ANALYSIS OF DIVERSITY AMONG EAST AFRICAN SWEET POTATO CULTIVARS (*Ipomoea batatas*) USING MORPHOLOGICAL AND SIMPLE SEQUENCE REPEATS DNA MARKERS

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DECLARATION

I **GICHURU GATHONI VIRGINIA** hereby declare that this thesis is from my original research, and has not been submitted for the award of a degree in any University.

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DEDICATION

To my dad Patrick Gichuru, to my dear mum Florence Gichuru and to my beloved sisters; Jennifer and Rose, my brother John Paul. For all you have been in my life!

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LIST OF ABBREVIATIONS AND ACRONYMS

DNA	Deoxyribonucleic Acid
CIP	International Potato Centre
SSR	Simple Sequence Repeats
UPGMA	Unweighted Pair Group Method of arithmetic averages
AMOVA	Analysis of Molecular Variance
SPVD	Sweet potato Virus Disease
RAPD	Random Amplified Polymorphic DNA
ISSR	Inter-Simple Sequence Repeats
AFLP	Amplified Fragment length Polymorphism
cpDNA	Chloroplast DNA
mtDNA	mitochondrial DNA
FAO	Food and Agricultural Organisation
PRAPACE	Regional Potato and Sweet potato Improvement Programme for Eastern and Central Africa
RDA	Recommended Daily Allowance
PVP-25	Polyvinylpyrrolidone
EDTA	Ethylene diamine tetra-acetic acid
RT	Room temperature
C-TAB	N-Cetyl-N,N,N-trimethyl-ammonium bromide
TE	Tris-EDTA storage buffer
TBE	Tris-borate-EDTA electrophoresis buffer
UPGMA	Unweighted pair-group method of arithmetic averages
bp	Base pairs
KARI	Kenya Agricultural Research Institute
ANOVA	Analysis of Variance
rpm	Revolutions per minute
AVRDC	Asian Vegetable Research and Development Centre

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SUMMARY

East Africa is considered to be a secondary centre of origin of sweet potato and it is suspected that the wide morphological variation observed indicates wide genetic diversity in the region. To conserve and utilize the germplasm, it is important that proper assessment of the diversity of the East African sweet potato germplasm be made. Identification by molecular technologies is more commonly used over morphological characters since the latter can be influenced by environmental factors. In this study, we used molecular and morphological markers to study the genetic diversity of the germplasm in the region. Collections of cultivars were made from selected locations of Uganda, Kenya and Tanzania and subsequently established in pots in a screen house at Makerere University. A total of 266 cultivars were collected. After 3 weeks, the cultivars were screened for morphological characters using the CIP Research Guide. Cluster analysis was done using UPGMA in Treecon (Version 1.3). Based on morphological grouping, 57 cultivars, which were morphologically diverse, were randomly selected for DNA extraction and further analysis was done. Cluster analysis revealed only two major groupings (A & B) of sweet potatoes with very low bootstrap support of 0-54 %. The key distinguishing morphological markers were triangular leaf outline and a cordate shaped leaf outline for group A & B respectively. In addition, there were no geographical distinct morphological types identified. No population structure was detected. However, within each country, a high variation was observed (97.65%), suggesting that a wide range of cultivars is being grown in each country. Microsatellite (SSR) reactions were performed using four SSR primer combinations. The polymerase chain reaction (PCR) products were resolved using a high resolution metaphor agarose gel electrophoresis. Genetic

distance data matrices were subjected to Unweighted pair-group method of arithmetic averages (UPGMA) clustering using TREECON phylogenetic program Version 1.3 b. Two major sub-clusters were found by UPGMA at a bootstrap value of 54 %. Low bootstrap values (0-55 %) indicate absence of clusters and close genetic relationships among the cultivars. The majority of cultivars were in the range of 0.1-0.3 Nei's genetic distance from each other, which also shows close genetic relatedness. The clustering of sweet potato cultivars based on SSR markers showed that cultivars from Kenya, Uganda and Tanzania were grouping in group A. In sub-cluster B the cultivars were from Uganda and they seemed to form a unique group. However the Tanzanian cultivars seem to cluster closely together in various sub-clusters. Analysis of Molecular Variance (AMOVA) indicated that there is statistically measurable divergence between the sweet potato of Uganda-Kenya and the other East-African country, Tanzania with detectable difference between the cultivars of the three sources. The largest source of diversity comes from within-population variation, which accounts for 88.91 % of the total variance. The data from AMOVA analysis also indicated an F_{st} value >0.05 which seems to suggest great genetic differentiation amongst the cultivars in the East African region and hence presence of a population structure. The gene flow values > 1 shows that there is high genetic drift amongst the cultivars in this region. In this study, the morphological analysis of sweet potato landraces indicated that there was not much variation in the East African sweet potato. However the investigation at genome level using PCR-based SSR markers was able to identify significant variation amongst the landraces and existence of a population structure. The major results in this study indicate that SSR markers are appropriate for the genotyping and revealing genetic relationship of East African sweet

potato cultivars. In addition, morphological characterisation should be complemented with DNA –based characterisation using SSR markers to reveal genetic diversity of East African sweet potato cultivars.

CHAPTER ONE

INTRODUCTION

1.1 Importance of sweet potato

About 75 % of the annual African production of sweet potato is in East Africa, mainly around Lake Victoria with altitude regions of 1000-2000 metres above sea level. Uganda is the largest sweet potato producer in Africa and the second largest in the world after China (FAO, 2002). Sweet potato is usually intercropped with starchy staples such as bananas, cassava, maize etc. Sweet potato is regarded as a food security crop, mainly because of its reliable yields, its ease of propagation, and low requirements for production inputs (Aritua and Gibson, 2002). Among the major starch staple crops such as bananas, potatoes, cassava sweet potato has one of the highest rates of dry matter production per unit area and unit time (Woolfe, 1992), thus making it attractive to farmers who have small areas of land. Rural women grow it near their homes to feed their families and may provide them with a source of income if they produce more than the family needs (Hakiza *et al.,* 2000; Scott *et al.,* 2000). Its short growing season allows it to fit into different cropping systems, and it can be harvested piecemeal to provide fresh daily food for a family (Karyeija *et al.*, 1998). Orange-fleshed sweet potato varieties are rich sources of pro-vitamin A (Anonymous, 2000) which further adds to the benefits of the crop in the countries where high proportions of the population, particularly women and children, suffer from vitamin A deficiency. Sweet potato also has other nutritional qualities; it is a good source of energy, vitamin C, thiamine, riboflavin and minerals (Tardif-Douglin, 1991; Woolfe, 1992). The roots provide 100% recommended daily allowance (RDA) for vitamin A, 79% for vitamin C, 10% for iron and 15 % for potassium (Food and Nutrition

Board, 1980). Sweet potato leaves an important vegetable is mainly consumed on the Kenyan and Tanzanian coastal regions (Mutuura *et al.,* 1992; Ndunguru, 1992). Sweet potato tubers are used as vegetables, eaten boiled, baked, fried, or dried and ground into flour to make biscuits, bread, and other pastries, dehydrated chips, canned, cooked and frozen, creamed and used as pie fillings much like pumpkin. Dry vines have feed value for farm animals, which compares favorably with alfalfa hay as forage (Tewe, 1992).There are a number of production constraints, which are severely affecting sweet potato in this region including sweet potato virus disease (spvd), sweet potato weevils, low soil fertility, high costs of transport and labour and unimproved cultivars. Other constraints include lack of market, vertebrate pests, shortage of farm implements and butterfly caterpillars (Bashaasha *et al.,* 1995).

1.2 The distribution of sweet potatoes in East Africa

Sweet potato occurs in a wide range of agro-ecologies in sub-Saharan Africa (Doku, 1988; Ewell and Kirkby, 1991). Sweet potato is grown in areas that are 500-2000 metres above sea level and receive sufficient rainfall for plants to survive (Carey *et al.,* 1997; Low, 1997). Four main agroecologies for sweet potato growing in East Africa are: moist warm environments which receive bimodal rainfall and are major production zones of Kenya, Uganda and Western Tanzania, dry, warm environments with unimodal rainfall of North-Eastern Uganda, parts of Kenya and Tanzania, moist, cool environments-higher elevations with bimodal rainfall and include highland production zones of Southwestern Uganda where cooler temperatures, however, tend to prolong the plants vegetative cycle and diminish yields.

1.3 Problem statement

Most sweet potato cultivars in East Africa are local landraces or farmers' varieties (Bashaasha *et al*., 1995; Kapinga *et al*., 1995). About 2000 landraces exist under different names and these varieties are constantly being collected for breeding programs assuming that they are genetically variable. Conventionally when characterisation has been done, it has used morphological and agronomic characters coupled with reaction to pests, diseases and other stresses. Sweet potatoes vary in their growth habit, leaf shape, leaf size, flesh colour, vine root ratio, level of anthocyanin, and pigmentation among other morphological characters. Agronomic characters like tuber yield, size, shape and number also vary greatly. Besides, they are strongly influenced by the environment. The interaction of all these markers with the environment is difficult to quantify or control so that their use has not given consistent results.

DNA fingerprinting using Polymerase Chain Reaction (PCR) based methods has proven to be a powerful tool for characterising germplasm. The PCR methods that have been used to characterise sweet potato include: Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) (Connolloy *et al.,* 1994; Zhang *et al.,* 1998; Huaman *et al.,* 1999), Inter Simple Sequence Repeats (ISSR)(Huang and Sun, 2000), and Simple Sequence Repeats (SSR) (McGregor *et al.,* 2000; Hwang *et al.,* 2002). Within East Africa, there appears to be three major groupings of sweet potato germplasm with one group being closely related to the original introductions from the America (Gichuki, 2001). Within this group are representative genotypes from all the

major sweet potato growing regions in East Africa. This group appears to have originated from the coastal regions of Tanzania and then spread northwards to Eastern Uganda and the Western and Central Highlands of Kenya. The second major grouping consists of Central and Western Ugandan varieties together with the Northern Tanzania-Lake Victoria zone germplasm (Gichuki 2001). The third major group is mostly varieties from the Nyanza region of Kenya, Western Kenya Highlands and Eastern Uganda (Gichuki, 2001). The latter two regions differ geographically yet show similar agro-ecologies and socio-cultural backgrounds. These two major groups likely represent the evolutionary adaptation of sweet potato to Africa.

Characterisation of some sweet potato cultivars in East Africa has been done using AFLP and RAPD techniques (Gichuki *et al.,* 2000; Nakattude 2002). However the majority of the landraces have not undergone DNA fingerprinting and there is, therefore, need to characterise more landraces in East Africa to sample the genetic diversity of the sweet potatoes germplasm of East Africa.

1.4 Justification of study

A large number of sweet potato cultivars exist varying in taste, food value, root size and shape (Bashaasha *et al.,* 1995). This has mainly arisen through natural hybridization and selection. In many households sweet potato is often grown as a mixture of two or three cultivars either planted as a patchwork or intermingled (Karyeija *et al.,* 1998). Farmers usually identify varieties by their local names. Farmers prefer to grow more than one cultivar for various reasons such as varietal preference, lack of enough planting materials

of any one cultivar, food security, spreading of yield over time, and/or guarding against losses from storage and pests or diseases (Bashaasha *et al.,* 1995; Kapinga *et al.,* 1995).

The identification and characterization of these landraces is important for purposes of conservation of genetic diversity. There is, a need to understand the genetic base of sweet potato in East Africa by characterising and evaluating the germplasm so as to make the best use of them by the regional sweet potato breeders. Identification by molecular technologies is more commonly used over morphological characters since the latter can be influenced by environmental factors (Jondle, 1992;Sosinski and Douches, 1996; Smith, 1998). A study done in South Africa on the identification of sweet potato cultivars using Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats markers (SSR) revealed that a high degree of variation exists in the sweet potato germplasm (McGregor *et al.,* 2000). The SSR technique enables direct comparisons between different laboratories possible, reveals high levels of polymorphism *(*Wu and Tanksley, 1993; Morgante and Oliveri, 1994; Salimath *et al.,* 1995; Powell *et al.,* 1996) and requires small amounts of DNA. This, together with the fact that sweet potatoes are vegetatively propagated simplifies the use of DNA fingerprinting for identification of accessions (McGregor *et al.,* 2000). In recent years it has become well recognised that PCR-based DNA markers make powerful tools for genetic analysis and for breeding programs because of their simplicity and ease of handling. Gichuki *et al*. (2000) reported that several accessions clustered together based on their geographic origin including those from South America and New Guinea, suggesting an evolutionary relatedness, but, some New Guinea sweet potatoes dispersed across many clusters, indicating some

genetic divergence in this cluster, probably caused by adaptation to isolated highland ecological conditions (Jarret and Austin, 1994; He *et al.,*

1995; Zhang *et al.,* 1996).

Although investment in East African sweet potato research and extension is low, considerable efforts have been made in the region towards germplasm collection, characterization and evaluation. In Kenya, modest germplasm collections have been established at Kakamega Research Station. The Tanzanian collection initially had a total of 81 accessions maintained at AVRDC, Arusha representing the three most important sweet potato production regions. The main Ugandan collection is at Namulonge Research Institute (NAARI). In Uganda a germplasm collection had been established in the Eastern region but due to mislabeling the accessions were destroyed (Mwanga, personal communication). The gene bank at CIP in Lima, Peru now maintains 5,526 cultivated accessions of sweet potato comprising 4,168 accessions of native and advanced cultivars from 57 countries 822 in the Americas, 26 in Asia, and 9 in Africa), and 1,358 breeding lines (Table 1).

Breeding and evaluation research of sweet potatoes has been going on in the region for quite some time. Most of this material was obtained from farmers in different locations in the region (Mwanga *et al.,* 1995; Carey *et al.,* 1999; Hagenimana *et al.,* 1999). Due to the variations in environmental conditions across locations some morphological features of these materials varies a lot. This therefore means that a number of these entries were potentially uniform genetically, though catalogued under different names depending on where they were collected. This results in multiple entries of the same cultivar leading to their misuse in breeding programmes, which costs valuable time and resources. This

study therefore was aimed at characterizing sweet potato germplasm in the region using both morphological and molecular markers, compare results and eliminate possible multiple entries.

Table 1: Geographic coverage of *Ipomoea batatas* conserved in the gene bank maintained at CIP in Lima, Peru

Area and Country	**Number of accessions**	**Country**	**Number of accessions**
Asia			
Bangladesh (BGD)	4	Burma (BUR)	3
China (CHN)	38	Morocco (MAR)	1
Hong Kong (HKG)	1	Korea(KOR)	10
Indonesia (IDN)	31	Japan (JPN)	142
Lao Peoples Republic (LAO)	8	Malaysia (MYS)	12
Philippines (PHL)	51	Papua New Guinea (PNG)	474 5
Singapore (SGP)	3	Sri Lanka (LKA)	94
Taiwan (TWN)	324	Thailand (THAI)	3
Vietnam (VNM)	2	Australia (AUS)	4
Cook Islands (COK)	6	Fiji (FJI)	2
New Caledonia (NCL)	2 7	New Hebrides (NHB)	63 5
New Zealand (NZL)	18	Solomon Islands (SLB)	
Tonga (TON)		Nike (NIU)	

Africa			
Burundi (BDI)	5	Cameroon (CMR)	4
Egypt (EGY)	2	Madagascar (MDG)	2
Kenya (KEN)	2	Nigeria (NGA)	18
Rwanda (RWA)	4	South Africa (ZAF)	2
Uganda (UGA)	4		
Others			
Unknown country	11	RCB (Peru) hybrids	282
AVRDC hybrids	38	IITA hybrids	1,038
Subtotal	**2,440**	**Subtotal**	**3,086**

1.5 Objectives of the study

To main objective of the study reported in this thesis was to characterise sweet potato cultivars from selected locations in East Africa

The specific objectives included:

1. Characterising sweet potato cultivars from East Africa using morphological characters.
2. Characterisng sweet potato cultivars using Simple Sequence Repeats (SSRs).
3. Determining genetic diversity of sweet potato germplasm and relatedness of the cultivars.

It was hypothesized that the genetic base for sweet potato in East Africa was narrow and most cultivars were closely related or indeed were homologs.

