blood levels of vitamin C.

5.2.1.3 Sources and roles of iron

Iron (Fe) is an essential nutrient that carries oxygen and forms part of the oxygen-carrying proteins, hemoglobin in red blood cells, myoglobin in muscle and a necessary component of various enzymes (Beard and Dawson, 1997). The major function of Fe is to combine with protein and copper in making hemoglobin. Hemoglobin transports oxygen in the blood from the lungs to the tissues which need oxygen to maintain basic life functions; iron builds up the quality of the blood and increases resistance to stress and disease; it is also necessary for the formation of myoglobin which supplies oxygen to muscle cells for use in the chemical reaction that results in muscle contraction (Yip and Dallman, 1996). Iron deficiency is considered as a micronutrient deficiency of public health significance largely because it is widespread; nearly two billion persons (both adults and children) are affected worldwide. Nutritional iron deficiency, or habitual iron intake that is insufficient to cover requirements, is the most common cause of iron deficiency (FAO/WHO, 2002). Dietary sources of iron occur in two forms, haem and non-haem iron. While haem iron, found in animal-source foods such as meat, poultry and fish, has greater bioavailability, the non-haem iron, found in cereals, pulses, fruits and vegetables is not. Iron deficiency is the most common cause of anaemia, which can also occur as a result of vitamin B12 or folate deficiencies, congenital hereditary defects in red cells, reproductive blood losses, or from infection by malarial parasites or infestations of the gut by parasites such as hookworm (FAO/WHO, 2002).

Table 5.3 RDA for Iron (Food and Nutrition Board, 2001)

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	0.27	0.27
Infants	7-12 months	11	11
Children	1-3 years	7	7
Children	4-8 years	10	10
Children	9-13 years	8	8
Adolescents	14-18 years	11	15
Adults	19-50 years	8	18
Adults	51 years and older	8	8
Pregnancy	all ages	-	27
Breastfeeding	19 years and older	-	9

Table 5.3 presents the RDA for iron revised in 2001 based on the prevention of iron deficiency and maintenance of adequate iron stores in individuals (Food and Nutrition Board, 2001). Iron deficiency is more prevalent in women than in men and more particularly during pregnancy leading to serious consequences for both mother and baby; women experience higher mortality risk during childbirth and an increased incidence of low-birth-weight babies (FAO/WHO, 2002). Increased iron utilization by the developing fetus and placenta, as well as blood volume expansion significantly, increases the iron requirement during pregnancy. Infants and children between the ages of 6 months and 4 years also have high iron requirements due to the rapid growth rates sustained during this period. Menstruation in female adolescents adds to the increased iron requirement due to rapid growth and the blood loss that occurs.

5.2.1.4 Sources and roles of Zinc

Zinc (Zn) is an essential trace mineral highly concentrated in specialized areas of the brain, pancreas and adrenal gland, but is present in all cells. Zinc has structural, catalytic (enzymatic) and regulatory roles (Cousins, 1996). Zinc function as an antioxidant nutrient; necessary for protein synthesis; wound healing; vital for the development of the reproductive organs, prostate functions and male hormone activity; it governs the contractility of muscles; important for blood stability; maintains the body's alkaline balance; helps in normal tissue function; aids in the digestion and metabolism of phosphorus (Cousins, 1996). Consequences of Zn deficiency includes the slowing or cessation of growth and development, delayed sexual maturation, characteristic skin rashes, chronic and severe diarrhea, immune system deficiencies, impaired wound healing, diminished appetite, impaired taste sensation, night blindness, swelling and clouding of the corneas, and behavioral disturbances (Brown and Wuehler, 2000). Zinc deficiency is prevalent among women, infants and children when they do not access dietary sources of the most bioavailable iron (animal meats) which are also the most important sources of bioavailable Zn in peoples' diets (Shrimpton, 1993; Gibson, 1994). Important plant sources for zinc include cereals, nuts, legumes and soy products.

Table 5.4 RDA for Zinc (Food and Nutrition Board, 2001).

Life Stage	Age	Males (µg /day)	Females (µg /day)
Infants	0-6 months	2	2
Infants	7-12 months	3	3
Children	1-3 years	3	3
Children	4-8 years	5	5
Children	9-13 years	8	8
Adolescents	14-18 years	11	9
Adults	19 years and older	11	8
Pregnancy	19 years and older	-	11
Breastfeeding	19 years and older	-	12

The RDA (Table 5.4) for zinc is listed for all age groups because infants, children, pregnant and lactating women are at increased risk of zinc deficiency (Food and Nutrition Board, 2001). Zn supplements offer approaches to reducing Zn deficiency and frequency of diarrhoea in infants and children (Hambidge, 1992; Sazawal *et al.,* 1996). A number of zinc supplements are available, including zinc acetate, zinc gluconate, zinc picolinate and zinc sulfate (Food and Nutrition Board, 2001). When Zn supplements are given to poor children, growth rates increase demonstrating that Zn deficiency may be widespread (Gibson, 1994).

5.2.1.5 *Sources and roles of Copper*

Copper (Cu) is a trace element that is essential in most animals, including humans. The influence of copper upon human health is due to the fact that it is part of enzymes, which are proteins that help biochemical reactions occur in every cell (Turnlund, 1999).

Table 5.5 RDA for Copper (Food and Nutrition Board, 2001)

Life Stage	Age	Males (µg /day)	Females (µg /day)
Infants	0-6 months	200	200
Infants	7-12 months	220	220
Children	1-3 years	340	340
Children	4-8 years	440	440
Children	9-13 years	700	700
Adolescents	14-18 years	890	890
Adults	19 years and older	900	900
Pregnancy	all ages	-	1,000
Breastfeeding	all ages	-	1,300

Copper is involved in the absorption, storage and metabolism of iron and therefore the symptoms of a copper deficiency are similar to those of iron deficiency anemia (Fosmire, 1990). Individuals at high-risk due to copper deficiency include: premature infants, especially those with low-birth weight, infants with prolonged diarrhea, infants and children recovering from malnutrition and individuals with mal-absorption syndromes (Turnlund, 1999). The essentiality of copper in human nutrition was associated with the discovery of Menkes disease, a genetic copper-deficiency syndrome (Danks *et al.,* 1972). Excessive high copper intakes also increase risk of death from cancer and cardiovascular disease. Copper may be absorbed by both the stomach and small intestinal mucosa, with most absorbed by the small intestine. Copper is found in the blood bound to proteins. Meat organs, nuts, chocolate and shellfish have the highest copper content (Murphy *et al.,* 1975; Olivares and Uauy, 1996). Most of these rich food sources however are not commonly consumed in developing countries resulting in the copper provided by plant-based foods than would be expected considering their relatively low contents of the mineral and also poor bioavailability (Wright *et al.,* 1991; Subar *et al.,* 1998). The RDA for copper presented in Table 5.5 reflects the results of depletion-repletion studies to prevent deficiency (Food and Nutrition Board, 2001). Supplements are available as cupric oxide, copper gluconate, copper sulfate and copper amino acid chelates (Hendler and Rorvik, 2001).

5.2.1.6 Interactions between the nutrients under study

Studies in humans (Hodges *et al.,* 1978) have showed that vitamin A deficiency causes abnormalities in iron metabolism and that supplementation improves iron status as measured by hematological indexes (Bloem *et al.,* 1990). Zinc deficiency is commonly associated with low plasma concentrations of vitamin A, even when hepatic vitamin A stores are normal, suggesting that there is a defect in mobilization of vitamin A rather than in its absorption or transport to the liver. Zinc deficiency causes impairement in the synthesis of proteins that turnover rapidly, such as retinol binding protein (RBP). This impairment affects retinol transport from the liver to the blood circulation and other tissues because retinol is transported as a retinol-RBP complex in association with transthyretin. Shingwekar *et al.* (1979) indicated beneficial effects of zinc supplementation on vitamin A metabolism in malnourished children, preterm infants and adults with alcoholic cirrhosis (Hustead *et al.,* 1988). The amount of iron absorbed by young women from two different bean varieties of similar iron density was greater for the beans with higher zinc density (King *et al.,* 2000). Such a synergistic effect strongly indicates breeding crops for both iron and zinc density in order to address iron-deficiency anaemia effectively.

In iron deficient individuals, vitamin C greatly enhances iron absorption from foods when consumed in the same meal (Monsen, 1982). The absorption-enhancing effect is much smaller when the vitamin C is taken 4 to 8 hours before the meal. Both synthetic and dietary ascorbic acid enhance iron absorption (Hallberg *et al.,* 1989). Ascorbic acid also increases the iron bioavailability of those iron compounds used in fortification (Hurrell, 1997) and it is also a promoter for zinc uptake in human. Adequate copper appears to be necessary for normal iron metabolism and red blood cell formation. Anemia is a clinical sign of copper deficiency. Animal studies demonstrate a role for copper in iron absorption (Vulpe *et al*., 1999) which has been found to accumulate in the livers of copper deficient animals, indicating that copper is required for iron transport to the bone marrow for red blood cell formation (Turnlund *et al*., 1999). Infants on a high iron formula food base absorbed less copper than a low iron formula, suggesting that high iron intakes may interfere with copper absorption (Food and Nutrition Board, 2001).

5.2.2 Extent of micronutrient deficiencies in the world and Malawi

Statistics on the extent of micronutrient deficiencies have been provided by various reports on important elements and vitamins that call for health attention. Figure 5.1 shows that more than two-thirds of the world population- for the most part women and children suffer from at least one micronutrient deficiency: 4-5 billion people are iron deficient (WHO, 2003), 2 billion are iodine deficient, about 150 million are vitamin A deficient (UN-SCN, 2004) and as many as 3 billion people are at risk of zinc deficiency (Hotz and Brown, 2004).

Table 5.6 Malawi demographic indicators (GoM, 2000)

Indicator	Malawi	Urban	Rural
Total fertility rate (children per woman)	6.3	4.5	6.7
Infant mortality (infant deaths per 1,000 births)	104	83	117
Child mortality (child deaths per 1,000 births)	95	71	106
Under 5 mortality rate (< 5 deaths per 1,000 births)	189	148	210

In Malawi, the prevalence of iron and Zinc deficiency among pre-school children are 80% and 34.2% respectively (Hotz and Brown, 2004). The country also continues to experience food insecurity and malnutrition which results in high infant and child mortality rates (Table 5.6). Infant and under five mortality rates were estimated to be 104 and 189 deaths per 100,000 live births, respectively in 2000. The maternal mortality rate was 1,120 deaths per 100,000 live births.

The Malawi Demographic and Health Surveys (GoM, 2000) showed that the proportion of children who are under-weight is more than 12 times the level expected in a healthy well-nourished population (Table 5.7). Wasting affects 6 % of the children, three times the level expected in a healthy population.

Table 5.7 Demographic indicators for children under-five years (GoM, 2000)

Indicator	1992	2000
Underweight (too thin for age)	27%	25%
Wasting (too short for height)	5%	6%
Stunting (too short for age)	49%	49%

The proportion of stunted children (49 % in 2000) is almost 25 times the level expected in a healthy well-nourished population. In addition, the poor consume only 66 % of the recommended daily calorie requirements, implying that malnutrition affects the adults too. The consequences of micronutrient deficiencies are profound as they diminish the health, livelihood, and well being of all those afflicted and the productivity and stability of the societies in which these people live in ways that are not always obvious (Combs *et al.,* 1996; Welch *et al.,* 1997).

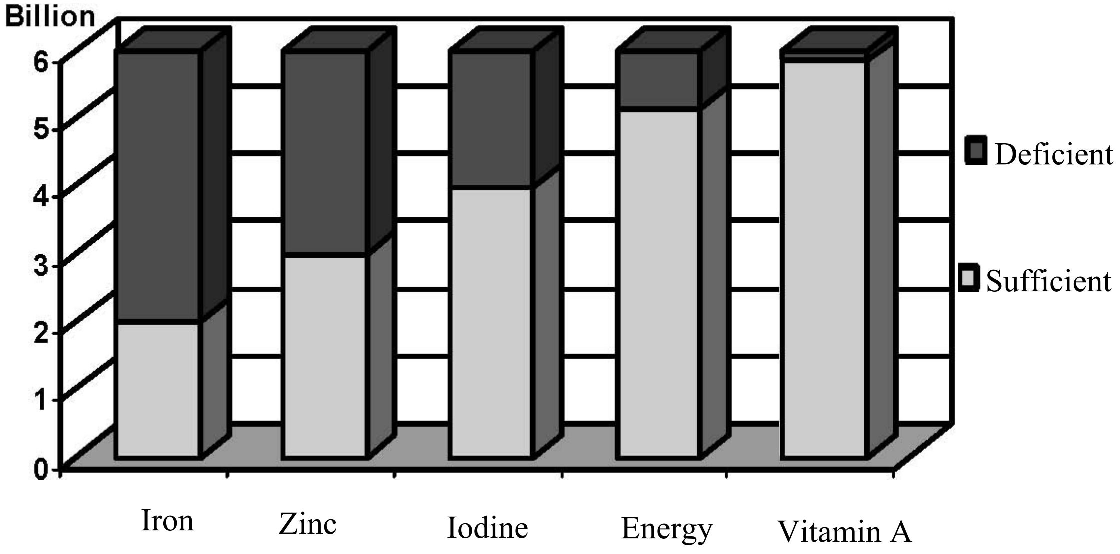

Figure 5.1 Number of people suffering from malnutrition worldwide (Hotz and Brown, 2004; UN-SCN, 2004; WHO, 2002)

Given these large figures (Figure 5.1, Tables 5.6 and 5.7) and the general agreement on the individually debilitating and economically damaging effects of micronutrient malnutrition, focusing public attention and research on this more subtle form of malnutrition is certainly warranted (Black, 2003). These three elements (iodine, iron and zinc) and vitamin A are of first priority in primary health care.

5.2.3 Role of agriculture in micronutrient malnutrition intervention

Situations of food insecurity, where populations do not have enough to eat, will inevitably result in micro nutrient deficiency. Apparently, agriculture's primary focus on producing more cereals which are rich in calories to meet growing energy demands from population pressures has resulted in reduced food supplies that are high in micronutrients, especially for the world's poor. The use of modern cereal cropping systems in many developing nations has been paralleled by decreased per capita production of traditional foods having higher micronutrient density (*e.g.,* legume seeds and pulses). In Malawi, maize contributes 80 per cent of the daily food calorie-intake and occupies approximately 76 per cent of Malawi's total cultivable land (Malawi Government, 1999). Increased cereal production has resulted in lower availability of foods rich in micronutrients for the poor at least in some regions of the world. Legume seeds are rich sources of micronutrients in diets than cereal grains for two reasons. First, most commonly eaten pulse seeds (beans, lentils, chickpeas, peas, *etc.*) contain higher concentrations of micronutrients when compared to whole rice and wheat grain (Doesthale *et al.,* 1979). Second, pulse seeds are normally prepared and eaten without removal of seed parts while both rice and wheat grain are normally processed (*i.e.,* milled and/or polished) before cooking and consumption which removes various grain parts (*e.g.,* the germ and aleurone layer) that are rich sources of micronutrients. Many nutritionists and social scientists believe that the integration of food rich in micronutrients into the diet is the only sustainable way to improve micronutrient status in the human body (Ali and Tsou, 1997). The more diverse the sources of food in a diet, the more likely balanced amounts of all micronutrients will be consumed (Anonymous, 1993). Interventions using micronutrient supplements and food fortificants are important programs currently being used to provide adequate micronutrient nutrition in many nations. These programs are very important in treating micronutrient deficiencies to large numbers of people relatively quickly; they should continue to be used as short- to medium-term interventions for decreasing micronutrient malnutrition. However, agriculture should become a primary intervention tool to eliminate "hidden hunger" in sustainable ways (Welch *et al.,* 1997). Thus food diversity is the traditional means of ensuring a balanced diet containing all these micronutrients, but this is becoming increasingly difficult as the global population continues to increase. In announcing the urgency of addressing dietary deficiencies in human populations, WHO/FAO (United Nations, 1992) called on governments to fund food-based solutions. Plant foods are able to supply all these micronutrients in adequate amounts with the exception of cobalamin that comes mostly from animal products and bacterial contaminants on plant food.

5.2.4 Iron, zinc and copper uptake from the soil

Since iron, zinc and copper are usually present in soil in adequate to excess amounts, deficiency is caused by their presence in an unavailable form rather than by their lack, and a plant can improve its iron, zinc and copper uptake by using strategies solubilize these mineral elements present in the soil (Rengel, 2001). For the most part, plants acquire micronutrients by absorbing them from the soil solution; therefore, the availability of micronutrients to plants is closely related to the solubility of the forms in which they appear (Aquaah, 2002). Several environmental factors can affect the solubility of micronutrients. Leached, acid, sandy soils, organic soils, soils that have supported intensive cropping, soils with high pH, and eroded soils all tend to be low in available iron and zinc (Brady and Weil, 2002). Uptake efficiency of soil-grown plants may consist of increased capacity to solubilize non-available nutrient forms into forms that are available to the plant, and/or increased capacity to transport nutrients across the plasma membrane. However, it appears that increased conversion capacity is of greater importance for efficient uptake, especially for nutrients that are transported to roots by diffusion (Rengel, 2001). Tolerance to micronutrient-deficient soils, termed *micronutrient efficiency*, is a genetic trait of a genotype or phenotype that causes a plant to be better adapted or to produce higher yields in a micronutrient-deficient soil than the average cultivar of the species (Graham and Rovira, 1984). For instance, growing zinc- efficient plants on zinc-deficient soils, "tailors the plant to fit the soil" instead of "tailoring the soil to fit the plant" (Foy, 1983). These efficient genotypes exude substances from their roots that chemically unbind trace minerals from other binding elements and make trace minerals available to the plant. Other micronutrient-efficient varieties grow deeper roots in mineral deficient soils thereby tapping subsoil water and minerals (Grubb, 1994).

5.2.5 Role of plant breeding in micronutrient malnutrition intervention

Prioritising plant-breeding in food-based strategies is important as it increases (1) the concentration of certain trace minerals and vitamins, (2) the bioavailability of micro nutrients by reducing the concentration of anti-nutrient factors (inhibitors of absorption) or (3) the concentration of absorption promoters. Fortified plants provide a delivery system for nutritional supplements that would have few recurring costs. Once farmers have the appropriate seeds, they could continue to grow them year after year. In contrast, supplementation or fortification programs require ongoing expenditures to keep delivering required nutrients. Furthermore, if the nutrient dense crops are unchanged in appearance, processing and cooking qualities, and taste, they could provide higher levels of micronutrients in the diet without requiring any apparent dietary change. The role of plant breeding in combating micronutrient deficiency is therefore to identify, select and recommend efficient genotypes that are self fortifying.

5.2.6 Rationale underlying sweetpotato dietary intervention strategy

In a food-based approach to nutrition improvement, the choice of crops is important. Sweetpotato is a logical choice for such an intervention as the crop is already widely cultivated in Malawi as a secondary dietary source of calories. Further, among plant sources of vitamin A from dark orange fleshy fruits and vegetables (such as ripe mango, papaya, or sweetpotato, but not oranges), appears to be more bio-available (absorbed and utilized) than the vitamin A from dark leafy greens (de Pee *et al.,* 1995; Khan *et al.,* 1997; Jalal *et al.,* 1998). The commonly used conversion factors for estimating vitamin A intake are based on two studies conducted in the 1940s and reflect the higher bio-available of animal sources of vitamin A over plant sources:

- Retinol-rich foods (animal sources): 1 μg retinol = 1 RE
- Fruits, pumpkin, orange-fleshed sweet potatoes: 12 μg beta-carotene = 1 RE

- Dark green leafy vegetables or carrots: 26 μg beta-carotene = 1 RE

Absorption varies considerably depending on the initial health status of the individual. For example, persons with very low serum retinol status initially absorb more than those with better status. The presence of inhibitors, such as fiber, and enhancers, such as fat, are also major factors determining differences seen in bioavailability among plant sources (de Pee, 1999). However, given the very high level of beta-carotene present in orange-fleshed varieties of sweetpotatoes, it still remains an excellent source of vitamin A. Cultivars that provide > 100 RE/100 grams) are adequate sources of vitamin A (Low *et al.,* 1997), even at half of the commonly used conversion rate and taking losses due to preparation into account. These results do indicate that emphasis should be placed on promoting sweetpotato roots over sweetpotato leaves. Further, the advantage with sweetpotato as a crop is that it can grow on soils of limited fertility, is relatively drought tolerant, provides good ground cover, and is usually cultivated without fertilizer or pesticide (Ewell, 1990); Sweetpotato is a classic food security crop in Malawi that once matured, roots are harvested over a period of several months. Sweetpotato is therefore ideal for gap filling in food availability from other sources. More importantly, studies have revealed that sweetpotato leaves which are widely consumed are an important source of several micronutrients.

5.3 Justification of the study

Bio-fortification (genetic micronutrient-enrichment) of a crop that is a staple and commonly used could be an important and sustainable means of reducing micronutrient malnutrition in Malawi. Available research has indicated that micronutrient enrichment traits are available within the genomes of crops as a result, improvements in micronutrient concentration can be made without adversely affecting yield and furthermore, enrichment traits appear to be stable across soil types and climatic environments (Welch and Graham, 2002).

Traditional breeding requires that genetic variation for the trait in question, in this case, pro-vitamin A, Vitamin C, iron, zinc and copper density exists in the germplasm for sweetpotato. An important initial step in developing a bio-fortified crop is the screening of germplasm to determine the existence of genetic variation. Studies with common bean (*Phaseolus vulgaris*), rice (*Oriza sativa*), wheat (*Triticum* spp.) have large (two- to three-fold) variation in iron and zinc concentration (Welch and Graham, 2004). Results of a study at CIMMYT showed that of the wheat varieties released by that organization over 40 years of breeding, the best had about

20% higher in iron and/or zinc concentration in grain than the lowest (Graham *et al.,* 1999). This chapter therefore reports on the genotypic variation in the nutrient levels of β-carotene and ascorbic acid in 15 sweetpotato genotypes.

The analysis of iron, zinc and copper was also necessary in light of the emerging evidence synergistic effects between carotene, iron and zinc. Genotypic screening for the five nutrients is the first of its kind in Malawi and provides the basis for sweetpotato bio-fortification program towards a sustainable approach to promoting the production and consumption of adequate quantities of good-quality, nutritious sweetpotato roots, especially among the nutritionally vulnerable groups. Filling this knowledge gap regarding the possible impact of bio-fortification is both vital and urgent. Neglecting a potentially effective intervention and any delays in its implementation may literally cost lives. Thus the knowledge base of the findings of this research work will contribute to nourishing Malawi as a nation through the promotion of the right cultivars and their incorporation in the breeding programs. People with poor health are less able to fend for themselves as well as care for others. Since agricultural production in Malawi is labour intensive, productivity of people with poor health is minimal, thereby perpetuating food insecurity and malnutrition and therefore poverty.

5.3.1 General objective

The general objective is to determine the existence of genotypic variation in β-carotene, Vitamin C, iron, zinc and copper among sweetpotato germplasm in Malawi.

5.3.2 Specific objectives

(i) To establish the existence of genotypic variation in content of iron, zinc, copper, pro-vitamin A and vitamin C in 15 sweetpotato genotypes

(ii) To establish genotypic variability in the retention of iron, zinc, copper, pro-vitamin A and Vitamin C as affected by age and peeling of the sweetpotato storage roots

5.4 Materials and Methods

5.4.1 Study cultivars

Fifteen sweetpotato cultivars from different sources (Table 5.8) comprising 4 released genotypes 2 local bred elite clones, 2 elite introductions and seven landraces (local) were planted at

Bvumbwe Research Station 19th December 2005. The landraces represented low to high yielding, early to late maturing, good to bad taste, texture, varying flesh colour, flavour and susceptibility and resistance to weevil attack as described by farmers during the IK survey. Released cultivars, locally bred clones and introductions represented breeders' selections. Experimental plots were arranged in a Randomized Complete Block Design (RCBD), replicated three times. A gross plot consisted of two ridges (4.8m long) spaced at 90 x 90 cm apart and planting stations of 30 x 30 cm apart. Weather details of Bvumbwe are presented in Figures 4.2 and 4.3. Sampling times were on 19th April, 19th May and 19th July representing 4, 5 and 7 MAP.

