CHARACTERIZATION OF KENYAN SWEETPOTATO [*Ipomoea batatas* (L.) Lam] GERMPLASM USING MORPHOLOGICAL AND MOLECULAR MARKERS

BY

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Characterization of Kenyan sweet potato
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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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We confirm that the candidate, under our supervision carried out the work reported in this thesis.

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DEDICATION

This thesis is dedicated to my parents Leah W. Njuguna and David W. Njuguna who have encouraged, supported and funded my education including my MSc studies.

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ABSTRACT

East Africa is the largest producer of Sweet potato in Africa with Uganda taking the lead. Sweet potato has higher genetic diversity than other root crops such as cassava, yams or cocoyams. The short tropical day lengths found in East Africa favours Sweet potato flowering leading to hybridisation and giving rise to new varieties. In addition, the nature of its dispersal and vegetative propagation has created a vast number of cultivated genotypes. Conservation of this germplasm is necessary for the improvement of agricultural production. Characterization of germplasm is the first step in establishing a core collection, which promotes easier management of the genetic diversity. In the present study, morphological characterization was done to eliminate the duplicates in the Sweet potato collection. Principle Component Analysis (PCA) was done to determine the descriptor that contributed the highest variation while DIVA-GIS version 4.2, a Geographical Information Systems software was used to analyse the Sweet potato collections with regard to their geographical locations. Four hundred and eighty three varieties were identified as unique varieties, while there were one hundred and fifty seven duplicates in the collection using a sorting method. There were also some varieties that were initially identified as "unknown" because they did not have local names. Some of these were identified because they formed duplicates with some of the known varieties. The descriptor, Shape of Central Leaf Lobe was found to have the highest influence in discriminating among the varieties. In the present study morphological and ISSR (Inter Simple Sequence Repeats) profiles for twenty-two most popular farmer varieties of Sweet potato in Kenya were generated. Both techniques identified the different varieties as unique. However ISSR gave low variation as compared to morphological markers from the bootstrap values calculated for the trees generated. The morphological markers separated the twenty-two varieties into five groups each having a different shape of Central Leaf Lobe. Farmer varieties are the greatest source of genetic variation and therefore need to be conserved and used in breeding. It has been noted that even when improved cultivars are readily available, farmers continue to rely on landraces because of lack of economic and institutional resources to suit the requirements of modern cultivars. Introduction of new varieties into smallholder cropping systems can lead to rapid loss of valuable traditional varieties. Continued erosion of germplasm indicates the need to secure long-term preservation of landraces.

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ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorpism
AVRDC	Asian Vegetable Research and Development Center
AYT	Advanced Yield Trial
CIP	Centro Internacional De La Papa (International Potato Center)
CTAB	Cetyltrimethyl ammonium bromide
DNA	Deoxyribonucleic Acid
DNTP	Deoxynucleoside triphosphate mix
EDTA	Ethyleneditetraacetate
GIS	Geographical Information Systems
IITA	International Institute of Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute (formerly IBPGR)
ISSR	Inter-Simple Sequence Repeats
KARI	Kenya Agricultural Research Institute
KEPHIS	Kenya Plant Health Inspectorate Service
Kb	Kilobase
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PYT	Preliminary Yield Trial
PVP	Polyvinyl Pyrolidone
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SSR	Simple Sequence Repeats

Taq	DNA polymerase enzyme from thermus aquaticus
TE	Tris/EDTA buffer
TBE	Tris/Borate/EDTA buffer
TRIS	N-tris[hydroximethyl] methyl-2-aminosulfonic acid
V	Volts
OD	Optical Density
UPGMA	Unweighted pair-group method using arithmetic averages

ACRONYMS

Genetic Diversity	The variation in the genetic composition of individuals within or among species
Core collection	A limited subset of accessions derived from a larger germplasm collection, chosen to represent the genetic spectrum of the whole collection.
Accession	Plant sample, variety or population held in a gene bank or breeding program for conservation or use.
Cultivar	A cultivated variety of a domesticated crop or plant; synonymous to variety
Landrace	Farmers varieties; farmer developed varieties of crop plants that are heterozygous, adapted to local environmental conditions and have their own local names.
Germplasm	A set of genotypes that may be conserved or used; synonymous to genetic resource
Center of Diversity	A region is termed a region/centre of diversity due to rich morphological variation in cultivated Sweet potato.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Sweet Potato (*Ipomoea batatas*)

1.1.1 Background Information on Sweet Potato

Sweet potato [*Ipomoea batatas* (L.) Lam] is a dicotyledonous plant that is classified in the taxonomic group *Ipomoea* section *batatas* in the Convolvulaceae family (Varadarajan and Prakash, 1992; Missah and Kissiedu, 1994). Amongst the approximately 50 genera and more than 1000 species of this family, only *Ipomoea batatas* is of major economic value owing to its edible tubers (Janssens, 2001; Woolfe, 1992).

Sweet potato is an important food crop particularly in developing countries where 98% of it is grown (Scott *et al.*, 2000; Woolfe, 1992) with China as the largest producer of sweet potato in the world. It is grown in 101 developing countries (Woolfe, 1992) as a valuable food resource and is adaptable to a wide range of agro-ecological conditions including tropical and sub-tropical climates. Sweet potato is widely grown in Eastern, Central, and Southern Africa, where it is a reliable, low input, food security crop for resource-poor farmers, with increasing commercial potential (Minde *et al.*, 1998). Out of an approximate 1.5 million hectares of sweet potato grown in sub-Sahara Africa, East and Central Africa accounts for about 80% (FAO, 1997). In Kenya, it is one of the most important food and fodder crops (Gethi and Kidanemarian, 1994; Gatumbi *et al.*, 1994).

Mutuura *et al.*, (1992) observed that 33% of rural households in Kenya eat sweet potatoes two to three times a week during the dry season.

Sweet potato has the potential for expanded production in stressful environments because of its ability to give reasonable root yield under adverse climatic conditions or poor soils (Kasele, 1998), with low pH and low fertility (IITA, 1982). Fast growing varieties have the capacity to produce ground cover that can be used to prevent soil erosion (Gregory, 1992). The failure of cassava and cereal crops, and the ability of sweet potato to withstand locust attack and drought has increased its importance among farmers and has consequently led to an increase in the production of sweet potato (Jeremiah, 1994; Scott *et al.,*1998) and more so as a food security crop (Kasele, 1998).

1.1.2 Origin

The oldest remains of cultivated sweet potato so far discovered have been in the Chilca Canyon of Peru (Engel, 1970), which have been radiocarbon-dated at 8,000-10,000 years old. Sweet potato remains from the Aasma valley of Peru dated approximately 2000 B.C. have been discovered (Ugent *et al.,* 1983). Archaeological, linguistic and historical evidence have established that the sweet potato originated either in the Central or South American lowlands with subsequent dispersal to North America, Europe, Africa, Asia and the Pacific (O' Brien, 1972). This is supported by the high genetic diversity in this region (Austin,

1988). Austin (1977) suggested that sweet potato originated in a region bounded by the Yucatan Peninsula to the north and the Orinoco River to the south. In 1492, during the first voyage of Columbus to Hispaniola and Cuba he introduced it into Spain. From there it spread to the rest of Europe. Further spread of the sweet potato was mainly by two lines of dispersal. The first was the batatas line which followed Spanish introduction into Europe, continuing after 1500 A.D by the transfer of European grown clones into Africa, India and the East Indies through Portuguese exploration. The second was the kamote line where Mexican clones were carried to the Philippines by Spanish trading galleons (Yen, 1974). Several introductions into Polynesia and New Zealand took place prior to the 18th century (Janssens, 2001).

However, recent evidence strongly suggests that Central America may be the actual center of origin of the sweet potato. According to a study carried out by Gichuki *et al,* (2003), the Central American/Caribbean genotypes showed a close relationship with sweet potato genotypes from all the regions outside the Americas.

It is concluded that the Portuguese through Mozambique and possibly Angola introduced the sweet potato into Africa in the 16th century directly from Portugal. Linguistic evidence suggests a later introduction of sweet potato by British colonialists and missionaries around 1662 (Woolfe, 1992).

1.1.3 Uses

Sweet potato is used as human food and animal feed (vines are fed to cattle and pigs) (Janssens, 2001; Scott and Maldonado, 1999; Gregory, 1992). In tropical Africa, sweet potato is used mainly for human consumption. The tuber is eaten boiled, fried or braised. When boiling sweet potato tubers, the carbohydrates are converted to amylopectin, which is then digested to glucose. In Tanzania the sweet potato is processed into chips and flour (Jeremiah, 1994). It is also suitable for the manufacture of syrup, alcohol or starch. The young leaves can be consumed as a vegetable; the leaves also constitute an important supplement in animal feed (Janssens, 2001; Gregory, 1992).

1.1.4 Composition

The water content of the tuberous root of sweet potato varies from 57 to 78 % of the fresh weight. The remainder is made up mainly of starch (13-33 %), sucrose (2.6 - 6.0 %), reducing sugar (0.3 - 0.8 %), mineral (0.8 - 2.2 %) as well as protein (0.8 - 2.2) and cellulose (0.9 - 1.2). Carotene content of the tubers varies between 0 and 24 mg per 100 g of fresh tuber. The ascorbic acid content (vitamin C) varies between 23 and 43 mg per 100 g of fresh tuber (Janssens, 2001).

1.1.5 Nutritional Content

Sweet potato contains Vitamin A, B_1 C and E, which are antioxidants that may offer protection against heart disease and cancer. It also provides calcium, iron and

some minerals and is high in carbohydrates (Ambeh-Tumanthe, 1994; Gregory, 1992). Substantial amount of protein present in the crop has been attributed to the high consumption level in west, equatorial and semi arid Africa (Gregory, 1992).

1.1.6 Production

Sweet potato has the highest solar energy fixing efficiency amongst food crops primarily because of its capacity to produce dry matter for a long time (Hahn, 1977). It is therefore the world's highest yielding food crop in terms of production per unit area. It is ranked seventh in production among world's foods because of its versatility and adaptability (Scott and Maldonado, 1996). Africa is documented to be the only world region in which sweet potato production increased throughout the period between 1961/63-1983/85 (Woolfe, 1992). This can be attributed to factors such as drought, diseases and pests affecting alternative staple foods like maize, cassava and millet. For example in Namibia, during drought prone growing seasons, shortages of pearl millet (staple food) led to increased sweet potato production according to the 1994/5 Namibia Agricultural Census (Rusch, 1998).

In Africa production is concentrated in East Africa, especially the area around Lake Victoria, with Uganda being the largest producer in Africa and fourth worldwide (Janssens, 2001; FAO 1995). Uganda produces an average output of nearly 2 million tons (Scott and Maldonado, 1999). Production tends to be concentrated in those countries with low per capita incomes like Kenya (Scott and

Maldonado, 1998; Mutuura *et al.*, 1992). Sweet potato ranks as the third most important food crop in Kenya compared to 20 other major food crops (FAO, 1998; Gatumbi *et al.*, 1994). In Kenya about 74,000 ha is planted with sweet potato annually with 90% produced in Nyanza, Western, Central and Eastern provinces, while the rest of the country accounts for the other 10% (Shakoor *et al.,* 1987).

1.1.7 Production Constraints

Viruses that limit sweet potato production are widespread, of which seven different strains have been identified in Kenya (Wambugu, 1991). Aphids (*Myzus persicae* and *Aphis gossypii*) transmit the sweet potato feathery mottle potyvirus (SPFMV) in a non-persistent manner. Sweet potato mild mottle Potyvirus (SPMMV) is transmitted by the whiteflies *Bemisia tabaci* and *Trialeurodes abutilonea* (Janssens, 2001).

Sweet potato weevil (*Cylas puncticolis, Cylas brunneus, and Cylas formicarius*) is the major pest, which cause leaves to shrivel and die. According to Smit (1997), *C. brunneus* and *C. puncticollis* are most widespread in all sweet potato growing regions particularly in Western Kenya. Yield losses of up to 50% have been reported during the dry season (Jeremiah, 1994). Infestations of sweet potato weevils have been reported in about 65% of Kenyan sweet potato farms (Mutuura *et al*, 1992).

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Mole rats and other rodents cause significant damage (Ewell, 1992). Damage by nematodes (*Meloidogyne spp.*, *Rotylenchulus reniformis*, *Ditylenchus spp.*) is frequent because these nematodes are found in many regions. The root-knot nematode (*M. incognita*) is the most harmful to the crop (Janssens, 2001). Other constraints of sweet potato production are the lack of disease free vines for planting at the beginning of the rains, non-availability of improved varieties, and drought (Ewell, 1992; Jeremiah, 1994). Ndolo *et al,* (1998) also indicated that most of the varieties planted by the Kenyan farmer are inferior and therefore low yielding.

1.1.8 Growing Conditions

Sweet potato is very adaptable to a wide range of agro-ecological conditions but yields are higher under favourable conditions. It requires high humidity only at the beginning of its growth. Any moisture after production of roots can cause them to rot. Short days (11 hours) and cool nights favour formation of tubers. Optimal growth is between 21 and 28°C (Janssens, 2001).

Sweet potato can colonize marginal soils because it readily produces adventitious roots and trailing vines. It however needs a light, fairly permeable loam, without excess of nitrogen, which stimulates development of the aerial parts at the expense of the tuberous roots. Sweet potato is tolerant to aluminium-rich, phosphorous

poor acid soils. However excess nitrogen and hydromorhpic soil conditions promote the issuing of fibrous roots (Janssens, 2001).

1.1.9 Genetic Diversity

1.1.9.1 Variability and Conservation

Genetic diversity is the variation in the genetic constitution of individuals within or among species (Lanaud and Vincent, 1997). Assessment of genetic variability is important for conservation of genetic resources and broadening the genetic base of varieties (Lefebvre *et al.*, 2001). It is important to conserve the genetic diversity for availability and utilization by the community and users (plant breeders, agronomists, and farmers) (Ayad *et al.*, 1997). The success of any genetic conservation program is dependent on understanding the amount and distribution of genetic diversity present in the gene pool (Zhang *et al.,* 2000) and it creates a basis for improvement of agricultural production. Data from genetic diversity studies may be used in establishing core collections. Maximum genetic diversity is the key to establishing a good core subset (Zhang *et al.*, 1998). Core collections are usually 10% of the total collection (Lanaud and Vincent, 1997). Cultivars are identified based either on morphological, biochemical or DNA profiling (Lefebvre *et al.*, 2001).

Over time, natural hybridization and selection have resulted in the evolution of hundreds of native sweet potato cultivars in South and Central America, Africa,

Asia and Oceania. Central and South America have been identified as the primary centre of diversity of sweet potato with East Africa, Asia and Oceania suggested as secondary centres of diversity (Huamán *et al.*, 1999).