CHAPTER TWO

LITERATURE REVIEW

2.1 The sweet potato crop

2.1.1 Historical dispersal of sweet potato

Sweet potato (*Ipomoea batatas* L. Convolvulaceae) originated in South America around 8,000-6,000 B.C. (Austin, 1988). Abundant evidence shows that sweet potato was spread widely through the migration routes of people in the New World tropics before the discovery of America (Austin, 1988). The main hypothesis for the rapid spread of the sweet potato in the sixteenth century is that the Portuguese voyagers following the track of Vasco da Gama carried the plant eastwards from the Caribbean, Brazil and Europe to Africa, India, Southeast Asia and Indonesia (Simmonds, 1976). Europeans introduced the crop into Africa in the early 1500's (O'Brien, 1972; Yen, 1982). Secondary introductions were made from India between the seventeenth and nineteenth centuries (O'Brien, 1972; Yen, 1982). By 1900, sweet potato was already an important food crop in the East African region (McMaster, 1962).

The crop's primary center of diversity is in Northwestern South America and parts of Central America where a great diversity of native sweet potatoes, weeds, and wild Ipomoea exists (Huaman, 1999). Based on the presence of large numbers of varieties,

East Africa, Asia and Oceania (Australia, New Zealand and the Pacific Islands) are suggested as secondary centers of sweet potato diversity (Gichuki *et al.,* 2000) (Table 2).

Table 2: Major sweet potato producing countries in the world (FAO, 2002)

Country	Production (Metric tones)
China	114,289,100
Uganda	2,515,000
Nigeria	2,503,000
Indonesia	1,746,311
Vietnam	1,725,100
Japan	1, 030,000
India	1, 200,000
Rwanda	1, 292,361
Tanzania	950,100
Burundi	780,859
Madagascar	525,700

2.1.2 Evolution of sweet potato

The sweet potato belongs to the species; *Ipomoea batatas* (L.) Lam. Linnaeus described the cultivated sweet potato in 1753 as *Convolvulus batatas*. *Ipomoea batatas* is a dominantly ”domesticated” species. It, however, has wild ancestors that bear morphological resemblance to the crop. One such species was reported to be *I.trifida* collected in Mexico and reported as a 6x *I.trifida* (accession K 123) (Huaman, 1999). The ploidy level of *I. batatas* is as yet undefined. Nevertheless polyploidization is thought to have facilitated the evolution of *I. batatas* to the hexaploid level (Nishiyama, 1971), though cytogenetic evidence suggested an autohexaploid structure with a B genome

(Shiotani, 1988). From 2x *I.leucantha*, 4x *I. littoralis* was produced. 2x x 4x crosses between these two species then gave 3x *I. trifida*, from which 6x *I.trifida* were derived. Further selection and domestication of these wild plants ultimately led to the formation of *I.batatas*. This is supported by the findings of Orjeda *et al.* (1990), Freye *et al.* (1991 and Bohac *et al.* (1992) who reported formation of unreduced pollen in diploid *I.trifida* and some tetra and *I.batatas*.

The growth habit of sweet potato is typically herbaceous and perennial. However, it is grown as an annual plant by vegetative propagation using either storage roots or stem cuttings. The crop is predominantly prostrate with a vine system that expands rapidly horizontally on the ground. Variations from this include the erect, semi-erect, spreading, and extremely spreading types (Huaman, 1999).

The root system is fibrous with lateral projections that serve as storage organs. Roots grown from vegetatively propagated plants; start with adventitious roots that develop into primary fibrous roots that are branched into lateral roots. As the plant matures, lignifications occur. However storage roots remain unlignified. Plants grown from true seed form a central axle with lateral branches. The former later functions as a food storage root (Huaman, 1999).

The stem of the sweet potato is cylindrical. Its overall length and that of the internodes depends on the growth habit of the cultivar and the availability of water. Erect cultivars are approximately 1 m long, while extremely spreading ones can grow to more than 5 m long. Some cultivars have stems with twining characteristics. Depending on the cultivar, the stem colour varies from green to the red-purple colours that are totally pigmented with anthocyanins. Apical shoots and stem hairiness varies from none to very pubescent (Huaman, 1999).

The leaves are simple and spirally arranged alternately on the stem in a pattern known as 2/5 phyllotaxis (i.e. there are 5 leaves spirally arranged in 2 circles around the stem for any two leaves and are located in the same vertical plane on the stem). The lamina edge may be entire, toothed or lobed. The base of the leaf lamina generally has two lobes that are either straight or rounded. Leaf shape varies from rounded to reniform (kidney-shaped), cordate (heart-shaped), triangular, hastate (trilobular and spear-shaped with the two basal lobes divergent), lobed and almost divided. Lobed leaves differ in the degree of the cut, ranging from superficial to lobed. The number of lobes generally ranges from 3 - 7 and can be easily determined by counting the veins that go from the junction of the petiole up to the edge of the leaf lamina.

2.2 Germplasm characterization

As a result of the asexual propagation of sweet potato cultivars, numerous duplicate accessions are normally found in the collections. A list of sweet potato descriptors has been used to select key morphological descriptors that adequately describe each accession (CIP/AVRDC/IBPGR, 1991). A colour chart for the characterization of storage-root skin and flesh colour has also been produced to obtain more consistent data. Computerized systems for multivariate analyses have been used to group morphologically similar accessions grown side by side in the field. Molecular markers have also been employed to compare morphologically identical accessions. Those that produce similar DNA fingerprints are considered duplicates. One accession from each duplicate group is selected to represent the group on the basis of its data on reaction to pests and diseases. All duplicate samples are then converted into true seed and their clonal forms discarded. Using this approach, the number of Peruvian accessions in the collection was reduced from 1,939 to 673 (Huaman, 1999). The number of duplicates of the same cultivar ranged from 1 to 99 accessions. A Peruvian sweet potato core collection comprising 85 accessions (12% of 673) was selected to enhance the utilization of this

germplasm (Huaman, 1999). Similar efforts are underway in various centers of diversity in collaboration with CIP (Huaman, 1999). Genotypes with good breeding potential and desirable traits have also been identified during these exercises (Huaman, 1999).

DNA based markers have been found to be more reliable than morphological and agronomic characters in the sweet potato. The RAPD marker is the least expensive and quickest of the molecular marker techniques (Gichuki *et al.,* 2000) but has problems of reliability and reproducibility (Ellsworth *et al*., 1993). Gichuki *et al.* (2000), reported RAPD analysis on 74 sweet potato varieties originating from 23 countries within eight geographical regions of South America, Central America, United States of America (USA), East Africa, South Asia, East Asia and Oceania. Out of the 52 primers, eleven of the primers generated 71 polymorphic markers indicating that significant differences existed among the genotypes in different regions as well as among varieties within each region. Gichuki *et al.* (2000) suggested that there was some relationship between genetic diversity and the geographic distribution of genotypes. Clustering patterns within East African varieties were detectable using 71 loci.

Gichuki *et al. (*2000) reported AFLP analysis of 158 sweet potato genotypes from East Africa, 3 from South and Central America and 4 from other African regions. Using two AFLP primer combinations, it was possible to get a high number (100-200) of informative products within the 50-500 base pair range per single primer combination. Gichuki *et al.* (2000) suggested that there was some relationship between genetic diversity and the geographic distribution of genotypes.

ISSR, which involves the PCR amplification of DNA using single primers composed of microsatellite sequences, overcomes many of the technical limitations of RFLP and RAPD analyses (Tsumura *et al.*, 1996). These primers target microsatellites that are abundant throughout the eukaryotic genome (Tautz, 1989; Kijas *et al.*, 1995). Genetic diversity and the relationships of sweet potato and its wild relatives in Ipomea series Batatas (Convoluvulaceae) was determined using this marker and on average, 52 bands per accession and 207 bands per species were amplified with 15 primers, ranging from 2-5 bands per accession per primer. Each of the 15 primers used were able to detect numerous polymorphisms among accessions (Hwang, 2002).

Microsatellite markers exhibit high levels of polymorphism and have been successfully used in the study of genetic diversity and genotype identification in barely (Saghai-Maroof et al., *1994), wheat (Plaschke* et al., *1995), and rice (Xiao* et al., *1996). Microsatellite variation results from differences in the number of repeat units. These differences are thought to be caused by errors in DNA replication when the DNA polymerase "slips" when copying the repeat region, changing the number of repeats (Jarne and Lagoda, 1996; Moxon and Willis, 1999). Such differences are detected on polyacrylamide gels, where repeat lengths migrate different distances according to their sizes. Furthermore, the ability of the method to differentiate individuals when a combination of loci is examined makes the technique very useful for gene-flow experiments, cultivar identification and paternity analyses (Hokanson* et al., *1998).*

The extraordinary discriminatory capacity of microsatellite markers was observed in 113 Latin America sweet potato samples using six primer pairs that generated scorable information revealing a total of 70 alleles, with allele size ranging from 102 to 173 base pairs (Zhang *et al*., 2001). Both the richness and the evenness of the alleles showed a significant geographical pattern in the Latin American sweet potato gene pool (Zhang *et al*., 2000). In South Africa, microsatellite analysis (SSR) was done with five SSR primers on 21 known accessions from the germplasm collection and the technique was able to distinguish these accessions (McGregor *et al.*, 2000). There are, however, some problems encountered with microsatellites in that unless useful primers have been designed in previous studies, it is necessary to screen an organism for microsatellites. Microsatellite analyses assume that co-migrating fragments are homologous and whereas there are few *a priori* reasons to assume this, non-homology, which can be divided into that occurring within the SSR flanking, and the SSR repeat regions can occur. Several studies have sequenced amplified microsatellites to test homology and the mechanisms of microsatellite mutation (Blanquer-Maumont and Crouau-Roy, 1995; Grimaldi and Crouau-Roy, 1997; Buteler *et al.*, 1999). Buteler *et al.* (1999) characterised microsatellites in diploid and polyploidy sweet potatoes (*Ipomoea trifida* and *I. batatas*), and found "instability" in the microsatellite flanking regions. The "instability" in the non-repeated flanking regions consisted of both point mutations and indels. They suggested that caution should be used when relying exclusively on band size in the interpretation of SSR length polymorphisms. Mutations in the binding region of one or both of the microsatellite primers may inhibit annealing that may result in the reduction or loss of the PCR product (Callen *et al*., 1993). Such products are termed null alleles and are

comparable to the null alleles identified by allozymes in their effects. Null alleles may be manifested as fewer heterozygotes than expected in a randomly mating population or by the appearance of "empty" lanes (Morgante and Oliveri, 1994). That is, in a heterozygote of two different microsatellite alleles, if one of these alleles cannot be amplified due to primer annealing difficulties, then the phenotype (on the SSR gel) will appear as a single banded homozygote. Primers designed to flank simple sequence repeats (SSRs) loci were developed (Jarret and Bowen, 1994) and used to fingerprint sweet potato cultivars (Zhang *et al.,* 2000; Hwang *et al.,* 2002).

Microsatellite markers have their own limitations when used on polyploid species. The most important limitation is that the real genotype of a hexaploid individual cannot be revealed because the PCR-based nature of SSRs cannot differentiate the dosage effect of a given allele (i.e. it cannot differentiate between simplex and duplex). Buteler *et al*. (1999) reported that in sweet potato, insertions/deletions and base substitutions occurred in the microsatellite flanking regions, meaning SSR markers may not be appropriate for the study. The occurrence of null alleles is another possible problem with the use of microsatellite markers in highly out breeding, heterozygous species (Powell *et al.,* 1996). Because of these limitations, the allele frequency of a given germplasm pool cannot be calculated, and classical population genetics cannot be fully applied but selective amplification of microsatellite polymorphic loci (SAMPL) markers is a remedy to the insertions/deletions and base substitutions inherent in microsatellites flanking regions (Buteler *et al.,* 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Morphological characterisation

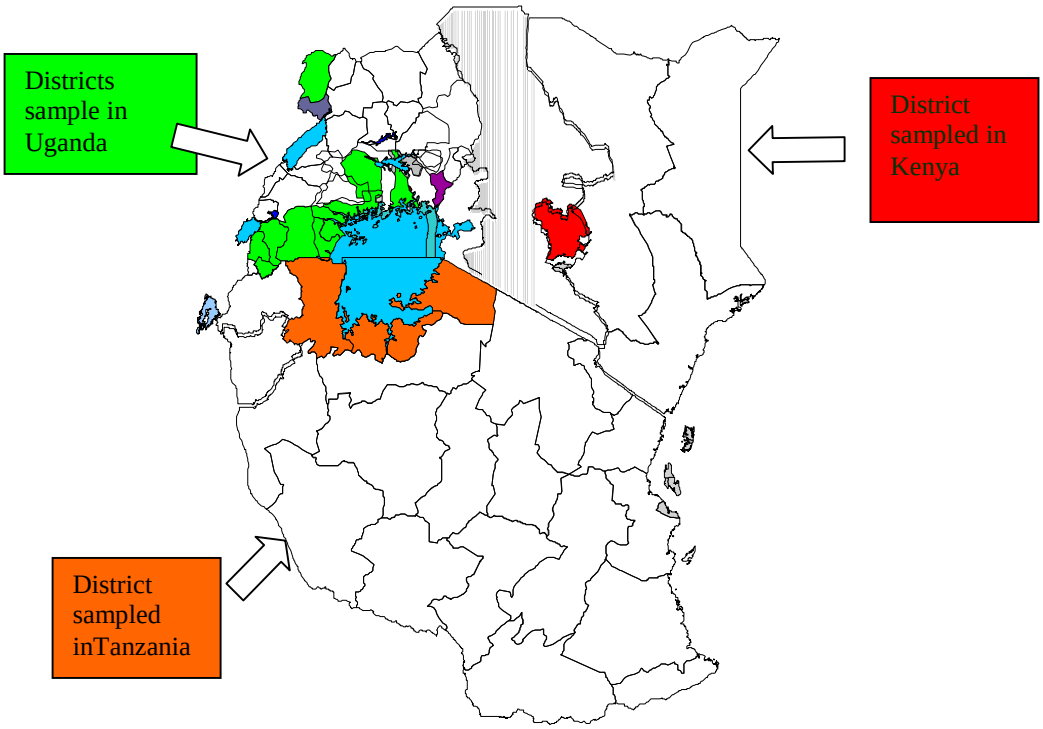
3.1.1 Survey

Four hundred and ninety one sweet potato cultivars were obtained during a random sampling survey carried out in Mbarara, Kisoro and Kabale districts in Western Uganda, Luwero, Mukono, Mpigi, Masaka districts in Central Uganda, Soroti district in Eastern Uganda, Arua and Masindi districts in Northwestern Uganda, Bukoba and Muleba districts in Tanzania and Kiambu and Tigoni districts in Kenya. The sites in Kenya do not indicate where sweet potato is popularly grown but were selected on the basis of convenience. The survey was carried out on 13 farms in each of the districts. Using a questionnaire, data on latitude, longitudes and altitudes of the sites of collection were recorded (Appendix 1). In addition the farmers were asked to identify each of the various cultivars they had on the farm by name.

3.1.2 Establishment in the screen house

For every sweet potato cultivar found in the farmers' field, vines were sampled. In total, two hundred and sixty-six vines were planted in pots in the screen house at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) between February and May 2003 after elimination of duplicates. During the growing period, the cultivars were screened on the basis of morphological characteristics using the CIP Research Guide 36

(Huaman, 1992) using a scale of 0-9 for 13 key sweet potato descriptors selected from an internationally accepted list (CIP/AVRDC/IBPGR, 1991; Huaman *et al*., 1992).



Key:

Luwero, Mukono, Mpigi, Masaka districts in Central Uganda, Soroti district in Eastern Uganda, Arua and Masindi districts in Northwestern Uganda

Bukoba and Muleba districts in Tanzania

Kiambu and Tigoni districts in Kenya

Figure 1: A map of East Africa showing the sites where sweet potato cultivars were collected during the course of this study. The details of the samples and sites are to be found in the Appendix section of this thesis.

3.2 Samples used for DNA extraction

Fifty-seven accessions representing the different geographical sites (Table 3-5) were chosen from distinct morphological clusters of the 266 accessions defined by the UPGMA method based on passport data and morphological dendogram clusters.