Table 5.8 Origin and status of the study cultivars

Cultivar	Origin	Status
Zondeni	Local	Wide spread, low to medium yield and maturity
LU96/374	Local bred	Elite clone, high yield, early maturing
Yoyera	Local	Being eroded due to very low yields and late maturing,
Kamchiputu	Local	Being eroded due to very low yields and late maturing,
Tainoni (Tainon 57)	AVRDC[a]	Released in 1999, low adoption, early maturing
Pepu	Local	High yield, not widely grown
Babache	Local	Grown in Lower Shire, Medium yield, late maturity
Mfumu	Local	Grown in Lower Shire, Medium yield, medium maturity
LU96/303	Local bred	Elite clone, high yield and early maturity
Kenya (Kemb 10)	Tanzania	Wide spread, released 1988, high yield, early maturity
Lunyangwa	Local bred	Released in 1990, high yield, early maturity, low adoption
Semusa (Cemsa 74-228)	CIP[b]	High yield, early maturity, released 1999 increased adoption
A45	RSA[c]	Preliminary evaluation trials, high yield, medium maturity
Mzungu	Local	High yield, early maturity
Mafutha	RSA	Promising cultivar, high yield and early maturity

[a] =Asian Vegetable Research and Development Center; [b] = International Potato Centre; [c] =Republic of South Africa

To put the mineral results in the genotypes into perspective, composite top and sub soil samples (30 cm deep) from the experimental plots was collected for pH, zinc, iron and copper determination (AOAC, 1990) on the planting day. Soil technicians from Bvumbwe assisted with the analysis of the soil whose results are presented in Table 5.9

Table 5.9 Status of Fe, Zn and Cu at Bvumbwe research station in 2005

	Analite (PPM)				
Soil sample	**Fe**	**Zn**	**Cu**	**pH**	**Soil type**
Top soil	8.27	9.96	1.52	5.56	Sandy clay
Sub soil (30cm deep)	6.35	8.08	1.28	5.78	loam

5.4.2 Determination of dry matter content

Dry matter content was measured by random sampling of five storage roots from each plot and chopped into 2 cm thick strips. Two hundred grams of the chopped samples in three replicates were oven-dried at 105°C until weight remained constant (AOAC, 1990).

The percentage of dry matter is calculated as:

$$\% \text{ DM} = \frac{\text{Dry weight}}{\text{Fresh weight}} \text{ X } 100 \qquad (1)$$

5.4.3 Preparation of samples for nutrient analysis

Mature roots from six randomly selected plants were harvested at 4, 5 and 7 MAP with each sample split into two batches. One batch was washed, chopped into 1-cm cubes, mixed, packed in well sealed plastic bags and stored at -20^{0}C until extraction time. The second batch was left peeled (by removing the epidermis and cortex) but treated similarly. Analysis of nutrients was undertaken within 2-months of harvesting.

5.4.4 Determination of β-carotene

5.4.4.1 Preparation of β-carotene standard stock and working solutions (1.7695 x 10^{-3}M)

β-carotene (0.100g, 95%) was dissolved in petroleum ether and diluted to the mark in a volumetric flask (100mL). Aliquots of this stock solution (0.1, 0.2, 0.3, 0.4 and 0.5mL) were pipetted into a set of 50mL amber volumetric flasks and diluted to give a set of standard solutions with absorbance readings between 0 and 1.0. The absorbances of the standard solutions were determined at 450nm.

5.4.4.2 β-carotene extraction and determination

Beta-carotene content was determined spectrophotometrically as described by Imungi and Wabule (1990). Fresh peeled and unpeeled samples were initially homogenized using a blender. Exact portions (2.00g) in duplicates were weighed in a 50mL extraction conical centrifuge tube and mixed with cold acetone (40mL). The samples were centrifuged for 60 seconds before filtering with suction through a buchner funnel. The flask and the residues were washed with acetone while receiving the washing in the funnel. The filtered residues were white in colour, which indicated that all β-Carotene had been extracted. Petroleum ether (40 mL) was put in a separatory funnel. The resultant acetone extract was then added into the separatory funnel. Distilled water was slowly added while letting it flow along the walls of the funnel. The mixture was not shaken to avoid formation of an emulsion. The two phases were allowed to separate and the lower aqueous acetone phase was discarded. The resultant was washed first with distilled water (300mL) and three times with distilled water (200mL) to remove the acetone completely. The petroleum ether phase was collected in a 50mL volumetric flask. The ethereal extract was made to pass through the funnel containing filter paper and anhydrous sodium sulphate. The washing solvent was also collected into the volumetric flask and the volume was made to the mark. The absorbance of the β-Carotene ethereal extract was read at 450nm on UV/VIS spectrophotometer. β-carotene content was calculated using the following formula:

$$\mu g/g = (A*Volume*10^4)/(2592*Sample\ weight), \text{ where } A= Absorbance \qquad (2)$$

5.4.5 Extraction and determination of ascorbic acid

A 5 g of sample was homogenised in a solvent system (100 mL) for two minutes at medium speed, and allowed to stand for 30 minutes in a refrigerator for the supernatant to settle before determination. Ascorbic acid was determined according to AOAC (1997). The 2,6 dichlorophenolindophenol titrimetric (0.250g) method described in AOAC was used for detrmination. The dye was standardized by titrating it against ascorbic acid standard (2.0mL) in solvent system (5.0mL) before determination. The supernatant (10.0mL) was transferred in a well-rinsed and dried conical flask, titrated in triplicate against 2,6 -dichlorophenolindophenol until end point (pink coloration). Blank determinations were done to correct sample and standard titre values. Ascorbic acid concentration (mg/100g) was calculated using the equation:

$$\text{Concentration of ascorbic acid (mg/100g)} = (X- B) \times (F/E) \times (V/Y) \times 100 \qquad (3)$$

Where X= average mL for sample titration, B = Average mL for sample blank titration, F = mg ascorbic acid equivalent to 1.0 mL indophenol standard solution, E = number of grams, V = initial volume of assay solution and Y = volume of sample aliquot titrated.

5.4.6 Extraction and determination of metals (Zn, Fe and Cu)

This was done as described in AOAC (1990). Sweetpotato chips (1.00g) were dried in an oven (110 ^{0}C) for 12 hours and the dried material was ashed in a furnace for 5 hours at 500 ^{0}C. The samples were cooled to room temperature in dessicator and subsequently weighed using an analytical balance. To the ash, some more deionised water (10 drops) and nitric acid (5.85M, 4drops) were added as the excess acid evaporated in the oven (110 ^{0}C) for 30 minutes. The residues in crucibles were ashed for an additional 1 hour at 500 ^{0}C, cooled and ash taken up in hydrochloric acid (5.09M, 10.0mL). The resultant mixture was diluted to the mark in a 50.0 mL volumetric flask with de-ionised water. Standard solutions (50.0mL; 0.0, 0.05, 0.10, 0.20, 0.30, 0.40 and 0.5 ppm) of each sample were prepared and their absorbances read at 422.7, 248.3 and 213.9 nm for copper, iron, and zinc respectively. The absorbance of sample solutions was read immediately after each standard and their concentration C (ppm) calculated from standard calibration plot as follows:

$$\text{C for Iron} = \text{Absorbance} - (0.001/0.023) \tag{4}$$

$$\text{C for Zinc} = \text{Absorbance} - (0.0077/0.1098) \tag{5}$$

$$\text{C for Copper} = \text{Absorbance} - (0.0042)/0.0413) \tag{6}$$

$$\text{Concentration of metal (mg/100 g)} = \frac{\text{C x 100 }(\mu\text{g})}{\text{Sample weight (g)}} \tag{7}$$

5.4.7 Data analysis

The effects of cultivar, maturity age and preparation form (peeled and unpeeled) were determined using the ANOVA analysis using AGROBASE software (Agrobase, 1999) package for analysis. Genotypes were assumed to be fixed, and time of harvesting and sample type effects to be random. Least significant difference ($p<0.05$) was used to compare means.

5.5 Results and Discussion

5.5.1 Dry matter content of 15 genotypes

The dry matter content of sweetpotato storage roots at 4, 5 and 7 MAP are presented in Table 5.10. The DM results demonstrate significant variation among the genotypes at $p \leq 0.001$.

Variation in percent DM content across harvesting times and in both sample types; peeled and unpeeled were not significant at $p \leq 0.05$. Across the harvesting times, cultivar Yoyera a landrace exhibited the highest content (36.25%) followed by Babache and Kenya (36%) a landrace and introduction respectively. Mzungu (23.33%) and A45 (25.17%) were relatively low in percent DM content. Acceptable levels of dry matter content in preferred cultivars is 30% and above in Malawi where Kamchiputu and Yoyera are among the best cultivars (Chipungu *et al.,* 1999).

Table 5.10 DM content (%) of 15 sweetpotato genotypes at 4, 5 and 7 MAP

Genotype	4 MAP	5 MAP	7 MAP	Mean
Yoyera	35.50	36.50	36.00	**36.25**
Kamchiputu	35.50	36.50	36.00	**36.00**
Babache	36.50	36.50	35.00	**36.00**
Kenya	33.00	35.00	34.00	**34.00**
LU96/303	31.50	32.00	33.00	**32.17**
Semusa	32.00	33.50	31.00	**32.17**
Zondeni	31.50	32.50	30.00	**31.33**
Lunyangwa	33.00	32.50	28.50	**31.33**
Mfumu	30.50	32.00	31.50	**31.33**
LU96/374	30.00	32.00	31.50	**31.17**
Pepu	27.50	30.50	31.00	**29.67**
Tainoni	27.50	28.50	27.00	**27.67**
Mafutha	27.00	27.00	27.00	**27.00**
A45	25.00	26.00	24.50	**25.17**
Mzungu	22.50	23.50	24.00	**23.33**
Coeffient of Variation (%)	**6.60**	**7.48**	**7.77**	**5.36**
Least Significant Difference	**3.52**	**4.40**	**4.15**	**2.37**
Genotype G	* * *	* * *	* * *	* *
Peeling	**ns**	**ns**	**ns**	**ns**
MAP T				**ns**

Key: ns= not significant, ***= Significant at $p \leq 0.001$

5.5.2 Variation in β-carotene content

Results on the analysis of variance on β-carotene content in 15 sweetpotato genotypes, harvested at three different times (MAP) and in two sample types (peeled and unpeeled) are presented in Table 5.11. The variables under study; genotype, harvesting times and sample type results and their first order interactions; genotype x storage root age, genotype x sample type and storage root age x sample type and second order interactions (genotype x storage root age x sample type) were all highly significant at $p \leq 0.001$.

5.5.2.1 Effect of genotype and flesh colour on β-carotene content

The reference harvesting time which is recommended for sweetpotato in Malawi is at 5 MAP. The reference sample type being used is the unpeeled storage roots. At this reference harvesting time and sample type, the results indicated large variation in β-carotene content ($p \leq 0.001$) among the genotypes (Table 5.11). This is probably due to the wide spectrum of storage root flesh colour where carotenoids are responsible for the yellow, orange, and red colours of fruits, roots, flowers, fish, invertebrates, and birds (Rodriguez-Amaya, 1993). Zondeni, an orange fleshed cultivar provided the highest β-carotene content (13.93 mg/100g) followed by A45 (4.39 mg/100g) which is also an orange fleshed cultivar at 5 MAP. There was a general down ward trend in β-carotene levels in the genotypes from orange, pale orange to yellow root flesh colour registering colour category means of 7.47±5.60, 2.76±0.44 and 1.65±0.66mg/100g respectively at the reference harvesting time (5 MAP) and sample type (unpeeled storage roots). Very low β-carotene levels were registered in unpeeled white/cream cultivars where cultivar Yoyera (0.13mg/100g) registered the lowest levels at 5 MAP.

The present findings suggest that qualitatively, colour seems to indicate relative β-carotene levels: the orange ones having highest levels. The results agree with earlier findings that β-carotene are largely responsible for the orange flesh colour in sweetpotato (K'osambo *et al.,* 1998; Picha, 1985; Huang *et al.,* 1999). High variation was noted within the colour categories where for instance Zondeni had four times more than the rest of the genotypes in the orange category. Further, it is shown in Table 5.11 that the yellow fleshed cultivar Mzungu is not significantly different ($p \leq 0.05$) from Kamchiputu a pale orange fleshed cultivar.

Table 5.11 Effect of Genotype and age on β-carotene values (mg/100 g fresh tissue)

		Unpeeled root samples				**Peeled root samples**				**Grand**
Cultivar	**Flesh colour**	**4 MAP**	**5 MAP**	**7 MAP**	**Mean**	**4 MAP**	**5 MAP**	**7 MAP**	**Mean**	**Mean**
Zondeni	Orange	10.29	13.93	8.49	10.90	7.73	6.59	6.07	6.80	8.85
A45		4.32	4.39	4.38	4.36	3.26	3.49	2.36	3.04	3.70
LU96/374		3.62	4.09	2.92	3.54	2.36	2.16	1.93	2.15	2.85
Mean ±SD		**6.08±3.67**	**7.47±5.60**	**5.26±2.89**	**6.27±4.03**	**4.45±2.88**	**4.08±2.27**	**3.45±2.28**	**3.99±2.47**	**5.13±3.25**
Kamchiputu	Pale orange	1.26	2.29	2.38	1.98	1.02	1.57	1.24	1.28	1.63
Tainoni		2.18	2.83	1.49	2.17	1.81	1.87	0.98	1.55	1.86
Mafutha		3.44	3.17	3.39	3.33	2.15	2.96	3.15	2.75	3.04
Mean ±SD		**2.29±1.09**	**2.76±0.44**	**2.42±0.95**	**2.49±0.73**	**1.66±0.58**	**2.13±0.73**	**1.79±1.18**	**1.86±0.78**	**2.18±0.76**
Mzungu	Yellow	0.33	2.11	1.19	1.21	0.23	0.27	0.28	0.26	0.74
Kenya		1.01	1.18	1.03	1.07	1.45	2.16	0.89	1.50	1.29
Mean ±SD		**0.67±0.48**	**1.65±0.66**	**1.11±0.11**	**1.14±0.10**	**0.84±0.86**	**1.22±1.34**	**0.59±0.43**	**0.88±0.88**	**1.01±0.39**
Yoyera	White/cream	0.74	0.13	0.41	0.43	0.47	0.33	0.44	0.41	0.42
LU96/303		0.61	0.39	0.77	0.59	0.63	0.89	0.96	0.83	0.71
Lunyangwa		0.44	0.28	0.35	0.36	0.31	0.43	0.21	0.32	0.34
Mfumu		0.62	0.76	0.86	0.75	0.59	0.45	0.81	0.62	0.68
Semusa		0.63	0.45	0.35	0.48	0.44	0.50	0.19	0.38	0.43
Babache		0.23	0.19	0.14	0.19	0.10	0.15	0.06	0.10	0.15
Mean ±SD		**0.55±0.18**	**0.37±0.23**	**0.48±0.28**	**0.46±0.19**	**0.42±0.20**	**0.46±0.25**	**0.45±0.37**	**0.44±0.25**	**0.45±0.21**
Pepu	Purple	0.93	0.94	0.86	0.91	0.67	0.16	0.24	0.36	0.63
Mean		**1.99**	**2.45**	**1.85**	**2.07±0.31**	**1.53**	**1.58**	**1.32**	**1.47±0.14**	**1.77**
CV %		**16.36**	**21.56**	**19.97**	**13.25**	**10.25**	**21.81**	**15.87**	**12.58**	**21.42**
LSD		**0.89**	**0.91**	**0.46**	**0.86**	**0.34**	**0.63**	**0.38**	**0.82**	**0.64**
Cultivar (C)		* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
Sample type S)										* * *
MAP (T)					* * *				* * *	* * *
S * T										* * *
C * T					* * *				* * *	* * *
C * S										* * *
C * T * S										* * *

CV%= coefficient of variation; LSD= Least Significance Difference, ***= Significant at p ≤0.001

The high variation of β-carotene levels in cultivars is well established for example, K'osambo *et al.* (1998) observed a wide variation in β-carotene within the orange fleshed categories ranging from 1 to 8 mg/100g fresh weight). Apart from the similarities with other studies in β-carotene levels as regards the effect of colour and their variation within colour categories, the current study however, makes an important revelation where higher levels of β-carotene in cultivar Zondeni (13.93 mg/100g) being reported is among the highest than results by similar studies in Eastern and Southern Africa Region (K'osambo *et al.,* 1998; Carey *et al.,* 1999; Manrique and Herman, 2000). This genotype is therefore an important resource for direct use and breeding programs.

5.5.2.2 Effect of peeling on β-carotene content at 5 MAP

Figure 5.2 and Table 5.11 reveal that at the reference harvesting time (5 MAP) peeling of sweetpotato root samples significantly ($p \leq 0.001$) reduced β-carotene levels in most cultivars.

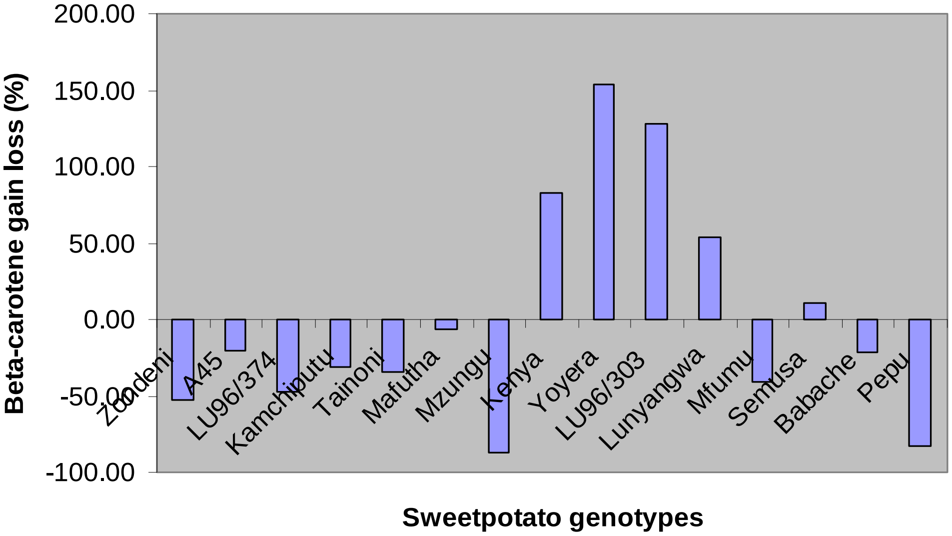

Figure 5.2 Gain/loss (%) of β-carotene levels at 5 MAP due to peeling

All the orange fleshed cultivars consistently lost β-carotene when peeled. However, Kenya in the yellow fleshed category interestingly showed increased levels due to peeling at 5 MAP. Variation in gain or loss resulting from peeling was also observed in the white/cream fleshed category where some genotypes such as Yoyera, LU96/303, Lunyangwa and Semusa gained while Mfumu and Babache lost β-carotene levels at 5 MAP. Peeling the cultivar Pepu, (purple), also resulted in loss of β-carotene.

The genotypes lost varying levels of β-carotene due to peeling (Figure 5.2) at 5 MAP. Mzungu experienced the highest loss (-87.20%) followed by Pepu (-82.98%). Zondeni (-52.69%) also lost a considerable amount of β-carotene. Yoyera followed by LU96/303 and Kenya gained 153.85%, 128.21% and 83.05% respectively due to peeling. In the rural settings of Malawi, sweetpotato roots are mostly boiled without peeling unlike in urban areas where the boiling is done after peeling. During the month of Ramadhan in the Moslem community of the country, sweetpotato roots are peeled for the preparation of *futali* a sweetpotato delicacy in groundnut paste/flour. Woolfe (1992) indicated that sweetpotato processing for human consumption in some countries is remarkably diverse and widespread more especially in China where sweetpotato products like noodles, candies, drinks, ketchup *etc.* have been commercialised. Peeling is an important processing activity for most of these products and consequently in the utilisation diversification and value adding, which Malawi as a country is looking forward to. The knowledge gap on genotypic variation on the retention of nutrients under study after peeling is necessary in the efforts of value adding and commercialisation of sweetpotato in Malawi while maintaining quality in terms of nutrients.

In the current study, peeling of storage roots generally resulted in β-carotene loss averaging 36.63%, 93.60% and 48.85% at 4, 5 and 7 MAP, respectively. An important finding in the current study indicates that at five months old, the β-carotene in sweetpotato storage roots is concentrated in the peel. Although the present findings agree with earlier observations by Rodriguez-Amaya (1993) that most fruits and vegetables have higher carotenoid levels in the peel than the pulp, increases in β-carotene levels for cultivars Yoyera, Kenya, LU96/303 and Lunyangwa have been observed due to peel retention contradicting the earlier findings. Although the β-carotene levels of these four cultivars is low, the verification of the trend and isolation of the responsible trait for its incorporation in genotypes with high β-carotene potential is an important challenge in sweetpotato bio-fortification research and development. Alternatively, skinning after boiling is recommended.

5.5.2.3 Effect of root age on β-carotene content in unpeeled samples

It was observed in Figure 5.3 that except for Kamchiputu, all orange, pale orange and yellow fleshed genotypes gained and lost β-carotene as the roots aged from 4 to 5 MAP and 5 to 7 MAP respectively in unpeeled samples. Contrastingly, the very opposite was exhibited in white/cream fleshed cultivars where losses and gain in β-carotene was observed as the roots aged from 4 to 5 MAP and 5 to 7 MAP respectively in unpeeled samples.

It is well established that plant age affects the composition of food nutrients including β-carotene levels. Carey *et al.* (1999) reported increasing β-carotene levels with time; the values peaked at 5 MAP and declined thereafter. Also, in leafy vegetables, the β-carotene content of mature leaves of lettuce (12µg/g) and endive (14µg/g) were nearly three times higher than those of the young leaves (Ramos and Rodriguez-Amaya 1987). In the present study, there was variation in β-carotene levels as affected by age. Some genotypes experienced gains and others losses due to root age. The various genotypes would therefore have various recommended time of harvesting for maximum use of the nutrient. For instance, the appropriate time for Zondeni, Tainoni and Mzungu would be at 5 MAP when roots are used without peeling.

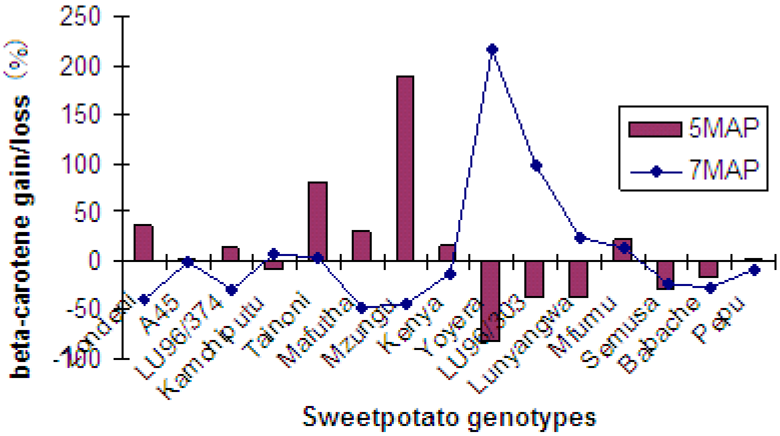

Figure 5.3 Gain/loss (%) in β-carotene content in unpeeled samples of storage roots of 15 sweetpotato genotypes due to plant age

5.5.2.4 Percent contribution of variables to the total sum of squares

Since all the variables and their interactions under study showed significant contributions to β-carotene content at $p \leq 0.001$ (Table 5.10), an estimate of the contribution of each variable to the Total Sum of Squares (TSS) is an important assignment to isolate variables of importance in recovering β-carotene in sweetpotato genotypes.

Table 5.12 Contribution of variables to Total Sum of Squares (TSS)

Source	Degrees of freedom	% Contribution to TSS
Genotype G	14	47.23
Sample type S	1	20.56
G*S	14	7.12
MAP T	2	8.11
G*T	28	5.39
T*S	2	4.14
G*T*S	28	3.84
REP (G*S)	12	2.52
Error	84	1.09

The difference in performance among the genotypes in β-carotene content is largely due to genetic variability which accounts for 47.23% to the TSS (Table 5.12). As discussed in section 5.5.1.1, the variability in the genotypes is attributed to their flesh colours, the orange being superior to the rest (Table 5.11). The second variable of importance is the sample type, which contributes 20.56 to the TSS; peel retention being the superior sample type for β-carotene content. The contribution of harvesting time and each of the interactions to TSS was less than 10% and may therefore be regarded as less important to the recovery of β-carotene in this study.