1.1.9.2 Erosion of Genetic Diversity

Causes of erosion of plant genetic resources include the introduction of exotic crops and germplasm, spread of monocultures resulting in a narrowing of the genetic resource base (Mugabe *et al.*, 1998). There is greater need for conservation of sweet potato and other cultivated plants because of change in socio-economic activities resulting in irreversible degradation of biodiversity. These changes are associated with the rapidly growing populations. Kenya's population is estimated to grow at over 3.5% per annum (Republic of Kenya, 1989).

1.1.9.3 Gene Banks

The International Potato Centre (CIP) holds one of the largest sweet potato gene banks with more than 5,000 cultivated varieties from America, Africa, Asia and Oceania (Huamán *et al.*, 1999). This includes over 1671 accessions from over 30 countries of Asia and the Pacific transferred from the Asian Vegetable Research and Development Centre (AVRDC) after 1991 (AVRDC, 1992). Several national programs in the major sweet potato growing countries hold smaller germplasm collections.

In Kenya, sweet potato germplasm certified to be disease and pest free is stored at the Plant Quarantine Station (PQS) in Muguga and is normally distributed to plant breeders, national research stations and universities. Duplicates of some accessions are stored at the Gene Bank of Kenya based at Muguga. Due to financial and technical limitations, conservation of germplasm often experiences problems. For example between 1980 and 1983 the PQS {an institutional organ of National Agricultural Research System (NARS)} refrigeration equipment broke down, resulting in the loss of a wide range of accessions (Kenya Energy and Environment Organization (KENGO), (1989).

Sweet potato collections were made from early 2002 from major sweet potato growing regions in Kenya, mainly Western, Nyanza, Eastern, and Central provinces. These sweet potato germplasm are currently conserved at the University of Nairobi in Kabete Campus where evaluation and multiplication is taking place. This collection, evaluation and conservation is funded by the Collaborative Crop Research Programme of the McKnight Foundation through a grant to the East African Sweet potato project.

1.1.9.4 Measurement of Genetic Diversity

1.1.9.4.1 Morphological Characterization

Morphological characterization in sweet potato is achieved by checking variations in vine, leaf and storage root characters of collections. This is followed by data

analysis and duplicate verification (CIP, AVRDC & IBPGR, 1991). Morphological descriptors for sweet potato are mostly applied to sort out duplicates, both in collections at the experimental and farmer fields (Ewell and Mutuura, 1991; Zosimo, 1992; Mok and Schmiediche, 1998). Morphological characters are also the principle descriptors in germplasm collections (Russell *et al.*, 1997). Traditionally, morphological markers have been used to provide descriptors for identifying sweet potato cultivars (Huamán *et al.*, 1991). Morphological characterization has also been used to assess genetic diversity and establish core collections, for example in Indian mung bean (Bisht *et al.*, 1998) and sweet potato (Mok and Schmiediche, 1998).

However morphological descriptors do not always allow the quantification of the genotypic difference or similarity between cultivars as do genetic distances based on DNA polymorphism (Lefebvre *et al.*, 2001). In addition, they are unreliable due to their paucity and their vulnerability to environmental influence (Gepts, 1993; Vosman, 1998). Cultivars need to be grown to full maturity to carry out all necessary observations, which makes the process time consuming and expensive (Vosman, 1998). Despite the disadvantages associated with morphological characterization, a candidate variety by definition must differ from protected ones on the basis of phenotypic traits (morphological). Therefore molecular markers will only provide complementary information in cases of phenotypic similarity or proximity (Lefebvre *et al.*, 2001). The use of molecular markers to supplement

and refine the morphological-based classification has been recommended by Russell *et al*, (1997). Soller and Beckman (1983) proposed using molecular markers as an additional tool for varietal description because they have the advantage of being independent of environmental effects and providing direct information of the genome of each individual.

1.1.9.4.2 DNA-based Molecular Markers

1.1.9.4.2.1 Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) is defined as the variation(s) in the length of DNA fragments produced by a specific restriction endonuclease from genomic DNAs of two or more individuals of a species (Kahl, 2001). RFLP was first developed in the early 1980s for use in human genetic applications for determining genetic relationships (Botstein *et al.*, 1980) and was later applied to plants (IAEA, 2002). RFLPs are detected by hybridizing labelled DNA clones to southern blots containing DNA digested with endonuclease restriction enzymes. Southern hybridization analysis of RFLP requires skill and is time consuming (IAEA, 2002). With Polymerase Chain Reaction-RFLP (PCR-RFLP) the RFLP differences are visualized with locus specific PCR amplification products. Amplification plus digestion has often detected intra and inter specific variations (Gielly and Taberlet, 1994).

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1.1.9.4.2.2 Random Amplified Polymorphic DNA (RAPD)

The RAPD technique described by Williams *et al,* (1990) makes use of arbitrary short oligomers (usually 10-mer) which anneal to a certain sequence of random homologous target sites within the genome allowing the generation of a product only if the configuration of adjacent target sites satisfies the requirements of PCR. These sequences are both abundant and highly polymorphic in plants (Tautz, 1989; Saghai-Maroof *et al.,* 1994). Polymorphism is based on the disruption or displacement of homologous target sites between individuals, which results in the loss of a product. They are an attractive choice for determining genetic relationships because they are simple to detect and do not require DNA sequence information or synthesis of specific primers. The technique uses short oligodeoxynucleotide primers of arbitrary nucleotide sequence (amplifiers) and PCR procedures. RAPD generates dominant markers although polymorphism caused by small insertions/deletions can occur at low frequencies (2-5%). However, because the fragments are amplified based on homology to a very short, random DNA sequence used to prime the PCR, there is some uncertainty about the genetic relationship of fragments from different genotypes and about the genome origin of the fragments. RAPD markers cannot distinguish the heterozygote from the homozygote and are irreproducible across different laboratories (Jones *et al.*, 1997). RAPD markers have been successfully used to assess genetic diversity in sweet potato (Gichuki *et al.*, 2003).

1.1.9.4.2.3 Amplified Fragment Length Polymorphism (AFLP)

The AFLP technique is a PCR-based fingerprinting technique developed mainly to overcome the disadvantages of other PCR based genotyping techniques such as RAPDs which suffer from sensitivity to reaction conditions, DNA quality and PCR temperature profiles that limit their application (Vos *et al.*, 1995, Jones *et al.*, 1997). The steps involved in AFLP are restriction digestion/ligation, preselective amplification, selective amplification and visualization. The technique has become an attractive tool because it generates a high number of polymorphic products (Powell *et al.*, 1996), and is reproducible across laboratories (Jones *et al.,* 1997). In a study on *Sorghum bicolor* accessions from South Africa, AFLP revealed higher bootstrap values as compared to RAPD and SSRs suggesting that AFLP revealed higher variation between the accessions (Uptmoor *et al.*, 2003). AFLP markers have mainly been used for phylogeny analysis, cultivar/accession identification and genetic variation studies (Robinson and Harris, 1999). It has been used to assess genetic diversity of maize inbred lines (Vuylsteke *et al.*, 2000) and *Moringa Oleifera* Lam (Muluvi *et al.*, 1999). AFLP has previously been used successfully in genetic diversity studies in sweet potato from South America and Papua New Guinea (Zhang *et al.,* 1998) and among East African sweet potato cultivars (Gichuki, 2000). This study found AFLP to be more informative when compared to other molecular markers like RAPD.

1.1.9.4.2.4 Simple Sequence Repeats (SSR) or Microsatellites

Simple Sequence Repeats (SSRs) are hyper-variable tandem repeats of DNA motifs 2-5 bases long, common in eukaryotic and prokaryotic genomes (Zhu *et al.*, 2000). They are widely distributed in higher plants (Morgante and Olivieri, 1993; Wang *et al.,* 1994). Although they are ubiquitous (Kijas *et al.,* 1995), retrieval of SSRs has not been easy in plants because of their relatively low abundance compared with animal/human genomes. The variation in size of SSRs comes from differences in number of repeat units originating from error in copying of DNA during replication by DNA polymerase (Robinson and Harris, 1999). SSRs are highly reproducible, polymorphic and are co-dominant molecular markers (Lelley *et al.*, 2000). Co-dominancy allows them to identify heterozygotes and therefore are more accurate compared to RAPD and AFLP markers (Robinson and Harris, 1999). The SSR technique requires prior screening for these SSRs which makes it more practically complex and may yield a small number of potential SSR loci. However, a simple method for development of SSR markers using amplified fragments of Inter-Simple Sequence Repeats (ISSR) has been described by Lian *et al*, (2001). This method does not require the prior enrichment process and screening procedures.

SSR has been used in genetic diversity analysis and genotype identification in barley (Saghai-Maroof *et al.,* 1994), wheat (Plaschke *et al.,* 1995) and rice (Xiao *et al.,* 1996). SSR primers and sequences have already been developed for some

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plants; peach and pear (Hayashi and Yamamoto, 2002) and sweet potato (Jarret and Bowen, 1994). Sequence characterization of SSR in sweet potato has been carried out by Buteler *et al,* (1999). In sweet potato, SSR has revealed high levels of polymorphism (Hwang *et al.*, 2002) and has been successfully used to assess genetic diversity in East African sweet potato (Gichuru, 2004).

1.1.9.4.2.5 Inter-Simple Sequence Repeats (ISSR)

Inter-SSR amplification is a variant of the PCR that uses SSR primers (e.g. [AC] n) to amplify regions between their target sequences (Kahl, 2001). ISSR amplification is a technique that encompasses both the applicability of RAPD where no prior knowledge of the analyzed genome is required and the advantage of targeting multiple SSR loci (Parsons *et al.*, 1997; Zietkiewicz *et al.*, 1994). It overcomes many of the technical limitations of RFLP and RAPD analyses (Tsumura *et al.,* 1996). These primers target microsatellites that are abundant through out the eukaryotic genome (Kijas *et al.,* 1995). ISSR are dominant markers and are known to be highly polymorphic, robust in usage, can be automated and have a higher reproducibility than RAPDs (Huang and Sun, 2000). They have a wide range of application such as fingerprinting, varietal/line identification, genetic maps, F1 identification, gene tagging, breeding, bulk segregant analysis, diversity studies, marker-assisted selection, high-resolution mapping and seed testing (IAEA, 2002). They have previously been used successfully in sweet potato genetic diversity studies (Huang and Sun, 2000).

ISSR have also been used to assess the genetic diversity in dent corn and popcorn (Kantenty *et al.*, 1995), to study relationship among Pansies (Yockteng *et al.*, 2003), to identify cultivars of chrysanthemum (Wolff *et al.*, 1995) and citrus, (Fang and Roose, 1997).

1.1.9.5 Measurement of Genetic Diversity Distribution using Geographical Information Systems (GIS)

For the success of any genetic conservation and breeding program, it is important to not only assess the amount of genetic diversity, but also its distribution (Zhang *et al.*, 2000). Geographical Information Systems (GIS) is a computer system for capturing, managing, integrating, manipulating, analyzing and displaying data which is spatially referenced to the earth (McDonnell and Kemp, 1995). Spatial information uses location within a coordinate system as its reference base. Locational reference used are latitude and longitude or a grid system like the national grid (www.DIVA-GIS.org). GIS has been used by scientists to study the global geographical distribution of different plant varieties. For example, Hijmans and Spooner (2001) used GIS to study the global geographical distribution of potato and Huaccho and Hijimans (2000) used it on sweet potato. GIS software can be used to assess diversity of species similarity and distance between accessions, variation in the range of a particular character and variation between sets of descriptors (Jagtap and Ng, 1999).

In the Daily Nation Newspaper of 17th December, 2004 it was reported that the Lands and Housing ministry recognizes the need for geographically referenced data that would support decision making for planning and management of natural resources and the environment. This has been triggered by the poorly maintained maps, outdated statistics, inaccurate information and limited sharing of data.

1.2 Problem Statement and Justification

1.2.1 Problem Statement

Owing to the greater distribution and importance given to sweet potato, a larger number of varieties exist than for cassava, yam, or cocoyam (Robert, 1985; Woolfe, 1992). Africa represents a unique secondary site of genetic diversity for sweet potato due to the nature of its dispersal from the Americas because it has its own unique sweet potato gene pool with unique desirable characteristics (Gichuki *et al.,* 2003). These characteristics include: high storage root dry matter (DM) content, high resistance to virus diseases and vigorous foliage growth and ground cover. Though an introduced crop in Africa, numerous varieties are grown (Ewell and Mutuura, 1991). In East Africa this diversity has been increased by natural hybridization, selection, systematic breeding efforts and mutations (Onwueme, 1982).

Cross-fertilization is the general rule as most varieties of sweet potato are self-incompatible but in some cases cross incompatibility occurs (Wilson *et al.*, 1989).

Most sweet potato varieties flower naturally in the Pacific islands and other tropical countries (Wilson *et al.*, 1989). Short tropical day lengths (11-12 hours of daylight) favor sweet potato flowering in East Africa (Janssens, 2001). This implies that there probably exist a number of clones in East Africa which would have a combination of superior characteristics that would qualify them to be officially recommended as varieties. Previous evaluation has revealed that Kenyan germplasm has a combination of superior characteristics such as resistance to important diseases and pests, adaptation to environments where sweet potato is grown and desirable characteristics such as high storage root dry matter content (Ndolo *et al.*, 1998).

Karp *et al,* (1997) emphasized the importance of conservation of genetic resources for improvement of agricultural production. Most Kenyan farmers preserve sweet potato collections on their farms for different uses such as planting in the next season, exchange with other neighbouring farmers and processing. These collections are prone to environmental changes and effects like adverse weather as well as regional conflicts which can lead to loss of entire germplasm collections. This is demonstrated well in Mozambique and Rwanda. In Mozambique, after the civil war ending in 1992, the El Nino season hit during the 1991/1992 cropping season that led to the entire destruction of sweet potato collections in the fields. Since then, trying to replace the local germplasm through sustainable food security measures has strained the Government of Mozambique financially (Andrade *et al,*

1998). For Rwanda a sweet potato improvement program was developed in 1983 which resulted in the production of superior varieties to address constraints of leaf blight, the virus complex and sweet potato weevils. Sadly the political events in 1994 that led to the genocide of more than a million Rwandans with another million displaced from their homes reversed these crop improvement strategies and loss of important varieties. Rwandans were known to eat more sweet potato than anyone else (Machuka, 2003).

Through breeding programs, new and improved varieties are introduced which lead to the erosion of those with superficially less desirable characteristics. In Zambia, for example, an improved variety, "Chingovwa", was introduced in 1993/4 by RTIP (Root and Tuber Improvement Program) and replaced most of the local varieties (Chiona, 1998). The Kenyan sweet potato germplasm collections have not been well characterized. There has, however, been considerable effort carried out using S-SAPs (Simple Sequence Amplified Polymorphism), AFLPs, SSRs and RAPDs on the sweet potato varieties in East Africa and it has been concluded that some East African germplasm are unique from those of Asian, American and Oceania germplasm (Gichuki *et al.*, 2003). In Kenya, germplasm evaluation carried out by Ndolo *et al,* (19987) indicated significant variation in yield, dry matter content and culinary quality among varieties. Root dry matter content is associated with the culinary qualities preferred by consumers. Farmers have selected sweet potato varieties for their ability to yield storage roots of

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acceptable consumer quality, and to produce planting material for continued propagation (Carey *et al.*, 1998).

