CHARACTERIZATION OF UGANDAN SWEETPOTATO GENETIC POTENTIAL USING MORPHO-AGRONOMIC AND MOLECULAR APPROACHES

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DECLARATION

I, **YADA BENARD** 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

I dedicate this thesis to the Almighty God, my parents, brothers, sisters and friends.

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ABSTRACT

Sweetpotato (Ipomoea batatas (L.), a hexaploid, self-incompatible species with 2n=6x=90 chromosomes, is the seventh most important food crop in the world. Uganda is the third largest global producer after China and Nigeria, and a secondary center of diversity where subsistence farmers mostly grow it as a starchy staple for food security. Through a purposive sampling of 21 districts of Uganda, a total of 1303 sweetpotato accessions with 565 vernacular names were collected from farmers' fields in 171 sub-counties. Great variations were observed in the numbers of accessions grown in the different districts and morphological characteristics in the farmers' fields.

The level of morphological variation as estimated using the Shannon Weaver diversity index (H') for the traits ranged from 0.10 to 0.99 with an overall mean of 0.71±0.03, an indication of high diversity. Cluster analysis using the unweighted pair-group method using arithmetic averages (UPGMA) grouped 1256 of the accessions into 20 major clusters and many sub-clusters and classified 899 accessions as morphologically distinct and 357 as duplicates.

The morphologically distinct genotypes differed significantly for yield, dry matter content, sweeetpotato virus disease and Alternaria blight resistance (p=0.05). Most of the genotypes had mean total root yields of less than 20.0 t/ha, low specific gravity values of less than 2.0, and low disease resistance. A total of 192 superior genotypes were selected for further evaluation and use in hybridization schemes and variety development.

Ten fluorescent-labeled simple sequence repeat (SSR) markers were used to assess the genetic relationships among the superior genotypes. The mean pair-wise genetic distance among the 192 genotypes was 0.57, an indication of high genetic diversity. The 192 genotypes were grouped into four major clusters. The SSR markers used in the study were highly informative with average polymorphic information content (PIC) of 0.62. Two duplicate genotypes were identified through SSR genotyping. The genetic potential of sweetpotato germplasm in Uganda has been understood through this study.

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CHAPTER ONE

1.0 Introduction

1.1 Origin and spread of sweetpotato

Sweetpotato (*Ipomoea batatas* (L.) Lam, the world's seventh most important food crop after wheat, rice, maize, potato, barley and cassava (FAO, 2007) is currently grown most in developing countries with many genotypes being maintained by the farmers. Sweetpotato and its closely related wild species are classified in the family *Convolvulaceae*, genus *Ipomoea*, subgenus *Eriospermum*, section *Eriospermum* and series *batatas*. It is a hexaploid self-incompatible species with 2n = 6x = 90 chromosomes (Jones, 1964). Some plants morphologically similar to *I. batatas* with 2n=6x=60 considered as synonyms of this species have been described and named (Austin, 1977).

It is generally accepted that sweetpotato originated from South America in the region between the Yucatán peninsula of Mexico and the Orinoco River in Venezuela, where *I. trifida* and *I. triloba* might have crossed to produce the wild ancestor of *I. batatas* (Austin, 1988). The four major American taxa of the *batata* group are spread in this region (Austin, 1988). Native people in the area may have discovered sweetpotato and brought it into cultivation. Two main groups of sweetpotato were known during that period: the a*je* group, which was starchy and had a slightly sweet taste, and the *batata* group, which was also starchy but markedly sweet in taste and economically important as

food (Austin, 1988). The greatest diversity of sweetpotato is found in the countries of Guatemala, Colombia, Ecuador and Peru (Austin, 1983). Abundant evidence shows that sweetpotato was spread widely through migration routes of people in the New World tropics before the discovery of America. Linguistic evidence also shows that the dispersal of the *batata* group from America dates from the first voyage of Columbus in 1492, which resulted in the introduction of West Indian sweetpotatoes to Western Europe. Portuguese and Spanish explorers transferred sweetpotatoes grown in Europe to Africa by 1500 (Yen, 1982). However, recent molecular analyses on the genetic relationships among sweetpotato genotypes from South America and Central America using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers revealed that there is a greater molecular variability among the genotypes from Central America as compared with those from South America (Zhang *et al.*, 1998; Zhang *et al.*, 2000b; Zhang *et al.*, 2004). The geographical origin of sweetpotatoes might be probably somewhere in the Caribbean or Central American region though not fully confirmed.

1.2 Sweetpotato production in Uganda

Sweetpotato has been increasing in importance since its introduction; it was already an important crop in the Central and Western parts of Uganda by 1900 (McMaster, 1962). Greenway (1944) suggested that the crop must have entered Uganda from both the east and western parts of Africa. Sweetpotato occupies a national status as the third most important crop in Uganda after banana and cassava (Mwanga and Wanyera, 1988; Bashaasha *et al.*, 1995; Mwanga *et al.*, 2001b). Sweetpotato cultivation extensively

increased from 473,000 to 590,000 hectares in the past decade with an annual production of 1.7 million Mt (FAO, 2007). However, the root yields are still as low as 4.0 Mt/ha, due to subsistence production techniques characterized by heavy reliance on low yielding indigenous cultivars (Bashaasha *et al.*, 1995), which are highly susceptible to pests and diseases, and no or minimal use of agricultural inputs (Carey *et al.*, 1999; FAO, 2007). The Eastern region is the greatest sweetpotato producer in Uganda, followed by the Western, Northern and lastly the Central region (MAAIF, 2007). Uganda is now the third largest global sweetpotato producer after China and Nigeria (FAO, 2007), and a secondary center of diversity (Austin, 1988; Huamán and Zhang, 1997). Ugandan farmers consequently grow and maintain a large number of sweetpotato genotypes from which they obtain planting material for seasonal clonal propagation (Bashaasha *et al.*, 1995).

1.3 Importance of sweetpotato in Uganda

Ugandan farmers regard sweetpotato as a food security crop because of high yields and low input requirements for its production (Bashaasha *et al.*, 1995; Aritua and Gibson, 2002), and the high rates of production per unit area and time (Woolfe, 1992). The rural poor grow it near their homes to feed their families and also provide income from the sale of the excess produce (Hakiza *et al.*, 2000; Mwanga *et al.*, 2001b). The crop is mainly harvested piecemeal for providing fresh daily food for the family almost throughout the year (Bashaasha *et al.*, 1995). However, during the long dry season in northeastern Uganda, harvested roots are sun-dried to products such as amukeke (dry-sliced form) or inginyo (dry-chunk form) for storage (CIP, 1998; Owori and Hagenimana, 2000).

Sweetpotato is also widely used as an animal feed and compares favorably with alfalfa as forage (Woolfe, 1992; Bashaasha *et al.*, 1995).

1.4 Sweetpotato germplasm collections and gene banks

Two major sweetpotato germplasm gene banks exist in the world today. The International Potato Center (CIP) in Lima, Peru, holds the largest number of accessions in the world with 7,000 accessions mainly from Latin America and the Caribbean (Zhang *et al.*, 1996; Huamán and Zhang, 1997; Zhang *et al.*, 2000a). The second largest collection of over 700 sweetpotato accessions is maintained *in vitro* at the United States Department of Agriculture - Agricultural Research Service Plant Genetic Resources Conservation unit (USDA-ARS PGRCU), at Griffin, Georgia.

The Ugandan germplasm is minimally represented in these large collections (Huamán and Zhang, 1997) due to quarantine restrictions to move vegetatively propagated crops across countries and continents. Due to field maintenance, the earlier collected 433 sweetpotato accessions assembled and duplicated at the National Semi-Arid Resources Research Institute (NaSARRI), Serere, and the National Crops Resources Research Institute (NaCRRI), Namulonge, respectively by the National sweetpotato program in 1986 were lost to viruses (Mwanga *et al.*, 1992). Subsequent collections of 206 accessions from northeastern Uganda for variety development in 1999 (Abidin *et al.*, 2001), and of 266 accessions from selected parts of East Africa for characterization at

Makerere University Agricultural Research Institute, Kabanyolo in 2003 (Gichuru *et al.*, 2006) were also lost to similar causes.

1.5 Justification of the study

Genetic diversity is the foundation on which selection of traits for crop improvement is based (Guarino *et al.*, 1995; Tseng *et al.*, 2002). Genetic improvement of sweetpotato is a continuous process due to the constantly occurring environmental changes and the evolution of pests and diseases. A narrow germplasm base limits the development of new varieties. Thus plant breeders need a variable genetic base of material including wild ancestry to draw upon. Despite the large numbers of sweetpotato landraces with varying taste, food value, root size and shape grown by Ugandan farmers, the representation of Ugandan sweetpotato germplasm in the national and global gene banks is minimal (Huamán and Zhang, 1997). Yet many of the Ugandan sweetpotato farmers are loosing their landraces predominantly because of their ever declining agronomic potential, acquisition of improved varieties and other natural disasters due to reasons proposed by Kapinga *et al.*, 1995 under similar conditions in Tanzania.

Utilization of the new sweetpotato genetic resources has been limited to only the adaptable, higher yielding and early maturing genotypes resulting in consistent genetic erosion in Uganda. To mitigate this genetic erosion, and the increasing need to find germplasm with high resistance to the multiple disease and pest pressures on the crop, it is necessary to collect, characterize, evaluate and conserve the farmers' genotypes. The

first step towards the diversification of sweetpotato genetic base is the characterization of the collected sweetpotato germplasm at both morphological and molecular levels followed by rigorous multi-location field evaluations for identifying superior genotypes fwith desirable traits. The use of morphological traits in sweetpotato diversity analysis has been limited over the years by the high morphological plasticity of these traits, making the accessions with the same genotype to vary in morphological characters in the varying environmental conditions. Yet due to parallel evolution (Austin, 1977), two genotypes that are phenotypically similar could still be very different genetically having developed the similar character over time due to environmental effects. The SSR markers can be used to overcome the influence of environment on the accuracy of the characterization of a germplasm collection (Morakinyo and Ajibade, 1998).

1.6 Objectives of the study

The main objective of the study was to collect, characterize and evaluate sweetpotato germplasm for conservation and utilization in sweetpotato variety development.

The specific objectives were to:

1. Establish and document sweetpotato genotypes and related indigenous knowledge in Uganda.
2. Characterize sweetpotato germplasm using morpho-agronomic traits.
3. Genotype superior germplasm using SSR markers for conservation and use.

It was hypothesized that:

1. Ugandan farmers do not maintain a large number of sweetpotato grmplasm and do not have any indigenous knowledge on sweetpotatoes.

2. The Ugandan sweetpotato germplasm is not morphologically diverse.

3. SSR markers cannot detect differences in sweetpoatato germplasm.

CHAPTER TWO

2.0 Literature Review

2.1 Evolution and biology of sweetpotato

Sweetpotato (2n=6x=90) belongs to a single species, *Ipomoea batatas* (L.) Lam. The cultivated sweetpotato was described by Linnaeus in 1753 as *Convolvulus batatas*. *Ipomoea batatas* is a dominantly domesticated species although wild ancestors that bear morphological resemblance to the crop also exist. Nishiyama (1971) reported one of such species to be *I. trifida* collected in Mexico as a 6x *I. trifida*. Sexual polyploidization through the production of unreduced gametes is thought to have facilitated the evolution of *I. batatas* to the hexaploid level (Nishiyama, 1971). From 2x *I. leucantha*, 4x *I. littoralis* was produced. Crosses between the two species 2x and 4x resulted in 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*. Orjeda *et al.*, 1990 also reported the formation of unreduced pollen in diploid *I. trifida* and some tetra and hexaploid *I. batatas*.

The ploidy level coupled with self-incompatibility, and high heterozygosity, makes the genetics of inheritance of many important traits in sweetpotatoes unclear (Huamán and Zhang, 1997). Both autopolyploidy and allopolyploidy types of inheritance though not yet clear have been observed in sweetpotato. There could have been a chance of

chromosomal doubling of a single species in sweetpotato, leading to individuals with several copies of the same genome. The corresponding homologous chromosomes could have paired randomly between each other resulting in autopolyploidy in sweetpotato (Huamán and Zhang, 1997). On the other hand, the existence of allopolyploidy could have originated from the abnormal hybridization between two different species, such that the resulting individual had two complete and different genomes and their chromosomes could have paired preferentially to their homologs from the same genome, and in lower frequency to an homologous chromosome (Sybenga, 1996; Ramsey and Schemske, 2002). Cytological studies have confirmed allopolyploidy in sweetpotatoes (Jones, 1965), but Ukoskit and Thomson, (1997) suggested that sweetpotatoes are autopolyploids.

The hypothesis of autopolyploidy has further been supported by the study of the inheritance of beta-amylase in storage roots of sweetpotato from which either hexasomic or tetrasomic mode of inheritance was concluded (Kumagai *et al.*, 1990). Molecular studies using random amplified polymorphic DNA (RAPD) also confirmed this hypothesis of autopolyploidy (Ukoskit *et al.*, 1997). However, studies using amplified fragment length polymorphism (AFLP) revealed that sweetpotato is an autopolyploid showing some preferential pairing (Kriegner *et al.*, 2000). Further, recent studies on the development of a genetic linkage map and identification of homologous linkage groups using multiple-dose AFLP markers support the hypothesis that sweetpotato is an autopolyploid (Cerventes-Flores *et al.*, 2008).

2.2 Ecology of sweetpotato

Swetpotato grows widely in the tropics; subtropics and warm temperate regions between latitudes 40°N to 32°S of the equator and takes 3-6 months to produce mature roots (Purseglove, 1991; Woolfe, 1992). Sweetpotatoes perform best in well drained light and medium textured soils with a pH range of 4.5-7.0 (Woolfe, 1992), and are grown from sea level to 3000 m.a.s.l. Poor soil aeration retards storage root formation (Woolfe, 1992). Best growth is obtained under high light intensity and temperatures above 24°C, yet temperatures above 35°C or below 12°C tend to retard growth. Dry matter production increases with increasing soil temperatures between 20-30°C and declines above 30°C. Annual rainfalls of 750-1000 mm are most conducive for sweetpotato growth, with a minimum of 500 mm in the growing season.

2.3 Germplasm diversity

High phenotypic and genotypic diversity occurs in sweetpotato as shown by the root skin or flesh color, size and shape, leaf shapes, colors and pigmentation (Austin *et al.*, 1970), the depth of rooting, and time to maturity, resistance to pests and diseases, and even the flavor and texture of cooked roots (Huaman, 1992; Woolfe, 1992). Sweetpotato is generally an unstable crop with high tendency to mutate (Collins *et al.*, 1999), and is also a highly outcrossing and heterogeneous crop (Jones, 1965). These characteristics naturally give rise to large numbers of sweetpotato genotypes (landraces) grown by farmers under traditional agricultural systems (Carey *et al.*, 1998). More diversity is

identify duplicates. This enhanced reduction of the collection to 673 unique accessions (Huamán *et al.*, 1999). With the aid of 14 vegetative and root descriptors, the phenotypic diversity in the sweetpotato landraces collected in the agricultural systems of the Vale do Ribbeira in Brazil was evaluated. A high morphological variation was found in the collection with Jaccard similarity index (0.12 to 1.0) though the diversity was not structured in space as no defined groups were clustered (Veasey *et al.*, 2007).

In other crops, morphological and agronomic traits proved useful in the assessment of variation among Russian wildrye accessions in which 69 entries consisting of 62 diploid ($2n = 2x = 14$), 3 tetraploid ($2n = 4x = 28$) and 4 plant introductions were grouped into 10 clusters (Berdahl *et al.*, 1999). Morphological traits have also enabled the analysis of diversity in garlic germplasm (Panthee *et al.*, 2006), *Amygdalus* L. fruit tree species (Talhouk *et al.*, 2000), cultivated hexaploid oat (Diederichsen, 2008), Tef (*Eragrostis tef*) collection (Kefyalew *et al.*, 2000), maize in the Archipelago of Madeira (Pinheiro de Cavalho *et al.*, 2008), wheat germplasm in Ethiopia (Hailu *et al.*, 2006) and pigeon pea core collection at ICRISAT (Upadhyaya *et al.*, 2007). However, the genetic make up, environment and field management practices influence the morphology of a crop (Sigh and Rachie, 1985), limiting the accuracy of morphological germplasm characterization.

2.5 Principal component and cluster analysis

Cluster analysis is the classification of similar objects into different groups so tha data in each group or subset ideally share some common trait often proximity according to some

defined distance measure (Milligan, 1980). Pricnipal component analysis aims at reducing the dimensionality of multivariate data while preserving as much of the relevant information as possible (Jackson, 1991). Principal component analysis (PCA) is most times used prior to cluster analysis to determine the relative importance of classification variables (Jackson, 1991). The use of multivariate analysis has been employed in several cross-fertilized crops to group cultivars into clusters (Steiner *et al.*, 1998; Berdahl *et al.*, 1999). The eign-values normally show the relative discriminating power of the principal axes. Principal component analysis has also been used as a linear dimensionality reduction technique to identify orthogonal directions of maximum variance in the original data set and to project the data into lower dimensions of the highest variance components.

Five sweetpotato cultivars (Kemb10, SPK004, KSP20, Yan Shu 1 and Zapallo) were analyzed by Quirien *et al.*, 2003 for their sensory characteristics and changes during storage under tropical conditions using nine sweetpotato root descriptors: floury, smooth, soft, chestnutty, sweet, fibrous, grainy, moist and discoloration. Principal component analysis (PCA) revealed two principal components (PC1 and PC2) explaining in total 68% of the variation. PC1 explained 52% and correlated with textural properties, and PC2 explained 16% of the variation and correlated with flavor components. The results suggested that differences between sweetpotato cultivars in root organoleptic properties are mainly determined by textural components.

PCA was also applied in the morphological characterization of a germplasm collection of *Carthamus tinctorius* in the Middle East (Jaradat and Shahid, 2006). In this case, four

principal components ably explained up to 80.0% of the total variation in the whole germplasm collection, with the first and second principal components explaining 26.0% and 16.9% variance, respectively. Four traits in decreasing order (number of capitula per plant, days to maturity, seed yield per plant and capitula diameter) accounted for most of variance in the collection. In the Russian wildrye collection, the first four principal components explained 41, 16, 10 and 7% of the total variance (Berdahl *et al.*, 1999). In diversity analysis among garlic collections available in Nepal, the identification of a principal component was based on the correlation between different traits, their eign values, and eign-vectors of the principal components (Panthee *et al.*, 2006). Traits that show high positive correlation can be used in preliminary selection programs. Principal component analysis revealed that the four principal components explained 86% of the total morphological variation in the garlic accessions, while cluster analysis showed that there were three distinct major clusters with no relation with geographical locations. The garlic accessions collected from high hills and plain areas formed major clusters each with a lot of overlapping. Getting three clusters from a large collection indicated the occurance of several duplicate accessions (Panthee *et al.*, 2006). Cluster analysis based on 21 morphological descriptors scored on 673 Peruvian unique sweetpotato accessions also facilitated the selection of a core subset of 85 accessions (Huamán *et al.*, 1999).

2.6 Core collection development

A core collection is a limited subset (about 10%) of accessions derived from the entire germplasm collection chosen to represent most of the available genetic diversity of the

whole collection (Brown, 1989). The core collection can be extensively evaluated under controlled experiments to generate information needed to plan a more efficient utilization of the germplasm (Tohme *et al.*, 1995). A core collection for the Peruvian sweetpotato germplasm was selected on the basis of morphological, eco-geographical and disease and pest reaction. Duplicate identification using morphological characters and electrophoretic banding patterns of total proteins and esteraces reduced the number of accessions from 1939 to 673. On the basis of the square root of the number of accessions from each Peruvian department and respective cluster as defined by unweighted pair group method of arithmetirc averages (UPGMA) the Peruvian core collection was formed of 85 accessions (12.6%) of the entire collection (Huamán *et al.*, 1999).