5.5.2.5 Ranking of genotypes, harvesting time and sample type for β-carotene content

In Table 5.11, the mean β-carotene of the genotypes ranged from 0.06 mg/100g (Babache) to 13.93 mg/100g (Zondeni) and the coefficients of variance (CV) ranged from 10.25 to 21.56 for the variables. These CVs are acceptable and reflects to reliability of the study results in field sampled materials. The relative β-carotene levels of the 15 genotypes (Table 5.11) show that Zondeni a landrace is the most superior cultivar that ranked highest across harvesting times and the two sample types providing and average of 8.85 mg/100g followed by A45 (3.7 mg/100g) an introduction. Across sampling times and between sample types, the β-carotene means for the orange fleshed cultivars were the highest providing a grand mean of 5.13 mg/100g of β-carotene levels seconded by the pale orange (2.18 mg/100g) and thirdly, the yellow (1.01 mg/100g) fleshed Genotypes. Except in peeled samples, the means for Pepu (purple coloured) were higher than the white/cream fleshed means; thus on overall, Pepu was superior (0.63 mg/100g) to the white/cream (0.45 mg/100g) in unpeeled samples. β-carotene content in the unpeeled samples were consistently superior (providing an average of 2.07 mg/100g across all colour categories) to the peeled samples (1.47 mg/100g). Among the harvesting times and within a sample type, 5 MAP in both, unpeeled (2.48 mg/100g) and peeled (1.60 mg/100g) was the best time for β-carotene recovery.

The current study therefore makes an important revelation where Zondeni is a superior cultivar followed by A45 in terms of β-carotene content. Highest potential of β-carotene content in the two genotypes is obtained at 5 MAP and in unpeeled samples. Nutritionally, unpeeled Zondeni and A45 are therefore very important sources of β-carotene for sustainable vitamin A deficiency programs in Malawi. In terms of quality improvement for nutrients in the breeding programs, Zondeni and A45 are important parents in the Malawi germplasm worth exploiting.

5.5.2.6 Adequacy of the dietary pro-vitamin A

The underlying purpose of this research is to identify the genotypic range of β –carotene content in sweetpotato for human nutrition. To attempt to put this data into perspective, an estimate on how much of a general daily requirement for the nutrient in retinol equivalent (RE) units will be provided by eating one hundred grams of each of these genotypes. The RE estimate was based on the conversion ratio of 12:1 β -carotene (μg) to RE units (Food and Nutrition Board, 2001) and the results are presented in Table 5.13. At all harvesting times, Zondeni consistently showed to supply the highest RE units from a 100g fresh sample. Woolfe (1992) indicated that small quantities of orange fleshed cultivars which contain 300 >3000 μg RE/100g fresh weight can easily provide the RDA. In Table 5.1, the RDA (RE) indicated for different age groups range from 300 (children of 1-3 years) to 1,300 (breast feeding mothers). One hundred grams of unpeeled storage roots of Zondeni demonstrate to adequately supply RE to all age groups except lactating mothers. However, at 5 MAP unpeeled Zondeni supplies 89% of the RE daily requirements to the lactating mothers. Unpeeled A45 and LU96/374 have also shown to be good sources of pro-vitamin A although more quantities have to be consumed than Zondeni). Although the pale orange and yellow genotypes may provide a reasonable percentage of the pro-vitamin A, the white/cream and purple fleshed cultivars provides very low to negligible percentages. The retention of peelings would ensure maximum pro-vitamin A access.

Table 5.13 Amounts of RE (µg) in 100g of each of the 15 genotypes

Flesh colour/ Cultivar	Unpeeled root samples			Peeled root samples		
	4 MAP	5 MAP	7 MAP	4 MAP	5 MAP	7 MAP
Zondeni	857.50	1160.83	707.50	644.17	549.17	505.83
A45	360.00	365.83	365.00	271.67	290.83	196.67
LU96/374	301.67	340.83	243.33	196.67	180.00	160.83
Kamchiputu	105.00	190.83	198.33	85.00	130.83	103.33
Tainoni	181.67	235.83	124.17	150.83	155.83	81.67
Mafutha	286.67	264.17	282.50	179.17	246.67	262.50
Mzungu	27.50	175.83	99.17	19.17	22.50	23.33
Kenya	84.17	98.33	85.83	120.83	180.00	74.17
Yoyera	61.67	10.83	34.17	39.17	27.50	36.67
LU96/303	50.83	32.50	64.17	52.50	74.17	80.00
Lunyangwa	36.67	23.33	29.17	25.83	35.83	17.50
Mfumu	51.67	63.33	71.67	49.17	37.50	67.50
Semusa	52.50	37.50	29.17	36.67	41.67	15.83
Babache	19.17	15.83	11.67	8.33	12.50	5.00
Pepu	77.50	78.33	71.67	55.83	13.33	20.00

5.5.3 Ascorbic acid content in sweetpotato genotypes

The levels of ascorbic acid in 15 genotypes assessed at 4, 5 and 7 MAP in unpeeled and peeled storage root samples of sweetpotato are provided in Table 5.14.

5.5.3.1 Genotypic variability of ascorbic acid content

At the reference harvesting time (5 MAP) and in unpeeled samples, the results showed that significant variation ($p \leq 0.001$) in ascorbic acid content existed among the genotypes at 5 MAP. Genotypic variability contributed the highest percent to the TSS (36.32%) an indication that most of the variability observed in ascorbic acid content is as a result of the genetic constitution of the genotypes. The cultivar Semusa (22.43 mg/100g fresh weight) gave the highest ascorbic acid content and was not significantly different from Mfumu (22.42 mg/100g) and Babache (21.88 mg/100g) while the least was LU96/303 (10.94 mg/100g). Across harvesting times and sample types, different genotypes exhibited different rankings for ascorbic acid content. For instance, in unpeeled samples, Mzungu (36.18 mg/100g) ranked highest at 7 MAP while

Mafutha was lowest (15.74 mg/100g). At 4 and 5 MAP, different genotypes ranked highest and lowest. A similar observation also exists in peeled samples, where different genotypes ranked highest and lowest at the different harvesting times. Comparing the findings with those by Bradbury and Singh (1986) who reported ascorbic acid levels of four Solomon Island sweetpotato cultivars ranging from 19.8 to 32.9 mg/100g fresh weight, a wider range exist in the present study which is attributed to the large sample of genotypes. However, although some genotypes in the current study have exhibited higher levels of ascorbic acid than those reported by Bradbury (1986), the inconsistence across harvesting times is a challenge to isolate genotypes that are relatively superior in terms of quantity and stability of ascorbic acid content over time. Increasing the sampling level in order to identify and isolate stable genotypes with high ascorbic acid levels may therefore be an important assignment. Otherwise, introductions will be required for the improvement of ascorbic acid content in the Malawian cultivars.

Table 5.14 Effect of genotype and age on ascorbic acid values (mg/100 g fresh tissue)

Genotype	Unpeeled root samples				Peeled root samples				
	4 MAP	5 MAP	7 MAP	Mean	4 MAP	5 MAP	7 MAP	Mean	
Babache	15.99	21.88	33.64	**23.84**	7.27	16.73	21.21	**15.07**	
Mzungu	16.01	17.79	36.18	**23.33**	5.82	13.41	13.28	**10.84**	
Kamchiputu	24.69	17.24	20.45	**20.79**	11.63	11.72	15.18	**12.84**	
Zondeni	17.45	18.61	25.16	**20.41**	5.82	10.04	21.22	**12.36**	
Lunyangwa	14.39	16.83	27.23	**19.48**	10.94	15.41	11.64	**12.66**	
Kenya	21.81	16.41	19.67	**19.30**	11.63	13.39	11.93	**12.32**	
A45	17.01	19.64	19.67	**18.77**	9.73	10.79	14.56	**11.69**	
Mfumu	7.85	22.42	25.73	**18.67**	9.73	9.25	15.99	**11.66**	
Yoyera	16.01	16.41	29.86	**20.76**	5.81	11.71	10.60	**9.37**	
Semusa	11.77	22.43	24.21	**19.47**	6.08	12.32	11.63	**10.01**	
LU96/303	20.36	10.94	26.74	**19.35**	5.82	10.04	13.26	**9.71**	
Pepu	11.64	13.67	30.25	**18.52**	4.86	11.71	14.58	**10.38**	
LU96/374	14.38	11.21	27.21	**17.60**	6.08	13.86	13.09	**11.01**	
Tainoni	11.78	16.83	24.22	**17.61**	6.08	9.24	14.54	**9.95**	
Mafutha	13.09	11.22	15.74	**13.35**	9.72	7.71	11.65	**9.69**	
Mean	**15.62**	**16.90**	**25.73**	**19.42**	**7.80**	**11.82**	**14.29**	**11.30**	
CV %	**11.23**	**14.72**	**13.96**	**11.03**	**10.28**	**10.85**	**11.18**	**13.68**	**Contribution to TSS (%)**
LSD	**1.73**	**1.98**	**2.65**	**1.98**	**1.17**	**1.58**	**1.66**	**1.77**	
Genotype (G)	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	**36.32**
Sample type S)									**26.35**
MAP (T)				* * *				* * *	**18.96**
S * T									**6.35**
G * T				* * *				* * *	**3.27**
G * S									**1.27**
G * T * S									**2.11**

CV%= coefficient of variation; LSD= Least Significance Difference

5.5.3.2 Effect of peeling on ascorbic content

Removing peels in sweetpotato storage roots resulted in loss of ascorbic acid amounts as exhibited by Figure 5.4. At the reference harvesting time (5 MAP), all genotypes experienced losses due to peeling except LU96/374 which gained 26.64% of ascorbic acid. Retention/removal of peelings contributed highly (26.35%) to the total variability observed in the analysis.

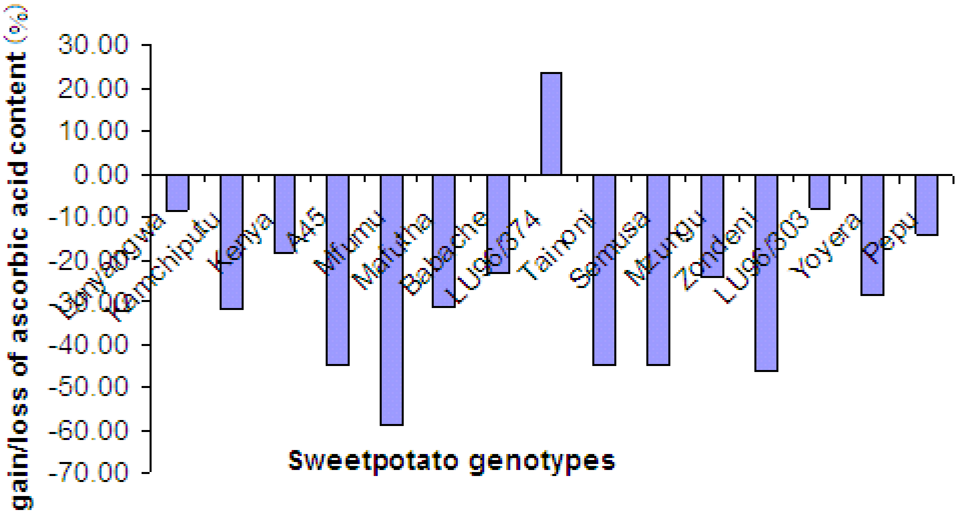

Figure 5.4 Gain/loss (%) in ascorbic acid amounts of storage root samples in 15 sweetpotato genotypes due to peeling at 5MAP

Variation in percent loss among the genotypes is also depicted in Figure 5.4 where Mfumu was the most affected loosing up to 58.74% followed by Zondeni (46.05%). Effect of peeling on vitamin C has been observed elsewhere. Ezell *et al.* (1948) reported that ascorbic acid vary considerably within as well as between cultivars; 160% variation for 30 individual roots of cultivar ‘Porto Rico’ was observed. Eaks (1996) also observed that the rind of citrus fruits has high ascorbic acid than the pulp. While a general recommendation to use unpeeled storage roots to make use of the available ascorbic acid in the 14 of the 15 genotypes is necessitated, further studies are required for LU96/374 for its exploitation in the breeding programs since some consumers opt to peel the storage roots before use. Industrialization of the crop in value addition shall also require the peeling of the storage roots and therefore posing a challenge on the retention of ascorbic acid.

5.5.3.3 Effect of age on ascorbic content

The performance of 15 genotypes in ascorbic acid content as affected by delayed harvesting in unpeeled samples is presented in Figure 5.5. Variation due to storage root age presented in Table 5.14 is 18.96% and is considered high. A one month delay (4 to 5 MAP) in harvesting resulted in the accumulation of ascorbic acid levels in 10 of the 15 genotypes with highest accumulation percent in cultivar Mfumu (186%) and Semusa (90.57%).

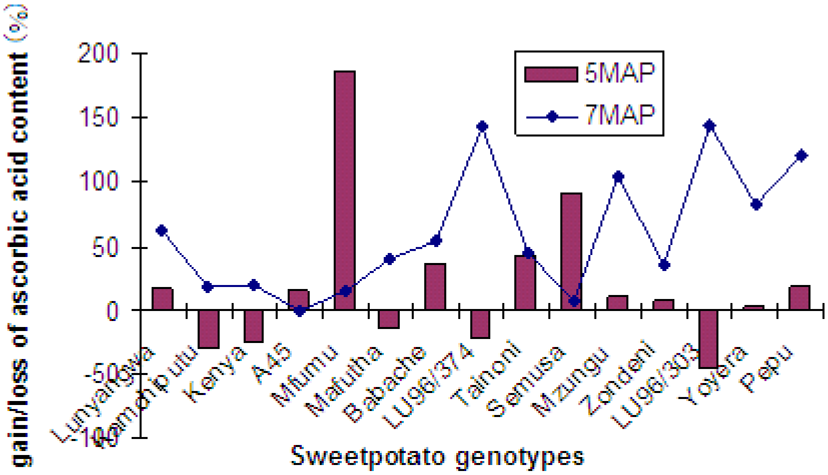

Figure 5.5 Gain/loss (%) in ascorbic acid amounts in unpeeled storage root samples of 15 sweetpotato genotypes due to age variation

The genotype LU96/303 (-46.27%) experienced the highest percent loss at 5 MAP from 4 MAP, followed by Lunyangwa (-30.17%). An average accumulation of 19.23% was observed due to age from 4 to 5 MAP. A two months delay from 5 to 7 MAP resulted in further accumulation of ascorbic in all genotypes. The genotype LU96/303 which experienced the highest loss due to one month delay in harvesting from 4 to 5 MAP, showed the highest percentage gain (144.42%) in ascorbic acid due to two months delay from 5 to 7 MAP followed by LU96/374 (142.73%). The lowest percentage gain was exhibited by Tainoni (0.15%). A two months delay from 5 to 7 MAP gave an average percent increase from 19.23% to 59.33%. Based on unpeeled samples, delays in harvesting of sweetpotato roots resulted in increased ascorbic acid content such that where possible, delayed lifting is recommended for maximum exploitation of available ascorbic acid.

5.5.3.4 Adequacy of the dietary Vitamin C

The RDA for various individuals of different sex, age groups and activities range from 15g (1-3 years of age) to 125g (smokers) a day (Table 5.5). Based on these recommended RDAs the present findings of ascorbic content may provide sufficient levels depending on the variety, age, processing and amounts of sweetpotato consumed. For instance, the amounts of sweetpotato which provide the adequate RDA for different individuals using the cultivar Semusa which gave the highest ascorbic acid amount (22.43mg/100g) at five months in unpeeled storage roots are provided in Table 5.15.

Table 5.15 Amounts (g) of unpeeled roots of cultivar Semusa required to provide adequate RDA for vitamin C to various individuals at 5 MAP

Age	Vitamin C requirements (mg/day)		Amounts to be consumed (g)	
	Males	**Females**	**Males**	**Females**
0-6 months	40	40	178	178
7-12 months	50	50	223	223
1-3 years	15	15	67	67
4-8 years	25	25	111	111
9-13 years	45	45	201	201
14-18 years	75	65	334	290
19 years and older	90	75	401	334
19 years and older	125	110	557	490
18 years and younger	-	80		357
19-years and older	-	85		379
18 years and younger	-	115		513
19 years and older	-	120		535

Using the cultivar Semusa, the highest amount that can be consumed to satisfy the RDA for a 19 year old male who has the highest requirement is only 557 grams of sweetpotato (Table 5.15). For adequacy, the use of unpeeled sweetpotato storage roots to meet the recommended dietary allowance for ascorbic acid should be encouraged. Further, the cultivation and consumption of different cultivars at different times may also satisfy the RDAs for ascorbic acid as different genotypes have different threshold times.

5.5.4 Mineral content (Iron, Zinc and Copper)

The contents of the three mineral elements; Fe, Zn and Cu of peeled and unpeeled samples at 4, 5 and 7 MAP are presented in Tables 5.16, 5.17 and 5.18. The sweetpotato cultivars exhibited significant variations within their respective mineral elements at 5% level of significance at 3 different harvesting times and two preparation forms. Iron, zinc and copper are usually present in the soil in adequate to excess amounts (Table 5.9) such that a deficiency is caused by their presence in an unavailable form rather than by their lack, and a plant can improve its uptake by using strategies to solubilize the iron, zinc and copper present in the soil (Rengel, 2001). Apart from environmental factors that affect the solubility of micronutrients such as leached, acid, sandy soils, organic soils, soils that have supported intensive cropping, soils with high pH, and eroded soils which all tend to be low in available iron, zinc and (Brady and Weil, 2002), the genotypic variation in mineral content is attributed to uptake efficiency of the individual genotypes by increased capacity to solubilize non-available into available nutrient forms (Rengel, 2001).

5.5.4.1 Effect of genotype, age and peeling on iron content

Results in Table 5.16 indicate that the highest iron content (0.67 mg/100g) in unpeeled samples was recovered at 4 MAP in genotype Mzungu followed by LU96/374 (0.46mg/100g) and Mfumu (0.43mg/100g). Unpeeled Semusa at 4 MAP recorded the least amount (0.09 mg/100g). Upon peeling of samples at 4 MAP, LU96/303 significantly retained the highest amount (0.29 mg/100g) while Pepu retained the least (0.03 mg/100g). Delays in lifting of storage roots reduce the iron content in all cultivars and in both peeled and unpeeled samples significantly, the highest value at 5 MAP being unpeeled cultivar A45 (0.11 mg/100g). Iron values reviewed by Bradbury and Holloway (1988) were 1.1, 1.0, 0.70, 0.62, 0.49 and 0.40mg/100g. Although Mzungu gave the highest content of iron at 4 MAP, the genotypes in the current study indicate low levels when compared with 1.1, 1.0 and 0.7 mg/100g by Bradbury and Holloway (1988). The lowest RDA value for iron (Table 5.3) was 0.27 mg/100g for 1-6 months old babies who upon analysis need 40g of unpeeled Mzungu at 4 MAP. In Malawi, it is recommended that up to six months, babies have to be on exclusive breast feeding, and therefore feeding of six months babies on sweetpotato is not applicable.

A further analysis was therefore on 1-3 year old infants who need to eat 1045 grams of cultivar Semusa to meet their RDA (7 mg) which may be too much for these infants. The present findings have therefore revealed the necessity for further screening of the germplasm available to identify iron dense cultivars comparable with the results of earlier findings that recorded higher values than the current study. The studies should also include the identification of genotypes with stable amounts with root age.

Table 5.16 Iron content (mg/100g) of 15 sweetpotato cultivars

	Unpeeled root samples			Peeled root samples		
Cultivar	**4 MAP**	**5 MAP**	**7 MAP**	**4 MAP**	**5 MAP**	**7 MAP**
Babache	0.32	0.09	0.03	0.19	0.08	0.01
Mzungu	0.67	0.06	0.04	0.20	0.06	0.01
Yoyera	0.15	0.06	0.06	0.12	0.03	0.01
Kamchiputu	0.19	0.03	0.03	0.15	0.03	0.01
Kenya	0.21	0.08	0.06	0.17	0.04	0.01
Zondeni	0.23	0.04	0.02	0.14	0.04	0.01
LU96/303	0.47	0.04	0.03	0.29	0.04	0.01
Pepu	0.15	0.04	0.02	0.03	0.03	0.01
Mafutha	0.20	0.06	0.02	0.17	0.05	0.01
A45	0.17	0.11	0.04	0.16	0.1	0.02
Lunyangwa	0.16	0.05	0.02	0.09	0.04	0.01
LU96/374	0.46	0.04	0.02	0.16	0.05	0.01
Tainoni	0.14	0.05	0.02	0.12	0.05	0.01
Mfumu	0.43	0.07	0.04	0.19	0.04	0.01
Semusa	0.09	0.06	0.03	0.10	0.04	0.01
Mean	**0.27**	**0.06**	**0.03**	**0.15**	**0.05**	**0.01**
CV (%)	**25.63**	**24.23**	**15.67**	**21.36**	**18.91**	**19.2**
Significance	* * *	*	* *	* * *	* *	*
LSD	**0.06**	**0.02**	**0.01**	**0.05**	**0.01**	**0**

CV (%)= coefficient of variation; LSD= Least Significance Difference

5.5.4.2 Effect of genotype, age and peeling on zinc content

The levels of zinc in the 15 genotypes under study are provided in Table 5.17. The highest level of zinc in unpeeled storage roots was recovered in cultivar Mzungu (0.63 mg/100g) at 4 MAP followed by Kenya and Kamchiputu (0.59 and 0.58 mg/100g respectively). In peeled samples, cultivar Mzungu remained the highest (0.62 mg/100g) followed by Kamchiputu and Lunyangwa (0.53 mg/100g). Babache is the only cultivar that showed an increase in Zn content from 0.16mg/100g to 0.60g/100g in peeled samples and from 0.23 to 0.36 mg/100g in unpeeled samples when lifting was delayed by a month (from 4 to 5 MAP) in peeled samples. Across genotypes the results indicate that when storage roots are peeled and harvesting delayed, Zn levels decreases. At 4 MAP, potential genotypes in Malawi that are higher in Zinc levels than earlier values of 0.24 by Woolfe (1992) and comparable with 0.59 mg/100g reported by Bradbury and Holloway (1988) have been isolated.

Table 5.17 Zinc content (mg/100g) of 15 sweetpotato genotype

	Unpeeled root samples			Peeled root samples		
Genotype	**4 MAP**	**5 MAP**	**7 MAP**	**4 MAP**	**5 MAP**	**7 MAP**
Babache	0.23	0.36	-0.3	0.16	0.60	-0.49
Mzungu	0.63	0.07	-0.31	0.62	-0.91	-1.45
Yoyera	0.47	0.16	-0.18	0.43	-0.50	-0.38
Kamchiputu	0.58	0.16	-0.26	0.53	-0.22	-0.38
Kenya	0.59	0.45	-0.31	0.34	-0.21	-0.30
Zondeni	0.35	0.31	-0.12	0.21	-0.27	-0.78
LU96/303	0.43	0.19	-0.26	0.24	-0.29	-0.63
Pepu	0.54	0.24	-0.25	0.23	-0.38	-0.79
Mafutha	0.33	0.35	-0.36	0.29	-0.51	-1.14
A45	0.43	0.39	-0.35	0.17	-0.25	-0.86
Lunyangwa	0.50	0.14	-0.46	0.53	-0.32	-1.06
LU96/374	0.33	0.12	-0.22	0.20	0.11	-0.39
Tainoni	0.46	0.19	-0.40	0.31	-0.27	-0.94
Mfumu	0.50	0.23	-0.39	0.42	-0.53	-0.64
Semusa	0.51	0.31	-0.41	0.22	-0.08	-0.47
Mean	**0.46**	**0.24**	**-0.31**	**0.33**	**-0.27**	**-0.71**
CV %	**18.24**	**21.54**	**22.54**	**23.17**	**19.65**	**19.88**
Significance	* * *	* * *	*	* * *	* *	* * *
LSD	**0.07**	**0.01**	**0.02**	**0.15**	**0.05**	**0.03**

CV%= coefficient of variation; LSD= Least Significance Difference

In terms of Zn adequacy, 100 grams of unpeeled root samples of all the genotypes at 4 and 5 MAP are adequate to meet the RDA requirements for all age and sex groups (Table 5.4). In peeled samples Zn adequacy is met only at 4 MAP and only by Babache at 5 MAP. Regardless of sample type, all the genotypes do not meet the RDA requirements for any individual at 7 MAP. Although a general dwindling trend has been observed up to undetectable levels regardless of sample form at 7 MAP, the current study has however isolated the cultivar Babache which exhibited a unique characteristic of increasing Zn levels from 4 to 5 MAP. Such a unique trait is worth exploiting in the breeding programs and therefore calls for further research.

5.5.4.3 Effect of genotype, age and peeling on copper content

The range of copper levels recovered as presented in Table 5.18 was from 0.43 (Lunyangwa) to 0.61 mg/100g (Yoyera) in unpeeled sample roots at 4 MAP. At the same harvesting time, the range was lower in peeled samples, which was from 0.28 (A45) to 0.49 mg/100g (LU96/303). A general dwindling trend in copper content from 4 to 5 MAP was exhibited in both sample types resulting from age of the storage roots except for Kenya and Semusa (unpeeled samples) and Zondeni and LU96/374 (peeled samples).

