CHARACTERIZATION AND SELECTION OF KENYAN SWEET POTATO (*Ipomoea batatas* L.) GENOTYPES FOR SWEET POTATO VIRUS DISEASE RESISTANCE AND HIGH DRY MATTER

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DECLARATION

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

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To my parents who have directed every step of my life

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
bp	Base pair(s)
BCIP	5-bromo-4-chloro-3-indolyl phosphate
CIAT	International Center for Tropical Agriculture
CIP	International Potato Center
CTAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTP	2', 3'-deoxyribonucleoside 5'-triphosphate
EDTA	Ethylendiamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food Agriculture Organisation
GAR	Goat anti rabbit
IgG	Immunoglobulin G
IITA	International Institute for Tropical Agriculture
NBT	Nitroblue tetrazolium
NCM	Nitrocellulose membrane
NTSYS	Numerical taxonomy multivariate analysis system
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidone
RAM	Rabbit anti-mouse

RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNAse	Ribonuclease
Rpm	Revolutions per minute
SSR	Simple sequence repeats
Taq	Thermophilus aquaticus
TBE	Tris borate EDTA
Tris	Tris (hydroxymethyl) aminomethane
SPCaLV	Sweet potato caulimo-like virus
SPCFV	Sweet potato chlorotic fleck virus
SPCSV	Sweet potato chlorotic stunt virus
SPFMV	Sweet potato feathery mottle virus
SPMMV	Sweet potato mild mottle virus
SPVD	Sweet potato virus disease
SwPLV	Sweet potato latent virus
T-TBS	Tris buffered saline supplemented with tween 20
UPGMA	Unweighted pair group method of arithmetic averages
UV	Ultraviolet

ABSTRACT

Sweet potato virus disease (SPVD) is a major constraint to sweet potato production in Kenya. In addition to SPVD, low production of sweet potato in Kenya is also due to lack of cultivars with consumer quality attributes such as high dry matter content. Use of resistant cultivars is the most effective means of controlling the disease. This study aimed at characterizing Kenyan sweet potato genotypes for SPVD resistance and high dry matter content using morphological and simple sequence repeat (SSR) markers. A total of 314 genotypes were collected, established in a screenhouse and evaluated for their reaction to SPVD using symptom severity. Severity of SPVD in each genotype was determined using a scale of 1-5; where 1= no symptoms and 5=very severe symptoms. Serological assays were done on 89 genotypes with a symptom severity score of between 1.00 and 1.50. Analysis of variance of the symptom severity scores showed that the genotypes responded differently ($P < 0.001$) to SPVD in the screenhouse. Twenty genotypes tested negative for both SPFMV and SPCSV and were considered resistant/tolerant to SPVD.

Three hundred and fourteen genotypes were planted in the field and characterized using 42 morphological traits based on the CIP Research Guide 36 followed by cluster analyses of the scored traits using unweighted pair group method with arithmetic means (UPGMA). Tuber dry matter content was determined 5 months after planting in the field. Phylogenetic analysis using morphological descriptors grouped the genotypes into two

major clusters. None of the clusters clearly distinguished the 20 resistant genotypes from the 294 susceptible ones. The tuber dry matter content significantly ($P<0.001$) varied among the sweet potato genotypes. Genotypes with highest and lowest tuber dry matter content were not distinguished from each other using UPGMA phenogram generated. This indicates that morphological markers are not reliable in identifying and classifying sweet potato genotypes into phenotypic groups based on their resistance to SPVD and high dry matter. Therefore, morphological markers supplemented with molecular markers need to be investigated for their potential application in identification of sweet potato genotypes with SPVD resistance and high dry matter content.

Eighty nine sweet potato genotypes were selected following graft inoculation with SPVD-infected scions and characterized using 6 SSR primer pairs. The amplified DNA fragments were screened by capillary electrophoresis on the ABI 3730 genetic analyzer and analysed using the Genemapper v3.7 software. Cluster and principal component analysis (PCA) were done using NTSYS-pc version 2.11T. Six primer pairs were highly polymorphic among the genotypes and polymorphic information content (PIC) varied from 0.33 to 0.81 with an average of 0.47. The number of alleles within the 89 genotypes across the 6 loci ranged from 10 to 17, with an average of 13.52. Cluster analyses showed Jaccard's coefficient from 0.5 to 1, with an average of 0.75 accounting for 50% variation among the 89 genotypes. The phylogenetic and PCA analysis clustered 89 genotypes into 2 main clusters and 5 subclusters. The dendrogram did not reveal any unique clustering of the sweet potato genotypes according to dry matter content or reaction to SPVD. The genetic differences among the SPVD resistant and high dry matter content genotypes

revealed by the clustering into distinct groups suggest the presence of different sources of resistance to SPVD and high dry matter. This study therefore indicates that there is a high level of genetic diversity in sweet potato genotypes that are SPVD resistant and have high dry matter. These genotypes can be used as parents in breeding programmes aimed at improving the crop for the two traits.

CHAPTER ONE

1.0 INTRODUCTION

1.1 General introduction

Sweet potato (*Ipomoea batatas* L.) is an important starchy tuberous root crop grown in many tropical and subtropical regions of the world. In sub-Saharan Africa, sweet potato plays a major role in providing food for the population and is the second most important root crop after cassava (Hijmans *et al*., 2001). About 75% of African sweet potato production is in East Africa, especially around Lake Victoria, where it is a basic subsistence crop mainly grown by women (Gibson and Aritua, 2002; Karyeija *et al.,*1998). Sweet potato performs well in infertile soils, is relatively drought-insensitive and has a short growing period. Among the major staple crops, it has the largest production per unit area per unit time (Woolfe, 1992), making it attractive to farmers with limited resources. However, its productivity is limited by biotic constraints including viruses which cause substantial losses worldwide (CIP, 2000).

Viruses are the second most important biotic constraint after insects (weevils), which limit sweet potato production both in Africa and worldwide (Geddes, 1990). Greater than 50% of production losses are attributed to virus infections (Ngeve and Bouwkamp, 1991; Gutiérrez *et al.,* 2003). More than 20 viruses have been reported to infect sweet potato worldwide (Brunt *et al.*, 1996). Viruses reported to infect sweet potato in Africa include Sweet potato feathery mottle virus (SPFMV), Sweet potato chlorotic stunt virus (SPCSV), Sweet potato mild mottle virus (SPMMV), Sweet potato virus G (SPVG), Cucumber mosaic virus (CMV), Sweet potato chlorotic fleck virus (SPCFV), Sweet

potato virus 2 (SPV-2), Sweet potato latent virus (SwPLV) and Sweet potato caulimo-like virus (SPCaLV) (Hollings *et al*., 1976; Mukiibi, 1977; Hahn, 1979; Geddes, 1990; Wambugu, 1991; Gibson *et al,* 1998). The most devastating virus-induced syndrome of sweet potato is sweet potato virus disease (SPVD). It is the most economically important disease because the diseased plants produce almost no usable yield (Gibson *et al*., 1998; Karyeija *et al*., 1998).

Apart from viral diseases, low production of sweet potato in some parts of Kenya is due to lack of high yielding genotypes with consumer acceptable attributes, such as high dry matter content. Kenyan consumers prefer sweet potato genotypes with high dry matter as indicated in a survey carried out in different Kenyan villages (Ndolo *et al*., 2006). Therefore, there is a need to identify sweet potato cultivars with high dry matter content or genotypes that are SPVD resistant and have high dry matter.

Accurate assessment of levels and patterns of genetic diversity can be invaluable in crop breeding for various purposes, including analysis of genetic variability of cultivars (Cox *et al*., 1986), identification of diverse parental combinations to develop segregating progenies with maximum genetic variability for further selection (Barrette and Kidwell, 1998) and introgression of desirable genes or chromosome segments from diverse sources into elite genotypes (Thompson *et al*., 1998). Understanding genetic relationships among genotypes can be particularly useful in planning crosses, in defining heterotic pools, assigning lines to specific heterotic groups (Hallauer and Miranda, 1998), as well as for distinctiveness, uniformity and stability testing (Heckenberger *et al*., 2002, 2003).

Furthermore, the knowledge of genetic diversity in the available genotypes is of indispensable importance for plant genetic resources management in genebanks. Analysis of genetic diversity in genotypes collections facilitates classification of accessions, detection of duplicates and identification of useful accessions for specific breeding purposes (Mohammadi and Prasanna, 2003). Numerous studies have so far been conducted to analyze genetic diversity in sweet potato genotypes (Zhang *et al*., 1996; Gichuki *et al*., 2003; Gichuru *et al*., 2004; Njuguna, 2005), but none of the studies focused on identification of genotypes resistant to SPVD and contain high dry matter. Diversity analysis of traits such as SPVD resistance and high dry matter is important in efforts aimed at increasing sweet potato yield.

Comprehensive genetic diversity studies have been conducted in major crops including sweet potato using passport data, morphological (Ben-Har *et al*., 1995), and biochemical data obtained by analyses of isozymes or storage proteins. Nevertheless, their usefulness in obtaining reliable estimates of genetic similarity is limited because of the small number of marker loci available and the low degree of polymorphism generally found in elite breeding materials (Messmer *et al*., 1991). The major strength of molecular markers is their ability to detect genetic diversity at levels of resolution that exceed by far those achievable with other previously applied methods such as use of biochemical markers (Karp, 1997). Regarding their nature, DNA-assays are most robust and independent of developmental and environmental conditions (Bernatzky and Tanksley, 1989). Nevertheless, the extent of their utility may depend on the nature of the marker system, their number, genome coverage, and the population under investigation (Karp, 1997).

Restriction fragment length polymorphism (RFLP), Random amplified length polymorphism (RAPD), Amplified fragment length polymorphism (AFLP) and Simple sequence repeats (SSRs) are the most common marker systems used to assess genetic diversity present in plant populations. SSRs are considered the most efficient markers for genetic diversity studies in many plants (Rakoczy-Trojanowska and Bolibok, 2004) including sweet potato (Zhang *et al*., 2000). This is because of their high levels of allelic variation and their co-dominant character which means that they deliver more information per unit assay than any other marker system (Rakoczy-Trojanowska and Bolibok, 2004).

1.2 Problem statement and justification

Sweet potato is one of the most important staple food crops consumed throughout East Africa and plays a significant role not only as a food security crop but also as a potential commercial and subsistence crop (Karyeija *et al*., 1998). The crop is extremely important to developing countries, which produce 95% of the global production. However, virus diseases are one of the major factors that greatly reduce yields particularly SPVD which is an African-wide menace to sweet potato production. SPVD drastically reduces both quality and quantity of tubers and vines (Gibson *et al*., 1998). Although SPVD can be controlled by healthy stock programmes, phytosanitation and cultural measures, these are difficult to integrate within subsistence production systems common with resource-poor farmers (Gibson *et al*., 2004).

Use of resistant genotypes is therefore the most effective means of reducing sweet potato losses due to virus infection. It significantly decreases production costs by cutting down the use of pesticides which are also potential pollutants to the environment.

Development of resistant genotypes takes time and is made difficult by the genetic nature of sweet potato. Early identification of resistant sweet potato would save time in selecting materials for use in breeding programmes. Therefore, the use of morphological and molecular marker technology in identifying Kenyan sweet potato cultivars resistant to SPVD will greatly help in early identification of resistant genotypes and also accelerate the time of development of resistant cultivars. In addition, molecular characterization followed by cluster analysis for the national germplasm will lead to selection of representative landraces for conservation. This will promote conservation of SPVD resistant genotypes and provide genetic material to restore accessions that may be lost or reduced due to SPVD.

In Kenya, farmers grow a wide range of sweet potato cultivars depending on the needs of a particular group and attributes of the genotypes, and one of this attributes is high dry matter content (Ndolo *et al.*, 2006). Some sweet potato cultivars may have desirable agronomic traits but low dry matter content make farmers cultivate inferior cultivars that contain high dry matter. Other undesirable attributes that make farmers grow low yielding genotypes with high dry matter content is that the high moisture content in genotypes with low dry matter make the crop susceptible to pathogens during storage and unsuitable for making processed products (Woolfe, 1992). Breeding for quantitative traits

such as root dry matter content in hexaploid sweet potato is inhibited by the significant genotype by environment interaction and the complex polyploid genome of sweet potato. The net effect is that controlled crossing programs are slow. Due to the importance of sweet potato in Kenyan households there is need to fast track development of genotypes that are high in dry matter content by characterization.

Sweet potato is said to have higher genetic diversity than other root crops such as cassava and yams (Woolfe, 1992). Kenyan sweet potato genotypes has a combination of superior characteristics such as resistance to diseases and pests, tolerance to stress and high storage root dry matter content and significant variation in these attributes has been reported (Ndolo *et al*., 1998). Developing a thorough understanding of the range of phenotypic and genotypic diversity in cultivars exhibiting SPVD resistance and high dry matter will be a valuable step towards efforts aimed at improving the crop for these traits.

Morphological characterization is an important first step in assessment of sweet potato diversity but has certain limitations due to morphological plasticity and parallel evolution (Prakash and He, 1996). Therefore, genetic differences exhibited as presence/absence of polymorphisms that exist between accessions can be combined with phenotypic analyses to augment germplasm characterization. In addition, molecular markers obtained can be associated with economically important traits (Bruckner, 2004). For genetic analysis of sweet potato SSR markers produce easily scorable, unique alleles and/or allele combinations, which makes them an ideal system for cultivar identification.

1.3 Objectives

1.3.1 Overall objective

To increase sweet potato yields through identification of genotypes that are resistant to SPVD and contain high dry matter content.

1.3.2 Specific objectives

The specific objectives of the study were:

1. To assess the reaction of sweet potato (*Ipomoea batatas* L.) genotypes to sweet potato virus disease in the greenhouse

2. To characterize Kenyan sweet potato genotypes resistant to SPVD and high in dry matter content using morphological markers

3. To characterize Kenyan sweet potato genotypes resistant to SPVD and high in dry matter content using SSR markers

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sweet potato

2.1.1 Origin and distribution of sweet potato

Current scientific evidence suggests that sweet potato is of American origin (Central or South America) where it was widely established by the time the first Europeans arrived. Sweet potato may be one of the earliest domesticated plants (Yen, 1976). It is not clear whether sweet potato reached Polynesia through human contact or by chance (e.g. washing ashore (Woolfe, 1992; Yen, 1976). Sweet potato could have been introduced into Polynesia before the 8th century AD, and named *kumara*. The evidence for prehistoric spread of sweet potato include, the recovery of storage roots from archaeological sites in Hawaii, New Zealand, and Easter Island; the presence of prehistoric root storage facilities in temperate New Zealand; the fact that sweet potato germplasm is less diverse outside of the Americas (Yen, 1974); and lexical parallels between a Quechua (the Inca language) name (*apichu*) for sweet potato and the Polynesian *kumara* (Yen, 1982). Two main groups of sweet potato, the *aje* (an Arawakan word) group (starchy and slightly sweet) and the *batata* (a Spanish word) group (starchy and very sweet) were known and are evidence of the widespread distribution of sweet potato through the migration routes in the New World tropics before the discovery of America (Austin 1987). O'Brien (1972) showed linguistic and historic evidence indicating that sweet potato had reached southern Peru and southern Mexico around 2000-2500 B.C.

According to linguistic evidence there are three lines of dispersal of sweet potato. The *kumara* line is prehistoric and is based on lexical parallels between the Quechua name and the Polynesian word, *kumara*. This could explain the movement of sweet potato by Peruvian or Polynesian voyagers from northern South America to eastern Polynesia around 400 AD. The *batata* line, which dates back to the first voyage of Columbus in 1492, resulted in the introduction of West Indian sweet potato to western Mediterranean Europe. The Portuguese explorers had introduced sweet potato from western Mediterranean Europe to Africa, India, South East Asia, Indonesia, the East Indies and South China by the 16th century, and Southern Japan by 1698 (Onwueme, 1978; Bassett, 1986). The *camote* (derived from *camotli* in the Mayan language Nahuatl) line was directly introduced from Mexico by Spanish trading galleons between Acapulco, and Manila, the Philippines, and Guam, in the 16th Century (Yen, 1982). Today, sweet potato is grown in nearly all parts of the tropical world, and in the warmer areas of the temperate regions (Onwueme, 1978).

2.1.2 Botany of sweet potato

Sweet potato is a perennial dicot, which is cultivated as an annual for vines and storage roots. Genotypes are broadly grouped into bush, intermediate and vining types, which may vary greatly in branching pattern and overall stem length. Latex is present in all parts of the plant. Leaves are spirally arranged and have long petioles measuring 5-30 cm. They are broad, entire or lobed with a more or less pronounced leaf incision. The plants produce both tuberous and fibrous roots (Onwueme, 1978). The campanulate, violet or white flowers are grouped in cymose inflorescences and are hermaphroditic.

The flowers are complete with a compound superior pistil, five separate stamens attached to the corolla and with petals united into a trumpet- or bell-shaped corolla. The corolla is usually white at the margin and pink to purple in the throat. Seeds have a hard seed coat and develop within a capsule. The plants usually set few viable seeds, many genotypes do not readily flower, others are sterile and most are self-incompatible (Bassett, 1986; Thompson *et al.,* 1997).

Sweet potato is sensitive to light with photoperiod of 11.5 hrs day length or less promoting flowering, while at 13.5 hrs day light, flowering ceases although storage root yield is not affected (Kay, 1985). Short days with low light intensity promote root development. Flowers are perfect and produce capsules with 1-4 seeds after pollination and seed set. Complex sporophytic self- and cross-incompatibility cause serious problems in breeding (Nakanishi and Kobayashi 1979). Sweet potato is hexaploid with 2n = 6x = 90 chromosomes, and although some plants morphologically similar to *I. batatas* with 2n = 4x = 60 have been described and named, they are considered synonyms of this species. Storage root initiation varies from 21-35 days from planting (Austin *et al.*, 1970; Bhattachary *et al.,* 1985).

2.1.3 Dry matter content in sweet potato

Sweet potato has high moisture content, resulting in relatively low dry matter content. The average dry matter content is approximately 30%, but varies widely depending on such factors as cultivar, location, climate, day length, soil type, incidence of pests and diseases, and cultivation practices.

An increase in water stress has been shown to result in an increase in root dry matter content. Dry matter production increases with increasing soil temperatures between 20 - 30^0 C and declines above 30^0 C (Woolfe, 1992).

Mcharo (2001) suggests that root dry matter content is an important trait depending on the market being targeted. While some communities prefer low dry matter coupled with high sugar content others prefer starchy genotypes with high sugar content. However, most Kenyan consumers prefer sweet potato genotypes that have high dry matter, making increasing dry matter content the primary objective for sweet potato breeding in many regions such as Southeast Asia (Mok *et al*., 1997) and Africa where regional breeding efforts aim at selecting local farmers' genotypes that are high in dry matter (Carey *et al*., 1997; Ndolo *et al*., 2006).

To enhance sweet potato utilization, consumers reduce the moisture content through processing of the harvest product to a drier state. In the tropics, solar drying is the most economic method used but the energy input and product quality cannot be controlled. Furthermore, the drying methods used predispose the sweet potato to contamination by dust, insects and microbial organisms which thrive on the high soluble carbohydrate medium provided by the moisture in sweet potato (Tewe, 1994). High moisture content in sweet potato also results in low energy density which means that persons especially children have to consume large amounts of sweet potato for their energy supply. Large quantities of sweet potato are too bulky for most people to digest and they would therefore require energy-rich supplements (Woolfe, 1992).

The composition of sweet potato dry matter (Table 1) which is a trait influenced by several loci is extremely variable and the concentration of each component depends on one or more of the same factors that influence dry matter content (Woolfe, 1992). Many agronomically important traits in sweet potato such as root dry matter is quantitative. Breeding for such quantitative traits in hexaploid sweet potato has been inhibited by the significant genotype by environment interaction and by the complex polyploid genome of sweet potato (Jones *et al*., 1986). Marker systems such as RAPD, AFLP and SSR have provided easier protocols for genome analysis of such quantitative traits.

Table 1. The composition of raw sweet potato

Constituent	**% dry matter**
Starch	70
Total sugars	10
Total proteins	5
Lipids	1
Ash	3
Total fibre (Non-starch polysaccharide+lignin)	10
Vitamins, organic acids and other components in low concentration	< 1

Source: Woolfe (1992)

2.2 Production and importance of sweet potato

Sweet potato is among the world's most important versatile and important food crops. With an annual production of more than 133 metric tons globally (FAOSTAT, 2006), sweet potato ranks as the world's seventh most important food crop on fresh-weight basis and fifth in developing countries after rice, wheat, maize and cassava. Production is concentrated in East Asia, the Caribbean and tropical Africa, with the bulk of the crop (88%) being grown in China. In sub-Saharan Africa sweet potato plays a major role in providing food for the population and is the second most important root crop after cassava (Hijmans *et al*., 2001). Eastern Africa produces only 5% of the world total production although this comprises 75% of sweet potato produced in Africa. Uganda is the leading eastern Africa producer contributing 38% while Kenya ranks fifth contributing only 8% (FAOSTAT, 2006). Although the total area under sweet potato in eastern Africa is only a quarter of that under cereal production, sweet potato yields five times more on unit area basis compared to cereals (FAOSTAT, 2006).

Sweet potato is an important carbohydrate source and popular within regions with high per capita consumption of fresh roots i.e Rwanda 160kg/yr compared to Asia 18 kg/yr, Latin America 5kg/yr and USA 2kg/yr (CIP, 2000). It has a great potential as an industrial crop and some processed products include starch, noodles, candy, desserts, flour and beverage (CIP, 2000). In view of both high food and energy demands within the region due to increasing population, sweet potato has high potential as food, fuel and feed. It is a source of carbohydrates, high quality protein (e.g lysine, thiamine, riboflavine, niacin, pyridoxine, folic acid and ascorbic acid) (CIP, 2000). Other important

nutrients include calcium, phosphorus, iron, sodium and potassium. Due to the high beta-carotene content of the yellow and orange fleshed tubers, they are being promoted to alleviate vitamin A deficiency in East Africa (CIP, 1999).