In the present work, morphological descriptors were used to characterize Kenyan sweet potato collections to eliminate duplicates. Duplicates increase the cost of maintenance of collections and time when the germplasm needs to be screened for various characteristics important for breeding and distribution of planting material to farmers. This was done as a first step in creating a core collection of the Kenyan sweet potato germplasm. Secondly, morphological and ISSR markers were used to characterize and generate profiles for sweet potato varieties identified as the most popular by farmers. Information generated will be useful in protecting farmer preferred varieties. The two technologies used were also evaluated for suitability in discriminating between varieties.

1.2.2 Research Questions

i. Are there any duplicates in the Kenyan sweet potato germplasm collection?

ii. Is there any variation in the sweet potato varieties grown by Kenyan farmers?

iii. Are the sweet potato varieties given different names and grown by Kenyan farmers genetically unique?

1.2.3 Null Hypotheses

There is no genetic difference in the sweet potato cultivars grown by Kenyan farmers

1.2.4 Objectives

1.2.4.1 General Objective

To characterize Kenyan sweet potato germplasm using morphological and molecular markers.

1.2.4.2 Specific Objectives

i. To identify and eliminate duplicates in collections of sweet potato germplasm identified only on the basis of morphological attributes.

ii. To create morphological and ISSR molecular profiles for the selected most popular varieties of sweet potato in Kenya.

1.2.5 Justification

Sweet potato is a short season crop (four-month growth cycle) which reliably provides food on marginal and degraded soils (Hahn, 1977). It requires little labour with few or no inputs from outside the farm (Ewell and Mutuura, 1991). The cultivated germplasm of sweet potato provides the foundation for advances in modern sweet potato breeding. Regional conflicts and adverse weather threaten *in*

situ germplasm collections, which increase the risk of loss of total germplasm and farmer knowledge of traditional conservation methods (Karp *et al.*, 1997).

In this project sweet potato germplasm were collected from major sweet potato growing regions and characterized morphologically, as a first step to establishing a core collection. Constructing a core collection is important for successful genetic conservation of any given gene pool (Zhang *et al*, 1998). Core collections promote easier management of the maximum genetic diversity and allow easier usage and availability of resources to plant breeders for the development of new varieties and also for distribution of planting material to farmers (Diwan *et al.*, 1994). Assessment of genetic variability between cultivars is of interest not only for cultivar protection but also for practical applications such as conservation of genetic resources and broadening the genetic base of the varieties (Lefebvre *et al.*, 2001)

Farmers identify varieties using local names and their varieties are the greatest source of genetic variation and therefore need to be conserved and used in breeding (Eyzaguirre and Iwanaga, 1996). It has been noted that even when improved cultivars are readily available, farmers continue to rely on landraces (Brush, 1992), because of lack of economic and institutional resources to suit the requirements of modern cultivars (Eyzaguirre and Iwanaga, 1996). Farmers therefore carry out traditional breeding or management of diversity by maintaining

and selecting among their landraces (Riley, 1996). When these farmer varieties are compared with improved cultivars they are often better performers (Eyzaguirre and Iwanaga, 1996). Introduction of new varieties into smallholder cropping systems can lead to rapid loss of valuable traditional varieties (Kapinga *et al.*, 1995; Franzen *et al.,* 1996). Continued erosion of germplasm indicates the need to secure long-term preservation of landraces.

In this study, morphological and ISSR molecular profiles for selected most popular farmer varieties of sweet potato in Kenya were generated. Fingerprinting is important for breeders to estimate similarities between different cultivars for crossing purposes and also for protection of their own varieties (Milbourne *et al.*, 1997).

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Sweet Potato Varieties Used

The sweet potato varieties used in this study were collected from major sweet potato growing regions in Kenya, namely, Western, Nyanza, Eastern, and Central provinces, which produce 90% of sweet potato nationally. A total of 564 accessions were collected from these regions.

2.2 Sweet Potato Samples Collection

The identification of high sweet potato production areas and the actual collection was carried out with the assistance of agricultural extension officers. The farmers were selected at random by moving at least half a kilometre and entering any homestead on the route. In every household, a questionnaire was given where pedigree information about the varieties was recorded by the farmers (see Appendix 2). Efforts were made to avoid the collection of duplicate accessions. Also included in the study were varieties from Tanzania, Uganda, South Africa and Central America which were obtained through CIP Nairobi, Kenya. The areas where the sweet potato varieties were collected and the details of the accessions are shown in Table 2.1.

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Table 2.1 Summary of sweet potato collection sites and number of accessions collected from different farmers

Province	Districts	Number of Accessions
Western	Teso	21
	Busia	36
	Kakamega	15
	Bungoma	4
	Vihiga	5
Nyanza	Kisumu	21
	Siaya	71
	Migori	24
	Rachuonyo	54
	Homa Bay	60
	Kisii	8
Central	Mukuruweini	42
Eastern	Machakos	42
Unknown*		69
Exotic material		18
Advanced Yield Trial Field		30
Preliminary Yield Trial Field		44
TOTAL		564

- Varieties that do not have farmer names assigned to them. This is either because they are recent introductions, or have characteristics that are not desirable to farmers

The sweet potato varieties used for fingerprinting were obtained from farmers who had identified them as the most popular. Details of the sweet potato varieties used for fingerprinting are shown in Table 2.2. Exotic varieties were also included as reference material.

Table 2.2 Farmer preferred varieties for fingerprinting

Accession number	Accession name	Origin of Accession
1	Nyathi odiewo	Western (Kenya)
2	Spongi	Tanzania
3	SPK 013	Western (Kenya)
4	Zapallo	South America
5	Maria Angola	South America
6	Bungoma	Western (Kenya)
7	Ex Shimba	Coast (Kenya)
8	Sinia	Tanzania
9	Budagalla	Tanzania
10	Namaswakhe	Western (Kenya)
11	K 117	South Nyanza (Kenya)
12	Beuregard	U. S. A
13	Kindolo	Eastern (Kenya)
14	Nyatonge	South Nyanza (Kenya)
15	Wera	South Nyanza (Kenya)
16	Odinga	South Nyanza (Kenya)
17	Cheglina Mowar	South Nyanza (Kenya)
18	Nemanete	South America
19	Pedro	Papua New Guinea
20	Ngorobu	Central (Kenya)
21	Amina	South Nyanza (Kenya)
22	Kilungu	Eastern (Kenya)
Total accessions	22	

After collection, the varieties were planted in experimental fields at the University of Nairobi (Kabete Campus) and also at KARI (Kenya Agricultural Research Institute) Biotechnology Center's experimental field (Kabete).

2.3 Morphological Characterization

For the elimination of duplicates morphological characterization was carried out as described by CIP AVRDC & IBPGR (1991). The number of morphological characters checked in every variety was 24 (see Appendix 1). Morphological characterization was carried out three months after planting when most of the varieties were mature enough for observation. The morphological descriptors used for the characterization included vine, leaf and storage root characters. Figure 2.1 shows the leaf variation of different sweet potato varieties.

(a) (b) (c)

Figure 2.1 Leaf descriptors used to characterize different varieties. (a) The leaves have 5 lobes, are slightly lobed with a semi elliptic shape of the central lobe. (b) The leaves are 5 lobed, are very deeply lobed with an elliptic shape of the central lobe. (c) The leaves have no lateral lobes and have a triangular shape of the central leaf lobe.

Different sweet potato root characters exhibited by different varieties are shown in Figure 2.2. Examples of sweet potato root descriptors used in the morphological characterization include: storage root flesh colour, storage root cortex thickness,

storage root defects and predominant skin colour. For a complete list of all the sweet potato descriptors used for morphological characterization refer to Appendix 2

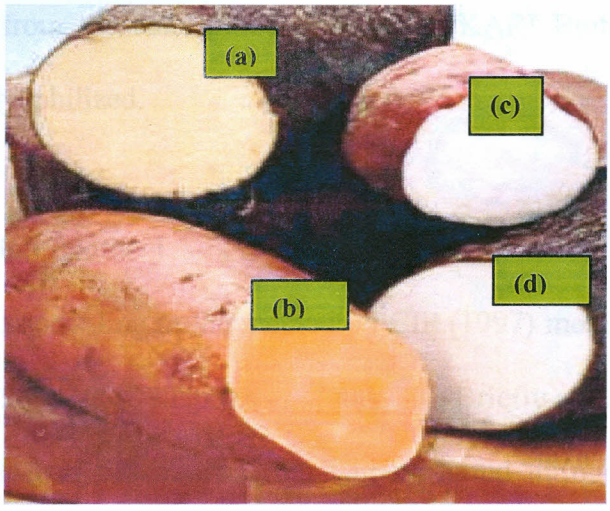

Figure 2.2 Variation of sweet potato flesh colour. (a) Pale yellow (b) orange fleshed (c) **and** (d) White flesh color.

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2.4 Sweet Potato Database

A database containing sweet potato passport data and the morphological characters was created in Microsoft Excel file format to allow analysis with Geographical Information Systems (GIS). The presence of coordinate data allowed the analysis of the database with GIS. DIVA-GIS version 4.2 software (Hijmans and Spooner, 2001) was used for this analysis.

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2.5 DNA Extraction

2.5.1 Sample Collection

About 5-7 very young leaves were harvested from 1 month old vigorously growing plants in the field. The leaves were immediately dipped in liquid nitrogen, placed in well-labeled glass bottles and stored in cold boxes with dry ice. Samples were then transported from the collection points to the KARI Biotechnology Center where they were lyophilized.

2.5.2 DNA Isolation Protocol

DNA was isolated according to the method by CIP (1997) modified from Murray and Thompson (1980) and Dellaporta *et al.*, (1983). Briefly about 100 mg of each lyophilized sample leaf tissue was ground using liquid nitrogen and 700 µl of cetyltrimethyl ammonium bromide (CTAB) extraction buffer added and the mixture placed in water-bath at 65°C for 45 minutes. Seven hundred micro litres of chloroform:isoamyl alcohol (24:1) was added to each sample, vortexed briefly and centrifuged for 5 min at 14000 revolutions per minute (rpm). The aqueous layer was removed and transferred into a new-labelled tube. Fifty micro litres of 10% CTAB (in 0.7M NaCl), was added and vortexed briefly. Seven hundred micro litres of chloroform:isoamyl alcohol (24:1) was again added and briefly vortexed. This was centrifuged for 5 minutes at 14000 rpm, the aqueous layer removed and transferred into another labelled tube. Five hundred micro litres of chilled isopropanol was added to each sample and manually inverted several times.

The tubes were then placed at 4 °C for 1-2 minutes. The pellet was washed first in 70 % ethanol and then repeated with 90 % ethanol. The samples were spun for 40 minutes at 14000 rpm, the ethanol poured off and the pellets dried overnight. Each DNA pellet was then dissolved in a 150 µl of Tris/EDTA (Ethylenediaminetetraacetic acid) buffer. Two micro litres 10mg/ml DNAse-free RNAseA was then added, vortexed briefly and this was incubated at 37 °C for 1 hour. The DNA samples were then stored at 4 °C.

2.5.2.1 Determination of DNA Quantity

DNA samples were electrophoresed in a 1.0% agarose gel in 1 X TBE buffer (0.045 M Tris-Borate and 0.001 M EDTA) at 100 V for 1 hour. Each DNA sample (5 µl) was mixed with 6 X gel loading dye (0.25 % bromophenol blue, 0.25 % xylene cyanol and 30 % glycerol) prior to loading. The quantity of DNA in the sample was then estimated by comparing the florescent yield of the sample with that of a series of standards. A series of DNA standards of known concentrations (15, 31, 63, 125, 250, 500 ng/6µl) were run alongside the DNA samples.

2.5.2.2 Determination of DNA Quality

The spectrophotometer method was used for checking the purity of DNA extracted. The Optical Density (OD) was taken at 260nm and 280nm and the ratio OD 260: OD 280 calculated. This ratio provided an estimate of the purity of

nucleic acid. Pure preparations of DNA have OD260: OD 280 values of 1.8 (Sambrook *et al.*, 1989; Hoisington *et al.*, 1994).

2.6 Fingerprinting of Farmer-preferred Varieties

2.6.1 ISSR Primers

Eight ISSR primer sequences were screened (Milbourne *et al.*, 1997) and the best 5 that gave highest variation were selected for fingerprinting. The sequences of the best 5 primers are shown in Table 2.3.

Table 2.3 The ISSR primer sequences used in this study

Primer code	Primer Sequence
ISSR 1	5' CAC ACA CAC ACA GG 3'
ISSR 2	5' GTG GTG GTG GC 3'
ISSR 11	5' GTG TGT GTG TGT 3'
ISSR 14	5' CTC CTC CTC GC 3'
ISSR 15	5' CTC TCT CTC TCT CTC TCT 3'

2.6.2 ISSR protocol

Polymerase Chain Reactions (PCR) were carried out in a total volume of 20 µl consisting of 2 µl of X 10 PCR buffer, 2 µl of 25 mM of $MgCl_2$, 0.4 µl of 200mM dNTPs, 0.2 µl 100 ng/µl ISSR primer, 0.2 µl of Taq, 2 µl 20 ng DNA, and 13.2 µl sterile distilled H_2O. The amplification conditions used an initial denaturing at 94°C for 5 minutes, followed by 45 cycles at 94°C for 45 seconds, 52°C for 45 seconds, 72°C for 1.5 minutes and a final 7 minute extension at 72°C.

2.6.3 Gel electrophoresis

The PCR products were resolved on a 1.5 % agarose gel using 1 X TBE buffer electrophoresed at 150 V for 1 hour. PCR products were mixed with 6 X loading dye before loading into the wells. One Kb DNA ladder was included in the gel to help in deducing the size of amplified fragments.

2.7 Data Analysis

2.7.1 Elimination of Duplicates from Sweet Potato Collection

Elimination of duplicates was carried out as described by CIP, AVRDC & IBPGR (1991). The accessions of the collection were grouped according to their similarities by sorting based on those descriptors that are least affected by the environment (Table 2.4).