Table 5.18 Copper content (mg/100g) of 15 sweetpotato genotypes

	Unpeeled root samples			**Peeled root samples**		
Cultivars	**4 MAP**	**5 MAP**	**7 MAP**	**4 MAP**	**5 MAP**	**7 MAP**
Babache	0.56	0.44	0.08	0.48	0.21	0.57
Mzungu	0.54	0.19	0.08	0.46	0.28	0.50
Yoyera	0.61	0.51	0.09	0.38	0.19	-0.10
Kamchiputu	0.46	0.43	0.10	0.30	0.17	0.00
Kenya	0.53	0.54	0.06	0.48	0.39	-0.11
Zondeni	0.48	0.35	0.18	0.48	0.52	0.27
LU96/303	0.51	0.33	0.57	0.49	0.27	-0.28
Pepu	0.56	0.44	0.26	0.45	0.13	0.49
Mafutha	0.59	0.31	0.31	0.45	0.25	-0.09
A45	0.59	0.37	0.50	0.28	0.60	0.63
Lunyangwa	0.43	0.23	0.31	0.30	0.08	0.53
LU96/374	0.59	0.37	0.34	0.40	0.49	0.05
Tainoni	0.53	0.33	0.51	0.36	0.33	-0.08
Mfumu	0.50	0.34	0.64	0.36	0.21	-0.28
Semusa	0.46	0.48	0.40	0.45	0.23	0.23
Mean	**0.53**	**0.38**	**0.3**	**0.41**	**0.29**	**0.16**
CV (%)	**15.69**	**19.63**	**13.54**	**21.36**	**18.97**	**24.59**
Significance	*	*	* *	*	*	* *
LSD	**0.06**	**0.06**	**0.02**	**0.02**	**0.02**	**0.04**

CV (%)= coefficient of variation; LSD= Least Significance Difference

However, as the root aged from 5 to 7 MAP, a few genotypes of unpeeled samples (LU96/303, A45, Tainoni and Mfumu) showed an increase in Cu content while the majority showed a decreasing trend. In peeled samples, Babache, Pepu, A45 and Lunyangwa showed increases in Cu content from 5 to 7 MAP, Semusa maintained its levels while others decreased to undetectable levels. Thus peeling of storage roots of some genotypes resulted in total loss of copper at 7 MAP. While Woolfe (1992) reported copper values of 0.16 and 0.26, Bradbury Holloway (1988) reported copper values of 0.15, 0.16 and 0.17 mg/100g. Most of the genotypes in the current study have exhibited high levels of copper content than the earlier findings indicative of potentiality in the Malawian germplasm when compared with reports else where.

Based on the RDA for copper (Table 5.5), 100 grams of unpeeled sweetpotato roots for all genotypes would adequately meet the requirements of most human individuals at 4 and 5 MAP. However, doubling the amounts to be consumed (200 grams) would adequately meet the requirements for all ages, including pregnant but excluding breast feeding women who need to triple the amount. Peeling and increase in age of roots to 5 and 7 MAP result in increasing the amounts to be consumed.

5.6 Conclusions

Promotion and development of plant foods with increased density of micronutrients and nutritional quality constitutes a sustainable approach to alleviating the "hidden hunger" of micronutrients in developing countries. Sweetpotato genotypes such as Zondeni are an excellent source of β-carotene that can meet the RDA more especially when unpeeled roots are used at all maturity ages. Utilization of unpeeled storage roots increases the amount of β-carotene. White, cream, yellow and purple cultivars are generally not good sources of pro-vitamin A. Generally genotypes exhibited increase in ascorbic acid content with age. Different genotypes ranked highest at different sampling times and sample types. At 7 MAP, the consumption of 200 to 300 g of unpeeled roots for most of the genotypes would meet the RDA for most human individuals.

The genotypes Mzungu, followed by LU96/374 and Mfumu in the current study appear to be good sources of iron when unpeeled storage root samples of 4 MAP are used. However, for all peoples' age groups and genotypes under study, more than 1000 grams are required to meet the RDA for iron. All genotypes can adequately meet the zinc RDA when 100 grams of unpeeled roots are utilized at 4 and 5 MAP for all humans while the same amount can only provide adequate copper to infants and only at 4 MAP. Age and peeling of roots seemed to reduce mineral content of the cultivars as such, variation in planting and consumption of sweetpotato of early maturity and utilization of unpeeled roots should be recommended. Although most genotypes exhibited the tendency to loose the zinc and copper elements with time, a few genotypes showed constant and increasing characteristics indicative for potential to select traits for mineral retention with age.

On overall, the results have shown that the genotypes under study can reliably be differentiated by dry matter, β-carotene, ascorbic acid, iron, zinc and copper contents. However, age had different effects on the content of the different nutrients. As β-carotene and ascorbic acid exhibited increasing trends, all the minerals decreased in content with age. Peeling had adverse effects on the contents of all nutrients. The current study has therefore established that genetic variation exist for these nutritional traits entailing the potential to breed for higher levels of the nutrients.

CHAPTER 6

SWEETPOTATO MANAGEMENT AND EVALUATION IN MALAWI-CONSOLIDATING FARMERS' PREFERENCE, INDIGENOUS KNOWLEDGE AND GERMPLASM DIVERSITY ANALYSIS

Abstract

A preference study by trained and naive panelists was conducted on 15 sweetpotato cultivars at Bvumbwe Research Station and Chancellor College in Malawi to identify and describe agronomic and organoleptic attributes that are considered valuable by farmers in sweetpotato storage roots. Using a preference scoring scale of 1-5, different cultivars were preferred for agronomic (yield, root size and number of roots per plant and weevil tolerance/resistance) and organoleptic (texture, taste, flavour and flesh colour) characteristics under study. For instance, local cultivars Yoyera (2.08) and Kamchiputu (2.23) ranked high for organoleptic preference but were among the least for agronomic traits. Mzungu a local cultivar was highly preferred agronomically (2.06) but was the least preferred organoleptically (3.36). The naive panel was capable of scoring attributes independently and is therefore the best panel to consider for quantitative evaluation of cultivars and associated attributes. On the basis of indigenous knowledge and farmers' preference, it appears to be a challenge to develop and identify an ideal genotype that contains all of the preferred traits. Researchers can therefore easy their work, by combining attributes into different genotypes with the knowledge base that farmers will select, manage and maintain multiples of cultivars. Further, the study has revealed that although researchers concentrate on introducing germplasm for adaptability evaluation, local cultivars have been shown to be a good source for valuable attributes including yield which have not been exploited in trait combination to date.

Key words: naive, trained panels, sweetpotato, organoleptic, agronomic attributes, cultivar preference

6.1 Introduction

The evidence in chapter five of this dissertation strongly supports the contention that there is enough genetic diversity as regards the levels of zinc, iron, copper, β-carotene and vitamin C in sweetpotato germplasm available in Malawi. Succeeding in promoting such nutrient dense genotypes would dramatically contribute to improving the health, livelihood and felicity of numerous resource-poor, micronutrient-deficient people in the country and would contribute greatly to sustaining national development. The current work in chapter five mainly identified the genotype Zondeni to significantly contain high levels of β-carotene and its exploitation by the nutrition and public health communities to combat micronutrient malnutrition is recommended. However, due to the high levels of β-carotene, Zondeni is orange fleshed in colour, a deviation from the white and cream fleshed cultivars prevalently grown in Malawi (Table 2.13). A strategy of breeding plants that enrich themselves and load high amounts of minerals and vitamins into their edible parts has the potential to combat micronutrient malnutrition only if farmers and/ or consumers are willing to adopt such varieties and make use of them. Improved mineral content, which generally does not alter appearance, taste, texture or cooking quality, is far less complicated in adoption than improving provitamin A content that alter the colour of food. Replacing white-fleshed sweetpotato varieties with high ß-carotene varieties could benefit people currently at risk of diseases associated with vitamin A deficiency. Evidence exist that the consumption of diets containing mostly plant sources of ß-carotene, the primary source being red and orange sweetpotato, increased serum retinol concentrations in Indonesian children who were marginally deficient in vitamin A (Jalal *et al.,* 1998).

To identify and relate preference with major attributes that affect sweetpotato cultivar selection by farmers, an organoleptic and agronomic preference study was conducted in Malawi through the use of trained and naive (experienced growers and consumers) panels, mainly to define desirable organoleptic (colour, texture, taste, aroma and fiber content) and agronomic attributes (yield, yield components and weevil damage) of 15 representative cultivar. Although yield is a very important characteristic in sweetpotato, but so is the maturity time, taste, aroma, colour *etc*. It is envisaged that through the trained and naive panel evaluations, the relative importance of the different cultivars and their major characteristics which are very important to farmers, will be depicted which will ultimately assist in quantification of decision making criteria and to explain underlying objectives and strategies in sweetpotato research and development to enhance adoption and production of the crop in Malawi. Further, the knowledge base that farmers use a wider range of criteria than breeders for selection of varieties is investigated.

6.2. Literature review

6.2.1 Variation in consumer preference

It has been well established that farmers' selection concerns are not homogenous, and may vary with agro-ecological, socio-economic and cultural conditions they face. Rich and poor farmers in a region probably have very different concerns. Likewise, two poor farmers in a marginal area may differ in perspectives. Even in a farming household, there may not be similarities in the males and females concerns (Bellon *et al.,* 1997). Farmers choose varieties for a number of reasons such as gastronomic considerations, early maturity and other agronomic criteria. The breeding program of International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in northern Namibia showed that farmers' preferences for pearl millet varieties were based on early maturity, drought tolerance, large grains and good threshing characteristics (Monyo *et al.,* 1998). In Zimbabwe, small-scale farmers favour sorghum varieties with consistent, reliable and stable yield rather than high yields (Oosterhout, 1993). Kapinga *et al.* (2000) reported that in Tanzania, farmers, traders and consumers have their own criteria for selecting sweetpotato roots where, among the traits selected, farmers were looking for high yielding ability, early maturity and potential to produce high forage for livestock, while consumers were looking for keeping quality (up to 21 days) after harvest but both groups considered dry matter content as being crucial. In breeding programs, these diverse concerns are therefore important considerations to appropriate answers are to be provided based on the needs and preferences of various farmers.

It has been reported in section 2.5.1.8 that in general, the selection criteria for genotypes in Malawi is similar across the study sites and is mainly based on organoleptic attributes. However, the importance and prioritization of the selection criteria differed among farmers within and between study sites based on agronomic, post-harvest and market demands. Chapter two also highlighted that in Mulanje and Mzimba, Kenya, a modern cultivar is grown for commercial purposes in pure stands, while in the other sites Kenya is grown in association with other farmers' varieties for its high yields and early maturity while the other genotypes are grown for their longer shelf life and tolerance to sweetpotato weevil. In these other sites, farmers grow both modern and farmers' varieties to increase yield while avoiding high losses due to rotting (poor shelf life in storage) and weevil damage before lifting. The multiple cultivar scenario has been reported in Mexico where some farmers maintain both modern and local varieties of maize to reduce overall variability in yield at the farm level in flat and sloping field plots respectively (Bellon, 1991). The understanding of farmers' selection criteria in order to address their concerns in genotype selection is therefore an important consideration.

6.2.2 Soliciting farmers' concerns

Agricultural research has often failed to achieve the required impact for many resource-poor farmers especially in Africa (Chambers and Jiggins, 1987). Yield maximization and agro-climatic stability are the main selection objectives for food self sufficiency at the household level. Thus conventional practice assumes that researchers are the custodians of knowledge and the source of technical innovations; that extension is a delivery mechanism and that farmers are the ultimate users (Bruin and Meerman, 2001). It has been argued by many that the most promising way to make research findings acceptable by farmers is to base research assumptions on their needs (Chambers *et al.,* 1989; Ashby, 1991). At present, there are on-going initiatives to decentralize agricultural research by involving farmers at different stages of the breeding process as alternative approaches of making agricultural research effective and sustainably relevant for small scale farmers. These are broadly categorized into Participatory Varietal Selection (PVS) and Participatory Plant Breeding (PPB) since they define two different approaches. PVS involves the selection by farmers of non-segregating, characterized products from plant breeding programmes such as released genotypes, varieties in advanced stages of testing and advanced non-segregating lines. In contrast, PPB involves farmers selecting the genotypes from genetically variable, segregating materials (Witcombe and Joshi, 1995).

An effective method for identifying valuable new germplasm which meet farmers' needs is participatory variety selection (PVS). This technique allows farmers to test, assess and select new materials (Soleri and Cleveland, 2000). Examples of a PVS program is the work in Rwanda by Sperling *et al.* (1993) who proceeded by involving farmers in the selection of bean varieties in on-station trials and then later grow them in their own fields. PVS has also proved useful for enhancing diversity in production systems where traditional genotypes have been lost (Joshi and Witcombe, 1998; Bellon, 2001). Participatory plant breeding (PPB) programmes have been established by a number of agencies and the CGIAR has played an important role in supporting them. PPB allows farmers to contribute their knowledge and expertise to crop breeding for their environments (Ceccarelli and Grando, 2000). Participatory plant breeding can involve scientists, farmers, consumers, extension agents, vendors, industry, and rural cooperatives. Some PPB work was also done by Sthapit *et al.* (1995) in Nepal where segregating lines of F5 bulk families of cold-tolerant rice were distributed to farmers for decentralized selection. PPB has been applied primarily as a crop improvement strategy for non-commercial crops and difficult environments.

6.3 Justification of the study

It is a known fact that conventional breeding programs are seldom designed to meet the specific requirements of different farmers and agro-ecologies, who, due to many reasons, may have contrasting preferences, which might require different breeding approaches. Adoption and maintenance of genetic resources that are adapted to local conditions and meet specific needs can ultimately be enhanced by consolidating farmers' knowledge and skills on germplasm evaluation with genetic diversity distribution and quantification analysis. An analysis of how farmers contribute to the enhancement of sweetpotato germplasm provides valuable insights into the effect of farmers' selection pressure to increase and maintain genetic variation. This chapter consolidates the process of conventional sweetpotato improvement and adaptation programs by researchers with the understanding of farmers' preference concerns and skills in order to design appropriate scientist-farmer collaboration in breeding, evaluation and management of sweetpotato genetic resources.

6.3.1 General objective

The general objective was to determine farmers' desiderata and skills underlying sweetpotato agronomic and organoleptic variation, evaluation, adoption and maintenance in Malawi.

6.3.2 Specific objectives

(i) To identify and determine the major agronomic and organoleptic attributes of sweetpotato cultivars and their value to evaluation, selection and maintenance.

(ii) To explore possible relationships between nutritional content (Fe, Zn, Cu, β-carotene and vitamin C), agronomic and organoleptic preference in sweetpotato accessions.

(iii) To consolidate the role of the farmer and researchers in cultivar development, evaluation, adoption and germplasm diversity management and strategise the way forward to sweetpotato germplasm maintenance in Malawi.

6.4 Materials and Methods

6.4.1 Study genotypes

Fifteen sweetpotato genotypes (Table 5.8 in chapter five) from different sources have been described in detail in section 5.4.1 and are comprised of low to high yielding, early to late maturing, good to bad taste, texture, varying flesh colour, flavour and susceptibility and resistance to weevil attack as described by farmers during the IK survey (chapter two) for local varieties. The experimental layout and environment in which they were grown at Bvumbwe Research Station has also been described in the same section 5.4.1 of chapter five. The sampling times to conduct preference testing were in April, May and July representing 4, 5 and 7 Months after planting.

6.4.2 Descriptive analysis of attributes

The organoleptic analysis was done to identify important properties (attributes) of the genotypes and facilitate the relation of preference with the genotypes. To this end, the naive panel through group discussion provided the important attributes to be considered for preference studies. Organoleptic attributes listed by farmers were texture, taste, aroma, fibre content and flesh colour of boiled roots in that order of importance (Table 6.1).

Table 6.1 Classes of attributes for organoleptic description (1-5)

	Attribute description classes				
Attribute	**1**	**2**	**3**	**4**	**5**
Texture	too mealy	somewhat mealy	medium mealy	slightly moist	too moist
Taste	Flat	somewhat sweet	pleasingly sweet	somewhat too sweet	too sweet
Flavour	no smell	somewhat a nice smell	pleasingly a nice smell	somewhat a strong smell	too strong a smell
Fibre content	absent	slight presence	moderate presence	more present	too much
Colour	Actual flesh colour				

A scale of one to five (Table 6.1) which described the attributes in their respective classes was formulated by farmers and scientists on a questionnaire which was pilot tested while training the trained panel to identify problems associated with design, ambiguity, interpretation or bias. The organoleptic analysis by the trained panel was an important analysis where the attributes of a sweetpotato genotype were identified using human subjects who have been specifically trained for this purpose (ASTM, 1995). The trained panel comprised of 8 female and 4 male home economics students in their final year at Chancellor College, University of Malawi. The age range of the trained panel was 22 to 26 years.

Sweetpotato genotypes were also described agronomically (yield, root size, number of roots per plant and weevil damage) to establish the possibility of identifying ideal genotype (s) carrying both important agronomic and organoleptic attributes. Data on yield (t/ha), root size and number of roots per plant on marketable roots was collected by researchers at harvesting times. Weevil severity on a score scale of 1-5 was used where; 1= absent, 2= present with very little damage, 3= present with moderate damage, 4= present with high damage and 5= present with severe damage. Percent incidence of damaged roots per plot was calculated by researchers at each harvesting time.

6.4.3 Preference scoring by naive and trained panels

The hedonic preference scoring is one of the subjective tests which measures the level of liking for foods or any other product where an affective tone is necessary (ASTM, 1995). This test relies on peoples' ability to communicate their feelings of like or dislike. Hedonic testing is popular because it may be used with untrained people as well as with experienced panel members (OMahony, 1986). The naive panel in the current study was also comprised of 8 females and 4 males. These were sweetpotato growers and consumers from surrounding villages of Bvumbwe Research Station. The age of the naive panel ranged from 40 to 68 years. Preference scoring was conducted by both panels using a questionnaire. Naive panelists were however conducted through the samples by scientists as most of them were illiterate. A hedonic preference score scale of one to five was used in the study where 1, 2, 3, 4, and 5 were; like very much, like slightly, neither like nor dislike, dislike slightly and dislike very much respectively. Preference scoring on agronomic and organoleptic attributes by the naive panel was done at Bvumbwe Research Station while the organoleptic attribute testing by trained panel was conducted at Chancellor College, Zomba, Malawi.

Figure 6.1 Some of the trained panelists in session at Chancellor College

6.4.5 Data analysis

Descriptive data on organoleptic attributes was analysed using SPSS version 12.0 to classify the main differences between the genotypes. Preference scores for organoleptic and agronomic attributes were subjected to analysis of variance to get the means for the variables using AGROBASE software (Agrobase^{TM} 1999). Correlations on scores between panels and among attributes were determined by spearman rank correlations using the SPSS software. The data on preference scores was transformed into a binary data matrix. The presence of a score at a particular class of an attribute was recorded as 1 and absence as 0 for all genotypes. Based on the presence/absence, dissimilarity coefficients for data were generated using the SIMINT module (NTSYS pc 2.11c software (Rolhf, 2001). Principal component analysis (PCA) was performed using NTSYSpc 2.1 where eigen values and vectors were generated following Pearson's correlation matrix.

6.5 Results and Discussion

6.5.1 Organoleptic description of genotype attributes

The organoleptic classification by colour, texture, taste and flavour of freshly boiled sweetpotato storage roots are presented in Table 6.2. There was a wide representation for the colour classes in the study genotypes although the orange fleshed colour dominated. However due to the small size (n) of panel describing the genotypes, the degree of colours were confounded and therefore collapsed to main colours only, for instance, deep or pale yellow colours were collapsed to yellow, as such, deepness of colours is not reflected in the genotypes.

Slight changes in flesh colours were observed with time in some genotypes due to root age such as Babache (white to cream), Mzungu (cream to yellow) and Tainoni (yellow to orange). There was a wide representation of texture among the genotypes ranging from too mealy to too moist. Texture description was straight forward as it discriminated the genotypes into too main categories thus either incriminating towards the moist or mealy categories. The texture changed with root age. However, four local genotypes Babache, Kamchiputu, Zondeni and Yoyera and one introduction Kenya, were described to be mealy to too mealy while two local genotypes, Pepu and Mzungu varied between moist and too moist. The rest of the genotypes were either moderately mealy or some what mealy.

Table 5.10 presented results of dry matter content which provided an idea of texture (mealiness) for each genotype at the different times. In general (except for Lunyangwa), the genotypes that had dry matter content of 30% and above were rated too mealy (tm), medium mealy (mm) and some what mealy (swm) while those under 30% were rated too moist (to) or medium moist (mo). The description by the trained panel was therefore in agreement with the dry matter content of the genotypes. Slight changes in dry matter content were in some cases picked by the trained panel for instance Mzungu was too moist at 4 MAP but medium moist at 5 and 7 MAP. Babache was categorized to be flat while Mafutha was more sweet. Most of the genotypes were rated to be somewhat and medium sweet. While A45 and Zondeni had a strong flavour, most of the genotypes were rated to be somewhat and medium flavoured. No significant differences were detected for fibre content among the genotypes as the genotypes had no fibres hence results are not reported.

Table 6.2 Description of sweetpotato genotypes by flesh colour, texture, taste and flavour at the various MAP

	Flesh colour			Texture			Taste			Flavour		
Genotypes /time	**4**	**5**	**7**	**4**	**5**	**7**	**4**	**5**	**7**	**4**	**5**	**7**
Babache	white	cream	cream	mm	tm	mm	flat	flat	flat	mdf	swn	swn
Mzungu	cream	yellow	yellow	tmo	mo	mo	sws	sws	sws	nf	swn	swn
Yoyera	white	white	white	tm	tm	swm	mds	mds	mds	mdf	mdf	mdf
Kamchiputu	orange	orange	orange	swm	tm	mm	sws	ms	mds	mdf	mdf	mdf
Kenya	yellow	yellow	yellow	swm	tm	swm	mds	mds	mds	swn	mdf	swn
Zondeni	orange	orange	orange	swm	tm	swm	mds	mds	mds	swn	sf	sf
LU96/303	cream	cream	cream	mo	swm	mm	mds	sws	mds	swn	sf	swn
Pepu	purple	purple	purple	tmo	mo	mo	sws	sws	mds	nf	sf	nf
Mafutha	orange	orange	orange	mo	mo	mo	sws	ms	ms	swn	swn	mdf
A45	orange	orange	orange	mo	mo	mo	sws	mds	mds	sf	tsf	sf
Lunyangwa	cream	cream	yellow	mo	mo	mo	ms	mds	ms	mdf	swn	swn
LU96/374	yellow	yellow	yellow	mm	mm	mm	mds	mds	mds	swn	swn	swn
Tainoni	yellow	yellow	orange	mo	mo	tmo	mds	ms	mds	mdf	swn	nf
Mfumu	cream	cream	cream	mo	mm	mo	mds	sws	mds	swn	swn	swn
Semusa	white	cream	cream	mm	swm	mm	mds	mds	ms	swn	mdf	swn

Key: texture, tm=too mealy, swm=somewhat mealy, mm=medium mealy, mo=moist, tmo=too moist; taste, sws=somewhat sweet, mds=medium sweet, ms=more sweet; aroma, nf=no flavour, swn=somewhat nine flavour, mds=medium flavour, sf=strong flavour, tsf=too strong flavour

6.5.2 Preference for organoleptic attributes by naive and trained panels

ANOVA identified high significant differences in preferences among genotypes for texture, taste, flesh colour and aroma at $p \leq 0.001$ (Table 6.3). No significant differences ($p \leq 0.05$) were however detected between panels for texture while highly significant differences ($p \leq 0.001$) were observed between the same for taste, flesh colour and aroma (Table 6.3). The panels demonstrated agreement on texture where Yoyera was highly preferred by both naive (2.11) and trained (1.74) panels. However, on the rest of the attributes, preferential scoring on genotypes for attributes differed, for instance, as the young and learned panel showed a high preference for whitish colour (Yoyera, (1.7) the older and less educated panel opted for the orange colour (Zondeni (1.64) and Mafutha (1.75).