2.7 Molecular characterization of germplasm

Molecular markers have proved to be more reliable than morphological and agronomic characters in sweetpotato germplasm characterization (Zhang *et al.*, 2000a). Many molecular markers have been used to simplify sweetpotato genetic diversity assessment (Zhang *et al.*, 2000a), genetic linkage map construction (Kriegner *et al.*, 2000), core collection establishment and management (Zhang *et al.*, 2000a) and sequencing.

Random amplified fragment length polymorphism (RAPD) markers

Random amplified fragment length polymorphism (RAPD) marker, the cheapest and quickest of the molecular marker techniques has problems of reliability and

reproducibility. Gichuki *et al.* (2003) used RAPDs to analyze diversity among 74 sweetpotato 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. Of the 52 primers used in their study, eleven generated 71 polymorphic markers indicating that significant differences existed among the genotypes in different regions as well as among varieties within each region.

Amplified fragment length polymorphism (AFLP) markers

The AFLP technology is a powerful fingerprinting technique (Vos *et al.*, 1995). Zhang *et al.*, 2000b used AFLP markers to analyze the genetic diversity of 69 sweetpotato clones from Tropical America. Their result supported the hypothesis that Central America is the primary center of diversity and most likely the center of origin of sweetpotato, and that the Peru-Ecuador region should be considered a secondary center of sweetpotato diversity. The evidence of a Mesoamerican center of origin for sweetpotato was strengthened because this region ranked highest in terms of total number of alleles, number of region-specific alleles, and actual heterozygosity confirmed by AFLPs (Zhang *et al.*, 2004), supporting their hypothesis from earlier work (Zhang *et al.*, 2000a) that Mesoamerica is the primary center of sweetpotato diversity. AFLP molecular markers were also used to study sweetpotato genetic diversity (Rossel *et al.*, 2000) and to study the historic dispersal of sweetpotato, and analyze sweetpotato germplasm from Papua New Guinea in the form of botanical seed (Fajardo *et al.*, 2002). The genetic diversity in the Tanzanian sweetpotato germplasm collection analyzed by AFLPs revealed a low

genetic diversity among the accessions and a low genetic distance among the regions of origin as well (Elameen *et al.*, 2008). The major advantage of AFLPs over other molecular techniques is the short time required to assay large numbers of DNA loci to give highly reproducible results (Majer *et al.*, 1996).

Simple sequence repeat (SSR) markers

The SSRs or microsatellite markers have an extraordinary discriminatory capacity for genotyping sweetpotato germplasm. This was observed when six primer pairs used in the genotyping of 113 Latin America sweetpotato genotypes that revealed a total of 70 alleles, with allele sizes ranging from 102 base pairs to 173 (Zhang *et al.*, 2000a). Five SSR primers were successfully used to distinguish 21 known sweetpotato accessions from the germplasm collection held in South Africa (McGregor *et al.*, 2001). A lower molecular diversity was revealed among East African landraces assessed by SSR markers in a preliminary diversity study (Gichuru *et al.*, 2006). Powell *et al.*, 1996 reported the occurrence of null alleles as another possible problem with the use of microsatellite markers in highly out breeding heterozygous species. Buteler *et al.*, 1999 reported that insertions/deletions and base substitutions occurred in the microsatellite flanking regions, and proposed the selective amplification of microsatellite polymorphic loci (SAMPL) markers as a useful marker technique for sweetpotato genotyping. The SAMPL markers proved to be the most efficient technique in assessing genetic relationships among sweetpotato genotypes (Tseng *et al.*, 2002).

Analysis of genetic relationships

The first step in analyzing genetic relationships among samples is the construction of a matrix specifying the character state (binary or multi-state) of each marker for each sample, which is then used to construct a sample genetic distance matrix. The genetic distance between two samples can be calculated on the basis of the allelic differences between them (Hendrick, 1974), or the Nei's genetic distance (D) (Nei, 1973). The resulting genetic distance matrix may be analyzed and displayed in form of Principal Component Analysis (PCA), which produce a 2 or 3-dimensional scatter plot of samples such that the geometric distances among samples in a plot reflect the genetic distances among them with minimum distortion; aggregation of samples in such a plot will reveal sets of genetically similar material. Secondly, a dendrogram linking together cluster samples that are more genetically similar to each other than to samples in other clusters can be produced. The algorithms widely used for clustering include Unweighted Pair Group Method with Arithmetic Averages (UPGMA), Neighbour-joining methods and Ward's method (Mohammadi and Prasanna, 2003).

2.8 Germplasm releases in Uganda and their origin

The main emphasis for sweetpotato variety improvement in Uganda is the development of varieties with high yield potential, high beta-carotene content, resistance to sweetpotato virus disease (SPVD) and Alternaria blight disease and minimum sensitivity to seasonal fluctuations over a wide range of environments (Turyamureeba *et al.*, 1997;

Mwanga *et al.*, 2001b). However, the development of varieties has predominantly relied on the use of local germplasm collections and the exchange of germplasm between National Agricultural Research Organization (NARO), International Potato Centre (CIP) and the International Institute of Tropical Agriculture (IITA). Most of the germplasm received from IITA in West Africa with high resistance to SPVD caused by sweetpotato chlorotic stunt virus (SPCSV) and Nigerian isolates of sweetpotato feathery mottle virus (SPFMV) succumbed severely to SPVD in Uganda (Mwanga *et al.*, 1991). This same trend of reaction was also observed on many of the sweetpotato virus disease resistant materials received from CIP (Turyamureeba, person. comm.). The very high pressure of SPVD in Uganda often led to the break down of resistance in released varieties.

Six sweetpotato cultivars, Bwanjule, New Kawogo, Tanzania, Wagabolige, Sowola and Tororo 3 were released in Uganda in 1995 on the basis of their superior performance and consumer acceptance (Mwanga *et al.*, 2001a). These were the first sweetpotato cultivars to be officially released in Uganda. Sowola is a seedling selection from the sweetpotato program at NaCRRI, and was selected from bulked seed of a polycross of 18 parents made from 1989 to 1990 (Mwanga *et al.*, 2001a). The rest are superior farmers' cultivars selected from a collection of 380 landrace accessions assembled at NaCRRI in 1987 and subsequently evaluated in trials in the main agro ecologies in Uganda. Six other sweetpotato cultivars NASPOT 1, NASPOT 2, NASPOT 3, NASPOT 4, NASPOT 5 and NASPOT 6 selected from bulked seed of a poly-cross nursery of 24 parents, made in 1991 at NaCRRI were also released by the sweetpotato program in Uganda (Mwanga *et al.*, 2003). These cultivars all have good storage root shapes, high dry matter content,

excellent consumer acceptance, moderate to high levels of field resistance to SPVD and high storage root yield compared to the national average of 4.0 t/ha (Mwanga *et al.*, 2003). In 2004, two orange-fleshed cultivars, SPK004 (Kakamega) and Ejumula with high beta-carotene and dry matter content were released by the sweetpotato program in Uganda (Mwanga *et al.*, 2007b). In 2007, five cultivars, NASPOT 7, NASPOT 8, NASPOT 9 O, NASPOT 10 O and Dimbuka were also released (Mwanga *et al.*, 2007c).

The incomplete understanding of the ploidy level in cultivated sweetpotato had always presented a challenge in the understanding of the inheritance of traits of importance to the breeders as well as molecular characterization of the crop. Germplasm characterization has therefore predominantly relied on the use of morphological traits, which are highly influenced by the environmental changes. The recent confirmation that sweetpotato is an autopolyploid at molecular level (Cerventes-Flores *et al.*, 2008) will enhance the study of inheritance of several traits of importance. The use of automated sequencers like ABI3730 will enhance accurate allele sizing for large-scale genotyping of sweetpotato germplasm collections at relatively low costs (Weising *et al.*, 1998; Wenz *et al.*, 1998).

CHAPTER THREE

3.0 ESTABLISHMENT AND DOCUMENTATION OF SWEETPOTATO GENETIC RESOURCES AND RELATED INDIGENOUS KNOWLEDGE IN UGANDA

3.1 Introduction

Uganda is the third largest global producer of sweetpotato [*Ipomoea batatas* (L.) Lam] after China and Nigeria (FAO, 2007), and is a secondary center of diversity (Huamán and Zhang, 1997; Mwanga *et al.*, 2001a; Gichuki *et al.*, 2003) where the crop is mostly grown as a starchy staple and source of income for the rural poor (Hakiza *et al.*, 2000; Mwanga *et al.*, 2001b). The ability of sweetpotato to grow with minimal external inputs has led to the extensive increase of its cultivation from 473,000 to 590,000 hectares in the past decade, with an annual production of 1.7 million Mt (FAO, 2007). The root yields are as low as 4.0 Mt/ha, because of subsistence production techniques that use low yielding indigenous cultivars (FAO, 2007). Like the rest of the sub-Saharan Africa region, Ugandan farmers maintain large numbers of different sweetpotato genotypes in their fields (Kapinga *et al.*, 1995; Villordon *et al.*, 2006).

In 1986 the national sweetpotato program collected 433 sweetpotato cultivars, assembled and duplicated them at the National Semi-Arid Resources Research Institute (NaSARRI), Serere, and National Crops Resources Research Institute (NaCRRI), Namulonge, respectively, however, these were subsequently lost to viruses due to field maintenance (Mwanga and Otim-Nape, 1992). A survey on the sweetpotato farming systems in some

regions of Uganda in the 1990s led to identification of up to 183 local names of farmer varieties (Bashaasha *et al.*, 1995). A collection of 206 sweetpotato accessions from northeastern Uganda in 1999 (Abidin and Carey, 2001), and 266 cultivars from selected parts of East Africa in 2003 (Gichuru *et al.*, 2006) were also lost to similar causes.

The genetic base of Uganda's sweetpotato germplasm is therefore not known due to inadequate collection, characterization and conservation. This has resulted in the minimal representation of Ugandan germplasm in the global gene banks (Huamán and Zhang, 1997). Yet the heterogeneous nature of the crop (MacDonald, 1965) and rampant mutations (Hernandez *et al.*, 1964), has led to the development of many new varieties over the years. Many low performing farmer varieties (landraces) have been abandoned resulting in genetic erosion. Therefore, the objective of this study was to establish and collect sweetpotato germplasm maintained by Ugandan farmers and document the indigenous knowledge and complete germplasm passport data for conservation and use.

3.2 Materials and Methods

Trips were made for collecting sweetpotato germplasm along with farmers' knowledge on the varieties to the farmers' fields in the main sweetpotato producing districts from the five major sweetpotato producing agro-ecological zones in Uganda, that is; the high altitude eastern zone, southwestern high land zone, the northern and eastern short grassland zone, southern tall grassland zone which occupies large areas along Lake Victoria, and western tall grassland zone. The districts included: Arua, Lira, Apac,

Masindi, Hoima, Mbale, Pallisa, Iganga, Kamuli, Soroti, Kumi, Rakai, Luwero, Mukono, Masaka, Mpigi, Kisoro, Kabale, Kabarole, Bushenyi and Mbarara. The districts were purposively selected on the basis of sweetpotato production (tons) and area (ha) under production statistics from the Ministry of Agriculture, Animal Industry and Fisheries.

Sweetpotato fields were inspected in the ten leading sweetpotato producing sub-counties in the districts. The unit of sampling in the field was the sweetpotato plot rather than the farmer from whom the accession was collected. Attempt to spread the plots across different parts of the village to ensure ecological diversity and maximize cultivar range was achieved by driving 5-10 km apart. Local collection site information, latitude, longitude and altitude were obtained using a Garmain GPS V2.0 to produce detailed passport data. Ten to thirty cuttings, about 30-40 cm long of each morphologically distinct cultivar (landrace) were collected from the farmers' fields and assembled in a screen house and a field at NaCRRI for multiplication. The farmers were interviewed to obtain indigenous knowledge related to these accessions using an individual guided questionnaire. The vernacular name, place of origin, duration of maturity, foliage traits, root skin and flesh color among others were used to distinguish the accessions from the collection sites. We avoided the collection of duplicate accessions from the same plot but made little attempt to avoid accessions that looked similar from different sample plots, as duplication could not be conclusively determined at this point. Farmers' varieties were collected in a collaborative activity of the National Sweetpotato Program with the zonal agricultural research development institutes (ZARDIs) and the district local governments. The statistical data was analyzed using SPSS software. The passport information was

collated to a data base system using dBASE for windows v. 5.0 (Borland, 1994) and the data analyzed using geographic information system, the Arc view V. 3.0 (ESRI, 1996).

3.3 Results and Discussions

3.3.1 Number of sweetpotato accessions collected

A total of 1303 accessions with 565 vernacular names were collected from farmers' fields in 171 sub-counties (SCs) in 21 districts from January-July, 2005 (Table 3.1). Great variations were observed in the numbers of accessions grown in the different districts. The number of accessions collected per district ranged from 30 in Kisoro district to 102 in Masindi district. This variation can be attributed to the relative importance of the crop in the districts to other staples like cassava, maize, sorghum and banana. The commercial importance of the crop also influences the numbers of cultivars grown per district. For instance, in Soroti and Kumi districts most farmers grow few commercial varieties like SRT43 (Osukut) commonly called Tanzania for the Kampala market. Except for Kisoro district, all the other districts had more than 40 accessions collected. This large number of accessions confirms the importance of sweetpotato as a food security crop in most Ugandan households (Mwanga *et al.*, 2001b) and Uganda as a secondary center of diversity for the crop (Huamán and Zhang, 1997; Gichuki *et al.*, 2003). The high number collected also agrees with increased production of sweetpotato in Uganda over the past decade (FAO, 2007). A large amount of landrace genetic diversity is still in existence in Uganda. The passport data for the accessions recorded at each site including

morphological descriptors is available at: http://www.viazitamu.org/ugasp_db/gis.htm. A similar web accessible database for the Kenyan sweetpotato germplasm collection was developed using the open source software (Villordon *et al.*, 2007).

Table 3.1. Summary of collected Ugandan sweetpotato germplasm from 171 sub-counties (SCs) in 21 districts

District	No. of SCs	Accession Codes	No. of acccessions	Varieties		Altitued m.a.s.l	Region
				Weevil resistant	Orange flreshed		
Soroti	9	SRT01-SRT52	52	19	8	1133-1255	Eastern
Kumi	9	KMI53-KMI93	41	14	3	1159-1254	Eastern
Pallisa	13	PAL94-PAL161	69	11	1	1150-1250	Eastern
Mbale	10	MLE162-MLE207	46	2	2	1244-1678	Eastern
Kamuli	8	KML872-KML964	91	4	0	1117-1227	Eastern
Iganga	11	IGA963-IGA1020	58	5	0	1163-1778	Eastern
Arua	9	ARA208-ARA256	49	13	2	1215-1383	Northern
Lira	6	LIR257-LIR322	66	5	1	1148-1215	Northern
Apac	6	APA323-PA379	57	7	2	1142-1180	Northern
Masindi	9	MSD380-MSD481	102	10	1	1129-1313	Western
Hoima	4	HMA482-HMA523	42	4	1	1188-1302	Western
Mbarara	11	MBR524-MBR610	87	8	1	1260-2060	Western
Kabale	6	KBL611-KBL651	43	14	1	1982-2617	Western
Kisoro	9	KSR652-KSR681	30	2	0	1852-2168	Western
Kabarole	9	KRE682-KRE739	58	6	0	1183-1830	Western
Bushenyi	7	BSH740-BSH778	39	1	0	1558-1803	Western
Rakai	8	RAK779-RAK871	93	0	0	1285-1393	Central
Masaka	6	MSK1023-MSK1098	76	3	1	1267-1441	Central
Mpigi	7	MPG1099-MPG1166	68	2	2	1267-1392	Central
Mukono	6	MKN1167-MKN1224	58	1	0	1234-1384	Central
Luweero	8	LUW1225-LUW1302	78	1	0	1194-1315	Central
Total	171		1303	132	26		

3.3.2 Ecology of sweetpotato landraces in Uganda

Most of the sweetpotato germplasm (80%) in Uganda was collected from altitudes of between 1117 to 1500 m.a.s.l, 15.2% was from 1501-2000 m.a.s.l, and only 4.8% was collected from altitudes from 2001 to 2617 m.a.s.l. These high altitude areas in Kisoro and Kabale districts have predominantly low temperatures and sweetpotato takes 7 to 9

months to mature, making farmers invest more in early maturing crops for food security. Most of the sweetpotatoes are grown in the low land districts around the Lake Victoria basin. Through GIS-based distribution modeling, the areas around the Lake Victoria basin were predicted to have great diversity of sweetpotato in sub-Saharan Africa and suitable for germplasm collection (Villordon *et al.*, 2006). Hijmans *et al.*, 2002 also reported high concentrations of global sweetpotato germplasm in the low lands of China and the mid-elevations of the Lake Victoria area in Africa. The Arc View based spatial distribution of sweetpotato germplasm collection sites in Uganda is shown in Fig. 3.1.

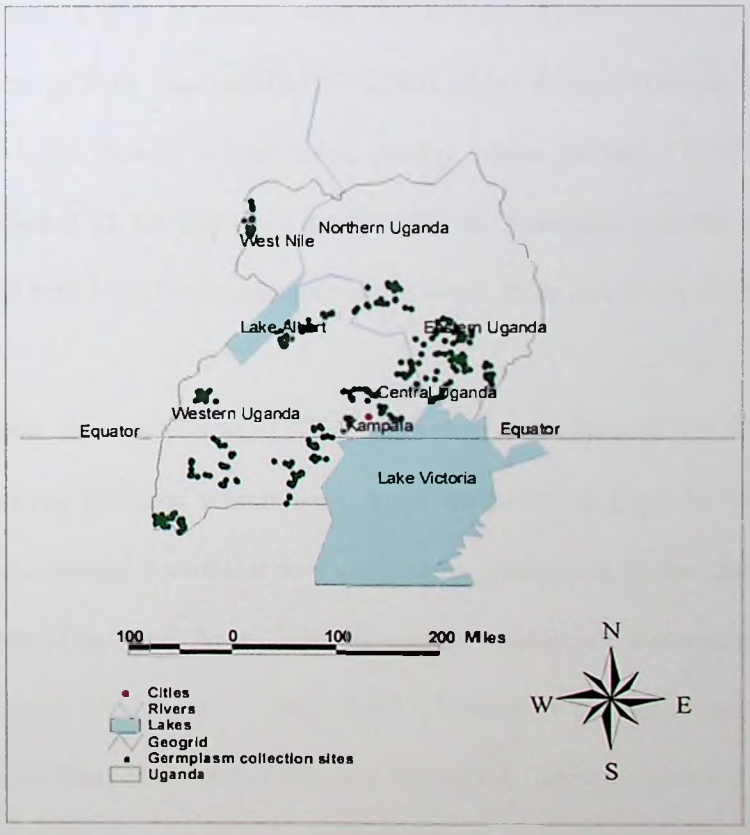

Fig 3.1. Arc view based map of sweetpotato germplasm collection sites in Uganda

3.3.3 Traits with agronomic and economic importance

Twenty-six orange-fleshed accessions were collected most of which had light orange flesh color (Table 3.1). Most of the accessions were growing on a few mounds in the fields of non-orange flesh sweetpotato crops, while others were volunteer crops in the main field. Many farmers were not aware of the health benefits of consuming orange-fleshed sweetpotatoes, though the National sweetpotato Program has released OFSPs; NASPOT 5 (Mwanga *et al.*, 2003), Ejumula and SPK004 (Mwanga *et al.*, 2007b), NASPOT 7, NASPOT 8, NASPOT 9 O, NASPOT 10 O (Mwanga *et al.*, 2007c) to fight Vitamin A deficiency. Most of the collected OFSP accessions were referred to as Kala. Other OFSPs collected were: SRT49 (Sanyuza meza) from Soroti, KBL640 (Africare) from Kabale and MBR558 (Food kala) from Mbarara. Pallisa district farmers liked PAL161 (Misi), a hard flesh variety when cooked. OFSPs, SRT33 (Abuket 1), SRT34 (Abuket 2), SRT35 (Anyumel), SRT38 (Rwanda), and the introductions SRT36 (Resisto) and SRT37 (Dar-es-salaam) carrot were collected from a farmer in Soroti district.