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Table 2.4 Descriptors Least Affected by the Environment

Letter Code	Sweet Potato Descriptor
B	Vine internode diameter
D	Vine pigmentation
F	General leaf outline
G	Type of leaf lobes
H	Number of leaf lobes
I	Shape of central leaf lobe
J	Abaxial leaf vein pigmentation
N	Petiole pigmentation
S	Storage root skin colour
V	Storage root flesh colour

2.7.2 Principle Component Analysis (PCA)

A principle component analysis was carried out using GENSTAT (Genstat Statistical Package, 1995) according to variance/covariance method to determine the one variable that can be used to assess the differences among the sweet potato varieties. PCA explores common patterns of variation among groups and subgroups of accessions based on their variance/covariance structure and reduces the observed variation to a few (usually 2 or 3) linear combinations of the original variables (Ortiz, 1999).

2.7.3 Fingerprinting of Farmer Preferred Varieties

Visual observation of the morphological and ISSR matrices was carried out to check whether each of the two methods were able to distinguish between the different farmer varieties. The morphological data was then converted to binary data format (presence =1; absence = 0) while each individual amplification product was considered to represent the dominant allele at an ISSR locus. POPGENE 1.32 (Nei, 1978) was used to generate similarity matrices. Treecon version 1.3b (Van de Peer and De Wachter, 1993) was used to generate trees with bootstrap values. The clustering technique used was unweighted pair-group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

2.7.4 Analysis of Sweet Potato Database using GIS

DIVA-GIS version 4.2 software (Hijimans *et al.*, 2001, www.diva-gis.org) was used to preview and create shapefiles from DBF (Data Base File) formats. Simple spatial analysis was performed using DIVA-GIS to identify potential collection "hotspots" as well as provide a graphical analysis of the accession data. A map was also generated to show the distribution of genetic diversity in Kenya.

CHAPTER 3

3.0 RESULTS

3.1 Morphological Characterization

3.1.1 Morphological Characterization of Sweet potato Collection

3.1.1.1 Elimination of Duplicates

Those varieties that had all of the descriptors similar were classified as duplicates, however, some that differed slightly in one or two descriptors were also classified as duplicates. Using this criterion, 483 varieties were identified as unique out of the total 564 varieties analyzed. One hundred and fifty seven of the varieties had duplicates in the collection. Table 3.1 shows how varieties were grouped as duplicates and some of the duplicates found in the collection. Some of the duplicates had the same farmer local names, for example, Kalamb Nyerere (sample 5 and 6), Mar Ooko (sample 7 and 8) and Sinia (samples 35, 36, 37). Most of the duplicates did not have similar local names, for example, sample 33 (Nyathi Odiewo) and 34 (Ombeko Leso); 31 (Kindolo 1) and 32 (Sipogi). Sixty-nine varieties were found in the collection without any farmer names and were therefore identified as unknown varieties during collection. Some of these varieties formed duplicates with varieties that have local names and could therefore be identified and given local names. For example unknown sample 2 clustered with Nya Kamungalo and unknown sample 3 clustered with Bulindo.

Table 3.1 Grouping of sweet potato collection duplicates

SAMPLE NUMBER	LOCAL NAME OF VARIETY	DESCRIPTORS♦										No OF SAMPLES
		B	D	F	G	H	I	J	N	S	V	
1	NYA KAMUNGALO	1	3	4	0	1	1	5	1	2	1	1
2	UNKNOWN	1	3	4	0	1	1	5	1	2	1	
3	UNKNOWN	1	4	6	5	5	6	3	1	2	4	2
4	BULINDO	1	4	6	5	5	6	3	1	2	4	
5	KALAMB NYERERE	1	7	6	7	5	5	5	5	8	1	3
6	KALAMB NYERERE	1	7	6	7	5	5	5	9	8	1	
7	MAR OOKO	3	3	3	0	1	1	5	3	2	1	4
8	MAR OOKO	3	3	3	0	1	1	5	1	2	1	
9	38.MUNYILIA WA NGITHO	3	4	6	7	5	4	3	3	2	1	5
10	27.KANYIKI	3	4	6	7	5	4	3	5	2	1	
11	CIEMSA	3	4	6	7	5	4	3	5	2	1	
12	4.KANJAMANI	3	4	6	1	3	1	3	5	8	1	6
13	41.MIEZI TATU	3	4	6	1	3	1	5	3	8	1	
14	45.GATUMBI	3	4	6	9	5	6	5	5	2	4	7
15	KEMB 10	3	4	6	9	5	6	5	3	2	4	
16	KASONGO	3	4	6	5	5	4	5	5	8	1	8
17	JABER	3	4	6	5	5	4	5	5	9	1	
18	WERA	3	4	6	5	5	4	5	5	8	1	
19	POLISTA	3	4	6	5	5	4	3	5	8	1	9
20	SPOUNGE	3	4	6	5	5	4	3	5	9	1	
21	YALA SWAMP	3	5	3	0	1	1	5	1	2	1	10
22	KEMB 33	3	5	3	0	1	1	5	4	2	1	
23	UNKNOWN 3	3	5	3	0	1	1	5	9	2	1	
24	ODINGA	3	6	6	5	5	4	5	9	9	4	11
25	SINIA	3	6	6	5	5	4	5	9	9	1	
26	34.SINIA	3	6	6	5	5	4	3	9	9	4	
27	EX-SHIMBA	3	7	6	5	5	4	5	9	9	1	12
28	JAYALO	3	7	6	5	5	4	3	9	9	1	
29	JAYALO	3	7	6	5	5	4	5	9	9	1	
30	NYALEWE KWAR	3	7	6	5	5	4	5	9	9	2	
31	KINDOLO 1	3	7	6	7	5	4	5	5	2	1	13
32	SIPOGI	3	7	6	5	5	4	5	5	5	1	
33	NYATHI ODIEWO	3	7	6	5	5	4	5	9	7	1	14
34	OMBEKO LESO	3	7	6	5	5	4	5	9	8	1	
35	SINIA	3	7	6	5	5	4	5	9	8	4	15
36	SINIA	3	7	6	5	5	4	5	9	8	4	
37	SINIA	3	7	6	5	5	4	5	9	8	4	
38	ODINGA	3	8	6	5	5	4	5	9	-	-	16
39	ODINGA	3	8	6	5	5	4	3	9	-	-	

♦ The letters (B, D, F, G, H, I, J, N, S, V.) represent the descriptors least affected by the environment used to group the duplicates. The numbers assigned to each sample variety refer to scores describing a particular quality of a variety with regard to the descriptor.

3.1.1.2 Principle Component Analysis (PCA)

The character found to have the highest influence in discriminating between varieties was the shape of the central lobe followed by the type of leaf lobes. The shape of the central leaf lobe can therefore be used to assess differences among different sweet potato varieties. Table 3.2 shows the results obtained from Principle component analysis.

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Table 3.2Principle component analysis generated using GENSTAT (Fifth Edition) using the variance/covariance method

Descriptor	code	LV 1*	LV 2*
Plant type	A	0.02027	-0.05394
Vine internode diameter	B	-0.02553	-0.01187
Vine internode length	C	-0.00363	-0.03354
Predominant vine color	D	0.02328	0.21720
Secondary vine color	E	-0.03854	0.18820
General leaf outline	F	0.00429	0.21505
Type of leaf lobes	G	0.01833	**0.63948**
Number of leaf lobes	H	0.01566	0.30919
Shape of central leaf lobe	I	**0.03202**	0.30267
Mature leaf size	J	0.00462	0.00828
Abaxial leaf vein pigmentation	K	0.01996	0.09004
Mature leaf color	L	0.01009	0.09501
Immature leaf color	M	0.01729	0.38934
Petiole pigmentation	N	0.00676	0.26758
Petiole length	O	-0.00282	-0.00139
Storage root shape	P	-0.61289	-0.04210
Storage root defects	Q	-0.16728	-0.01816
Storage root cortex thickness	R	-0.40042	-0.05529
Predominant color of root skin	S	-0.57438	0.14497
Intensity of predominant skin color	T	-0.17478	0.01594
Secondary skin color	U	-0.03465	0.00059
Predominant flesh color	V	-0.17987	0.01976
Secondary flesh color	W	-0.12123	0.03857
Distribution of secondary flesh color	X	-0.14744	0.04633
	% variation	22.36	14.46

*LV refers to Latent Vector, which represent linear combinations of the original variables and can be used to assess differences among two individuals.

3.1.2 Morphological Characterization on Farmer-preferred Varieties

3.1.2.1 Morphological Descriptors

The morphological descriptors used were able to distinguish between all the varieties of sweet potato. None of the varieties had an identical combination of scores. Table 3.3 shows the morphological characterization of the farmer selected varieties.

Table 3.3 Morphological characterization of farmer-preferred varieties

	DESCRIPTORS*																							
VAR_NAME	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
EX-SHIMBA	7	3	1	7	2	6	5	5	4	5	7	3	3	9	3	2	0	3	8	1	2	1	0	0
BEUREGARD	7	3	1	1	0	6	3	3	2	3	2	2	3	1	1	3	0	5	5	3	0	7	2	2
ZAPALLO	5	1	1	9	0	6	7	5	5	3	7	3	6	9	1	6	0	5	4	2	0	8	4	9
NAMASWAKHE	7	5	1	5	4	6	5	5	4	5	8	2	6	3	3	2	0	7	7	2	0	4	4	3
MARIANGOLA	7	3	1	9	0	6	5	5	4	3	8	5	7	9	3	5	0	3	9	1	0	4	8	6
NYATHIODIEWO	9	1	3	4	0	4	0	1	1	3	6	2	3	3	3	3	0	5	8	3	0	3	3	3
ODINGA	7	3	1	6	2	6	5	5	4	5	7	3	9	9	3	8	0	1	8	2	0	4	4	8
*K-117	7	5	1	3	2	4	0	1	1	5	2	2	3	1	3	2	0	7	2	1	0	1	0	0
NEMANETE	7	3	1	5	4	6	7	5	5	3	5	6	9	5	3	4	0	7	6	1	0	4	0	0
SPONGI	7	3	1	4	0	6	5	5	4	3	3	3	6	5	3	3	0	3	8	2	0	1	4	3
KSP-013	7	5	1	4	6	6	5	5	4	3	8	3	9	5	1	8	0	3	8	2	0	1	0	0
BUNGOMA	7	3	1	5	6	6	5	5	4	5	8	3	6	3	3	8	0	3	8	2	0	4	3	9
SINIA	9	3	3	6	2	6	5	5	4	5	8	3	9	9	3	8	5	5	8	2	0	1	2	3
PEDRO	5	3	1	4	0	6	7	5	6	5	7	3	6	3	1	4	0	5	4	2	0	7	3	3
AMINA	7	3	3	4	0	4	0	1	8	3	8	2	3	5	1	4	3	5	8	3	0	8	2	3
KILUNGU	7	3	3	4	0	5	5	3	4	5	4	3	6	4	3	8	0	3	9	3	0	2	0	0
BUDAGALLA	7	5	1	4	0	6	7	5	4	5	4	2	6	5	3	6	0	5	2	3	0	2	4	3
CHEGLINA	7	3	1	5	0	6	5	5	4	3	4	6	3	5	3	8	0	5	2	2	0	2	0	0
MOWAR NGOROBU	9	5	3	3	0	6	1	5	1	3	4	3	1	5	1	8	2	5	2	3	0	1	0	0
WERA	7	3	1	4	2	6	5	3	4	1	2	2	1	1	3	2	0	7	2	3	0	5	0	0
KINDOLO	7	3	1	4	6	6	5	5	4	3	7	3	6	3	3	4	0	5	2	3	0	1	0	0
NYATONGE	5	3	1	4	0	6	7	5	6	5	2	3	1	3	3	3	0	7	2	2	0	4	4	3

* The letters A –X refer to the vine, leaf and storage root descriptors used to characterize the farmer selected varieties and the sweet potato collections. For a complete list of these descriptors see appendix 1. The numbers assigned to each sample variety refer to scores describing a particular quality of the variety with regard to the specific descriptor.

3.1.2.2 Dendrogram from Morphological Data

From dendrogram generated using the UPGMA method (Figure 3.1) all the varieties clustered as unique varieties within an evolutionary distance of 30 – 70 %. A hundred bootstrap replicates were analysed using Treecon (Van de Peer and De Wachter, 1993) to check the quality of the branching in the dendrogram generated. The bootstrap values ranged from 0 – 93 %, while most of the values were below 50 %. There was no distinct clustering according to geographic location. However, Maria Angola and Nemanete (both from South America) clustered together from a genetic distance of approximately 0.55 with a bootstrap value of 23 %, K117 and Wera (both from South Nyanza) formed a cluster at an evolutionary distance of approximately 45 % from each other and a bootstrap value of 27 %. One distinct feature about the dendrogram is that it separated the different varieties into five groups with regard to the descriptor, shape of central leaf lobe. The shape of the central leaf lobe was found to have the highest influence in discriminating among the different varieties. Group A had an elliptic shape, Group B had the semi – elliptic shape, Group C had the Lanceolate Shape, Group D had varieties with triangular and teeth shapes of the central lobe while E (Amina) has linear shape of the central lobe. The varieties Beuregard and Wera, both indicated with "(A)" had elliptic shapes but did not separate into the "A" group.