Table 3: Source of twenty five Tanzanian sweet potato cultivars characterized using morphological descriptors

Country	Field code	Cultivar name	District	GPS reading
Tanzania	BK 1-3	Kombegi	Bukoba	$01^0$21.21S, $031^0$45.41E, 4327m
Tanzania	BK 1-4	Unknown	Bukoba	$01^0$21.21S, $031^0$45.41E, 4327m
Tanzania	BK 1-10	Kagole	Bukoba	$01^0$21.21S, $031^0$45.41E, 4327m
Tanzania	BK 3-1	Vumilia	Bukoba	$01^0$14.37S, $031^0$23.56E, 4098m
Tanzania	BK 3-2	Zerida (D)	Bukoba	$01^0$14.37S, $031^0$23.56E, 4098m
Tanzania	BK 3-3	Zerida	Bukoba	$01^0$14.37S, $031^0$23.56E, 4098m
Tanzania	BK 3-4	Kalebe	Bukoba	$01^0$14.37S, $031^0$23.56E, 4098m
Tanzania	BK 3-5	Unknown	Bukoba	$01^0$14.37S, $031^0$23.56E, 4098m
Tanzania	BK 5-2	Regania	Bukoba	-
Tanzania	BK 5-3	Kigambile nyoleo	Bukoba	-
Tanzania	BK 5-4	Kagore	Bukoba	-
Tanzania	BK 5-5	Unknown	Bukoba	-
Tanzania	BK 7-2	Alinyiikira	Bukoba	$01^0$27.38S, $031^0$36.06E, 4017m
Tanzania	BK 7-5	Kamogoli	Bukoba	$01^0$27.38S, $031^0$36.06E, 4017m
Tanzania	BK 8-1	Unknown	Bukoba	$01^0$26.44S, $031^0$46.51E, 4032m
Tanzania	BK 8-3	Unknown	Bukoba	$01^0$26.44S, $031^0$46.51E,

				4032m
Tanzania	BK 8-4	Sinia nyempe	Bukoba	$01^0$26.44S, $031^0$46.51E, 4032m
Tanzania	BK 9-2	Madebe	Bukoba	$01^0$26.08S, $031^0$46.31E, 4032m
Tanzania	BK 9-4	Simba eichuu	Bukoba	$01^0$26.08S, $031^0$46.31E, 4032m
Tanzania	BK 9-7	Unknown	Bukoba	$01^0$26.08S, $031^0$46.31E, 4032m
Tanzania	BK 10-1	Chamusoma	Bukoba	$01^0$25.09S, $031^0$46.47E, 4042m
Tanzania	MLB 2-2	Ruganza	Muleba	$01^0$47.07S, $031^0$30.18E, 4401m
Tanzania	MLB 2-5	Kalebe	Muleba	$01^0$47.07S, $031^0$30.18E, 4401m
Tanzania	MLB 4-2	Mwasa	Muleba	$01^0$39.57S, $031^0$34.57E, 4420m
Tanzania	MLB 5-1	Tuula omushako	Muleba	$01^0$37.37S, $031^0$39.36E, 4393m

Table 4: Source of seven Kenyan sweet potato cultivars characterised using morphological descriptors

Country	Field code	Cultivar name	District	GPS reading
Kenya	TG 1-2	Unknown	Tigoni	
Kenya	TG 2-1	Unknown	Tigoni	
Kenya	KB 2-unk	Unknown	Kiambu	-
Kenya	KB 3-unk	Unknown	Kiambu	-
Kenya	KB 4-unk	Unknown	Kiambu	-
Kenya	KB 6-1	Mwibai	Kiambu	-
Kenya	KB 6-2	Kiganda	Kiambu	-

Table 5: Source of twenty five Ugandan sweet potato cultivars characterized using morphological descriptors

Country	Field code	Cultivar name	District	GPS reading
Uganda	MSK 9-2	Sifumba nagayaba	Masaka	$00^0$08.81S, 031045.39E, 1220m
Uganda	MSK 14-9	Sukaali	Masaka	$00^0$10.99S, 031045.61E, 1165m
Uganda	MSK 19-12	Unknown	Masaka	$00^0$10.72S, 031051.17E, 1192m
Uganda	LUW 5-4	Silk	Luwero	$00^0$40.35N,$032^0$42.03E,1081m
Uganda	LUW 7-4	Kateeteyi	Luwero	$00^0$45.67N,$032^0$40.61E,1100m
Uganda	LUW 13-2	Kawongo	Luwero	$00^0$56.79N, 032037.22E, 1130m
Uganda	MUK 5-2	Nantongo	Mukono	$00^0$0.40S, 031044.50E, 1196m
Uganda	MUK 5-5	Munyera	Mukono	$00^0$0.40S, 031044.50E, 1196m
Uganda	MUK 6-2	Bunduguza	Mukono	$00^0$01.37S, 031044.42E, 1220m
Uganda	MUK 20-3	Dimbuka	Mukono	00^0 09.99S, 031051.63E, 1209m
Uganda	MPG 5-2	Kajereje	Mpigi	$00^0$14.75N,$032^0$17.98E,1224m
Uganda	MPG 5-4	Soroti	Mpigi	$00^0$14.75N,$032^0$17.98E,1224m
Uganda	MPG 15-4	Dimbuka	Mpigi	$00^0$14.25N,$032^0$10.65E,1240m
Uganda	S-3	Kitunde	Mpigi	$00^0$11.34N,$032^0$04.29,1312m
Uganda	S-26	Cheparonk1	Soroti	-
Uganda	S-36	XKp5	Soroti	-
Uganda	S-36	Ateseke	Soroti	-
Uganda	G-1	Kampala	Masindi	-

Uganda	G-9	Kalobo	Masindi	-
Uganda	G-12	Nailoni	Masindi	-
Uganda	MBA 6-2	Kyitabira	Mbarara	00^{0}40.82S, 030024.01E, 1445m
Uganda	KAB 16-2	Koshokonyokozi	Kabale	01^{0}07.96S, 029052.16E, 2025m
Uganda	KAB 22-1	Mukazi anura	Kabale	01^{0}11.95S, 029055.82E, 1876m
Uganda	KAB 23-2	Egumura	Kabale	01^{0}13.20S, 029057.09E, 1876m
Uganda	A-24	Dele (B)	Arua	-

3.2.1 DNA extraction

For each of the 57 sweet potato cultivars (Table3-5), young leaves about three weeks old were ground into powder in liquid nitrogen for the extraction of genomic DNA based on a modified C-TAB method (Kiprop, 1998; Rubaihayo and Wasike, unpublished). Ground leaf material (1.0 g) was placed in 4 ml of extraction buffer (2 % CTAB, 1.4M NAACO, 0.2 %(w/v), 2-mecarptoethanol, 20mM EDTA, 100mM Tris-HCl (pH 8.0), 1% polyvinylpyrodilone (PVP-25) and incubated at 65^0C for 45 minutes followed by extraction with 3 ml of phenol: chloroform: isoamyl alcohol (25:24:1) at room temperature (r.t.) for 10 minutes, and centrifuged at 5,000 r.p.m for 20 minutes at 25^0C, so as to separate the phases. This extraction removes CTAB-protein/polysaccharide complexes. Three mls of the aqueous phase was recovered and further extraction was done using an equal volume of chloroform: isoamyl alcohol (24: 1), followed by an incubation period of 10 minutes at r.t. and then centrifugation at 5,000 r.p.m for 20 minutes at 25^oC. Fifty microlitres of the aqueous phase was transferred into a fresh eppendorf tube and 1000 µl of cold isopropanol was added and the tube gently shaken back and forth until a stringy white DNA precipitate became clearly visible. This was incubated for 1-2 hours at r.t. so as to precipitate the nucleic acids followed by centrifugation at 3,000 r.p.m for 10 minutes at r.t. The supernatant was gently poured off so as not to loose the pellet. One ml of the washing buffer (76 % v/v ethanol, 10mM ammonium acetate) was added to the pellet and swirled gently and left at r.t for 60 minutes while on a vertical shaker at 15-20 rpm. This was followed by centrifugation at 2,500 r.p.m for 10 minutes at r.t and the supernatant was discarded, and the pellet dried for 30 minutes. The pellet was resuspended in 400 µl of TE buffer. One µl RNase A

(conc. 20µg/µl) was added to the sample and incubated for thirty minutes at 37^0C. Four hundred µl of chloroform: isoamyl alcohol was added and after gentle inversion for 5 minutes, centrifuged at 12,000 rpm for 10 minutes at 4^oC. The upper aqueous phase was transferred to a new eppendorf tube and 1/10 volume of 3M sodium acetate (PH 8.0) and 2 volumes of cold 100 % ethanol added and shaken to precipitate DNA and kept for 1 hour at -70^0C. Then centrifugation was done at 13,000 r.p.m for 15 minutes at 4^0C and the supernatant discarded. The pellet obtained was washed twice in 700 µl of cold 70 % ethanol to dissolve the remaining salts. The ethanol was poured off and the remainder removed using a micropipette, and the pellet air-dried for 10 minutes. The pellet was then dissolved in 100 µl of TE buffer and the DNA stored in TE at -20^0C. The DNA concentration was determined using spectrophotometer (wavelength 3000, Biorad) to establish DNA purity and run on a 1.5 % agarose gel to determine its quality.

3.2.2 Polymerase Chain Reaction (PCR)

Four pairs of microsatellites (SSR primers) (Table 6) designed for sweet potatoes (McGregor *et al.,* 2000) were used in this study and first subjected to annealing temperature screening using a thermocycler (BIORAD). The 57 sweet potato cultivars, which were subjected to SSR analysis and subsequently to gel electrophoresis, are as indicated in Table7.

Table 6: SSR primers used in PCR (McGregor *et al*., 2000)

Primer Name	Sequence 5' TO 3'	Annealing Temperature ^{o} C
IB-242 F	GCG GAA CGG ACG AGA AAA	57
IB-242 R	ATG GCA GAG TGA AAA TGG AAC A	
IB-316 F	CAA ACG CAC AAC GCT GTC	51
IB-316 R	CGC GTC CCG CTT ATT TAA C	
IB-318 F	AGA ACG CAT GGG CAT TGA	55
IB-318 R	CCC ACC GTG TAA GGA AAT CA	
IB-248 F	GAG AGG CCA TTG AAG AGG AA	55
IB-248 R	AAG GAC CAC CGT AAA TCC AA	

Table 7: Nomenclature, codes and origin of samples analysed in Figure 3

Field code	Code on gel	Cultivar name	Origin
BK 1-10	A 1- 10	Kagole	Tanzania
BK 5-2	A 5- 2	Regania	Tanzania
BK 8-1	A 8-1	Unknown	Tanzania
MLB 5-1	B 5-1	Tuula Omushako	Tanzania
MLB 2-5	B 2-5	Kalebe	Tanzania
BK 9-2	A 9-2	Madebe	Tanzania
MLB 2-2	B 2-2	Ruganza	Tanzania
KAB 23-2	O 23-2	Egumura	Uganda
MUK 20-3	G 20-3	Dimbuka	Uganda
MUK 5-2	G 5-2	Nantongo	Uganda
MPG 11-5	K 11-5	Dimbuka	Uganda
S-26	L-26	XKp5	Uganda
A-24	P-24	Dele (B)	Uganda
KAB 22-1	O 22-1	Mukazi Anura	Uganda
KB 6-1	D 6-1	Mwibai	Kenya
TG 1-1	C1-1	Unknown	Kenya
KB 2-unk	D 2-unk	Unknown	Kenya
TG 1-2	C 1-2	Unknown	Kenya
KB 6-2	D 6-2	Kiganda	Kenya
TG 2-1	C 2-1	Unknown	Kenya
KB 4-unk	D 4-unk	Unknown	Kenya

3.2.3 Optimal PCR conditions

The optimal reaction conditions for PCR were set as follows:

Item	**Volume in microlitres (µl)**
10 PCR Buffer	2.5
Taq polymerase (5 units/µl)	0.3
50 mM Magnesium chloride	1.25
100 µM forward primer	0.5
100 µ M reverse primer	0.5
10mM dNTP mix	0.4
Genomic DNA **(**500 ng/ml)	1.5
Water (pH 7)	13.05
Total	20

The PCR amplification procedure was based on Welsh and McClelland (1990) and Williams *et al*. (1990). The PCR conditions were cycle 1: (1x) step 1: 94^0C for 3 minutes followed by cycle 2:(10 x) consisting of denaturation for 1 min at 94^0C, annealing at 65^0C for 1 min, after that decrease temperature after cycle 1 by 0.5^0C and the last step was polymerization at 72^0C for 1 min and 50 s. Cycle 3(20 x) consisted of denaturation for 1 min at 94^0C,annealing at

60 ^{0}C for 1 min and the last polymerization step at 72^0C for 1 min 50 s. The last cycle was the final extension for 5 min at 72^0C.

3.2.4 Electrophoresis of PCR products

Five µl of bromophenol blue was added to 12 µl of the PCR products, which were subsequently analyzed by electrophoresis in a 4 % metaphor agarose gel (FMC Products,

Rockland, ME, USA) in 1 X TBE buffer at 90V. The gel was allowed to run until the moving dye front had migrated ¾ of the way down the gel for 45 minutes. DNA was stained by soaking the gel in 1 X TBE buffer containing 7 µl of ethidium bromide(10mg/ml) solution, and visualised under ultraviolet light. All the analyses included a negative control in which no DNA template was included in the amplification reaction mixture. The positive control was a sweet potato sample of DNA, which had been used in a previous study using AFLP.

3.3 Data analysis

3.3.1 Cluster analysis

Cluster analysis of both the morphological data and molecular was performed by UPGMA using TREECON Version 1.3 b phylogenetic program for Window-based environment (Van de Peer and De Wachter, 1994). Each SSR fragment was treated as a unit character and scored as binary codes (1/0= +/-). The binary data were then pooled together to constitute multiple loci genotypic data. Each lane was compared with all other lanes for each 'locus' for absence or presence of an amplified fragment. Genetic distance data matrices were constructed for both morphological and molecular data using the method of Nei and Li (1979) where genetic distances between the i^{th} and j^{th} cultivar was computed as follows: Where: Eij = average genetic distance between OTUs *i* and *j,* n_i and $n_{j=}$ total number of characters (fragments) for i and j respectively used in a particular comparison, $n_{loci\ =}$ total number of loci, x_{ki} and x_{kj}= the value of character (fragment) k for OTU*i* and OTU*j* respectively. Bootstrapping, a method of re-sampling data (100 times) to infer the variability of the estimate was used. In this analysis, each accession was treated as an operational taxonomic unit (OTU), which is the object being studied. This generated matrices of genetic distances between the i^{th} and j^{th} cultivars for all possible pair-wise comparisons, which were subjected to cluster analysis. In this analysis, cultivars are grouped in a cluster so that those in one cluster are more similar to one another than they are to other objects in different clusters. By sequence fusions between OTUs (accessions) or groups of OTUs, this analysis generates a hierarchic dendogram. The agglomerative clustering technique employed was group average clustering, UPGMA (Unweighted pair-group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973). In this method, genetic distance between an OTU and an established cluster is the average genetic distance of that OTU with all OTUs in the cluster. This means that after fusion of two most similar OTUs clustering continues between two next closest OTUs or between any unplaced OTU and the established cluster. For an unplaced

OTU to join a cluster, its average genetic distance from all member of the cluster must be short enough in comparison with any other pairs of unplaced OTU. The process is repeated until all the clusters join into one cluster. The method is called Unweighted because it gives equal weight of each OTU within a cluster. Rooted analyses were performed.

3.3.2 Anova and Amova analysis

The morphological and molecular data was tested for presence of population or genetic structure by Analysis of Molecular Variance (AMOVA) (Excoffier *et al.,* 1992) using Arlequin version 2 (Schneider *et al.,* 2000).

CHAPTER FOUR

4.1 RESULTS AND DISCUSSION

4.1.1 Cluster analysis of morphological characters

The analysis was based on many variations such as plant type, leaf shape and vine length. Results of UPGMA clustering of 57 genotypes of East African sweet potato cultivars are presented in Figure 2.