From the collection, 132 accessions were reported by the farmers to be resistant to weevils most of which were from northeastern Uganda. Weevils were reported to be a very serious constraint to sweetpotato production in the districts of Soroti, Kumi, Pallisa, Lira, Apac and Arua. Weevils cause significant sweetpotato yield loss in northeastern Uganda (Abidin and Carey, 2001). Weevils were not a very big constraint to sweetpotato production in the other districts surveyed. Farmers reported that weevil damage increased

with the onset of drought and associated long maturity period, quantity of sap, depth of rooting, thickness of cortex and high dry matter with weevil resistance (Table 3.2).

Table 3.2. Farmers' understanding for sweetpotato weevil resistance in Uganda

Reason for resistance	Variety
Long maturity period and drought tolerant	SRT35 (Anyumel), APA335 (Oleke), MSD431 (Kalobo), KRE696 (Rwabuganda), KRE723 (Kibogo), KRE734 (Yello), BSH741 (Mugiga)
Produces a lot of sap	SRT21 (Gunyombekere), KMI56 (Opila), KMI57 (Kassim), KRE695 (Kasoga)
Deep rooting	SRT28 (Epura Amojong), KMI88 (Opade)
Low or no cracking	LIR279 (Imatojony)
Thick/ Hard root cortex	KMI59 (Kampala), PAL108 (Dagada), MLE199 (Bungoma), APA323 (Lira Lira)
High dry matter (hard flesh)	ARA230 (Andinyaku), ARA231 (Mbutra), LUW1296 (Bunduguza)

3.3.4 Farmers' perception on the origin of sweetpotato varieties

The farmers mostly got their new varieties from their friends who could not give them detailed information about their places of origin. Certain varieties originated from the neighboring villages, sub-counties and districts within the country, whereas others came from the neighboring countries. Those from neighboring countries were commonly collected from the boarder line districts of Arua, Kisoro, Kabale and Rakai. They also had some experiences with certain varieties like MSK1040 (Dimbuka obuleku) in Masaka, IGA994 (Bunduguza empyaka) in Iganga and RAK798 (Nantongo) in Rakai that had appeared spontaneously in their fields and were also morphologically distinct from their previous ones. This could be explained by the rampant mutations in the crop (Hernandez *et al.*, 1965) or due to chance seedlings growing in farmers' fields (Aldrich, 1963; MacDonald, 1965; Carey *et al.*, 1998; Mwanga *et al.*, 2001b).

3.3.5 Farmers' preference for varieties grown in Uganda

The farmers identified the most popular genotypes grown in the districts during the collection. In all the districts farmers preferred varieties that were high yielding (96%), early maturing for food security (81%) with high dry matter content (75%), appreciable resistance to pests and diseases (63%), and sweet taste (48%). Using participatory variety selection, Gibson *et al.*, 2008 also observed that, Ugandan farmers preferred the same traits. The following genotypes were identified to be high yielding: SRT24 (Bunduguza2), SRT48 (Eyambi), SRT30 (Anyara), SRT40 (Mary), KMI56 (Opila), KMI57 (Kassim1), KMI72 (Obure), KMI73 (Eritu), KMI74 (Calvin), PAL133 (Tengerera), PAL153 (Abukoki1), LIR257 (Otada), APA352 (Oketo Dede), APA371 (Korina), APA377 (Oluli), MSD431 (Nakasongola), KSR681 (Musazaryakimwe), BSH740 (Kibanda), RAK867 (Bumbakali), IGA981 (Sarah), a high dry matter content variety, IGA986 (Shiphon), a very sweet, soft and shining variety on cooking and MSK1025 (Bitambi). The early yielding varieties were given names that reflected earliness: SRT2 (Araka White), SRT3 (Araka Red), LIR287 (Dwe achel), LIR307 (Arakaraka), LIR320 (Duachel), MLE189 (Mwezigumu), ARA252 (Mba alua), MSD461 (Myezimbili), KRE726 (Kwezikumwe), RAK843 (Mwezigumu), the oldest variety in Rakai, BSH765 (Kwezikumwe), and MBR596 (Kwezikumwe). Table 3.3 shows the most popular varieties preferred by the farmers in each district.

Table 3.3. Preferred farmer varieties in each district and the desirable characteristics

District	Variety	Desirable characteristics
Soroti	Osapat (SRT1)	Highest yielding variety and high dry matter content, sweet taste, and resistant to weevils
Kumi	Osukut (SRT43)	Highest yielding, sweet, fairly high dry matter content and good weevil resistance
Pallisa	Silk omuyaka (PAL94)	High yielding, relatively early maturing (3 moths after planting) very sweet taste, fairly resistant to weevils that are not even a very big problem in the district
Mbale	Kyebandula (MLE163)	High yielding, very sweet, high dry matter and fairly early maturing
Arua	Koromojo (ARA209)	High yielding, early maturing, very sweet taste with high dry matter content, consumed in fresh boiled form as well as porridge, though susceptible to weevil
Lira	Tedolo Kereni (LIR258)	High yielding and early maturing, quite resistant to weevils, good for making cakes
Apac	Lira Lira (APA323)	High yielding, early maturing (3-4 months), sweet taste, resistant to weevils and high dry matter content
Masindi	Malagalia (MSD380)	High yielding and early maturing, sweet and fairly high dry matter, though susceptible to weevils
Hoima	Dimbuka (HMA496)	High yielding, high dry matter and sweet taste, but susceptible to weevils
Mbarara	Kahungezi (MBR552)	High yielding, sweet taste, resistant to weevils and fairly early maturing
Kabale	Burundi (KBL611)	Very high yielding and vigorous, sweet taste, fairly resistant to diseases and rotting
Kisoro	Ndambi (KSR656)	High yielding, sweet and does not rot easily
Kabarole	Kalebe (KRE691)	High yielding, early maturing (3 months), high dry matter and sweet
Bushenyi	Kibanda (BSH740)	High yielding and early maturing, but susceptible to rotting
Rakai	Kyebandula-omupya (RAK862)	Very high yielding and early maturing (3 months)
Kamuli	Bunduguza (KML872)	Relatively high yielding, high dry matter, early maturing and sweet taste
Iganga	Bunduguza (IGA994)	Highest yielding variety though yield is declining, high dry matter and good texture, early maturing (2-3 months), sweet taste and not severely damaged by weevils
Masaka	Bitambi (MSK1025)	Fairly new variety, high yielding (bumper harvest), early maturing (3 months), fairly resistant to pests and diseases, sweet with high dry matter content
Mpigi	Unknown556 (MPG1107)	Highest yielding, fairly resistant to pests and diseases.
Mukono	Dimbuka-omupya (MKN1171)	Very high yielding, early maturing, high dry matter content and sweet taste, resistant to diseases and pests
Luwero	Silk (LUW1254)	Fairly high yielding and early maturing, sweet taste and fine texture, relatively resistant to diseases and pests

3.3.6 Naming of farmers' sweetpotato varieties in Uganda

The farmers named only 565 of the collected accessions. Abidin and Carey, 2001 reported that the name of variety influenced its market demand. Some varieties were named according to their place of origin. Some local names indicated the usage and agronomic traits like extent of earliness, hairiness, twinning, vigor, skin color, and flesh texture of a variety. A number of varieties were given the names of the people who first introduced them into an area. In some cases, different local names had the same meaning in different languages or dialects as obersve in Indonesia by Mok and Schneider. (1994). For instance the names Duachel (LIR320), Dwe achel (LIR287, Mba alua (ARA252), Kwezikumwe (KRE726), and Mwezigumu (RAK843) all mean 'one month' implying the variety takes one month to mature (Table 3.4). Morphologically distinct varieties with the same name were encountered in different locations.

Table 3.4. Meaning of the names of farmers' varieties

Genotype name	Meaning/Description
Dwe achel (LIR287), Duachel (LIR320), Mwezigumu (MLE189), Mba alua (ARA252)	Takes one month to mature (early)
Myezimbili (MSD461)	Takes two months to mature
Kampala (IGA995, SRT29), Bugerere (PAL109), Mukono (KBL641), Kawanda (MBR581)	Place of origin
Dimbuka omupya (MKN1171)	Bumper harvest
Ocaka amani (SRT31)	Very low yield
Sanyuza meza (SRT49)	Decorates the table (orange fleshed)
Osukari (KMI78), Sukari (MBR554)	Very sweet taste
Susan (PAL137), Mushemeza (MBR597), Nafutali (LUW1298), Ogwang James (APA339)	Persons who brought the varieties
Tedolo Keren (LIR258)	Keren failed to cook the potato to readiness (high dry matter)
Musazaryakimwe (KSR681)	Old man can eat one (yields one big root)
Muganda Red (BSH757	Skin color

3.3.7 Abandoned sweetpotato varieties in Uganda

Although varieties like KBL644 (Makara), MKN1174 (Unknown607), MBR561 (Ngarozabigarire), MBR580 (Nylon), MPG1150 (Namubiru) have been grown in the districts for more than thirty years, many varieties that used to be widely grown have now been abandoned. Their yield potential declined over time and with the arrival of better varieties they automatically abandoned them as in sorghum in Ethiopia (Teshome *et al.*, 2007). The abandoned varieties included: Alugbea, Delea, and Saulo maku in Arua district due to low yields, Kawogo mukadde, Kisagadi, Somba obusero, and Kalebe in Masaka district because of declining yields and viruses (yellowing). Kawa, Kareebe, Kahungezi, Kalingu became threadlike textured on continued cultivation, whereas Kyebandula, Dagada, Namujuna, Bitambi, Kigaire also declined in yields and were abandoned in Iganga district. The farmers in Mukono district abandoned Kawogo due to very low yields, likewise Kisakyamaria, Kareebe omukadde were abandoned in Rakai district due to low yields. Kahungezi, Kyebandula, Kalebe, Kisakyamaria, Karooli and Sukari were abandoned from Mpigi district because of their high susceptibility to viruses. Ntamankusimire, Kabonge, Wazala, Nakasoma, Walioka and Nakulyaku have been abandoned in Kamuli district predominantly due to low yields and increased susceptibility to pests and diseases. This is a strong indication of sweetpotato genetic erosion in Uganda as observed in Ethiopian sorghum by Tsegaye and Berg. (2007).

However, we observed that some varieties abandoned in one place were still popular in others. For instance Kyebandula that had been abandoned from Iganga, Mpigi and

Mukono districts was still being grown in Kabarole (KRE682) where the viruses were not a big constraint. Kahungezi has been abandoned in Iganga, Masaka and Mpigi but was being grown in Mbarara (MBR552); Kareebe already abandoned in Mukono is still grown in Mbarara (MBR536).

3.3.8 Ranking of sweetpotato in the Ugandan food system

The ranking of sweetpotato with cassava, maize, sorghum, millet and banana that are considered key in the food system of Uganda is shown in Fig 3.2. Most respondents (50.1%) ranked sweetpotato as their number one crop whereas 0.1% ranked it as their number five. Sweetpotato was earlier on ranked third after banana and cassava in Uganda (Mwanga and Wanyera, 1988; Bashaasha *et al.*, 1995). Farmers attributed the high ranking to the various forms in which the crop is consumed, ease of production with minimum input requirements and also high yields in poor agro-climatic conditions.

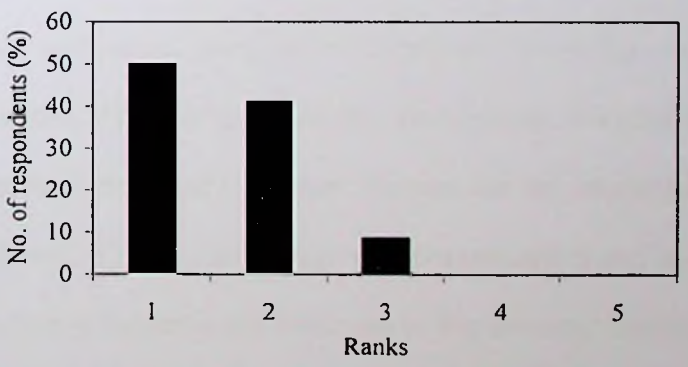

Fig 3.2. Ranking of sweetpotato in the food system of Uganda

Sweetpotato was ranked as the number one crop by 61.0% of the farmers in Eastern Uganda, 56.1% in central region, 45.6% in northern Uganda, and 36.7% of the farmers in western region who predominantly depend on banana for food where banana bacterial wilt is not yet a problem. The farmers in the north attributed the gain in importance of sweetpotato to the destruction of cassava by the cassava mosaic virus in the 1990s. Sweetpotato has been reported first as a food crop in Soroti district (Smit *et al.*, 1996), and as a major crop in the farming system in Kumi district (Mudiope *et al.*, 2000) and Eastern and Southern Africa (Ewell and Mutura, 1994) and this agrees with the findings of this research.

3.4 Conclusion and Recommendations

Detailed information on sweetpotato germplasm across Uganda has been collected and documented in this study. Up to 1303 accessions with 565 vernacular names were collected from 21 districts of the major sweetpotato agroecologies in Uganda. Passport data for collections along with indigenous knowledge on the varieties has been fully documented. There is no doubt that the existing diversity in the sweetpotato germplasm of Uganda is the most important resource for the improvement of the crop in the region and beyond. The characterization at morphological and molecular levels and evaluation to effectively conserve and make use of this resource is recommended.

CHAPTER FOUR

4.0 MORPHOLOGICAL CHARACTERIZATION OF SWEETPOTATO GERMPLASM IN UGANDAN

4.1 Introduction

Sweetpotato (*Ipomoea batatas* (L.) Lam) is a multipurpose hexaploid (2n = 6x = 90) (Kumagai *et al.*, 1990; Austin and Huamán, 1996) root crop of great economic importance in Uganda. It is mainly harvested piecemeal for providing fresh daily food for the family almost throughout the year (Bashaasha *et al.*, 1995). Sweetpotato is also widely used as an animal feed (Woolfe, 1992; Bashaasha *et al.*, 1995). Uganda is the second largest producer of sweetpotato in the world after China (FAO, 2007) and is a secondary center of diversity of the crop (Austin, 1988) where a large number of sweetpotato landraces are grown and maintained by farmers in their natural environments (Huamán and Zhang, 1997; Mwanga *et al.*, 2001a). Frequent mutations take place in the crop (Hernandez *et al.*, 1964) resulting in numerous new genotypes in the farmers' fields, and presenting a challenge to conservation (Collins and Cannon, 1983). Sweetpotato displays a great morphological diversity in Uganda, which has developed following a long time of cultivation and selection across diverse environments coupled with accumulated mutations throughout the history of its cultivation. Gichuru *et al.*, 2006 observed distinct clones of sweetpotato on the basis of morphological and DNA characterization in some East African genotypes.

A total of 1303 accessions of Ugandan sweetpotato germplasm with complete passport data have been assembled at the National Crops Resources Research Institute (NaCRRI). Although high phenotypic variations were observed in farmers' varieties during germplasm collection, numerous duplicate accessions are expected in this collection due to asexual propagation of sweetpotato cultivars and rampant mutations (Huamán *et al.*, 1999). Such accessions just like in other crops are likely to be polyclonal as farmers may not have made a deliberate effort to separate phenotypically similar genotypes (Elias *et al.*, 2001; Veasey *et al*, 2007). The phenotypic diversity among these sweetpotato accessions maintained at NaCRRI for important morpho-agronomic traits is not known, yet phenotypic diversity usually reveals important traits of interest to the plant breeders (Singh, 1989). This collection needs to be morphologically characterized in order to identify and eliminate duplicate accessions and lower costs of conservation. The characterization of these accessions for important traits would also facilitate the efficient synthesis of breeding populations that are designed to accomplish specific objectives. The objective of this study was to characterize the Ugandan sweetpotato germplasm collection using morphological traits in order to eliminate duplicate accessions.

4.2 Materials and Methods

4.2.1 Plant material and study description

A total of 1,303 accessions of the Ugandan sweetpotato germplasm being maintained at NaCRRI, Namulonge were examined in a rain fed trial. The entries were planted in the

field in September 2005 at Namulonge (0° 32'N, 32° 35'E, 1150 meters above sea level) characterized by a bimodal rainfall pattern and tropical wet and mild dry climate with slightly humid conditions (average 65%). Ten clean vines (30 cm long) per accession were planted per plot (ridge) measuring 3 m long and 1 m apart. Five plants per plot were assessed for morphological traits. The trial was weeded manually thrice with no fertilizer and chemical application for disease and pest control.

4.2.2 Evaluation of morphological traits

Fourty morphological characteristics for the key sweetpotato descriptors were scored using a scale of 0-9 (Anonymous, 1991; Huamán, 1992), 90-100 days after planting (DAP). These traits included, twining (TNG), plant type (PTP), vine internode length (VIL), vine internode diameter (VID), predominant color of vine (PVC), secondary color of vine (SVC), vine tip pubescence (VTP), general leaf outline (GOL), type of leaf lobes (LLT), number of leaf lobes (LLN), shape of central leaf lobe (SCL), mature leaf size (MLS), abaxial leaf vein pigmentation (ALP), mature leaf color (MLC), immature leaf color (ILC), petiole length (PTL), petiole pigmentation (PPP), storage root shape (SRS), storage root defects (SRD), storage root cortex thickness (SRT), predominant skin color (PSC), intensity of predominant skin color (IPC), secondary skin color (SSC), predominant root flesh color (PFC), secondary flesh color (SFC), distribution of secondary flesh color (DSF), flowering habit (FHT), flower color (FCL), flower length (FLT), flower width (FWT), shape of limb (SLB), equality of sepal length (ESP), number of veins (NVS), sepal shape (SSP), sepal apex (ASP), sepal color (SLL), sepal

pubescence (SPN), color of stigma (CST), color of style (CSL) and seed capsule set (CSC). Morphological traits were recorded for 1256 accessions, whereas the others that succumbed severely to SPVD were not characterized.

4.2.3 Standardization of characters

The original classification data values were measured in different scales. Standardization allowed each character to contribute toward the overall semblance inversely in proportion to its variability among the entire set of operational taxonomic units (OTUs) used in the study, so that a character with a small range of variation could contribute as much as another character with a large range of variation (Williams, 1976; Sneath and Sokal, 1973). The data was standardized by ranging to equalize both data size and variability (Milligan and Cooper, 1987), as the characters did not have normally distributed states. Ranging was done by subtracting the minimum value of each character amongst all OTUs from the value of that character for a given OTU and then dividing the result by the range for that character over all OTUs (Rohlf, 1993) given as:

$y' = (y-a)/b$

Where; y' is the standardized value, y is the original value of the character, a is the minimum value of that character and b is the range of that character. The values among the OTUs were standardized from 0 to 1.