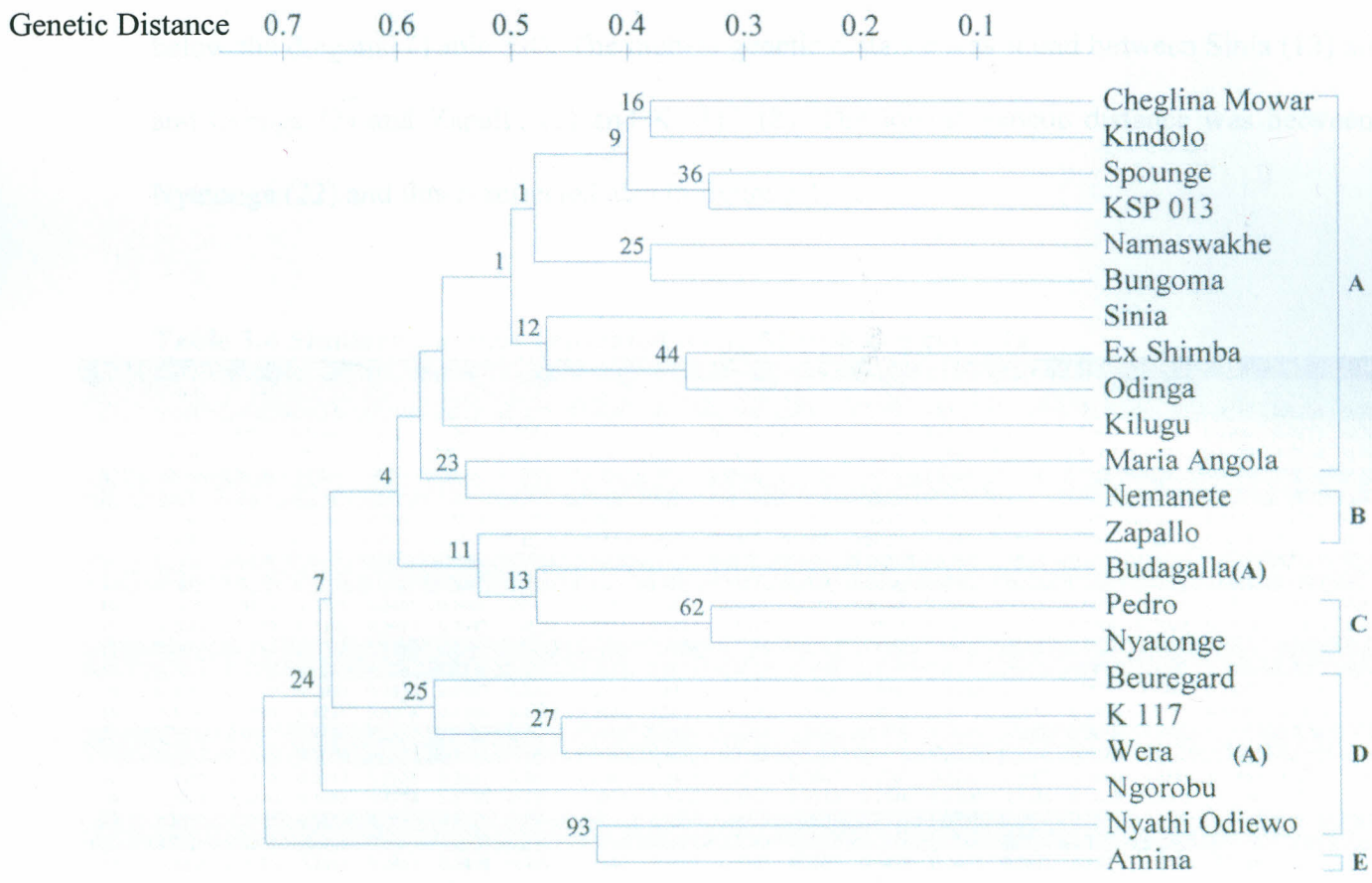

Figure 3.1 Dendrogram generated from morphological data

3.1.2.3 Similarity Matrix from Morphological data

A similarity matrix was generated using Popgene version 1.32 according to Nei's original measures of genetic identity and genetic distance (Nei, 1972). Nei's genetic identity is shown above the diagonal and genetic distance is shown below the diagonal (Table 3.4). The highest genetic distance was found between Sinia (13) and K -117 (8), Amina (15) and Odinga (7) and Zapallo (3) and K -117 (8). The lowest genetic distance was between samples Pedro (14) and Nyatonge (22) and this is reflected also in figure 3.1.

Table 3.4 Similarity matrix generated using Morphological data

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	****	0.6182	0.6818	0.7455	0.7091	0.7273	0.7636	0.7818	0.7091	0.7091	0.7636	0.7091	0.7545	0.7000	0.6636	0.7273	0.6909	0.7273	0.7545	0.6909	0.7545	0.6545
2	0.4810	****	0.7182	0.6727	0.7091	0.7091	0.6727	0.7091	0.6909	0.7273	0.6909	0.6727	0.6455	0.7182	0.7364	0.6909	0.7273	0.7273	0.6818	0.7818	0.7182	0.7273
3	0.3830	0.3310	****	0.7182	0.7364	0.6636	0.7545	0.6273	0.7182	0.7727	0.7364	0.7364	0.6545	0.8182	0.6545	0.6636	0.7364	0.7182	0.7091	0.6636	0.7273	0.7545
4	0.2938	0.3964	0.3310	****	0.7636	0.6727	0.8000	0.7455	0.7636	0.7818	0.7636	0.8364	0.7364	0.7545	0.6455	0.6909	0.8000	0.7455	0.6455	0.7455	0.7364	0.7818
5	0.3438	0.3438	0.3060	0.2697	****	0.6545	0.7818	0.6727	0.7636	0.7818	0.7636	0.7818	0.6818	0.7000	0.6636	0.7273	0.7273	0.7636	0.6636	0.7273	0.7364	0.7091
6	0.3185	0.3438	0.4100	0.3964	0.4238	****	0.6364	0.7273	0.6364	0.7091	0.6545	0.6545	0.6818	0.7182	0.8091	0.6727	0.7091	0.6909	0.7000	0.6727	0.6818	0.6909
7	0.2697	0.3964	0.2816	0.2231	0.2461	0.4520	****	0.6909	0.7455	0.8000	0.8000	0.8364	0.8091	0.7545	0.6273	0.7273	0.7273	0.7636	0.6636	0.7273	0.7545	0.7636
8	0.2461	0.3438	0.4664	0.2938	0.3964	0.3185	0.3697	****	0.7273	0.6545	0.7091	0.6545	0.6273	0.6455	0.6818	0.6727	0.7091	0.7091	0.7000	0.8000	0.7000	0.6909
9	0.3438	0.3697	0.3310	0.2697	0.2697	0.4520	0.2938	0.3185	****	0.7273	0.7636	0.7273	0.6455	0.7182	0.6636	0.6727	0.7273	0.8000	0.7000	0.7455	0.7545	0.7273
10	0.3438	0.3185	0.2578	0.2461	0.2461	0.3438	0.2231	0.4238	0.3185	****	0.8545	0.8364	0.7727	0.7909	0.7182	0.7818	0.8000	0.8182	0.7182	0.7636	0.8273	0.8182
11	0.2697	0.3697	0.3060	0.2697	0.2697	0.4238	0.2231	0.3438	0.2697	0.1572	****	0.8364	0.7727	0.7364	0.7000	0.7636	0.7455	0.8182	0.7909	0.7636	0.8273	0.7091
12	0.3438	0.3964	0.3060	0.1787	0.2461	0.4238	0.1787	0.4238	0.3185	0.1787	0.1787	****	0.8091	0.7909	0.6455	0.7818	0.7636	0.8000	0.6636	0.7273	0.8091	0.7818
13	0.2816	0.4378	0.4238	0.3060	0.3830	0.3830	0.2118	0.4664	0.4378	0.2578	0.2578	0.2118	****	0.7455	0.6909	0.7182	0.7182	0.7364	0.7273	0.6818	0.7636	0.7545
14	0.3567	0.3310	0.2007	0.2816	0.3567	0.3310	0.2816	0.4378	0.3310	0.2346	0.3060	0.2346	0.2938	****	0.7091	0.7182	0.7909	0.7364	0.7091	0.6818	0.8000	0.8455
15	0.4100	0.3060	0.4238	0.4378	0.4100	0.2118	0.4664	0.3830	0.4100	0.3310	0.3567	0.4378	0.3697	0.3438	****	0.6818	0.7364	0.7000	0.7091	0.6818	0.6909	0.6455
16	0.3185	0.3697	0.4100	0.3697	0.3185	0.3964	0.3185	0.3964	0.3964	0.2461	0.2697	0.2461	0.3310	0.3310	0.3830	****	0.7636	0.7818	0.7364	0.7818	0.7909	0.7091
17	0.3697	0.3185	0.3060	0.2231	0.3185	0.3438	0.3185	0.3438	0.3185	0.2231	0.2938	0.2697	0.3310	0.2346	0.3060	0.2697	****	0.8000	0.7545	0.7636	0.7909	0.8000
18	0.3185	0.3185	0.3310	0.2938	0.2697	0.3697	0.2697	0.3438	0.2231	0.2007	0.2007	0.2231	0.3060	0.3060	0.3567	0.2461	0.2231	****	0.7909	0.8000	0.8273	0.7455
19	0.2816	0.3830	0.3438	0.4378	0.4100	0.3567	0.4100	0.3567	0.3567	0.3310	0.2346	0.4100	0.3185	0.3438	0.3438	0.3060	0.2816	0.2346	****	0.7182	0.7636	0.7000
20	0.3697	0.2461	0.4100	0.2938	0.3185	0.3964	0.3185	0.2231	0.2938	0.2697	0.2697	0.3185	0.3830	0.3830	0.3830	0.2461	0.2697	0.2231	0.3310	****	0.8273	0.7636
21	0.2816	0.3310	0.3185	0.3060	0.3060	0.3830	0.2816	0.3567	0.2816	0.1896	0.1896	0.2118	0.2697	0.2231	0.3697	0.2346	0.2346	0.1896	0.2697	0.1896	****	0.7727
22	0.4238	0.3185	0.2816	0.2461	0.3438	0.3697	0.2697	0.3697	0.3185	0.2007	0.3438	0.2461	0.2816	0.1679	0.4378	0.3438	0.2231	0.2938	0.3567	0.2697	0.2578	****

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3.2 Molecular Profiling of Farmer-preferred Varieties

3.2.1 DNA Extraction

The DNA extraction profile of the samples used is shown in Figure 3.2 below.

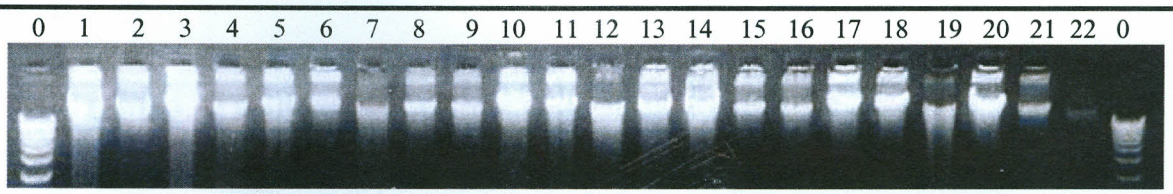

Figure 3.2 DNA gel electrophoresis.
Lane 0 = 1Kb DNA ladder. For the identity of the samples labelled 1 – 22 refer to Table 3.5.

3.2.1.1 DNA Quantification using Ethidium Bromide Staining

The quantity of the DNA samples was estimated to be 20 ng/µl using Ethidium Bromide staining and a series of DNA standards of known concentration (Figure 3.3).

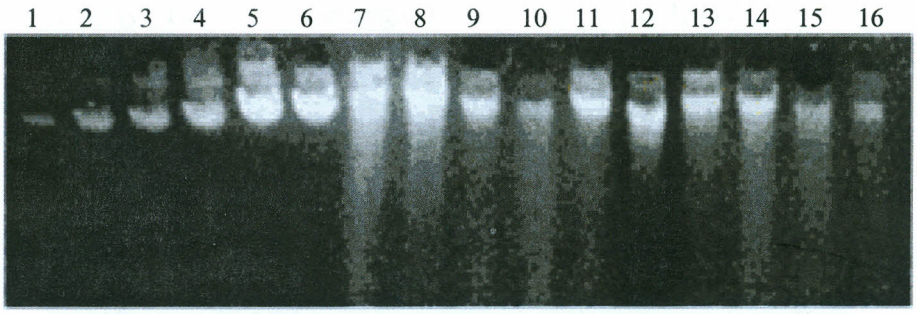

Figure 3.3 DNA gel electrophoresis with a series of DNA standards.
Lanes 1 – 6 are DNA standards (15, 31, 63, 125, 250, 500 ng/6µl respectively); Lanes 7 – 16 are representative DNA samples.

3.2.1.2 Assessment of DNA Quality Using Spectrophotometric Method

The DNA samples differed in their purity as evidenced by the different ratios of OD 260: OD 280 which ranged approximately from 1.7 -2.0 (Table 3.5).

Table 3.5 Quality of DNA using Optical Density (OD) Ratios

code	Sample identity	OD 260/OD 280
1	Nyathi odiewo	1.82
2	Spongi	1.92
3	SPK 013	1.94
4	Zapallo	1.989
5	Maria Angola	2.016
6	Bungoma	1.805
7	Ex Shimba	1.922
8	Sinia	2.00
9	Budagalla	1.87
10	Namaswakhe	1.79
11	K 117	1.839
12	Beuregard	1.847
13	Kindolo	1.837
14	Nyatonge	1.881
15	Wera	1.898
16	Odinga	1.896
17	Cheglina Mowar	1.695
18	Nemanete	1.878
19	Pedro	1.91
20	Ngorobu	1.864
21	Amina	1.892
22	Kilungu	1.775

3.2.2 ISSR

3.2.2.1 Number of Phenotypes Identified by ISSR Primers

Primers differed in the ability to identify unique multiband phenotypes among the 22 sampled varieties. Primer ISSR 1 identified 16 phenotypes, primer ISSR 2 identified 11, primer ISSR 11 identified 20, primer ISSR 14 identified 9 and primer ISSR 15 identified 15 phenotypes. None of the ISSR primers was able to discriminate between the 22 varieties. Table 3.6 shows the number of products (total and polymorphic bands) generated by each primer across the 22 varieties and it suggests that there were no monomorphic bands, no conserved alleles across the different varieties of sweet potato.

Table 3.6 Amplification products observed in 22 Ipomoea *batatas* varieties used for fingerprinting based on five ISSR primers.

Primer	Total no. of bands	Polymorphic bands	No. of phenotypes
ISSR 1	16	16	16
ISSR 2	11	11	11
ISSR 11	13	13	20
ISSR 14	7	7	9
ISSR 15	11	11	15
TOTAL	**58**	**58**	
MEAN	**11.6**	**11.6**	

The number of individual specific bands generated by the primers was also checked visually. Primer ISSR 11 did not generate any individual specific bands while primer ISSR 1 generated 4 specific bands for the variety Cheglina Mowar and 2 specific bands for Nemanete. ISSR 14 generated one specific band for the

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variety Pedro, ISSR 2 generated one specific band for variety Zapallo and ISSR 15 produced 2 individual specific bands for the variety Nyathi Odiewo. The distribution of individual specific ISSR markers is shown in Table 3.7.

Table 3.7 Primers that revealed individual specific bands

	Nyathi odiewo	Zapallo	Cheglina Mowar	Nemanete	Pedro
ISSR1	0	0	**4**	**2**	0
ISSR14	0	0	0	0	**1**
ISSR2	0	**1**	0	0	0
ISSR15	**2**	0	0	0	0

3.2.2.2 Gel Scoring

From the gel scoring none of the ISSR primers was able to discriminate among the 22 varieties. An ISSR profile generated with one of the primers is shown in Figure 3.4.

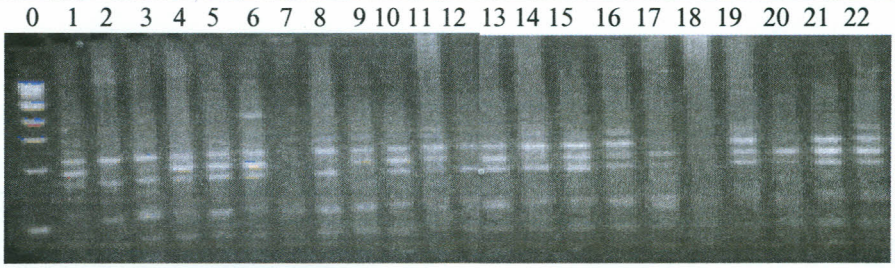

Figure 3.4 Amplification products using primer ISSR 11. Lane 0 – 1Kb DNA ladder; Lane 1 – 22 refer to amplification products of farmer selected varieties and are listed in Table 2.2.