Table 6.3 Preference scores for texture, taste, aroma and flesh colour by trained (TR) and naive (NV) panels

	Texture		Taste		Aroma		Colour		**Overall**
Genotype	**NV**	**TR**	**NV**	**TR**	**NV**	**TR**	**NV**	**TR**	**Mean**
Yoyera	2.11	1.74	1.67	2.67	2.03	2.41	2.3	1.7	**2.08**
Kamchiputu	2.11	2.37	2.22	1.67	2.44	2.33	2.1	2.63	**2.23**
Zondeni	2.53	2.30	2.28	2.56	2.25	2.15	1.6	2.15	**2.23**
Kenya	2.19	2.26	2.44	3.00	1.89	2.63	2.3	2.44	**2.39**
Mafutha	2.47	2.78	3.19	1.89	2.14	2.44	1.8	3.22	**2.49**
Semusa	2.50	2.59	2.97	2.33	1.92	2.67	2.6	3.37	**2.62**
LU96/303	2.53	2.85	2.56	2.67	2.56	2.67	2.4	3.04	**2.66**
LU96/374	3.00	2.67	3.17	2.89	2.22	2.78	2.4	3.00	**2.77**
Lunyangwa	2.50	2.93	3.47	2.78	2.31	2.67	2.5	3.22	**2.80**
Babache	2.19	2.15	4.44	3.00	2.36	2.63	2.7	2.94	**2.80**
A45	2.44	2.70	3.61	3.67	2.53	3.04	2.3	2.19	**2.81**
Mfumu	2.81	2.93	3.22	2.78	2.33	2.74	3.2	2.74	**2.84**
Tainoni	2.78	2.70	3.42	3.89	1.97	3.11	2.4	3.00	**2.91**
Pepu	3.00	3.44	3.53	2.56	2.33	2.93	2.3	3.78	**2.98**
Mzungu	3.85	4.52	1.86	4.44	2.36	3.78	2.6	3.48	**3.36**
Mean	2.60	2.73	2.94	2.85	2.24	2.73	2.37	2.86	2.67
LSD (cultivars)	0.45	0.47	0.42	0.44	0.36	0.46	0.43	0.46	**0.31**
CV (%)	5.95	23.24	6.50	25.90	2.04	27.15	7.3	23.75	**18.56**
Significance (cultivars)	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
Significance (panels)	ns		* * *		* * *		* * *		

*** p≤0.001; CV= coefficient of variation; LSD= least significant difference

6.5.3 Comparison of preference on organoleptic attributes by panels

Texture was the only attribute that had a strong correlation (r=0.80) between the two panels, thus 64% (r^2=0.64) preference agreement was observed between panels indicating that either of the panels can be used for texture assessment (Table 6.4).

Table 6.4 Correlation coefficients (r) of organoleptic attributes

		Texture		**Taste**		**Aroma**		**Colour**
		naive	**trained**	**Naïve**	**trained**	**naive**	**trained**	**naive**
	trained	0.80**						
Taste	**naive**	-0.09	0.11					
	trained	0.60*	0.68**	0.04				
Aroma	**naive**	0.16	0.29	0.16	0.03			
	trained	0.80**	0.88**	0.06	0.84**	0.14		
Colour	**naive**	0.29	0.25	0.27	0.38	0.08	0.48	
	trained	0.57*	0.52*	0.36	0.05	0.08	0.47	0.25

* and * * significant at $p \leq 0.05$ and 0.01 respectively

Texture was highly linked with the other attributes (Table 6.4) as observed by its association with taste between panels (r=0.60) and within trained (r=0.68) panel; aroma between panels (r=0.80) and within the trained (r=0.88) panel; colour between panels (r=0.57) and within the trained panel (r=0.52). Surprisingly, the correlation results indicate that the naive panel demonstrated an understanding of the attributes and scored them independent of each other. Thus, there was no significant association between attributes within the naive panel while the trained panel associated texture with colour, aroma and taste. In addition, the results by the naive panel were more reliable as shown by the lower percent of coefficient of variation (% CV) for all attributes (Table 6.3) as opposed to the high CV of the trained panel group. Frequent consumption of sweetpotato by naïve panelists is attributed to the high agreement of the results.

6.5.4 Variation in importance of organoleptic attributes

Variance of attributes was further studied by principal component analysis (PCA). A principal component matrix is provided in Table 6.5 and it revealed that three of the four attributes under study had eigenvalues >1 and contributed significantly to the variability (100%). However, texture alone accounted for 50.45% of the total variation from the four attributes. The next important attribute was taste which accounted for 32.34% of the variation followed by flavour (16.66%).

Table 6.5 Degree of importance of individual attributes determined by the % variability proportion accounted for by the first four principal components

	Attributes			
Eigen vectors	**Texture**	**Taste**	**Flavour**	**Colour**
PC 1	-0.05	0.17	-0.02	-0.03
PC 2	0.14	0.23	0.28	-0.07
PC 3	0.00	0.00	0.00	0.00
PC 4	-0.10	-0.02	-0.07	-0.05
Eigenvalue	99.39	64.79	32.81	0.03
IVE %	50.45	32.89	16.66	0.00
CVE %	50.45	83.34	100	0

PC = Principal Component; IVE = Individual Variability Explained; CVE = Cumulative Variability Explained.

The study revealed that colour was not an important attribute at all such that the high frequency of cream and white fleshed sweetpotato landraces in Malawi (Table 2.13) could be due to the selecting for other attributes such as texture, taste, flavour and other organoleptic (fiber content), agronomic (yield and yield components, diseases, pests, root shape), post harvest (shelf life) and market value (demand) attributes other than colour. The current results which have revealed that colour was not an important attribute considered for selection simplifies that bio-fortification efforts aimed at releasing genotypes with high dense β-carotene content will have no or less barriers in adoption. Results in Table 6.3 also revealed that the naive panel who were the growers and consumers of sweetpotato preferred the orange fleshed as opposed to the trained who preferred the white fleshed genotypes.

6.5.5 Preference for agronomic attributes by naive panelists

Preference results in the current study on yield by the naive panel confirms the obvious fact that farmers preferred high yielding cultivars, such that at 4 and 5 MAP Mzungu a landrace (1.9) and Semusa (1.4) an introduction which were highest yielding (Table 6.7) respectively were the most preferred (Table 6.6). However, at 7 MAP, LU96/303 which was not among the top six highest yielding (Table 6.7) was the most preferred with a preference score of 1.5 (Table 6.6). These results suggest that in the presence of several high yielding cultivars, preference for other agronomic attributes play important roles in cultivar selection. Such attributes apart from root size and number of roots per plant may be root shape, root form and colour. LU96/303 has attractive smooth, white skinned and medium sized roots.

During discussion, when formulating the most important agronomic attributes, farmers indicated that root shape, colour and form were among the least important. Across the harvesting times, Semusa and Mzungu which both had mean scores of 1.83 and were the overall preference for yield while local landraces Kamchiputu and Yoyera were the least preferred (3.9 and 3.93 respectively) for the same. Results on root size (Table 6.6) showed that Mzungu (2.4), LU96/374 (1.8) and Kenya (1.4) were highly preferred at 4, 5 and 7 MAP. Mzungu attained an overall highest score of 2.03 followed by Kenya (2.17). Semusa had the highest preference for mean number of roots per plant (2.13) across times with Kenya (2.4), LU96/374 (1.7) and LU96/303 and Lunyangwa (1.4) attaining high preference at 4, 5 and 7 MAP respectively. Mzungu (2.06) and Semusa (2.09) were the overall preferred genotypes for yield and yield components while Kamchiputu (3.69) and Yoyera (3.98) were the overall disliked cultivars across harvesting times. The findings imply that no genotype was ideal that satisfied farmers' preferences and needs.

Table 6.6 Preference scores for yield, root size and number of roots per plant by the naive panel

	Yield				**Root size**				**Root number/plant**				
Genotype	**4**	**5**	**7**	**Mean**	**4**	**5**	**7**	**Mean**	**4**	**5**	**7**	**Mean**	**Overall mean**
Mzungu	1.9	1.9	1.7	**1.83**	2.4	1.9	1.8	**2.03**	2.5	2.5	1.9	**2.30**	**2.06**
Semusa	2.4	1.4	1.7	**1.83**	2.9	2.0	2.0	**2.30**	2.9	1.8	1.7	**2.13**	**2.09**
Kenya	2.9	2.4	2.0	**2.43**	3.1	2.0	1.4	**2.17**	2.4	2.2	2.3	**2.30**	**2.30**
Lu96/374	2.4	1.9	3.3	**2.53**	2.5	1.8	3.3	**2.53**	2.5	1.7	2.5	**2.23**	**2.43**
Mfumu	3.3	1.8	2.2	**2.43**	3.4	2.4	2.0	**2.60**	3.2	2.2	2.7	**2.70**	**2.58**
Mafutha	2.5	2.3	2.0	**2.27**	2.9	2.8	3.1	**2.93**	2.6	2.9	2.3	**2.60**	**2.60**
Lu96/303	2.8	3.6	1.5	**2.63**	3.1	3.7	1.8	**2.87**	2.8	2.9	1.4	**2.37**	**2.62**
Lunyangwa	2.9	3.1	2.0	**2.67**	3.4	3.5	2.3	**3.07**	3.0	3.3	1.4	**2.57**	**2.77**
Pepu	2.6	3.0	2.6	**2.73**	2.6	3.3	3.3	**3.07**	2.9	2.9	2.6	**2.80**	**2.87**
Tainoni	2.9	3.1	3.0	**3.00**	3.0	2.1	2.8	**2.63**	3.2	3.4	2.7	**3.10**	**2.91**
Babache	3.5	3.6	2.3	**3.13**	3.6	3.2	2.4	**3.07**	3.6	3.3	2.4	**3.10**	**3.10**
Zondeni	3.6	3.7	2.4	**3.23**	3.4	3.5	2.4	**3.10**	3.6	3.0	2.7	**3.10**	**3.14**
A45	3.5	3.4	3.2	**3.37**	3.3	3.1	3.2	**3.20**	3.4	2.7	2.5	**2.87**	**3.14**
Kamchiputu	3.6	4.3	3.8	**3.9**	3.7	3.5	3.6	**3.60**	3.5	3.5	3.7	**3.57**	**3.69**
Yoyera	4.2	4.1	3.5	**3.93**	3.9	4.0	3.8	**3.90**	4.1	4.2	4	**4.10**	**3.98**
Mean	**3.00**	**2.91**	**2.48**	**2.79**	**3.15**	**2.85**	**2.61**	**2.87**	**3.08**	**2.83**	**2.45**	**2.79**	**2.82**
% CV	**28.36**	**24.24**	**29.31**	**22.16**	**23.54**	**21.22**	**25.43**	**23.91**	**19.96**	**27.83**	**21.34**	**19.16**	**18.72**
LSD	**0.56**	**0.48**	**0.42**	**0.46**	**0.52**	**0.49**	**0.39**	**0.45**	**0.56**	**0.44**	**0.41**	**0.41**	**0.51**
Significance	***	***	***	***	***	***	***	***	***	***	***	***	***

*** p≤0.001; LSD= least significant difference

6.5.6 Selection for agronomic attributes by researchers

Table 6.7 presents the agronomic evaluation results of the genotypes whose yield and root sizes were significantly different at $p \leq 0.05$ within and between harvesting times. In a normal evaluation and selection process by researchers, genotypes considered for advancement from advanced yield trial (Figure 6.2) are the ones that give yields of not less than 20t/ha at 5 MAP across evaluation locations and seasons as highlighted in section 4.5.1

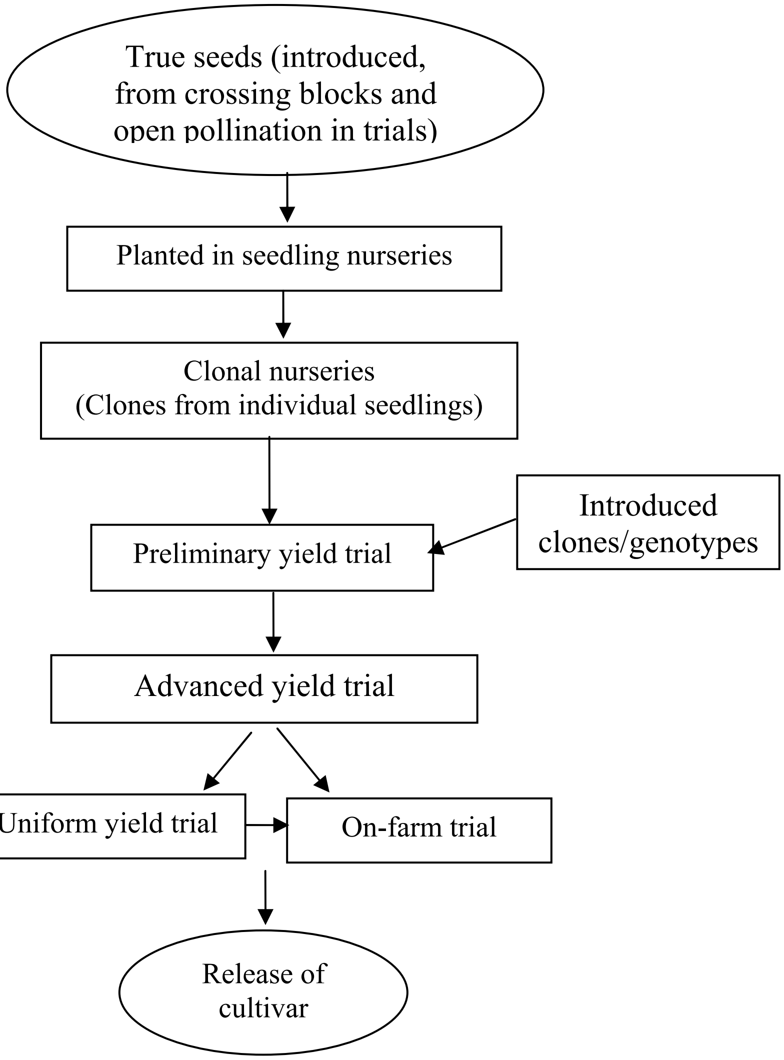

Figure 6.2 Schematic routine for sweetpotato evaluation in Malawi

In unfavourable environments, the performance of testing genotypes is compared with checks. In the early stages of evaluation (seedling and clonal nurseries, Figure 6.2) the number of entries may be in hundreds and screening of the materials is mainly based on resistance/tolerance to diseases and pests, root shape and form among others. Yield evaluation replicated over several sites starts at the preliminary stage such that by uniform yield trial stage, there are a few (normally less than ten in number), high yielding, early maturing genotypes that are tolerant/resistant to pests and diseases with attractive root shape and form, high dry matter content *etc.* and being evaluated in parallel at on-farm.

Table 6.7 Root yield, size and number of roots per plant as assessed by researchers

	Total yield (t/ha)			**Root sizes (g/root)**			**Root number/plant**		
Genotype	**4**	**5**	**7**	**4**	**5**	**7**	**4**	**5**	**7**
Mzungu	19.44	21.53	23.50	139.37	189.34	180.00	5	5	6
LU96/374	11.44	17.42	17.50	110.59	95.20	121.50	5	5	5
LU96/303	16.33	18.21	19.50	111.77	136.12	111.00	5	5	6
Pepu	15.48	18.50	20.34	210.30	234.00	230.11	4	4	7
Kenya	15.43	17.89	21.00	134.20	125.73	153.50	4	4	5
Mafutha	13.83	13.81	21.00	88.24	85.28	119.00	5	6	6
A45	13.35	15.30	19.00	79.62	137.11	114.50	5	6	6
Tainoni	12.58	14.87	17.50	95.41	99.78	104.50	4	5	6
Babache	12.11	10.65	17.00	99.91	102.12	144.00	4	4	6
Lunyangwa	11.88	15.58	18.00	91.25	84.04	209.00	5	7	4
Mfumu	11.39	18.13	23.00	121.55	132.51	228.50	4	5	4
Semusa	20.60	27.16	26.50	168.21	192.41	237.00	5	5	4
Zondeni	8.41	16.36	14.50	90.19	91.00	106.67	3	6	7
Kamchiputu	6.79	5.710	9.00	84.33	79.44	103.00	3	3	3
Yoyera	3.70	8.10	17.50	78.37	116.05	257.00	2	3	5
Mean	**12.85**	**15.95**	**18.99**	**113.55**	**126.68**	**161.29**	**4.20**	**4.87**	**5.33**
LSD	**2.34**	**3.43**	**1.87**	**18.95**	**23.56**	**26.63**	**0.91**	**1.21**	**1.37**
% CV	**22.46**	**26.28**	**29.36**	**22.96**	**25.34**	**20.56**	**19.86**	**19.36**	**24.28**
Significance	* * *	* * *	* * *	* * *	* * *	* * *	* *	* *	* *

***p≤0.001; ** p≤ 0.01; %CV= percent coefficient of variation

Evaluation of yield and yield components is done at 5 MAP. Genotypes such as Mzungu (21.53 t/ha) a landrace and Semusa (27.16 t/ha) a released introduction in Table 6.7 qualify the description as high yielding and early maturing at 5 MAP assuming they are at advanced stage of the schematic diagram (Figure 6.1). Genotypes such as Mzungu (21.53 t/ha) a landrace and Semusa (27.16 t/ha) a released introduction in Table 6.7 qualify the description as high yielding and early maturing at 5 MAP assuming they are at advanced stage of the schematic diagram (Figure 6.2). The majority of the genotypes in the current study were however early maturing and medium yielding giving yields between 15 and 19 t/ha at 5 MAP. Such genotypes may be advanced for further evaluation depending on performance in comparison with the medium yielding check Kenya and other attributes such as acceptable palatability, stability, resistance to pests and diseases *etc*. Genotypes giving less than 15t/ha are considered late maturing and low yielding and are therefore dropped. The present selection criterion which is based on yield level at 5 MAP is important but need modifications when evaluating landraces which have undergone through evolution under farmers' fields where environmental conditions are highly variable and unfavourable. Some landraces may be important sources for adaptation traits other than yield

6.5.7 Comparison of agronomic preference by farmers and selection by researchers

The preference ranking for yield by farmers was highly correlated with both root size (r=0.71) and root numbers per plant (r=0.65) at $p \leq 0.01$. Thus, upon selecting for large roots there was 54% ($r^2=0.54$) chances that farmers will select for high yielding genotypes and $r^2=0.42$ indicates that 42% high yielding genotypes will be selected if selection criteria is based on high root numbers/plant. Agronomic measurement by researchers also showed a high correlation between root yield and size ($r^2=0.58$) than that of yield and number of roots per plant ($r^2=0.24$) both at $p \leq 0.01$ was established in the study. The correlation results indicate that root size accounts for 58% of total yield while number of roots account for 24% of root yield. Thus, in large population and early stage selection like in a clonal evaluation, selection on root size leads to indirect selection of tuberous root yield.

On yield, it has been shown that as farmers select for high yields based on root size, ($r^2=0.58$ for yield and root size preference), so are researchers based on weight of marketable roots ($r^2=0.54$ for yield (t/ha) and root size (g/root). Thus in both cases, high correlations between yield and root size were observed. Lower associations for root numbers per plant with yield were also observed by both farmers (42%) and researchers (24%). However, while as researchers' base yield levels at 5 MAP, farmers grow multiples of cultivars that differ in yield and attain maturity levels at different times (Table 6.4) with the main purpose of spreading consumption. At 5 MAP only two genotypes (Semusa and Mzungu) meet the researchers' selection criterion. However, at 7 MAP, six genotypes have yields that are over 20t/ha and therefore qualify for selection (Table 6.4). Selection consideration at 5 MAP implies that many potential genotypes are discarded. Furthermore, Yoyera at 7 MAP is no longer a low but a medium yielding genotype.

Table 6.8 present results on weevil assessment at 5 and 7 MAP. No weevil damages were detected at 4MAP which showed no significant differences among genotypes for weevil severity damage at $p \leq 0.05$ at 5 MAP.

Table 6.8 Weevil damage severity scores and percent incidence

	5 MAP			7 MAP		
Genotypes (G)	**Severity**	**Number of roots/ha**	**% incidence**	**Number of roots /ha**	**Severity**	**% incidence**
Kamchiputu	1.00	28703	0	45673	2.00	2
Lu96/374	1.67	94135	11	93891	2.50	7
Yoyera	1.00	26346	0	58923	2.00	7
Lunyangwa	2.00	89691	11	65432	2.00	31
Kenya	2.00	84876	9	89172	3.00	36
Babache	2.00	69444	2	75617	3.00	16
Lu96/303	2.00	80246	9	81926	3.00	22
Zondeni	2.33	57098	15	63323	4.00	14
Semusa	2.33	97826	20	103864	3.00	36
Mfumu	2.00	77160	2	81234	2.00	4
Tainoni	2.00	109567	13	98762	3.00	47
Pepu	2.33	41666	16	47650	3.00	5
A45	2.00	78703	31	86549	2.00	8
Mafutha	2.33	94135	20	123657	3.50	17
Mzungu	2.00	98346	3	109636	2.00	8
Mean	1.93	75196.13	10.80	81687.27	2.67	17.33
LSD	**0.56**	**12561**	**10.91**	**9861**	**1.26**	**23.91**
CV %	**15.63**	**21.36**	**25.36**	**24.09**	**26.58**	**24.29**
Significance (G)	**ns**	***	*	***	*	*

However, significant differences at p≤0.05 were detected for percent incidences at second and third harvesting and for severity at 7 MAP. Table 6.8 is very important to researchers as the severity and percent incidence data depicts the level of resistance/tolerance to sweetpotato weevil which is a crucial selection factor as farmers prefer in ground storage of sweetpotato roots. At 5 MAP Kamchiputu (1.0) was not yet attacked by weevil while at 7 MAP, the cultivar was moderately attacked. Zondeni (4.0) showed high susceptibility to weevil attack when harvesting is delayed by two months. The results have showed that the late maturing and low yielding cultivars, Yoyera and Kamchiputu are tolerant to sweetpotato weevil (Table 6.8). In their study, Kabi *et al.* (2001) indicated that root size was the only sweetpotato rooting characteristic that significantly influenced tuber infestation and damage by weevils. The resistance/tolerance of cultivars to weevil attack in the current study is therefore attributed to small root sizes. The cultivars that had no or very little weevil damage were highly preferred by farmers at harvest.

6.5.8 Attribute variation, cultivar preference and selection by farmers

The current results have shown that regardless of the evaluating panel group, different cultivars are identified as the best cultivars for different attributes. Mzungu which was the best agronomically was the last preferred gastronomically while both Kamchiputu and Yoyera which were disliked agronomically (yield and yield components) were respectively the second and first best preferred gastronomically in addition to high preference for weevil tolerance/resistance. The inclusion of the three cultivars Mzungu, Kamchiputu and Yoyera in the study was purposeful and a verification of indigenous knowledge documentation from farmers, where mzungu 'white man' is actually an insult for demonstrating high yielding traits, early maturing and smooth attractive roots but watery (moist) when eating while Yoyera and Kamchiputu, varieties which were officially recommended varieties for production in Malawi in the early 1980s and were commonly grown in Lilongwe, are characterised to be mealy, tasty with a good flavour. However, due to late maturity and low yielding characteristics, apparent erosion of Kamchiputu and Yoyera has been observed. As such, the cultivars were collected for conservation at Bvumbwe Research Station and are normally included in research trials as checks for palatability and as breeding parents.

An important emerging issue on the agronomic assessment study is that although the results are preliminary and need several seasons for validation, some local landraces have the potential to contribute considerably to the sweetpotato improvement program in Malawi by providing new varieties for selection and parent stocks for trait combinations. Examples of such genotypes are mzungu for yield, Zondeni for beta-carotene, Yoyera and kamchiputu for texture and taste. Information gathered during the documentation of indigenous knowledge was that some attributes are dependent on each other in terms of genotype preference. For instance, it was revealed that yield alone is not indicative to earliness as is the case to breeders. Taste, latex production and colour intensity entails maturity according to the farmers. Kenya and Zondeni are some of the genotypes which were highlighted to be early in terms of yield at 3 MAP, but tasteless at this age because they are not mature. Immature roots of Kenya for instance were described to be cream coloured with high latex production and tasteless while matured roots are yellow to deep yellow with no or less latex produced and very tasty. The texture was also described to be too mealy after Kenya roots are matured. It was therefore revealed that among the early bulking genotypes, not all can be used during the early months. While some genotypes need to mature, others do not have this problem and can therefore be used as long as the root sizes are usable. It was revealed in Table 6.2 that Mzungu and Semusa apparently changed colours from cream and white to yellow and cream respectively with age entailing maturity.

The aspect of growing multiples of cultivars in a mixture has been well covered in chapter two, and the current results therefore reveal and confirm that no single genotype contain all the preferred attributes understudy to address varied farmer and consumer concerns. Thus in response to variability of local conditions and production objectives, farmers grow several varieties (Matlon, 1991). Farmers' multiple criteria to select what varieties to plant, as well as where, when and how to do it, has been well established and reflect their concerns (Brush *et al.,* 1981; Bellon, 1991). These selection criteria by farmers have been reported to be quite different from those of the breeders (Sperling *et al.,* 1993). Ethno-botanically, the findings in chapter two revealed high diversity at field level as the genotypes on-farm were composed of varying attributes. In chapters two and three, morphological and DNA analysis (SSR) respectively also exhibited a high level of diversity, all stemming from varied attribute composition of genotypes.