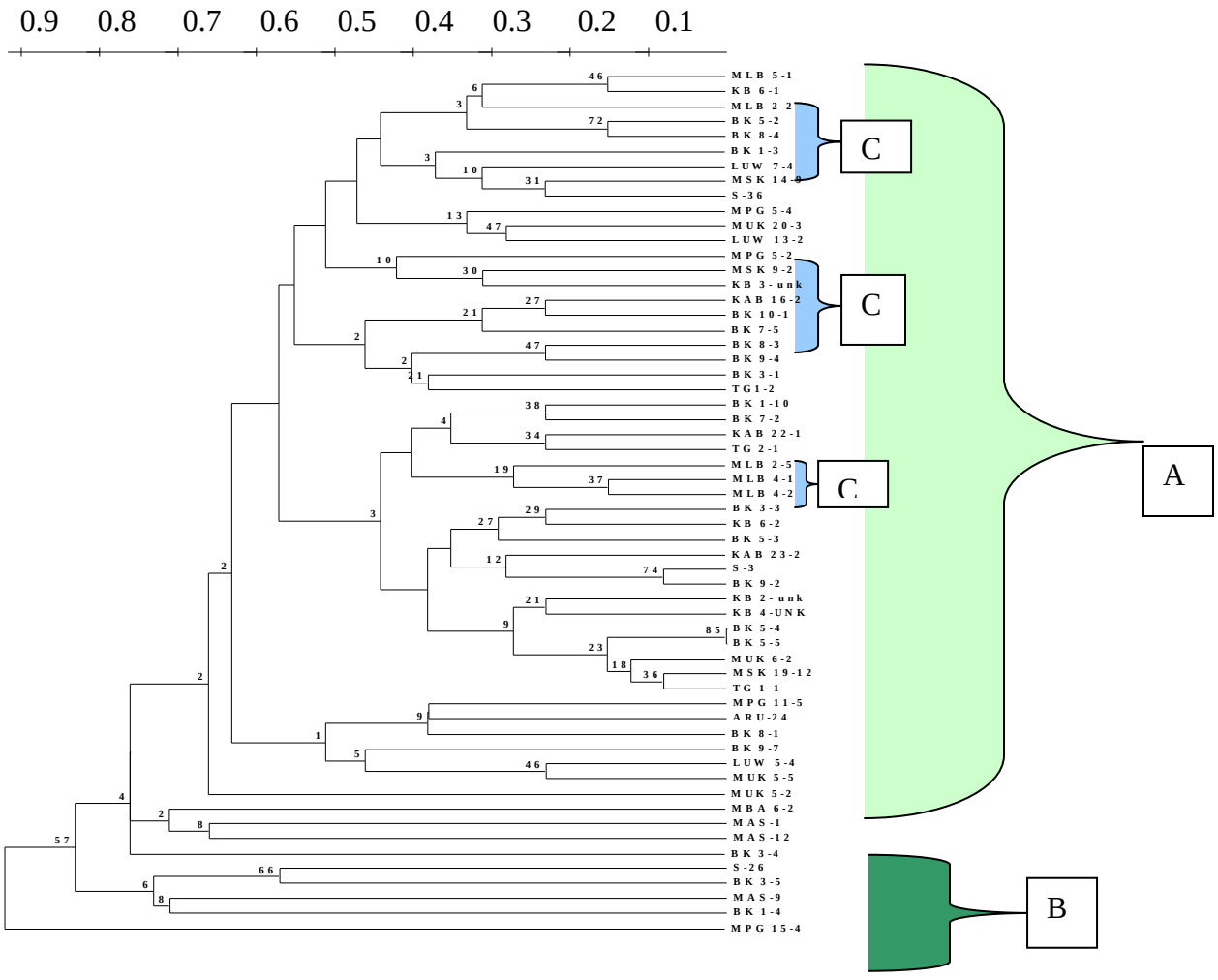


Figure 2: Relationships among 57 East African sweet potato cultivars using morphological characters. The scaled bar on top of the phenogram refers to Nei's genetic distance, labels at the root of the phenogram on the right refer to the cultivars in question and numbers in the phenogram are bootstrap support values generated using TREECON Version 1.3 b phylogenetic program for Window-based environment (Van de Peer and De Wachter,1994).

Most of the cultivars were showing low bootstrap values (0-85 %). This indicates absence of distinct clusters amongst these cultivars and close evolutionary relationships between them. The lack of distinct clusters could be attributed to the fact that in East Africa sweet potato is a vegetatively propagated crop and this leads to most of the cultivars showing genetic uniformity (Ortiz, 1995). The majority of the cultivars were in the range of 0.1-0.4 Nei's genetic distance from each other. Nei's genetic distance scale is a scaled bar which appears on top of the phenogram in Figure 2. This suggests very close genetic relatedness amongst the cultivars, which may be due to selections arising from the same or closely related parents. At a bootstrap value of 85 %, the analysis was able to distinguish synonyms i.e. Bk 5-4 (Kagore) and Bk 5-5 (Unknown). The synonyms revealed by this analysis seem to suggest that these cultivars are the same clone although they had been given different names. Bashaasha *et al,* (1995) and Kapinga *et al*. (1995) found that over time farmers in the East African region have selected a number of sweet potato varieties and identified them by their local names. Synonyms could arise because farmers base this nomenclature on varietal characteristics such as yield, maturity period, root size and shape, leaf size and shape, and other factors such as place of origin and person who introduced the cultivar into a particular location. There were two major sub-groupings at a bootstrap value of 57 %. Majority of the cultivars belonged to group A. These have a green predominant colour of vine, triangular leaf outline, one leaf lobe and green mature leaf lobe. Only 9 cultivars belonged to sub-cluster B. Of these 3 cultivars originated from Tanzania and 6 cultivars were from Uganda. The cultivars in group B have a green predominant colour of vine, cordate leaf outline, one leaf lobe and a green mature leaf colour. The distinguishing morphological character between the two main sub-groupings (A&B) was the leaf outline. Group A had a triangular leaf outline while group B had a cordate leaf outline. Results also indicate cultivars grouping together irrespective of geographical location. However the Tanzanian cultivars indicated within sub-group A, showed slight clustering together but still scattered within the variation of the Kenyan and Ugandan cultivars. The cultivars grouping together irrespective of geographical location seems to suggest that these cultivars could have come from the same breeding program. They also could have had the same origin or could have shared parents. There is a lot of cross-border movement of people between the three countries and many times sweet potato vines are exchanged during this movement. The fact that the Tanzanian accessions showed slight clustering together seems to suggest that there has not been very recent exchange of planting materials between Tanzania and the other two countries due to separation by distance. One of the ways in which farmers name their cultivars is by the source of origin of that cultivar (Bashaasha *et al*., 1995; Kapinga *et al*., 1995). From the survey done in this study, a cultivar called Kiganda (D 6-2) which was obtained from Kiambu (Kenya) suggests that the cultivar may have originated from Uganda perhaps brought in through farmer-farmer exchange. This is further evidence that there is a lot of movement of material across the border.

4.1.2 Analysis of Variance of morphological data

Analysis of variance of morphological data on the basis of geographical distribution revealed an F value < 0.05,which indicates absence of a population structure in the East African sweet potato. These data seems to suggest that these cultivars are not distinct. The percentage variation among groups was (0.49 %). The percentage variation within groups was 1.86 percent. The highest percentage variation was within populations (97.65 percent) (Table 8).

Table 8: Analysis of variation (ANOVA) for East African sweet potato cultivars

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**% Variation**	**F-value**
Among groups	1	12.786	0.03789 Va	0.49	**0.02353**
Among pop. Within groups	1	9.284	0.14476 Vb	1.86	
Within populations	66	500.278	7.57996 Vc	**97.65**	
Total	**68**	**522.348**	**7.76261**		

Group 1: Uganda, Kenya

Group 2: Tanzania

The high percentage variation within-populations (97.65 %) is an indicator of the fact that distinct cultivars from each country are quite varied when examined on their own. This could be attributed to the breeding programs, which have been going on in these individual countries for the last 25-30 years (Kapinga *et al.*, 1995; Mwanga *et al.*, 1995). Usually the varieties grown by farmers are released varieties that are newly bred (Mwanga *et al.*, 2001). For example in Uganda,'Sowola'made from a polycross of 18 parents consisting of farmer's cultivars from various parts of Uganda was released in

1995 (Mwanga *et al*., 2001). The low values for percentage variation shown among-groups and within-groups data seems to suggest that when the Uganda-Kenya accessions are compared to the Tanzanian accessions, they do not seem to be distinctly different. This implies that the spatial distribution of diversity among and within the East African sweet potato populations was limited.

4.1.3 Gene flow within cultivars using morphological data

The gene flow values (Table 9) are > 1 also indicating potential of high gene flow within these cultivars owing to the high exchange of planting material in the region and probably alluding to the fact that sexual reproduction may be taking place in this crop resulting in exchange of genetic material within the crop.

Table 9: Matrix of Mean values showing gene flow within the East African sweet potato cultivars

	Tanzania	Uganda	Kenya
Tanzania	0.00000	0.00000	0.00000
Uganda	22.77910	0.00000	0.00000
Kenya	14.25349	26.65435	0.00000

This could probably explain the reason why the cultivars in this region are not very distinct because they have probably been selected from one clone or parent. It has been shown that N_m values greater than 1, imply that gene flow is strong enough to prevent substantial fixation of selectively neutral alleles in populations, preventing development

of genetic structures (Wright 1951; Slatkin and Barton, 1989). However the highest gene flow was found between the Ugandan and Kenyan cultivars (26.65435). The least gene flow was occurring between the Kenyan and Tanzanian cultivars (14.25349). This could be due to the distance between the two sites where the collections were obtained from. The gene flow values between the Kenyan and Ugandan populations was the highest (8.72716), implying a strong gene flow may have occurred or is prevalent between these two populations

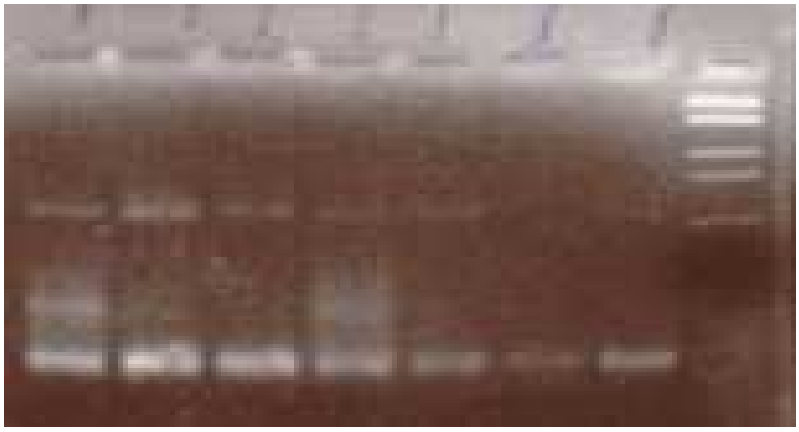
4.2 Results of molecular characterization

4.2.1 Polymorphisms

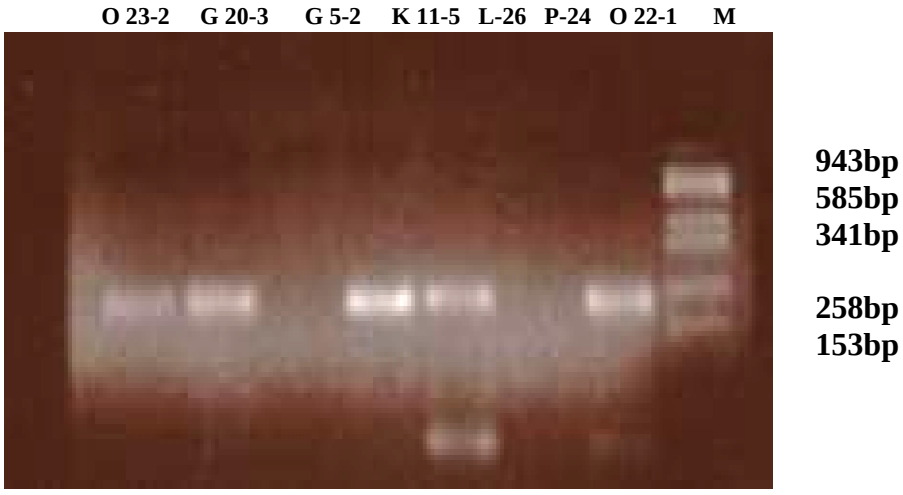
The DNA analysis using four pairs of SSR primers (Table 6) gave between 1 to 3 polymorphic bands (Figure 3). The nomenclature, codes and origin of the samples in Figure 3 are given in Table 7. The size range of the amplified products differed according to the primers used, but all were in the range of 36-943 bp.The SSR banding patterns in Figure 3 b confirm that cultivars G 20-3 and K 11-5 as amplified by primer IB-316 are the same cultivar because they both amplified the same locus. Both of these cultivars were given the name Dimbuka when they were collected from the farmers' fields. This shows the value of DNA analysis in identifying duplicates in germplasm collections. Cultivars that are morphologically identical and produce the same total protein and esterase electrophoretic banding patterns or DNA fingerprints are considered as duplicates (Huaman and Zhang 1997; Zhang *et al*., 1997). The cultivars from Kenya (D 6-1, C 1-1, D 2-unk, and C 2-1) also gave an identical banding pattern (Figure 3 c). The gel picture in Figure 3 c illustrates the usefulness of PCR-SSR for differentiating the cultivars that had been identified as morphologically different but at molecular level are s given a name by farmers; the rest of the clones had no local names specified for them although they differed in morphological features. However from DNA analysis it is clear that all these cultivars are actually the same clone. A similar principle was applied by researchers at CIP (Kenya) to classify four cultivars that could not be separated on morphological basis. These four cultivars namely SPN/O, Chingowva, KEMB 10 and Tanzania were classified as the same cultivar because they gave an identical banding pattern using SSR primer IB-255 (McGregor, 2000).

a)

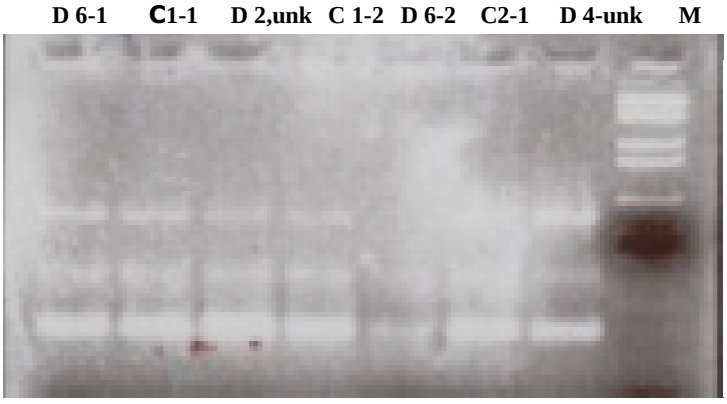
A 1- 10 **A 5- 2** **A 8-1** **B 5-1** **B 2-5** **A 9-2** **B 2-2**


943bp
585bp
341bp
153bp
36bp

b)


O 23-2
G 20-3
G 5-2
K 11-5
L-26
P-24
O 22-1
M
943bp
585bp
341bp
258bp
153bp

c)


D 6-1
C1-1
D 2,unk
C 1-2
D 6-2
C2-1
D 4-unk
M
943bp
585bp
341bp
258bp
153bp

ırs

18

However, identifying duplicates in sweet potato is complicated by the fact that somaclonal mutation is frequent in this crop (Zhang, 1996). Therefore, it is important to know whether these mutations are detectable by DNA fingerprinting methods. The cultivars (G 5-2,P-24) as shown in Figure 3 b gave no amplification product, therefore their specific loci might have been mutated such that they could not be amplified using primer IB-316. This may suggest that primer IB-316 might not be used universally in phylogenetic analysis.Using primer combination IB-318 (Figure 3 a), the cultivars A 5-2, A 8-1 and B 2-5 gave 2 bands of size 155 base pairs (bp) and 36 bp whereas cultivars A 1-10 and B 5-1 gave three bands of size 153 bp, 78 bp and 36 bp and cultivars A 9-2 and B 2-2 gave 1 band of size 36 bp. Using primer combination IB-316 (Figure 3 b), the following results were observed: cultivars O 23-2, G 20-3, K 11-5 and O 22-1 gave one band of size 258bp, cultivar L-26 gave two bands of 258bp and 36 bp and cultivars G 5-2 and P-24 gave no amplification product. When primer IB-242 was used on the Kenyan cultivars (Figure 3 c) it gave similar bands for cultivars D 6-1, C1-1, D 2, unk C 1-2, C2-1 and D 4-unk. The band sizes were 153 bp, 105 bp and 36 bp respectively. However cultivar D 6-2 gave only one band of size 36 bp.Thus amplification of 57 different cultivars with these four primers yielded a total of 14 alleles. Polymorphism of the alleles was analysed and 13 out of the 14 alleles were found to be polymorphic (Table 10). This means that a high level of SSR polymorphism (93%) was obtained. An allele is considered to be polymorphic when it is present in one cultivar but absent in another. In this study, primer pairs IB-316, IB-248 and IB-318 amplified 2, 3 and 4 alleles out of 2,3 and 4 alleles obtained. This indicates that from these primers there was 100 % polymorphism. Primer pair IB-242 amplified the highest number of alleles (5) with 4 alleles being found to be polymorphic. This indicates 80% polymorphism. On average, 93 % polymorphism was found for sweet potato based on SSR markers. The high level (93%) of SSR polymorphism in this study is comparable to that seen by RAPD analysis of sweet potato, 77.6 % (Connolly *et al*., 1994), 51.7 % (Zhang *et al*., 1998) or ISSR analysis of 5 cultivars of allohexaploid sweet potato (Huang and Sun, 2000). The SSR studies seem to suggest that there is distinct genetic variation in the East African sweet potato genomes. It is likely that the large genome size, allopolyploidy and heterozygosity of sweet potato are the reasons for its high levels of polymorphism (Hwang *et al*., 2002). It was reported by He *et al*. (1995) that high levels of polymorphism among sweet potato cultivars were fixed through vegetative reproduction and maintained through high level of gene flow due to self-incompatibility.