3.2.2.3 Dendrogram from ISSR Data

From the tree generated using Treecon version 1.3b (Figure 3.4) all the varieties clustered as unique varieties within an evolutionary distance of 10 – 60 % from each other. Cheglina Mowar showed similarity to Ngorobu from a genetic distance of approximately 0.45. The bootstrap values ranged from 0 – 53 % but most were below 50 %. There was no distinct clustering with regard to geographical location; however two Tanzanian varieties, Sinia and Budagalla, clustered together from an evolutionary distance of approximately 30 % from each other at a bootstrap value of 12%.

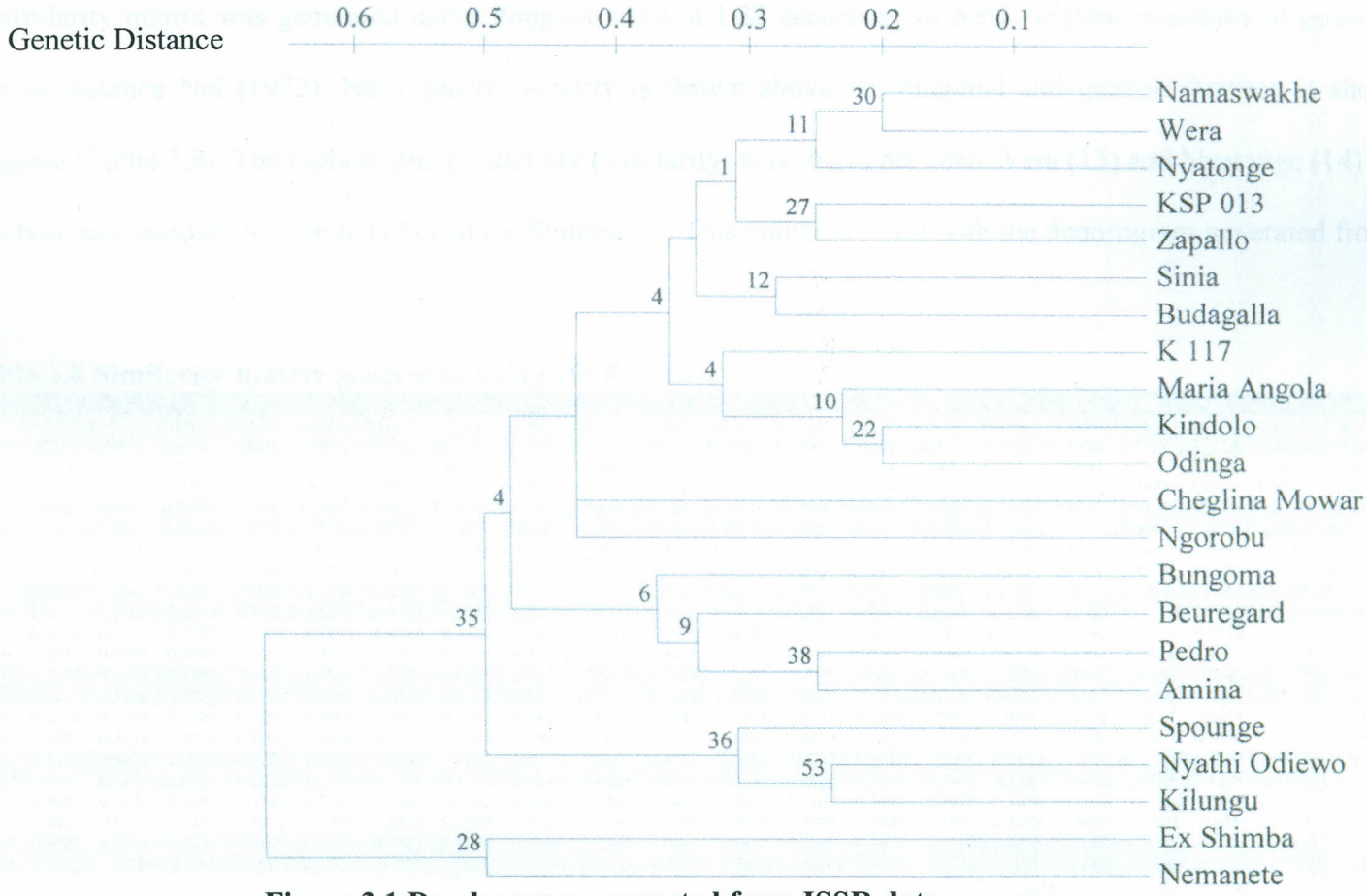

Figure 3.1 Dendrogram generated from ISSR data

3.2.2.5 Similarity Matrix from ISSR Data

A similarity matrix was generated using Popgene version 1.32 according to Nei's original measures of genetic identity and genetic distance Nei (1972). Nei's genetic identity is shown above the diagonal and genetic distance is shown below the diagonal (Table 3.8). The highest genetic identity (similarity) was found between Wera (15) and Nyatonge (14) and the lowest was between samples Nyatonge (14) and Ex Shimba (7). This coincides well with the dendrogram generated from ISSR data.

Table 3.8 Similarity matrix generated using ISSR data

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	****	0.6379	0.6034	0.5690	0.5172	0.6552	0.4655	0.5172	0.5862	0.6724	0.5345	0.6207	0.4310	0.6552	0.6724	0.5690	0.5862	0.4655	0.4828	0.6034	0.6379	0.7759
2	0.4495	****	0.4483	0.5517	0.5000	0.5000	0.8276	0.5345	0.5345	0.5172	0.3793	0.5000	0.7241	0.4310	0.5172	0.5172	0.4655	0.5172	0.7414	0.4483	0.4483	0.4828
3	0.5051	0.8023	****	0.7241	0.7414	0.6379	0.4483	0.6724	0.6724	0.7586	0.6207	0.6034	0.5172	0.6724	0.6897	0.7241	0.5690	0.5172	0.5000	0.5862	0.5862	0.6897
4	0.5639	0.5947	0.3228	****	0.7759	0.6724	0.5862	0.6724	0.7069	0.7586	0.4828	0.6724	0.6207	0.6034	0.6552	0.6552	0.4655	0.4483	0.6034	0.5517	0.5862	0.5862
5	0.6592	0.6931	0.2992	0.2538	****	0.7241	0.5345	0.7586	0.7241	0.7069	0.6724	0.7931	0.6724	0.6897	0.6724	0.7414	0.4828	0.5345	0.5517	0.6034	0.6379	0.6379
6	0.4229	0.6931	0.4495	0.3969	0.3228	****	0.6034	0.6552	0.7241	0.6379	0.3966	0.7241	0.4655	0.6207	0.6724	0.5345	0.5517	0.5345	0.4828	0.5345	0.8793	0.7759
7	0.7646	0.1892	0.8023	0.5341	0.6265	0.5051	****	0.5345	0.5690	0.4828	0.2414	0.4655	0.7241	0.3621	0.5172	0.4828	0.4655	0.5862	0.7069	0.4138	0.5517	0.4828
8	0.6592	0.6265	0.3969	0.3969	0.2763	0.4229	0.6265	****	0.7586	0.7414	0.6379	0.6897	0.5690	0.7241	0.7414	0.8103	0.4828	0.4655	0.4828	0.6724	0.5690	0.6034
9	0.5341	0.6265	0.3969	0.3469	0.3228	0.3228	0.5639	0.2763	****	0.7414	0.5000	0.6552	0.5690	0.6897	0.7069	0.7414	0.5172	0.5000	0.5172	0.6034	0.6724	0.6379
10	0.3969	0.6592	0.2763	0.2763	0.3469	0.4495	0.7282	0.2992	0.2992	****	0.5862	0.6724	0.5517	0.7759	0.8276	0.7241	0.5000	0.4138	0.5345	0.6207	0.5862	0.7241
11	0.6265	0.9694	0.4769	0.7282	0.3969	0.9249	1.4214	0.4495	0.6931	0.5341	****	0.5690	0.4138	0.7069	0.5517	0.6897	0.5690	0.5517	0.4310	0.6552	0.3793	0.5172
12	0.4769	0.6931	0.5051	0.3969	0.2318	0.3228	0.7646	0.3716	0.4229	0.3969	0.5639	****	0.7069	0.7586	0.7069	0.6379	0.3793	0.3621	0.6552	0.6724	0.7414	0.5690
13	0.8416	0.3228	0.6592	0.4769	0.3969	0.7646	0.3228	0.5639	0.5639	0.5947	0.8824	0.3469	****	0.5345	0.5862	0.6552	0.3966	0.4138	0.8103	0.5172	0.4828	0.3793
14	0.4229	0.8416	0.3969	0.5051	0.3716	0.4769	1.0159	0.3228	0.3716	0.2538	0.3469	0.2763	0.6265	****	0.8448	0.8103	0.5517	0.4310	0.5517	0.7414	0.5690	0.6379
15	0.3969	0.6592	0.3716	0.4229	0.3969	0.3969	0.6592	0.2992	0.3469	0.1892	0.5947	0.3469	0.5341	0.1686	****	0.7931	0.6034	0.5517	0.6034	0.7931	0.6207	0.6897
16	0.5639	0.6592	0.3228	0.4229	0.2992	0.6265	0.7282	0.2103	0.2992	0.3228	0.3716	0.4495	0.4229	0.2103	0.2318	****	0.5690	0.5517	0.5690	0.7241	0.4828	0.5862
17	0.5341	0.7646	0.5639	0.7646	0.7282	0.5947	0.7646	0.7282	0.6592	0.6931	0.5639	0.9694	0.9249	0.5947	0.5051	0.5639	****	0.6724	0.4483	0.5690	0.5690	0.6379
18	0.7646	0.6592	0.6592	0.8023	0.6265	0.6265	0.5341	0.7646	0.6931	0.8824	0.5947	1.0159	0.8824	0.8416	0.5947	0.5947	0.3969	****	0.4655	0.5862	0.5172	0.5517
19	0.7282	0.2992	0.6931	0.5051	0.5947	0.7282	0.3469	0.7282	0.6592	0.6265	0.8416	0.4229	0.2103	0.5947	0.5051	0.5639	0.8023	0.7646	****	0.6034	0.5000	0.3966
20	0.5051	0.8023	0.5341	0.5947	0.5051	0.6265	0.8824	0.3969	0.5051	0.4769	0.4229	0.3969	0.6592	0.2992	0.2318	0.3228	0.5639	0.5341	0.5051	****	0.5862	0.6207
21	0.4495	0.8023	0.5341	0.5341	0.4495	0.1286	0.5947	0.5639	0.3969	0.5341	0.9694	0.2992	0.7282	0.5639	0.4769	0.7282	0.5639	0.6592	0.6931	0.5341	****	0.7931
22	0.2538	0.7282	0.3716	0.5341	0.4495	0.2538	0.7282	0.5051	0.4495	0.3228	0.6592	0.5639	0.9694	0.4495	0.3716	0.5341	0.4495	0.5947	0.9249	0.4769	0.2318	****

3.3 Analysis of Sweet potato Database File using GIS

3.3.1 Mapped Sweet Potato Collection Sites

The locations of the sweet potato collection sites were well recorded and GIS (latitude and longitude co-ordinates) were assigned to the collections. These readings were used to generate a map with DIVA-GIS software (Figure 3.6).

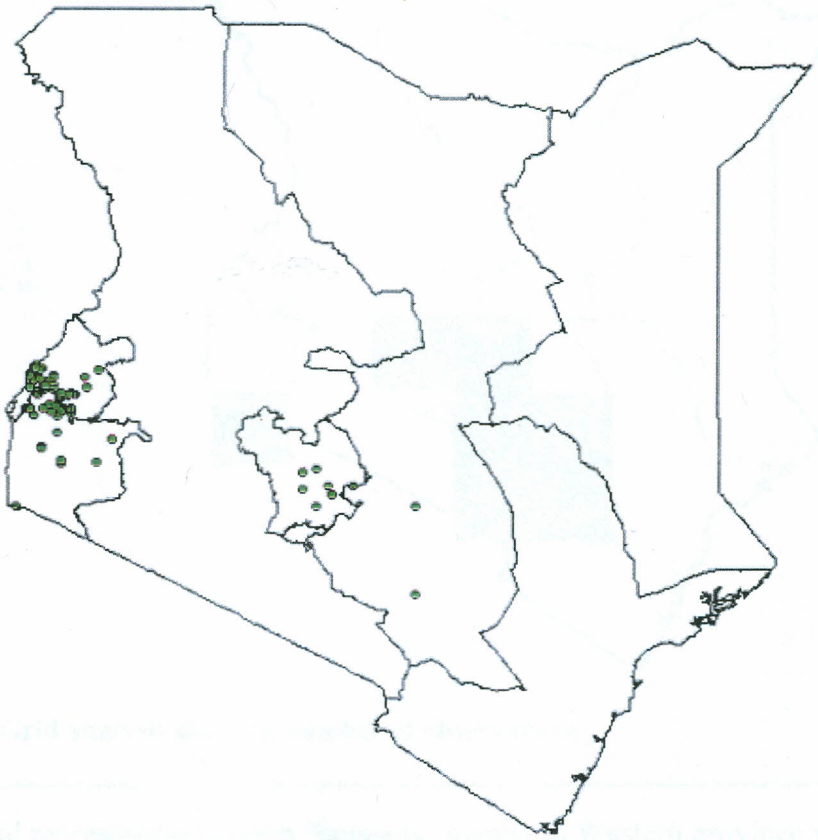

Figure 3.6 Map of Kenya generated by DIVA-GIS 4.2 showing collection sites of sweet potato varieties

Each point represents a unique record, i.e., variety or accession. For a map showing provinces in Kenya refer to Appendix 3.

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3.3.2 Grid analysis

3.3.2.1 Number of Observations (Richness)

The different colours represent the varying number of collections per given area as revealed by DIVA-GIS analysis (Figure 3.7).