6.5.9 Value of the naive panel

Apart from texture, the results have exhibited significant variation on the organoleptic assessment by the two panels (Table 6.3). Influences on consumer choice have been outlined by Randall and Sanjur (1981) which include "people factors" such as age, education, nutritional knowledge; environmental influences such as marketing variables, and intrinsic sensory influences such as flavour, texture and appearance. The preferential differences between the trained and naive on organoleptic variables are attributed to the age, education and organoleptic experience (naive) on the attributes in the present study.

It has been observed in the present study that through experience, the naive panel is capable of scoring and isolating attributes independent of each other and can therefore be involved in genotype evaluation and selection. Since farmers are expert experimenters in genotype selections, scientists can improve the relevance of their research by drawing their knowledge. The use of a trained panel in genotype selection was not effective in this study as the panelists associated texture with all other attributes. However, the trained panel competently described the organoleptic attributes.

6.5.10 Consolidating nutritional, agronomic and organoleptic with ethno-botanical values

Increased food production and access are crucial to achieving major nutritional improvement. Selecting micronutrient dense food crops and cultivating micronutrient-dense staple plant food varieties should be a major goal of agriculturalists in developing countries where micronutrient deficiencies among people are common. In chapter five, the nutrient levels of three mineral elements (iron, zinc and copper) and two vitamins (beta-carotene and ascorbic acid) were reported. The nutrient of interest among the five evaluated in the current study was beta-carotene. Zondeni was the superior genotype for beta-carotene content ranging from 6.07 and 13.93 mg/100g fresh weight in peeled (7 MAP) and unpeeled (5 MAP) samples respectively. Other genotypes (unpeeled) which followed Zondeni at 5 months were A45 (3.49), Mafutha (2.96), LU96/374 (2.16), Tainoni (1.87) and Kamchiputu (1.57). Although the content of beta-carotene fluctuated with peeling and maturity age in genotypes, Zondeni remained relatively high to adequately supply the recommended daily allowance for all age groups. It was however observed that at every assessment period; 4, 5 and 7 months after planting, Babache was consistently the lowest in beta-carotene content, whether peeled or unpeeled.

Babache and Mfumu have been well documented in chapter two, section 2.5.1.6 as genotypes prevalently grown in the Lower Shire districts of Malawi. Zondeni was also well documented in section 2.5.1.3 as the second prevalent genotype after Kenya that was sampled in most sites of collection (Chitipa, Karonga, Mzimba, Nsanje, Mulanje and Phalombe). However, while as Zondeni was sampled twice in Nsanje under the name 'Nyamatanga' (pumpkin like colour), Babache and mfumu were commonly grown genotypes in these districts. In the present study, both mfumu and Babache registered low levels of beta-carotene. The morphological analysis, Table 2.11 (chapter two) showed that the accessions from alluvial soils which are Nsanje and Chikwawa districts were composed of only 1% dark yellow, 1% intermediate orange, 0% orange and 1% dark orange fleshed genotypes. Yet it is well established that orange fleshed sweetpotato genotypes are good sources of beta-carotene (Woolfe, 1992; Low *et al.,* 1997).

It has been observed that the prevalent genotypes grown in the Lower Shire districts (Chikwawa and Nsanje) for time in memorial (as farmers inherited the white fleshed genotypes) are very low in beta-carotene content. In Malawi, the area that is highly prone to vitamin A deficiency is this Lower Shire area where Tielsc *et al.* (1986) reported that prevalence of active xerophthalmia in 1983 was 3.9% in children under six years of age. Rates for night blindness and active corneal disease were more than five times the World Health Organization criterion for a problem of public health importance. Xerophthalmic corneal scarring occurred at a rate of 5.9/1,000, more than 10 times the World Health Organization criterion. All cases of bilateral blindness in this age group were considered to be due to vitamin A deficiency. Sixteen years later, Courtright *et al.* (2003), found that overall blindness in the Lower Shire Valley was reduced by 31% due to intervention services. However, the study in 1999 further reports that there was little change in excess blindness among women, suggesting that the same barriers that prevented utilisation of services (by ministry of healthy) in 1983 probably persist in 1999.

The current study on β-carotene content determination on prevalent, released and elite genotypes will enormously contribute to reducing vitamin A deficiency problems in Malawi. The promotion of Zondeni in the lower Shire is highly recommended in order to complement the efforts by ministry of healthy in vitamin A reduction. Since the current study has also established that Zondeni was one of the two genotypes that came second in terms of organoleptic preference scores (Table 6.3) after Yoyera promotion of the genotype in non traditional growing area will not be a problem. In addition, Table 6.5 has shown that flesh colour of roots is of less importance as far as preference is concerned. Thus in Malawi, the prevalently grown genotypes are cream and white fleshed as depicted in Table 2.11 of chapter 2. Zondeni genotype is prevalently grown in other study areas, an indication that organoleptically, the genotype is good. Ethno-botanically, Zondeni was also documented under the name 'Kampalendo', implying 'don't give it to visitors', meaning the cultivar is too good for visitors to take leave. The promotion of the orange fleshed genotype is therefore expected to have no organoleptic set backs as it is convincingly good by naming, prevalence (grown widely in other areas) and organoleptically (results in Table 6.6).

The elite clone in yield (LU96/374) and the released genotype Tainoni are also good sources of vitamin A mainly for adults who can manage to eat larger quantities. However, on the premise that farmers grow multiples of genotypes in mixtures, the percentage of RDA per day for vitamin A that these genotypes can provide may be reduced due to multiple varieties in use. It is therefore recommended that while these genotypes can be released and make a difference to vitamin A contribution, efforts should be done to release such genotypes as Zondeni that can contribute enormously to reduce the vitamin A deficiency. Ascorbic acid content exhibited high genotype variation that showed no consistency in response to variation in root age and processing (peeling). One hundred grams of unpeeled samples for all genotypes can however provide reasonable percentages of the RDA for ascorbic acid which increased with age. The results on iron, zinc and copper analysis indicate that Mzungu had the highest iron (0.67mg/100g) and zinc (0.63mg/100g) content while Yoyera had the highest copper (0.61mg/100g) at 4 MAP but the levels of all these elements decreased tremendously with time.

6.5.11 Challenges to sweetpotato improvement in Malawi

The importance of the agricultural sector in Malawi is that its performance has a direct impact on the overall economic growth of the country. For this reason, policies that affect growth of the sector are more important since they similarly affect national economic growth of the country (Agricultural Extension, 2000). In this case, it may not be easy to release and promote Zondeni through the technology clearing committee, since the yields are lower than the 20t/ha. According to Agricultural research master plan (MoALD, 1995) demand driven, high yielding, early maturing, widely adapted and acceptable genotypes that are resistant / tolerant to major pests and diseases are supposed to be released and recommended for production in Malawi.

Thus, the yields of Zondeni (16.36t/ha at 5 MAP) presented in Table 6.7 are a true representation of the genotype although they are for a single season and one site in this study. The genotype is low to medium yielding. On the basis of its orange colour Zondeni was evaluated on a wider scale both on-farm and on-station in years 2000 to 2002 for yield. The aim was to officially release the genotype in trying to address vitamin A deficiency problems in Malawi. However, the genotype exhibited high instability characteristics on yield levels both, within and between sites and seasons. At M'deka in 2001, the genotype was evaluated on-farm where the yields were embarrassingly low (less than 5 tons/ha), while as in Mulanje the yields were as high as 17t/ha. Since the yields of Zondeni vary even within sites, the recommendation to specific environment is also a problem as the genotype probably responds to both, macro as well as micro-environments. Contrastingly, Zondeni a local genotype was evaluated to replace a high yielding clone TBI 11 (CIP440057), which was introduced from CIP, Nairobi for its high β-carotene content (deep orange coloured) but did not advance in the evaluation system due to high water content (moist texture). The genotype was therefore not released due to poor gastronomic characteristics. Researchers are therefore frustrated with the low adoption of genotypes such as Tainoni which can not proliferate in the farming community due to poor texture qualities (moist). In the present studies, results have shown that Tainoni is also a good source of pro-vitamin A. Lobbying for the partial release of Zondeni is however suggested. On the basis that farmers grow multiples of genotypes of both desirable and undesirable attributes (section 2.5.1.13), Zondeni can be promoted for production in Malawi to address vitamin A deficiency problems. The theory behind the partial release is that where the genotype performs, it will be retained.

The production and therefore consumption of Zondeni along with other genotypes will not adversely affect the amounts of RE (µg) required per day as less than 100g of the fresh storage root (unpeeled samples at 5 MAP) is required in all age groups (Tables 5.1, 5.11 and 6.9) except in pregnant and breast feeding mothers. Thus small amounts of Zondeni are adequate to meet the RDA for all age groups while huge and impossible amounts are required for genotype Babache to meet the required RE levels. Kenya, a prevalently grown genotype has also shown to contain some reasonable amounts of β-carotene but huge amounts are required to meet the RDA (Table 6.9), such that in situations where the genotype is grown in a mixture, the amounts required per person are even more. Partial release will allow time for researchers to improve on the genotype through crosses to come up with genotypes that will replace Zondeni for both yield and vitamin A contribution.

Table 6.9 Amounts of unpeeled fresh storage root of 5 sweetpotato genotypes required to meet the RDA for Vitamin A at 5 MAP

		Amounts (g)					
Individuals	**RE (µg) requirement**	**Zondeni**	**LU96/374**	**Tainoni**	**Mafutha**	**Kenya**	**Babache**
Infants (7-12 months)	500	43.07	146.70	212.01	189.27	508.49	3158.56
1 to 3 years	300	25.84	88.02	127.21	113.56	305.10	1895.14
4 to 8 years	400	34.46	117.36	169.61	151.42	406.79	2526.85
9 to 13 years	600	51.69	176.04	254.42	227.13	610.19	3790.27
Adults males	900	77.53	264.06	381.63	340.69	915.29	5685.41
Adult females	700	60.30	205.38	296.82	264.98	711.89	4421.98
Pregnancy < 18 years	750	64.61	220.05	318.02	283.91	762.74	4737.84
Pregnancy > 18 years	770	66.33	225.92	326.50	291.48	783.08	4864.18
Breast feeding < 18	1,200	103.37	352.08	508.83	454.25	1220.38	7580.54
Breast feeding >18	1,300	111.99	381.42	551.24	492.11	1322.08	8212.26

This includes the evaluation of introductions. However, the development of a new sweetpotato genotype in Malawi is a long term process attributed to staff turnover and poor funding for research due to low prioritization of the crop in importance. This trend is also applicable when it comes to extension services at community level. Maize, the staple is a political and a priority crop which is well funded for research and extension services throughout the year as opposed to these other food crops. The importance of sweetpotato is only recognised in times of disaster as late rains, dry spells, floods, and the response in such times is normally the distribution of planting material and non for research programs.

Another institutional set back is the lack of seed multiplication schemes and companies willing to deal with smallholder food crops and diversity issues. The situation becomes more difficult with sweetpotato multiplication as it has to be done during the dry season, near a water source, securely fenced to avoid animals and has to be kept in vegetative form until a buyer is sought and ready to transport the materials. The multiplication of sweetpotato is therefore private and costly attracting high prices that are only afforded by NGOs whose agenda may only be food security other than nutrition. The importance of the informal sector in meeting seed needs has been confirmed by most of the work on crop diversity management (Hardon and de Boef, 1993). Improving on-farm seed storage was important in the maintenance of traditional genotypes in the Philippines (Morin *et al.,* 1998). Such systems are essential elements of the security and sustainability of many farming systems. Only a few and modern varieties are formally multiplied by private individuals. Largely, the multiplication system is informal and does not promote diversity. Technological information on maintaining vines of improved genotypes during the dry period needs to be transmitted to the farmers. Unfortunately, sweetpotato is given least priority among crops, when it comes to extension aimed at helping farmers improve production practices.

6.5.12. Enhancing germplasm diversity development, evaluation and management

The importance of diversity in plant genetic resources (PGR) cannot be understated. It is the raw material on which agriculture develops crops, utilizing important characteristics for pest resistance, nutritional quality and other factors, to meet both predictable and unforeseen ecological and economic circumstances (Cooper *et. al.,* 1992). Genetic diversity provides security against pests and unexpected changes in climatic conditions, ensures maximum production in highly variable environments and provides plant material of varied usefulness. Farmers often manage a combination of cultivars and multiply or eliminate varieties as they evaluate their performance over time (Brush *et al.,* 1991). Researchers can therefore easy their work by combining attributes into different cultivars with the knowledge base that farmers will manage and maintain multiples of cultivars.

Several studies recognize farmers' role in the management of diversity (Conklin, 1957; Bellon and Brush, 1994). In communities, the crops and varieties that exist are part of an evolutionary process of cultivation by farmers to continually adapt to the changing environment and their needs. This was a result of the interaction between people and the environment (Maurya, 1989; Soleri and Cleveland, 1993). In rice for example, the thousand varieties stored at the International Rice Research Institute (IRRI) gene bank in the Philippines is an indication of these diverse genetic resources. Farmers have created and managed the environment where plants could evolve under selective pressure creating a diversity of crops, varieties and agro-ecosystems (Richards, 1985).

In farming communities, there are no distinctions between conservation and development. On-farm conservation of local varieties is an existing strategy for food security. It is a potential strategy for genetic conservation. By its very nature, on-farm conservation is dynamic because the varieties that farmers manage continue to evolve in response to natural and human selection (Bellon *et al.,* 1997). Mende rice farmers in Sierra Leone are successful in creating and maintaining their rice varieties over the years. Their method of harvesting, breaking off panicles one-by-one by hand, gives farmers the option to reject off-types as they harvest. Panicle harvesting results in the stabilization of the main seed types and also brings about a systematic grouping among off-types: early-ripening types will be rogued as they ripen by farmers anxious to secure a little extra for consumption in the hungry-season, and longer duration types will be left in the field to the gleaners. This method of panicle selection has resulted in the differentiation of Mende rice germplasm into three distinct duration classes (Longley and Richards, 1993).

6.6 Conclusions

Crop diversity is a valuable resource for both farmers and plant breeders. It has been observed in the current study that some landraces are worth preserving as they are a valuable source of important traits for either on-farm production or plant breeding. To isolate a few, such genotypes are like Zondeni which is very rich in beta-carotene, Mzungu a high yielding, early maturing and good source of iron and zinc, Yoyera and Kamchiputu, which have good organoleptic attributes (texture, taste, aroma and fibre content). These genotypes are therefore a valuable resource in the breeding program to improve on organoleptic, vitamin A content and yield.

Consolidating results on ethno-botanical, morphological, molecular and nutritional analyses have shown high genetic diversity of sweetpotato germplasm in Malawi. The driving factors for the diversity are complex and in addition to production environment variation in sweetpotato is attributed to consumer preference to satisfy various needs.

CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Genetic diversity is vital to meet the ever-changing needs of growers and consumers of sweetpotato in Malawi in the face of changing and unpredictable environmental challenges. High genetic diversity implies sustainable and stable productivity, buffering various biotic and abiotic stresses. Science-based information on the levels and patterns of genetic diversity is valuable for efficient management of germplasm and for effective utilization of the materials in breeding programs. The Convergence of Sciences however advocates interactive science by which the research agenda is set and implemented through the systematic participation of all stakeholders. Interactive science suggests the need for an approach that will make research more useful for farmers in their own local context.

In Malawi sweetpotato is one of the important secondary staple food crops that partly forms the basis of the farming systems. Apart from being a food source, sweetpotato is a very versatile and traditional crop that is largely grown by subsistence farmers and contributes to income source and employment to many. Until recently, low priority has been given to the crop and it has received comparatively little attention from national research institutions when compared with cereals like maize and rice. However, in the private sector there is interest in sweetpotato and this, in turn, provides opportunities to raise the research profile of the crop. Effective crop improvement however depends on the existence of genetic diversity. Therefore, it seems necessary to understand the levels and distribution of genetic diversity in existing gene pools, as a basis for developing strategies of sweetpotato resource management and exploitation in Malawi. To this end sweetpotato germplasm was collected from seven districts where information related to cultivars grown was gathered and later various studies aimed at determining genetic diversity were undertaken. Some of the observations highly relevant to sweetpotato improvement in Malawi and further recommendations follow:

7.1.1 Sweetpotato germplasm and associated indigenous knowledge

An understanding of farmers' cultivars and present diversification strategies is of fundamental importance to researchers who need to consider farmer preferences carefully when developing new cultivars. IK analysis revealed wide genetic diversity based on cultivar naming by farmers in ten different ethnic groups. Although the naming of cultivars was found to be imprecise and confusing within and between ethnic groups, their meanings however revealed important insights of sweetpotato attributes that attracts further science-based exploration in order to promote production while addressing the needs of the grower and consumer.

It was revealed that farmers grow a diverse of cultivars that are characterised by agronomic (yield, earliness to maturity, root size, root numbers *etc.*), post-harvest (shelf life), gastronomic (taste, texture, aroma) and market (earliness to maturity, shelf life and gastronomic) values and differences. Farmers grow the different cultivars in mixtures to satisfy their requirements as determined by the environmental and socio-economic differences. However an important revelation was that the number of attributes that form the selection basis are too many and therefore calls for the end user's participation as a way forward in the improvement program. In terms of cultivar variability, results showed that the aged farmers (over 40 years) who live in the most rural study areas were the custodians of sweetpotato genetic diversity. Such a situation poses a threat to sweetpotato diversity and therefore calls for immediate interventions. *Ex situ* conservation appears to be more realistic though expensive than *in situ* conservation due to the rapidly changing environmental and socio-economical conditions of Malawi

7.1.2 Morphological characterisation

In consistence with high ethno-botanical cultivar variability at farm level, studies on morphological traits also showed high variability within and between sources of origins. The results of the character distribution pattern permitted some broad generalisation about the collection and conservation of sweetpotato germplasm in Malawi. It was concluded that there is high phenotypic diversity among and within populations of different geographical origins for some traits and not for others. The Shire Valley population has the highest diversity and therefore suitable site for exploration and *in situ* conservation.

7.1.3 Genetic diversity analysis using SSR

A diversity analysis performed on 59 sweetpotato accessions grouped into three eco-geographical populations namely; the Northern, South Eastern and the Lower Shire Valley populations using ten SSR primers revealed high genetic diversity within and among populations. As in morphological analysis, Nei's genetic diversity was found to be highest in the Shire Valley population suggesting an area of most diversity. However, an analysis of genetic relationship among accessions revealed that the level of variation for SSR was higher than that of morphological traits.

Five most important primers namely; IB-297, IB-242, IB-S09, IB-324 and IB-CIPR 5 revealed high polymorphism levels and therefore high discrimination capacity in the characterisation of sweetpotato accessions. These primers are therefore recommended for SSR characterization of sweetpotato in Malawi. However the screening of more primers in order to identify a few loci with even higher discrimination capacity is suggested for cost effective while maintaining reliable results.

7.1.4 Performance of genotypes under different environments

A combined analysis of variance on yield across six localities (Bvumbwe, Makoka, Chitedze, Chitala, Lunyangwa and Baka) and three seasons (2004, 2005 and 2006) displayed highly significant differences ($p<0.001$) among six testing genotypes namely: LU96/374, LU96/303, LU96/220, LU96/274, LU96/334 and Mugande against two checks; Semusa and Kenya due to partly edaphic and climatic factors. Significant differences were also exhibited between localities and seasons and their first order interactions (genotype x season; genotype x location and season x location) and second order interaction (genotype x season x location). While the genotypes only accounted for less than a fifth, most of the variance was attributed to environment necessitating stability analysis. This suggests that in evaluating for yield, diversifying seasons and locations is recommended.

Stability analysis revealed that LU96/303 was the most stable genotype. Chitedze as an evaluation site revealed conducive conditions for yield evaluation more especially in preliminary stages where many entries are handled to exploit those with high yield potential. However, Lunyangwa should also be used as results will provide indicative performance potential in worst conditions. In the past, Bvumbwe and Lunyangwa were used for preliminary evaluations due to administrative siting of scientists. Yield potential might have been suppressed and therefore eliminating important and valuable genotypes. At advanced stages of evaluation, it has however been shown that Baka and Chitedze, and Bvumbwe and Makoka respectively are similar for yield evaluation. The economic implication is that use of one site between each of the two pairs is adequate. ANOVA also revealed that Semusa the check was the highest yielding cultivar. The test clones which yielded above average were LU96/303, LU96/220 and LU96/374. Although Mugande yielded slightly below average, the genotype however yielded above the low yielding check, Kenya and in addition, it also proved to be moderately stable than both checks. These genotypes are possible candidates for release assuming their performance is satisfactory in equally other important traits.

7.1.5 Genotypic variation for nutritional contents

Micronutrient-enriched staple plant foods provide a powerful intervention tool that target the most vulnerable resource-poor people as women and children. Development and promotion of plant foods with increased density of micronutrients and nutritional quality constitutes a sustainable approach to alleviating micronutrient malnutrition in developing countries. The current results have shown that the 15 genotypes under study can reliably be differentiated by β-carotene, ascorbic acid, iron, zinc and copper contents which entails the potential to breed for higher levels of the nutrients.

β-carotene analysis on 15 genotypes revealed that Zondeni a landrace has the highest content of the nutrient. Consumption of unpeeled storage roots of 5 months old amounting to 100 grams provides the RDA for all ages. Generally the genotypes exhibited increase in ascorbic acid content with age increase of the storage roots. Consumption of 100 to 500 grams of all study genotypes provides 50 to 100% of RDA depending on human age group and storage root age. The genotypes Mzungu, LU96/374 and Mfumu appear to be good sources of iron although consumptions of over 1000 grams are required to meet the RDA. All genotypes can adequately meet the zinc RDA when 100 grams of unpeeled roots are utilized at 4 and 5 MAP for all humans while the same amount can only provide adequate copper to infants and only at 4 MAP. Age of storage roots has different effects on the content of the different nutrients; while as most of the genotypes exhibited increasing trends for β-carotene and ascorbic acid contents, all the minerals decreased as the storage roots aged. Peeling has adverse effects on the contents of all nutrients. Use of unpeeled storage roots entails maximum exploitation of the nutrients. However, further research on nutrient retention as affected by peeling is required as this is a set back to industrialization of the crop since peeling alone means removal of more than 50% of the nutrients. A few genotypes exhibited characteristics to retain nutrients with age indicative of the presence of such traits with further screenings. The nutrient results obtained in this study will however need a compliment analysis on nutrient retention as affected by prcessing.

7.1.6 Selection of cultivars by farmers

It has been revealed in the preference studies that different genotypes have different characteristics which form the basis for its selection. No single genotype was identified to contain all the preferred organoleptic and agronomic attributes understudy. An important emerging issue in the current studies is that some local landraces have the potential to contribute considerably to the sweetpotato improvement program in Malawi by providing new varieties for selection as well as parent stocks for trait combinations.

7.2 Recommendations

In Malawi, the genetic diversity studies on sweetpotato as reported in this thesis is the first of its kind. Results on ethno-botanical, morphological, molecular and nutritional analyses have shown high diversity of sweetpotato germplasm in Malawi. The driving factors for the diversity are complex and in addition to production environment, variation in sweetpotato cultivars at farm level is attributed to consumer preference to satisfy various needs.

A recommendation from the issues arising is the need for developing appropriate research and development strategies that are based on farmer selection criteria. Farmers often manage a combination of cultivars and multiply or eliminate varieties as they evaluate their performance over time. The process of selection and maintenance exist since farmers' concerns are varied, and that a good performance with respect to certain concerns often implies poorer performance with respect to others, therefore, several varieties are maintained to satisfy the various concerns. As such, researchers can easy their work by combining attributes into different cultivars with the knowledge base that farmers will select, manage and maintain multiples of cultivars. Farmers' knowledge and skills in enhancing and creating diversity is important to local crop development. In order to maintain and improve genetic diversity, formal plant breeding and local crop development must be linked and built upon farmers' knowledge and their participation is therefore highly recommended.

Modern bio-technologies such as marker assisted selection (MAS), in combination with conventional breeding have been successfully used for genetic enhancement of crop species in other countries. The establishment of marker assisted selection protocols that will assist in the enhancement of sweetpotato research and development and consequently contributing to overall productivity is highly recommended in Malawi. Future research is needed to identify molecular markers linked to important traits and locating quantitative trait loci (QTL). Appropriate mapping populations must be developed for markers linked to traits, followed by screening of markers for usefulness in development of sweetpotato varieties with improved yield, resistance (pests and diseases) and adaptability to the environment.