4.2.4 Data analysis

A variable reduction technique was used to select the most discriminating variables among the 40 measured traits and also to eliminate the redundant traits. Stepwise discriminant analysis (SDA) using the PROC STEPDISC procedure (SAS, 2001) was used to select traits that were included in the classification model. A significance level of 0.01 of an F test from an analysis of covariance was imposed to choose the most discriminating descriptors (SAS, 1990). Wilk's lambda (λ) was used as the criterion to determine the classification efficiency with the entry of each trait. The selected traits were then used in the subsequent analyses. Principal component analysis (PCA) was used on the ranged data as a linear dimensionality reduction technique to identify orthogonal directions of maximum variance in the original data set and to project the data into lower dimensions of the highest variance components, and to examine the percentage contribution of each trait to variation using the PROC PRINCOMP subprogram of SAS. Correlations between the first two PCs and the selected traits were calculated by PROC CANCORR subprogram to aid in interpretation of the analysis. The accessions were clustered by a dissimilarity distance matrix and the UPGMA (unweighted pair group method using arithmetic averages) clustering algorithm procedure (PROC FASTCLUST & PROC CLUSTER) to index similarities between the sweetpotato accessions. The categorical nature of the data could not allow assumption of normal distribution to be made hence a nonparametric method; the k-means neighbor method was used to estimate the group specific densities that produced a classification criterion.

The phenotypic diversity for the recorded traits was analyzed by the Shannon Weaver diversity index (H'), given as:

$$H' = -\sum_{i=1}^{s} pi \ln (pi)$$

Where s is the number of phenotypic classes for a character and pi is the relative proportion of the total number of entries (N) in the ith class. The closely related accessions from the clusters were planted side by side in the field and compared after 90 days for highly correlated morphological traits for duplicate identification.

4.3 Results and Discussions

4.3.1 Phenotypic character distribution

Individual characters differed in their patterns of distribution and amount of variation among the 1256 accessions. Most of the accessions (68%) had short (3-5 cm) vine internode lengths (VIL), 23% had very short (<3 cm) VIL, whereas 9% had intermediate (6-9 cm), none had a long (10-12 cm) VIL, and only 0.1% had very long (>12 cm) VIL. The predominant vine color observed was green (64%), while 21% of the accessions had green vines with few purple spots, 7% had green vines with many purple spots, and a few of them had totally dark purple vines. Most of accessions (58%) had no secondary vine color, 26% had purple nodes, 10% had purple bases and 5% purple tips. Vine tip pubescence was absent in 52% of the accessions, whereas sparse, moderate and heavy pubescence was observed in 31%, 13% and 5% of the accessions, respectively.

The general outline of the leaves was spread among the seven different classes. Most (60%) of the accessions had lobed leaves, an adaptation probably for reducing insect pest damage, 15% had triangular leaves, 9% had hastate leaves, 8% had almost divided leaves, 7% had cordate leaf outlines, 0.1% had rounded and reniform leaf outlines. Insect pest attacks were more severe on leaves with large surface area. The sweetpotato butterfly (*Acreae acerata*) and weevils rarely attacked plants with highly divided leaf surfaces. Sixteen percent of the accessions had no lateral lobes, 6% had very slight leaf lobes, 14% had slight lobes, and 36% had moderate lobes, whereas 23% and 5% had deep and very deep lobes, respectively. Most accessions (64%) had 5 leaf lobes, and 20% of them had 1 leaf lobe, 10% had 3 leaf lobes, 5.6% had 7 lobes and 0.4% and 9 lobes. However, most of the accessions (80%) had no central leaf lobe. The predominant (69%) leaf size observed was the medium one (8-15 cm), and 31% of the accessions had small leaves (<8 cm). Large (16-25 cm) and very large (>25 cm) leaves were not observed among the accessions. Abaxial leaf pigmentation was distributed among the different phenotypic classes. Most accessions (32%) had leaves with green abaxial veins. Nine percent of the accessions had yellow abaxial veins, while 6% had purple spots in the base of several veins, 12% had purple spots in the base of the main rib, 9% had partially purple main ribs, 4% had totally purple main ribs, 15% had all partially purple veins, 14% had all totally purple veins and 0.2% had lower surface and veins totally purple.

The predominant mature leaf color was green (81%), and others were; green with purple edge (9%), slightly purple (6%), green with purple vein on the upper surface (1.8%),

mostly purple and green upper, purple lower (both 1%), and yellow (0.2%), whereas no plants with purple leaves on both surfaces were observed. This is probably an adaptation for increasing the photosynthesis rate. The accession SRT333 has yellow leaves, and could probably be developed as an ornamental genotype (Owings *et al.*, 2000). A similar distribution was found in the immature leaf color of the accessions. However, 41% of the accessions had green immature leaves with purple edges, and 23% had green immature leaves. The 11 and 12% of the accessions had slightly purple and mostly purple immature leaves, respectively. The 8% of the accessions had immature leaves that were purple on both surfaces. The petiole lengths varied in the order; 29% very short (<10 cm), 68% short (10-20 cm), 3% intermediate (21-30 cm) petioles. There were no accessions with long (31-40 cm) and very long (>40 cm) petioles. Thirty five percent of the accessions had green petioles, whereas 32% had green petioles with purple near leaf, 19% had green petioles with purple at both ends, 6% had green petioles with purple spots throughout the petiole, 3% had totally purple petioles, 2% had green petioles with purple strips, 1% had some petioles purple, others green, and 1% had green petioles with purple near stem.

The predominant storage root shape was round elliptic (25%), followed by elliptic (21%), round (15%), long elliptic (15%), long irregular or curved (10%), ovate (7%), obovate (4%), oblong (2%), and long oblong (1%). Twenty eight percent of the accessions had no storage root defects. The defected accessions mostly had shallow horizontal constrictions on the skin (35%). Other defects observed were shallow longitudinal grooves (21%), veins (10%), and alligator-like skin (6%). The 45% of the accessions had thin (1-2 mm) root cortex, whereas 36% had intermediate (2-3 mm) cortex, 10% had thick cortex, 6%

had very thin (<1 mm) cortex, and 4% had very thick (>4 mm) cortex all of which were varieties collected from northeastern Uganda. This could be an evolutionary adaptation to resist weevil infestation in the region that has a high weevil pressure (Story *et al.*, 1999). The different skin colors observed among the accessions were cream (46%), purple-red (39%), yellow (8%), brownish orange (6%), and white (1%). The intensity of the predominant skin color was distributed as pale (23%), intermediate (48%), and dark (29%). Secondary skin was absent in most accessions. The predominant flesh color observed among these Ugandan accessions was cream (49%). Some varieties (21%) had white flesh, pale yellow (15%), dark yellow (10%), pale orange (2%), intermediate orange (1%), dark cream (2%), and dark orange and strongly pigmented with anthocyanins (0.2%). Sweetpotato in Uganda is mainly produced as a source of food. Farmers normally prefer high dry matter varieties. Most of the white and cream fleshed cultivars are high dry matter materials unlike the orange-fleshed types. The large proportion of cream and white-fleshed accessions could have been a result of farmer selection in search of high yielding and dry matter cultivars over the years (Gibson *et al.*, 2008). Most of the accessions (80%) were non-flowering during the morphological characterization. Huamán, 1992 reported that sweetpotato cultivars vary in their ability to flower, and under normal field conditions, some cultivars do not flower, and others produce very few flowers, whereas others flower profusely. The adaptive significance and patterns of distribution of most these traits could have been due to the effect of long term selection practice of the farmers combined with the patterns of changes in the climatic conditions that prevailed over the years of selection in Uganda.

4.3.2 Stepwise discriminant analysis (SDA)

The discriminating power of 20 morphological traits was strong enough to differentiate among the sweetpotato genotypes (Table 4.1). The significant results ($P<0.001$) of using fewer descriptors confirmed the usefulness of the STEPDISC procedure in selecting a critical subset of descriptors. Concentrating on the selected descriptors could reduce the cost and time for investigating sweetpotato morphological relationships without compromising on the information gained. Flowering traits did not account significantly to discrimination of the genotypes as such were eliminated in the STEPDISC procedure.

Table 4.1. Morphological descriptors in sweetpotato selected by the STEPDIC procedure, Shannon Weaver diversity index (H') estimates

Step	Descriptor	STEPDISC procedure			Diversity index
		Partial R-square	Wilks' Lambda (λ)	Pr < Lambda	H'
1	General leaf outline (GOL)	0.14	0.86	<.001	0.99
2	Predominant color of vine (PVC)	0.07	0.80	<.001	0.98
3	Storage root cortex thickness (SRT)	0.07	0.75	<.001	0.97
4	Number of leaf lobes (LLN)	0.06	0.70	<.001	0.97
5	Petiole pigmentation (PPP)	0.06	0.66	<.001	0.97
6	Abaxial leaf vein pigmentation (ALP)	0.05	0.63	<.001	0.96
7	Mature leaf color (MLC)	0.05	0.60	<.001	0.95
8	Predominant skin color (PSC)	0.04	0.52	<.001	0.94
9	Secondary skin color (SSC)	0.04	0.50	<.001	0.93
10	Predominant root flesh color (PFC)	0.04	0.48	<.001	0.92
11	Vine internode length (VIL)	0.04	0.44	<.001	0.90
12	Intensity of predominant skin color (IPC)	0.04	0.41	<.001	0.89
13	Storage root defects (SRD)	0.03	0.40	<.001	0.87
14	Storage root shape (SRS)	0.03	0.38	<.001	0.86
15	Petiole length (PTL)	0.03	0.37	<.001	0.85
16	Secondary color of vine (SVC)	0.03	0.36	<.001	0.83
17	Immature leaf color (ILC)	0.03	0.35	<.001	0.76
18	Vine tip pubescence (VTP)	0.03	0.34	<.001	0.74
19	Mature leaf size (MLS)	0.03	0.33	<.001	0.73
20	Type of leaf lobes (LLT)	0.03	0.32	<.001	0.64

4.3.3 Diversity analysis

The Shannon Weaver diversity index (H') (Shannon and Weaver, 1949) values for the landrace traits ranged from 0.1 for vine internode diameter to 0.99 for general outline of the leaf (Table 4.1). For all the landraces, the mean diversity index calculated from the frequencies of the forty morphological traits was 0.71±0.03, indicating a high sweetpotato landrace diversity in Uganda. Except for the 20 morphological descriptors that significantly accounted for the variability in the germplasm, the rest of the traits had very low diversity index estimates ranging from 0.1 to 0.2. A low H' is an indicator of an extremely unbalanced frequency of classes for an individual trait and a lack of genetic diversity. Abidin and Carey, 2001 also reported relatively high morphological diversity among the sweetpotato accessions from northeastern Uganda. Mutations could be a probable source of variation in this collection (Hernandez *et al.*, 1964). Another probable cause of the variation could be rampant hybridizations as it is a common practice of the Ugandan farmers to take volunteer plants from the previous season to use as planting material (Carey *et al.*, 1998), coupled with the heterogeneous nature of the crop which could have enhanced chances of out crossing.

4.3.4 Phenotypic trait correlation

The correlation matrix showed that some characters had high positive pair-wise correlation coefficients (Table 4.2), meaning that these traits could be used for future characterization and selection programs. Predominant vine color was highly positively

correlated with petiole pigmentation ($r=0.59$; $p\leq0.05$). Secondary vine color was highly correlated with abaxial leaf pigmentation ($r=0.52$; $p\leq0.05$). Very strong positive correlation was found between general leaf outline and leaf lobe type ($r=0.79$; $p\leq0.05$) and leaf lobe number ($r=0.80$; $p\leq0.05$). Leaf lobe type was strongly and positively correlated with leaf lobe number ($r=0.77$; $p\leq0.05$) and mature leaf size ($r=0.70$; $p\leq0.05$). Abaxial leaf pigmentation was correlated with petiole pigmentation ($r=0.58$; $p\leq0.05$). Petiole pigmentation was positively correlated with predominant skin color ($r=0.50$; $p\leq0.05$). Correlations between the storage root traits and vine traits were generally low or negative. Leaf traits could be effectively used with other highly correlated vine and root traits in the future efficient morphological characterization of sweetpotato germplasm to minimize the costs of morphological characterization and save time and labor.

Table 4.2. Correlation coefficient between 20 morphological traits used for distinguishing 1256 accessions of Ugandan sweetpotato germplasm collection

	VIL	PVC	SVC	VTP	GOL	LLT	LLN	MLS	ALP	MLC	ILC	PTL	PPP	SRS	SRD	SRT	PSC	IPC	SSC	PFC
Vine internode length (VIL)	1.00																			
Predominant color of vine (PVC)	0.00	1.00																		
Secondary color of vine (SVC)	-0.02	0.19	1.00																	
Vine tip pubescence (VTP)	0.08	-0.01	0.08	1.00																
General leaf outline (GOL)	-0.07	0.01	-0.07	-0.07	1.00															
Type of leaf lobes (LLT)	-0.13	0.02	-0.02	-0.09	0.79	1.00														
Number of leaf lobes (LLN)	-0.08	0.01	-0.09	-0.04	0.80	0.77	1.00													
Mature leaf size (MLS)	0.02	0.06	0.01	0.00	0.13	0.70	0.13	1.00												
Abaxial leaf vein pigmentation (ALP)	-0.05	0.36	0.52	0.08	-0.01	0.02	-0.01	-0.02	1.00											
Mature leaf color (MLC)	-0.06	0.43	0.15	0.01	-0.03	-0.03	0.00	0.00	0.33	1.00										
Immature leaf color (ILC)	-0.02	0.36	0.17	0.10	0.03	0.03	0.03	0.01	0.24	0.38	1.00									
Petiole length (PTL)	0.09	0.09	0.02	-0.05	0.02	0.01	0.05	0.40	0.02	0.02	0.04	1.00								
Petiole pigmentation (PPP)	-0.01	0.59	0.41	0.05	-0.02	-0.02	-0.06	-0.01	0.58	0.48	0.22	0.01	1.00							
Storage root shape (SRS)	-0.06	-0.01	-0.02	-0.03	0.03	0.02	0.06	0.00	-0.05	0.06	0.00	-0.01	-0.01	1.00						
Storage root defects (SRD)	-0.05	-0.07	0.05	-0.01	0.01	0.03	0.00	-0.03	0.02	-0.04	-0.08	-0.05	0.01	-0.01	1.00					
Storage root cortex thickness (SRT)	-0.02	-0.05	-0.03	0.02	0.06	0.27	0.03	0.02	-0.04	-0.05	-0.01	0.03	-0.04	-0.18	0.14	1.00				
Predominant skin color (PSC)	-0.05	0.31	0.30	0.08	0.01	-0.03	0.00	0.02	0.20	0.24	0.30	0.06	0.50	0.03	-0.06	-0.06	1.00			
Intensity of predominant skin color (IPC)	-0.05	0.04	0.02	-0.03	0.03	0.03	0.03	0.02	0.02	0.08	0.06	0.00	-0.01	0.06	-0.02	-0.03	0.08	1.00		
Secondary skin color (SSC)	0.08	-0.21	-0.09	-0.01	0.03	0.07	0.02	-0.03	-0.16	-0.17	-0.23	-0.07	-0.17	0.01	-0.01	0.02	-0.48	-0.11	1.00	
Predominant root flesh color (PFC)	-0.03	-0.01	-0.09	-0.15	0.08	0.13	0.10	-0.01	-0.11	-0.01	-0.07	-0.02	-0.07	0.00	0.08	0.10	-0.14	0.09	0.08	1.00

$P \leq 0.05$

4.3.4 Principal component analysis (PCA)

Principal component analysis was used prior to cluster analysis to determine the relative importance of the 20 classification traits (Jackson, 1991). The correlation between the 20 traits, and their eign-values was used as a basis for the identification of the principal components (PCs) in this study (Jaradat and Shahid, 2006; Panthee *et al.*, 2006). The first seven PCs with eign values greater than 1.0 accounted for 89.2 % of the total variance and their respective eign vectors are shown in Table 4.3. The relative discriminating power as shown by the eign values was high for principal axes 1 (3.20) and 2 (2.66). The first PC that explained the highest proportion (30.1%) of total morphological variance was correlated with the general leaf outline, and the coloration traits like petiole pigmentation, abaxial leaf pigmentation, predominant vine color, mature leaf color, predominant skin color and immature leaf color. However, the second PC axis explained 27.4% of the total variance and was related mainly with leaf lobe types and leaf lobe number. It is clear from this principal component analysis that much of the variance in this Ugandan germplasm collection was explained by the first two PCs though a large number of PCs was needed to explain total variance in the germplasm. This implies that there is a high variability in relation to the traits correlated with the first two PCs. The large number of traits needed to explain total variance could probably be an indication of a large number of duplicate accessions in the collection (Berdahl *et al.*, 1999). This was probably because of the very highly heterogeneous nature of the sweetpotato crop with high hybridization levels (Jones, 1965). As a result of segregation, which takes place in F1in farmers' fields, many of the genotypes collected could be closely related.

Table 4.3. Eign-vectors from the first seven principal component axes for traits used to classify 1256 sweetpotato accessions

Character	Principal component axis						
	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7
Vine internode length (VIL)	-0.03	-0.11	0.18	0.24	-0.24	0.03	0.40
Predominant color of vine (PVC)	0.39	0.09	0.03	0.01	0.07	0.18	0.28
Secondary color of vine (SVC)	0.30	0.00	-0.21	0.30	-0.10	0.09	-0.33
Vine tip pubescence (VTP)	0.07	-0.07	0.00	0.13	-0.33	-0.45	0.00
General leaf outline (GOL)	0.56	-0.08	-0.04	0.01	-0.10	-0.09	0.02
Type of leaf lobes (LLT)	-0.08	0.55	-0.10	0.03	-0.07	-0.03	0.00
Number of leaf lobes (LLN)	-0.08	0.55	-0.01	-0.02	-0.11	-0.07	0.02
Mature leaf size (MLS)	0.01	0.14	0.55	0.29	0.06	0.14	-0.19
Abaxial leaf vein pigmentation (ALP)	0.39	0.06	-0.23	0.26	-0.08	0.10	-0.15
Mature leaf color (MLC)	0.34	0.06	-0.01	-0.15	0.10	0.16	0.26
Immature leaf color (ILC)	0.31	0.09	0.09	-0.15	0.01	-0.15	0.30
Petiole length (PTL)	0.05	0.07	0.59	0.30	0.11	0.18	-0.12
Petiole pigmentation (PPP)	0.42	0.05	-0.17	0.18	-0.03	0.16	0.00
Storage root shape (SRS)	0.00	0.04	0.03	-0.37	-0.21	0.35	-0.36
Storage root defects (SRD)	-0.04	0.01	-0.25	0.19	0.38	-0.11	-0.39
Storage root cortex thickness (SRT)	-0.06	0.04	-0.07	0.32	0.47	-0.35	0.16
Predominant skin color (PSC)	0.32	0.05	0.20	-0.26	0.06	-0.32	-0.12
Intensity of predominant skin color (IPC)	0.05	0.06	0.07	-0.30	0.25	0.11	-0.10
Secondary skin color (SSC)	-0.25	-0.02	-0.22	0.28	-0.24	0.36	0.19
Predominant root flesh color (PFC)	-0.11	0.10	-0.13	-0.03	0.48	0.33	0.24
Eign value	3.20	2.66	1.46	1.37	1.23	1.19	1.08
% variation	30.1	27.4	7.3	6.8	6.2	6.0	5.4
Cumulative % variation	30.1	57.5	64.8	71.6	77.8	83.8	89.2

4.3.5 Cluster analysis

The proc fastclust sub-program of SAS grouped the 1256 accessions into 20 clusters with the numbers of accessions per cluster ranging from 15 to 166 (Table 4.4). Cluster 19 with highest number of accessions consisted predominantly of accessions from the eastern and central districts. The accessions in this cluster were very closely related to those in cluster 16 (s=0.6416) though most of the accessions grouped in this cluster were from western Uganda. Despite the large number of accessions in this cluster, the distance between the cluster centroids was relatively low (s=0.7789), implying that these could be very closely related accessions. The distance between cluster centroids (s), which is an indication of the level of diversity among the

accessions within each cluster ranged from 0.6109 for cluster 18 and 20 to 0.8964 for accessions in cluster 14 (Table 4.4). There was no distinct relationship between clusters generated and the distribution of accessions from the regions. A similar pattern in clustering and geographic distribution was observed among some east African sweetpotato cultivars (Gichuru *et al.*, 2006), and the accessions collected from some districts of northeastern Uganda (Abidin and Carey, 2001). Similarly, diversity among the Brazilian sweetpotato germplasm was not structured in space (Veasey *et al.*, 2007). The Ugandan sweetpotato planting material might have been routinely moved from region to region.