As a food crop, sweet potato combines a number of advantages that gives it an exciting potential role in combating the food shortages and malnutrition that may be occasioned by population growth and pressure on land (Woolfe, 1992). The crop has a short growing season which allows it to fit into many different cropping systems; it can be harvested piecemeal to provide fresh daily food for a family (CIP, 1998; Karyeija *et al.,* 1998). Sweet potato has a high productivity per unit area of crop, performs well in infertile soils with few inputs and is relatively drought-insensitive. These features make sweet potato an ideal crop to provide a secure food supply for millions of resource-poor farmers in developing countries.

2.3 Constraints to sweet potato production

Sweet potato is subject to attack by storage root feeders, stem borers and feeders, foliage feeders, virus transmitters, mites and natural enemies. The crop is also affected by viral, fungal, bacterial diseases which cause substantial yield losses. Other diseases are caused by nematodes and some disorders of unknown origin such as fasciation also occur (Ames *et al*., 1997). Sweet potato weevils (*Cylas puncticollis* and *C. brunneus*) have been reported to cause yield losses of up to 90% (IITA, 1985). Weevil infestation of sweet potato in Kenya is estimated to cause yield reductions of up to 20% on a national scale and 80% in individual fields (Qaim, 1999). Viruses are the second most important biotic

constraints to sweet potato production in Africa (Geddes, 1990) after the sweet potato weevil. Sweet potato viruses are a production constraint in Kenya (Kabira, 1994) with SPVD being a major production constraint in major sweet production zones. (Carey *et al*., 1997).

2.3.1 Sweet potato viruses

There are several viruses associated with sweet potato worldwide but the major ones infecting sweet potato in Eastern Africa include sweet potato feathery mottle virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV), sweet potato mild mottle virus (SPMMV) and sweet potato chlorotic fleck virus (SPCFV). Sweet potato feathery mottle virus (genus *Potyvirus*, family *potyviridae*) occurs in all sweet potato growing areas and is the most widespread in Eastern Africa (Ateka *et al*., 2004; Mukasa *et al*., 2003; Tairo *et al*., 2004). Although infection of sweet potato plants with SPFMV may cause mild or no symptoms, infection may cause cracking and necrosis of the tubers (Kreuze, 2002). The symptoms on the leaves include vein clearing, vein feathering and chlorotic spots mainly on older leaves (Karyeija *et al*., 1998). The sweet potato chlorotic stunt virus (genus *Crinivirus*, family *Closteroviridae*) is the second most widespread virus in Eastern Africa. The symptoms due to SPCSV infection include stunting and changes in leaf pigmentation depending on the variety. A survey on the distribution of sweet potato viruses in Kenya (Ateka *et al*., 2004) indicated that virus incidence was highest in Nyanza and Western provinces and low in Eastern, Central and Coast provinces.

2.3.2 Sweet potato virus disease (SPVD)

SPVD is the most harmful disease of sweet potato in Africa and elsewhere (Geddes, 1990; Gibson *et al.,* 1998; Carey *et al.,* 1999; Gibson and Aritua, 2002). The disease was first reported in 1939 in Central Africa near the eastern border of the Democratic Republic of Congo and in East Africa 14 years later (Sheffield, 1953). However, the first published description of SPVD was from Uganda (Hansford, 1944). SPVD symptoms result from infection caused by two viruses namely SPCSV and SPFMV (Schaefers and Terry, 1976; Gibson *et al.,* 1998).

Most African sweet potato cultivars infected with SPFMV show no symptoms but co-infection with SPCSV leads to SPVD (Gibson, *et al.,* 1997). Although symptoms in sweet potato plants affected by SPVD differ with genotype, infected plants generally appear very stunted and have small distorted leaves, which are often narrow (strapped) and crinkled with a chlorotic mosaic especially on mature leaves often with vein clearing and vein feathering (Gibson *et al.,* 1998; Karyeija *et al.,* 2000). In other *Ipomoea* species, including the indicator species *I. setosa* and *I. nil*, symptoms of SPVD are more pronounced and include necrosis, vein-clearing, mosaic, leaf stunting and distortion. More recent studies indicate that the SPCSV is limited to the phloem and that it unusually mediates an increase in SPFMV titres perhaps by suppressing the plant's resistance to SPFMV (Karyeija *et al.,* 2000).

There have been many appraisals on SPVD-associated production losses, but only a few reliable estimates of such loss are available. Yield reductions exceeding 50% resulting from infection of sweet potato by SPVD in Africa and elsewhere have been documented (Hahn, 1979; Gibson *et al.,* 1998; Gutièrrez *et al.,* 2003). SPVD causes yield losses through reductions in growth of above ground parts, and the number and weight of tuberous roots (Hahn, 1979). These data indicate that SPVD diminishes tuberous root yields in sweet potato and therefore constitutes a serious constraint to sweet potato production. In Kenya, loss in production of three commonly grown cultivars is estimated at 92% (Njeru *et al*., 2004).

2.4 Sweet potato resistance to viruses

SPVD resistant landraces are known to occur in East Africa (Aritua *et al*., 1998). Resistance to SPVD in sweet potato clones seems to be of a type that restricts the number of plants that develop the disease (Alicai *et al*., 1999) with sweet potato genotypes showing large differences in their susceptibility to SPVD (Aritua *et al*., 1998). Since SPVD is caused by a dual infection with SPCSV and SPFMV, resistance to the disease could be as a result of resistance to SPFMV, SPCSV, or a mixed infection of these diseases (Gibson *et al*., 1998). Several sweet potato cultivars seem to be naturally resistant to SPFMV strains, showing only mild initial symptoms, from which they usually recover. East African sweet potato cultivars express resistance to SPFMV, which is characterized by extremely low titres of SPFMV and a lack of symptoms (Gibson *et al*., 1998). Symptoms of SPCSV alone are the first sign of eventual development of SPVD and without preinfection with SPCSV, SPFMV has difficulty in infecting at least some East African sweet potato genotypes (Gibson *et al*., 1997; Aritua *et al*., 1998).

This suggests that resistance to SPCSV is the key feature of the current SPVD resistant cultivars (Gibson and Aritua, 2002).

2.5 Genetic diversity of sweet potato

A comprehensive analysis of the extent and distribution of the genetic variation in sweet potato is essential for sound genetic conservation strategies (e.g. sampling of extant genetic resources in germplasm collections and at successive stages of development in breeding programmes, identification of duplicates, selection for core collection, future exploration planning). This approach helps in planning an efficient search for unique and favourable alleles (Swaminathan, 1997). Conservation and sustainable use of genetic resources is essential to meet the demand for future food security. Successful conservation of any given gene pool is largely dependent on understanding the diversity and its distribution in a given region (Zhang *et al*., 1999). Studying the diversity of important crops enables identification of land marks for in situ germplasm conservation, the creation of core genotypes for genetic analysis and the extension of knowledge, useful for breeding programs.

South America and parts of Central America are the primary centres of diversity, considering the many wild relatives of sweet potato (Zhang *et al*., 2000). Secondary centers of sweet potato diversity outside of the Americas are in China, Southeast Asia, New Guinea and East Africa. Natural cross-pollination in these centres of diversity may have contributed to the wide array of genotypes in one location. The cultivated genotypes of *I. batatas* display important morphological polymorphism.

Among the species within the genus *Ipomoea* series *Batatas,* 13 are considered to be closely related to sweet potato (Austin, 1987). Sweet potato exhibits great phenotypic and genotypic diversity as reflected by the skin and flesh color of the tubers, the size and shape of roots, leaves and branches the depth of rooting, and maturity period, resistance to pests and diseases, dry matter content of the tubers and the flavor and texture of cooked roots (Austin and Huaman, 1996). Studies suggest that cultivated sweet potato could either be allopolyploid or autopolyploid (Kriegner *et al*., 2003). The complex genome of sweet potato, and the fact that it is extremely heterozygous, exhibiting multiple combinations of chromosomes and genes due to its ploidy, contributes to the complexity of the crop as well as its molecular diversity. The crop's ploidy level significantly increases the possibilities for novel phenotypes. The crop also naturally mutates for traits like root and skin color, leaf and vine characteristics. In addition, sweet potato is asexually propagated via stem cutting and adventitious buds arising from storage roots which result in the accumulation of random mutations. These factors contribute to the crop's diversity and complexity (Villordon and Labonte, 1996). Various classical breeding techniques aimed at exploiting sweet potato genetic diversity have been employed but the complicated nature of sweet potato genetics (polyploid nature, genetic incompatibility and high rates of mutation within the species) make controlled crossing programs expensive and time consuming.

Molecular markers have been utilized to assess sweet potato genetic diversity. Gichuki *et al*. (2003) used randomly amplified polymorphic DNA (RAPD) to assess genetic diversity of sweet potato in relation to geographic sources.

Zhang *et al*. (1996) used RAPD markers to identify duplicates in the CIP sweet potato germplasm collection then later Zhang *et al*. (1999) used Amplified fragment length polymorphisms (AFLP) markers to analyze the genetic diversity of 69 sweet potato clones from Tropical America. Zhang *et al*. (2000) used 6 SSR markers which identified a total of 70 alleles to study the New World origins of sweet potato. Rossel *et al*. (2000) used AFLPs to study the historic dispersal of sweet potato whereas Fajardo *et al*. (2002) used AFLPs to analyze sweet potato germplasm from Papua New Guinea in the form of botanical seed.

Kenyan sweet potato germplasm has been characterized using simple sequence amplified polymorphism (S-SAP), AFLP, Inter simple sequence repeats (ISSR), SSR and RAPD. Njuguna (2005) used ISSR markers to fingerprint 22 popular sweet potato genotypes from Kenya but none of the ISSR primers was able to discriminate the genotypes. Gichuru *et al*. (2004) analyzed the diversity among sweet potato cultivars from distinct agro ecologies in Kenya, Uganda and Tanzania using morphological and simple SSR markers, the cultivars from Tanzania were found to cluster close to each other suggesting that they are slightly morphologically and genetically distinct from the Kenyan and Ugandan cultivars. However, only four SSR primers were used and increasing the number of SSR primers may yield more polymorphic DNA fragments. Germplasm characterization in Kenya has mainly focused on diversity analysis of sweet potato genotypes that are preferred by farmers. Assessment of variation in SPVD resistance and high dry matter is important in efforts aimed at increasing sweet potato yield.

2.6 Characterization of sweet potato

2.6.1 Use of morphological markers

Phenotypic identification of plants is based on morphological traits recorded in the field. It has been used as a powerful tool in the classification of genotypes and to study taxonomic status. However, traditionally these assessments depended on botanical traits (Stegemann, 1984; Zacarias, 1997). Most characteristics of agronomic importance are controlled by multiple genes and are subjected to varying degrees of environmental modifications and interactions, hence are ambiguous and have limited use for cultivar identification. Morphological characterization has been used for various purposes including identification of duplicates, studies of genetic diversity patterns, and correlation with characteristics of agronomic importance. These methods involve a lengthy survey of plant growth that is costly, labour intensive and vulnerable to environmental conditions (CIAT, 1993). Sweet potato cultivars are generally distinguished on the basis of morphological traits and have a wide variability of botanical characteristics. Morphological and agronomic characters coupled with reaction to pests, diseases and other stresses have been used by scientists to characterize sweet potato. Phenotypic characterization in sweet potato is done by assessing variations in the vine, leaf, flower and storage root characteristics (CIP, AVRDC, IBPGR, 1991) and it has been traditionally used for identification of sweet potato cultivars. Therefore, there is need to characterize Kenyan sweet potato genotypes resistant to SPVD and high in dry matter content using morphological markers in order to allow for faster selection.

2.6.2 Use of molecular markers

The use of DNA markers is widespread among plant geneticists because of the substantial amount of useful information that can be gathered from these markers. DNA markers are a popular tool for examining genetic diversity of organisms and generating genetic maps for tagging traits of interest for germplasm conservation and genetic enhancement. In plant breeding, superior cultivars of higher productivity can be detected by identifying quantitative traits loci (QTL) manifested with DNA markers. The markers provide a linkage framework and an estimate of similarity and difference among individuals (Stuber *et al.*, 1999). Based upon the principles of marker assisted systems, a gene or genes conferring traits of interest are expected to be associated with sets of markers. Thus, selection can target the molecular markers rather than for the trait itself (Karp and Edwards, 1997).

2.6.2.1 Biochemical markers

The term “biochemical markers” was first introduced by Markert and Moller (1959) often referred to as allozyme or isozyme markers. They were the first to describe the differing forms of bands that they visualized with specific enzyme stains. Isozymes are functionally similar forms of enzymes (Murphy *et al*., 1990). Allozymes on the other hand are different forms of the same enzyme resulting from allelic variation (Crozier, 1993). Biochemical studies had considerably more success distinguishing genotypes than previously using morphological markers.

However, isozymes are difficult to work with due to their limited amount of polymorphism, low levels of reproducibility since they are influenced by tissue type and developmental stage of the plant (Zacarias, 1997) and they are unevenly distributed throughout the genome (Neilsen and Scandalios, 1974).

2.6.2.2 DNA based markers

DNA markers make use of the variation in nucleotide sequence of the DNA to produce characteristic fingerprints or band patterns. The value of the DNA marker analysis is determined to a large extent by the technology that is used to reveal DNA polymorphisms. Many studies have aimed at assessing the genetic diversity in germplasm collections of crops using allozyme markers or various types of molecular markers such as RFLP, RAPD and AFLP. Recently, microsatellites or SSR, which correspond to tandemly repeated DNA sequences with a very short repeat unit, have been introduced as powerful genetic markers in plants (Morgante and Olivieri, 1993).

In sweet potato molecular markers have been used in phylogenetic studies and gene pool evaluation (Jarret *et al*., 1992; Jarret and Bowen 1994; He *et al*., 1995; Prakash *et al*., 1996; Buteler *et al*., 1999; Zhang *et al*., 1999; Huang and Sun, 2000), genome characterization (Villordon and La Bonte, 1995; 1996), fingerprinting (Connolly *et al*., 1994); linkage mapping (Ukoskit and Thompson, 1997; Kriegner *et al*., 2003); markers for root-knot nematode resistance (Ukoskit *et al.,* 1997), and SPFMV and SPCSV resistance (Mwanga *et al*., 2002).

2.6.2.3 Use of SSR markers for diversity assessment

SSRs are abundantly distributed throughout the nuclear genomes of all studied plant species, which makes them useful both for genetic mapping and for the study of natural populations. They have several advantages over other DNA markers such as Restriction fragment length polymorphism (RFLPs), RAPDs and AFLPs. They are co-dominant, highly informative giving high levels of polymorphism and are amenable to automated genotyping strategies. Finally, radioisotopes are not required in the detection of SSR markers, because sequence polymorphism can be detected by separation in agarose gels. SSRs have become the molecular markers of choice for a wide range of applications including genetic mapping and genome analysis, genotype identification and variety protection, seed purity evaluation and germplasm conservation, diversity studies, paternity determination and pedigree analysis, gene and quantitative trait locus analysis and marker assisted breeding (Chen *et al*., 1997). For measuring genetic diversity, assigning lines to heterotic groups and genetic fingerprinting, SSR markers provide a power of discrimination equal to or greater than that of RFLP in a more cost effective manner. Studies have shown that SSR loci give good discrimination between closely related individuals in some cases even when only a few loci were employed. The analysis of SSRs has been automated, thereby facilitating data exchange among researchers (Powell *et al*., 1996).

SSR markers have been useful for integrating the genetic, physical and sequence-based linkage maps in plant species and have simultaneously provided an efficient tool to link phenotypic and genotypic variation.

They have been identified in many plant genomes including those of maize, soybean, barley, sorghum, pearl millet, winter rye, wheat, potato, sunflower, olive and sweet potato. The result of studies using SSR markers in these species suggest that they may provide an outstanding tool for genetic analysis of plant species. The possibility to detect several alleles at a high frequency makes SSRs an ideal tool for identifying individuals and establishing genetic diversity between them (Prasad *et al*., 2000). SSRs in plants have been shown to be up to ten-fold more variable than other markers and have been highly recommended for genetic diversity analysis (Rakoczy-Trojanowska and Bolibok, 2004).

Dominant molecular markers, such as RAPD and AFLP have been proved to be genetically informative for sweet potato but they do not properly contribute to understanding of the allelic diversity of the crop. Lack of sequence specificity in RAPD and AFLP markers limits their cross-laboratory application for variety identification. Therefore, a polymerase chain reaction (PCR)-based, sequence-tagged, co-dominant marker system such as SSRs is needed to play a complementary role (Zhang *et al*., 2000). SSR markers for sweet potato have been developed (Jarret and Bowen, 1994) and have been successfully tested for diversity of cultivars from Oceania and Latin America and in paternity analysis of sweet potato and its wild relatives (Buteler *et al*., 1997, 1999). A total of forty six microsatellites have also been isolated from analysis of 1200 sweet potato expressed sequence tags (ESTs). SSR's multiallelic nature, relative abundance, and extensive genome coverage make it a unique tool for sweet potato cultivar identification, diversity assessment, and linkage mapping (Zhang and Kapinga, 2002).

CHAPTER THREE

3.0 RESPONSE OF KENYAN SWEET POTATO GENOTYPES TO SWEET POTATO VIRUS DISEASE (SPVD)

3.1 Introduction

Viruses cause the most important diseases of sweet potato in East Africa (Geddes, 1990; Gibson *et al*., 1997). The most devastating virus-induced syndrome of sweet potato is sweet potato virus disease (SPVD). The disease is characterized by a range of symptoms including chlorosis, small deformed leaves and severe stunting. It is the most important disease economically because the diseased plants produce almost no usable yield (Gibson *et al*., 1998; Karyeija *et al*., 1998). Yield reductions exceeding 50% resulting from infection of sweet potato by SPVD in Africa and elsewhere have been documented (Hahn, 1979; Mukiibi, 1977; Gibson *et al.,* 1998; Gutièrrez *et al.,* 2003). SPVD causes yield losses through reductions in growth of aboveground parts, and the number and weight of tuberous roots (Hahn, 1979). These reports indicate that SPVD diminishes tuberous root yields in sweet potato and therefore constitutes a serious constraint to sweet potato production. In Kenya, loss in production of three commonly grown cultivars was established to be as high as 92% (Njeru *et al*., 2004).

Although SPVD can be controlled by healthy stock programs, phytosanitation and cultural measures, these are difficult to integrate with subsistence production systems used by resource poor farmers (Gibson *et al*., 2004). Therefore, the use of resistant cultivars offers an attractive option for SPVD management, since it would be less costly

to implement, environmentally friendly and the most practical (would have no problems with reinfection) means of control of viral diseases. Identification of resistant cultivars to SPVD would also provide possible source of resistance which can be incorporated in the existing high yielding but susceptible genotypes. Thus, it is vital to screen germplasm collections to identify sources of resistance to SPVD. The objective of this study was to determine the reaction of Kenyan sweet potato genotypes to SPVD under glasshouse conditions.

3.2 Materials and Methods

3.2.1 Collection of sweet potato cultivars

Sweet potato cultivars were collected from major sweet potato growing areas namely, Kakamega and Busia districts in Western province, Homabay, Migori, Kisii and Rachuonyo districts in Nyanza province, Thika and Kirinyaga districts in Central province, Embu and Machakos districts in Eastern province and Kwale and Kilifi districts in Coast province. Sweet potato fields along rural roads and paths were sampled at approximately 1km intervals. A total of 314 sweet potato cultivars were collected as vine cuttings and transferred to a screenhouse at Kenya Agricultural Research Institute-National Agricultural Research Laboratories for subsequent experiments. The top 20-30cm of the apical sweet potato cuttings were planted in 15cm diameter pots containing a sterile soil mixture enriched with diammonium phosphate fertilizer in an insect-proof screenhouse.

3.2.2 Response of sweet potato genotypes to SPFMV and SPCSV inoculation.

This experiment was conducted to evaluate 314 sweet potato genotypes for response to SPVD. Five plants per genotype were planted in sterilized soil in perforated 15cm diameter pots. The plants were arranged in a completely randomized design with five replications. Once established, the apical portion of each plant was side grafted (Beetham and Mason, 1992) with scions from sweet potato plants pre-infected with SPVD.

To graft, a scion (with leaves removed) having two nodes was cut with a sharp blade to make a wedge shaped cut end. A 5 to 10-mm slit was cut longitudinally along the lower portion of stems of a 3-week-old plant with a sterile scalpel. The tip of the scalpel was inserted into the slit in order to insert an infected scion. All scions had two nodes to allow for growth, which would indicate graft establishment. Cutting and insertion manipulations were kept to a minimum to minimize damage to the graft area. The incision and the lower part of the scion were wrapped with parafilm to secure scions and decrease dehydration around the graft union. The whole plant was covered with a transparent plastic bag for at least 6 days to minimize moisture loss. The grafted plants with successful graft unions were examined for the development of SPVD symptoms. This was done for a period of 6 weeks starting from 3 weeks after inoculation. Symptoms and SPVD severity were recorded for each genotype using score scale of 1-5 according to Njeru *et al*. (2004), (Table 2).

Sweet potato genotypes (89) showing a mean symptom severity score of between 1 and 1.5 were selected for reinoculation.

Out of the 89 genotypes, 20 showed a mean SPVD severity of between 1.00 and 1.50 and tested negative for both SPFMV and SPCSV in nitro-cellulose membrane enzyme-linked immunosorbent assay (NCM ELISA). These genotypes were reinoculated with SPVD in 20 replications.