Morphological analysis in the present study was done for two seasons; 2004 and 2005 under field conditions. The work on SSR data scoring took about one and half years, due to delays in importation of reagents. The theoretical advantages of marker assisted selection have been long recognized although the facilitating technology has been too expensive. Thus the major set back of using molecular markers in developing countries for characterisation of germplasm is the initial costs and sustainability due to poor maintenance and expensive laboratory consumables which have to be imported from overseas. Nevertheless, the impact of molecular breeding such as SSR offers to dramatically enhance the efficiency of plant breeding where genetic variability and how it can be efficiently manipulated are an important resource. Although both morphological and SSR analyses have set backs, their use should however complement each other. Morphological and SSR analyses showed different amounts of patterns of genetic diversity and different accessions were found to be distantly related in the different methods. Combining the two methods of genetic diversity could therefore help in acquiring more reliable genetic information for discriminating germplasm and cultivars.

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Appendix I Questionnaire for sweetpotato collection and cultivar identification at Bvumbwe

Name of farmer	**Sex**	**Age**	**Date**
Village	**T/A**	**District**	
Ethnic group	**Number of years in sweetpotato production**		
Crops grown in order of importance for :	**Food** **Cash**		

Variety name	**Cultivar identification process- characteristics**											
	Source of origin for the variety	Capacity to sprout after harvest	Grown in pure/ mix stand	Propor-tionate Area allocated to variety	Is the leaf edible	Months to root maturity	Yield capacity	Texture (Cooked)	Taste (cooked)	fibre content (cooked)	Flavour (cooked)	grown for food or sale

Check list

How much money did you get from sweetpotato in	2003	2002	2001	2000	1999

Proportionate land allocation to various crops
Which sweetpotato variety (ies) is/are on demand for sale and why?
Length of cultivars in storage facilities
Who does the cultivation for sweetpotato
Who does planting of sweetpotato, why?
When is planting done
Which variety(ies) can store underground after maturity without weevil damage and for how long?
Major forms of preparation
Major production problems
Major markets
Meanings of cultivar names
Cultivars that are no longer grown, why

Appendix II Landraces collected from seven Districts and introduced germplasm

CHITIPA

Dira
unknown 1
unknown 7
Kanonono 1
Kanono
Nyachusa
Chindere
Mwachusa 1
Kanonono 2
Mkhalabweka
Kasangani 1
Kavinthotwa
Kenya Mtuwa 1
Kenya Mtuwa 2
Kenya Mswesi
Mwambande
Kenya Mfipa
Yawaka
Kenya (mugamba)
Mbalizi
Chisungawalanda
Nachiwusu
Kenya Mswesi 2
Kenya mtuwa 3
Kenya yellow
Mwachusa 2
Mwenechipwera
Kasangani 2
Mwachusa Mchoko
Msungawapina
Chipapa
Lindali
Nasambo 2
Mwenemisuku
Chisungawalanda mtuwa
Chisungawalanda mswesi
Kenya
Nasambo
Chisungawapina
Mwachusa mchoko
Mwachusa mkulu
Mpeya
Vinthotwa
unknown (ID Kakoma)
Chisungawalanda

Kenya mswesi mkati
Kasangani 3
Mwachusa 3
unknown 2
Unknown 3
Unknown 4
Unknown 5
Unknown 6
Kenya
Tchuwa
Nachipapa
Nasambo 1
Kenya Mswesi kuwalo

KARONGA

Kasangani Mkulu
Bwetanyina
Mwakyusa
Yamasumbi
Kasangano Mchoko
Boma
Boma (Semusa)
John
Unknown 8
Kasangani
Mangochi
Karonga
Kenya (unknown 9)
Makanjira
Nyamulowe
Zambia
Mwanguku
Kenya (unknown 10)
Kasangani Mchoko mchoko
Pepu
Bwebwebwe
Muhungwa
Kanthongomala
Yamasumbi
Kasupuni
Bwetanyina mtuwa
Salima
Makanjira
Kacholola

MZIMBA

Sambalimodzi
Kamphunobi
Seven
Chilumba
Tchesama
Chaina
Makolo
Chabechabe
Makwakwa
Kautheka
Kachamba
Chilumba
Kacholola
Kamphunobi 2
John
Chililongwe
Unkown 11
Unkown 12
Zambia
Kamutabitabi
Nakakajoni
Bwatanyina
Kautheka 2
Yituba
Unkown 16
Katabitabi
Zambia mkulu
John 2
Chililongwe 2
Kauteka mchoko
Unknown 17
Katabitabi mutuba
Muswesi

NSANJE

Mozambique
Malawi
Demboka
Nyamatanga
Ngolezapaka 1
Thinda nchena 1
Folinda 1
Biliati
Gubu wa mkulu
Jipa Yekha
Phudzi la mfumu

Katabitabi muswesi
Kabula
Kadidimi
Machumi
Bwatanyina nfipa
Muswesi yamasumbi
Kasawaya
Daniel
Kautheka muswesi
Unkown 12
Unkown 13
Unkown 14
Unkown 15

NSANJE

Nyamanduwi
Thinda mzade
Ndabwera ndi nthumbo
China 2
Bangakhala
Panyo pa mzungu
Nyamajoya 1
Research
Dembuka 2
Madalena
Nyamadzoya
Nyamadzoya 2
Nkhodolede 1
Matako a nkazi Chimombo
Ndakwata banja ndi nzeru zanga
Folinda 2
Ntchayi
Nyamadzoya ya Mozambique
Unknown 27
Nkhodole dee 4
Unknown 30
Zimbabwe
Namakhoza
Folinda 3
Unknown 19
Demboke
Mugamba
Unkown 29
Njeru

Appendix II continued

NSANJE		CHIKWAWA	PHALOMBE	INTRODUCTIONS
Boma	Zambia	Phala la mwana	Kenya	Tainoni
Wakwata banja na nzeru zake	Semusa	Unkown 34	Kaulesi I	Kakoma
Gondolo	unknown 20	Unknown 35	Kaulesi II	LU96/296
Nkhodolede 3	Nkhodolede 2	Supuni	Sakana nthaka	1987-16-1
Koko	Unknown 18	Unknown 36	Nadzimbiri	Kemb 37
gubu wa ng'ono	Suga ntape	Baluwa	Nsanje	Naveto
Unknown 21	**CHIKWAWA**	Kenya	Nakajoni	Lunyangwa
Gubu	Mfumu	Unknown 37	**INTRODUCTIONS**	420240
Gubu wang'ono	Kenya	Unknown 38	Lu96/374	Salera
Limani	Babache	Chinese 4	Lu96/303	Kenya
Lopa	Mzungu	Unknown 42	Yanshu	Semusa
Jipe nunkhe	Chitute	Unknown 39	1990 – 4 - 2 .	Kandee
unknown 22	Mache	Unknown 40	Nemanete	
unknown 23	Dembuka 2	Mzungu 2	ST87-030	
unknown 24	Unknown 31	Namasupuni	BP-SP-2	
Kasungu	Nkhodole dee	Chinese 6	Natal Red	
Unkown 26	Chikhomeni	Unkown 42	Mafutha	
unknown 25	Unknwn 32	Folinda	Tresmesino	
Matakobalala	Unkown 33	koko	LU96/334	
Khomani	Thinda	**MULANJE**	A-45	
Nyamajoya 2	Thinda Mdzade	41	LU96/220	
Nzeru	Thinda mphiri	Vision	Salybro	
Unknown 26	Thinda 2	Makolo	Mugande	
Lilongwe	Mjipayekha 2	Zondeni	LU96/274	
nzeru zanga	Salera	**PHALOMBE**	Lu96/304	
makolo	Semusa	Lifebouy	BV95/25	
Unkown 29	Mugamba	Admarc	LU96/115	
Mugamba	Mfumu	Lingoni	Busbock	
Unknown 28	Chinese 3	sipiriti	LU96/158	
Namakhoza	Chinese 5	Makina	TIS 2498	
China 1				

Appendix III Accessions for SSR characterisation

Number	Ferruginous	Number	Lithosols	Number	Introductions
1	Chitsiru pa mchenga	27	Mbalizi	51	Mafutha
2	Chitute	28	Chisungabalanda	52	A45
3	Nadzimbiri	29	Katawitawi	53	Semusa
4	Molele	30	Mwakyusa	54	Mugamba
5	Zondeni	31	Msungabapina	55	Lunyangwa
6	Kamchiputu	32	Lindali	56	Kenya
7	Yoyera	33	mwambande	57	Tainoni
	Alluvial	34	Mwenemisuku	58	LU96/374
8	Njeru	35	Nasambo	59	LU96/303
9	Mfumu	36	Salima		
10	Babache	37	Zimbabwe		
11	Folinda	38	Kasangani		
12	Njipenunkhe	39	Nyamulowe		
13	Nyamajoya ya Malawi	40	Kanthongomala		
14	Nyamajoya Mozambique	41	Zganga mlima		
15	Sakananthaka	42	Pepu		
16	Njipayekha	43	Zambia		
17	Dembuka	44	Seven		
18	Mzungu	45	Tsambalimodzi		
19	Khomeni	46	Kasawaya		
20	Nyamandota	47	Zambezi		
21	Gondolo	48	Tchubatchuba		
22	Thindamphiri	49	Kadidimi		
23	Phalalamwana	50	Katawitawi mtuwa		
24	Chinese				
25	Thinda nchena				
26	Thinda mzade				

Appendix IV Genetic distances for SSR (below diagonal) and morphological (above diagonal) based on Dice similarity coefficients for 59 accessions

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1		0.45	0.51	0.36	0.46	0.46	0.45	0.38	0.39	0.39	0.29	0.41	0.35	0.35	0.43	0.43	0.42	0.32	0.32	0.36
2	0.53		0.53	0.43	0.39	0.46	0.45	0.39	0.45	0.45	0.44	0.39	0.43	0.43	0.47	0.42	0.46	0.45	0.45	0.46
3	0.69	0.66		0.53	0.53	0.51	0.52	0.52	0.51	0.52	0.49	0.50	0.52	0.49	0.51	0.46	0.51	0.48	0.52	0.51
4	0.63	0.45	0.69		0.47	0.49	0.48	0.39	0.47	0.42	0.39	0.42	0.38	0.45	0.43	0.46	0.46	0.41	0.42	0.36
5	0.57	0.66	0.63	0.69		0.39	0.45	0.41	0.32	0.43	0.46	0.32	0.43	0.41	0.47	0.42	0.47	0.43	0.41	0.46
6	0.63	0.72	0.57	0.75	0.63		0.45	0.46	0.42	0.48	0.44	0.41	0.46	0.45	0.46	0.39	0.43	0.42	0.42	0.47
7	0.63	0.53	0.63	0.49	0.57	0.75		0.42	0.46	0.45	0.40	0.42	0.45	0.41	0.45	0.42	0.41	0.42	0.42	0.42
8	0.85	0.72	0.63	0.69	0.69	0.57	0.69		0.41	0.39	0.34	0.36	0.39	0.35	0.48	0.43	0.42	0.38	0.38	0.33
9	0.66	0.63	0.45	0.66	0.60	0.66	0.66	0.77		0.43	0.43	0.39	0.38	0.36	0.46	0.45	0.43	0.41	0.38	0.45
10	0.66	0.80	0.60	0.72	0.66	0.66	0.60	0.66	0.69		0.36	0.38	0.38	0.36	0.43	0.43	0.43	0.33	0.39	0.36
11	0.60	0.69	0.60	0.77	0.60	0.53	0.77	0.72	0.57	0.75		0.39	0.34	0.32	0.4	0.41	0.390	0.310	0.340	0.320
12	0.63	0.77	0.57	0.85	0.63	0.57	0.75	0.75	0.60	0.53	0.60		0.38	0.30	0.46	0.46	0.430	0.360	0.390	0.390
13	0.66	0.57	0.72	0.72	0.66	0.60	0.60	0.60	0.75	0.69	0.63	0.60		0.32	0.45	0.42	0.390	0.360	0.260	0.300
14	0.63	0.66	0.57	0.63	0.63	0.75	0.49	0.75	0.66	0.53	0.66	0.57	0.63		0.45	0.46	0.360	0.280	0.330	0.360
15	0.72	0.63	0.53	0.53	0.60	0.72	0.45	0.66	0.49	0.57	0.69	0.66	0.66	0.72		0.42	0.350	0.390	0.410	0.460
16	0.72	0.75	0.53	0.72	0.66	0.66	0.72	0.66	0.63	0.63	0.63	0.60	0.49	0.69	0.66		0.420	0.410	0.390	0.420
17	0.66	0.75	0.72	0.82	0.60	0.60	0.72	0.66	0.75	0.69	0.69	0.60	0.72	0.35	0.69	0.53		0.33	0.36	0.41
18	0.60	0.57	0.60	0.53	0.60	0.60	0.45	0.72	0.57	0.63	0.63	0.72	0.60	0.77	0.75	0.53	0.63		0.33	0.38
19	0.63	0.72	0.75	0.80	0.63	0.63	0.63	0.63	0.77	0.53	0.60	0.57	0.77	0.72	0.57	0.77	0.75	0.69		0.33
20	0.60	0.69	0.53	0.66	0.60	0.72	0.60	0.72	0.63	0.63	0.63	0.60	0.45	0.72	0.69	0.53	0.49	0.63	0.75	
21	0.69	0.60	0.49	0.57	0.63	0.69	0.49	0.63	0.60	0.60	0.60	0.63	0.57	0.69	0.45	0.57	0.66	0.66	0.53	0.66
22	0.53	0.63	0.53	0.60	0.35	0.60	0.53	0.66	0.57	0.63	0.57	0.66	0.66	0.66	0.69	0.45	0.63	0.49	0.63	0.63
23	0.66	0.49	0.60	0.53	0.60	0.72	0.53	0.66	0.57	0.63	0.63	0.72	0.40	0.75	0.60	0.40	0.35	0.53	0.72	0.53
24	0.69	0.53	0.57	0.63	0.63	0.69	0.49	0.63	0.60	0.72	0.60	0.75	0.60	0.72	0.63	0.60	0.57	0.63	0.63	0.57
25	0.77	0.69	0.60	0.60	0.66	0.60	0.66	0.53	0.57	0.63	0.69	0.66	0.45	0.66	0.63	0.60	0.49	0.69	0.85	0.57
26	0.63	0.66	0.57	0.57	0.57	0.69	0.49	0.69	0.60	0.53	0.66	0.69	0.57	0.69	0.66	0.57	0.53	0.66	0.72	0.45
27	0.60	0.57	0.66	0.72	0.53	0.60	0.60	0.72	0.63	0.75	0.63	0.66	0.60	0.66	0.69	0.72	0.49	0.75	0.69	0.57
28	0.77	0.63	0.53	0.66	0.60	0.77	0.60	0.66	0.57	0.69	0.57	0.72	0.49	0.63	0.77	0.49	0.53	0.66	0.77	0.45
29	0.75	0.66	0.69	0.63	0.49	0.75	0.57	0.57	0.60	0.60	0.72	0.69	0.60	0.72	0.57	0.66	0.63	0.69	0.57	0.57
30	0.72	0.69	0.60	0.72	0.72	0.60	0.66	0.66	0.57	0.63	0.57	0.66	0.60	0.77	0.69	0.53	0.57	0.57	0.75	0.63
31	0.63	0.60	0.57	0.57	0.69	0.69	0.57	0.75	0.53	0.53	0.66	0.63	0.63	0.69	0.60	0.69	0.53	0.72	0.66	0.66
32	0.63	0.72	0.63	0.69	0.63	0.63	0.69	0.69	0.66	0.66	0.53	0.63	0.60	0.72	0.63	0.72	0.63	0.75	0.69	0.63
33	0.69	0.60	0.63	0.63	0.69	0.69	0.63	0.63	0.66	0.72	0.60	0.75	0.49	0.75	0.72	0.49	0.53	0.60	**0.87**	0.53
34	0.66	0.75	0.66	0.77	0.53	0.60	0.60	0.66	0.63	0.63	0.69	0.66	0.57	0.57	0.60	0.63	0.66	0.60	0.66	0.66
35	0.69	0.60	0.57	0.69	0.57	0.63	0.63	0.63	0.60	0.66	0.60	0.63	0.63	0.63	0.66	0.63	0.66	0.77	0.72	0.60
36	0.60	0.63	0.45	0.66	0.60	0.66	0.66	0.72	0.49	0.63	0.63	0.60	0.66	0.72	0.57	0.77	0.63	0.75	0.49	0.69
37	0.66	0.63	0.53	0.66	0.53	0.66	0.45	0.60	0.63	0.57	0.69	0.60	0.49	0.69	0.66	0.63	0.53	0.72	0.72	0.66
38	0.63	0.60	0.69	0.63	0.63	0.69	0.57	0.75	0.60	0.72	0.60	0.75	0.66	0.72	0.75	0.60	0.63	0.57	0.63	0.63
39	0.69	0.77	0.69	0.80	0.63	0.63	0.69	0.63	0.82	0.66	0.66	0.69	0.53	0.66	0.57	0.53	0.49	0.57	0.63	0.63
40	0.63	0.66	0.63	0.57	0.57	0.69	0.49	0.75	0.66	0.60	0.77	0.69	0.63	0.63	0.72	0.63	0.66	0.77	0.82	0.53
41	0.57	0.66	0.69	0.75	0.63	0.63	0.69	0.69	0.66	0.72	0.45	0.63	0.75	0.69	0.72	0.69	0.77	0.60	0.60	0.66
42	0.69	0.60	0.63	0.69	0.75	0.63	0.57	0.63	0.66	0.66	0.72	0.69	0.57	0.63	0.77	0.57	0.53	0.66	0.66	0.45
43	0.60	0.69	0.60	0.66	0.60	0.60	0.53	0.72	0.57	0.57	0.57	0.60	0.63	0.75	0.60	0.57	0.72	0.66	0.72	0.60
44	0.66	0.63	0.60	0.66	0.53	0.60	0.53	0.60	0.57	0.57	0.57	0.66	0.63	0.63	0.60	0.69	0.66	0.72	0.60	0.60
45	0.57	0.66	0.63	0.75	0.63	0.63	0.63	0.69	0.66	0.66	0.53	0.63	0.35	0.60	0.63	0.53	0.49	0.63	0.69	0.49
46	0.69	0.72	0.63	0.69	0.63	0.57	0.57	0.63	0.66	0.53	0.72	0.63	0.45	0.66	0.63	0.60	0.49	0.63	0.69	0.40
47	0.57	0.60	0.63	0.57	0.57	0.63	0.57	0.63	0.66	0.53	0.60	0.63	0.63	0.69	0.66	0.57	0.66	0.66	0.77	0.53
48	0.69	0.72	0.49	0.75	0.57	0.63	0.75	0.69	0.53	0.60	0.53	0.49	0.57	0.75	0.66	0.63	0.60	0.60	0.66	0.45
49	0.60	0.57	0.60	0.60	0.66	0.66	0.60	0.66	0.57	0.63	0.63	0.66	0.49	0.69	0.66	0.49	0.53	0.60	0.77	0.53
50	0.63	0.66	0.49	0.69	0.57	0.49	0.63	0.63	0.66	0.60	0.53	0.63	0.63	0.80	0.72	0.57	0.60	0.60	0.72	0.66

51	0.69	0.72	0.69	0.69	0.57	0.57	0.69	0.57	0.60	0.66	0.60	0.63	0.66	0.66	0.63	0.66	0.63	0.80	0.75	0.57
52	0.63	0.72	0.49	0.69	0.63	0.69	0.57	0.80	0.53	0.53	0.72	0.49	0.57	0.69	0.66	0.63	0.60	0.66	0.72	0.53
53	0.66	0.63	0.53	0.66	0.60	0.72	0.72	0.72	0.57	0.75	0.63	0.60	0.63	0.75	0.60	0.69	0.60	0.72	0.60	0.66
54	0.57	0.60	0.63	0.57	0.63	0.69	0.49	0.63	0.77	0.60	0.77	0.69	0.57	0.63	0.77	0.49	0.53	0.66	0.77	0.60
55	0.66	0.69	0.60	0.66	0.66	0.72	0.72	0.66	0.69	0.63	0.57	0.72	0.72	0.72	0.69	0.66	0.63	0.63	0.63	0.75
56	0.72	0.63	0.45	0.53	0.66	0.72	0.45	0.66	0.49	0.57	0.69	0.72	0.63	0.63	0.66	0.57	0.60	0.66	0.66	0.60
57	0.60	0.75	0.66	0.77	0.60	0.66	0.60	0.72	0.63	0.49	0.63	0.60	0.66	0.72	0.75	0.66	0.63	0.63	0.63	0.69
58	0.63	0.66	0.49	0.69	0.57	0.49	0.63	0.63	0.66	0.60	0.53	0.63	0.45	0.72	0.75	0.45	0.40	0.57	0.80	0.40
59	0.69	0.72	0.69	0.69	0.57	0.57	0.69	0.57	0.60	0.66	0.60	0.63	0.53	0.60	0.63	0.60	0.63	0.69	0.63	0.63