Table 10: Polymorphism of the alleles (bands) amplified from the SSR locus

Primer pairs	**Total alleles amplified**		**Polymorphic alleles amplified**		**Non-polymorphic alleles**	
	Total	**Sizes (bp)**	**Number**	**Sizes**	**Number**	**Size**

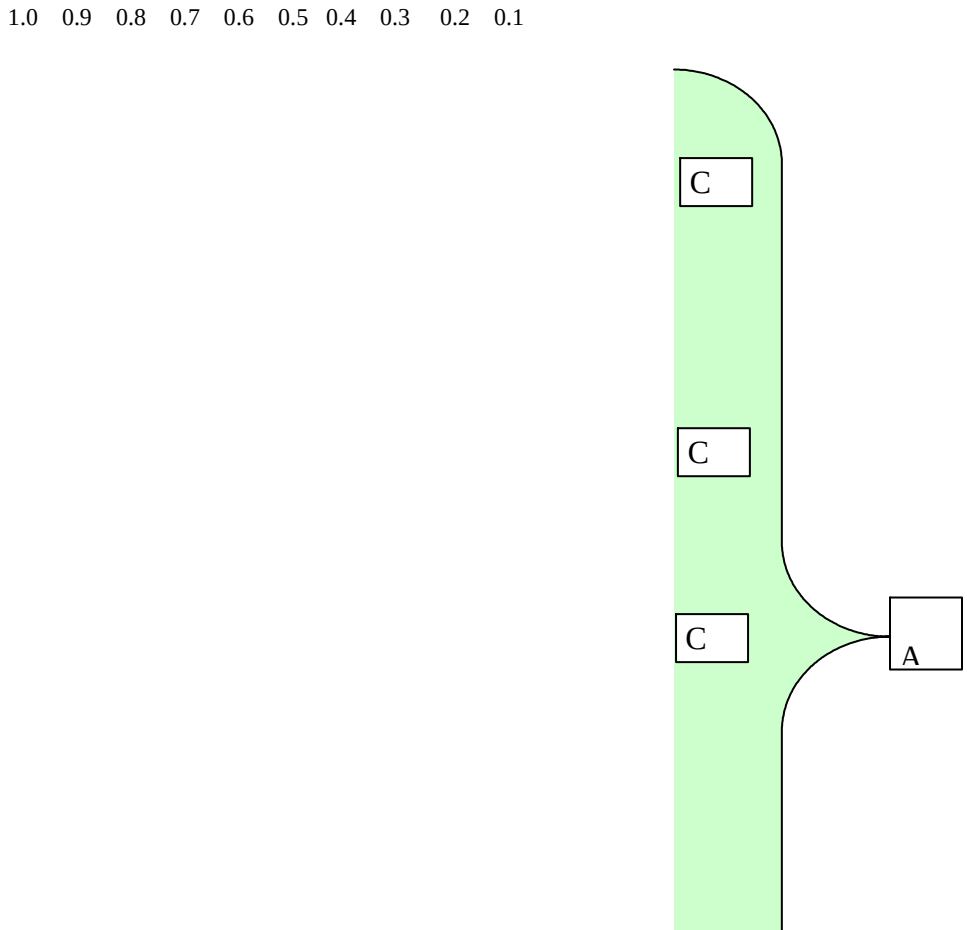
	number					
IB-316	2	943 and 36	2	943 and 36	-	-
IB-318	4	943, 585, 341 and 153	4	943,585,341 and 153	-	-
IB-248	3	153, 105 and 36	3	153,105 and 36	-	-
IB-242	5	258,153,78, 36 and 36	4	258,153,46 and 36	78	-
Total	14		13			
Average	3.5		3.25			

The results indicated in Table 10 show that 2-5 alleles per SSR locus were amplified in this study. However, Buteler *et al*. (1999) reported that in sweet potato 3-10 alleles per SSR primer pair was amplified. The differences in the range of alleles amplified could be due to the non-denaturing polyacrylamide gel used by Buteler *et al.* (1999) for separation of the PCR products. Acrylamide gels have greater resolving power than agarose gels. The increased resolution of acrylamide over agarose gel separation could result in the detection of larger number of alleles per locus (Agrama, 2003). However, one SSR primer pair IB-242 amplified 5 alleles both in this study and according to Buteler *et al*. (1999). SSR primer pairs IB-316, IB-248 amplified 7 and 8 alleles respectively in the Buteler *et al*. (1999) study but only 2 and 3 alleles respectively in this study. The reason for the smaller number of alleles amplified with these two primer pairs in this study was probably due to the higher annealing temperature used in this study. The annealing

temperature for IB-316 and IB-248 primer pairs was 65-60^{0}C (touch down annealing profile) in this study; however, Buteler *et al*. (1999) used 56^{0}C and 59^{0}C as the annealing temperature for IB-316 primer pair and IB-248, respectively**.** A higher annealing temperature is preferable in phylogenetic studies because it increases the number of specific amplification products.

4.2.2 Cluster analysis of molecular data

Cluster analysis was based on similarity matrices obtained with the unweighted pair group method using arithmetic averages (UPGMA) and relationships between cultivars were visualised as dendograms (Fig 4)



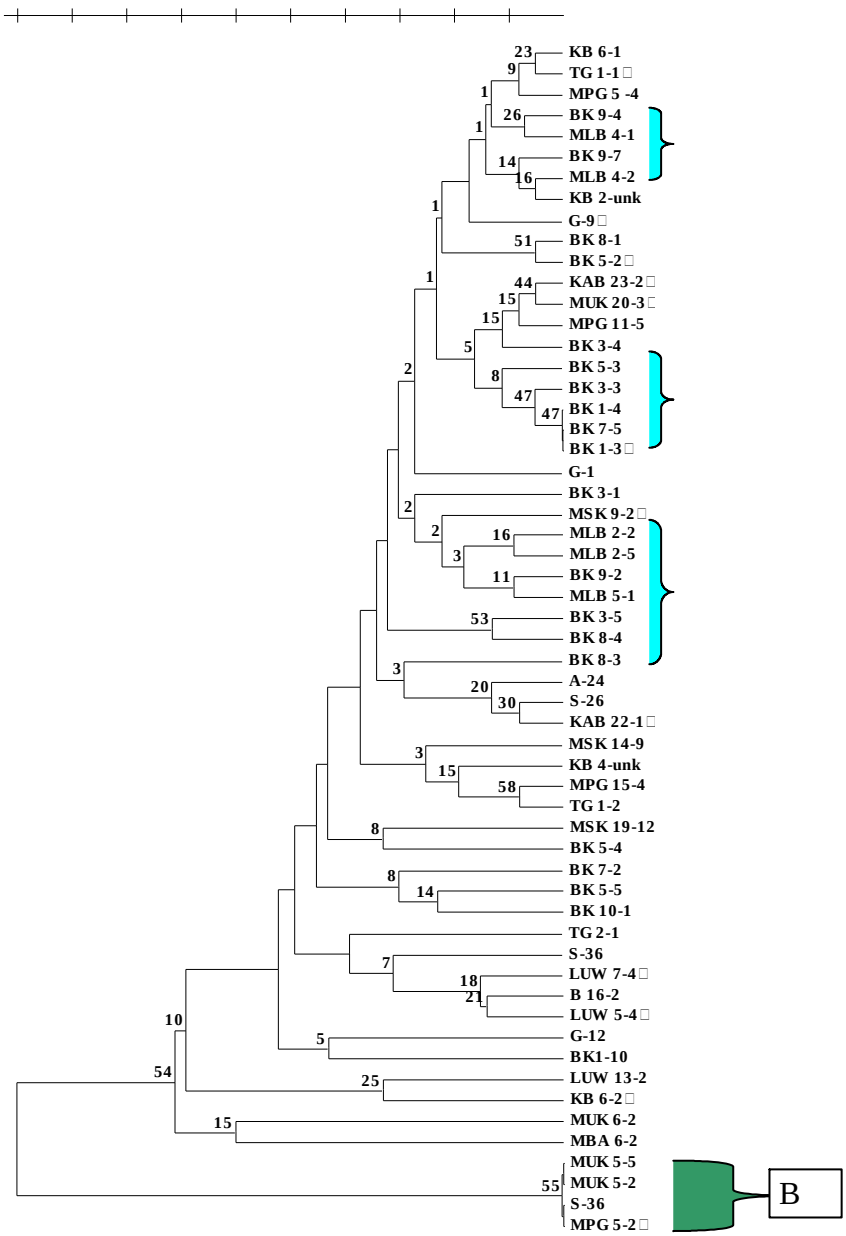


Fig 4: **Relationships among the 57 East African sweet potato cultivars using simple sequence repeats DNA markers.**The scaled bar on top of the phenogram refers to Nei's genetic distance, labels at the root of the phenogram on the right refer to the cultivars in question and numbers in the phenogram are bootstrap support values generated using TREECON Version 1.3 b phylogenetic program for Window-based environment (Van de Peer and De Wachter,1994).

The results showed no major grouping of the cultivars, owing to the low bootstrap values (0-55 %), which indicates absence of distinct clusters amongst these cultivars suggesting close genetic relationships among them. Majority of accessions separated from each other at a range of 0.1- 0.3 Nei's genetic distance, which also suggests very close genetic relatedness amongst these cultivars, which may be due to selections arising from same or closely related parents. This low diversity observed could also arise from the narrow original genetic base, presumably derived from introductions into the Indian Ocean ports (Gibson, personal communication). Two major subclusters A and B were found by UPGMA at a bootstrap value of 54 %. The UPGMA clustering of sweet potato cultivars based on SSR markers showed that landraces from Kenya, Uganda and Tanzania were occurring in group A. Subcluster B comprised of cultivars from Uganda only. These data indicated that cultivars grouped together irrespective of geographical location. However the Tanzanian cultivars indicated as C, showed slight tendency of clustering together but still scattered within the variation of the Kenyan and Ugandan accessions. The cultivars grouping together irrespective of geographical location seems to suggest that these cultivars could have come from the same breeding program. Also they could have had the same origin or could have shared parents. The fact that the Tanzanian accessions showed slight clustering together seems to suggest that there has not been very recent exchange of planting materials between Tanzania and the other two countries due to separation by distance. However, Gichuki (2001) was able to detect more distinct clustering patterns among East African sweet potato cultivars, which correlated with the geographic source of sweet potato cultivars.

4.2.3 Analysis of molecular variance

This analysis revealed the presence of a population structure (F value=0.11088) because F value >0.05. The percentage variation among groups was (5.14 %). The percentage variation within groups was 5.95 %. The highest percentage variation was within populations (88.91 %) (Table 11).

Table 11: Analysis of Molecular Variance (AMOVA) of East African sweet potato cultivars

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**% variation**	**F- value**
Among groups	1	11.40	0.15762 Va	5.14	**0.11088**
Among populations within groups	1	4.724	0.18252 Vb	5.95	
Within populations	54	147.280	2.72741 Vc	**88.91**	
Total	**56**	**163.404**	**3.06755**		

Group 1: Uganda, Kenya

Group 2: Tanzania

d.f. -degrees of freedom

In order to test for genetic variation present in the population, it was necessary to complement analysis by UPGMA with the AMOVA.The results of AMOVA analysis indicated presence of a population structure suggesting that there were some slight genetic differences in the East African sweet potato cultivars not distinguishable by morphological analysis. The presence of gene flow was an indicator of sexual reproduction that occurs in the crop resulting in the genetic differences observed. This is contrary to the result obtained using morphologi**c**al analysis (Table 9) whereby no

population structure was found to exist amongst the East African sweet potato cultivars. This supports the belief (Hwang, 2002) that the SSR marker technique is highly polymorphic and is highly sensitive so as to resolve subtle relationship among sweet potato cultivars which morphological characterisation cannot do. The percentage variation among groups was low both in molecular analysis (5.14 %) and in morphological analysis (0.49 %). However the fact that the value obtained for morphological analysis was less than that of molecular analysis, suggests that DNA analysis is more sensitive for differentiating plant varieties. The percentage variation within-populations (88.91%) by molecular analysis and by morphological analysis (97.61 %) indicates that cultivars from each country are quite varied when examined on their own. This could be attributable to the breeding programs, which have been going on in these individual countries for the last 25-30 years (Kapinga *et al.,* 1995; Mwanga *et al.*, 1995). Usually the varieties grown by farmers are released varieties that are newly bred (Mwanga *et al*., 2001). For example in Uganda, 'Sowola' (a variety released in 1995) from a polycross of 18 parents made from 1989 to 1990, consisted of farmer's cultivars from various parts of Uganda (Mwanga *et al*., 2001). Also the fact that the variation within-populations seen by morphological analysis (88.9 %) is higher than that observed by molecular analysis (97.6%), can be attributed to the wide phenotypic variations that are known to exist in farmer's varieties of sweet potato in the East African region (Bashaasha *et al.,* 1995; Kapinga *et al.,* 1995).

4.2.4 Gene flow within cultivars using molecular data

Gene flow among the East African sweet potato cultivars was investigated by estimating N_m values that is the number of immigrants per generation from Wright's F_{st} (Slatkin and

Barton, 1989). N_m values were computed according to an "island model" using Arlequin (Schneider et al., 2000) and the results are presented in Table 12. The gene flow values were an indicator of high gene flow within the cultivars. This could be attributable to the fact that sexual reproduction may be occurring in the crop. The highest gene flow was found between the Ugandan and Kenyan cultivars (8.72716) implying a strong gene flow may have occurred or is still occurring among the cultivars from these two countries. This can be attributed to the fact that these countries share breeding materials a lot. The least gene flow was occurring between the Kenyan and Tanzanian cultivars (3.77641), which suggest that these populations are quite far apart geographically and hence, have little likelihood of genetic exchange through crossing of clones or parents.

Table 12: Matrix of Mean values showing gene flow values within East African sweet potato cultivars

	Tanzania	Uganda	Kenya
Tanzania	0.00000	0.00000	0.00000
Uganda	3.95460	0.00000	0.00000
Kenya	3.77641	8.72716	0.00000

However, the likelihood of gene flow occurring is very minimal because this crop is harvested often piece meal to provide fresh daily food for a family (Karyeija *et al*., 1998). In that case it does not stay in the field long enough to allow genetic recombination to take place through sexual reproduction.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 GENERAL DISCUSSION

In this study, UPGMA analysis using both morphological and molecular analysis indicated low bootstrap values (0-85 %) as well as most of the cultivars separated by 0.1-0.4 Nei's genetic distance. This result indicates that there is close genetic relatedness amongst the East African sweet potato which suggests a common source of introduction of the crop as well as arising from the high exchange of sweet potato vines in the East African region. Similar work done on selected sweet potato cultivars in Uganda using AFLP indicated that the sweet potato had low bootstrap values suggesting lack of distinct cultivars (Nakattude, 2002). The analysis also indicated random variation amongst the cultivars irrespective of geographical location. Gichuki et al. (2000) reported significant genetic diversity of sweet potato cultivars grown in the same growing region. However from both morphological and molecular analysis, it was clear that the cultivars from Tanzania displayed sub-clustering within the major sub-group A thus suggesting that these cultivars maybe distinct from the Kenyan and Ugandan cultivars. This suggests that there has not been recent exchange of material between Tanzania and the other two countries: Kenya and Uganda.