Table 2. Disease severity scale used for sweet potato virus disease assessment

Rating	Symptoms manifested
1.	No visible symptoms.
2.	Very mild symptoms on leaves, few leaves purpling / yellowing or mosaic, little distortion of leaf, apparent but negligible stunting.
3.	Moderate symptoms of purpling / yellowing or mosaic on leaves, moderate distortion of leaves shape and moderate stunting
4.	Severe symptoms of purpling /yellowing or mosaic on leaves, severe distortion of leaves with reduced size, plant partially stunted (very short internodes) but apparently still growing.
5.	Very severe symptoms of purpling / yellowing or mosaic on leaves, severe leaf distortion, reduced leaf size, plant severely stunted (stem extension more or less stopped)

Source: Njeru *et al*. (2004)

3.2.3 Virus assays

Presence of SPFMV and SPCSV were assayed using NCM-ELISA using kits and antisera obtained from the International Potato Center (CIP), Lima, Peru. The kit contained polyclonal antibodies to SPFMV and SPCSV as well as NCM strips pre-spotted with sap from virus-infected and healthy control plants. To test the plants, two leaf discs (1cm in diameter) excised from a composite sample of two leaves taken from different points (middle and top) of a sweet potato plant were ground in 1ml of Tris-buffered saline

(TBS) pH 7.5 containing 0.2% sodium sulfite (Na_2SO_3) in plastic bags. The ground sample was allowed to stand for 30-45 min at room temperature for the sap to phase out. Using a clean pipette each time, 15 µl of clear supernatant of each sample was blotted at the centre of a square made on the nitrocellulose membrane. The membrane was allowed to dry at room temperature for 15-30 min. Once dry, the membrane was immersed in 30ml blocking solution (TBS containing 2% powdered milk and 2% Triton X-100) in a Petri dish and incubated for 1 h. The blocking solution was discarded and the membrane immersed in a Petri dish containing the primary antibody diluted (1:1000, v/v) in antibody buffer. The membranes were incubated at room temperature overnight with constant agitation on an orbital shaker at 50 rpm. The primary antibody solution was discarded and unbound antibodies removed from the membranes by washing in Tris buffered saline supplemented with tween 20 (T-TBS) four times for 3 min each time with constant agitation at 100rpm. The membranes were then immersed in 30ml of alkaline phosphate-labelled goat anti-rabbit (GAR-AP) in conjugate buffer (1:1000 v/v) in a Petri dish for 1 h. The conjugate buffer was discarded and unbound antibodies removed from the membranes by washing in T-TBS four times for 3 min each time with constant agitation at 100rpm. The substrate solution (NBT/BCIP) was added and the reaction allowed to proceed for 5 to 30 min. Positive and negative reactions were determined by visual assessments with different grades of purple colour indicating positive reactions. The substrate solution was discarded after 30min incubation and the membranes washed twice with distilled water to stop the reaction.

3.2.4 Data analysis

The SPVD severity data were subjected to analysis of variance (ANOVA) and means were separated using least significant differences.

3.3 Results

3.3.1 Response of sweet potato genotypes to SPCSV and SPFMV inoculation

Analysis of variance showed highly significant ($p \leq 0.001$) differences in symptom severity among the 314 sweet potato genotypes. Average symptom severity of all the genotypes ranged from 1.00 to 4.06. Symptom variation among the sweet potato genotypes was not apparent, however, various combinations of SPVD symptoms were exhibited by the sweet potato genotypes depending on the degree of susceptibility or resistance. Symptoms generally consisted of vein clearing, chlorotic mosaic, chlorotic spots, reduced leaf size, purpling of older leaves, narrowing of leaf lamina (strapping), leaf distortion (crinkling) and stunting (Plate 1). Most of the genotypes (>71%) were highly susceptible to SPVD. Of the 314 genotypes evaluated for SPVD resistance, 89, 211 and 14 had mean SPVD severity scores of between 1.00 and 1.50, 1.6 and 3.00 and 3.01 and 5.00, respectively.

Plate 1. Symptoms observed in different sweet potato genotypes graft inoculated with Sweet potato feathery mottle virus (SPFMV) and Sweet potato chlorotic stunt virus (SPCSV).

(A) Sweet potato virus disease (SPVD) symptoms in Kemb-10, 8 weeks post inoculation, (B) purpling of older leaves observed in variety SPK 013, (C) vein clearing observed in variety OR-Nyasi and (D) vein clearing, chlorotic spots and stunting observed in Mugande.

Following re-inoculation of these 89 genotypes, 20 and 69 had mean SPVD severity scores of between 1.00 and 1.50, and 1.6 and 3.00, respectively.

There were significant ($p \leq 0.001$) differences in symptom severity of the 89 genotypes (Appendix 1). Serological indexing showed that 49 out of the 89 genotypes were infected with both SPFMV and SPCSV. Sixty two (62) genotypes tested positive for SPFMV whereas 55 tested positive for SPCSV (Appendix 2). Twenty (20) genotypes tested negative for both SPFMV and SPCSV in NCM-ELISA. Three genotypes namely BGM/02/2007, Kikanda (2) and MCK/21/2007 did not express any symptoms but tested positive for both SPFMV and SPCSV in NCM-ELISA (Table 3). The twenty genotypes with SPVD severity scores ranging from 1.00 and 1.50 and tested negative for both SPFMV and SPCSV by NCM-ELISA were regarded as resistant to SPVD.

Table 3. Reaction of resistant sweet potato genotypes to infection with SPCSV and SPFMV

No.	Variety	SPVD severity	Serological test*	
			SPFMV	SPCSV
1	OP-LNA-006-08	1.4	-	-
2	TVT/02/2007	1.1	-	-
3	WFTC/03/2007	1.3	-	-
4	YS sopalla	1.4	-	-
5	Marooko (1)	1.4	-	-
6	KKFS Mwavuli	1.2	-	-
7	YS Kemb 10	1.2	-	-
8	YS Nyanguyegwo	1.1	-	-
9	Marooko (3)	1.4	-	-
10	KAK/04/2007	1.0	-	-
11	KKFS 56682/03 (1)	1.1	-	-
12	Kamau (1)	1.4	-	-
13	Naspot	1.4	-	-
14	MKN/04/2007	1.5	-	-
15	Katumani (2)	1.5	-	-
16	Kikuyu (3)	1.4	-	-
17	Katumani (7)	1.5	-	-
18	Kikanda (1)	1.0	-	-
19	Kikamba (2)	1.0	-	-
20	SPK 004 (Katumani)	1.2	-	-

*NCM-ELISA. SPVD severity score determined on a scale of 1-5 where; 1 = no visible symptoms, 2 = very mild symptoms on leaves consisting mainly of chlorotic and/or purple spots; 3 = moderate symptoms of chlorotic spots, vein clearing, interveinal chlorosis, mottling, and mosaic; 4 = severe symptoms of purpling/yellowing or mosaic on leaves, moderate distortion of leaves shape and moderate stunting and 5 = very severe symptoms of purpling / yellowing or mosaic on leaves, severe leaf distortion, reduced leaf size, plant severely stunted.

3.4 Discussion

Identification of sources of resistance to SPVD from the available sweet potato germplasm is an important contribution to the genetic improvement of sweet potato. Several sweet potato genotypes commonly grown by farmers in Kenya are susceptible to SPVD. Following graft-inoculation with SPVD, the genotypes were found to differ greatly in the severity of SPVD symptom expression in the screenhouse. In the 314 genotypes evaluated for SPVD resistance, severity ranged from 1.0 to 5.0. These results indicate that the search for genotypes with moderate to high SPVD resistance is achievable. Some of the differences in SPVD symptom severity could be associated with differences in virus concentration although some genotypes modify expressed symptoms independently of the rate of virus replication (Kuhn *et al*., 1981). Only 20 (6%) of 314 genotypes showed mean SPVD severity scores of between 1.00 and 1.50 and tested negative for both SPFMV and SPCSV, indicating their relative resistance to SPVD and therefore have potential for sweet potato improvement. It is likely that these genotypes either suppressed the rapid titer rise which occurs in other genotypes or have a mechanism of lowering virus titre during progress of the SPVD.

More genotypes tested positive for SPFMV (69%) than for SPCSV (61%). The reasons for this is unclear, but Winter *et al*. (1992), Cohen *et al*. (1992) and Karyeija *et al*. (2000) have suggested that during co-infection, SPCSV titer does not increase as much as SPFMV, or it remains constant or even decreases slightly (Gibson *et al*., 1998). Kokkinos and Clark (2006) confirmed this by observing that the titers of SPCSV decline in the presence of SPFMV.

It is likely that SPCSV may have been undetected in SPVD-infected plants in NCM-ELISA. Seventeen out of the twenty genotypes that tested negative for SPVD had a severity score of between 1.01 and 1.47 possibly because of the presence in plant tissue of phenolic compounds and latex that inhibits and adversely affects the serological detection and symptoms caused by non-viral factors (Esbenshade and Moyer, 1982; Abad and Moyer, 1992).

Three genotypes namely BGM/02/2007, Kikanda (2) and MCK/21/2007 showed no symptoms of infection but were confirmed to be infected with both SPFMV and SPCSV when indexed serologically by NCM-ELISA. The lack of symptoms in these genotypes would be due to reduced levels of virus multiplication or low levels of SPFMV and SPCSV that are not enough to trigger the cascade of events associated with symptom induction (Maule *et al*., 2000). These genotypes, although without symptoms, could be reservoirs of inoculum and source of infection to susceptible genotypes when planted in the field.

Grafting was used to inoculate the sweet potato genotypes with the viruses because SPCSV is not mechanically transmitted. However, when a virus is transmitted by an insect, resistance may be expressed at the insect-plant interface (VidaVsky and Czosnek, 1998) during the short time the insect is feeding unlike in grafting where the virus is directly delivered into the vascular system as long as the scion remains alive. In addition, the graft scion is usually from a susceptible host, which ensures virus replication regardless of the resistance in the test plant stock.

While insect transmission might give a more natural inoculation, graft-inoculation has the advantage that the survival of the scion may be used as an indicator of successful inoculation and this minimizes false resistant genotypes. The results from these screening experiments indicate that 20 out of the 314 genotypes possess good sources of resistance to SPVD, and they therefore have great potential for utilization in sweet potato improvement.

CHAPTER FOUR

4.0 IDENTIFICATION OF PHENOTYPIC MARKERS FOR SPVD RESISTANCE AND FOR HIGH DRY MATTER IN KENYAN SWEET POTATO GENOTYPES

4.1 Introduction

Sweet potato expresses a diverse range of phenotypes in its foliage and storage roots as exhibited in different shapes, colours and sizes. Mutations in sweet potato result cultivars with different phenotypes (Hernedez *et al*., 1964). The phenotypic diversity of sweet potato is also evident in traits such as disease resistance and quality attributes such as dry matter and β-carotene content (Austin and Huaman, 1996). Such characters have been used to identify the centre of origin and evolution of *Ipomoea batatas* (Austin, 1987), duplicates in sweet potato germplasm (Zhang *et al.*, 1996), establishment of core collections (Mok and Schmiendiche, 1999) and in identification of markers associated with resistance to SPVD (Gasura *et al*., 2007).

Kenyan sweet potato germplasm has been characterized phenotypically to eliminate duplicates in collections (Njuguna, 2005) and for diversity analyses (Gichuru *et al*., 2004). Studies done in Kenya (Ndolo *et al*., 1998) showed that Kenyan sweet potato germplasm has desirable characteristics such as disease resistance and high dry matter content compared to exotic genotypes. Kenyan sweet potato genotypes show marked differences in susceptibility to viral diseases and are good sources of resistance (Miano *et al*., 2008).

Since the use of resistant cultivars is the most effective means of reducing sweet potato losses due to SPVD, and is compatible with subsistence agriculture (Mwanga *et al*., 2001), there is need to identify cultivars resistant to SPVD. Low production of sweet potato in Kenya is also due to lack of cultivars with consumer quality/acceptable attributes such as high dry matter content. Sweet potato consumers and processing industries prefer genotypes with high dry matter (Ndolo *et al.*, 1998). However, no studies have been done to identify morphological markers linked to SPVD resistance or high dry matter content. Therefore, the investigation aimed at identifying morphological markers linked to SPVD resistance and high dry matter content which will greatly help in early identification of genotypes with these desirable traits.

4.2 Materials and Methods

4.2.1 Collection and characterization of sweet potato genotypes

The 314 sweet potato genotypes were collected as detailed in section 3.2.1. and established in a field at Kenya Agricultural Research Institute, National Agricultural Research Laboratories (KARI-NARL). For each genotype, six cuttings were planted in the field in single rows on ridges spaced 1m apart and 0.3m within a row. The plants were allowed to grow to maturity. Morphological characterization of the above and the below ground parts was conducted using CIP, AVRDC, IBPGR, (1991) guide at 3 and 5 months after planting, respectively. A total of 42 characters/descriptors were used in the evaluation of each genotype (Table 4; Appendix 3). The phenotypic data of 18 aerial, 16 storage root and 8 floral descriptors was converted into a binary data matrix.

Cluster analysis was done using the Nei and Li coefficients (Nei and Li, 1979) and the uweighted pair group method with arithmetic means (UPGMA) algorithm (Sneath and Sokal, 1973) using Treecon version 1.3b (Van de Peer and De Wachter, 1994). Principal component analysis (PCA) was done using XLSTAT 2008 (Agresti, 1990, New York).

PCA is used to simplify the data by reducing the number of variables into a smaller number of orthogonal variables which are linear combinations of the original variables and maximize the variation within them thereby displaying most of the original variability in a smaller number of dimensions.

Table 4. Vegetative, floral and storage roots characteristics used for evaluation of sweet potato genotypes

Plant part	**Observed character**
Vine	Twining, plant type, ground cover, vine internode, vine pigmentation, vine tip pubescence
Leaf	General outline of the leaf, leaf lobes type, leaf lobe number, shape of central leaf lobe, mature leaf size, abaxial leaf vein pigmentation, foliage color, petiole pigmentation
Storage root	Root shape, root surface defects, root skin color, root flesh color (predominant and secondary flesh color, distribution of secondary flesh color) root formation, root cracking, latex production and oxidation in roots, quality characteristics of boiled storage root (consistency, undesirable color, texture and sweetness of boiled storage root)
Flower	Flower color, shape of limb, equality of sepal length, sepal pubescence, sepal color, color of stigma and style, stigma exertion

Source: CIP, AVRDC, IBPGR (1991)

4.2.2 Determination of dry matter content

The dry matter content was determined in freshly harvested roots. For each genotype, five roots were randomly selected washed, dried and then peeled. The medium sections of the roots were sliced and 25g in three replicates (fresh weight) were dried at 80^0C for 20 h in a heating cabinet. After drying the samples were weighed immediately (dry weight). The percentage dry matter content (% dry matter) was calculated as follows:

% dry matter =dry weight/ fresh weight X 100

The dry matter content data obtained was subjected to Analysis of variance (ANOVA) and means were separated using least significant differences.

4.3 Results

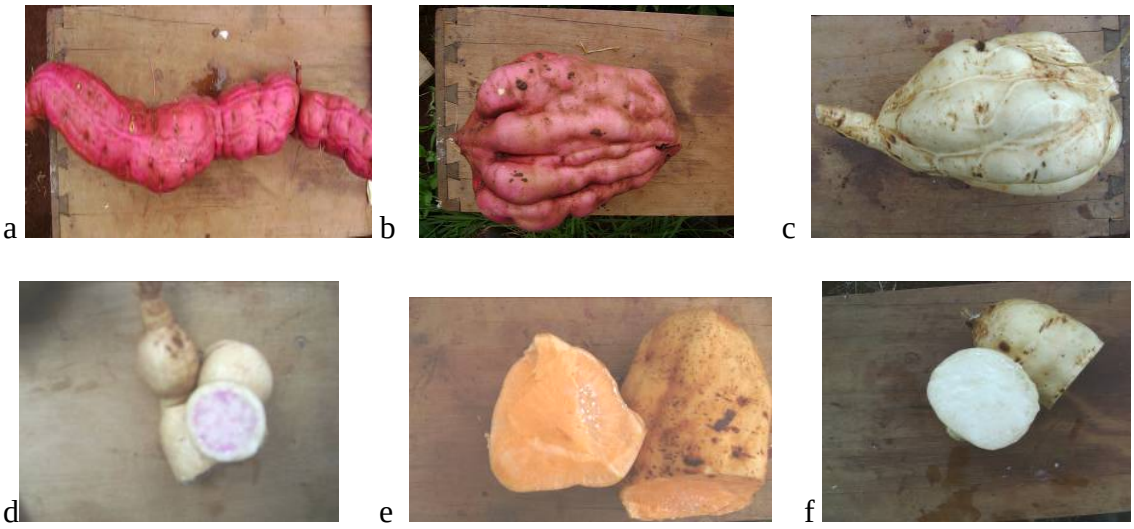
4.3.1 Morphological description of 314 genotypes

The 314 sweet potato genotypes characterized using 42 morphological traits showed significant variation in vine, leaf, root and flower characters (Plate 2 and 3).

Plate 2. Morphological diversity observed in the leaves of different sweet potato genotypes

(a) moderately lobed outline and a semi elliptic central leaf lobe in KAK/03/2007, (b) an almost divided outline and an elliptic central leaf lobe in genotype Ganchurere, (c) very deep leaf lobes and a linear (narrow) central leaf lobe in KAK/04/2007, (d) moderately lobed with a triangular central leaf lobe in genotype Riziki, (e) a triangular outline and no lateral lobes in genotype Odinga, and (f) a chordate outline and lack of lateral leaf lobes in 102019-3.

Plate 3. Morphological diversity observed in sweet potato roots



(a) purple red skin colour and long irregular shape in genotype Nyathi odiewo, (b) an ovate root shape and deep longitidunal grooves in genotype KRG/02/2007, (c) white skin and veins on the root surface in genotype Oyieo, (d) strongly pigmented with anthocyanins flesh in genotype YS/07/2007, (e) orange skin and dark orange flesh colour in genotype Tainung and (f) white storage root flesh in genotype Amina.

4.3.2 Principal component analysis

The principal component analysis revealed 13 principal components which had eigen values greater than 1 and accounted for 68.7% of the total variation (Table 5). The first 3 principal components accounted for 28.2% of the variation. The leaf lobe type, general outline of the leaf, shape of the central leaf lobe, leaf lobe number, twining and predominant flesh colour were the most important characters associated with the first component axis. The first principal component accounted for 12.7% of the variation. In comparison the second principal component accounted for 8.6% of the variation. Petiole pigmentation, abaxial leaf vein pigmentation, predominant vine and root skin colour, secondary vine colour, and immature leaf colour were the major characters associated with the second principal component. The third principal component accounted for 6.9%

of the variation with the most important characters being vine internode length, plant type, twining, predominant flesh colour, secondary vine colour and abaxial leaf vein pigmentation.

Table 5. Eigen values and total variation of 13 principal components for 314 Kenyan sweet potato genotypes

Principal component	Eigen value	Variation (%)	Cumulative variation (%)
1	4.3	12.7	12.7
2	2.9	8.6	21.2
3	2.3	6.9	28.2
4	2.2	6.4	34.5
5	1.9	5.6	40.1
6	1.5	4.3	44.4
7	1.4	4.0	48.4
8	1.3	3.8	52.3
9	1.2	3.6	55.9
10	1.2	3.5	59.4
11	1.1	3.3	62.7
12	1.1	3.1	65.8
13	1.0	3.0	68.7

4.3.3 Phylogeny of sweet potato genotypes

Phylogenetic analysis of the sweet potato genotypes resulted in two major clusters A and B (Fig. 1). Cluster A was further sub-divided into 7 sub-clusters, whereas cluster B was sub-divided into two sub-clusters (Table 6). The phenogram did not reveal any unique clustering of the sweet potato genotypes according to dry matter content and/or reaction to SPVD.

Genotypes with high dry matter content were grouped in different sub-clusters as were those with low dry matter. Similarly, SPVD resistant genotypes were grouped in different sub-clusters throughout the phenogram and together with susceptible ones (Fig. 1).