Table 4.4. Clustering summary for 1256 sweetpotato accessions and regional distribution of accessions

Cluster	Proc fastclust			Regional distribution				Proc cluster	
	Frequency	Nearest cluster	Distance between cluster centroids	East	North	West	Central	Average distance between clusters	No. of Duplicates
1	39	5	0.8480	12	7	6	14	0.0-1.4	13
2	22	12	0.7714	5	4	11	2	0.0-1.3	5
3	112	16	0.7738	28	9	35	40	0.0-1.4	37
4	15	11	0.8315	5	0	5	5	0.0-1.3	5
5	51	18	0.6251	11	13	17	10	0.0-1.4	12
6	97	16	0.6416	21	12	35	29	0.0-1.3	28
7	23	5	0.8085	7	1	7	8	0.0-1.2	4
8	33	11	0.8502	13	3	10	7	0.0-1.3	16
9	39	17	0.7454	7	1	20	11	0.0-1.4	24
10	40	15	0.6534	13	4	9	14	0.0-1.3	11
11	58	16	0.6905	16	9	22	11	0.0-1.1	11
12	70	2	0.7714	18	16	28	8	0.0-1.2	26
13	37	17	0.7405	12	3	16	6	0.0-1.4	22
14	41	20	0.8967	17	3	6	15	0.0-1.5	16
15	56	10	0.6534	15	8	15	18	0.0-1.3	2
16	122	6	0.6416	28	14	34	46	0.0-1.2	37
17	75	13	0.7405	18	9	34	14	0.0-1.3	19
18	88	20	0.6109	29	14	22	23	0.0-1.4	18
19	166	16	0.7789	50	28	38	50	0.0-1.4	36
20	72	18	0.6109	18	9	18	27	0.0-1.3	15

4.3.6 Identification of duplicates or nearly related accessions

Relatedness among the accessions was visualized by Proc cluster sub-program of SAS. The average distance between the cluster points revealed by Proc cluster ranged from 1.1 among the accessions in cluster 11 to 1.5 among the accessions of clusters 14. The 20 clusters had several sub-clusters with no relation to the district of origin. The number of duplicate or closely related accessions per cluster ranged from 2 in cluster 15 to 37 in clusters 3 and 16 (Table 4.4). Cluster analysis therefore led to the identification of 357 duplicate accessions, and 899 morphologically distinct accessions. The number of duplicates of the same accession ranged from 1 to 24. In the Peruvian sweetpotato germplasm collection that contained 1939 accessions, the number of duplicates of the same cultivar ranged from 1 to 99 (Huamán *et al.*, 1999). Abidin and Carey (2001) found 18 duplicates out of 206 northeastern Uganda sweetpotato accessions.

Most of the duplicates identified had been collected from different districts. For instance, duplicate accessions SRT21 (Gunyombekere), ARA226 and MSD434 in cluster 1 were collected from Soroti, Arua and Masindi districts, respectively. This could be a result of regular movement of sweetpotato planting material across districts, as sweetpotato vines can be kept for up to one week in a cool place before planting. This enhances the transit of vines over long distances. Duplicates collected from the same district included: KML921 (Nyindoyamulalo enkaire) and KML962 (Museveni) from Kamuli district, MBR576 and MBR577 (Nkaijaomunyonyi) from Mbarara district among others. Some accessions that were given the same name by the farmers for instance SRT29, KMI84, MSD441 and IGA995 (Kampala) were morphologically different. On the other hand some accessions with similar names like Nylon (KRE698 and MBR580) were

duplicates. One representative accession was selected from the duplicate sets and added to the morphotypes to constitute a collection of 946 accessions (Ortiz *et al.*, 1998).

4.4 Conclusion and Recommendations

Significant morphological variations were observed among the 1256 Ugandan sweetpotato accessions. The discriminating power of 20 morphological traits was strong enough for distinguishing among the 1256 accessions. The general outline of the leaf accounted for the highest amount of variation in the germplasm. It was not surprising that high positive correlations were observed among the other leaf traits and the general outline of the leaves. This implies that future morphological characterization of sweetpotato germplasm in Uganda could be effectively and cheaply done with much emphasis on the leaf traits with less emphasis on the flowering traits. The high morphological diversity observed among the sweetpotato accessions may not be conclusive as variations in environmental conditions such as soil types and fertility levels, light, temperature and moisture regimes (Morakinyo and Ajibade, 1998) could still allow for different results to be obtained if this morphological characterization is repeated in time and space. The need to evaluate the morphologically distinct accessions for identifying superior genotypes for use in hybridization schemes for variety development in Uganda is highly recommended. The use of molecular characterization of the germplasm to confirm the superior genotypes is also strongly recommended.

CHAPTER FIVE

5.0 FIELD EVALUATION OF UGANDAN SWEETPOTATO GERMPLASM FOR YIELD, DRY MATTER AND DISEASE RESISTANCE

5.1 Introduction

The enhancement of sweetpotato (*Ipomoea batatas* (L.) Lam) breeding program requires a comprehensive collection and evaluation of native germplasm from different locations to provide a wide genetic base. Uganda, the third largest producer of sweetpotato in the world after China and Nigeria (FAO, 2007), and a secondary center of diversity has a large number of genotypes being maintained by farmers in their natural environments (Huamán and Zhang, 1997; Mwanga *et al.*, 2001a). Sweetpotato is a hardy crop, able to grow in arid conditions and with little demand for water and fertilizers (Bohac *et al.*, 1995). It is as a very important crop in Uganda where the crop is grown up to 5,900 ha per season (FAO, 2007), predominantly by the rural poor for food security and source of income (Hakiza *et al.*, 2000). However, the root yields are low (4.0 Mt/ha) as a result of subsistence production that heavily relies on low yielding cultivars (Bashaasha *et al.*, 1995; Grüneberg *et al.*, 2004). Production is also constrained by pests (*Cylas spp)* and diseases mainly SPVD (Aritua *et al.*, 2000; Gibson *et al.*, 2000; Mwanga *et al.*, 2002a) and Alternaria blight. SPVD causes up to 90% yield loss (Gibson *et al.*, 1997; Gibson *et al.*, 1998) and over the years led to the loss of earlier collections at the National Crops Resources Research Institute (NaCRRI), Namulonge (Mwanga *et al.*, 1991).

Sweetpotato genotypes being maintained in their natural environments when collected possess many desirable traits such as high yield potential, vigorous growth, disease and pest resistance,

and stress tolerance. Such collections constitute valuable and diverse genetic materials for a breeding program. The sweetpotato variety improvement in Uganda has focused on the development of varieties with high yield potential high provitamin A and dry matter contents, resistance to SPVD, Alternaria blight, weevils and minimum sensitivity to seasonal fluctuations over a wide range of environments (Turyamureeba *et al.*, 1997; Mwanga *et al.*, 2001a).

Efforts to broaden the germplasm base of sweetpotato in Uganda led to the identification of 946 morphologically distinct accessions out of a collection of 1303 accessions being maintained at NaCRRI. The identification of germplasm with specific and broad adaptability and stable agronomic performance from this collection would be helpful in the development of improved sweetpotato cultivars (Grüneberg *et al.*, 2005). The evaluation of potential varieties should form an important constituent of these collection efforts because of their in built genetic variability due to several generations of growing and selection by farmers. The data derived from trait evaluation trials of these collections could be useful in the selection of a diverse array of superior genotypes. These selections can then be available to incorporate into the long-term goals of the sweetpotato genetic improvement program in Uganda. This especially entails the development of genetically diverse populations with greater expression of traits that contribute to their usefulness in pest and disease resistance, high yields and dry matter content highly preferred by Ugandan farmers (Gibson *et al.*, 2008), high beta-carotene contents, vegetative vigor, and broad adaptation. The objective of this study was to evaluate sweetpotato germplasm collection from Uganda for yield, SPVD and Alternaria blight disease resistance so as to select superior genotypes for hybridization schemes.

5.2 Materials and Methods

5.2.1 Source of plant material and study area description

A total of 946 morphologically distinct genotypes obtained from the sweetpotato germplasm collection being maintained at the National Crops Resources Research Institute (NaCRRI) were planted at three sites, NaCRRI, National Semi-Arid Resources Research Institute (NaSARRI), Serere, and Kachwekano Zonal Agricultural Research and Development Institute (KAZARDI) described in Table 5.1.

Table 5.1. Description of study sites

Site	Location	Altitude (m.a.s.l)	Mean Temperature (°C)	Mean Rainfall (mm)	Soils	Main constraint
NaCRRI	0° 32'N, 32° 35'E	1150.0	22.0	1170.0	Sandy clay	SPVD
NaSARRI	1° 32'N, 3° 27'E	1140.0	26.8	1114.3	Sandy loam	Weevils
KAZARDI	10° 16'S, 290° 57'E	2150.0	16.3	1100.0	Sandy clay loam	Alternaria blight

The genotypes were evaluated for two consecutive seasons at each site. The first season trials were planted in September 2005 and harvested in March 2006 at NaCRRI and NaSARRI, June 2006 at KAZARDI. The second season's trials at NaCRRI and NaSARRI were planted in May 2006 and harvested in September 2006, whereas at KAZARDI planted in September 2006 and harvested in June 2007. For each genotype 15 vines measuring 30 cm long were planted on three 1.5 m long ridges with 5 vines per ridge and weeded three times.

5.2.2 Data Collection and analysis

The accessions were left under field infection and rated for SPVD and Alternaria blight disease severity using a scale of 1-5 (1=no apparent damage/not present, 2=very little damage, 3=moderate damage, 4=considerable damage, and 5=severe damage). At harvest, data was recorded on plant stand, number of marketable and non marketable roots, and weight of marketable and non marketable roots for storage root yield determination. Dry matter content was determined using specific gravimetric method (Fonseca *et al.*, 1994). One to three kilograms of marketable roots packed in a mesh bag were weighed in air, and then weighed when submerged in a bucket of water placed in a wind free area.

$$\text{Specific gravity} = \frac{\text{Weight in air}}{(\text{Weight in air} - \text{Weight in water})}$$

Data were summarized and analysis of variance (ANOVA) for the measured trait means was done by the generalized linear model (GLM) of the Statistical Analysis System version 8.1 statistical package (SAS, 2001). Where "F" statistics were significant, separation of means was by Least Significance Difference (LSD) of General Linear Model (GLM) procedure of SAS.

5.3 Results and Discussions

5.3.1 Reaction to sweetpotato virus disease (SPVD)

There was a significant difference in the reaction of the different sweetpotato genotypes to SPVD ($P \leq 0.05$), with SPVD severity scores ranging from 1.0 to 5.0 with a mean of 2.6 (Table 5.2). The

highest mean SPVD severity (3.1) was observed at NaCRRI and the lowest was at KAZARDI. This confirmed the findings by Mukasa *et al.* (2003) and Bua *et al.* (2005) that the highest incidence of SPVD in Uganda was observed in the shoreline of Lake Victoria and the lowest was in the northern region. High SPVD pressure at NaCRRI earlier on led to the loss of the sweetpotato germplasm collection assembled at NaCRRI (Mwanga *et al.*, 1992).

The reaction of the genotypes to SPVD at NaCRRI, the hotspot for the disease is shown in Fig 5.1. Most genotypes (87.8%) had mean SPVD scores ranging from 3.1 to 5.0, implying that most of the germplasm is susceptible to SPVD. Only 4 genotypes had mean severity scores of 1.0-2.0. A similar trend in the frequency distribution of SPVD severity was observed among 15 promising diallel families (1352 genotypes) at NaCRRI in 1998-2000 (Mwanga *et al.*, 2002b).

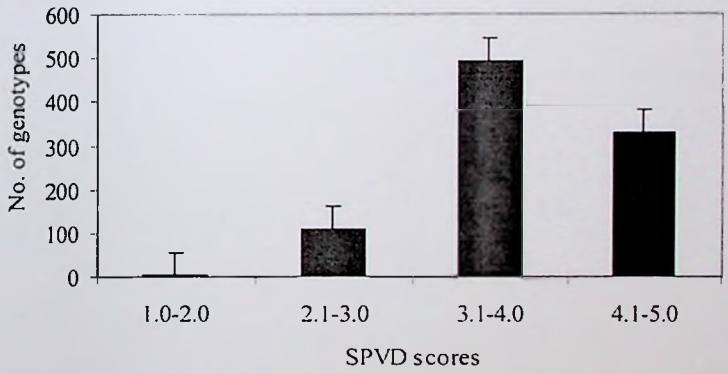

Fig 5.1. Distribution of 946 genotypes based on mean SPVD ratings at NaCRRI

The selection for SPVD resistance was based on genotype disease reaction at NaCRRI. Twenty-two genotypes with mean SPVD severity scores of 2.5 and below at NaCRRI were selected as resistant (Table 5.2). KMI76 (Mukoma) from Kumi district was the most resistant variety with a mean off 1.8 across sites. SRT27 (Kigaire) also performed well across sites. Most of the resistant

varieties were those from areas with low SPVD pressure, though it is actually expected that the genotypes from low SPVD pressure areas should have succumbed more to the disease (Mwanga *et al.*, 1991). These genotypes probably have the SPVD resistance genes that have not been subjected to high SPVD pressure. However, SRT27 (Kigaire), KMI76 (Mukoma), KMI83 (Kala 2), PAL102 (Tanzania1), PAL120 (Muyambi) and KRE687 (Kahungera) were reported by the farmers to have been introduced into those districts from the high SPVD pressure districts of Mukono and Luwero. Three of the resistant varieties (MKN1169, LUW1239 and LUW1257) were collected from the Lake Victoria basin districts of Mukono and Luwero. Some of the landraces that have been established in the Central Uganda have been reported to be relatively resistant to SPVD (Aritua *et al.*, 1998; Alicai *et al.*, 1999). To obtain good yields from these superior sweetpotato germplasm in central Uganda, clean planting material must be used (Mwanga *et al.*, 2007a).

Table 5.2. Mean sweetpotato virus disease scores of superior 22 and bottom 5 sweetpotato genotypes at three sites (NaCRRI, NaSARRI and KAZARDI) in the seasons 2005B-2006A

Code	Name	District of	Site			Mean
		Collection	NaCRRI	NaSARRI	KAZARDI	Across sites
Superior 22 genotypes						
SRT1	Osapat	Soroti	2.5	2.0	1.5	1.8
SRT27	Kigaire	Soroti	2.0	2.5	1.5	1.8
SRT32	Unknown6	Soroti	2.0	3.5	2.0	2.3
KMI76	Mukoma	Kumi	1.5	2.0	2.0	1.8
KMI83	Kala 2	Kumi	2.0	2.5	2.5	2.2
PAL102	Tanzania 1	Pallisa	2.5	1.5	2.0	1.8
PAL120	Muyambi	Pallisa	2.5	1.5	1.5	1.7
ARA232	Unknown75	Arua	2.5	1.5	2.0	1.8
ARA237	Unknown77	Arua	2.5	1.5	1.5	1.7
KSR661	Kasherengenyi	Kisoro	2.5	2.5	1.0	1.8
KSR676	Makara	Kisoro	2.5	2.0	1.5	1.8
KRE687	Kahungera	Kabarole	2.5	2.0	1.5	1.8
KRE734	Yello	Kabarole	2.5	1.5	2.0	1.8
BSH762	Unknown346	Bushenyi	2.5	2.5	1.0	1.8
RAK808	Dduka enzala	Rakai	2.5	2.0	1.5	1.8
KML873	Silk	Kamuli	2.5	2.0	1.5	1.8
KML962	Museveni	Kamuli	2.5	2.0	1.5	1.8
IGA971	Unknown482	Iganga	2.5	2.0	1.5	1.8
IGA983	Silimu	Iganga	2.5	2.0	1.5	1.8
MKN1169	Unknown603	Mukono	2.5	2.0	1.5	1.8
LUW1239	Unknown664	Luwero	2.5	2.0	1.5	1.8
LUW1257	Unknown667	Luwero	2.5	1.5	1.5	1.7
Bottom 5 Genotypes						
KBL620	Unknown290	Kabale	5.0	3.5	3.5	3.8
KBL643	Kashusha	Kabale	5.0	3.5	1.5	3.8
KBL626	Unknown291	Kabale	5.0	3.5	2.0	4.2
KBL619	Kamamanzi	Kabale	5.0	4.5	4.0	4.5
MBR580	Nylon	Mbarara	5.0	5.0	5.0	5.0
Mean			3.1	2.5	2.2	2.6
CV (%)			29.1	27.6	32.3	30.8
LSD (0.05)			1.5	1.3	1.2	3.0

5.3.2 Reaction to Alternaria blight disease

The genotypes varied significantly ($p \leq 0.05$) in their reaction to Alternaria blight (Table 5.3). Alternaria blight severity ranged from 1.0-5.0, with an overall mean of 2.2 across sites and seasons. The highest mean Alternaria severity of 2.8 was observed at KAZARDI and the lowest was at NaSARRI. At KAZARDI, most genotypes (80.8%) were highly susceptible to Alternaria

blight with mean severity scores of 3.1-5.0 and only 32 genotypes (4.4%) had mean severities of 1.0-2.0 (Fig 5.2). Turyamureeba *et al.*, 1999 reported that sweetpotato genotypes succumbed more severely to Alternaria blight at high agro ecologies.

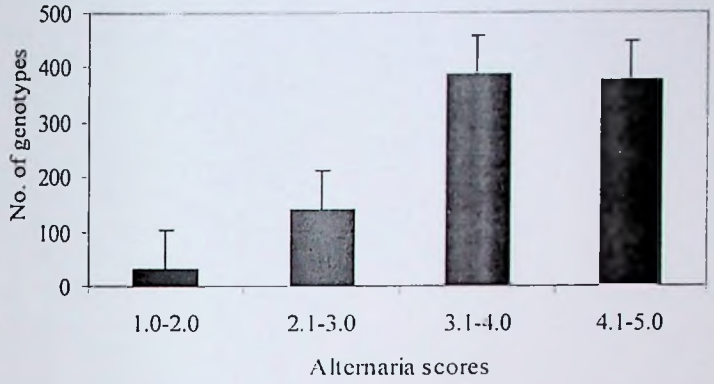

Fig 5.2. Distribution of 946 genotypes based on mean Alternaria ratings at KAZARDI

Twenty one genotypes were identified as sources of resistance to Alternaria blight disease (Table 5.3). SRT24 (Bunduguza) and SRT26 (Igang amalayan), both introductions to Soroti district from Mbale district, a high altitude area with high Alternaria pressure were the most resistant varieties. A number of these resistant varieties were collected from the leading sweetpotato producing districts in Uganda where the farmers over the years of cultivation have selected well adapted genotypes to a range of constraints, including Alternaria disease (Carey *et al.*, 1998). Most of the genotypes collected from Kabale district with high Alternaria severities produced appreciably high yields, suggesting high resistance to the disease in these genotypes. Apart from possessing Alternaria resistance genes, some of the genotypes with unkown names could probably have been introductions from high Alternaria pressure areas like Kabale, Kisoro and Mbale districts.