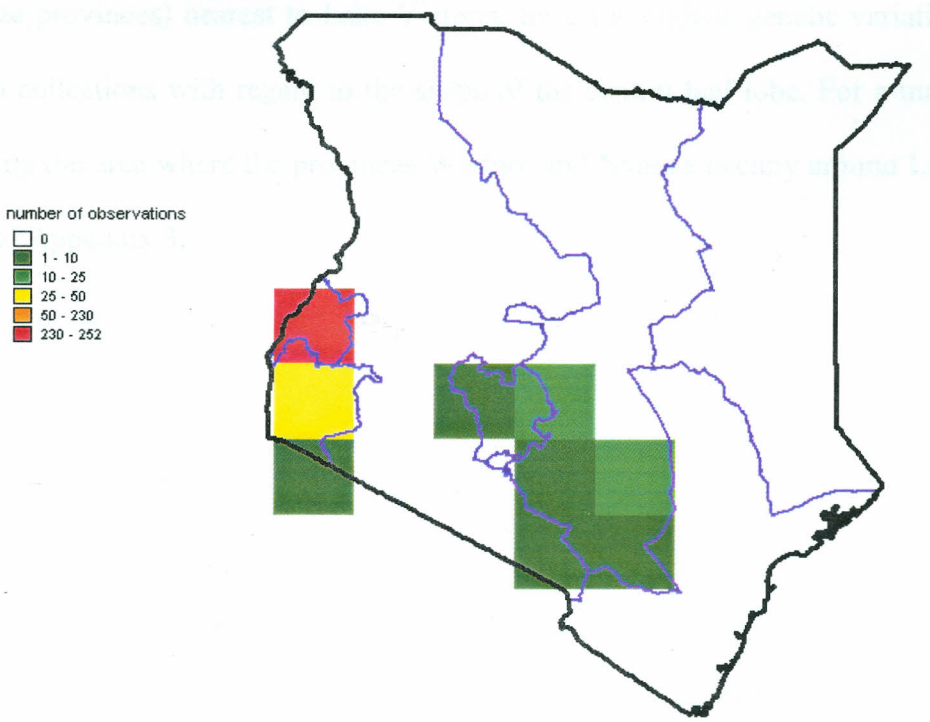

Figure 3.7 Grid analysis showing number of observations

The red grid represents collection "hotspots" mainly in Western province whereas yellow grids in Nyanza province. The green grids represent collection sites with only 1 – 25 varieties per grid which were distributed mainly in Central and Eastern Provinces.

3.3.2.2 Genetic Variation using Shape of the Central Leaf Lobe

3.3.2.2 Genetic Variation using Shape of the Central Leaf Lobe

The shape of the central leaf lobe was found to have the highest influence in discriminating among varieties (see Table 3.3). DIVA-GIS was used to visualize the diversity of the shape of central leaf lobe descriptor in relation to collection points of sweet potato varieties (Figure 3.8). The different colors represent the genetic variation in the collection of sweet potato with regard to their geographical collection sites. The legend on the left side of the map indicates that the Western part of Kenya (Western and Nyanza provinces) nearest to Lake Victoria, have the highest genetic variation of sweet potato collections with regard to the shape of the central leaf lobe. For a map of Kenya showing the area where the provinces Western and Nyanza occupy around Lake Victoria refer to Appendix 3.

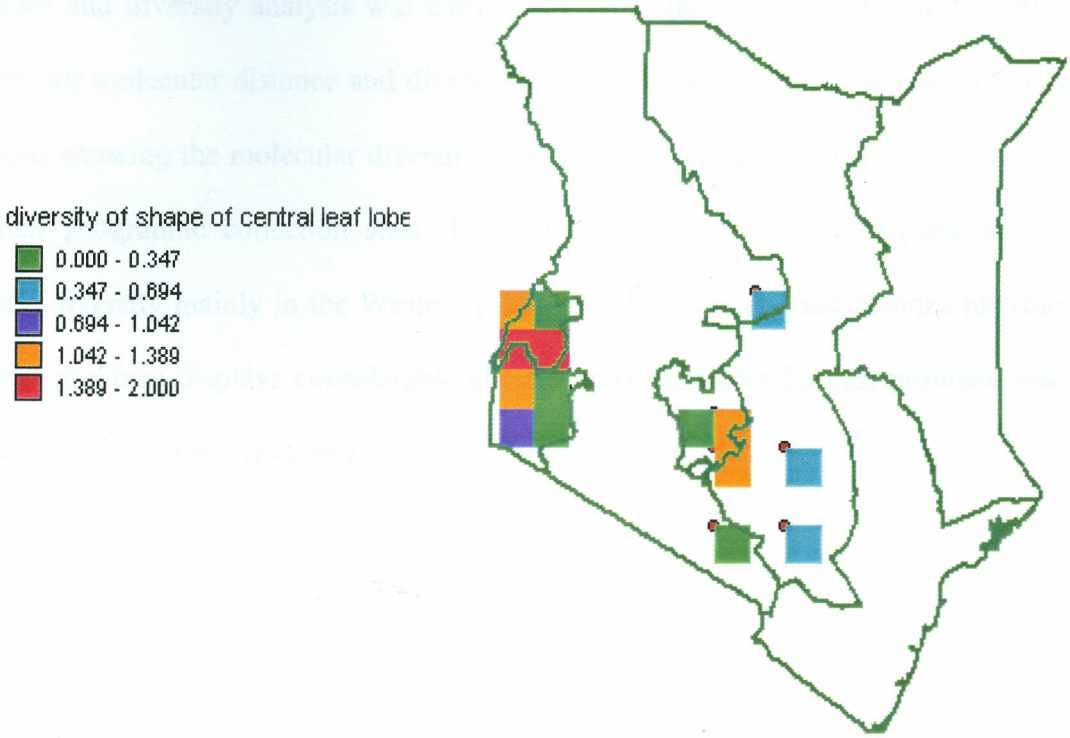

Figure 3.8 Grid analysis showing diversity of Sweet potato Varieties using Shape of Central leaf lobe

3.3.2.3 Molecular Distance using DIVA-GIS 4.2

DIVA-GIS was also used in molecular analysis of the sweet potato collections. Molecular distance and diversity analysis was carried out using Jaccard's coefficient of similarity matrix for molecular distance and diversity estimates (J). Figure 3.9 represents the grid analysis showing the molecular diversity of Kenyan sweet potato collections with regard to their geographic collection sites. The red boxes/grids represent regions with high genetic diversity mainly in the Western part of Kenya (Western and Nyanza provinces). Central province displays considerable genetic diversity while Eastern province has the lowest level of genetic diversity.

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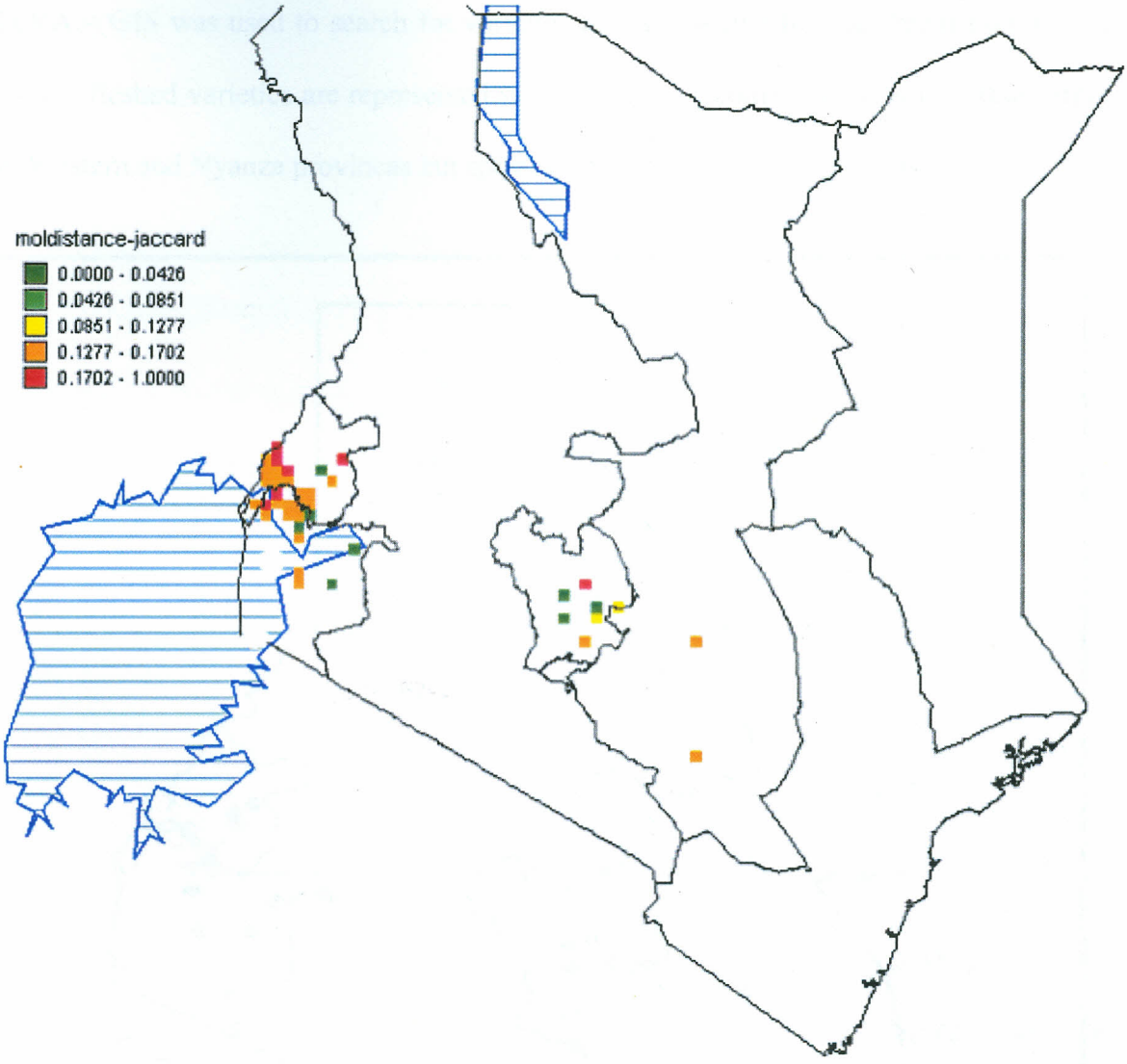

Figure 3.9 Grid analysis showing molecular distance and diversity of Kenyan sweet potato collections

3.3.2.4 Search Query Tool of DIVA-GIS

DIVA – GIS was used to search for varieties that are orange fleshed. From Figure 3.10 orange fleshed varieties are represented with the yellow points and are found abundantly in Western and Nyanza provinces but sparsely in Central province of Kenya.

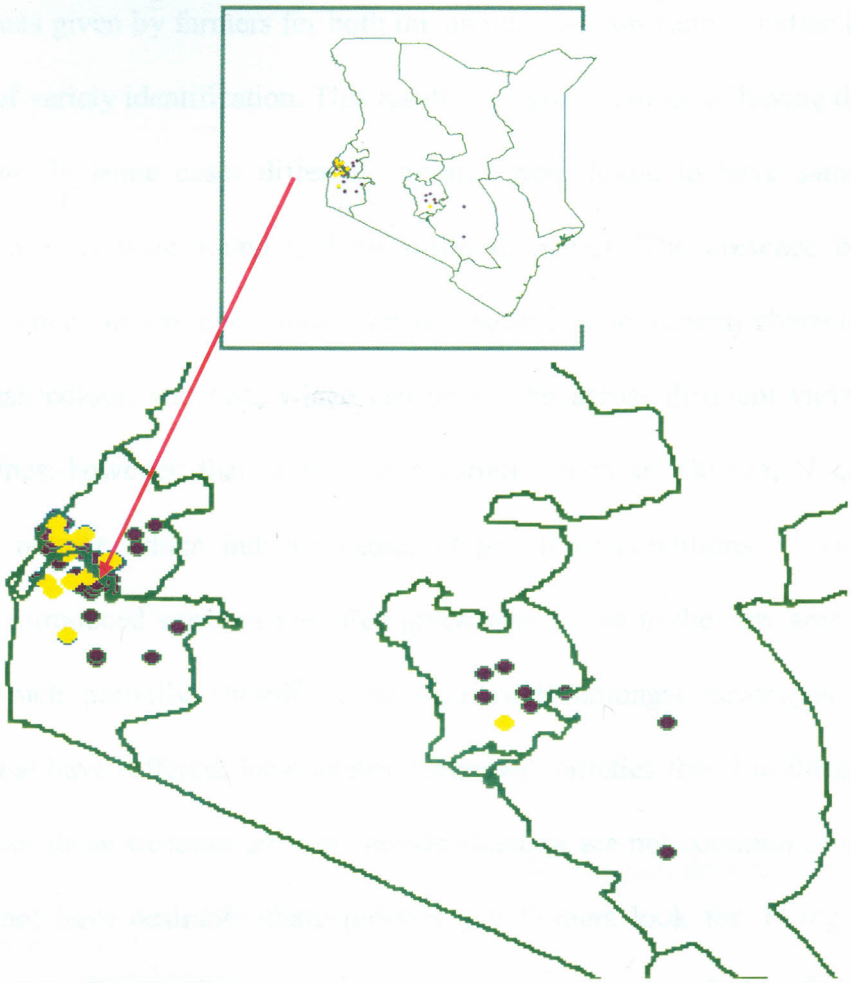

Figure 3.10 Orange-fleshed varieties displayed by DIVA-GIS analysis

CHAPTER 4

4.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 DISCUSSION

Four hundred and eighty three sweet potato varieties were found unique and one hundred and fifty seven varieties had duplicates in the collections. It was found that the use of local names given by farmers for both the unique and duplicate varieties is not a reliable method of variety identification. This results in higher chances of having duplicates in the collections. In some cases different varieties were found to have same names while similar varieties were found to have different names. The presence of synonyms is expected since farmers name most varieties according to varietal characteristics such as yield, flesh colour, and taste which can be similar across different varieties. There are some names, however, that farmers give varieties such as; Odinga, Nyathi odiewo and Cheglina mowar, which indicate names of people or conditions of human beings. In addition, introduced varieties are often given new names in the new area where they are grown which partially contributes to similarities amongst genotypes from different regions that have different local names. Unknown varieties found in the collection could mean either those varieties are new introductions, or are not common or still further that they do not have desirable characteristics that farmers look for. Using the method of eliminating duplicates, these unknown varieties could be identified by forming duplicates with other known and named varieties. From the principle component analysis, the shape of the central leaf lobe had the highest influence in discriminating varieties. Using DIVA-GIS to assess the level of variations of shape of central leaf lobe in relation to

geographical collection site, Western Kenya showed highest variation compared to the other regions. This implies that certain factors have contributed to higher variation of the sweet potato varieties in Western Kenya compared to the other sweet potato growing regions in Kenya. The dendrogram generated using morphological characterization separated the 22 varieties into five groups each having a different shape of the central leaf lobe. This indicates that this descriptor had a higher influence in distinguishing the varieties.

In the cluster analysis of the morphological and ISSR data of farmer varieties, all the varieties clustered as unique varieties. The clustering of two South American varieties, and two Kenyan ones from Nyanza province, by morphological and molecular analysis respectively, shows similarities between varieties from similar geographical locations. The geographical clustering found in this study was not distinct and this could be due to the extensive exchange of germplasm between farmers and research iInstitutions (e.g KARI) in the region. Yockteng *et al,* (2003) also observed lack of correlation between these components using ISSR markers in studying the relationships among pansies.