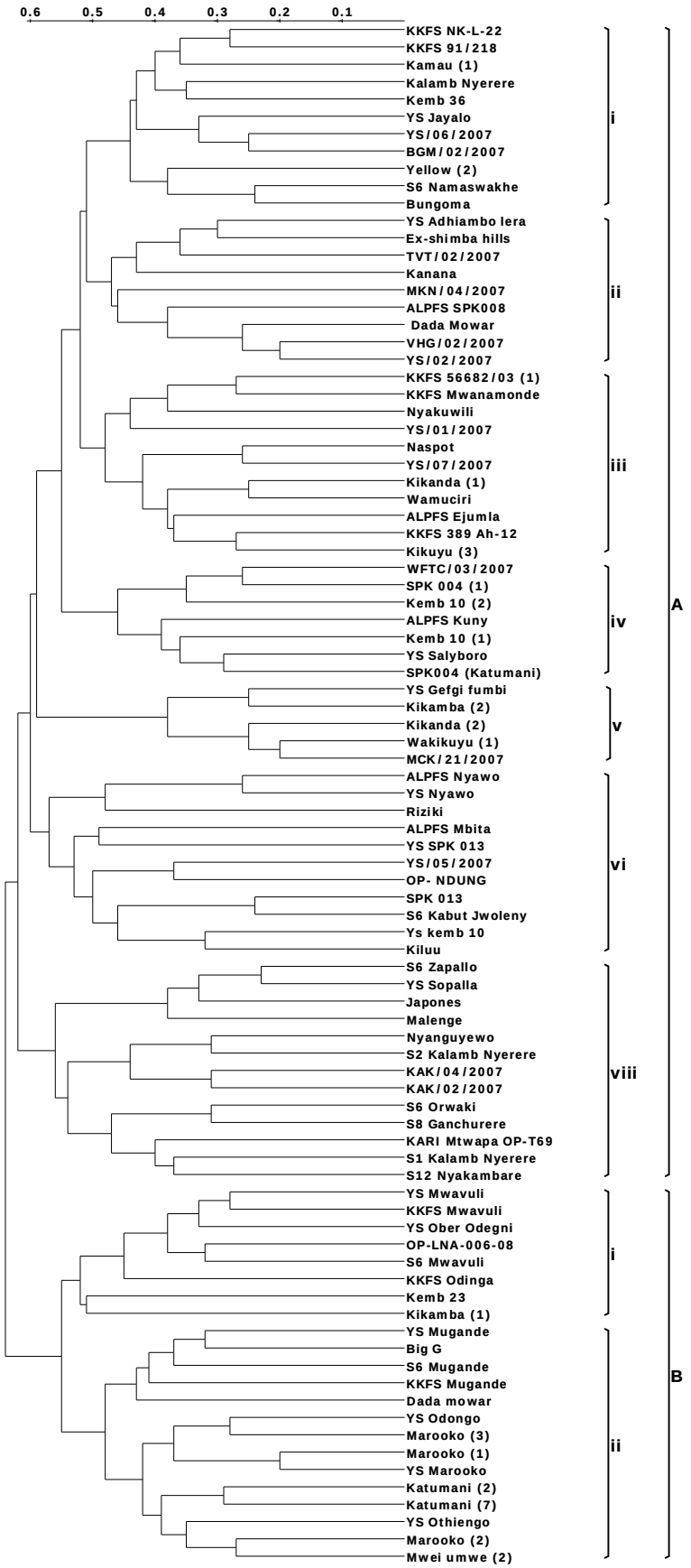


Fig.1. Cluster analysis of sweet potato genotypes using morphological traits

Table 6. Phenotypic characters separating sweet potato genotypes

Cluster	Sub-cluster	Resistant/tolerant genotypes	Phenotypic characters
A	I		Green mature leaves with five lobes and semi-elliptic central leaf lobe, an erect plant type with thin vines and purple nodes as the secondary vine colour and green petioles with purple at both ends
	II	TVT/02/2007, MKN/04/2007	Green mature leaves moderately lobed with five leaf lobes and semi-elliptic central lobe and storage roots that were slightly sweet when cooked
	III	KKFS 56682/03 (1), Naspot , Kikanda (1), Kikuyu (3)	Green mature leaves, moderately lobed with a semi-elliptic central lobe. Absence of secondary vine colour and storage roots were soft when cooked
	IV	WFTC/03/2007, SPK 004 (Katumani)	Green mature leaves, moderately lobed with green abaxial veins and short green petioles. Non-twining green vines with very short vine internode length
	V	MCK/21/2007, Kikamba (2).	Green mature leaves with five lobes, semi-elliptic central lobe, green petioles were green with purple near the leaf and abaxial veins with a purple spot at the base of the main rib. Very thin green vines with few purple spots and no secondary colour. White fleshed storage roots formed in a dispersed manner and soft and sweet when boiled.
	VI	YS Kemb 10	Green mature leaves, moderately lobed with five leaf lobes. Storage roots that were soft with no undesirable colour when boiled.
	VII	KAK/04/2007, YS Sopalla, Nyanguyegwo	Green mature leaves that were almost divided with five leaf lobes and an elliptic central leaf lobe. Absence of secondary vine colour.
B	I	OP-LNA-006-08, KKFS Mwavuli	Green mature leaves with a single leaf lobe, triangular outline, toothed central leaf lobe and no lateral leaf lobes. Short petioles with purple colour at both ends, thin vine internode diameter, purple nodes as the secondary vine colour
	II	Marooko (1), Marooko (3), Katumani (2) and Katumani (7)	Green mature leaves with a single leaf lobe, triangular leaf outline, toothed central lobe and green petioles. Non-twining vines with no secondary colour

4.4 Determination of dry matter content

A total of 314 sweet potato genotypes were used for dry matter content analysis (Appendix 4). There was high variation in the dry matter content as well as the predominant flesh colour of storage roots. Analysis of variance showed significant ($p<0.001$) differences in the dry matter content among the 314 sweet potato genotypes (Appendix 5). The dry matter content ranged from 20.0 to 37.8%. Out of the 314 genotypes 72.9% had white/cream root flesh colour with a dry mater content ranging from 20.0 to 37.8%, 19.1% had yellow flesh colour (dry matter content 23.1-35.6%), 7.3% were orange fleshed (dry matter content 22.5-32.9%) and 0.6% were strongly pigmented with anthocyanins (dry matter content 29.2-34.9%). Most of the white/cream (70.3%) and yellow fleshed genotypes (61.7%) had dry matter content greater than 35% (Fig. 2). The orange fleshed genotypes had relatively low dry matter content compared to the rest of the genotypes and they were also easily infected with SPVD with a mean severity score of 2.5. Dry matter content ranged from 22.5 to 32.9%.

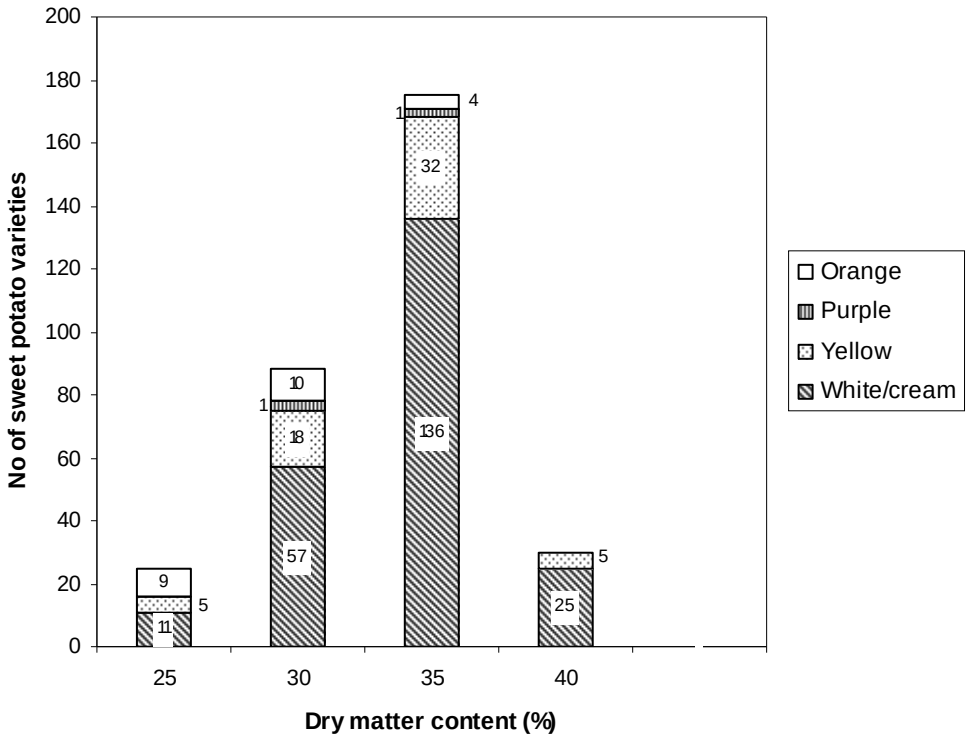


Fig. 2. Frequency distribution of dry matter content for 314 sweet potato genotypes

4.4 Discussion

The 314 genotypes commonly grown by farmers were characterized morphologically in order to identify markers linked to SPVD resistance and high dry matter. Following the PCA, vegetative descriptors that contributed to the diversity of sweet potato included predominant vine colour, leaf lobe type, shape of central leaf lobe, abaxial leaf vein pigmentation, and immature leaf colour and petiole pigmentation. This confirm earlier reports by Njuguna (2005) and Tairo *et al*. (2008) that variation in Kenyan and Tanzanian sweet potato genotypes, respectively, is expressed based on the shape of the central leaf lobe. Two storage root descriptors namely the predominant root skin and flesh colour are other expressions of the crop's diversity.

This confirms earlier report by Oliveira *et al.* (2000) that the predominant root flesh colour highly contributed to the diversity of sweet potato.

The phylogeny of the sweet potato genotypes using 42 traits was mainly influenced by the general outline of the leaf. This confirmed an earlier report by Gichuru *et al*. (2004) that the general leaf outline separated cultivars from Kenya, Uganda and Tanzania into two clusters using UPGMA. In this study, cluster analysis showed no formation of distinct groups based on resistance to SPVD and high dry matter content. For instance, the expectation that the genotypes with SPVD resistance should be classified in a separate sub-cluster was not observed as resistant genotypes were present in 8 out of the 9 sub-clusters formed. Similarly, genotypes (KKFS NK-L-22, Kemb 36, S6 Namaswakhe, YS/01/2007, ALPFS Nyawo, S2 Kalamb Nyerere and S6 Mugande) with high dry matter (>35%) were grouped in different sub-clusters together with genotypes (YS/02/2007, KKFS Mwanamonde, Riziki, YS Sopalla, Malenge, Big G and Marooko (2)) with low dry matter (less than 30%).

Dry matter content is an important quality attribute in sweet potato as it is directly linked to Kenyan consumers' preference for a particular genotype. Farmers grow a wide range of sweet potato cultivars depending on the needs of a particular market segment including attributes of the genotypes such as high dry matter content. Determination of the dry matter content of the 314 genotypes revealed a significantly high variation among the genotypes that ranged from 20.0 to 37.8%.

Dry matter content greater than 35% was observed in the white/cream and yellow fleshed genotypes whereas orange fleshed genotypes which consisted of mainly exotic genotypes had dry matter less than 30% confirming earlier reports by Brabet *et al*. (1998) that orange fleshed sweet potato genotypes have low dry matter content as compared to the white/cream and yellow fleshed genotypes. Of the 20 sweet potato genotypes apparently resistant to SPVD, nine had dry matter content of less than 30%. The genotypes that had high dry matter content were severely affected by SPVD, for instance Mugande had the highest dry matter content but was severely affected by SPVD with a score of 5. Since dry matter content is an important quantitative trait of direct interest to the Kenyan consumer, there is need to breed for high dry matter content and SPVD resistance.

From this study, no relationship between the reaction to SPVD, dry matter and morphological markers in the 314 genotypes was observed confirming earlier reports by Ivancic and Lebot (2000) that agronomically desirable traits such as disease resistance are not always expressed as morphological characters or linked to them. This indicates that morphological markers may not be reliable in identifying and classifying sweet potato genotypes into phenotypic groups as resistant or susceptible to SPVD or as having high and low dry matter content. Consequently, the use of molecular markers may be a more reliable way to identify genotypes resistant to SPVD and with high dry matter.

CHAPTER FIVE

5.0 CHARACTERIZATION OF KENYAN SWEET POTATO GENOTYPES FOR SPVD RESISTANCE AND FOR HIGH DRY MATTER CONTENT USING SSR MARKERS

5.1 Introduction

Relationships among genotypes can be assessed based on information of the geographic origin of genotypes, pedigree, and on plant characteristics. Geographic information is specifically useful when other information on the genotypes is either not available or is very sparse, while pedigree information may sometimes not be available or may consist of erroneous or inadequate historical records (Ajmone-Marsan *et al*., 1992; Schut *et al*., 1997). Morever, farmers are known to exchange and distribute material among themselves, and different ethnic groups assign different vernacular names to similar genotypes or similar names to different genotypes (Mignouna *et al*., 1998). This result in duplication of collected genotypes and makes the information on the geographic origin unreliable in assessing relationships.

Plant characteristics for any set of genotypes used to assess their relationship (Schut *et al*., 1997) include agronomic and morphological (phenotypic) characters, and biochemical (e.g. storage proteins, isozymes) markers and genetic or molecular (DNA) markers. Genetic markers have shown important and critical application in the assessment and conservation of genetic variation.

Amplified fragment length polymorphism (AFLP) has been used for studying the historic dispersal of sweet potato (Zhang *et al.,* 2004) as well as for assessing the genetic diversity of cultivars and landraces (Zhang *et al.*, 2000; Fajardo *et al.*, 2002). Huang and Sun (2000) and Hu *et al.* (2003) used inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA to investigate the genetic relationships between cultivated sweet potato and its wild relatives. In sweet potato DNA markers have been identified for economically important traits such as resistance to SPVD (Mwanga *et al*., 2002, Miano *et al*., 2008) and root knot nematodes (Mcharo *et al*., 2005b), dry-matter and starch content, yield and beta-carotene content (Cervantes-Flores, 2006).

Microsatellite or simple sequence repeat (SSR) markers exhibit high levels of polymorphism, and several such markers have been developed for sweet potato (Jarret and Bowen, 1994; Buteler *et al.*, 1999; Hu *et al.*, 2004) and used successfully for determining the genetic relationship between cultivars derived from hybrid or polycross breeding programs (Hwang *et al.*, 2002) and for analyzing the genetic diversity of sweet potato landraces (Zhang *et al.*, 1999; Gichuru *et al.*, 2006; Veasey *et al*., 2008).

Comparative studies in crop plants have shown that simple sequence repeat (SSR) or microsatellite markers, which are single locus markers with multiple alleles are more variable than other markers and provide an effective means of discriminating between genotypes (Morgante and Olivieri, 1993; Powell *et al*., 1996).

To ensure that durable resistance to SPVD is maintained within the Kenyan sweet potato germplasm, there is a need to increase the levels of resistance within the genepool using additional sources of resistance with a wider genetic base. Therefore, the aim of this chapter was to identify genetic markers for SPVD resistance and for high dry matter content.

5.2 Materials and methods

5.2.1 Plant materials

Eighty nine sweet potato genotypes were selected following graft inoculation with SPVD-infected scions as described in chapter 3 (section 3.2.2) were used for DNA extraction and genetic characterization.

5.2.2 Extraction of DNA

Genomic DNA was extracted from fresh leaves of each genotype using a modified CTAB protocol (CIP, 2000). One young leaf lobe, approximately 100 mg was harvested and ground in liquid nitrogen using a pestle and pre-chilled mortar into a fine powder. The powder was transferred to a frozen 2.0 ml eppendorf tube, 800 µl of pre-heated CTAB buffer was added and the mixture shaken vigorously until the tissue became dispersed in the buffer. The homogenate was incubated at 65°C in a water bath for 45 min, while shaking tubes every 15 min. The samples were removed from the water bath and allowed to cool at room temperature for 5 min. Solvent extraction was done by adding 800 µl chloroform: isoamylalcohol (24:1) to each tube and inverted 50 times to mix. The tubes were centrifuged at 14,000 rpm for 10 min in a microcentrifuge to separate the phases.

A fixed volume of the aqueous phase (700µl) was carefully removed and transferred to new, labeled eppendorf tubes. To each tube, 50 µl 10% CTAB (in 0.7 M NaCl) was added, vortexed gently, and mixed. About 800 µl chloroform: isoamylalcohol (24:1) was added to each tube and inverted 50 times to mix. The tubes were spun at 14,000 rpm for 10 min and 500 µl of the aqueous phase was carefully transferred to a new eppendorf tubes. An equal volume of 4°C isopropanol (500 µl) was added to each tube, inverted several times, incubated at 4°C for 30 min. and then centrifuged at 12,000 rpm for 20 min. The supernatant was decanted and the DNA pellet was washed with 1 ml of 70% ethanol (for 3 min) and centrifuged at 14,000 rpm for 30 min followed by another wash in 1 ml of 90% ethanol, spun for 30 min at 14,000 rpm, and ethanol poured off carefully. The tubes were inverted and pellet air-dried for 30 min. One hundred microliters low salt TE buffer was added to each sample and then 2 µl RNase (10mg/ml) was added to each of the samples and incubated at 37°C for 1 hr. The resulting DNA was stored at 4°C till use

5.2.3 DNA quantification and quality checking

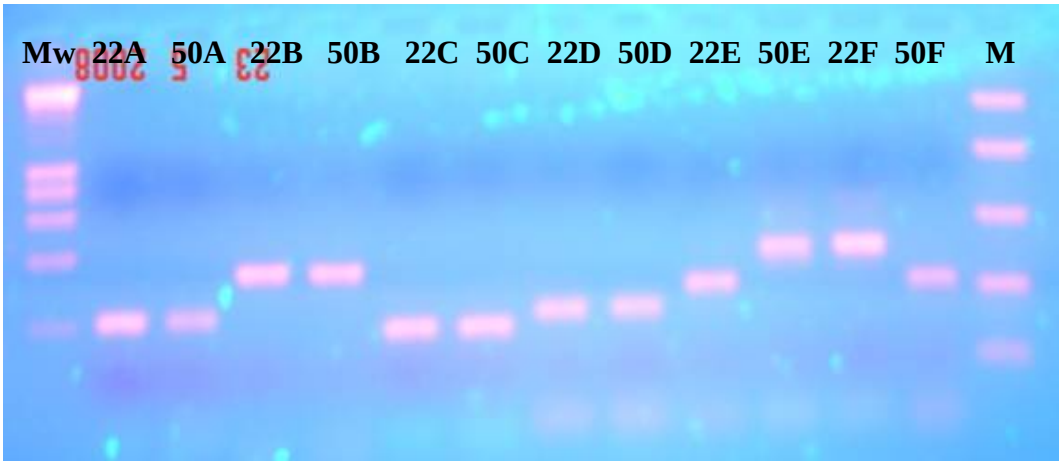
Agarose powder was dissolved in Tris Borate EDTA (TBE) buffer (1% w/v) by slowly boiling in a microwave oven. The agarose was allowed to cool to about 50^0C and ethidium bromide was added to the gel at a concentration of 1 mg/ml. While the agarose was cooling, the gel tray was prepared by sealing the open edges of a clean, dry glass tray with autoclave tape so as to form a mold to avoid leakage and so that the tray could accommodate the desired thickness of the gel. The warm agarose solution was then poured into the gel tray in which a comb was inserted to form sample slots.

The gel was allowed to set for 30 minutes before removing the autoclave tape, and immersing the gel in the electrophoresis tank containing TBE buffer. The combs were removed and 7µl of each DNA sample containing 2 µl of loading solution (Biolabs England) was loaded to the wells of the gel. DNA lambda (Biolabs England) digested with E*cor*I and Hind III restriction enzymes was used as a molecular weight marker that was run in parallel i.e. in one lane of each gel. The gel was run at a constant voltage of 100 volts until the bromo-phenol blue migrated almost to the end of the gel. The gel was then removed from the rig, placed in a UV trans-illuminator and photographed. DNA quantities ranged from 28.1 to 99.2 ng/ul. It was then diluted to give a concentration of about 10ng/µl.

5.2.4 PCR with SSR primers

A total of 6 polymorphic microsatellite primers (Source, International potato centre and Louisiana University) were used for PCR amplification of the DNA samples (Table 7). The forward primers for each of the 6 markers were labeled at the 5' end of the oligonucleotide using fluorescent dyes for screening by capillary electrophoresis on the ABI prism and 3730 genetic analyzer (Applied Biosystems). The fluorescent capillary based dyes were 6FAM (Blue), PET (Red), VIC (Green) and NED (Yellow) (Table 7). After screening of the 6 pairs of SSR primers, all were found to amplify scorable and reproducible banding profiles. Each of the 6 pairs of SSR markers was successfully optimized (Plate 4) and PCR reactions were set up in 20 µl volumes in 0.2 ml PCR tubes.

Plate 4. Amplification from the optimized conditions of six SSR primers



DNA used was from sweet potato genotype 22 (YS/07/2007) and 50 (S5 Nyatonge 1).

Table 7. Sweet potato microsatellite primers used in the study

Marker name	**Dye**	**Primer forward 5'-3'**	**Primer reverse 5'-3'**	**Repeat motif**	**Tm (^{0}C)**	**Expected product size**
IB-R03	PET	GTAGAGTTGAAGAGCGAGCA	CCATAGACCCATTGATGAAG	(GCG)5	73	243-258
1B-S07	FAM	GCTTGCTTGTGGTTCGAT	CAAGTGAAGTGATGGCGTTT	(TGTC)7	69	162-178
IB-R12	NED	GATCGAGGAGAAGCTCCACA	GCCGGCAAATTAAGTCCATC	(CAG)5A	71	303-342
IB-R16	VIC	GACTTCCTTGGTGTAGTTGC	AGGGTTAAGCGGGAGACT	(GATA)4	76	201-213
1B-R19	PET	GGCTAGTGGAGAAGGTCAA	AGAAGTAGAACTCCGTCACC	(CAG)5b	76	190-208
IB-CIP13	NED	CGTGCTTGAGGTCTGAGTAGAA	TTCCCTAGAAGCTGCGTGAT	ACC)3+(CCG)2+(TGC)3+(GTC)2	68	196-373
IB-RO8*	PET	GGCGACACCTTAGAGTAT	CACCCCCTATTCACAA	(T3A)4	-	204-216
IB-S09*	NED	GCTGCTCAATCCCTCTCCTT	GGAACTCGATACAGCGTGGT	(AT)11	-	193-203
IBCIP-7*	PET	GGTTTGACCGTGGAGTTGTT	GGACGAACTTTCCCAAATCA	(CCA)2+3+(CCG)2+1+3(CCA)3+CG4	-	39-99
IB-CIP9*	FAM	AGACTGCTAGGGTTATCTCTCCA	GACATTGCCAAGGACACTGA	(ACC)3+(CCG)2+(TGC)3+(GTC)2	-	42-63

*SSR primers which did not produce amplification.

5.2.5 DNA amplification

PCR conditions for each of the 6 SSR markers were optimized and PCR reactions were set up in a 20 µl volume. Each PCR reaction contained 10pmol/µl of each primer, 2.5mM $MgCl_2$, 10mMdNTPs, 0.1U Amplitaq Gold Polymerase (Applied Biosystems) and 5X PCR buffer (Applied Biosystems). Amplification was carried out using the Gene-Amp PCR system 9700 (Applied Biosystems) following thermocycling conditions; 1 cycle of 94°C for 2 min, followed by 15 cycles of 94°C for 1 min, 60°C for 2 min, 72°C for 1.5 min, 94°C for 1 min, 50°C for 2 min and 72°C for 1.5 min. After completion of the 15 cycles, a final extension of 10 min at 72°C was used to reduce the probability of false scoring of stutter bands as alleles.

5.2.6 Gel electrophoresis of PCR products

Following amplification, PCR products were stored at 4°C prior to electrophoresis. Agarose gel electrophoresis was carried out as outlined in section 5.2.3. Five microlitres of the PCR product was run on 2% agarose gel. To 5 µl of each PCR product, 3 µl of sample loading buffer was added and mixed by pipetting before loading the resulting mixture in the pre-formed sample wells on the gel. The samples were run alongside 1.0 µl 1kb DNA ladder at 100 volts for 45 min. After the run, the gel was viewed under UV light and photographed. Each amplified SSR fragment was visualized as a distinct band.

5.2.7 Capillary electrophoresis

The amplified DNA fragments were screened by capillary electrophoresis on the ABI 3730 genetic analyzer (Applied Biosystems). The capillary electrophoresis runs were post-PCR co-loaded in 2 groups on the basis of dye colour and fragment size and group 3 had only one primer (IB-R03). Co-loading within dyes was done only when the expected fragment sizes were different.