Table 5.3. Mean Alternaria blight scores of superior 21 and bottom 5 sweetpotato genotypes at three sites (NaCRRI, NaSARRI and KAZARDI) in the seasons 2005B-2006A

Code	Name	District of	Site			Mean
		Collection	NaCRRI	NaSARRI	KAZARDI	Across sites
Superior 21 genotypes						
SRT24	Bunduguza2	Soroti	1.0	1.5	1.5	1.3
SRT26	Igang amalayan	Soroti	2.5	1.5	1.5	1.8
SRT27	Kigaire	Soroti	1.0	2.0	1.5	1.5
SRT32	Unknown6	Soroti	2.0	2.0	2.0	1.8
SRT35	Anyumel	Soroti	1.5	1.0	2.0	1.5
KMI86	Kassim 2	Kumi	1.5	1.5	2.0	1.5
PAL98	Unknown24	Pallisa	1.5	1.5	2.0	1.5
PAL125	Muiruki	Pallisa	2.0	1.0	2.0	1.5
PAL157	Unknown41	Pallisa	1.5	1.0	2.0	1.5
PAL158	Unknown42	Pallisa	1.5	1.5	2.0	1.5
MLE174	Nangumi 1	Mbale	1.5	1.5	2.0	1.5
ARA236	Tanzania	Arua	1.5	1.0	2.0	1.5
APA379	Unknown156	Apac	1.5	1.5	2.0	1.5
HMA487	Nyanya	Hoima	1.5	1.0	2.0	1.5
KML941	Unknown460	Kamuli	1.5	1.5	1.5	1.8
KML943	Unknown462	Kamuli	1.5	1.5	2.0	1.5
MSK1094	Unknown547	Masaka	1.5	1.5	2.0	1.5
KBL650	Unknown299	Kabale	1.5	1.5	2.0	1.5
LUW1254	Silk	Luwero	1.5	1.5	2.0	1.5
LUW1264	Unknown684	Luwero	1.5	1.5	2.0	1.5
KBL619	Kamamanzi	Kabale	1.5	1.0	2.0	1.5
Bottom 5 genotypes						
APA346	Twongwengo	Apac	2.5	2.0	5.0	2.8
KML953	Unknown472	Kamuli	1.5	2.0	5.0	2.8
LIR291	Unknown110	Lira	2.5	1.5	5.0	3.0
KML885	Unknown425	Kamuli	4.0	1.5	5.0	3.2
SRT08	Esamiat	Soroti	2.0	1.5	5.0	2.8
Mean			2.0	1.9	2.8	2.2
CV (%)			39.9	39.4	31.6	42.5
LSD (0.05)			1.9	2.1	1.7	2.1

5.3.3 Marketable root yield performance

The Ugandan sweetpotato genotypes differed significanlty in their marketable root yields ($p \leq 0.05$). Mean genotype marketable root yields ranged from 0.0 t/ha to 48.4 t/ha with an overal mean of 17.2 t/ha. Most genotypes had mean marketable root yield of less than 15.0 t/ha. Abidin *et al.*, 2002 also reported low mean marketable root yields in the sweetpotato genotypes from

northeastern Uganda. The highest mean marketable root yields of 22.1 t/ha was observed at NaCRRI while the lowest of 12.8 t/ha was at KAZARDI (Table 5.4). Byamukama *et al.*, 2002 observed that sweetpotato clones had higher yields at NaSARRI (15.6 t/ha) and lowest at KAZARDI (8.1 t/ha). The highest mean marketable root yield of 48.4 t/ha was observed in the genotype RAK835 and the lowest root yield of 0.0 t/ha was observed in ARA245.

Table 5.4. Mean marketable root yield (t/ha) values of superior 22 and bottom 5 sweetpotato genotypes at three sites (NaCRRI, NaSARRI and KAZARDI) in the seasons 2005B-2006A

Code	Name	District of	Site			Mean
		Collection	NaCRRI	NaSARRI	KAZARDI	Across sites
Superior 22 genotypes						
SRT32	Unknown6	Soroti	55.0	18.4	30.0	34.5
PAL148	Unknown37	Pallisa	5.0	12.2	7.0	44.6
MLE201	Unknown59	Mbale	44.4	17.8	44.2	35.5
LIR265	Unknown87	Lira	55.6	23.8	18.5	32.6
APA329	Unknown131	Apac	44.8	11.1	47.2	34.4
MSD382	Suwedi	Masindi	88.3	43.9	10.4	47.6
KBL650	Unknown299	Kabale	39.4	46.1	8.3	31.3
KRE713	Unknown320	Kabarole	59.6	40.2	2.2	34.0
KRE724	Unknown325	Kabarole	74.1	29.9	6.9	37.0
RAK808	Dduka enzala	Rakai	68.5	22.8	12.3	34.5
RAK835	Unknown400	Rakai	126.7	10.9	7.6	48.4
KML875	Mpaifumbiro	Kamuli	46.0	39.0	7.5	30.8
KML956	Duduma	Kamuli	23.7	10.9	3.5	32.9
KML959	Munyesa	Kamuli	57.4	34.0	12.6	34.7
KML962	Museveni	Kamuli	60.3	35.1	14.2	36.5
IGA998	Bunduguzaempyaka	Iganga	26.2	39.4	24.6	30.1
IGA1003	Unknown494	Iganga	80.4	36.1	3.5	40.0
MPG1122	Unknown565	Mpigi	56.4	11.7	34.0	34.0
MPG1128	Unknown571	Mpigi	25.9	21.3	44.2	30.5
MPG1134	Unknown577	Mpigi	70.8	14.0	19.2	34.7
MPG1146	Mpambire	Mpigi	55.6	5.9	44.6	35.4
MPG1158	Bikiramaria	Mpigi	15.0	25.9	56.8	32.6
Bottom 5 genotypes						
ARA245	Unknown81	Arua	0.0	0.0	0.0	0.0
LIR264	Unknown91	Lira	0.0	0.0	0.0	0.0
MSD423	Yetwala	Masindi	0.0	0.0	0.0	0.0
KBL647	Unknown296	Kabale	0.0	0.0	0.0	0.0
MPG1115	Ndikiryanomwami	Mpigi	0.0	0.0	0.6	0.2
Mean			22.1	16.3	12.8	17.2
CV (%)			31.8	15.9	10.7	35.5
LSD (0.05)			1.7	1.9	1.6	2.4

5.3.4 Total root yield performance

The mean total root yield of the 946 genotypes differed significantly ($p \leq 0.05$), and ranged from 0.0 to 52.5 t/ha with an overall average of 20.3 t/ha. The total root yields for the 19 released sweetpotato varieties in Uganda ranged from 14.9 t/ha for SPK004 to 31.0 t/ha for Dimbuka (Mwanga *et al.*, 2001a; Mwanga *et al.*, 2003; Mwanga *et al.*, 2007b, c). Mean root yields were higher at NaCRRI and lowest at KAZARDI (Table 5.5). The mean root yields varied with the site (environment), as earlier observed in Uganda and Peru (Byamukama *et al.*, 2002; Grüneberg *et al.*, 2005). RAK835 had the highest mean storage root yield of 53.0 t/ha. Thirty-three high yielding genotypes that could be used for variety development in hybridization programs were seelected. Abidin *et al.*, 2002 identified 10 superior varieties from the sweetpotato germplasm from northeastern Uganda. Six superior parental genotypes for hybridization programs were identified from the Indonesian germplasm (Hartana and Renwarin, 1998). HMA496 (Dimbuka) and IGA998 (Bunduguza empyaka) with relatively stable yields across sites could be used to develop broadly adapted genotypes for different areas in Uganda as earlier reported for the germplasm form northeastern Uganda by Abidin *et al.* (2005).

Table 5.5. Mean total root yield (t/ha) values of superior 33 and bottom 5 sweetpotato genotypes at three sites (NaCRRI, NaSARRI and KAZARDI) in the seasons 2005B-2006A

Code	Name	District of Collection	Site			Mean Across sites
			NaCRRI	NaSARRI	KAZARDI	
Superior 33 genotypes						
SRT32	Unknown6	Soroti	59.6	19.1	33.1	37.3
SRT41	Uganda mali	Soroti	57.2	11.8	23.6	30.9
PAL148	Unknown37	Pallisa	6.1	13.0	6.7	48.9
MLE201	Unknown59	Mbale	45.8	20.0	46.2	37.3
ARA224	Koromojo Red	Arua	73.5	17.6	5.5	32.2
LIR265	Unknown	Lira	55.9	24.4	19.7	33.4
APA329	Unknown92	Apac	44.8	12.2	49.5	35.5
APA349	Tutwech	Apac	34.3	117.0	6.1	52.5
APA354	Unknown145	Apac	40.9	10.9	42.0	31.3
MSD382	Suwedi	Masindi	90.0	44.8	11.0	48.6
HMA496	Dimbuka	Hoima	39.1	27.5	25.8	30.8
KBL650	Unknown299	Kabale	45.3	49.1	11.0	35.1
KSR663	Kijambire	Kisoro	86.7	11.6	1.0	33.1
KRE713	Unknown320	Kabarole	67.3	42.3	2.2	37.3
KRE724	Unknown325	Kabarole	75.9	30.8	8.2	38.3
BSH777	Unknown356	Bushenyi	52.5	18.9	6.1	30.7
RAK805	Dimbuka	Rakai	53.1	29.2	12.5	31.6
RAK808	Dduka enzala	Rakai	69.6	23.4	19.5	37.5
RAK815	Unknown382	Rakai	65.4	23.5	5.3	31.4
RAK835	Unknown400	Rakai	132.2	16.1	10.8	53.0
KML875	Mpaifumbiro	Kamuli	50.0	40.5	9.8	33.4
KML877	Unknown419	Kamuli	42.8	26.4	20.9	30.0
KML956	Duduma	Kamuli	89.8	11.0	6.9	35.9
KML959	Munyesa	Kamuli	63.3	42.9	16.0	40.7
IGA998	Bunduguzaempyaka	Iganga	27.0	39.6	26.6	31.1
IGA1003	Unknown494	Iganga	88.1	36.4	3.7	42.8
MSK1079	Munafudimbuka	Masaka	7.2	56.4	28.1	30.6
MPG1122	Unknown565	Mpigi	57.2	11.8	35.9	35.0
MPG1128	Unknown571	Mpigi	27.1	22.2	50.1	33.1
MPG1134	Unknown577	Mpigi	71.1	14.1	21.8	35.7
MPG1146	Mpambire	Mpigi	56.8	6.7	48.0	37.2
MPG1158	Bikiramaria	Mpigi	15.5	26.7	62.8	35.0
LUW1290	Rashid	Luwero	39.3	42.3	11.3	31.0
Bottom 5 genotypes						
ARA245	Unknown81	Arua	0.0	0.0	0.3	0.1
LIR264	Unknown91	Lira	0.4	1.0	0.1	0.5
MPG1115	Ndikiryanomwami	Mpigi	0.0	1.2	0.6	0.6
LUW1234	Unknown659	Luwero	0.0	0.0	1.8	0.6
MSD423	Yetwala	Masindi	0.4	0.2	1.4	0.7
Mean			26.2	18.3	16.5	20.3
CV (%)			24.2	16.5	29.8	27.4
LSD (0.05)			1.8	1.6	1.5	2.3

5.3.5 Root specific gravity performance

The Ugandan sweetpotato genotypes differed significantly in the specific gravity values ($p \leq 0.05$). Most of the genotypes had mean specific gravity scores ranging between 1.1 to 2.0 and only 11 genotypes had specific gravity values of greater than 2.0 implying medium to high dry matter content. This is possibly a result of farmer selection for high dry matter sweetpotato varieties as observed by Gibson *et al.* (2008). Most of the released varieties have high dry matter content of >30% (Mwanga *et al.*, 2001a, 2003, 2007b). The highest mean speicific gravity was observed at KAZARDI (Table 5.6). There was significant variation in mean specific gravity values at the sites. Grüneberg *et al.* (2005) observed significant genotype x environment interaction for sweetpotato storage root dry matter in Peru. Genotype PAL131 (Katiayime) had the highest mean specific gravity value while KBL639 had the lowest specific gravity. The orange-fleshed variety KMI81 (Kala1) also had a high mean specific gravity value of 2.0. This variety could be used in breeding for high dry matter orange fleshed cultivars for Uganda. Most of the high specific gravity genotypes were collected from northeastern and northwestern districts where drought is often severely experienced.

Table 5.6. Mean root specific gravity values of superior 11 and bottom 5 sweetpotato genotypes at three sites (NaCRRI, NaSARRI and KAZARDI) in the seasons 2005B-2006A

Code	Name	District of Collection	Site			Mean Across sites
			NaCRRI	NaSARRI	KAZARDI	
Superior 11 genotypes						
SRT1	Osapat	Soroti	2.8	2.1	1.2	2.0
SRT47	Unknown10	Soroti	3.2	2.2	1.3	2.2
KMI67	Igang Uganda	Kumi	1.1	1.2	1.2	2.3
KMI81	Kala 1	Kumi	2.3	2.2	1.6	2.0
PAL131	Katiayime	Pallisa	1.8	1.7	3.6	3.4
PAL148	Unknown37	Pallisa	3.1	1.9	2.9	2.8
ARA231	Mbutra	Arua	2.1	2.1	2.1	2.1
ARA242	Sanjemoko	Arua	2.6	3.3	1.1	2.3
LIR286	Unknown108	Lira	2.3	2.2	1.5	2.0
LIR288	Unknown109	Lira	1.6	2.9	1.2	2.4
LUW1257	Unknown677	Luwero	2.6	2.7	1.2	2.1
Bottom 5 genotypes						
KBL639	Unknown294	Kabale	0.6	0.2	0.3	0.4
MBR588	Kasanuka	Mbarara	0.4	0.4	0.3	0.4
MBR589	Unknown280	Mbarara	0.4	0.6	0.5	0.5
MBR549	Unknown261	Mbarara	0.1	0.4	1.2	0.6
KRE715	Kwezi kumu R	Kabarole	1.3	0.2	0.3	0.6
Mean			1.9	1.9	2.7	2.2
CV (%)			38.7	29.9	18.9	29.7
LSD (0.05)			1.6	1.2	1.0	1.3

5.3.6 Correlation among traits used for evaluating Ugandan sweetpotato germplasm

A significant positive correlation was found between SPVD and Alternaria (r=0.33) as shown in Table 5.7. This is expected since plants infected with one disease get weakened and severely succumb to the other, but it is not known whether control of the two diseases is genetically linked. There was negative relationship between disease reaction and all the yield traits. High disease pressure caused reduction in photosynthetic leaf area and stunting which led to reduction in vegetative and root yield. Diseases have been shown to significantly reduce yield and specific gravity in sweetpotato cultivars. Root yield losses by SPVD of up to 98% have been reported

(Gibson *et al.*, 1998; Aritua *et al.*, 2000). However, the results in this study showed significant positive correlation between genotype specific gravity and marketable root yield (r=0.74).

Table 5.7. Pearson correlation coefficients for sweetpotato virus disease, Alternaria blight, marketable root yield, total root yield and specific gravity among 946 sweetpotato genotypes

Variable	Sweetpotato virus disease	Alternaria blight	Marketable root yield	Total root yield	Specific gravity
Sweetpotato virus disease	1.00				
Alternaria blight	0.33**	1.00			
Marketable root yield	-0.13*	-0.09 ns	1.00		
Total root yield	-0.07*	-0.08 *	0.97**	1.00	
Specific gravity	-0.01 ns	-0.17 ns	0.74**	0.37**	1.00

N = 946, Prob > |r| under H0: Rho=0
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
ns Not Significant at the 0.05 probability level.

5.3.7 Selection of superior genotypes

Out of 946 genotypes evaluated, 192 superior genotypes were selected on the basis of storage root yield, specific gravity, sweetpotato virus disease and Alternaria blight disease resistance across sites and disease hotspots. Genotypes with mean total root yield of greater than 30.0 tons/ha and specific gravity values of greater than 2.0 were selected. Those genotypes with mean SPVD and Alternaria disease scores of 2.5 and 2.0 at NaCRRI and KAZARDI, respectively where the disease pressures are high were also selected.

5.4 Conclusion and Recommendations

Disease resistance, yield and specific gravity varied significantly among the genotypes. The variations were due to genetic variability among the genotypes. The germplasm has a high

potential for use in the breeding of new varieties for the region, as the collection contains materials of varied traits. Some of the superior genotypes selected for use in breeding might be duplicates due to limitations associated with morphological germplasm characterization. To enhance their proper use, it is crucial to characterize these selected genotypes using molecular markers to distinguish them at genetic level.

CHAPTER SIX

6.0 CHARACTERIZATION OF UGANDAN SWEETPOTATO GERMPLASM USING FLUORESCENT LABELED SSR MARKERS

6.1 Introduction

Successful conservation and genetic improvement of sweetpotato (*Ipomoea batatas* L.) is to a great extent dependent on the level of knowledge by scientists regarding germplasm genetic diversity. Sweetpotato (2n=6x=90), the world's seventh most important food crop after wheat, rice, maize, potato, barley and cassava (FAO, 2007) is widely cultivated in Uganda and world over as a food security crop (Diaz *et al.*, 1996; Aritua and Gibson, 2002). Uganda is Africa's leading sweetpotato producer and second in the world after China (FAO, 2007), and a secondary center of diversity for the crop (Huamán and Zhang, 1997). The ultimate goal of sweetpotato breeding in Uganda is to generate improved varieties for a combination of traits with specific and broad adaptation to overcome constraints in the different agro ecologies (Mwanga *et al.*, 2003). The new varieties released from breeding programs are often more vulnerable to pests, pathogens and environmental stresses (He *et al.*, 2006). The constant identification of parental genotypes with high disease (SPVD and Alternaria) and pest resistances, yield and dry matter content for breeding is critical in Uganda. He *et al.*, 2006 reported wild sweetpotato relatives and landraces as important gene sources for improving yield and resistance to stresses in sweetpotato.

The National Crops Resources Research Institute (NaCRRI), Namulonge, holds 1303 accessions of sweetpotato germplasm collected from 21 districts of Uganda, of which 946 morphologically distinct accessions have been identified after morphological characterization. Morphological

characterization has been predominantly employed for the analysis of diversity in sweetpotato germpasm collections (Huamán *et al.*, 1999; Abidin *et al.*, 2001; Gichuru *et al.*, 2006; Veasey *et al.*, 2007). Limited success has been achieved with morphological diversity analysis alone because of phenotypic plasticity and the environmental impact on morphological traits (Huang and Sun, 2000), thus limiting the selection of superior parental genotypes.

Molecular markers have become an important wide spread genetic diversity analysis tool for enhancing sweetpotato breeding efficiency. Many molecular techniques have been used in sweetpotato genetic diversity studies including: RAPDs (Connolly *et al.*, 1994; Jarret and Austin, 1994; Zhang *et al.*, 1998; He *et al.*, 2006), AFLPs (Zhang *et al.*, 2000b; Elameen *et al.*, 2008), ISSRs (Huang and Sun, 2000; Hu *et al.*, 2003), DNA amplification fingerprinting (DAF) (He *et al.*, 1995), SSRs (Gichuru *et al.*, 2006) and SAMPL (Tseng *et al.*, 2002). Microsatellites developed for genotyping sweetpotato (Jarret and Bowen, 1994; Buteler *et al.*, 1999; Hu *et al.*, 2004) have been widely used for diversity analysis (McGregor *et al.*, 2001; Hwang *et al.*, 2002). With the fluorescently labeled microsatellite primers and sequencing on automated sequencers such as the ABI 3730, multiplexing of many PCR products and the accuracy in allele sizing and data collection can be achieved (Coburn, *et al.*, 2002; Banjelj *et al.*, 2004). This technology is potentially useful for a detailed understanding of sweetpotato genetic diversity. The objective of the study was to characterize diversity among selected superior sweetpotato genotypes from the Ugandan germplasm collection using fluorescent labeled SSR markers for conservation and use in hybridization shemes.

6.2 Materials and Methods

6.2.1 Plant material

A total of 192 superior sweetpotato genotypes were selected from the evaluation of the morphologically distinct accessions of the Ugandan germplasm collection being clonally maintained at NaCRRI (Appendix 2). These genotypes were characteristically high yielding, SPVD and Alternaria disease resistant, while others had high dry matter content and could be useful as parental genotypes for hybridization.