In this study, the morphological analysis of sweet potato cultivars indicated that there was not much variation in the East African sweet potato. This was shown by an F-value<0.05 which also indicated a lack of population structure. Similar work done on East African

bananas showed no distinct clusters due to vegetative propagation of the crop (Tugume, 2002). Since sweet potatoes are also vegetatively propagated, this could be the reason for the low variation in the collected cultivars. However, the investigation at genome level, using PCR-based SSR markers was able to identify significant variation amongst the cultivars and the existence of a population structure (F value > 0.05). This result indicates that molecular analysis is more sensitive than morphological analysis in distinguishing sweet potato cultivars.The type of discrepancy between molecular characterisation and morphology-based characterisation is not likely to arise from a lack of sufficient variation within and between cultivars. It could be due to the fact that some genotypes of the East African sweet potatoes are morphologically similar therefore errors are likely to occur during their morphological characterisation and identification. This points to the value of molecular genetic characterisation of sweet potato germplasm resources (Huang and Sun, 2000).

There is a high percentage within-population variation therefore indicating that when cultivars from each country are examined individually (i.e. without making comparisons among country), they appear to be morphologically differentiated and genetically distinct. From this study, the existence of various varietal names in the East African region based on phenotypic variation could be an indication of their genetic variability. Although the bootstrap values were low, UPGMA clustering was able to distinguish the cultivars and also to identify synonyms.

5.2 Conclusions

This study was able to achieve the major objective of characterising some sweet potato cultivars from a few selected locations in East Africa mainly from 11 districts in Uganda, 2 districts in Kenya and 2 districts in Tanzania.

The conclusions from this study are:

1) Morphological characters are important in identifying, differentiating, assessing diversity and eliminating duplicates in East African sweet potato cultivars.

2) PCR-based SSR markers are more sensitive for identifying, differentiating, assessing genetic diversity and eliminating duplicates in East African sweet potato cultivars.

3) SSR markers are more appropriate for the genotyping and revealing genetic relationships of East African sweet potato cultivars and can be complemented with morphological characterisation.

Recommendations

Based on the results of this study, it is recommended that:

1. There is need for wider genome coverage and thus the need to employ more SSR primers that have been synthesised by CIP to complement the four primers used in this study.
2. Since there are about 2000 landraces in the East African region, a wider coverage of landraces should be considered in future morphological and DNA studies in order to assess the complete picture of the genetic diversity in the region.
3. Future studies should also consider including released varieties as well as the wild *Ipomoea* species and sweet potato landraces from other countries in the world in

order to compare them with those of the East African region so as to assess genetic diversity.

4. Sweet potato breeders should establish a well-characterised sweet potato germplasm complete with passport data, basing both on morphological and DNA characterisation.

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Appendix 1: Germplasm collection at Makerere University Agricultural Research Institute Kabanyolo (MUARIK)

Cultivars from Mukono District

Cultivar code	Local name	Sub-county	GPS reading
MUK 1,1	Dimbuka	Nakisunga	$00^0$18.86N,$032^0$46.30E,1201m
MUK 1,2	Silk	Nakisunga	$00^0$18.86N,$032^0$46.30E,1201m
MUK 2,1	Dimbuka	Nakisunga	$00^0$17.22N,$032^0$47.43E,1180m
MUK 2,2	New Kawogo	Nakisunga	$00^0$17.22N,$032^0$47.43E,1180m
MUK 3,2	Dimbuka	Nakisunga	$00^0$15.49N,$032^0$49.27E,1181m
MUK 3,3	Kimotooka	Nakisunga	$00^0$15.49N,$032^0$49.27E,1181m
MUK 3,4	Sukali	Nakisunga	$00^0$15.49N,$032^0$49.27E,1181m
MUK 4,1	New Kawogo	Nakisunga	$00^0$15.76N,$032^0$51.37E,1200m
MUK 4,2	Dimbuka	Nakisunga	$00^0$15.76N,$032^0$51.37E,1200m
MUK 4,3	Suula oluti	Nakisunga	$00^0$15.76N,$032^0$51.37E,1200m
MUK 5,1	Soroti	Nakisunga	$00^0$15.47N,$032^0$51.60E,1207m
MUK 5,2	Nantongo	Nakisunga	$00^0$15.47N,$032^0$51.60E,1207m
MUK 5,3	New Kawogo	Nakisunga	$00^0$15.47N,$032^0$51.60E,1207m
MUK 5,4	Silk	Nakisunga	$00^0$15.47N,$032^0$51.60E,1207m
MUK 5,5	Munyera	Nakisunga	$00^0$15.47N,$032^0$51.60E,1207m
MUK 5,6	Anonymous	Nakisunga	$00^0$15.47N,$032^0$51.60E,1207m
MUK 6,1	New Kawogo	Nakisunga	$00^0$14.83N,$032^0$52.07E,1195m
MUK 6,2	Bunduguza	Nakisunga	$00^0$14.83N,$032^0$52.07E,1195m
MUK 7,1	Kalebe	Sabagabo Nkokonjeru	$00^0$14.70N,$032^0$54.84E,1195m
MUK 7,2	Soroti	Sabagabo Nkokonjeru	$00^0$14.70N,$032^0$54.84E,1195m
MUK 7,3	New Kawogo	Sabagabo Nkokonjeru	$00^0$14.70N,$032^0$54.84E,1195m
MUK 7,4	Suula Oluuti	Sabagabo Nkokonjeru	$00^0$14.70N,$032^0$54.84E,1195m
MUK 7,5	Bunduguza	Sabagabo Nkokonjeru	$00^0$14.70N,$032^0$54.84E,1195m
MUK 7,6	Bitambi	Sabagabo Nkokonjeru	$00^0$14.70N,$032^0$54.84E,1195m
MUK 7,7	Kimotooka	Sabagabo Nkokonjeru	$00^0$14.70N,$032^0$54.84E,1195m
MUK 7,8	Ntudde buleku	Sabagabo Nkokonjeru	$00^0$14.70N,$032^0$54.84E,1195m
MUK 8,1	Silk	Sabagabo Nkokonjeru	$00^0$14.71N,$032^0$55.26E,1124m
MUK 8,5	New Kawogo	Sabagabo Nkokonjeru	$00^0$14.71N,$032^0$55.26E,1124m
MUK 9,1	Unknown	Ngogwe	$00^0$15.67N,$032^0$55.48E,1196m
MUK 9,2	Soroti(reddish)	Ngogwe	$00^0$15.67N,$032^0$55.48E,1196m
MUK 9,3	Soroti (pale green)	Ngogwe	$00^0$15.67N,$032^0$55.48E,1196m

MUK 9,5	Bunduguza	Ngogwe	$00^0$15.67N,$032^0$55.48E,1196m
MUK 10,2	Silk	Kawolo	$00^0$16.69N,$032^0$55.69E,1216m
MUK 10,3	Nakimese	Kawolo	$00^0$16.69N,$032^0$55.69E,1216m
MUK 11,1	Bitambi	Kawolo	$00^0$18.81N,$032^0$54.55E,1223m
MUK 11,2	Silk	Kawolo	$00^0$18.81N,$032^0$54.55E,1223m
MUK 11,4	Sitya musezi (Kimotooka)	Kawolo	$00^0$18.81N,$032^0$54.55E,1223m
MUK 11,5	Fumbura abaana	Kawolo	$00^0$18.81N,$032^0$54.55E,1223m
MUK 11,6	Somba obusero	Kawolo	$00^0$18.81N,$032^0$54.55E,1223m
MUK 11,7	Suula akati	Kawolo	$00^0$18.81N,$032^0$54.55E,1223m
MUK 11,8	Nvuunza	Kawolo	$00^0$18.81N,$032^0$54.55E,1223m
MUK 13,1	Silk	Kalungumira	$00^0$35.98N,$033^0$00.62E,1117m
MUK 13,2	Dimbuka	Kalungumira	$00^0$35.98N,$033^0$00.62E,1117m
MUK 13,3	Kimotooka	Kalungumira	$00^0$35.98N,$033^0$00.62E,1117m
MUK 16,2	Soroti	Ntenjeru	$00^0$40.91N,$032^0$53.89E,1086m
MUK 18,2	Silk	Kasano	$00^0$39.42N,$032^0$51.63E,1086m
MUK 18,3	Kateeteyi	Kasano	$00^0$39.42N,$032^0$51.63E,1086m
MUK 18,5	Kawungeezi	Kasano	$00^0$39.42N,$032^0$51.63E,1086m
MUK 20,1	Suula akati	Kasano	$00^0$37.77N,$032^0$49.61E,1091m
MUK 20,3	Nairobi	Kasano	$00^0$37.77N,$032^0$49.61E,1091m
MUK 21,2	Sifumba na ngajaba	Kasano	$00^0$36.53N,$032^0$48.70E,1090m

Cultivars from Luwero distict

Cultivar code	Local name	Sub-county	GPS reading
LUW 1,1	Dimbuka	Banunanika	$00^0$36.19N,$032^0$40.40E,1144m
LUW 1,2	New Kawogo	Banunanika	$00^0$36.19N,$032^0$40.40E,1144m
LUW 1,3	Bitambi	Banunanika	$00^0$36.19N,$032^0$40.40E,1144m
LUW 1,4	Mukutula	Banunanika	$00^0$36.19N,$032^0$40.40E,1144m
LUW 2,1	Dimbuka	Kalagala	$00^0$36.44N,$032^0$40.91E,1136m
LUW 5,2	Kateeteyi	Zirobwe	$00^0$40.35N,$032^0$42.03E,1081m
LUW 5,3	Naspot	Zirobwe	$00^0$40.35N,$032^0$42.03E,1081m
LUW 5,4	Silk	Zirobwe	$00^0$40.35N,$032^0$42.03E,1081m
LUW 5,5	Matugakibe	Zirobwe	$00^0$40.35N,$032^0$42.03E,1081m
LUW 5,6	Dimbuka(red)	Zirobwe	$00^0$40.35N,$032^0$42.03E,1081m
LUW 6,2	Mbale	Zirobwe	$00^0$41.19N,$032^0$41.95E,1115m
LUW 7,1	Dimbuka	Zirobwe	$00^0$45.67N,$032^0$40.61E,1100m
LUW 7,2	Soroti	Zirobwe	$00^0$45.67N,$032^0$40.61E,1100m
LUW 7,3	Katale Kake	Zirobwe	$00^0$45.67N,$032^0$40.61E,1100m
LUW 7,4	Kateeteyi	Zirobwe	$00^0$45.67N,$032^0$40.61E,1100m
LUW 7,5	Kyebandula	Zirobwe	$00^0$45.67N,$032^0$40.61E,1100m
LUW 11,2	Dimbuka	Wabusaana	$00^0$55.53N,$032^0$38.39E,1091m
LUW 11,3	New Kawogo	Wabusaana	$00^0$55.53N,$032^0$38.39E,1091m
LUW 12,2	Baganzimbire	Wabusaana	$00^0$56.28N,$032^0$37.97E,1123m
LUW 13,1	Dimbuka	Kamiura	$00^0$56.79N,$032^0$37.22E,1130m
LUW 13,2	Kawongo	Kamiura	$00^0$56.79N,$032^0$37.22E,1130m
LUW 13,4	Sukali	Kamiura	$00^0$56.79N,$032^0$37.22E,1130m
LUW 14,2	Silk	Kakooge	$00^0$59.36N,$032^0$32.81E,1093m
LUW 14,3	Old Kawogo	Kakooge	$00^0$59.36N,$032^0$32.81E,1093m

LUW 15,1	Muijjuza ddebe	Kakooge	$01^0$00.68N,$032^0$31.21E,1096m
LUW 15,3	Migeera	Kakooge	$01^0$00.68N,$032^0$31.21E,1096m
LUW 15,5	Kawongo	Kakooge	$01^0$00.68N,$032^0$31.21E,1096m
LUW 15,6	Kasifa	Kakooge	$01^0$00.68N,$032^0$31.21E,1096m
LUW 16,1	Kenya	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,3	Gaddumira	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,4	Bagamyombokere	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,6	Muijjuza ndebe	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,8	Ntudde buleku	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,9	Timba	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,10	Kaukunkumuke	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,11	Lunyonyi	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,12	Misaki	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,13	Nakato	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,14	Magabali	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 16,15	Mugeera	Kakooge	$01^0$02.29N,$032^0$28.87E,1082m
LUW 17,1	Nkoola onvirre	Kakooge	$01^0$03.35N,$032^0$28.02E,1082m
LUW 17,2	Baginyombokere	Kakooge	$01^0$03.35N,$032^0$28.02E,1082m
LUW 19,2	Silk	Butuntumilla	$00^0$54.82N,$032^0$28.35E,1112m
LUW 20,2	Mbale	Butuntumilla	$00^0$39.27N,$032^0$29.36E,1167m
LUW 20,3	Sukali	Butuntumilla	$00^0$39.27N,$032^0$29.36E,1167m
LUW 20,4	Nylon	Butuntumilla	$00^0$39.27N,$032^0$29.36E,1167m
LUW 21,3	Dimbuka	Nyimbwa	$00^0$38.56N,$032^0$31.11E,1157m
LUW 22,1	New Kawogo	Nyimbwa	$00^0$36.65N,$032^0$31.87E,1168m
LUW 23,2	Njule	Nyimbwa	$00^0$33.34N,$032^0$31.93E,1181m

Cultivars from Mpigi district

Cultivar code	Local name	Sub-county/village	GPS reading
MPG 3,2	Anonymous	Mayembe (v)	$00^0$13.97N,$032^0$18.91E,1248m
MPG 3,3	Kisa Kya Maria	Mayembe (v)	$00^0$13.97N,$032^0$18.91E,1248m
MPG 3,5	Busia	Mayembe (v)	$00^0$13.97N,$032^0$18.19E,1248m
MPG 3,6	Anonymous	Mayembe (v)	$00^0$13.97N,$032^0$18.19E,1248m
MPG 4,1	Soroti	Mbooza (v)	$00^0$14.75N,$032^0$17.98E,1224m
MPG 5,2	Kajereje	Kimbugu(v)	$00^0$14.75N,$032^0$17.98E,1224m
MPG 5,4	Soroti	Kimbugu(v)	$00^0$14.75N,$032^0$17.98E,1224m
MPG 7,1	Kavuza	Luggo (v)	$00^0$15.18N,$032^0$16.07E,1192m
MPG 7,2	Sukali	Luggo (v)	$00^0$15.18N,$032^0$16.07E,1192m
MPG 7,3	Kalebe	Luggo (v)	$00^0$15.18N,$032^0$16.07E,1192m
MPG 8,1	Kavuza	Kabasanda(v)	$00^0$15.46N,$032^0$13.88E,1177m
MPG 8,2	Mbale	Kabasanda(v)	$00^0$15.46N,$032^0$13.88E,1177m
MPG 8,5	Kawanda	Kabasanda(v)	$00^0$15.46N,$032^0$13.88E,1177m
MPG 9,2	Bitambi	Kalamba	$00^0$15.29N,$032^0$12.16E,1200m
MPG 9,3	Kisese	Kalamba	$00^0$15.29N,$032^0$12.16E,1200m
MPG 10,1	Kavuza	Kalamba	$00^0$15.01N,$032^0$11.23E,1221m
MPG 10,2	Dimbuka	Kalamba	$00^0$15.01N,$032^0$11.23E,1221m
MPG 10,3	Kyebandula	Kalamba	$00^0$15.01N,$032^0$11.23E,1221m