PCR products were co-loaded post-PCR, where a range of 0.5 and 1µl of the FAM, PET and VIC labelled products were mixed with their corresponding 9.0µl capillary electrophoresis cocktail (prepared by mixing 1ml of HIDI formamide and 12µl of Genescan™-500LIZ™ size standard (Applied Biosystems) for 96 reactions. DNA fragments were denatured and size-fractioned using capillary electrophoresis on an ABI prism 3730 automatic DNA sequencer (Applied Biosystems). Genotyping was carried out by capillary electrophoresis using the ABI PRISM 3730 (Applied Biosystems), a fluorescent based capillary detection system that uses polymer as the separation matrix at the International Livestock Research Institute (ILRI). The GeneMapper v3.7 software (Applied Biosystems) was used to size peak patterns, using the internal Genescan-500 LIZ size standard. This facilitated the accurate sizing of the microsatellite allele to within ± 0.3 base pairs (Buhariwalla and Crouch, 2004). Primers were optimized by running different ratios of PCR products and choosing the one giving the best signal profile (signal/noise ratio and relative fluorescent units (RFU)).

5.2.8 Fragment scoring

The amplified fragments were analysed using the Genemapper v3.7 software (Applied Biosystems). Size calling, which includes peak detection and fragment size matching were performed using the GeneMapper. Bins, which represent a fragment size or base pair range and dye colour that define an allele, were constructed from reference data. Algorithms were used to determine if peaks represented alleles. When a peak from a data sample matches the location of a bin, the software made an allele call. Alleles were automatically assigned allele calls based on the bin definitions. The results were stored in the GeneMapper data base. Allelobin software was used for checking the quality of the markers.

5.2.9 Statistical analysis

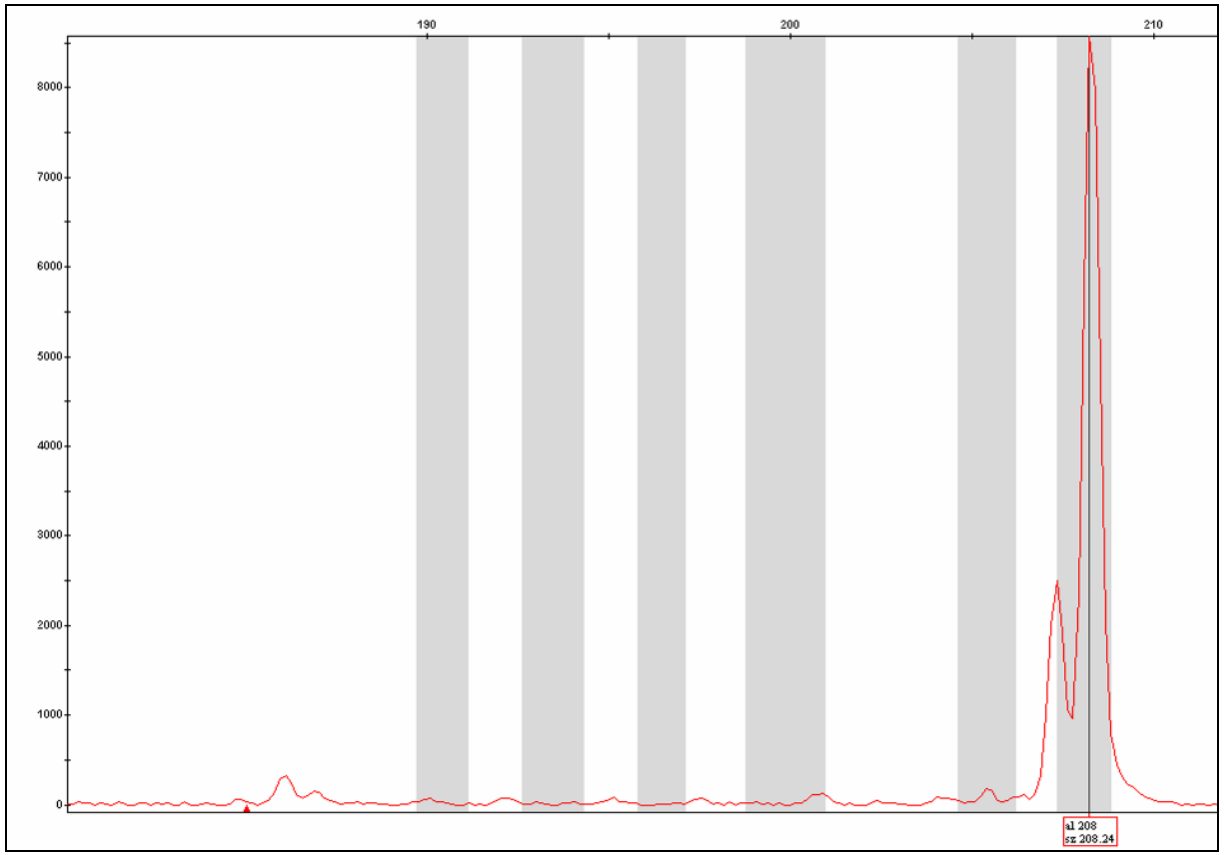
All the SSR markers showed high reproducibility and therefore the 6 markers were included in the analysis. The total number of alleles, the number of common alleles with allelic frequencies of at least 5% and the polymorphism information content (PIC) values (Bostein *et al.*, 1980; Smith *et al*., 2000) were determined for each SSR marker. The data was analysed using the SIMQUAL (Similarity for Qualitative Data) routine to generate jaccard similarity coefficients. These similarity coefficients were used to construct dendrograms using UPGMA and employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) parameters from the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) version 2.11T (Rohlf, 2000).

5.3 Results

5.3.1 Polymorphism of microsatellites used to characterize sweet potato genotypes

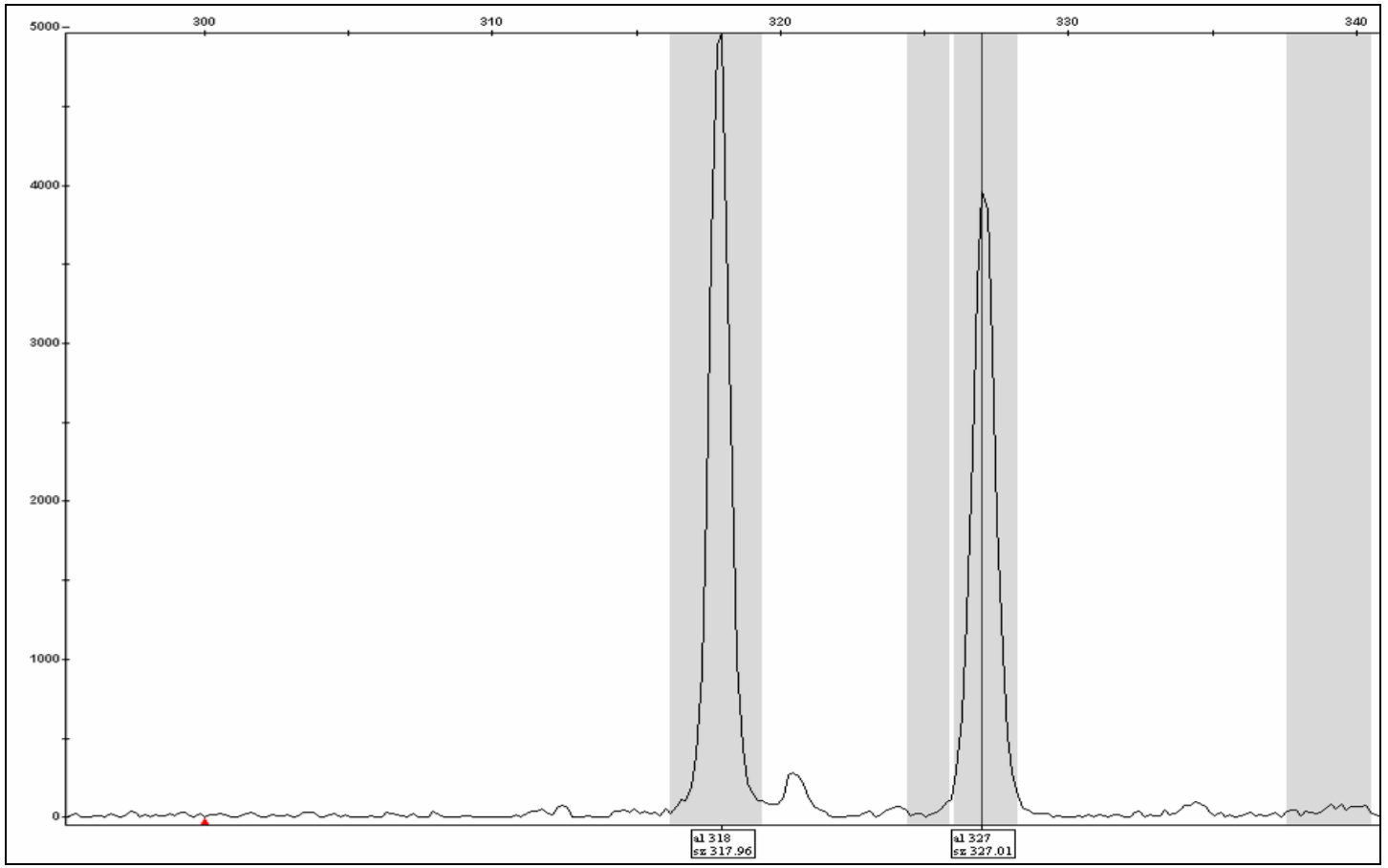
The 6 pairs of SSR primers used were polymorphic across all loci and had a polymorphic information index ranging from 0.33 to 0.81. A singe peak denoted homozygous genotypes while two clear peaks indicated heterozygous genotypes (Fig. 3 and 4).

Fig. 3. Electrophenogram showing homozygosity of genotype SP30 using marker IB-CIP13.



The X axis and Y-axis represents allele sizes and peak intensities, respectively.

Fig. 4. Electrophenogram showing heterozygosity of sweet potato genotype SP16 using marker IB-R12.



The X axis and Y-axis represents allele sizes and peak intensities, respectively.

5.3.2 Marker quality indices, allele variability, polymorphism and observed heterozygosity in the 89 genotypes

Six microsatellite markers for 6 loci were used to characterize 89 sweet potato genotypes. A total of 23 alleles were detected. The number of alleles per locus ranged from 3 for IB-CIP-13, IB-R12 and IB-S07 to 6 for IB-R19 with an average of 3.67 alleles per locus (Table 8). The maximum size range of 374 bp was observed with primer IBCIP-13 whereas the lowest of 175 bp was observed with primer pair IB-S07 (Table 9). The highest % of abundant alleles was observed in primer IB-S07 (87.91%) whereas the lowest % of abundant alleles was observed in primer IB-R19 (26.62%). The highest and lowest numbers of rare alleles at <=5% was 1 with primers IBCIP-13 and IB-S07 and none with primers IB-R16, IB-R19, IB-R12 and IB-R03, respectively. The lowest quality index was 0.0005, observed in markers IB-R16, IB-R19, IBCIP-13, IB-S07 and IB-R03 whereas the highest quality index of 0.001 was in marker IB-R12 with the average being 0.00058. The polymorphic information content (PIC) varied from 0.33 to 0.81 for genotypes with an average of 0.47. Marker IB-R19 revealed the highest PIC of 0.81 while marker IB-S07 had the lowest PIC of 0.33. Observed heterozygosity ranged from 0.21 to 1.0 with a mean of 0.75 across the six SSR loci. The highest observed heterozygosity was in marker IB-R03 with a value of 1.0 while the lowest was 0.21 in marker IB-S07 (Table 8).

Table 8. Characteristics of amplified fragments in 89 sweet potato genotypes using 6 SSR markers

Marker name	**Quality Index**	**Total no of alleles**	**Allele size range**	**Abundant Allele (%)**	**Rare Allele(s) (<=5%)**	**[a]PIC values**	**[b]Observed heterozygosity**
IB-R16	0.0005	4	202-214	41.55	None	0.69	0.99
IB-R19	0.0005	6	190-208	26.62	None	0.81	0.87
IBCIP-13	0.0005	3	206-374	56.08	206	0.53	0.87
IB-R12	0.001	3	318-339	41.18	None	0.65	0.56
IB-SO7	0.0005	3	175-191	87.91	191	0.33	0.21
IB-R03	0.0005	4	243-258	29.08	None	0.74	1.00
Mean	**0.00058**	**3.83**		**47.07**		**0.47**	**0.75**

[a] PIC=1-Σ(pi^2) (where Pi is the frequency of the i^{th} allele detected) and [b] Frequency at which heterozygous individuals occur in a population at a given locus

Table 9. Number and size of alleles detected in 89 sweet potato genotypes with 6 SSR markers

Marker name	[a]Alleles (base pairs)					
IB-R16	202	206	210	214		
IB-R19	190	193	196	199	205	208
IBCIP-13	206	338	374			
IB-R12	318	327	339			
IB-SO7	175	179	191			
IB-R03	243	249	252	258		

[a]Allele variants at a specific SSR locus

5.3.3 Genetic variability within the 89 sweet potato genotypes

5.3.3.1 Number of alleles

The 89 genotypes used were classified into two groups based on their response to SPVD inoculation in the screenhouse. The two groups were resistant and susceptible with 20 and 69 genotypes, respectively (Chapter 3). The number of alleles within the 89 genotypes across the 6 loci ranged from 10 to 17, with an average of 13.52 (Appendix 6). The highest (17) number of alleles was observed in genotypes SP33, SP66 and SP68, whereas the lowest was 10, which was observed in genotypes SP8, SP30, SP85, SP86 and SP87 all of which appeared resistant to SPVD except SP30. All the 89 genotypes (both resistant and susceptible groups) had the same alleles of 206, 374 and 175 at loci IB-R16, IBCIP-13 and IB-S07, respectively. At locus IB-R19, the most abundant alleles were 193 and 208 bp for the resistant and susceptible genotypes, respectively. At locus IB-R12, the

resistant and susceptible genotypes had 339 and 327 bp, respectively, as the most common allele. Also at IB-R03, the most common/abundant alleles were 252 and 258 bp for the resistant and susceptible genotypes, respectively. The rare allele of 191 bp at locus IB-S07 was observed in 6 genotypes namely SP79 (resistant), SP72 (resistant), SP78 (resistant), SP24 (susceptible), SP35 (susceptible) and SP40 (susceptible), whereas the rare allele of 206 bp at locus IBCIP-13 was observed in 5 genotypes namely SP54, SP42, SP55, SP53 and SP56, all of which were susceptible.

5.3.3.2 Allele frequencies

The allele frequencies ranged from 0.03 to 0.52 in marker IBCIP-13. The highest and lowest allele frequencies were observed in allele 374 and 206 bp, respectively, both at locus IBCIP-13 (Table 10).

Table 10. Allele frequencies of the SSR markers

Marker	Alleles (base pairs)	Allele frequency
IB-R16	202	0.26
	206	0.41
	210	0.06
	214	0.26
IB-R19	190	0.10
	193	0.18
	196	0.13
	199	0.28
	205	0.14
	208	0.17
IBCIP13	206	0.03
	338	0.45
	374	0.52
IB-R12	318	0.37
	327	0.41
	339	0.22
IB-SO7	175	0.81
	179	0.12
	191	0.07
IB-R03	243	0.16
	249	0.26
	252	0.28
	258	0.29

5.3.3.3 Polymorphism of the 89 sweet potato genotypes

Polymorphism within the 89 genotypes ranged from 43.48 to 73.91% with an average of 58.77% (Appendix 7). Three genotypes namely SP33, SP66 and SP68 showed the highest polymorphism of 73.91% across the 6 loci. The lowest level of polymorphism (43.48%) was observed in 5 genotypes namely SP8, SP30, SP85, SP86 and SP87; all of which appeared resistant to SPVD except genotype SP30. The 20 genotypes that appeared resistant to SPVD did not exhibit the same percentage polymorphism across the 6 SSR loci (Table 11).

Table 11. No. of genotypes and their corresponding percentage polymorphism across the 6 SSR loci

No. of genotypes	SPVD resistant genotypes*	% polymorphism
5	SP8, SP85, SP86, SP87	43.48
9	None	47.83
10	SP67, SP18, SP39	52.17
19	SP5, SP13, SP23, SP44, SP65, SP79, SP81, SP88, SP20,	56.52
14	SP38, SP72, SP78	60.87
24	SP75	65.22
2	None	69.57
3	None	73.91

* Resistant genotypes had a symptom severity score of between 1.0-1.5 and tested negative to SPFMV and SPCSV

5.3.4 Genetic relationships among the 89 sweet potato genotypes

The genetic similarity among the 89 genotypes ranged from 0.26 to 1.0 with an average of 0.62. The genetic similarity matrix showed that the most closely related genotypes were SP8, SP85, SP86 and SP87; SP48, and SP49; SP5, SP23, SP20 and SP81; SP1, SP25, SP31, and SP73; and SP33, SP36, SP70 and SP74; SP9 and SP49; and between SP7 and SP77. The most distantly related genotypes were observed between the genotypes SP5 and SP27, SP23 and SP28, SP5 and SP28, SP28 and SP81 and SP28 and SP20 with minimum (low) genetic similarity matrix of 0.26. The similarity matrices showed that the 20 genotypes that appeared resistant to SPVD were not genetically similar (Table 12). Similarly, the genotypes with very high dry matter were not genetically similar.

Table 12. Relationship among resistant and susceptible sweet potato genotypes based on microsatellite similarity matrix

Genotype	SP2	SP3	SP4	SP5	SP8	SP13	SP23	SP38	SP44	SP65	SP67	SP72	SP75	SP78	SP79	SP81	SP85	SP86	SP87	SP88	SP18	SP20	SP39
SP2*	1.00																						
SP3*	0.53	1.00																					
SP4*	0.69	0.71	1.00																				
SP5	0.39	0.42	0.40	1.00																			
SP8	0.57	0.41	0.47	0.53	1.00																		
SP13	0.79	0.69	0.75	0.30	0.44	1.00																	
SP23	0.39	0.42	0.40	1.00	0.53	0.30	1.00																
SP38	0.53	0.75	0.61	0.59	0.50	0.59	0.59	1.00															
SP44	0.56	0.80	0.56	0.53	0.53	0.63	0.53	0.93	1.00														
SP65	0.47	0.59	0.65	0.53	0.53	0.53	0.53	0.69	0.63	1.00													
SP67	0.50	0.73	0.50	0.56	0.57	0.56	0.56	0.86	0.92	0.67	1.00												
SP72	0.44	0.56	0.53	0.42	0.60	0.50	0.42	0.56	0.59	0.69	0.63	1.00											
SP75	0.42	0.61	0.50	0.65	0.39	0.47	0.65	0.81	0.75	0.65	0.69	0.61	1.00										
SP78	0.63	0.47	0.53	0.59	0.50	0.50	0.59	0.56	0.59	0.50	0.53	0.47	0.61	1.00									
SP79	0.39	0.59	0.40	0.53	0.53	0.44	0.53	0.69	0.73	0.53	0.79	0.80	0.75	0.50	1.00								
SP81	0.39	0.42	0.40	1.00	0.53	0.30	1.00	0.59	0.53	0.53	0.56	0.42	0.65	0.59	0.53	1.00							
SP85	0.57	0.41	0.47	0.53	1.00	0.44	0.53	0.50	0.53	0.53	0.57	0.60	0.39	0.50	0.53	0.53	1.00						
SP86	0.57	0.41	0.47	0.53	1.00	0.44	0.53	0.50	0.53	0.53	0.57	0.60	0.39	0.50	0.53	0.53	1.00	1.00					
SP87	0.57	0.41	0.47	0.53	1.00	0.44	0.53	0.50	0.53	0.53	0.57	0.60	0.39	0.50	0.53	0.53	1.00	1.00	1.00				
SP88	0.47	0.50	0.56	0.73	0.53	0.44	0.73	0.59	0.53	0.63	0.56	0.42	0.56	0.69	0.44	0.73	0.53	0.53	0.53	1.00			
SP18	0.50	0.44	0.59	0.56	0.69	0.39	0.56	0.53	0.47	0.79	0.50	0.63	0.50	0.53	0.47	0.56	0.69	0.69	0.69	0.67	1.00		
SP20	0.39	0.42	0.40	1.00	0.53	0.30	1.00	0.59	0.53	0.53	0.56	0.42	0.65	0.59	0.53	1.00	0.53	0.53	0.53	0.73	0.56	1.00	
SP39	0.50	0.44	0.69	0.47	0.57	0.47	0.47	0.53	0.47	0.79	0.50	0.63	0.50	0.53	0.47	0.47	0.57	0.57	0.57	0.56	0.85	0.47	1.00

* Sweet potato genotypes susceptible to SPVD.

NB: Similarity matrix was constructed from binary data with Jaccard's coefficients.

5.3.5 Phylogenetic analysis

The UPGMA analysis clustered 89 genotypes into 2 main clusters namely A and B and 5 sub-clusters (Fig. 5). The Jaccard's coefficient ranged from 0.5 to 1, accounting for 50% variation among the 89 genotypes. Cluster A contained 72 genotypes of which 6 had appeared resistant to SPVD, whereas cluster B contained 17 genotypes of which 14 had appeared resistant to SPVD. Cluster A, sub-cluster I contained 41 genotypes of which SP38, SP44, SP67, SP72, SP79 and SP75 were resistant to SPVD. This sub-cluster contained 3 (SP21, SP24 and SP33) and 12 genotypes with very high and high dry matter, respectively. Cluster A, sub-cluster II and III contained 7 and 24 genotypes, respectively, all of which were susceptible to SPVD. Cluster A sub-cluster III has 11 genotypes with high dry matter content. Cluster B, sub-cluster I had 9 genotypes of which 3 namely SP48, SP59 and SP71 were susceptible to SPVD. Cluster B, sub-cluster II contained 8 genotypes which had appeared resistant to SPVD with 4 genotypes namely SP19, SP39, SP65 and SP87 containing high dry matter. Generally, genotypes with high dry matter content were distributed across all the sub-clusters in the UPGMA dendrogram generated. Out of the 89 genotypes, 10 were resistant to SPVD and had high dry matter content and were not clustered together in the dendrogam.