6.2.2 Plant DNA extraction

Genomic DNA was isolated from tender fresh leaf samples using the CIP Molecular Biology laboratory protocols modified from the CTAB method (Doyle and Doyle, 1990). For each genotype, 100 mg of fresh leaf material from the screen house were ground in a pre-chilled mortar in liquid nitrogen in to powder, which was transferred to 1.5 ml eppendorf tubes. Thereafter, 700 µl of fresh extraction buffer (2% CTAB buffer and 2% β-mercaptoethanol) were added to the powder, vortexed, and the homogenate was incubated for 45 min at 65°C in a water bath. The samples were cooled to room temperature for 2 minutes.

Then 700 µl of chloroform: isoamylalcohol (24:1) were added to each tube and vortexed briefly, and the tubes were inverted several times. The homogenates were centrifuged for 5 minutes at 14000 rpm in a micro centrifuge. The aqueous phases were removed and carefully transferred

into new-labeled eppendorf tubes. Thereafter, 50 µl of 10% CTAB (in 0.7 M NaCl), were added to the samples and vortexed gently. Again 700 µl of chloroform: isoamylalcohol (24:1) were added to each tube and vortexed briefly, and the tubes were inverted several times. The homogenates were centrifuged for 5 minutes at 14000 rpm. The aqueous phases were removed and carefully transferred into new-labeled eppendorf tubes. To each tube were added 400-500 µl of isopropanol. The tubes were inverted several times and allowed to sit at 4°C for 30 minutes. The tubes were spinned at 14000 rpm for 20 minutes. The supernatants in each tube were poured off and the tubes were inverted and air-dried. The DNA pellets were washed in 1.0 ml 70% ethanol for 3 minutes and centrifuged at 14000 rpm for 30 minutes. The ethanol was carefully poured off and the pellets were washed in 1.0 ml of 90% ethanol and centrifuged at 14000 rpm for 30 minutes. The ethanol was poured off and the pelletes were dried overnight. The DNA samples were dissolved in 150 µl of T10E buffer. To the DNA samples 2.0 µl of DNAse-free RNAseA were added then incubated at 37 °C for 1 hour. Purified samples were stored at 4°C.

6.2.3 Determination of DNA concentration, purity and quality

One microlitre of each DNA sample was pipetted and put on the lower measurement pedestal of the NanoDrop ND-1000 spectrophotometer. The absorbance measurements at 260 nm (A260), and 280 nm (A280), the DNA concentration and purity (A260/ A280 ratios) were computed.

For quality analysis, 3.0 µl of the DNA samples were mixed with 2.0 µl of loading dye and electrophoresed for 1 hour at 100 volts in 1.2% TBE agarose gel stained with 10 µl of 10 mg/ml ethidium bromide. The DNA samples were run on the agarose gel alongside 4.0 µl of standard

molecular weight lambda DNA of 10 ng/µl. The gel images visualized under UV light were taken using a scion camera. The sample DNA concentration was visually estimated by comparison of the band intensity with standard digested lambda DNA. The DNA samples were then diluted to a working concentration of 1.0 ng/µl using double distilled water.

6.2.4 SSR primers and PCR conditions

A total of 10 polymorphic fluorescent-labeled SSR primers were used in this study. The forward primers were labeled at the 5` end with fluorescent dyes, PET (Red), 6FAM (Blue), VIC (Green) and NED (Yellow) (Table 6.1). The PCR was performed in a total reaction volume of 10 µl using the Accupower PCR premix tube containing lyophilized 1 U top Taq DNA polymerase, 250 µM dNTPs, 10 mM Tris-HCl (pH 9.0), 30 mM KCl, 1.5 mM $MgCl_2$, a tracking and stabilizing dye (Bioneer). To the premix was added 2.5 µl of 1.0 pmol of primers, 5.0 µl of 1.0 ng DNA template and 2.5 µl of double distilled water. The PCR program consisted of an initial denaturation at 94°C for 4 minutes, followed by 35 cycles each of denaturation at 94°C for 1 minute, 1 minute annealing at 58°C, 72°C for 1 minute, 20 minutes extension at 72°C and infinite 4°C hold. All the PCR programs were performed using a GeneAmp PCR system 9700 thermocycler (PerkinElmer, Wellesey, Mass).

Table 6.1. SSR primer sequences, dyes, coloadsets and PCR product dilutions

Primer	Forward	Reverse	Dye	Coload Set	Product Dilution
IB-R16	GACTTCCTTGGTGTAGTTGC	AGGGTTAAGCGGGAGACT	VIC	1	1:100
IB-R19	GGCTAGTGGAGAAGGTCAA	AGAAGTAGAACTCCGTCACC	PET	1	1:60
IBCIP-13	CGTGCTTGAGGTCTGAGTAGAA	TTCCCTAGAAGCTGCGTGAT	NED	1	1:60
IB-R03	GTAGAGTTGAAGAGCGAGCA	CCATAGACCCATTGATGAAG	PET	2	1:75
IBCIP-9	AGACTGCTAGGGTTATCTCTCCA	GACATTGCCAAGGACACTGA	6-FAM	2	1:50
IB-S09	GCTGCTCAATCCCTCTCCTT	GGAACTCGATACAGCGTGGT	NED	2	1:50
IB-R08	GGCGACACCTTAGAGTAT	CACCCCCTATTCACAA	PET	2	1:50
IB-R12	GATCGAGGAGAAGCTCCACA	GCCGGCAAATTAAGTCCATC	NED	3	1:100
IB-S07	GCTTGCTTGTGGTTCGAT	CAAGTGAAGTGATGGCGTTT	6-FAM	3	1:100
IBCIP-7	GGTTTGACCGTGGAGTTGTT	GGACGAACTTTCCCAAATCA	PET	3	1:50

6.2.5 Pooling of PCR products and SSR Fragment analysis

The amplified PCR products were diluted with double distilled water basing on the relative intensities of amplification resolved on a 2% TBE agarose gel and the relative fluorescence unit (rfu) on the ABI 3730 sequencer (Table 6.1) prior to the final capillary electrophoresis on the ABI 3730 genetic analyzer (Applied Biosystems). The capillary electrophoresis runs were post-PCR co-loaded in 3 sets on the basis of dye color and fragment size (Table 6.1). A volume of 5.0 µl of each diluted PCR products were mixed for co-loading. A standard cocktail mix for fragment analysis was prepared by thoroughly mixing 12.0 µl of 500 LIZ size standard and 1000 µl Hi-Di Formamide. From each pooled co-load PCR products, 1.0 µl was drawn and mixed with 9.0 µl of the cocktail mix in a 96 well PCR plate, gently vortexed and spinned down at 3000 rpm for 2 minutes. The mix was denatured at 95°C for 3 minutes and quickly chilled on ice for 5 minutes and then loaded into the ABI 3730 sequencer for fragment analysis.

6.2.6 Allele calling

The fragments were analyzed using the Genemapper v3.7 software (Applied Biosystems), which performed peak detection and fragment size matching from the reference data. Allele calls were automatically made when a peak from a data sample matched the location of a bin. Completed results were run in AlleloBin software (Prasanth *et al.*, 1997) to correct any errors in the scored alleles due to slippage of DNA polymerase during PCR resulting into stutter peaks (Schlotterer and Tautz, 1992). The allele calls were then converted to binary data using ALS-Binary software (Prasanth and Chandra, 1997) for the subsequent analyses.

6.2.7 Statistical data analysis

The polymorphic information content (PIC), the measure of the usefulness of each marker in distinguishing one individual from another was determined (Weir, 1996) as,

$PIC=1- \Sigma Pi^2$

Where Pi is the frequency of the i^{th} allele.

To investigate the diversity among the accessions, the number of alleles per locus was computed, and genetic distances (GD) among all pairs of individuals were calculated using the Nei and Li coefficient (Nei and Li, 1979). The distance matrix was subjected to cluster analysis using the neighbor-joining algorithm of the Numerical Taxonomic and Multivariate Analysis Systems (NTSYS) version 2.2 software (Rohlf, 1993) to generate a dendrogram (Sneath and Sokal, 1973). Principle component analysis (PCA) was used to graphically display genetic relationships (Gower, 1966).

6.3 Results and Discussions

6.3.1 DNA purity and concentration

The distribution of purity (A260/A280) ratios ranged from 0.6 to 2.5 (Fig 6.1). Most accessions had A260/A280 ratios of 1.6 to 2.0 indicating that most of the DNA samples were free from contaminants such as polyphenolics, polysaccharides and proteins. The NanoDrop DNA concentrations of samples revealed high concentrations of over 80 ng/µl. The concentration revealed by gel electrophoresis ranged from 10-100 ng/µl.

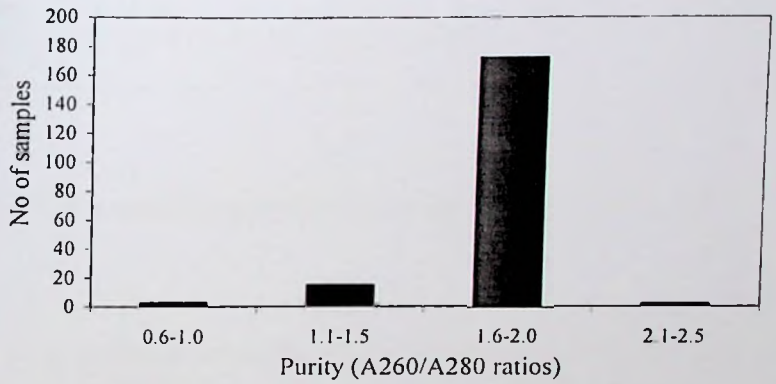

Fig 6.1. Distribution of DNA purity of the sweetpotato samples

6.3.2 Polymorphic information content (PIC)

The PIC value reflecting gene diversity of the 10 microsatellite loci ranged from 0.23 for locus IB-S07 to 0.76 for locus IB-R12 with an average of 0.62 (Table 6.2). Except for primers IB-S07 and IB-R08, the other primers were highly polymorphic with PIC values higher than 0.50. IB-

R12 was the most polymorphic primer. High average PIC value observed with our study can be attributed to the use of more informative markers.

Table 6.2. Primer motifs, dyes, annealing temperature (Tm), size range, alleles per locus, total number of alleles and polymorphic information content (PIC) of 10 primers

Primer	Motif	Dye	Tm (°C)	Size range	Alleles per locus	Total Alleles	PIC
IB-R16	(GATA)4	VIC	60	201-213	3	428	0.65
IB-R19	(CAG)5b	PET	60	190-208	4	392	0.68
IBCIP-13	(ACC)3+(CGG)2+(TGC)3+(GTC)2	NED	60	196-373	6	477	0.68
IB-R03	(GCG)5	PET	60	243-258	4	463	0.71
IBCIP-9	(CCA)2AC(ACC)6	6-FAM	50	42-63	4	461	0.68
IB-S09	(AT)11	NED	50	193-203	4	318	0.72
IB-R08	(T3A)4	PET	50	204-216	2	230	0.36
IB-R12	(CAG)5A	NED	60	303-342	5	496	0.76
IB-S07	(TGTC)7	6-FAM	60	162-178	4	193	0.23
IBCIP-7	(CCA)2+3+(CCG)2+1+3(CCA)3+(CG)4	PET	50	39-99	6	492	0.74
Mean					4	395	0.62

6.3.3 PCR amplifications and number of alleles detected

The PCR products revealed very high amplifications for most of the reactions with the 10 SSR primers used. The number of alleles detected per locus ranged from 2 for locus IB-R08 to 6 for IBCIP-13 with an average of 4 and the allele sizes ranged from 39 to 373 base pairs (Table 6.2). Sweetpotato has a hexaploid genome and the number of alleles per locus is expected to range from 1 to 6. However, the total number of alleles per locus detected in the 192 genotypes varied from 193 in locus IB-S07 to 496 in IB-R12 an indication of a high level of diversity. The number of alleles detected per genotype by the SSR primers ranged from 1 to 6 as shown by the electropherogram (Fig 6.2). There was no relationship between the number of repeats and polymorphism.

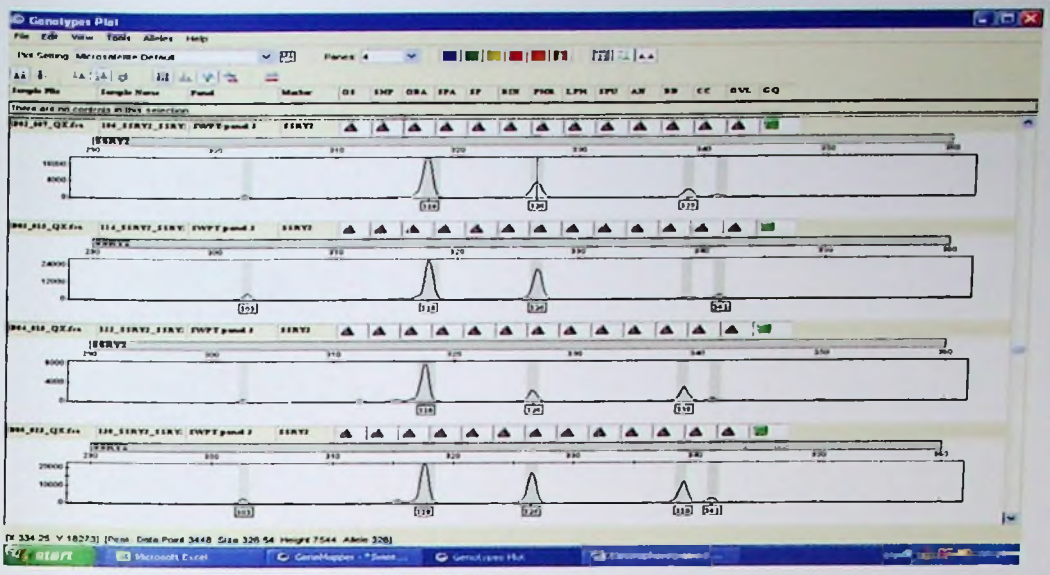

Fig 6.2. Genotype plot showing alleles detected in locus IB-R12

The 192 genotypes were clearly distinguished by the 10 SSR primers. The SSR markers have an extraordinary discriminatory power for genotyping sweetpotato germplasm. Only 6 SSR primer pairs generated scorable allelic information for typing 113 Latin American sweetpotato varieties (Zhang *et al.*, 2000a). Four SSR primer pairs also discriminated among 57 East African sweetpotato genotypes (Gichuru *et al.*, 2006). The level of polymorphism detected by SSRs in this study is higher than that detected by other SSRs (Zhang *et al.*, 2000a; Gichuru *et al.*, 2006), RAPDs (Connolly *et al.*, 1994; Jarret and Austin, 1994; Zhang *et al.*, 2001), SAMPL (Tseng *et al.*, 2002), and AFLP (Zhang *et al.*, 2004; Elameen *et al.*, 2008). This confirms the superiority of fluorescent-labeled microsatellite detection on ABI 3730 (Banjelj *et al.*, 2004).

6.3.4 Genetic distances among sweetpotato genotypes

The frequency of pair-wise genetic distance coefficients for the SSR analysis is shown in Fig 6.3. The SSR based genetic distance coefficients ranged from 0.0 to 1.0 with a mean of 0.57. The

high mean genetic distance and wide range of genetic distances among landraces indicated a high genetic diversity. Most distance coefficients were in the range of 0.5-0.6 accounting for 60.7% of the pair-wise distance coefficients in this study. Genotypes MLE191 (Tuulansime) and KML942 collected from the neighboring districts of Mbale and Kamuli respectively were identified as duplicates. MLE191 a popular variety from Mbale could have been brought to Kamuli for cultivation by a farmer who could not name it or vice versa (McGregor *et al.*, 2001). Likewise, genotypes ARA237 from Arua district and KSR663 (Kijambire) from Kisoro district also had a distance coefficient of 0.0 (duplicates). These districts are both bordered by the Democratic Republic of Congo from where the farmers could have brought this variety. The highest pairwise genetic distance was observed between genotypes BSH748 and MLE163 (Kyebandula) from Bushenyi and Mbale districts, respectively.

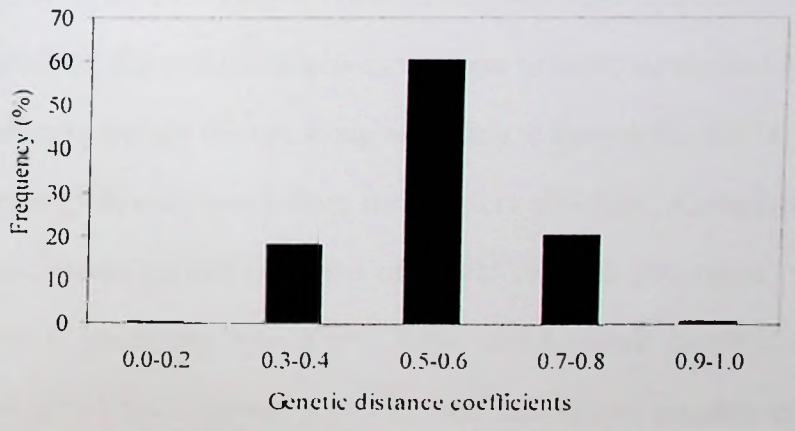

Fig 6.3. Frequency distributioon of of pair-wise genetic distance estimates among 192 sweetpotato genotypes

A high mean genetic distance of 0.58 was also found among the sweetpotato genotypes from China, the world's leading producer (He *et al.*, 2006). However, Gichuru *et al.*, 2006 observed a low diversity among some East African sweetpotato genotypes. Similar low diversities have

been observed among the sweetpotato genotypes from Tanzania (Elameen *et al.*, 2008), Papau New Guinea (Zhang *et al.*, 1998), and the U.S.A (He *et al.*, 1995). The high microsatellite based sweetpotato diversity in Uganda can be attributed to the self-incompatibility and vegetative propagation of the crop and directed selection in the crop for various uses (Tseng *et al.*, 2002), coupled with new introductions and mutations. High levels of polymorphism among sweetpotato plants is fixed through vegetative reproduction and maintained by high levels of gene flow due to self-incompatibility in the crop (He *et al.*, 1995). It also agrees with Uganda being a secondary center of diversity for sweetpotato (Huamán and Zhang, 1997).

6.3.5 Cluster analysis

The generated dendrogram grouped the genotypes into four major clusters (Fig 6.4). Apart from genotypes 1 (MLE191) and 90 (KML948), which had a similar coefficient and thus detected to be duplicates, the remaining genotypes were grouped as distinct cultivars. Within each cluster, the genotypes mainly did not group according to geographic origin. For instance, cluster 1 has 16 genotypes (1-86), collected from the districts of Mbale, Kamuli, Kumi, Rakai, Mpigi, Pallisa, Lira and Kisoro mainly consisted of SPVD resistant genotypes. Similarly, cluster 2 (15-189) consisted of genotypes from Kumi, Rakai and Luweero districts, mainly had Alternaria blight resistant genotypes. Cluster 3 (4-179) had the largest number of genotypes and many sub-clusters, and cluster 4 (19-146), likewise did not show geographic pattern but had high yielding genotypes mainly.