MPG 10,5	Kalebe	Mumu	$00^0$15.01N,$032^0$11.23E,1221m
MPG 10,6	Kimotooka	Mumu	$00^0$15.01N,$032^0$11.23E,1221m
MPG 11,1	Kavuza	Sabagabo	$00^0$14.25N,$032^0$10.65E,1240m
MPG 11,2	Kasenene	Sabagabo	$00^0$14.25N,$032^0$10.65E,1240m
MPG 11,4	Kalebe	Sabagabo	$00^0$14.25N,$032^0$10.65E,1240m
MPG 11,5	Dimbuka	Sabagabo	$00^0$14.25N,$032^0$10.65E,1240m
MPG 12,1	Old Kawogo	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,2	New Kawogo	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,3	Halima	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,4	Munyera	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,5	Kifuko	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,6	Mbikira	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,7	Mbakayabwe	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,8	Kifuta	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,9	Kyebandula	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 12,10	Dimbuka	Kibibi	$00^0$11.00N,$032^0$08.83E,1205m
MPG 13,1	Kawogo	Buggoye	$00^0$11.17N,$032^0$07.72E,1205m
MPG 13,2	Kavuza	Buggoye	$00^0$11.17N,$032^0$07.72E,1205m
MPG 14,2	Anonymous	Kayenje	$00^0$10.45N,$032^0$05.87E,1244m
MPG 15,1	Anonymous	Kayenje	$00^0$11.34N,$032^0$04.29E,1312m
MPG 15,2	Anonymous	Mpenya	$00^0$11.34N,$032^0$04.29,1312m
MPG 15,3	Kyevu	Mpenya	$00^0$11.34N,$032^0$04.29,1312m
MPG 15,4	Kitunde	Mpenya	$00^0$11.34N,$032^0$04.29,1312m
MPG 15,5	Kisa Kya Maria	Mpenya	$00^0$11.34N,$032^0$04.29,1312m
MPG 15,6	Kavuza	Mpenya	$00^0$11.34N,$032^0$04.29,1312m
MPG 15,8	Namubiru	Mpenya	$00^0$11.34N,$032^0$04.29,1312m
MPG 16,1	Buduka	Kyegonza	$00^0$11.13N,$031^0$58.74E,1250m
MPG 16,2	Kawogo	Kyegonza	$00^0$11.13N,$031^0$58.74E,1250m
MPG 16,3	Kavuza	Kyegonza	$00^0$11.13N,$031^0$58.74E,1250m
MPG 16,4	Soroti	Kyegonza	$00^0$11.13N,$031^0$58.74E,1250m
MPG 16,5	Anonymous	Kyegonza	$00^0$11.13N,$031^0$58.74E,1250m
MPG 16,6	Anonymous	Kyegonza	$00^0$11.13N,$031^0$58.74E,1250m
MPG 16,7	Anonymous	Kyegonza	$00^0$11.13N,$031^0$58.74E,1250m
MPG 17,1	Nassulu	Kimbo (v)	$00^0$11.40N,$031^0$58.30E,1228m
MPG 17,2	Dimbuka	Kimbo (v)	$00^0$11.40N,$031^0$58.30E,1228m
MPG 17,3	Kawogo	Kimbo (v)	$00^0$11.40N,$031^0$58.30E,1228m
MPG 18,1	Anonymous	Kyegonza	$00^0$10.82N,$031^0$57.03E,1228m
MPG 18,2	Anonymou	Kyegonza	$00^0$10.82N,$031^0$57.03E,1228m
MPG 18,3	Mulegerera nkofu	Kyegonza	$00^0$10.82N,$031^0$57.03E,1228m
MPG 18,4	Anonymous	Kyegonza	$00^0$10.82N,$031^0$57.03E,1228m
MPG 18,5	Anonymous	Kyegonza	$00^0$10.82N,$031^0$57.03E,1228m
MPG 18,6	Silk	Kyegonza	$00^0$10.82N,$031^0$57.03E,1228m
MPG 18,	Anonymous	Kyegonza	$00^0$10.82N,$031^0$57.03E,1228m
MPG 19,1	New Kawogo	Kyegonza	$00^0$10.46N,$031^0$55.63E,1258m
MPG 19,2	Kimotooka	Kyegonza	$00^0$10.46N,$031^0$55.63E,1258m
MPG 19,3	Somba obusero	Kyegonza	$00^0$10.46N,$031^0$55.63E,1258m
MPG 19,4	Kawogo	Kyegonza	$00^0$10.46N,$031^0$55.63E,1258m
MPG 19,5	Kawogo	Kyegonza	$00^0$10.46N,$031^0$55.63E,1258m
MPG 20,1	New Kalebe	Kyegonza	$00^0$10.28N,$031^0$54.68E,1212m

MPG 20,2	Sukaali	Kyegonza	$00^0$10.28N,$031^0$54.68E,1212m

Cultivars from Masaka District

Cultivar code	Local name	Subcounty/village	GPS reading
MSK 1,1	Sifumba nagayala	Bugumola (v)	$00^0$02.52N,$031^0$44.92E,1214m
MSK 1,2	Kabuusu	Bugumola (v)	$00^0$02.52N,$031^0$44.92E,1214m
MSK 1,3	Somba obusero	Bugumola (v)	$00^0$02.52N,$031^0$44.92E,1214m
MSK 1,4	Munafu adimbuka	Bugumola (v)	$00^0$02.52N,$031^0$44.92E,1214m
MSK 1,5	Kimotooka	Bugumola (v)	$00^0$02.52N,$031^0$44.92E,1214m
MSK 1,6	Kawogo	Bugumola (v)	$00^0$02.52N,$031^0$44.92E,1214m
MSK 2,1	New Kawogo	Rabwenge	$00^0$01.81N,$031^0$44.88E,1215m
MSK 2,2	Kalebe	Rabwenge	$00^0$01.81N,$031^0$44.88E,1215m
MSK 2,3	Nawmezigumu	Rabwenge	$00^0$01.81N,$031^0$44.88E,1215m
MSK 2,4	Kavuza	Rabwenge	$00^0$01.81N,$031^0$44.88E,1215m
MSK 2,5	Nylon	Rabwenge	$00^0$01.81N,$031^0$44.88E,1215m
MSK 3,1	Namwezigumu	Rabwenge	$00^0$01.81N,$031^0$44.88E,1215m
MSK 3,2	Old Kawogo	Rabwenge	$00^0$00.97N,$031^0$44.80E,1194m
MSK 3,3	Ntudde bulaku	Rabwenge	$00^0$00.97N,$031^0$44.80E,1194m
MSK 3,4	Dimbuka	Rabwenge	$00^0$00.97N,$031^0$44.80E,1194m
MSK 3,5	Anonymous	Rabwenge	$00^0$00.97N,$031^0$44.80E,1194m
MSK 4,1	Kavuza	Samba bukiri	$00^0$00.65N,$031^0$44.74E,1185m
MSK 4,2	New Kawogo	Samba bukiri	$00^0$00.65N,$031^0$44.74E,1185m
MSK 4,3	Anonymous	Samba bukiri	$00^0$00.65N,$031^0$44.74E,1185m
MSK 4,4	Kasanda	Samba bukiri	$00^0$00.65N,$031^0$44.74E,1185m
MSK 5,1	Dimbuka	Mbiwuula (v)	$00^0$0.40S,$031^0$44.50E,1196m
MSK 5,2	Matonde	Mbiwuula (v)	$00^0$0.40S,$031^0$44.50E,1196m
MSK 5,3	Somba obusera	Mbiwuula (v)	$00^0$0.40S,$031^0$44.50E,1196m
MSK 5,4	Soroti	Mbiwuula (v)	$00^0$0.40S,$031^0$44.50E,1196m
MSK 5,5	Kalebe	Mbiwuula (v)	$00^0$0.40S,$031^0$44.50E,1196m
MSK 5,6	Old Kawogo	Mbiwuula (v)	$00^0$0.40S,$031^0$44.50E,1196m
MSK 5,7	Bumbakali	Mbiwuula (v)	$00^0$0.40S,$031^0$44.50E,1196m
MSK 6,1	Kawogo	Rwabenge	$00^0$01.37S,$031^0$44.42E,1220m
MSK 6,2	Bitambi	Rwabenge	$00^0$01.37S,$031^0$44.42E,1220m
MSK 6,3	Kimtooka	Rwabenge	$00^0$01.37S,$031^0$44.42E,1220m
MSK 6,4	Kalebe	Rwabenge	$00^0$01.37S,$031^0$44.42E,1220m
MSK 7,1	Bumbakali	Kyamulibwa	$00^0$02.59S,$031^0$44.60E,1275m
MSK 7,2	New Kawogo	Kyamulibwa	$00^0$02.59S,$031^0$44.60E,1275m
MSK 7,3	Anonymous	Kyamulibwa	$00^0$02.59S,$031^0$44.60E,1275m
MSK 8,1	Kalebe	Kyamulibwa	$00^0$04.39S,$031^0$45.04E,1240m
MSK 8,2	Kimotooka	Kyamulibwa	$00^0$04.39S,$031^0$45.04E,1240m
MSK 8,3	Anonymous	Kyamulibwa	$00^0$04.39S,$031^0$45.04E,1240m
MSK 9,1	Kimotooka	Kalungu	$00^0$08.81S,$031^0$45.39E,1220m
MSK 9,2	Sifumbanagayaba	Kalungu	$00^0$08.81S,$031^0$45.39E,1220m
MSK 9,3	Anonymous	Kalungu	$00^0$08.81S,$031^0$45.39E,1220m
MSK 9,4	Anonymous	Kalungu	$00^0$08.81S,$031^0$45.39E,1220m
MSK 9,5	Anonymous	Kalungu	$00^0$08.81S,$031^0$45.39E,1220m
MSK 9,6	Anonymous	Kalungu	$00^0$08.81S,$031^0$45.39E,1220m

MSK 9,7	Anonymous	Kalungu	00^{0}08.81S,031^{0}45.39E,1220m
MSK 10,1	Kimotooka	Kabisa(v)	00^{0}09.46S,031^{0}45.34E,1241m
MSK 11,1	Kimotooka	Kabisa-lugazi(v)	00^{0}9.52S,031^{0}45.34E,1255m
MSK 11,2	Kawogo	Kabisa-lugazi(v)	00^{0}9.52S,031^{0}45.34E,1255m
MSK 11,3	Kisa Kya Maria	Kabisa-lugazi(v)	00^{0}9.52S,031^{0}45.34E,1255m
MSK 12,1	Kimotooka	Kalungu	00^{0}9.64S,031^{0}45.36E,1258m
MSK 12,2	Old Kawogo	Kalungu	00^{0}9.64S,031^{0}45.36E,1258m
MSK 12,3	Kayilu	Kalungu	00^{0}9.64S,031^{0}45.36E,1258m
MSK 12,4	Kyevu	Kalungu	00^{0}9.64S,031^{0}45.36E,1258m
MSK 12,5	Anonymous	Kalungu	00^{0}9.64S,031^{0}45.36E,1258m
MSK 13,1	Kalebe	Kalungu	00^{0}9.81S,031^{0}45.38E,1269m
MSK 13,2	髮▪▪edi	Kalungu	00^{0}9.81S,031^{0}45.38E,1269m
MSK 13,3	Sukaali	Kalungu	00^{0}9.81S,031^{0}45.38E,1269m
MSK 13,4	Kenya	Kalungu	00^{0}9.81S,031^{0}45.38E,1269m
MSK 13,5	Nganjaba	Kalungu	00^{0}9.81S,031^{0}45.38E,1269m
MSK 14,1	Kimotooka	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,2	New Kawogo	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,3	Ntudebuleku	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,5	Bumbakali	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,6	Anonymous	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,7	Anonymous	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,8	Anonymous	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,9	Sukaali	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,10	Anonymous	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,11	Anonymous	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,12	Magabali	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,13	Mbale	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,14	Anonymous	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,15	Old Kawogo	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 14,16	Anonymous	Kalungu	00^{0}10.99S,031^{0}45.61E,1165m
MSK 15,1	Damu lyamuzeeyi	Lukungwe	00^{0}10.99S,031^{0}45.61E,1165m
MSK 15,3	Old Kalebo	Lukungwe	00^{0}10.99S,031^{0}45.61E,1165m
MSK 15,4	Nsansa	Lukungwe	00^{0}10.99S,031^{0}45.61E,1165m
MSK 15,5	Timba	Lukungwe	00^{0}10.99S,031^{0}45.61E,1165m
MSK 15,6	Somba obusero(old)	Lukungwe	00^{0}10.99S,031^{0}45.61E,1165m
MSK 16,1	New Kawogo	Kyalusowei(v)	00^{0}17.22S,031^{0}47.13E,1224m
MSK 16,2	Kimtooka	Kyalusowei(v)	00^{0}17.22S,031^{0}47.13E,1224m
MSK 16,3	Mbigasso	Kyalusowei(v)	00^{0}17.22S,031^{0}47.13E,1224m
MSK 16,4	Nambale	Kyalusowei(v)	00^{0}17.22S,031^{0}47.13E,1224m
MSK 16,5	Kayinja	Kyalusowei(v)	00^{0}17.22S,031^{0}47.13E,1224m
MSK 17,1	Kyebandula	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m
MSK 17,2	New Kawogo	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m
MSK 17,3	Kimtooka	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m
MSK 17,4	Kuisaza	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m
MSK 17,5	Buganga	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m
MSK17,6	Sukaali	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m
MSK 17,7	Anonymous	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m
MSK 17,8	Anonymous	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m
MSK 17,9	Bufumbo butelede	Mukungwe	00^{0}15.92S,031^{0}48.97E,1235m

MSK 18,1	Dimbuka	Bukululа	$00^0$13.69S,$031^0$50.25E,1205m
MSK 18,2	Naluzala	Bukululа	$00^0$13.69S,$031^0$50.25E,1205m
MSK 18,3	Anonymous	Bukululа	$00^0$13.69S,$031^0$50.25E,1205m
MSK 18,4	Anonymous	Bukululа	$00^0$13.69S,$031^0$50.25E,1205m
MSK 19,1	Kawogo	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,2	Timba	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,3	Kyebandula	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,4	Bukululа	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,5	Sinfa	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,6	Anonymous	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,7	Anonymous	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,8	Anonymous	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,9	Anonymous	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,10	Anonymous	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,11	Anonymous	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 19,12	Anonymous	Bukululа	$00^0$10.72S,$031^0$51.17E,1192m
MSK 20,1	Kawogo(red)	Bukululа	00^0 09.99S,$031^0$51.63E,1209m
MSK 20,2	Soroti	Bukululа	00^0 09.99S,$031^0$51.63E,1209m
MSK 20,3	Dimbuka	Bukululа	00^0 09.99S,$031^0$51.63E,1209m
MSK 20,4	Bitambi	Bukululа	00^0 09.99S,$031^0$51.63E,1209m
MSK 20,5	Sinfa/Sadam	Bukululа	00^0 09.99S,$031^0$51.63E,1209m
MSK 21,1	New Kawogo	Bukululа	-
MSK 21,2	Anonymous	Bukululа	-
MSK 21,3	Anonymous	Bukululа	-
MSK 21,4	Anonymous	Bukululа	-

Cultivars from Arua district

Cultivar code	Local name	Subcounty/village	GPS reading
ARU-1	EwaMaku	Katrini	-
ARU-2	Karamoja (C)	Okaavia	-
ARU-3	Karamoja (B)	Katrini	-
ARU-4	Ombivu	Katrini	-
ARU-5	Ombivu	Pajulu	-
ARU-7	Andinyaku/Mbolo	Orraka	-
ARU-8	Nyaromiyo	Orraka	-
ARU-9	Mbutra	Katrini	-
ARU-10	Andinyaku (A)	Manibe	-
ARU-11	Andinyaku	Pajulu	-
ARU-12	Karamoja (A)	Pajulu	-
ARU-13	Sanja Moko	Katrini	-
ARU-15	Osisia	Adumi	-
ARU-16	Dacho	Katrini	-
ARU-17	Mba-Alua	Katrini	-
ARU-18	Araka	Offaka	-
ARU-19	Ladii	Offaka	-
ARU-20	Jopanzao	Offaka	-
ARU-21	Nakenya	Offaka	-
ARU-22	Acholi Maku	Katrini	-

ARU-23	Andinayku (B)	Katrini	-
ARU-24	Dele (B)	Vurra	-
ARU-25	Dele (A)	Vurra	-
ARU-26	Mba Alua	Adumi	-
ARU-27	Karamoja	-	-

Cultivars from Masindi District

Cultivar code	Local names	Subcounty/village	GPS reading
MAS-1	Kampala	-	-
MAS-2	Kyebandura	-	-
MAS-3	Kakobe	-	-
MAS-4	Kaahawa	-	-
MAS-5	Mwera debe	-	-
MAS-6	Rwabugerere	-	-
MAS-7	Tanzania	-	-
MAS-8	Ndabiryanda	-	-
MAS-9	Kalobo	-	-
MAS-10	MaraGalya	-	-
MAS-11	Kahogo	-	-
MAS-12	Nailoni	-	-