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
1	0.40	0.35	0.39	0.42	0.39	0.36	0.41	0.45	0.32	0.32	0.42	0.29	0.41	0.36	0.32	0.35	0.32	0.32	0.39	0.36
2	0.44	0.42	0.43	0.42	0.45	0.46	0.45	0.36	0.45	0.44	0.39	0.40	0.38	0.41	0.46	0.43	0.44	0.42	0.46	0.41
3	0.55	0.49	0.50	0.55	0.52	0.52	0.54	0.52	0.53	0.43	0.52	0.45	0.50	0.48	0.45	0.49	0.50	0.52	0.50	0.51
4	0.36	0.38	0.45	0.57	0.39	0.40	0.46	0.41	0.36	0.40	0.43	0.37	0.43	0.38	0.42	0.38	0.39	0.38	0.38	0.38
5	0.50	0.43	0.46	0.49	0.46	0.45	0.46	0.43	0.48	0.44	0.47	0.44	0.46	0.46	0.45	0.47	0.44	0.47	0.45	0.43
6	0.49	0.43	0.38	0.48	0.46	0.44	0.47	0.43	0.46	0.44	0.46	0.44	0.45	0.45	0.45	0.46	0.45	0.47	0.49	0.48
7	0.44	0.43	0.39	0.43	0.45	0.49	0.42	0.45	0.41	0.43	0.43	0.41	0.45	0.39	0.45	0.36	0.41	0.43	0.46	0.41
8	0.43	0.41	0.42	0.42	0.42	0.43	0.45	0.41	0.39	0.37	0.35	0.36	0.41	0.35	0.39	0.36	0.37	0.33	0.41	0.35
9	0.48	0.41	0.47	0.52	0.47	0.39	0.43	0.41	0.45	0.41	0.45	0.37	0.45	0.42	0.39	0.45	0.44	0.46	0.42	0.38
10	0.40	0.36	0.45	0.43	0.43	0.40	0.38	0.38	0.38	0.36	0.41	0.36	0.41	0.38	0.38	0.36	0.32	0.38	0.32	0.32
11	0.38	0.32	0.36	0.40	0.40	0.39	0.39	0.40	0.32	0.32	0.39	0.30	0.37	0.34	0.31	0.32	0.28	0.29	0.39	0.32
12	0.44	0.39	0.43	0.45	0.41	0.41	0.42	0.38	0.45	0.37	0.43	0.39	0.38	0.39	0.41	0.39	0.39	0.39	0.35	0.35
13	0.37	0.33	0.41	0.47	0.38	0.31	0.39	0.36	0.33	0.31	0.39	0.31	0.36	0.33	0.32	0.35	0.32	0.33	0.39	0.33
14	0.43	0.38	0.42	0.46	0.42	0.37	0.41	0.39	0.42	0.34	0.41	0.34	0.38	0.36	0.36	0.36	0.36	0.36	0.38	0.33
15	0.46	0.41	0.45	0.46	0.45	0.45	0.41	0.39	0.43	0.40	0.47	0.44	0.46	0.46	0.41	0.45	0.43	0.42	0.43	0.41
16	0.48	0.38	0.38	0.45	0.41	0.45	0.47	0.39	0.43	0.39	0.45	0.39	0.42	0.39	0.41	0.45	0.43	0.41	0.48	0.43
17	0.45	0.38	0.43	0.41	0.45	0.40	0.38	0.38	0.43	0.37	0.39	0.41	0.43	0.41	0.36	0.42	0.39	0.41	0.42	0.39
18	0.40	0.30	0.41	0.43	0.39	0.36	0.38	0.41	0.36	0.31	0.36	0.32	0.38	0.36	0.32	0.33	0.31	0.38	0.38	0.33
19	0.41	0.32	0.39	0.43	0.36	0.34	0.36	0.39	0.33	0.29	0.41	0.31	0.39	0.35	0.24	0.36	0.32	0.35	0.42	0.36
20	0.39	0.33	0.38	0.42	0.38	0.39	0.41	0.41	0.32	0.39	0.39	0.37	0.41	0.32	0.38	0.36	0.34	0.36	0.39	0.35
21		0.40	0.40	0.40	0.41	0.38	0.36	0.40	0.31	0.38	0.36	0.36	0.37	0.37	0.39	0.31	0.35	0.34	0.37	0.36
22	0.66		0.39	0.41	0.36	0.32	0.39	0.38	0.33	0.32	0.38	0.29	0.39	0.28	0.30	0.33	0.32	0.38	0.38	0.35
23	0.57	0.53		0.42	0.39	0.45	0.43	0.41	0.41	0.40	0.36	0.40	0.36	0.36	0.39	0.39	0.34	0.38	0.45	0.39
24	0.66	0.49	0.53		0.39	0.46	0.39	0.42	0.39	0.43	0.38	0.40	0.43	0.38	0.41	0.42	0.40	0.41	0.45	0.41
25	0.66	0.75	0.53	0.63		0.39	0.42	0.43	0.35	0.39	0.41	0.36	0.41	0.33	0.38	0.39	0.39	0.41	0.43	0.42
26	0.63	0.60	0.49	0.60	0.60		0.39	0.40	0.36	0.33	0.39	0.32	0.41	0.32	0.32	0.37	0.38	0.37	0.36	0.37
27	0.72	0.69	0.60	0.63	0.63	0.60		0.39	0.35	0.37	0.36	0.39	0.36	0.42	0.35	0.38	0.39	0.39	0.33	0.36
28	0.69	0.60	0.57	0.53	0.53	0.57	0.60		0.42	0.36	0.39	0.37	0.32	0.38	0.38	0.41	0.40	0.36	0.36	0.32
29	0.60	0.69	0.66	0.63	0.63	0.60	0.69	0.66		0.39	0.36	0.31	0.41	0.33	0.33	0.26	0.34	0.35	0.41	0.38
30	0.66	0.57	0.45	0.57	0.57	0.45	0.69	0.60	0.69		0.37	0.26	0.36	0.36	0.22	0.29	0.32	0.25	0.37	0.36
31	0.63	0.66	0.63	0.53	0.53	0.63	0.53	0.57	0.66	0.60		0.36	0.32	0.32	0.38	0.33	0.34	0.39	0.38	0.35
32	0.66	0.75	0.66	0.63	0.63	0.60	0.63	0.53	0.63	0.63	0.60		0.37	0.27	0.27	0.31	0.33	0.31	0.37	0.31
33	0.69	0.66	0.57	0.66	0.45	0.63	0.72	0.49	0.72	0.60	0.63	0.60		0.38	0.38	0.35	0.34	0.38	0.36	0.33
34	0.63	0.53	0.63	0.60	0.60	0.75	0.66	0.63	0.60	0.72	0.63	0.66	0.69		0.35	0.33	0.32	0.35	0.38	0.33
35	0.69	0.66	0.63	0.60	0.66	0.49	0.60	0.49	0.66	0.60	0.63	0.45	0.69	0.69		0.33	0.29	0.33	0.38	0.33
36	0.60	0.69	0.72	0.57	0.69	0.72	0.69	0.72	0.57	0.75	0.53	0.63	0.72	0.60	0.77		0.27	0.33	0.35	0.33
37	0.63	0.77	0.57	0.66	0.45	0.63	0.66	0.57	0.53	0.60	0.63	0.60	0.57	0.69	0.63	0.66		0.32	0.34	0.29
38	0.72	0.49	0.60	0.49	0.69	0.60	0.63	0.53	0.63	0.57	0.66	0.57	0.66	0.66	0.53	0.75	0.66		0.38	0.36
39	0.53	0.57	0.45	0.49	0.57	0.53	0.69	0.60	0.63	0.57	0.53	0.63	0.60	0.66	0.66	0.57	0.60	0.57		0.30
40	0.69	0.72	0.69	0.66	0.53	0.49	0.66	0.49	0.60	0.60	0.63	0.53	0.57	0.69	0.57	0.72	0.63	0.66	0.66	
41	0.63	0.66	0.69	0.66	0.77	0.63	0.77	0.69	0.77	0.66	0.75	0.77	0.75	0.75	0.75	0.66	0.75	0.77	0.66	0.75
42	0.75	0.60	0.63	0.60	0.66	0.63	0.60	0.40	0.53	0.72	0.63	0.66	0.63	0.63	0.63	0.66	0.63	0.60	0.66	0.63
43	0.57	0.60	0.63	0.60	0.66	0.63	0.72	0.57	0.60	0.66	0.69	0.60	0.63	0.57	0.57	0.72	0.63	0.66	0.72	0.57
44	0.63	0.66	0.69	0.72	0.66	0.57	0.66	0.57	0.53	0.72	0.63	0.45	0.63	0.63	0.57	0.60	0.63	0.60	0.60	0.57

45	0.53	0.63	0.53	0.57	0.57	0.60	0.63	0.53	0.57	0.69	0.66	0.57	0.60	0.53	0.60	0.63	0.60	0.57	0.49	0.60
46	0.53	0.63	0.53	0.57	0.49	0.45	0.49	0.53	0.57	0.57	0.53	0.63	0.60	0.60	0.66	0.63	0.60	0.63	0.57	0.53
47	0.63	0.60	0.63	0.60	0.66	0.49	0.66	0.57	0.60	0.66	0.69	0.60	0.63	0.63	0.57	0.72	0.69	0.66	0.66	0.49
48	0.63	0.72	0.63	0.60	0.66	0.63	0.60	0.49	0.60	0.72	0.63	0.53	0.63	0.69	0.63	0.66	0.69	0.60	0.60	0.63
49	0.57	0.60	0.49	0.53	0.53	0.57	0.66	0.49	0.72	0.60	0.57	0.66	0.40	0.69	0.63	0.72	0.57	0.66	0.53	0.63
50	0.69	0.60	0.57	0.60	0.60	0.69	0.60	0.57	0.66	0.53	0.63	0.66	0.57	0.63	0.69	0.72	0.49	0.60	0.72	0.69
51	0.72	0.63	0.66	0.57	0.57	0.60	0.49	0.45	0.69	0.69	0.53	0.49	0.60	0.60	0.45	0.69	0.66	0.57	0.69	0.53
52	0.69	0.66	0.57	0.53	0.60	0.57	0.60	0.57	0.60	0.60	0.69	0.60	0.63	0.63	0.63	0.66	0.57	0.66	0.66	0.63
53	0.57	0.66	0.63	0.53	0.72	0.63	0.53	0.63	0.60	0.66	0.49	0.53	0.69	0.69	0.57	0.60	0.63	0.60	0.60	0.63
54	0.75	0.60	0.63	0.66	0.60	0.69	0.66	0.49	0.66	0.72	0.69	0.66	0.49	0.63	0.69	0.66	0.57	0.60	0.60	0.63
55	0.77	0.57	0.60	0.57	0.63	0.72	0.63	0.60	0.63	0.63	0.60	0.63	0.72	0.53	0.60	0.69	0.60	0.49	0.63	0.72
56	0.69	0.53	0.57	0.53	0.72	0.63	0.66	0.57	0.66	0.72	0.69	0.72	0.69	0.69	0.63	0.72	0.69	0.60	0.60	0.75
57	0.66	0.57	0.53	0.57	0.69	0.60	0.63	0.60	0.75	0.49	0.66	0.63	0.72	0.66	0.60	0.75	0.66	0.57	0.69	0.72
58	0.72	0.49	0.45	0.57	0.57	0.45	0.57	0.45	0.69	0.49	0.60	0.63	0.45	0.66	0.60	0.69	0.66	0.57	0.57	0.60
59	0.45	0.63	0.66	0.63	0.63	0.66	0.69	0.66	0.63	0.69	0.66	0.69	0.66	0.60	0.72	0.57	0.66	0.63	0.57	0.66

1	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**
2	0.41	0.43	0.32	0.38	0.41	0.36	0.47	0.42	0.41	0.42	0.33	0.35	0.32	0.35	0.38	0.38	0.35	0.38	0.38
3	0.43	0.39	0.42	0.42	0.46	0.47	0.41	0.39	0.35	0.39	0.41	0.43	0.41	0.43	0.42	0.46	0.42	0.43	0.46
4	0.51	0.52	0.50	0.45	0.51	0.53	0.52	0.52	0.55	0.52	0.51	0.54	0.53	0.50	0.48	0.54	0.49	0.49	0.50
5	0.42	0.39	0.39	0.36	0.42	0.39	0.49	0.45	0.43	0.39	0.35	0.38	0.39	0.42	0.41	0.45	0.38	0.46	0.42
6	0.48	0.46	0.47	0.42	0.39	0.47	0.43	0.42	0.43	0.42	0.45	0.46	0.48	0.45	0.43	0.47	0.43	0.47	0.42
7	0.41	0.48	0.46	0.43	0.42	0.47	0.41	0.45	0.41	0.43	0.45	0.48	0.40	0.47	0.46	0.47	0.45	0.46	0.46
8	0.42	0.43	0.41	0.42	0.45	0.43	0.41	0.43	0.42	0.45	0.41	0.48	0.45	0.41	0.45	0.45	0.45	0.43	0.42
9	0.36	0.42	0.36	0.36	0.42	0.42	0.47	0.38	0.38	0.41	0.38	0.41	0.40	0.41	0.39	0.41	0.39	0.38	0.35
10	0.49	0.45	0.42	0.41	0.35	0.46	0.46	0.43	0.47	0.45	0.45	0.43	0.45	0.42	0.42	0.46	0.42	0.46	0.42
11	0.41	0.45	0.42	0.36	0.36	0.41	0.45	0.36	0.43	0.35	0.36	0.39	0.43	0.41	0.46	0.43	0.38	0.43	0.43
12	0.32	0.43	0.29	0.34	0.37	0.37	0.44	0.37	0.39	0.34	0.29	0.36	0.30	0.34	0.43	0.39	0.32	0.39	0.40
13	0.42	0.42	0.42	0.32	0.36	0.41	0.45	0.39	0.43	0.35	0.39	0.41	0.44	0.43	0.36	0.45	0.42	0.45	0.38
14	0.41	0.45	0.33	0.36	0.38	0.39	0.45	0.36	0.41	0.36	0.32	0.30	0.34	0.41	0.39	0.43	0.39	0.43	0.43
15	0.39	0.46	0.38	0.36	0.33	0.39	0.45	0.36	0.42	0.35	0.36	0.39	0.40	0.41	0.36	0.42	0.39	0.39	0.36
16	0.43	0.45	0.43	0.46	0.46	0.46	0.38	0.47	0.46	0.42	0.42	0.42	0.39	0.41	0.48	0.43	0.42	0.45	0.43
17	0.41	0.46	0.41	0.39	0.42	0.49	0.33	0.38	0.38	0.45	0.38	0.41	0.39	0.43	0.45	0.46	0.39	0.43	0.48
18	0.41	0.48	0.41	0.42	0.45	0.45	0.42	0.42	0.41	0.39	0.42	0.43	0.37	0.42	0.42	0.39	0.43	0.39	0.45
19	0.36	0.46	0.38	0.36	0.33	0.36	0.42	0.36	0.39	0.33	0.35	0.41	0.40	0.35	0.36	0.35	0.36	0.33	0.41
20	0.39	0.45	0.30	0.38	0.41	0.43	0.41	0.38	0.39	0.41	0.35	0.35	0.34	0.39	0.41	0.42	0.35	0.39	0.45
21	0.41	0.46	0.38	0.33	0.43	0.41	0.46	0.41	0.42	0.41	0.36	0.39	0.37	0.41	0.42	0.42	0.39	0.42	0.39
22	0.37	0.41	0.36	0.40	0.43	0.27	0.49	0.43	0.44	0.39	0.34	0.34	0.39	0.40	0.44	0.39	0.40	0.37	0.45
23	0.41	0.43	0.33	0.36	0.38	0.43	0.41	0.36	0.41	0.35	0.33	0.35	0.37	0.38	0.43	0.43	0.32	0.42	0.47
24	0.36	0.47	0.36	0.39	0.41	0.42	0.39	0.42	0.36	0.41	0.36	0.42	0.39	0.41	0.45	0.43	0.41	0.41	0.45
25	0.39	0.45	0.38	0.45	0.49	0.45	0.45	0.46	0.43	0.43	0.42	0.42	0.41	0.42	0.43	0.41	0.42	0.39	0.45
26	0.41	0.41	0.38	0.42	0.45	0.45	0.43	0.43	0.42	0.39	0.41	0.36	0.40	0.45	0.39	0.42	0.39	0.42	0.46
27	0.43	0.43	0.36	0.40	0.40	0.37	0.46	0.39	0.44	0.40	0.39	0.34	0.38	0.43	0.41	0.43	0.41	0.43	0.45
28	0.41	0.41	0.38	0.43	0.46	0.39	0.42	0.45	0.43	0.42	0.43	0.39	0.43	0.41	0.47	0.41	0.42	0.39	0.45
29	0.39	0.41	0.38	0.33	0.38	0.43	0.35	0.38	0.38	0.35	0.36	0.36	0.37	0.45	0.46	0.46	0.41	0.43	0.47
30	0.38	0.38	0.33	0.39	0.43	0.33	0.45	0.43	0.41	0.38	0.36	0.35	0.34	0.38	0.43	0.33	0.38	0.35	0.43
31	0.34	0.41	0.29	0.36	0.37	0.37	0.41	0.36	0.40	0.34	0.31	0.29	0.33	0.40	0.40	0.40	0.39	0.37	0.43
32	0.33	0.43	0.36	0.41	0.43	0.39	0.45	0.41	0.38	0.36	0.41	0.41	0.44	0.41	0.39	0.33	0.43	0.32	0.45
33	0.37	0.36	0.27	0.37	0.36	0.40	0.43	0.36	0.39	0.36	0.29	0.29	0.33	0.36	0.39	0.41	0.34	0.36	0.41
34	0.36	0.45	0.38	0.33	0.39	0.39	0.43	0.41	0.39	0.36	0.38	0.39	0.41	0.42	0.42	0.43	0.39	0.42	0.46
35	0.35	0.39	0.30	0.36	0.39	0.41	0.42	0.35	0.39	0.38	0.30	0.35	0.37	0.39	0.38	0.41	0.33	0.38	0.39
36	0.38	0.43	0.26	0.38	0.41	0.41	0.42	0.39	0.41	0.39	0.35	0.33	0.34	0.35	0.42	0.39	0.35	0.35	0.46
37	0.36	0.39	0.32	0.36	0.41	0.30	0.46	0.42	0.41	0.33	0.35	0.38	0.39	0.38	0.41	0.36	0.39	0.36	0.42
38	0.39	0.45	0.32	0.37	0.39	0.34	0.45	0.39	0.39	0.32	0.31	0.37	0.35	0.32	0.41	0.37	0.34	0.39	0.43
39	0.33	0.39	0.28	0.38	0.41	0.33	0.45	0.35	0.38	0.38	0.26	0.24	0.27	0.41	0.43	0.41	0.39	0.39	0.38

40	0.42	0.39	0.41	0.35	0.39	0.38	0.45	0.41	0.46	0.36	0.41	0.38	0.44	0.41	0.42	0.43	0.41	0.43	0.42
41	0.41	0.41	0.33	0.33	0.36	0.38	0.43	0.39	0.42	0.36	0.35	0.38	0.40	0.33	0.41	0.42	0.36	0.41	0.43
42		0.43	0.32	0.39	0.42	0.38	0.42	0.38	0.38	0.36	0.32	0.38	0.39	0.43	0.43	0.38	0.39	0.33	0.41
43	0.69		0.41	0.43	0.45	0.41	0.43	0.5	0.42	0.43	0.42	0.39	0.43	0.48	0.45	0.43	0.45	0.42	0.45
44	0.69	0.69		0.38	0.43	0.38	0.42	0.38	0.39	0.41	0.28	0.32	0.32	0.36	0.42	0.38	0.35	0.36	0.42
45	0.75	0.57	0.63		0.35	0.38	0.45	0.38	0.43	0.38	0.36	0.41	0.41	0.42	0.36	0.42	0.36	0.43	0.41
46	0.77	0.60	0.60	0.60		0.39	0.45	0.38	0.45	0.36	0.35	0.41	0.44	0.42	0.43	0.46	0.41	0.46	0.43
47	0.66	0.60	0.60	0.60	0.49		0.50	0.43	0.45	0.39	0.35	0.38	0.40	0.42	0.42	0.33	0.45	0.36	0.39
48	0.63	0.63	0.40	0.63	0.60	0.53		0.38	0.35	0.42	0.42	0.45	0.40	0.43	0.46	0.46	0.42	0.43	0.47
49	0.63	0.49	0.63	0.57	0.53	0.53	0.57		0.32	0.38	0.30	0.36	0.39	0.42	0.41	0.43	0.38	0.41	0.43
50	0.63	0.63	0.57	0.69	0.60	0.53	0.57	0.63		0.39	0.36	0.42	0.36	0.39	0.43	0.42	0.39	0.38	0.42
51	0.69	0.63	0.57	0.75	0.72	0.60	0.63	0.69	0.57		0.36	0.36	0.39	0.41	0.42	0.41	0.39	0.42	0.45
52	0.77	0.60	0.53	0.53	0.63	0.57	0.53	0.60	0.60	0.60		0.30	0.31	0.39	0.43	0.42	0.33	0.39	0.41
53	0.57	0.57	0.63	0.69	0.66	0.53	0.49	0.57	0.57	0.49	0.60		0.31	0.42	0.43	0.43	0.39	0.41	0.45
54	0.80	0.69	0.57	0.63	0.60	0.60	0.63	0.63	0.57	0.63	0.60	0.69		0.40	0.44	0.41	0.37	0.40	0.43
55	0.75	0.49	0.69	0.63	0.53	0.66	0.63	0.63	0.63	0.57	0.60	0.63	0.75		0.42	0.41	0.36	0.41	0.38
56	0.77	0.60	0.66	0.66	0.69	0.75	0.72	0.72	0.72	0.53	0.57	0.66	0.66	0.60		0.38	0.42	0.39	0.38
57	0.63	0.49	0.63	0.63	0.66	0.66	0.57	0.57	0.57	0.69	0.60	0.57	0.69	0.63	0.66		0.45	0.28	0.41
58	0.60	0.66	0.66	0.72	0.75	0.63	0.66	0.72	0.60	0.53	0.63	0.53	0.66	0.77	0.57	0.60		0.43	0.43
59	0.72	0.53	0.66	0.60	0.57	0.49	0.60	0.60	0.53	0.60	0.57	0.60	0.66	0.53	0.69	0.60	0.63		0.42

A CONSOLIDATED ANALYSIS OF FARMERS' SWEETPOTATO CULTIVARS IN MALAWI

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Abstract

In order to better understand farmers' sweetpotato varieties grown in Malawi for subsequent improvement and effective contribution of agricultural research to livelihood, a survey involving germplasm collection and indigenous knowledge documentation was conducted in 2003 in the Shire Valley Districts where vitamin A deficiency is prevalent. A total of 115 accessions were collected and planted at Bvumbwe Research Station in 2004 and 2005 seasons for morphological characterisation. A preference study by naive panelists was conducted at Bvumbwe Research Station to identify organoleptic attributes considered valuable by farmers on storage roots of 15 genotypes. Among other attributes, these 15 genotypes were selected to represent a wider range of flesh colour and those prevalently grown in Malawi and the Shire Valley. Beta-carotene determination on the 15 genotypes was conducted where Babache and Mfumu, white fleshed and the most grown cultivars in the Shire Valley Districts registered low levels (0.19 and 0.76 mg/100g fresh weight respectively). Morphological analysis indicated that Nsanje District has 4% pale orange cultivars, 1% intermediate orange and 1% dark orange, Chikwawa District has none at all in these character states of flesh colour. Using a preference score scale of 1-5, different cultivars were preferred most for texture, taste, flavour and flesh colour. Zondeni an orange fleshed colour and a local cultivar which registered the highest beta-carotene content (13.93mg/100g fresh weight) was the most preferred for colour at five months

old suggesting that colour may not be a barrier to promoting orange fleshed cultivars in the communities where these cultivars are less popular. However, since it appears to be a challenge to develop and identify an ideal genotype that contain all of the preferred traits, researchers can easy their work, by combining attributes into different genotypes with the knowledge base that farmers will select, manage and maintain multiples of cultivars.

An abstract of a paper presented at the International Society for Tropical Root Crops-Africa Branch (ISTRC-AB) Symposium at Joachim Chissano Conference Center, Maputo, Mozambique (8th to 12th October 2007)

SWEETPOTATO CULTIVAR MIXTURE BY FARMERS IN MALAWI: THE BASIS FOR LONGER HOUSEHOLD FOOD SECURITY

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Abstract

In Malawi, sweetpotato (*Ipomea batatas*) is the second important root crop after cassava (*Manihot esculenta* Crantz) and the most spread crop grown throughout the country for food and cash. At policy level, sweetpotato production is being encouraged as it is an important food security crop in times of maize failure and periods in between maize harvests. Documentation of indigenous knowledge of traditional varieties of crops is a valuable complement in the collection, maintenance, and evaluation of diversity representatives as working collections with purposes of improving traits. In order to collect and document the amount of cultivar diversity in Malawi and the traditional knowledge associated with the crop, a survey was conducted in 2003 in seven districts of the country, namely Chitipa, Karonga, Mzimba, Nsanje, Chikwawa, Mulanje and Phalombe In total, 268 landraces were collected. These accessions along with introductions were subjected to morphological characterisation at Bvumbwe Research Station where pre-selection representatives from the morphological studies were subjected to molecular and nutritional studies at Chancellor College, the University of Malawi. There was a marked difference in the maintenance of a variety of cultivars by gender, age, income group and proximity and access to urban centers. Farmers with low incomes, women and older people of above 50 years grow the most varied sweetpotato cultivars with the main purpose of reducing risks of food insecurity. Sweetpotato cultivar diversity in terms of maturity period, shelf life after harvest and resistance to sweetpotato weevil and mixtures enables household food security

over longer periods and farmers to sustain production to make use of different agro-ecological zones that significantly vary in terms of soils, topography, altitude, slope, water and fertility. However, in some areas of study areas which have direct access to urban markets, farmers concentrate on one high yielding and early maturing cultivar, Kenya whose roots are meant for sale.

An abstract of a paper presented at the 6th Symposium of the Southern African Plant Breeders' Association at Club Mykonos, Langebaan (RSA 13th to 15th March, 2006).

CONSUMER SELECTION OF SWEETPOTATO CULTIVAR BY TRAINED AND NAÏVE PANELS IN MALAWI

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Abstract

Fresh roots of sweetpotato of fourteen cultivars (comprising seven landraces, five introductions and three locally bred lines) were evaluated for organoleptic characteristics (texture, taste, colour and aroma) by trained (8 females and 4 males) and naïve (7 females and 5 males) panelists. At 5MAP, significant preferential differences were observed among cultivars for texture ($p \leq 0.05$), taste, flesh colour and aroma ($p \leq 0.001$) both panelists. Yoyera was highly preferred by both panels for texture and aroma. While Zondeni and Babache were the most preferred and most liked cultivars for taste by the trained panel, Yoyera was most preferred by the naïve panel. The least preferred cultivars for taste (scores ≥ 3.00) were LU/303, Tainoni, A45 and Pepu. A high and significant correlation on texture existed between trained and naïve panels ($r=0.62$: ($p \leq 0.05$) at 5 MAP.

An abstract of a paper presented at the International Conference of the Association of Professional Chemists and Chemical Engineers of Malawi at National Bank College, Blantyre, Malawi (19th to 23rd June 2006).

VITAMIN A CONTENT OF SWEETPOTATO CULTIVARS GROWING IN MALAWI

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Abstract

Roots of 14 sweetpotato cultivars comprising six landraces, five introductions and three locally bred lines planted at Bvumbwe Research Station in December, 2004 and harvested at 4, 5 and 7 months after planting (MAP) were analysed for Vitamin A content spectrophotometrically. The results indicate that cultivar and root age significantly affected the vitamin A content ($p \leq 0.05$), of sweetpotato. *Zondeni*, a local orange fleshed cultivar afforded the highest level (13918±40µg/100g fresh weight). Optimum concentrations of this vitamin in the roots were obtained at 4 MAP; the values declined thereafter in peeled roots. Peeling of roots significantly reduced Vitamin A levels ($p \leq 0.05$) resulting in 0.45, 34.3 and 50.5% losses at 4, 5 and 7 MAP respectively. Increased production and consumption of sweetpotato as alternative food sources to maize will contribute to combating vitamin A deficiency in Southern Africa including Malawi.

An abstract of a paper presented at the International Conference of the Association of Professional Chemists and Chemical Engineers of Malawi at National Bank College, Blantyre, Malawi (19th to 23rd June 2006).