Using morphological characterization, two varieties, Maria Angola and Nemanete, from South America which is a primary center of diversity, formed one cluster displaying their divergence from varieties from other regions. Gichuki *et al.,* (2003) concluded in a study with RAPD markers that South American varieties were significantly different from genotypes of other regions. Gichuki and co-workers also concluded furhter that East

African varieties are different from South American varieties. The clustering of the varieties as displayed by both morphological and ISSR markers was different indicating that the polymorphism displayed by the two techniques was different. Lelley *et al,* (2000) similarly concluded that morphological and molecular classification did not always correspond despite morphological characters being genetically controlled. This is because morphological traits are highly influenced by the environment. In addition a random whole genome molecular marker like ISSR will generate an overall genetic profile that is not necessarily related to the limited number of genes at the basis of a phenotypic or morphological difference. The ISSR tree was characterized by much lower bootstrap values compared to the morphological tree. This shows that ISSR markers revealed a lower level of variation than the morphological markers. This can be attributed to the few number of ISSR primers used and the dominant nature of ISSR markers.

The DNA extraction protocol used in this investigation is highlighted on page 30. However polyvinyl pyrolidone (PVP) added in the extraction buffer was to prevent the oxidation of the high amounts of polyphenols present in sweet potato. Where PVP was not added, the DNA obtained was brown in colour (due to oxidized polyphenols) which does not produce PCR product, presumably due to inhibition, an observation also made by Peterson *et al,* (1997) and Guillemaut and Marchal-Drouard, (1992). It was found that the crushing step (disruption of plant tissue) is vital when embarking on extraction because it determines the quantity of DNA. The quality of DNA was checked using the Optical Density (OD) ratio at 260nm and 280nm wavelengths. Most of the OD readings

were between 1.8 and 2.0, but some were lower than 1.8 or higher than 2.0 indicating the presence of contaminants from components of DNA extraction and RNA molecules respectively. These contaminants can limit PCR analysis as observed by Kidwell and Osborn, (1992) and Fang *et al,* (1992). The presence of contaminants could partially be the reason why ISSR exhibited lower variation than expected due to the low DNA quality of some of the samples.

The average number of bands generated per primer was 11.6 higher than 10.3 fragments found by Panda *et al, (*2003), and 4.3 found by Raina *et al,* (2001). The higher number of fragments observed in the present study can be attributed to the choice of primers. The primers used have been identified as SSR regions common in sweet potato (Buteler *et al.*, 1999). Primers ISSR 14 and ISSR 2 contained sequences especially important in sweet potato and are short tandem repeats (STR), which demonstrate higher polymorphism and mendelian inheritance. Amongst the most abundant dinucleotide repeats in plants are noted as $(GT)_n$, $(AC)_n$, $(CT)_n$ by Wang *et al,* (1994) and were used in this study. Trojanowska-Rakoczy and Bolibok, (2004) recommended using 16-18 bp primers; sequences used in this study were 14 oligomers and 12 oligmers in length. Raina *et al,* (2001) and Panda *et al,* (2003) used 16 -17 mers long primers. Four of the primers revealed individual specific bands which will form the basis of locus specific amplification that are variety specific. This will be useful in identification of varieties.

Figure 3.8 shows the mapped sweet potato collection sites. Using the software DIVA-GIS, one can easily click using the mouse on a computer, at any point within the map and get information about the variety/ies collected at any particular location. DIVA-GIS was also used to generate a map that gives a rapid visualization of the number of observations used in the study. Western province has the highest density of varieties. Scott and Maldonaldo, (1998) stated that sweet potato production in developing countries is concentrated in those regions with low per capita incomes such as Western part of Kenya.

Molecular distance and diversity analysis was carried out, which revealed a high genetic diversity in Western province as compared to Nyanza, Central and Eastern provinces. Nyanza had a few spots of high diversity near the Lake Victoria region. Central also had considerable genetic diversity while Eastern had the lowest. This coincides well with the number of observations and the level of diversity with regard to shape of central leaf lobe in the Western part of Kenya. According to FAO (1995) and Scott and Maldonado (1998) production in Africa is concentrated in East Africa, especially the area around Lake Victoria. The short tropical day lengths found in East Africa contribute to natural hybridization which leads to arising of new varieties. This could be the reason of high sweet potato diversity in the region around the Lake Victoria. Ndolo *et al* (1998), in an evaluation study of Western and Nyanza province germplasm indicated significant variation in yield, dry matter content and culinary qualities.

This software (DIVA-GIS) has a powerful query tool which has the ability to search for records that match specific criteria for example, varieties that are virus resistant or are high yielding. To demonstrate this ability, we selected records that were orange fleshed. Orange fleshed varieties contain high levels of ß-carotene which is the precursor for Vitamin A; its deficiency causes night blindness in children.

This study has demonstrated how to generate plant accession maps using DIVA-GIS which can be updated with the addition of new accession collections. With new and updated data it will eventually be possible to generate a map showing distribution of sweet potato in Kenya with information of a particular variety easily available to the user.

4.2 CONCLUSIONS

The present study was able to answer the three research questions namely, whether there are any duplicates in the Kenyan sweet potato collection, if there is any variation in the sweet potato varieties grown by the Kenyan farmer and if these farmer varieties are genetically unique.

It can be concluded that: 1) through morphological characterization of the collection, there are duplicates in the Kenyan sweet potato collection; 2) through both morphological and ISSR markers, there are variations among the sweet potato varieties grown by the Kenyan farmer; and 3) that sweet potato varieties grown by the farmers in Kenya are genetically unique.

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In the area of characterization of sweet potato more remains to be done. Kenyan sweet potato germplasm has not been characterized using molecular markers and to be able to make any advances in the increase of sweet potato production in Kenya there needs to be good germplasm evaluation and characterization. According to Dhillon and Ishiki (1999), comprehensive analysis of the extent and distribution of genetic variation in sweet potato is essential for sound genetic conservation strategies. Sufficient genetic variation is important to search and identify unique, elusive and favourable alleles for improvement of staple crops including sweet potato.

Unique varieties identified from this study need to be conserved to allow further and future research using molecular markers. Morphological characterization can, however, be used in preliminary identification of unknown varieties before any extensive work with molecular markers is embarked on. Varieties selected by farmers as most popular have now been fingerprinted using both molecular and morphological markers and will therefore be useful for breeding and conservation considerations.

GIS is an important tool in analyzing sweet potato collections with spatial reference to the earth. This study has demonstrated the usefulness of geographical analysis of sweet potato, which will aid in future decision making such as collection of varieties and selecting for varieties that match specific criteria and stakeholder needs.

4.3 RECOMMENDATIONS

Rakoczy-Trojanowska and Bolibok (2004), stated that SSR (microsatellites) are attractive in cases where species are derived from geographically close populations where differentiation differs using other approaches. This is because SSR flanking regions are usually much conserved. ISSR was unable to distinguish varieties from different regions in Kenya. SSR primers have not been screened for Kenyan varieties. In a method described by Lian *et al* (2001), ISSR primers can be used to study SSR abundance and distribution in the sweet potato genome.

It would also be important to characterize those varieties that have been identified as duplicates using molecular or biochemical methods to ensure that they are completely the same. This is because duplicates are varieties that are completely similar at all levels; morphological, biochemical and molecular. In addition there are more farmer selected varieties that were not included in the study, and should therefore be studied so as to be able to identify and use them in future.

The sweet potato germplasm studied was only from the major sweet potato production areas. But this crop is grown in other parts of Kenya as well. It is therefore necessary to collect from these areas in order to be able to generate a map showing the distribution of sweet potato germplasm in the entire country and publish an updated distribution map that is more accurate and one that allows analysis to be performed with reference to the earth. The molecular distance results from this study using DIVA-GIS software should be

verified using molecular analysis such as SSR or AFLP. It is also recommended that a publicly accessible database should be developed that will include the findings of this project where additional information can be progressively added to make it a valuable resource.

In CIP Lima, Peru, there are about 6,000 collections of sweet potato stored in the gene bank from around the world (Huamán *et al*., 1999). East Africa, the largest sweet potato growing region in Africa has only 6 of its varieties stored in this gene bank collection, with 2 from Kenya. Results of the current studies show that there are hundreds of unique varieties in Kenya. From the four hundred and eighty three varieties found to be unique further analysis needs to be done to assess the genetic diversity to aid in creating a core collection of which duplicates can be sent to CIP for conservation.

It is also recommended that better markers such as AFLP and SSR be used to carry out the fingerprinting study to get better and more reliable results about the differences in the farmer-preferred sweet potato varieties.

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APPENDICES

Appendix 1: Descriptors used for Morphological Characterization

The morphological characters that were observed for each variety are listed as follows.

Outline of Morphological Descriptors Used for Eliminating Duplicates:

1. Vine Characters

1.2 Plant type: The length of the main vines determined this

1.3 Vine inter node diameter and length: This was the average expression of at least three inter nodes located in the middle section of the vine.

1.3.1 Inter node diameter

1.3.2 Inter node length

1.4 Vine pigmentation: This refers to anthocyanin (purple) pigmentation present in the vines besides the green colour.

1.4.1 Predominant colour of vine: This was evaluated by checking the whole vine from base to tip

1.4.2 Secondary colour of vine: This was more easily evaluated by using the younger leaves

2. Leaf Characters

2.1 Mature leaf shape

2.1.1 General leaf outline

2.1.2 Type of leaf lobes

2.1.3 Number of leaf lobes

2.1.4 Shape of central leaf lobe

2.2 Mature leaf size: Involves checking the length from the basal lobes to the tip of the leaves. The average expression of three leaves was recorded.

2.3 Abaxial leaf vein pigmentation: This described the most frequent expression of the distribution of anthocyanin (purple) pigmentation shown in the veins of the lower surface of the leaves

2.4 Foliage colour: This was recorded after observing the overall foliage color considering the colour of fully expanded mature and immature leaves shown by several plants. Colour variation due to virus symptoms was not recorded.

2.4.1 Mature leaf colour

2.4.2 Immature leaf colour

2.5 Petiole pigmentation and length

2.5.1 Petiole pigmentation: The distribution of anthocyanin (purple) pigmentation in the petiole of leaves was recorded. The most predominant color was recorded first.

2.5.2 Petiole length: The average petiole length, from the base to the insertion with the blade, of at least 3 leaves in the middle portion of a main vine.

3.0 Storage Root Characters

3.1 Storage root shape:The storage root outline shown in a longitudinal section was recorded

3.2 Storage root defects

3.3 Storage root cortex thickness

3.4 Storage root skin colour

3.4.1 Predominant skin colour

3.4.2 Intensity of predominant skin colour

3.4.3 Secondary skin colour

3.5 Storage root flesh colour: The flesh colour was described from cross and longitudinal sections made about the middle of freshly harvested storage roots.

3.5.1 Predominant flesh colour

3.5.2 Secondary flesh colour

3.5.3 Distribution of secondary flesh colour

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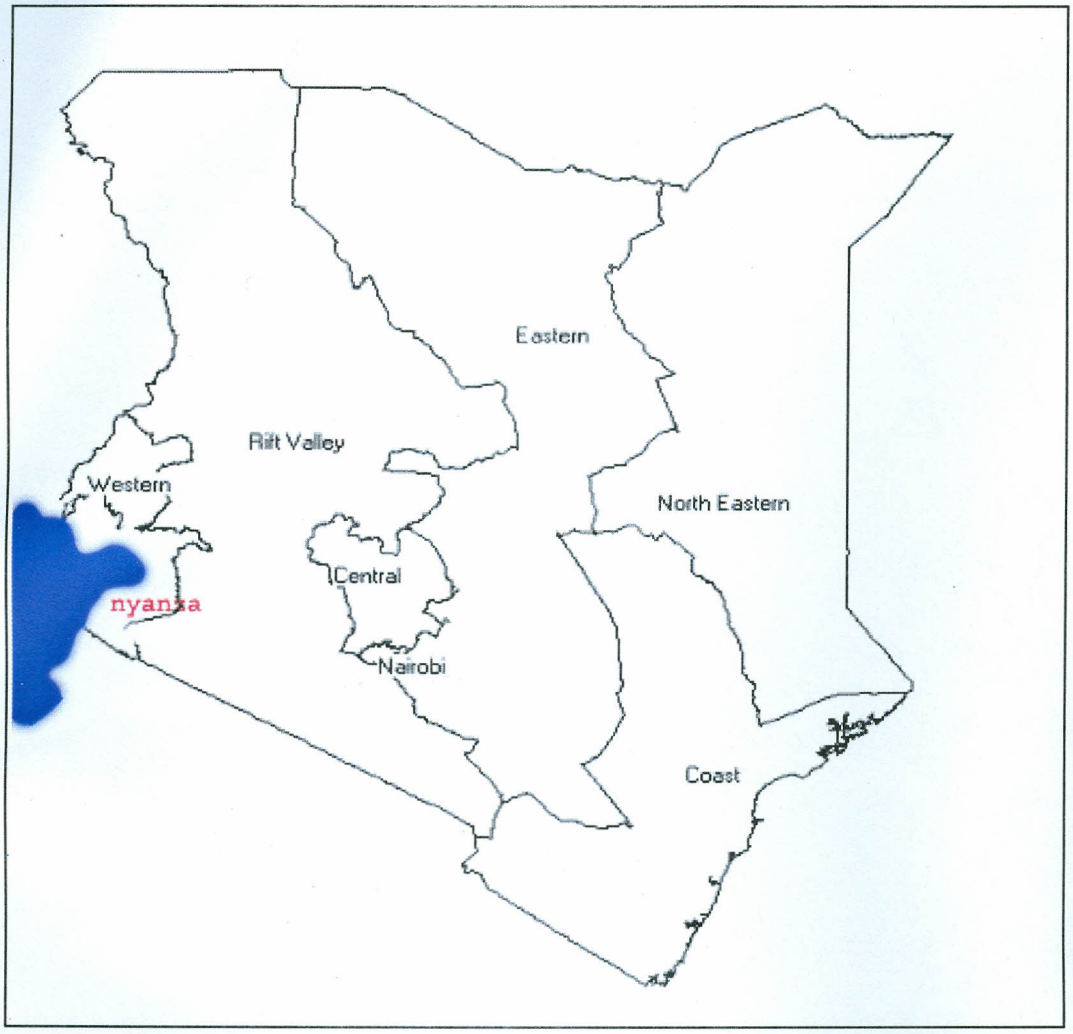
Appendix 2: Questionnaire used for Collection of Accessions from Farmers

1. Name of Farmer: .....
2. Local name of variety.....
3. Origin of variety.....
4. Village.....
5. Sub location.....
6. Location.....
7. Division.....
8. Province.....

Appendix 3: Map of Kenya showing Provinces and Lake Victoria



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