Cultivars from Mbarara district

Cultivar code	Local names	Subcounty	GPS reading
MBA 1,2	Rwasahansi	Ndeija	$00^0$44.69S,$030^0$20.53E,1876m
MBA 2,1	Kanyasi	Ndeija	$00^0$43.72S,$030^0$21.29E,1421m
MBA 2,2	Maria	Ndeija	$00^0$43.72S,$030^0$21.29E,1421m
MBA 2,3	Unknown	Ndeija	$00^0$43.72S,$030^0$21.29E,1421m
MBA 3,1	Kisa Maria	Ndeija	$00^0$43.59S,$030^0$21.51E,1455m
MBA 3,3	Kyitabi	Ndeija	$00^0$43.59S,$030^0$21.51E,1455m
MBA 3,4	Kyitekamajus	Ndeija	$00^0$43.59S,$030^0$21.51E,1455m
MBA 4,1	Kisa kya Maria	Ndeija	$00^0$43.57S,$030^0$21.58E,1437m
MBA 4,2	Kigabira	Ndeija	$00^0$43.57S,$030^0$21.58E,1437m
MBA 5,2	Kyitabi	Ndeija	$00^0$43.46S,$030^0$22.01E,1436m
MBA 5,3	Wandada	Ndeija	$00^0$43.46S,$030^0$22.01E,1436m
MBA 6,1	Kisa kya Maria	Ndeija	$00^0$40.82S,$030^0$24.01E,1445m
MBA 6,2	Kyitabira	Ndeija	$00^0$40.82S,$030^0$24.01E,1445m
MBA 7,1	Kanyasi	Ndeija	$00^0$40.48S,$030^0$25.31E,1432m
MBA 7,2	Kyitabi	Ndeija	$00^0$40.48S,$030^0$25.31E,1432m
MBA 8,1	Kyitabi	Ndeija	$00^0$40.35S,$030^0$25.76E,1448m
MBA 8,2	Kanyasi	Ndeija	$00^0$40.35S,$030^0$25.76E,1448m
MBA 9,2	Kyitabi	Ndeija	$00^0$40.34S,$030^0$25.79E,1428m
MBA 10,1	Mukyara tuba	Rungando	$00^0$39.19S,$030^0$29.01E,1438m
MBA 10,2	Kwasahansa	Rungando	$00^0$39.19S,$030^0$29.01E,1438m
MBA 10,3	Kyitabi	Rungando	$00^0$39.19S,$030^0$29.01E,1438m
MBA 11,1	Kitambi	Rungando	$00^0$38.64S,$030^0$29.85E,1429m
MBA 11,2	Mukazi Anura	Rungando	$00^0$38.64S,$030^0$29.85E,1429m

MBA 11,3	Mukabanyarwanda	Rungando	$00^0$38.64S,$030^0$29.85E,1429m
MBA 12,1	Kyiteka Maku	Rungando	$00^0$38.32S,$030^0$30.75E,1434m
MBA 12,2	Mukazi Ayuba	Rungando	$00^0$38.32S,$030^0$30.75E,1434m
MBA 12,3	Kahongo	Rungando	$00^0$38.32S,$030^0$30.75E,1434m
MBA 12,4	Katukura	Rungando	$00^0$38.32S,$030^0$30.75E,1434m
MBA 12,5	Kwasahansi	Rungando	$00^0$38.32S,$030^0$30.75E,1434m
MBA 13,1	Kebandira	Rungando	$00^0$38.18S,$030^0$31.08E,1440m
MBA 13,2	Kwasahansi	Rungando	$00^0$38.18S,$030^0$31.08E,1440m
MBA 14,1	Nderara	Rungando	$00^0$38.11S,$030^0$31.25E,1477m
MBA 14,2	Kyebadira	Rungando	$00^0$38.11S,$030^0$31.25E,1477m
MBA 14,3	Norah	Rungando	$00^0$38.11S,$030^0$31.25E,1477m
MBA 15,1	Kahongo	Rungando	$00^0$38.15S,$030^0$32.45E,1428m
MBA 15,2	Karebe	Rungando	$00^0$38.15S,$030^0$32.45E,1428m
MBA 16,1	Kahogezi	Nyakayojo	$00^0$38.57S,$030^0$35.18E,1415m
MBA 16,2	Kisa kya bikira maria	Nyakayojo	$00^0$38.57S,$030^0$35.18E,1415m
MBA 16,3	Kwasahansi	Nyakayojo	$00^0$38.57S,$030^0$35.18E,1415m
MBA 16,4	Karebekenda	Nyakayojo	$00^0$38.57S,$030^0$35.18E,1415m
MBA 17,1	Hotloaf	Nyakayojo	$00^0$38.49S,$030^0$35.62E,1435m
MBA 18,1	Kahongo	Nyakayojo	$00^0$38.80S,$030^0$36.07E,1438m
MBA 18,2	Kyebandira	Nyakayojo	$00^0$38.80S,$030^0$36.07E,1438m
MBA 19,1	Kanena	Bwekoma(v)	$00^0$39.09S,$030^0$35.90E,1443m
MBA 19,2	Kahongo	Nyakoni	$00^0$38.12S,$030^0$36.91E,1426m
MBA 20,1	Kisa kya bikira maria	Nyakoni	$00^0$38.12S,$030^0$36.91E,1426m
MBA 20,2	Kimotokaa	Nyakoni	$00^0$38.12S,$030^0$36.91E,1426m

Cultivars from Kabale District

Cultivar code	Local names	Subcounty	GPS reading
KAB 1,1	Kyigabari	Muko	$01^0$14.66 S, 029^0 48.75 E, 1865m
KAB 1,2	Ruhubura	Muko	$01^0$14.66 S, 029^0 48.75 E, 1865m
KAB 4,1	Nyirase	Muko	$01^0$12.49S, $029^0$48.48E, 2445m
KAB 6,1	Magabari	Muko	$01^0$12.22S,$029^0$48.67E,2220m
KAB 9,3	Kakoba	Muko	$01^0$12.07S,$029^0$50.22E,1997m
KAB 10,1	Mukazi Anura	Muko	$01^0$11.69S,$029^0$50.55E,1997m
KAB 11,2	Magabari	Muko	$01^0$11.04S, 029050.59E, 2039m
KAB 12, un	Unknown	Muko	$01^0$10.83S, 029050.51E, 2040m
KAB 12,1	Kanyasi	Muko	$01^0$10.83S, 029050.51E, 2040m
KAB 13,1	Karebe	Ikuba	$01^0$09.72S,$029^0$50.84E,1963m
KAB 14,3	Nyinarukamazi	Ikuba	$01^0$08.50S,$029^0$51.76E,2015m
KAB 15,5	Unknown	Ikuba	$01^0$08.31S,$029^0$52.10E,2015m
KAB 16,1	Nyirakamazi	Ikuba	$01^0$07.96S,$029^0$52.16E,2025m
KAB 16,2	Koshokonyokozi	Ikuba	$01^0$07.96S,$029^0$52.16E,2025m
KAB 17,2	Mukazi Anura	Ikuba	$01^0$07.52S,$029^0$53.14E,2035m
KAB 19,1	Kyindondo	Homurura	$01^0$07.10S,$029^0$53.63E,2009m
KAB 19,3	Magabari	Homurura	$01^0$07.10S,$029^0$53.63E,2009m

KAB 20,1	Mungurisi	Homurura	$01^0$7.06S,$029^0$53.73E,2009m
KAB 21,2	Magabari	Bubale	$01^0$10.94S,$029^0$55.62E,1865m
KAB 22,1	Mukazi Anura	Bubale	$01^0$11.95S,$029^0$55.82E,1876m
KAB 23,2	Egumura	Bubale	$01^0$13.20S,$029^0$57.09E,1876m
KAB 23,3	Bwajure	Bubale	$01^0$13.20S,$029^0$57.09E,1876m

Cultivars from Kisoro District

Cultivar code	Local name	Subcounty	GPS
KIS 3,1	Kanyasi	Nyakarebe Bukabiri	$01^0$10.18S,$029^0$45.36E,2335m
KIS 4,2	Kahira	Nyakarebe Bukabiri	01^0 09.17S,029^0 44.70E,2247m
KIS 5,1	Mugora	Nyakarebe Bukabiri	01^0 09.02S,029^0 44.65E,2237m
KIS 6,1	Murerabaana	Nyakarebe Bukabiri	01^0 08.59S,029^0 44.65E,2209m
KIS 10,1	Kanyasi	Nyakarebe Bukabiri	01^0 08.25S,029^0 43.84E,2010m
KIS 11,2	Murerabaana	Nyakarebe Bukabiri	01^0 08.21S,029^0 43.59E,2027m
KIS 14,1	Mugumira	Nyakarebe Bukabiri	01^0 07.96S,029^0 43.19E,1943m
KIS 15,1	Mugumira	Nyakarebe Bukabiri	01^0 07.94S,029^0 43.10E,1938m
KIS 20,1	Mvugumira	Kirundo	01^0 07.65S,029^0 41.92E,1850m
KIS 21,2	Murerarabaana	Rutaka	01^0 07.90S,029^0 41.77E,1979m
KIS 21,3	Mugora	Rutaka	01^0 07.90S,029^0 41.77E,1979m
KIS 22,1	Kanazi	Rutaka	01^0 07.82S,029^0 40.68E,1962m
KIS 24,1	Mureranbaana	Kirundo	-

Cultivars from Soroti District

Cultivar code	Local name	Subcounty	GPS
Sor-1	Ewaku	-	-
Sor-2	Bunchunguza7	-	-
Sor-3	Cheparon K1	-	-
Sor-4	Muyambi S 5	-	-
Sor-5	Magendo S7	-	-
Sor-6	Masara S1	-	-
Sor-7	Tanzania Kp 10	-	-
Sor-8	Katumani K3	-	-
Sor-9	Mwezi Gumu S5	-	-
Sor-10	Tula S6	-	-
Sor-11	Chedaron Kp 5	-	-
Sor-12	Cheparon Kp 7	-	-

Sor-13	Naspot 5	-	-
Sor-14	Asili NK 3	-	-
Sor-15	Daka daka S2	-	-
Sor-16	Edopelaap	-	-
Sor-17	Misaki	-	-
Sor-18	Aniyemo	-	-
Sor-19	Osapat	-	-
Sor-20	Sukari	-	-
Sor-21	Tat ok	-	-
Sor-22	Kawogo (AT) KP 14	-	-
Sor-23	Otto	-	-
Sor-24	Kawogo S5a	-	-
Sor-25	Katuman Kp9	-	-
Sor-26	XKp5	-	-
Sor-27	Chematui Kp4	-	-
Sor-28	Bugerere	-	-
Sor-29	Cheparon 1 cp sk dis	-	-
Sor-30	Katumani Kp 6	-	-
Sor-31	DS 12	-	-
Sor-32	Meumbe S 5a	-	-
Sor-33	Kakandet Kp 12	-	-
Sor-34	Iwoko	-	-
Sor-35	Letes-1	-	-
Sor-36	Ateseke	-	-
Sor-37	Araka	-	-
Sor-38	Entebbe	-	-
Sor-39	Letes	-	-
Sor-40	Ewela Gura	-	-
Sor-41	Achibiri	-	-
Sor-42	Dweachel	-	-
Sor-43	Enyou	-	-
Sor-44	Esirayiri	-	-
Sor-45	Tula	-	-
Sor-46	Lira	-	-
Sor-47	Entebbe kp1	-	-
Sor-48	S14 a Nabikerereko	-	-

Cultivars from Bukoba district-Tanzania

Cultivar	Local name	Subcounty	GPS reading

code			
BK 1,2	Naonao	Kalasagaine	$01^0$21.21S,$031^0$45.41E,4327m
BK 1,3	Kombegi	Kalasagaine	$01^0$21.21S,$031^0$45.41E,4327m
BK 1,4	Kombegi	Kalasagaine	$01^0$21.21S,$031^0$45.41E,4327m
BK 1,5	Njubu	Kalasagaine	$01^0$21.21S,$031^0$45.41E,4327m
BK 1,6	Kishuguti	Kalasagaine	$01^0$21.21S,$031^0$45.41E,4327m
BK 1,7	Ruganza	Kalasagaine	$01^0$21.21S,$031^0$45.41E,4327m
BK 1,8	Tuula	Kalasagaine	$01^0$21.21S,$031^0$45.41E,4327m
BK 1,10	Unknown	Kalasagaine	$01^0$21.21S,$031^0$45.41E,4327m
BK 2,1	Damu ya Mzee	Mwogo	$01^0$17.7S,$031^0$38.46E,4319m
BK 2,4	Kigambile nyoleo	Mwogo	$01^0$17.7S,$031^0$38.46E,4319m
BK 2,6	Polista	Mwogo	$01^0$17.7S,$031^0$38.46E,4319m
BK 2,8	Vumilia	Mwogo	$01^0$17.7S,$031^0$38.46E,4319m
BK 3,3	Zerida	Kyaka	$01^0$14.37S,$031^0$23.56E,4098m
BK 3,4	Kalebe	Kyaka	$01^0$14.37S,$031^0$23.56E,4098m
BK 3,5	Unknown	Kyaka	$01^0$14.37S,$031^0$23.56E,4098m
BK 3,6	Bitambi	Kyaka	$01^0$14.37S,$031^0$23.56E,4098m
BK 3,11	Kahogo	Kyaka	$01^0$14.37S,$031^0$23.56E,4098m
BK 4,1	Namba nana	Misenyi	-
BK 4,2	Hidaya	Misenyi	-
BK 4,3	Obote	Misenyi	-
BK 5,1	Silia	Misenyi-Nsunga	-
BK 5,2	Regania	Misenyi-Nsunga	-
BK 5,3	Kigambile nyoleo	Misenyi-Nsunga	-
BK 5,4	Kagore	Misenyi-Nsunga	-
BK 5,5	Unknown	Misenyi-Nsunga	-
BK 6,1	Kigambile nyoleo	Katoro	$01^0$22.28S,$031^0$28.18E,4044m
BK 6,2	Ruganza	Katoro	$01^0$22.28S,$031^0$28.18E,4044m
BK 6,5	Unknown	Katoro	$01^0$22.28S,$031^0$28.18E,4044m
BK 7,2	Alinyiikira	Nyakirimbiri	$01^0$27.38S,$031^0$36.06E,4017m
BK 7,3	Mugolia	Nyakirimbiri	$01^0$27.38S,$031^0$36.06E,4017m
BK 7,4	Kaisho ka Mugole	Nyakirimbiri	$01^0$27.38S,$031^0$36.06E,4017m
BK 7,5	Kamogoli	Nyakirimbiri	$01^0$27.38S,$031^0$36.06E,4017m
BK 8,1	Unknown	Ntoma	$01^0$26.44S,$031^0$46.51E,4032m
BK 8,3	Unknown	Ntoma	$01^0$26.44S,$031^0$46.51E,4032m
BK 9,1	Mavuno	Maruku	$01^0$26.08S,$031^0$46.31E,4032m
BK 9,2	Madebe	Maruku	$01^0$26.08S,$031^0$46.31E,4032m
BK 9,4	Simba eichuu	Maruku	$01^0$26.08S,$031^0$46.31E,4032m
BK 9,7	Unknown	Maruku	$01^0$26.08S,$031^0$46.31E,4032m
BK 10,1	Unknown	Maruku	$01^0$25.09S,$031^0$46.47E,4042m

Cultivars from Muleba district

Cultivar code	Local name	Subcounty	GPS

MLB 2,1	Mabungu	Kabirizi	$01^0$47.07S,$031^0$30.18E,4401m
MLB 2,2	Ruganza	Kabirizi	$01^0$47.07S,$031^0$30.18E,4401m
MLB 2,5	Kagore	Kabirizi	$01^0$47.07S,$031^0$30.18E,4401m
MLB 4,1	Kibuyu	Buganguzi	$01^0$39.57S,$031^0$34.57E,4420m
MLB 4,2	Mwasa	Buganguzi	$01^0$39.57S,$031^0$34.57E,4420m
MLB 5,1	Tuula Omushako	Kamachumu	$01^0$37.37S,031039.36E,4393m
MLB 5,2	Sinia	Kamachumu	$01^0$37.37S,031039.36E,4393m
MLB 5,3	Kigambile nyoleo	Kamachumu	$01^0$37.37S,031039.36E,4393m
MLB 5,4	Kigambile nyoleo	Kamachumu	$01^0$37.37S,031039.36E,4393m

Cultivars from Arua, Soroti, Kiambu and Bukoba districts did not have their GPS taken.