Phylogenetic analysis revealed strong genetic similarity between the genotypes SP8, SP85, SP86 and SP87, between SP48, and SP49, between SP5, SP23, SP20 and SP81, between SP1, SP25, SP31, and SP73, and SP33, SP36, SP70 and SP74. Strong similarities were also detected between SP9 and SP49, and between SP51 and SP 77 (Figure 5).

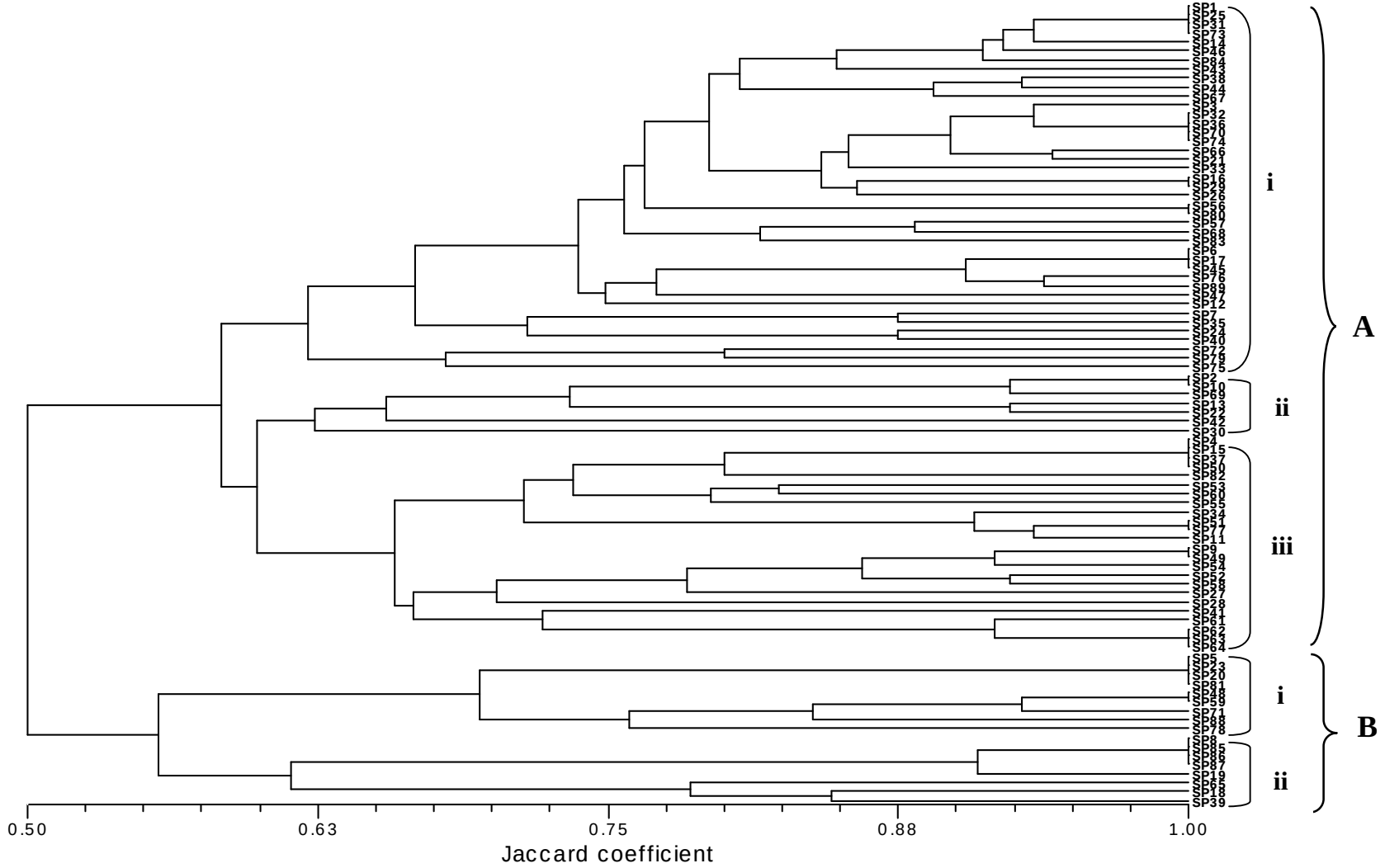


Fig. 5. UPGMA dendrogram of 89 sweet potato genotypes (Appendix 2) based on genetic similarity matrix calculated from SSR markers.

5.3.6 Principal Component Analysis (PCA) based on genetic distance estimates of 89 genotypes

The results of the PCA were consistent with those of UPGMA analysis. There was no clearly distinguishable pattern of clustering of genotypes on the basis of resistance to SPVD, however most of the genotypes that appeared resistant to SPVD grouped in the same area of the plot. The first and second principal components accounted for 24.7 and 13.8% of the total variation, respectively. The PCA scatter plot which gave the spatial representations of genetic distances among genotypes, revealed two major cluster groups (Fig. 6). The first group (A) made up of 72 genotypes with only 6 SPVD resistant genotypes, and the second group (B) comprised 14 resistant and 3 susceptible genotypes. Generally the PCA scatter plot, detected trends similar to the clustering illustrated in the dendrogram. For instance, the close associations among SP8, SP85, SP86 and SP87; SP5, SP23, SP20 and SP81; and between SP48, and SP49, revealed by the dendrogram were also detected in group B of the PCA.

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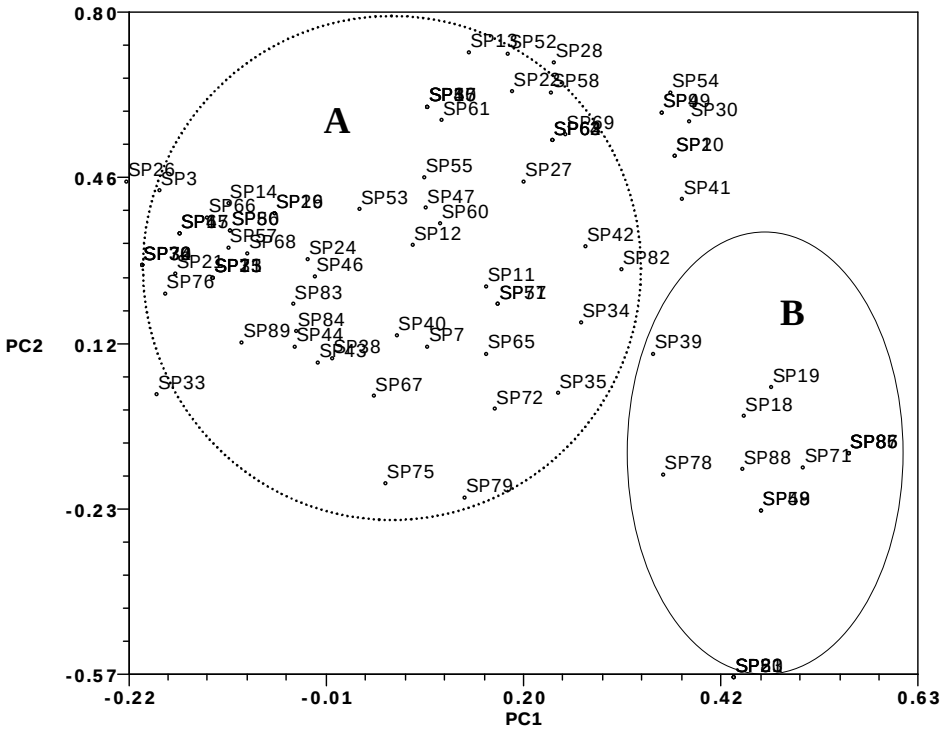

Fig. 6. Principal component analysis of 89 sweet potato genotypes using 6 SSR markers. PC1 and PC2 represent 24.72% and 13.82% of the total variation, respectively.

5.4 Discussion

Assessment of genetic diversity and relationships among genotypes is of interest not only for germplasm conservation but also for breeding purposes, particularly during the selection of parents resistant to diseases. The use of high yielding cultivars has led to considerable erosion of genetic diversity during the first few decades (Hawkes, 1987). Since the pool of available variation for disease resistance genes within cultivars is becoming limited, there is need to find novel sources of resistance (Callow *et al*., 1997), which would become the sources of new useful diversity and genetic resources (Swanson, 1996).

The present study is the first genetic evaluation of Kenyan sweet potato genotypes for resistance to SPVD and high dry matter content using microsatellite markers. Microsatellite markers have shown high levels of genetic polymorphism in many important crops. The present study showed that microsatellite markers were also highly polymorphic in sweet potato. The high level of polymorphism associated with SSR markers may be a function of unique replication slippage mechanism responsible for generating SSR allelic diversity (Pejic *et al*., 1998). It has also been suggested that high levels of polymorphism in microsatellite markers are related to the mechanism of mutations and the high rate at which they occur (Ashley and Dow, 1994). The polymorphic information content (PIC) of an SSR marker provides an estimate of the discriminatory power of that SSR marker by taking into account not only the number of alleles that are detected but also the relative frequencies of those alleles (Smith *et al*., 2000). Five out of the 6 SSR markers used in this study revealed a high discriminatory power (PIC of greater than 0.5) and hence were highly informative. The low PIC value of 0.33 for IB-SO7 could have been due to it being highly monomorphic.

Microsatellite markers have been used to investigate genetic diversity of a wide range of species in rice (Yang *et al*., 1994), wheat (Plaschke *et al*., 1995) and maize (Senior *et al*., 1998). The number of alleles amplified per primer pair ranged from 3 to 25 in rice, 3 to 16 in wheat and 2 to 23 for maize. In the present study 23 alleles were amplified from the 89 sweet potato genotypes. Thus the level of microsatellite polymorphisms in sweet potato is relatively high and similar to other out-crossing crops. One possible reason for the high degree of variation among the genotypes observed in this study may be related to

the mating system of sweet potato; a cross pollinating and hexaploid species (Ozias-Akins and Jarret, 1994). New genotypes would have arisen from seeds resulting from cross pollination, hence high genetic variation. The other possible reason is that the materials used in the present study were from diverse geographical areas and thus had a relatively wide genetic base. In this study, rare alleles were observed in several genotypes. The presence of these rare alleles may be explained by the relatively high rate of mutation in SSR loci (Henderson and Petes, 1992). Such alleles are important because they may be diagnostic for particular regions of the genome specific to a particular genotype of sweet potato. High levels of heterozygosity was observed in this study, and varied greatly across the six loci, ranging from 0.21 (IB-S07) to 1.00 (IB-R03), with a mean of 0.75. This could be attributed to the outbreeding nature of sweet potato, where the proportion of heterozygous loci is likely to be high. It has also been reported that self-incompatibility in the flowers (Martin, 1965) results in allogamy, increasing genetic heterozygosity (Thompson *et al.*, 1997).

Being a hexaploid species, each individual genotype could contain between one and six alleles at any one locus, assuming it is an autopolyploid. In this study, the number of alleles from any given variety ranged from 3 to 6. The presence of hexa-allelic genotypes indicates that sweet potato is an autohexaploid and the SSR alleles are in a pattern of tetrasomic inheritance. This agrees with previous findings that these SSRs were in tetrasomic segregation in sweet potato (Buteler *et al.*, 1999).

The mean genetic similarity of 0.62 obtained in this study is lower than values of 0.69 and 0.71 found among sweet potato cultivars in Taiwan and Tanzania, respectively, (Tseng *et al*. 2002; Elameen *et al*., 2008), and higher than that found among genotypes from South America (0.58) (Zhang *et al*., 2000). This is not surprising since the genetic diversity is supposed to be higher in the center of the diversity (South America), and the introductions both to Africa and Asia probably have involved just a few genotypes. SSR-based Jaccard's coefficient ranged from 0.5 to 1, with an average of 0.752, accounting for 50% variation among the 89 genotypes. These Jaccard coefficients are significantly higher than those obtained by Hwang *et al*. (2002), who used simple sequence repeats (SSR) to analyze sweet potato cultivars and found them to have an average similarity coefficient of 0.658, which was consistent with RAPD data (Connolly *et al*., 1994). He *et al*. (1995) reported that high levels of polymorphism among sweet potato plants are fixed through vegetative reproduction and maintained through high levels of gene flow because of the self-incompatibility of this plant. In the present study, the results of the PCA using SSR markers supported the result of the UPGMA clustering suggesting that SSR markers are efficient for detecting genetic relationships in sweet potato genotypes resistant or susceptible to SPVD and with high/low dry matter content.

The genetic differences among the SPVD resistant genotypes revealed by their clustering into distinct groups suggest the presence of different sources of resistance to SPVD. Hierarchical UPGMA analysis and PCA, revealed three groups of SPVD resistant genotypes. Generally, the relationship between genotypes in the cluster groups could not be attributed to their resistance to SPVD or high dry matter.

The PCA analysis further provides information about associations between genotypes, which are useful to formulate better strategies for breeding. The absence of strong associations among the genotypes in the groups implies significant diversity within each cluster group, and the dominant independent role in the cluster groups along each separate principal component implies significant diversity between the groups. It is therefore envisaged that, combining genotypes from the different groups as parents in breeding would result in diversifying SPVD resistance genes in the breeding population.

Previous studies have shown that the most preferred characters for the selection of specific cultivars by farmers are early bulking, tuber quality attributes and high storage root yield (Ndolo *et al.*, 1998). It is possible that whilst selecting, utilizing and distributing landraces with their preferred agronomic and quality traits, farmers have inadvertently added useful SPVD resistant genotypes to the germplasm available to them. This could explain the associations between the resistant and susceptible genotypes; for example SP5 and SP23, which clustered together despite their different SPVD resistance status, and SP38, SP43, SP44 and SP67, which clustered with the majority of susceptible genotypes.

Incorporating SPVD-resistant genotypes which have other desirable agronomic and consumer quality traits such as high dry matter content, from the different cluster groups into the breeding programmes as parents, would ensure the diversification of resistance to the disease while creating new genotypes.

In addition, by combining different genes that relate to different sources of resistance, epistatic interaction may be identified such that higher levels of resistance can be developed to protect the crop.

The high discriminatory capacity of microsatellite markers observed in other species has been confirmed in the present study. The SSR markers used in this study were informative in revealing the genetic relationships among 89 Kenyan sweet potato genotypes. The 89 sweet potato genotypes in this study can be fully identified with as few as three SSRs. The presence of easily scorable, unique alleles and/or allele combinations makes microsatellite markers an ideal system for genotype identification. The nature of microsatellites as being selectively neutral, co-dominant, and sequence tagged makes them a very useful tool for germplasm management, as well as for genome mapping of sweet potato. This study has shown that despite the damaging effects of the disease on the crop, there is a significant amount of genetic variability among the SPVD resistant genotypes, which could be utilized in breeding to diversify resistance to the disease.

CHAPTER SIX

6.0 GENERAL DISCUSSION AND RECOMMENDATIONS

Sweet potato (*Ipomoea batatas*) plays an important role as a major component of diets and as a food security crop in many Kenyan households. Sweet potato virus disease (SPVD) caused by the dual infection and synergistic interaction between sweet potato feathery mottle *potyvirus* (SPFMV) and sweet potato chlorotic stunt *crinivirus* (SPCSV) (Gibson *et al*., 1998) is a major constraint to sweet potato production since it can reduce yields of infected plants by up to 98% (Gutierrez *et al*., 2003). Since the use of resistant cultivars is the most effective means of reducing sweet potato losses due to SPVD, there is need to identify cultivars resistant to SPVD. The choice of sweet potato cultivars in Kenya is also determined by consumer acceptable attributes such as taste and dry matter content. Taste acceptability of sweet potato is dependent on the dry matter content with high dry matter being preferred.

Following graft-inoculation of the 314 sweet potato genotypes with scions pre-infected with SPVD, 20 (6%) genotypes showed mean SPVD severity scores of between 1.00 and 1.50 and tested negative for both SPFMV and SPCSV in NCM-ELISA. This indicates their relative resistance to SPVD and therefore could have potential for use in sweet potato genetic improvement. The 20 sweet potato genotypes should be planted in the field to confirm their actual resistance status. These genotypes namely; OP-LNA-006-08, TVT/02/2007, WFTC/03/2007, YS sopalla, Marooko (1), KKFS Mwavuli, YS Kemb 10, YS Nyanguyegwo, Marooko (2), KAK/04/2007, KKFS 56682/03 (1), Kamau (1),

Naspot, MKN/04/2007, Katumani (2), Kikuyu (3), Katumani (7), Kikanda (1), Kikamba (2) and SPK004 (Katumani) which have been regarded as resistant to SPVD can be used as parents for the development of high yielding virus resistant genotypes for the farmers.

Morphological characterization of the 314 sweet potato genotypes using 42 traits revealed significant variations in the vine, leaf, storage root and floral characters. Phylogenetic analysis of the sweet potato genotypes resulted in two major clusters and 9 sub-clusters. Determination of the dry matter content of the 314 genotypes revealed a significantly high variation among the genotypes that ranged from 20.0 to 37.8%. The phenogram generated using the scored traits did not reveal any unique clustering of the sweet potato genotypes according to dry matter content or reaction to SPVD. This indicates that morphological markers are not reliable in classifying sweet potato genotypes into groups based on their dry matter content or resistance to SPVD.

Molecular characterization of the 89 sweet potato genotypes revealed high genetic diversity among the genotypes. Phylogenetic and PCA analysis grouped the 89 genotypes into 2 main clusters and 5 sub-clusters. SPVD resistant genotypes were grouped in different sub-clusters throughout the dendrogram and together with the susceptible ones. Similarly, genotypes with high dry matter were grouped in different sub-clusters as were those with low dry matter. This indicates that there is a significant amount of genetic variability among the SPVD resistant and high dry matter genotypes. This molecular characterization provides valuable information for breeders and will lead to more efficient development of new cultivars resistant to SPVD and have high dry matter.

Since the SSR markers used in this study were developed to determine genetic diversity of sweet potato genotypes, there is need to use markers linked to the genes responsible for resistance to SPVD and high dry matter in order to facilitate the effective identification of quantitative trait loci linked to SPVD resistance and high dry matter. Results from this study have shown the efficiency of SSR markers in molecular characterization of sweet potato genotypes that are resistant to SPVD and have high dry matter. Although no unique allele (s) was identified in sweet potato genotypes exhibiting these two traits, the study has revealed their high genetic diversity and hence their potential as parents for genetic mapping studies.

Further work on resistance to SPVD and high dry matter in sweet potato should include i) Using the selected genotypes and a higher number of SSR markers to determine trait-marker association using discriminant analysis and logistic regression ii) Develop a genetic linkage map based on SSR markers using a mapping population segregating for SPVD resistance and high dry matter iii) Using the SSR markers and the linkage map generated to tag genes controlling SPVD resistance and high dry matter.

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APPENDICES

Appendix 1: Analysis of variance of symptom severity among 89 sweet potato genotypes

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	Variance ratio	F value
Variety	88	113.11	1.29	239.98	<0.001
Residual	178	0.95	0.005		
Total	266	114.01			

Appendix 2: Reaction of sweet potato genotypes after challenging with SPVD (SPFMV+SPCSV) by graft inoculation and their dry matter content.