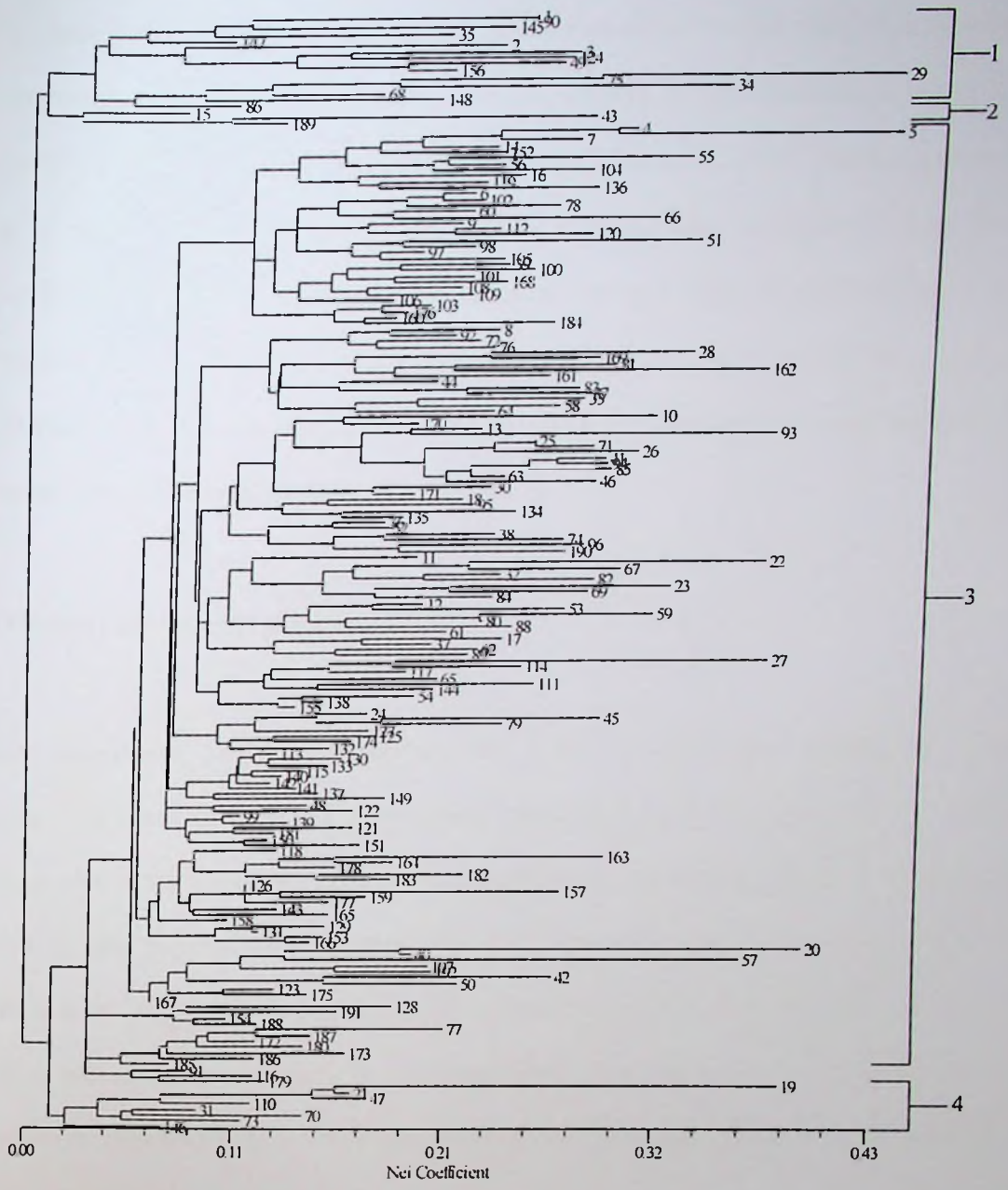

Fig 6.4. Dendrogram showing genetic relationships among 192 Ugandan sweetpotato genotypes

Clustering among some East African cultivars indicated a lack of geographical association as well (Gichuru *et al.*, 2006). However, clear regional patterns of clustering were observed among the sweetpotato genotypes from Latin America (Zhang *et al.*, 2000a) and tropical America (Zhang *et al.*, 2004). The lack of geographical associations among the Ugandan genotypes can be a result of regular gene flow as farmers routinely shared planting material over the years of sweetpotato cultivation in Uganda. Self-incompatibility and high out crossing favors gene flow in sweetpotato (He *et al.*, 1995). Many farmers could not accurately reveal the initial district of origin of these collected varieties; as such the only available information is on the location where the samples were collected (McGregor *et al.*, 2001).

6.3.6 Principal component analysis (PCA)

Principal component analysis displayed the genetic relationships among the individual accessions. The first two principal components accounted for 23.1% and 17.6% of the variance in the PCA plot of the genotypes respectively. Just like in cluster analysis, PCA did not group the genotypes by the regions of collection (Fig 6.5). However, genotypes 1 (MLE191) and 90 (KML948) were separately grouped closely together from the rest of the genotypes. In the genotyping and assessment of genetic relationships among elite polycross breeding cultivars of sweetpotato in Taiwan using SAMPL polymorphisms, PCA data supported UPGMA clustering (Tseng *et al.*, 2002).

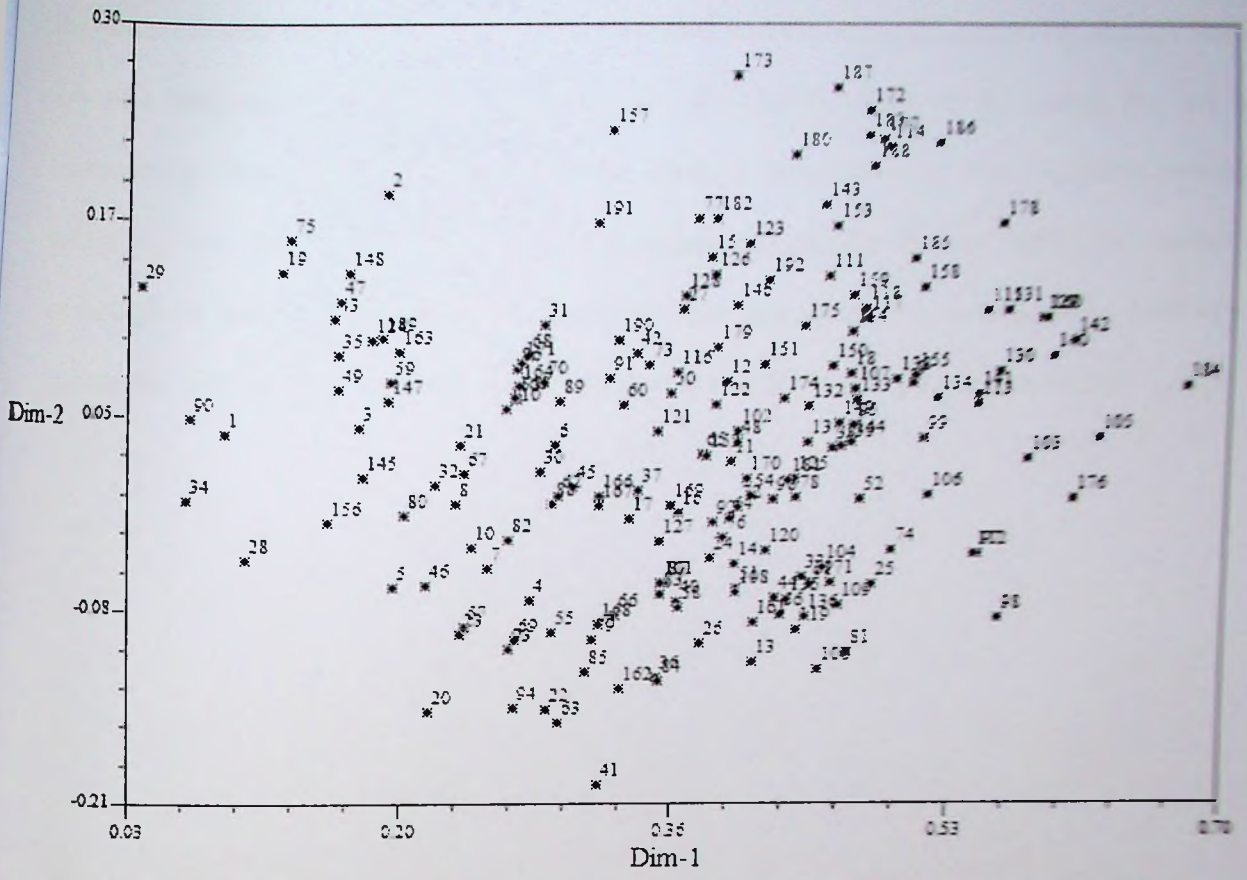

Fig 6.5. Two-dimension principle component analysis plot of 192 sweetpotato genotypes

6.4 Conclusion and Recommendations

The 10 fluorescent-labeled microsatellites detected on ABI 3730 have efficiently distinguished the 192 Ugandan sweetpotato genotypes. These primers have detected more polymorphisms and resulted in better resolution of the genetic relationships among these genotypes. The high level of genetic diversity is an indication of broad genetic base for sweetpotato in Uganda. The identification of 190 diverse parental genotypes of varying characteristics for use in hybridization schemes in the region will lead to the improvement of sweetpotato productivity.

The East African sweetpotato genotypes have several unique important characteristics like high dry matter content, high resistance to virus diseases and vigorous foliage cover, though they have low root beta-carotene contents (Gichuki *et al.*, 2003). Sources of genes for the above characteristics have been identified in the distinct genotypes of the Ugandan sweetpotato collection. Two duplicates identified from this morphologically distinct collection, confirms the advantage of molecular characterization over morphological characterization. This efficient genotyping will enhance low cost conservation and use of these superior genotypes.

CHAPTER SEVEN

7.0 GENERAL CONCLUSION AND RECOMMENDATIONS

In this study, an understanding of the Ugandan sweetpotato genetic potential was sought. A total of 1303 landrace accessions were collected from 21 Ugandan districts along with the farmer indigenous knowledge on these genotypes. From this collection, 946 accessions were morphologically distinct with a high mean morphological diversity (H'= 0.71±0.03). On multi location evaluation of the morphologically distinct genotypes, most genotypes were observed to be low yielding (64.4%), with low resistance to SPVD (87.8%) and Alternaria blight disease (80.8%). However, 192 superior genotypes were selected for high yield, high specific gravity, high resistance to SPVD and Alternaria blight disease. The superior genotypes were genetically diverse with average pair wise genotype genetic distance of 0.5. Only two duplicate genotypes were identified from the superior genotypes on molecular characterization using 10 fluorescent labeled SSR markers.

This research has led to a detailed understanding of the sweetpotato landrace genetic potential in Uganda. The study showed that Ugandan farmers maintain large numbers of sweetpotato landraces for food and feed grown in a wide range of altitudes. A lot of sweetpotato genetic erosion has been observed in the Ugandan sweetpotato genetic resource as revealed by many abandoned varieties. It is recommended that regular follow up germplasm collection expeditions be made to the farmers' fields to mitigate this rampant genetic erosion.

With time, it is recommended that those areas where sweetpotato is not an important crop should also be surveyed for collection of some of the landraces being grown by farmers. This will enhance further broadening of the sweetpotato genetic base for Uganda.

The study demonstrated that there was high morphological diversity in the Ugandan sweetpotato collection. Twenty morphological traits accurately distinguished variations among the accessions. In future, breeders should emphasize the use of leaf traits with less emphasis on the flowering traits in morphological characterization of sweetpotato germplasm collections, as these traits were highly correlated and accounted for most of the variability in this collection.

Molecular characterization should in future always follow morphological characterization as the sweetpotato genotypes are highly heterogenous and accessions differing by only one morphological trait are rampant in sweetpotato collections and are quite difficult to distinguish morphologically. A large number of molecular marker primers are highly recommended for more accurate genotyping of sweetpotato owing to the hexaploid genome ($2n = 6x = 90$) with 15 linkage groups. In this study, 10 SSR primers were used, however, in future at least 15 SSR primers are recommended. The use of more robust molecular markers like AFLP is also recommended for future genotyping.

The study demonstrated that the Ugandan sweetpotato germplasm has many genotypes that can be used as parental genotypes for developing high yielding, dry matter, sweetpotato virus disease and Alternaria blight disease resistance. These genotypes need to be further evaluated in

multilocation trials to screen their agronomic potential and adaptability for the selection of advanced cultivars to be released in future for the farming community in Uganda.

These superior genotypes should be entered into the crossing block for breeding superior varieties for the Uganda and the region.

Graft inoculation needs to be done to confirm SPVD resistance in the superior genotypes, as field resistance cannot be conclusive for the accurate selection of SPVD resistant cultivars.

The screening of orange-fleshed genotypes for quantification of the beta-carotene content is highly recommended. This will enhance their use in hybridization schemes for devdeloping high beta-carotene content sweetpotato varieties for alleviation of vitamin A deficiency in the region.

The conservation of the superior landraces should be done at NaCRRI and duplicated at Muguga and CIP, Lima for long-term storage both in the screen house and in vitro. However, the conservation of the entire morphologically distinct accessions should be done both in the field and screen house to minimize loss of accessions.

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APPENDIX

Appendix 1. Germplasm Collection Questionnaire

Passport Information

1. Accession number.....
2. Code name.....
3. Vernacular name.....
4. Origin of variety.....
5. Duration to maturity.....
6. Skin color.....
7. Root flesh color.....
8. Taste of storage roots.....
9. Date of collection.....
10. District.....
11. Sub County.....
12. Altitude of location.....
13. Latitude/Longitude of location.....
14. Collection source
    1) Wild habitat ☐
    2) Farm land
    3) Farm store
    4) Backyard
    5) Village market
    6) Commercial market
    7) Institute
    8) Others (Specify).....
15. Type of sample ☐
    1) Storage roots
    2) Stem cuttings
    3) *In vitro* culture
    4) Vegetative and seed
16. Status of sample ☐
    1) Wild
    2) Weedy
    3) Landrace
    4) Advanced cultivar/released variety
    5) Breeder's line
    6) Others (Specify)
17. Meaning of the name of variety.....
18. What is your number one sweetpotato variety in the district?.....
19. Which varieties have been abandoned?.....
20. What is the ranking of sweetpotato in the food system?.....

Appendix 2. List of 192 superior sweetpotato landrace cultivars selected and genotyped

No	Code	District	No	Code	District	No	Code	District	No	Code	District
1	MLE191	Mbale	49	RAK791	Rakai	97	MSK1020	Masaka	145	LUW1254	Luweero
2	PAL102	Pallisa	50	RAK829	Rakai	98	MSK1040	Masaka	146	LUW1243	Luweero
3	LIR279	Lira	51	RAK831	Rakai	99	MPG1118	Mpigi	147	MPG1146	Mpigi
4	MLE183	Mbale	52	RAK835	Rakai	100	MKN1173	Mukono	148	IGA1003	Iganga
5	PAL133	Pallisa	53	PAL1303	Pallisa	101	MKN1200	Mukono	149	MSK1094	Masaka
6	PAL148	Pallisa	54	KBL648	Kabale	102	MKN1180	Mukono	150	KML943	Kamuli
7	PAL94	Pallisa	55	KML932	Kamuli	103	MPG1151	Mpigi	151	HMA484	Hoima
8	PAL134	Pallisa	56	KML940	Kamuli	104	MKN1186	Mukono	152	KML960	Kamuli
9	SRT45	Soroti	57	KML924	Kamuli	105	LUW1238	Luweero	153	BSH761	Bushenyi
10	SRT1	Soroti	58	RAK846	Rakai	106	MKN1210	Mukono	154	BSH764	Bushenyi
11	BSH747	Bushenyi	59	KML915	Kamuli	107	MKN1212	Mukono	155	KBL616	Kabale
12	MLE171	Mbale	60	APA354	Apac	108	MKN1224	Mukono	156	IGA971	Iganga
13	PAL115	Pallisa	61	MBR535	Mbarara	109	LUW1250	Luweero	157	MPG1103	Mpigi
14	KMI83	Kumi	62	HMA490	Hoima	110	LUW1280	Luweero	158	KML916	Kamuli
15	KMI76	Kumi	63	HMA496	Hoima	111	LUW1286	Luweero	159	IGA982	Iganga
16	BSH740	Bushenyi	64	MBR530	Mbarara	112	LUW1285	Luweero	160	IGA983	Iganga
17	BSH748	Bushenyi	65	KSR659	Kisoro	113	LUW1302	Luweero	161	MPG1148	Mpigi
18	LIR276	Lira	66	BSH751	Bushenyi	114	KBL650	Kabale	162	MSK1026	Masaka
19	SRT24	Soroti	67	LUW1300	Luweero	115	KSR 663	Kisoro	163	MSK1045	Masaka
20	SRT47	Soroti	68	LUW1257	Luweero	116	KRE713	Kabarole	164	MPG1158	Mpigi
21	SRT32	Soroti	69	LUW1230	Luweero	117	KRE724	Kabarole	165	KML945	Kamuli
22	ARA222	Arua	70	MKN1188	Mukono	118	KRE734	Kabarole	166	KBL617	Kabale
23	MLE170	Mbale	71	MPG1150	Mpigi	119	BSH777	Bushenyi	167	SRT27	Soroti
24	KRE725	Kabarole	72	MPG1122	Mpigi	120	RAK815	Rakai	168	SRT41	Soroti
25	KRE693	Kabarole	73	IGA985	Iganga	121	RAK805	Rakai	169	MLE172	Mbale
26	RAK801	Rakai	74	MSK1072	Masaka	122	KML877	Kamuli	170	PAL153	Pallisa
27	MSD382	Masindi	75	KML944	Kamuli	123	KML875	Kamuli	171	KMI80	Kumi
28	ARA224	Arua	76	IGA972	Iganga	124	KSR661	Kisoro	172	KMI69	Kumi
29	MLE163	Mbale	77	IGA995	Iganga	125	ARA232	Arua	173	APA379	Apac
30	PAL120	Pallisa	78	MPG1128	Mpigi	126	ARA237	Arua	174	PAL158	Pallisa
31	SRT12	Soroti	79	MKN1168	Mukono	127	ARA 236	Arua	175	MBR609	Mbarara
32	MSD380	Masindi	80	MKN1195	Mukono	128	LIR265	Lira	176	APA352	Apac
33	ARA212	Arua	81	LUW1237	Luweero	129	LIR303	Lira	177	APA345	Apac
34	KML900	Kamuli	82	LUW1274	Luweero	130	MSD432	Masindi	178	APA373	Apac
35	RAK808	Rakai	83	MSK1079	Masaka	131	APA 349	Apac	179	MSD429	Masindi
36	KML882	Kamuli	84	MKN1169	Mukono	132	MBR509	Mbarara	180	KMI68	Kumi
37	RAK841	Rakai	85	IGA975	Iganga	133	MBR605	Mbarara	181	MBR546	Mbarara
38	RAK836	Rakai	86	KML956	Kamuli	134	MBR577	Mbarara	182	LIR295	Lira
39	RAK817	Rakai	87	KML941	Kamuli	135	MBR531	Mbarara	183	BSH754	Bushenyi
40	KML881	Kamuli	88	IGA978	Iganga	136	HMA512	Hoima	184	RAK796	Rakai
41	RAK819	Rakai	89	KML961	Kamuli	137	HMA495	Hoima	185	RAK811	Rakai
42	RAK821	Rakai	90	KML942	Kamuli	138	PAL159	Pallisa	186	MKN1219	Mukono
43	RAK786	Rakai	91	IGA963	Iganga	139	MLE201	Mbale	187	KML950	Kamuli
44	RAK787	Rakai	92	IGA998	Iganga	140	PAL125	Pallisa	188	SRT333	Soroti
45	KBL631	Kabale	93	MPG1112	Mpigi	141	PAL98	Pallisa	189	LUW1239	Luweero
46	KML876	Kamuli	94	HMA494	Hoima	142	SRT26	Soroti	190	MKN1215	Mukono
47	KML872	Kamuli	95	MPG1149	Mpigi	143	KMI86	Kumi	191	LUW1290	Luweero
48	KSR676	Kisoro	96	MPG1117	Mpigi	144	SRT35	Soroti	192	LUW1242	Luweero