Code	**Genotype name**	**Mean SPVD score**	**Symptoms**	***NCM ELISA**		Dry matter content
				SPFMV	SPCSV	(%)
SP1	TVT/07/2007	3	VC,P,LD,S	++	-	28.9
SP2	TVT/09/2007	2.8	VC,LD,CS	+++	++	30.26
SP3	TVT/03/2007	1.5	CS,VC,PS	+++	+	27.68
SP4	Kanini kaseo	1.6	CS,VC,P	++	-	24.52
SP5	OP-LNA-006-08	1.37	CS,P	-	-	28.95
SP6	MLD/05/2007	1.87	VC,Y,P,CS	-	+	23.26
SP7	Karoti (2)	2.57	VC,P,Ld	-	+	23.44
SP8	TVT/02/2007	1.13	P	-	-	28.19
SP9	TVT/12/2007	1.43	VC,P,CS	+	-	35.6
SP10	KARI Mtwapa OP-T21	2.2	PS,PRS	+++	-	28.58
SP12	MLD/01/2007	2.3	P,VC	++	-	25.41
SP13	Kikanda (2)	1	NONE	+++	+++	29.4
SP14	Kiazi cha nduma	2.53	CS,VC,P	+	+	28.33
SP15	Yellow 1	2.63	CS,VC,P	-	+	30.67
SP16	WFTC/02/2007	2.23	VC,P	+	+++	27.2
SP17	KWL/04/2007	2.07	VC,CS,P	+++	+++	21.61
SP23	Ys Kemb 10	1.23	VC,P	-	-	31.08
SP24	Farmer 5 Bungoma	1.4	P	++	+	27.8
SP25	ALPFS Were	1.9	VC,P	+	-	33.05
SP26	KAK/07/2007	1.93	VC,P	+	-	31.58
SP27	Dada Mowar	1.87	P	++	++	35.89
SP28	SYA/04/2007	1.73	P	+++	+++	35.89
SP29	Marooko (2)	2.37	P	+++	+++	29.94
SP30	ALPFS 2002/141	2.73	P	+	+	35.52
SP31	SYA/01/2007	1.77	P	+++	+++	33.76
SP32	Sadak	1.6	P	+	-	28.8
SP33	YS Masaba	1.57	P	-	+++	32.5
SP34	TS/01/2007	1.7	P	+	-	28.56
SP35	ALPFS Mbita	2.87	P	++	++	33.76
SP36	YS Sample 2	1.63	P	+	++	28.96

SP37	Big G	1.13	P	+	-	27.67
SP38	YS Nyanguyegwo	1.13	P	-	-	31.54
SP41	BGM/02/2007	1	NONE	+	+	35.68
SP42	KKFS Salyboro	1.67	VC,CS,P	+	++	33.61
SP43	MCK/21/2007	1	NONE	+++	+	34.41
SP44	KKFS 56682/03 (1)	1.07	VC	-	-	27.43
SP45	KRG/01/2007	1.51	P	+	+++	26.27
SP46	Kemb 10 (1)	1.8	P	++	+++	34.29
SP47	Msichana Nairobi	1.6	P	+	++	28.78
SP48	S13 Nyatonge (2)	1.87	P	+++	-	26.4
SP49	S4 Kuny kibuojo	1.53	P,VB	+	++	26.98
SP50	S5 Nyatonge (1)	1.57	P	+	+	32.92
SP51	S11 (Nyatonge (3)	1.51	P	-	+	32.08
SP52	Polista	1.52	P	+	+++	34.1
SP53	K9 (1V)	1.57	P,Fan L	+	+	28.71
SP54	K9 (2V)	1.73	P	++	++	29.28
SP55	Tainung	2.57	P,Fan L	++	+	30.11
SP56	S6 Mwavuli	2.37	P	+	+	29.07
SP57	SPK 004 (1)	1.54	P	-	+	25.08
SP58	K16 (1V)	1.53	P	++	++	34.15
SP59	S6 Ondiek chilo	1.53	P	+++	+++	28.96
SP60	Kemb 23	1.6	P	+++	+++	30.2
SP61	S1 Amina (2)	1.61	P	+++	+	28.3
SP62	K15	1.8	P,PRS	++	+++	34.02
SP63	Muibai	1.53	P	+++	+++	22.49
SP64	Bungoma	1.57	P,PRP	+++	+	29.67
SP65	Kamau (1)	1.37	P	-	-	32.91
SP66	Amina (1)	1.59	CS	+	-	25.6
SP67	Naspot	1.4	P	-	-	34.82
SP68	MCK/17/2007	2.47	P	+++	+++	34.24
SP69	MKN/08/2007	1.73	P	+++	++	29.66
SP70	Mwei umwe (4)	2.07	P,CS	+++	+++	30.11
SP71	MKN/07/2007	1.7	P	+++	++	34.93
SP72	MKN/04/2007	1.47	CS	-	-	35.22
SP73	MKN/06/2007	1.83	P	-	+	33.13
SP74	Kikuyu cha kikamba	1.53	VC,Fan L	+++	+++	25.43
SP75	Katumani (2)	1.47	LC,VC	-	-	27.42
SP76	Mwei umwe (5)	1.7	CS,IC,P	++	++	34.22

SP77	KBZ/01/2007	1.83	P	-	+	35.03
SP78	WFTC/03/2007	1.3	P	-	-	28.11
SP79	Kikuyu (3)	1.4	P	-	-	34.58
SP80	Katumani (5)	2.17	P	+++	+++	34.81
SP81	Katumani (7)	1.47	P,PRS	-	-	24.56
SP82	Kiluu	1.63	P	+	-	30.01
SP83	Ilukwasi	1.8	CS,P,IC	++	++	31.72
SP84	MKN/02/2007	1.87	P	+++	+++	33.76
SP85	Kikanda (1)	1	NONE	-	-	31.32
SP86	Kikamba (2)	1	NONE	-	-	34.66
SP87	SPK 004 (Katumani)	1.2	P	-	-	32.78
SP88	KAK/04/2007	1	NONE	-	-	31.23
SP89	MCK/23/2007	1.63	P	++	-	25.39
SP11	Nyakuwili	1.07	CS	++	-	33.99
SP18	YS Sopalla	1.43	LS,LC,P,LD	-	-	29.18
SP19	Marooko (1)	1.37	P	-	-	32.07
SP20	KKFS Mwavuli	1.2	P,VC	-	-	29.72
SP21	KKFS NK-L-22	2.5	P	++	++	28.02
SP22	YS/05/2007	2.63	VC,P	+++	++	32.44
SP39	Marooko (3)	1.36	P	-	-	26.27
SP40	BSA/02/2007	2.1	P	+	+	33.89

Key:

*NCM-ELISA. SPVD severity score determined on a scale of 1-5 where; 1 = no visible symptoms, 2 = very mild symptoms on leaves consisting mainly of chlorotic and/or purple spots; 3 = moderate symptoms of chlorotic spots, vein clearing, interveinal chlorosis, mottling, and mosaic; 4 = severe symptoms of purpling/yellowing or mosaic on leaves, moderate distortion of leaves shape and moderate stunting and 5 = very severe symptoms of purpling / yellowing or mosaic on leaves, severe leaf distortion, reduced leaf size, plant severely stunted.

Symptom expression: NS, no symptoms; CS, chlorotic spots; VC, vein clearing; IC, interveinal chlorosis; LC. Leaf curl; Fan L, fan shaped leaf; P, purpling; PRS, purple ring spots; VB, vein banding; Y, yellowing; LD, leaf deformation; S, stunting

- No purple colour
+ Faint purple colour
++ Moderate purple colour
+++ Intense purple colour

Appendix 3: Morphological characters used in phenotypic evaluation of sweet potato genotypes

A	**Twining**	0 Non twining 3 Slightly twining 5 Moderately twining 7 Twining 9 Very twining	**W**	**Secondary skin color**	0 Absent 1 White 2 Cream 3 Yellow 4 Orange 5 Brownish orange 6 Pink 7 Red 8 Purple red 9 Dark purple
B	**Ground cover**	3 Low (<50%) 5 Medium (50-74%) 7 High (75-90%) 9 Total (>90%)	**X**	**Predominant flesh color**	1 White 2 Cream 3 Dark cream 4 Pale yellow 5 Dark yellow 6 Pale orange 7 Intermediate orange 8 Dark orange 9 Strongly pigmented with anthocyanins
C	**Plant type**	3 Erect (<75 cm) 5 Semi-erect (75-100 cm) 7 Spreading (151-250 cm) 9 Extremely spreading (> 250 cm)	**Y**	**Secondary flesh color**	0 Absent 1 White 2 Cream 3 Yellow 4 Orange 5 Pink 6 Red 7 Purple-red 8 Purple 9 Dark purple
D	**Vine internode length**	1 Very short (<3cm) 3 Short (3-5cm) 5 Intermediate (6-9cm) 7 Long (10-12cm) 9 Very long (>12cm)	**Z**	**Distribution of secondary flesh color**	0 Absent 1 Narrow ring in the cortex 2 Broad ring in the cortex 3 Scattered spots in the flesh 4 Narrow ring in the flesh 5 Broad ring in the flesh 6 Ring and other areas in flesh 7 In longitudinal sections 8 Covering most of the flesh 9 Covering all flesh
E	**Vine internode diameter**	1 Very thin (<4mm) 3 Thin (4-6mm) 5 Intermediate (7-9mm) 7 Thick (10-12mm) 9 Very thick (>12mm)	**AA**	**Storage root formation**	1 Closed cluster 3 Open cluster 5 Dispersed 7 Very dispersed
F	**Predominant vine color**	1 Green 3 Green with few purple spots 4 Green with many purple spots 5 Green with many dark purple spots 6 Mostly purple 7 Mostly dark purple 8 Totally purple 9 Totally dark purple	**AB**	**Storage root cracking**	0 Absent 3 Few cracks 5 Medium number of cracks 7 Many cracks
G	**Secondary vine color**	0 Absent 1 Green base	**AC**	**Latex production in storage roots**	3 Little 5 Some

		2 Green tip 3 Green nodes 4 Purple base 5 Purple tip 6 Purple nodes 7 Other			7 Abundant
H	**Vine tip pubescence**	0 Absent 3 Sparse 5 Moderate 7 Heavy	**AD**	**Oxidation in storage root**	3 Little 5 Some 7 Abundant
I	**General leaf outline**	1 Rounded 2 Reniform 3 Cordate 4 Triangular 5 Hastate 6 Lobed 7 Almost divided	**AE**	**Consistency of boiled storage**	1 Watery 2 Extremely soft 3 Very soft 4 Soft 5 Slightly hard 6 Moderately hard 7 Hard 8 Very hard 9 Very hard and non-cooked
J	**Leaf lobes type**	0 No lateral lobes 1 Very slight 3 Slight 5 Moderate 7 Deep 9 Very deep	**AF**	**Undesirable color of boiled storage root**	0 None 1 Some beige 2 Much beige 3 Slightly green or grey 4 Green 5 Grey 6 Beige and green 7 Beige and grey 8 Beige and purple 9 Purple
K	**Leaf lobe number**	1,3,5,7,9	**AG**	**Texture of boiled storage**	1 Dry 3 Somewhat dry 5 Intermediate 7 Moist 9 Very moist
L	**Shape of central leaf lobe**	0 Absent 1 Toothed 2 Triangular 3 Semi-circular 4 Semi-elliptic 5 Elliptic 6 Lanceolate 7 Oblanceolate 8 Linear (broad) 9 Linear (narrow)	**AH**	**Sweetness of boiled storage root flesh**	1 Not at all sweet 3 Slightly sweet 5 Moderately sweet 7 Sweet
M	**Mature leaf size**	3 Small (<8cm) 5 Medium (8-15cm) 7 Large (16-25cm) 9 Very large (>25cm)	**AI**	**Flower color**	1 White 2 White limb with purple throat 3 White limb with pale purple ring and purple throat 4 Pale purple limb with purple throat 5 Purple 6 Other
N	**Abaxial leaf vein pigmentation**	1 Yellow 2 Green 3 Purple spot in the base of main rib 4 Purple spots in several veins 5 Main rib partially purple 6 Main rib mostly or totally purple 7 All veins partially purple 8 All veins mostly or	**AJ**	**Shape of limb**	3 semi-stellate 5 Pentagonal 7 Rounded

		totally purple 9Lower surface and veins totally purple			
O	**Mature leaf colour**	1 Yellow green 2 Green 3 Green with purple edge 4 Greyish green (due to heavy pubescence) 5 Green with purple veins on upper surface 6 Slightly purple 7 Mostly purple 8 Green upper, purple lower 9 Purple both surfaces	**AK**	**Equality of sepal length**	1 Outer two shorter 2 Equal
P	**Immature leaf color**	1 Yellow green 2 Green 3 Green with purple edge 4 Greyish green (due to heavy pubescence) 5 Green with purple veins on upper surface 6 Slightly purple 7 Mostly purple 8 Green upper, purple lower 9 Purple both surfaces	**AL**	**Sepal pubescence**	0 Absent 3 Sparse 5 Moderate 7 Heavy
Q	**Petiole length**	1 Very short (<10cm) 3 Short (10-20cm) 5 Intermediate (21-30cm) 7 Long (31-40cm) 9 Very long (>40cm)	**AM**	**Sepal color**	1 Green 2 Green with purple edge 3 Green with purple spots 5 Green with purple areas 6 Some sepals green, others purple 7 Totally pigmented-pale purple 9 Totally pigmented-dark purple
R	**Petiole pigmentation**	1 Green 2 Green with purple near stem 3 Green with purple near leaf 4 Green with purple at both ends 5 Green with purple spots throughout petiole 6 Green with purple stripes 7 Purple with green near leaf 8 Some petioles purple, others green 9 Totally or mostly purple	**AN**	**Color of stigma**	1 White 5 Pale purple 9 Purple
S	**Storage root shape**	1 Round 2 Round elliptic 3 Elliptic 4 Ovate 5 Obovate 6 Oblong 7 Long oblong 8 Long elliptic 9 Long irregular or curved	**AO**	**Color of style**	1 White 3 White with purple at the base 5 White with purple at the top 7 White with purple spots throughout 9 Purple
T	**Storage root**	0 Absent	**AP**	**Stigma exertion**	1 Inserted (shorter than

	surface defects	1 Alligator-like skin 2 Veins 3 Shallow horizontal constrictions 4 Deep horizontal constrictions 5 Shallow longitudinal grooves 6 Deep longitudinal grooves 7 Deep constrictions and deep grooves 8 Other			longest anther) 3 Same height as highest anther 5 Slightly exerted 7 Exerted (longer than longest anther)
U	**Predominant skin color**	1 White 2 Cream 3 Yellow 4 Orange 5 Brownish orange 6 Pink 7 Red 8 Purple-red 9 Dark purple			
V	**Intensity of predominant skin color**	1 Pale 2 Intermediate 3 Dark			

Appendix 4: Dry matter content of 314 sweet potato genotypes

Genotype	Dry matter content (%)	Flesh colour
Kambia mwongo (2)	20.0	White
WFTC/02/2007	21.6	White
BSA/02/2007	22.9	Cream
KWL/01/2007	23.0	White
Kanini kaseo	23.0	Cream
MLD/05/2007	23.3	White
WFTC/04/2007	23.4	White
WFTC/10/2007	23.8	Cream
MKN/06/2007	24.0	White
K5	24.3	White
Kanini kaseo	24.5	White
S4 Kuny Kibuojo	25.1	White
Katumani (7)	25.4	Cream
Nyakuwili	25.4	White
Amina 1	25.4	White
Mwei umwe (2)	25.5	Cream
MCK/20/2007	25.6	White
YS/04/2007	26.2	Cream
KAK/04/2007	26.3	Cream
Big G	26.3	White
K10	26.28	White
TVT/05/2007	26.4	White
KWL/02/2007	26.5	White
TS/03/2007	26.6	White
TVT/13/2007	26.9	White
VGH/03/2007	27.1	Cream
YS Sample 2	27.4	White
MCK/22/2007	27.5	White
S8 Nyamirare	27.5	Cream
K4	28	White
MCK/10/2007	28.0	White
Kikamba (2)	28.0	White
Mwei umwe (4)	28.1	White
TVT/02/2007	28.2	White
K9 (IV)	28.3	Cream
Kikanda (2)	28.3	White
Ex-Shimba Hills (2)	28.4	White
S6 K117	28.4	White

Mtwapa OP-T69	28.5	Cream
OR-Nyasi-022	28.5	White
Kikuyu	28.6	Cream
K 16 (IV)	28.6	White
Kasichana	28.6	White
Mtwapa OP-T21	28.6	Cream
KRG/01/2007	28.7	White
KKFS Mwanamonde	28.7	White
MLD/04/2007	28.8	White
TVT/07/2007	28.9	Cream
Nyathi Odiewo(2)	28.9	Cream
OP-LNA-006-08	29	Cream
KWL/07/2007	29	White
S11 Nyatonge (3)	29	Cream
S13 Nyatonge (2)	29.1	White
Ilukwasi	29.1	Cream
KKFS mwanamonde	29.2	Cream
YS Sample 4	29.2	White
Kemb 10 (1)	29.2	Cream
YS Salyboro	29.3	Cream
S1 Nyamvi	29.4	White
Kikamba (1)	29.5	White
MCK/12/2007	29.5	Cream
Local	29.5	White
S6 Mwavuli	29.6	Cream
K11	29.6	White
TVT/03/20007	29.71	White
Kikanda (1)	29.7	White
MCK/07/2007	29.7	Cream
KAK/01/2007	29.9	Cream
YS/05/2007	30.0	Cream
Kikuyu cha kikamba	30.0	Cream
KAK/03/2007	30.0	Cream
KKFS Odinga	30.0	Cream
Ex-Shimba Hills (1)	30.0	White
Kikuyu (2)	30.0	White
S12 Msichana Nai	30.1	Cream
K 15	30.1	White
TVT/08/2007	30.1	White
MKN/03/2007	30.1	Cream
MLD/03/2007	30.1	Cream
K 14	30.2	White
BGM/01/2007	30.2	White

KWL/03/2007	30.2	White
WFTC/09/2007	30.2	White
KARI Mtwapa SH93	30	White
Nyathi Odiewo (1)	30.5	White
BGM/03/2007	30.6	Cream
VHG/01/2007	30.6	Cream
YS Magereza	30.6	Cream
Kiazi cha nduma	30.6	White
MCK/19/2007	30.6	White
Polista	30.	Cream
YS Mwavuli	30.7	White
MCK/02/2007	30.7	Cream
YS Ober Odegni	30.7	Cream
Tororo	31.0	Cream
YS Gefgi fumbi	31.0	White
Kisumu (2)	31.0	Cream
KKFS Mugande	31.1	White
MKN/01/2007	31.2	White
Katumani (5)	31.2	White
Kimwendia	31.2	White
YS Nyar Bungoma	31.3	Cream
KBZ/01/2007	31.3	White
MCK/04/2007	31.4	White
Kambia Mwongo	31.4	Cream
Bau Dasa	31.4	White
KWL/06/2007	31.4	Cream
Sadak	31.5	Cream
KKFS Mwavuli	31.5	Cream
MCK/18/2007	31.6	White
Muka mukuvi (3)	31.6	Cream
TVT/04/2007	31.6	White
S12 Nyakambare	31.7	White
Katumani (2)	31.7	White
S1 Oyieo	31.7	White
Kazinga 11	31.7	Cream
OP/TORO-3-017-08	31.9	White
YS KK001	31.9	Cream
MKN/02/2007	32.0	White
WFTC/07/2007	32.0	White
MCK/21/2007	32.0	White
Kasinga 41	32.1	White
MCK/16/2007	32.1	Cream
TVT/01/2007	32.1	White

KKFS Nyambita	32.2	White
YS SPK 013	32.2	White
Muka mukuvi (2)	32.2	White
Obuogo	32.2	White
YS Adhiambo lera	32.3	Cream
Mshira	32.3	White
TVT/10/2007	32.4	White
Ex-shimba hills	32.4	Cream
SPK004 (Katumani)	32.4	Cream
Yellow (2)	32.4	Cream
K8	32.4	White
Mwezi moja	32.5	Cream
Katumani (4)	32.5	Cream
Kitoto	32.5	Cream
MKN/12/2007	32.6	White
S1 Kalamb Nyerere	32.6	Cream
S6 Orwaki	32.6	White
Kambia (1)	32.7	Cream
Kikuyu (3)	32.7	Cream
SPK 004(2)	32.8	Cream
Wakikuyu (1)	32.9	White
SYA/01/2007	32.9	Cream
YS Lupozi	33.0	White
WFTC/01/2007	33.0	White
MCK/25/20007	33.0	White
Mtwapa 8	33.0	Cream
Ex-Mukumeini	33.0	White
Kamau (1)	33.1	White
YS Marooko	33.1	Cream
MKN/11/2007	33.1	White
YS Nyandere	33.1	Cream
WFTC/06/2007	33.2	Cream
YS Odongo	33.3	Cream
SPK 013	33.3	White
Muka mkuvi (1)	33.3	White
KKFS 389 Ah-12	33.4	White
S6 Nyar Koyugi	33.4	White
MKN/05/2007	33.4	White
MCK/24/2007	33.5	White
MCK/05/2007	33.5	White
TS/01/2007	33.6	Cream
Mugamba	33.6	Cream
YS Mugande	33.7	White

Marooko (3)	33.7	Cream
Mwei umwe (5)	33.7	White
ALPFS were	33.7	White
MCK/13/2007	33.7	Cream
MKN/10/2007	33.	Cream
MCK/08/2007	33.8	Cream
MCK/23/2007	33.8	White
KAK/09/2007	33.9	Cream
S6 Kabut Jwoleny	33.9	White
MCK/15/2007	34.0	White
K9 (2V)	34.0	White
KAK/06/2007	34.0	Cream
BGM/04/2007	34.0	Cream
KKFS 56682/03 (1)	34.	Cream
Wakikuyu (2)	34.1	Cream
OP- NDUNG	34.1	Cream
MCK/17/2007	34.2	Cream
KKFS 56682/03 (2)	34.2	Cream
Kemb 23	34.2	Cream
Nyanguyewo	34.2	White
TVT/11/2007	34.3	White
ALPFS Mbita	34.4	Cream
WFTC/05/2007	34.4	White
YS Sample 3	34.5	Cream
KKFS Pipi	34.5	Cream
MKN/07/2007	34.5	White
Kalamb Nyerere	34.6	White
WFTC/03/2007	34.6	White
MCK/03/2007	34.6	White
Kinubi	34.7	White
LGL/01/2007	34.7	Cream
KRG/03/2007	34.8	White
MKN/04/2007	34.8	White
S6 Ondiek Chilo	34.8	White
Muibai	34.9	White
Katumani (6)	34.9	Cream
K 7 (iv)	34.9	White
MKN/08/2007	35.0	White
ALPFS SPK008	35.0	Cream
Kemb 36	35.0	White
KAK/02/2007	35.0	Cream
Mwei umwe (3)	35.1	Cream
Bungoma	35.2	Cream

K16 (2V)	35.2	White
S8 Ganchurere	35.3	White
MCK/11/2007	35.5	Cream
MCK/27/2007	35.5	Cream
MCK/14/2007	35.5	White
Nyamunyekera	35.5	Cream
TVT/12/2007	35.0	Cream
Kanana	35.0	White
KLF/02/20007	35.6	White
S2 Kalamb Nyerere	35.6	White
Mwei umwe (1)	35.6	Cream
MCK/09/2007	35.6	White
YS Masaba	35.6	Cream
S6 Namaswakhe	35.7	Cream
Kendo Okso	35.7	Cream
MKN/13/2007	35.8	White
KKFS NK-L-22	35.8	White
Wamuciri	36.0	White
S6 Mugande	37.8	White
TVT/06/2007	23.1	Yellow
Kambia (2)	23.3	Yellow
103033-8	23.5	Yellow
YS Othiengo	24.5	Yellow
BGM/02/2007	24.9	Yellow
KKFS Nyandere	26.2	Yellow
102019-3	26.0	Yellow
103014-33	26.4	Yellow
KRG/02/2007	27.0	Yellow
TVT/14/2007	27.1	Yellow
Sample 7 Misambi	27.1	Yellow
Yellow 1	27.2	Yellow
Marooko (2)	28.7	Yellow
Ukimwi	28.9	Yellow
YS/02/2007	29.1	Yellow
LGL/02/2007	29.2	Yellow
Kemb 10 (2)	29.2	Yellow
S6 Nyathi Odiewo(3)	29.3	Yellow
Wundanyi SPK004	29.3	Yellow
MLD/01/2007	29.4	Yellow
MLD/02/2007	29.5	Yellow
KLF/01/2007	29.7	Yellow
Ys kemb 10	29.9	Yellow
TVT/09/2007	30.2	Yellow

S6 Odinga	30.2	Yellow
YS Nyawo	30.3	Yellow
Mtwapa 1020019-17	30.6	Yellow
YS Butongwa	30.9	Yellow
YS Buziba	30.9	Yellow
KWL/04/2007	31.0	Yellow
Kikulu	31.1	Yellow
Kisumu (1)	31.1	Yellow
YS/03/2007	31.2	Yellow
YS/08/2007	31.4	Yellow
YS Kuny Kibuojo	31.5	Yellow
Agriculture	31.5	Yellow
BSA/03/2007	32.0	Yellow
YS Nameless	32.2	Yellow
YS Dada Mowar	32.3	Yellow
ALPFS Karat	32.4	Yellow
102018-11	32.4	Yellow
Dada mowar	32.5	Yellow
YS/06/2007	32.5	Yellow
Sponge	32.5	Yellow
MCK/26/2007	32.7	Yellow
YS Jayalo	33.0	Yellow
Marooko (1)	33.0	Yellow
Sya/02/2007	33.3	Yellow
LGL/03/2007	33.5	Yellow
ALPFS 2002/112	33.5	Yellow
TS/02/2007	33.8	Yellow
Kiluu	33.9	Yellow
Kamau (2)	34	Yellow
KAK/05/2007	34.0	Yellow
S5 Nyatonge (1)	34.1	Yellow
KAK/08/2007	35.0	Yellow
VHG/04/2007	35.1	Yellow
YS/01/2007	35.4	Yellow
VHG/02/2007	35.6	Yellow
ALPFS Nyawo	35.6	Yellow
Kikamba	29.2	Purple
YS/07/2007	34.9	Purple
Tainung	22.4	Orange
S6 Zapallo	22.9	Orange
Riziki	23.1	Orange
Karoti (2)	23.4	Orange
Malenge	23.7	Orange

Karoti (1)	23.9	Orange
BSA/01/2007	23.9	Orange
ALPFS Jewel	24.0	Orange
WFTC/08/2007	24.5	Orange
MCK/01/2007	25.2	Orange
MCK/06/2007	26.0	Orange
KAK/2004/215	26.1	Orange
Naspot	27.4	Orange
YS Sopalla	27.8	Orange
ALPFS Kuny	28.2	Orange
KAK/07/2007	28.8	Orange
ALPFS 2002/141	28.9	Orange
S1 Amina (2)	29.6	Orange
Japones	29.9	Orange
ALPFS Ejumla	30.0	Orange
S6 W-220	30.4	Orange
SPK 004 (1)	32.9	Orange
KKFS Salyboro	32.9s	Orange

Appendix 5: Analysis of variance of the dry matter content in the 314 sweet potato genotypes

Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean of squares**	**Variance ratio**	**F value**
Variety	313	1.10E+04	3.51E+01	6712.95	<0.001
Residual	628	3.29E+00	5.23E-03		
Total	941	1.10E+04			

Appendix 6: Alleles present in 89 sweet potato genotypes

Code	Genotype name	Locus name											
		IB-R16				IB-R19				IBCIP13			
SP1	TVT/07/2007	202	206	214	0	193	199	205	0	338	374	374	0
SP2	TVT/09/2007	202	206	0	0	190	196	0	0	338	374	0	0
SP3	TVT/03/2007	206	214	0	0	199	205	208	0	338	374	0	0
SP4	Kanini kaseo	202	206	0	0	196	199	208	0	338	374	0	0
SP5	OP-LNA-006-08	206	210	214	0	190	193	199	0	338	374	0	0
SP6	MLD/05/2007	206	214	0	0	196	199	205	0	338	374	0	0
SP7	Karoti (2)	202	206	214	0	190	193	196	199	338	374	0	0
SP8	TVT/02/2007	202	206	0	0	190	193	199	0	374	374	0	0
SP9	TVT/12/2007	202	206	0	0	208	208	0	0	338	374	0	0
SP10	KARI Mtwapa OP-T21	202	206	0	0	190	196	0	0	338	374	0	0
SP12	MLD/01/2007	206	206	0	0	199	205	0	0	338	374	0	0
SP13	Kikanda (2)	202	206	0	0	196	205	208	0	338	374	0	0
SP14	Kiazi cha nduma	202	206	214	0	199	205	0	0	338	374	0	0
SP15	Yellow 1	202	206	0	0	196	199	208	0	338	374	0	0
SP16	WFTC/02/2007	206	214	0	0	199	205	208	0	338	374	0	0
SP17	KWL/04/2007	206	214	0	0	196	199	205	0	338	374	0	0
SP23	Ys Kemb 10	206	210	214	0	190	193	199	0	338	374	0	0
SP24	Farmer 5 Bungoma	202	206	214	0	196	199	205	0	338	374	0	0
SP25	ALPFS Were	202	206	214	0	193	199	205	0	338	374	374	0
SP26	KAK/07/2007	206	214	0	0	199	205	208	0	338	374	0	0
SP27	Dada Mowar	206	214	0	0	208	208	0	0	338	374	0	0
SP28	YS unknown 1	202	206	0	0	208	208	0	0	374	374	0	0
SP29	Farmer 12 Marooko	206	214	0	0	199	205	208	0	338	374	0	0
SP30	ALPFS 2002/141	202	206	0	0	196	196	0	0	374	374	0	0
SP31	Farmer sya I unknown	202	206	214	0	193	199	205	0	338	374	374	0
SP32	Farmer sya sadak	206	214	0	0	193	199	205	208	338	374	0	0
SP33	YS Masaba	206	210	214	0	193	199	205	208	338	374	0	0
SP34	TS/01/2007	202	206	210	214	199	208	0	0	374	374	0	0
SP35	ALPFS Mbita	202	206	214	0	190	193	196	199	338	374	0	0
SP36	YS Sample 2	206	214	0	0	193	199	205	208	338	374	0	0
SP37	Big G	202	206	0	0	196	199	208	0	338	374	0	0
SP38	YS Nyanguyegwo	206	214	0	0	193	199	205	0	338	374	0	0
SP41	Farmer 9 Miezi sita	202	206	214	0	190	196	208	0	338	374	0	0
SP42	KKFS Salyboro	202	206	214	0	190	193	0	0	206	338	374	0
SP43	MCK/21/2007	202	206	214	0	193	199	205	0	338	374	0	0
SP44	KKFS 56682/03 (1)	206	214	0	0	193	199	205	0	338	374	0	0
SP45	KRG/01/2007	206	214	0	0	196	199	205	0	338	374	0	0
SP46	Kemb 10 (1)	202	206	214	0	193	199	0	0	338	374	0	0
SP47	Msichana Nairobi	202	206	214	0	196	199	0	0	338	374	0	0
SP48	S13 Nyatonge (2)	206	210	0	0	190	193	196	199	338	374	0	0
SP49	S4 Kuny kibuojo	202	206	0	0	208	208	0	0	338	374	0	0
SP50	S5 Nyatonge (1)	202	206	0	0	196	199	208	0	338	374	0	0
SP51	S11 (Nyatonge (3)	202	206	210	214	199	208	0	0	338	374	0	0
SP52	Polista	202	206	0	0	208	208	0	0	338	374	0	0

SP53	K9 (1V)	202	206	214	0	196	199	208	0	206	338	374	0
SP54	K9 (2V)	202	206	0	0	208	208	0	0	206	338	374	0
SP55	Tainung	202	206	214	0	199	208	0	0	206	338	374	0
SP56	S6 Mwavuli	202	206	214	0	193	199	205	208	206	338	374	0
SP57	SPK 004 (1)	206	214	0	0	193	196	199	208	338	374	0	0
SP58	K16 (1V)	202	206	0	0	208	208	0	0	338	374	0	0
SP59	S6 Ondiek chilo	206	210	0	0	190	193	196	199	338	374	0	0
SP60	Kemb 23	202	206	214	0	190	196	199	208	338	374	0	0
SP61	S1 Amina (2)	202	206	214	0	208	208	0	0	338	374	0	0
SP62	K15	202	206	214	0	208	208	0	0	338	374	0	0
SP63	Muibai	202	206	214	0	208	208	0	0	338	374	0	0
SP64	Bungoma	202	206	214	0	208	208	0	0	338	374	0	0
SP65	Kamau (1)	202	206	0	0	193	199	205	0	338	374	0	0
SP66	Amina (1)	202	206	214	0	193	199	205	208	338	374	0	0
SP67	Naspot	206	214	0	0	193	199	205	0	338	374	0	0
SP68	MCK/17/2007	202	206	214	0	193	196	199	208	338	374	0	0
SP69	MKN/08/2007	202	206	0	0	190	196	0	0	338	374	0	0
SP70	Mwei umwe (4)	206	214	0	0	193	199	205	208	338	374	0	0
SP71	MKN/07/2007	202	206	210	0	190	193	196	199	338	374	0	0
SP72	MKN/04/2007	202	206	206	0	193	199	205	0	374	374	0	0
SP73	Farmer 25 unknown	202	206	214	0	193	199	205	0	338	374	374	0
SP74	Kikuyu cha kikamba	206	214	0	0	193	199	205	208	338	374	0	0
SP75	Katumani (2)	206	214	0	0	193	199	205	0	338	374	0	0
SP76	Mwei umwe (5)	206	214	0	0	196	199	205	0	338	374	0	0
SP77	KBZ/01/2007	202	206	210	214	199	208	0	0	338	374	0	0
SP78	WFTC/03/2007	206	210	0	0	190	193	196	199	338	374	0	0
SP79	Kikuyu (3)	206	214	0	0	193	199	205	0	374	374	0	0
SP80	Katumani (5)	202	206	214	0	193	199	205	208	206	338	374	0
SP81	Katumani (7)	206	210	214	0	190	193	199	0	338	374	0	0
SP82	Kiluu	202	206	0	0	196	199	208	0	338	374	0	0
SP83	Ilukwasi	206	214	0	0	193	199	208	0	338	374	0	0
SP84	MKN/02/2007	202	206	214	0	193	199	205	0	338	374	374	0
SP85	Kikanda (1)	202	206	0	0	190	193	199	0	374	374	0	0
SP86	Kikamba (2)	202	206	0	0	190	193	199	0	374	374	0	0
SP87	SPK 004 (Katumani)	202	206	0	0	190	193	199	0	374	374	0	0
SP88	KAK/04/2007	206	210	0	0	190	193	199	208	338	374	0	0
SP89	MCK/23/2007	206	214	0	0	190	196	199	205	338	374	0	0
SP11	Nyakuwili	202	206	210	214	196	199	208	0	338	374	0	0
SP18	YS Sopalla	202	206	0	0	190	193	199	0	374	374	0	0
SP19	Marooko (1)	202	206	0	0	190	193	199	0	374	374	0	0
SP20	KKFS Mwavuli	206	210	214	0	190	193	199	0	338	374	0	0
SP21	KKFS NK-L-22	206	214	0	0	193	199	205	208	338	374	0	0
SP22	YS Unknown 2	202	206	0	0	196	205	208	0	338	374	0	0
SP39	Marooko (2)	202	206	0	0	193	196	199	0	374	374	0	0
SP40	Farmer 12 Unknown 1	202	206	214	0	193	196	199	0	338	374	0	0

Code	Genotype name	Locus name											
		IB-R12				IB-SO7				IB-R03			
SP1	TVT/07/2007	3 18	327	0	0	175	175	0	0	243	249	252	258
SP2	TVT/09/2007	3 18	327	0	0	175	175	0	0	249	252	258	0
SP3	TVT/03/2007	3 18	327	0	0	175	175	0	0	243	249	252	258
SP4	Kanini kaseo	3 18	327	339	0	175	175	0	0	243	249	252	258
SP5	OP-LNA-006-08	3 39	339	0	0	175	179	0	0	252	258	0	0
SP6	MLD/05/2007	3 18	327	0	0	175	175	0	0	243	249	252	258
SP7	Karoti (2)	3 18	318	0	0	175	175	0	0	243	249	252	258
SP8	TVT/02/2007	3 27	327	0	0	175	175	0	0	252	258	0	0
SP9	TVT/12/2007	3 18	339	0	0	175	175	0	0	249	252	258	0
SP10	KARI Mtwapa OP-T21	3 18	327	0	0	175	175	0	0	249	252	258	0
SP12	MLD/01/2007	3 18	327	0	0	175	175	0	0	249	252	258	0
SP13	Kikanda (2)	3 18	327	0	0	175	175	0	0	249	252	258	0
SP14	Kiazi cha nduma	3 18	327	0	0	175	175	0	0	243	249	252	258
SP15	Yellow 1	3 18	327	339	0	175	175	0	0	243	249	252	258
SP16	WFTC/02/2007	3 18	327	0	0	175	175	0	0	249	252	258	0
SP17	KWL/04/2007	3 18	327	0	0	175	175	0	0	243	249	252	258
SP23	Ys Kemb 10	3 39	339	0	0	175	179	0	0	252	258	0	0
SP24	Farmer 5 Bungoma	3 27	327	0	0	175	191	0	0	243	249	252	258
SP25	ALPFS Were	3 18	327	0	0	175	175	0	0	243	249	252	258
SP26	KAK/07/2007	3 18	327	0	0	175	175	0	0	243	249	258	0
SP27	Dada Mowar	3 18	339	0	0	175	175	0	0	249	252	258	0
SP28	YS unknown 1	3 18	327	0	0	175	175	0	0	243	249	252	258
SP29	Farmer 12 Marooko	3 18	327	0	0	175	175	0	0	249	252	258	0
SP30	ALPFS 2002/141	3 18	327	339	0	175	175	0	0	249	258	0	0
SP31	Farmer sya I unknown	3 18	327	0	0	175	175	0	0	243	249	252	258
SP32	Farmer sya sadak	3	327	0	0	175	175	0	0	243	249	252	258

		18											
SP33	YS Masaba	318	327	0	0	175	179	0	0	243	249	252	258
SP34	TS/01/2007	339	339	0	0	175	175	0	0	243	249	252	258
SP35	ALPFS Mbita	318	318	0	0	175	191	0	0	249	252	258	0
SP36	YS Sample 2	318	327	0	0	175	175	0	0	243	249	252	258
SP37	Big G	318	327	339	0	175	175	0	0	243	249	252	258
SP38	YS Nyanguyegwo	318	327	339	0	175	175	0	0	249	252	258	0
SP41	Farmer 9 Miezi sita	327	339	0	0	175	191	0	0	249	252	258	0
SP42	KKFS Salyboro	318	327	0	0	175	175	0	0	249	252	258	0
SP43	MCK/21/2007	318	327	0	0	175	179	0	0	249	252	258	0
SP44	KKFS 56682/03 (1)	318	327	0	0	175	175	179	0	249	252	258	0
SP45	KRG/01/2007	318	327	0	0	175	175	0	0	243	249	252	258
SP46	Kemb 10 (1)	318	327	0	0	175	175	0	0	243	249	252	258
SP47	Msichana Nairobi	318	327	0	0	175	175	0	0	249	252	258	0
SP48	S13 Nyatonge (2)	339	339	0	0	175	175	0	0	249	252	258	0
SP49	S4 Kuny kibuojo	318	339	0	0	175	175	0	0	249	252	258	0
SP50	S5 Nyatonge (1)	318	327	339	0	175	175	0	0	243	249	252	258
SP51	S11 (Nyatonge (3)	339	339	0	0	175	175	0	0	243	249	252	258
SP52	Polista	318	327	339	0	175	175	0	0	243	249	252	258
SP53	K9 (1V)	327	327	0	0	175	179	0	0	243	249	252	258
SP54	K9 (2V)	318	339	0	0	175	175	0	0	249	252	258	0
SP55	Tainung	327	339	0	0	175	175	0	0	243	249	252	258
SP56	S6 Mwavuli	318	318	0	0	175	175	0	0	243	249	252	258
SP57	SPK 004 (1)	318	327	0	0	175	175	0	0	243	249	252	258
SP58	K16 (1V)	318	339	0	0	175	175	0	0	243	249	252	258
SP59	S6 Ondiek chilo	339	339	0	0	175	175	0	0	249	252	258	0
SP60	Kemb 23	327	327	0	0	175	175	0	0	243	249	252	258
SP61	S1 Amina (2)	3	327	0	0	175	175	0	0	243	249	252	258

		27											
SP62	K15	327	0	0	175	175	0	0	249	252	258	0
SP63	Muibai	327	0	0	175	175	0	0	249	252	258	0
SP64	Bungoma	327	0	0	175	175	0	0	249	252	258	0
SP65	Kamau (1)	339	0	0	175	175	0	0	243	249	252	258
SP66	Amina (1)	318	327	339	0	175	175	0	0	243	249	252	258
SP67	Naspot	327	0	0	175	175	0	0	249	252	258	0
SP68	MCK/17/2007	318	327	0	0	175	179	0	0	243	249	252	258
SP69	MKN/08/2007	318	327	0	0	175	175	0	0	243	249	252	258
SP70	Mwei umwe (4)	318	327	0	0	175	175	0	0	243	249	252	258
SP71	MKN/07/2007	339	0	0	175	175	0	0	249	252	258	0
SP72	MKN/04/2007	327	0	0	175	179	191	0	243	249	252	258
SP73	Farmer 25 unknown	318	327	0	0	175	175	0	0	243	249	252	258
SP74	Kikuyu cha kikamba	318	327	0	0	175	175	0	0	243	249	252	258
SP75	Katumani (2)	318	339	0	0	175	179	191	0	249	252	258	0
SP76	Mwei umwe (5)	318	327	0	0	175	179	0	0	243	249	252	258
SP77	KBZ/01/2007	339	0	0	175	175	0	0	243	249	252	258
SP78	WFTC/03/2007	318	318	0	0	175	191	0	0	249	252	258	0
SP79	Kikuyu (3)	327	0	0	175	179	191	0	249	252	258	0
SP80	Katumani (5)	318	318	0	0	175	175	0	0	243	249	252	258
SP81	Katumani (7)	339	0	0	175	179	0	0	252	258	0	0
SP82	Kiluu	339	0	0	175	175	0	0	243	252	258	0
SP83	Ilukwasi	318	0	0	175	175	0	0	243	249	252	258
SP84	MKN/02/2007	327	0	0	175	175	0	0	243	249	252	258
SP85	Kikanda (1)	327	0	0	175	175	0	0	252	258	0	0
SP86	Kikamba (2)	327	0	0	175	175	0	0	252	258	0	0
SP87	SPK 004 (Katumani)	327	0	0	175	175	0	0	252	258	0	0
SP88	KAK/04/2007	3	339	0	0	175	175	0	0	249	252	258	0

		39											
SP89	MCK/23/2007	318	327	0	0	175	179	0	0	243	249	252	258
SP11	Nyakuwili	339	339	0	0	175	175	0	0	243	249	252	258
SP18	YS Sopalla	339	339	0	0	175	175	0	0	243	249	252	258
SP19	Marooko (1)	327	327	0	0	175	175	0	0	249	252	258	0
SP20	KKFS Mwavuli	339	339	0	0	175	179	0	0	252	258	0	0
SP21	KKFS NK-L-22	318	327	339	0	175	175	0	0	243	249	252	258
SP22	YS Unknown 2	318	318	0	0	175	175	0	0	249	252	258	0
SP39	Marooko (2)	339	339	0	0	175	175	0	0	243	249	252	258
SP40	Farmer 12 Unknown 1	327	327	0	0	175	191	0	0	243	249	252	258

Appendix 7: Percentage polymorphism of 89 sweet potato genotypes

Genotype	%polymorphism	Genotype	%polymorphism
SP16	56.52	SP62	47.83
SP55	65.22	SP52	56.52
SP6	60.87	SP2	52.17
SP7	65.22	SP14	60.87
SP4	65.22	SP17	60.87
SP73	65.22	SP80	69.57
SP49	47.83	SP77	60.87
SP81	56.52	SP32	65.22
SP11	65.22	SP20	56.52
SP66	73.91	SP75	65.22
SP58	52.17	SP84	60.87
SP37	65.22	SP43	65.22
SP88	56.52	SP87	43.48
SP40	65.22	SP27	47.83
SP41	65.22	SP79	56.52
SP15	65.22	SP57	65.22
SP67	52.17	SP42	60.87
SP36	65.22	SP19	47.83
SP31	65.22	SP65	56.52
SP3	60.87	SP34	56.52
SP18	52.17	SP25	65.22
SP86	43.48	SP29	56.52
SP70	65.22	SP76	65.22
SP8	43.48	SP89	69.57

SP53	69.57	SP82	52.17
SP13	56.52	SP54	52.17
SP28	47.83	SP44	56.52
SP10	52.17	SP50	65.22
SP45	60.87	SP68	73.91
SP39	52.17	SP60	65.22
SP26	56.52	SP38	60.87
SP1	65.22	SP35	65.22
SP5	56.52	SP71	60.87
SP30	43.48	SP78	60.87
SP51	60.87	SP72	60.87
SP48	56.52	SP59	56.52
SP83	56.52	SP63	47.83
SP46	60.87	SP69	56.52
SP12	47.83	SP64	47.83
SP61	52.17	SP24	65.22
SP56	69.57	SP9	47.83
SP85	43.48	SP33	73.91
SP23	56.52	SP21	69.57
SP74	65.22	SP22	52.17
SP47	56.52		

Appendix 8: Buffers and solutions

Buffers for DNA extraction

CTAB extraction buffer
1.0 M Tris HCl pH 8.0
5 M NaCl
2% CTAB
0.1% β-mercaptoethanol

Chloroform Isoamyl Alcohol 24:1

TE buffer

10 mM Tris HCl pH 8.0
1 mM EDTA

Buffers for NCM-ELISA

(i) TBS pH 7.5
- 0.02 M Tris base
- 0.5 M NaCl

(ii) T-TBS
- TBS

- 0.05, Tween-20

(iii) Substrate buffer pH 9.5 (0.5 l)

-Tris base	6.05 g	(0.1 M)
- NaCl	2.92 g	(0.1 M)
- $MgCl_2.6H_2O$	0.51 g	(0.005 M)

(iv) Substrate solution for NCM-ELISA

(a) NBT stock solution
- NBT 40 mg
- N, N-dimethylformamide (70%) 1.2 ml
- Mix well and store at 4^0c protected from light

(b) BCIP stock solution
- BCIP 20 mg
- N, N-dimethylformamide (70%) 1.2 ml
- Mix well and store at 4^0c protected from light

Preparation of the substrate solution

- Substrate buffer	30 ml
- NBT stock solution	90 µl
- BCIP stock solution	90 µl

Dissolve NBT in 30 ml of substrate then add BCIP drop wise.