PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF RELEASED AND ELITE SWEETPOTATO VARIETIES IN GHANA COMPARED WITH VIRUS-TESTED PUTATIVE RAMETS

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BY

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DECLARATION

I wish to declare to the best of my knowledge that the research presented in this thesis is original and conducted by myself under supervision and has not been presented for a degree award before.

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We declare that we have supervised the student in undertaking the study submitted herein and confirm that he has our permission to submit.

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DEDICATION

This thesis is dedicated to Dr. Marian Quain and Dr.Ted Edward Carey for their words of encouragement and serving as source of inspiration

ABSTRACT

Following release of Ghanaian sweetpotato varieties which are selections from exotic introductions, no effort was made to maintain true-to-type virus-tested foundation seed stocks. Original exotic virus-tested plants of Ghanaian released and elite varieties obtained from CIP were introduced and compared with putative ramets using morphological descriptors and molecular markers, yield and quality attributes and severity of virus symptoms with time. Planting materials of cultivars and introduced virus-tested materials of same genotypes were field multiplied at Fumesua, Ghana, before planting in replicated trials at two locations during the 2011 major growing season. Standard morphological descriptors and molecular markers were used to assess similarity of genotypes, while yield and virus symptoms during growth were used to determine benefits of using virus-tested planting material. The Ghanaian released varieties Otoo and Sauti were found to be closely related with original source material. Conversely, Faara and Okumkom were found not to be closely related with original source material. The recently introduced virus-tested genotypes, Mogamba and Kenya, yielded significantly ($p < 0.05$) higher than putative ramets and could be used as a source of clean planting material. On the contrary, TIS 3017 and TIS 8266 also introduced recently, produced significantly ($p < 0.05$) lower yields than putative ramets. These varieties should be cleaned locally using *in vitro* tissue culture techniques. Mogamba was found to be the genotype with the lowest expression of virus symptoms and recorded the highest yield. More attention should be given to maintenance of seed quality of virus-free stocks and continuous selection for trueness to type.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a tropical American crop belonging to the family Convolvulaceae. It is a hexaploid (2n=6x=90) (Austin and Huaman, 1996). Sweetpotato cultivars have wide variations in botanical characteristics and are readily distinguished on the basis of morphological traits. Most varieties of this crop are self-incompatible, and, because of the obligate outcrossing nature of the crop, have high levels of heterozygosity. Sweetpotato varieties vary considerably in horticultural and morphological characteristics with a wide range of yield potential, size, shape, flesh and skin colour of roots, as well as sizes, colours and shapes of leaves and branches. The highest diversity of sweetpotato was found in Central America based on the use of molecular markers which supports the hypothesis that Central America is the centre of origin of this crop (Zhang *et al*., 2000).

Worldwide, sweetpotato is the seventh most important food crop after rice, wheat, potatoes, maize, yam and cassava (Loebenstein, 2009). It is grown on about 8.2 million hectares worldwide, yielding about 102 million tons, with an average yield of about 12.1 tons/ha (FAOSTAT, 2010). The crop is mainly grown in developing countries, which account for over 95% of world production. Sweetpotato has low input requirements, is easy to produce and able to produce under adverse weather and soil conditions. The area harvested for sweetpotato in Ghana is 73,400 ha (FAOSTAT, 2010) which comes after cassava and yam in order of importance among root crops.

Sweetpotato root is an excellent source of vitamin A, if the flesh colour is orange. Vitamin C, vitamin B6, riboflavin, copper, pantothenic acid and folic acid are also contained in sweetpotato (Woolfe, 1992). Awareness of sweetpotato as a healthy food crop is increasing, especially the orange-fleshed sweetpotato which is rich in provitamin A carotenoids (Woolfe, 1992). Sweetpotato in Ghana is cultivated mainly for the carbohydrate-rich storage roots although the foliage has the potential for use as vegetable and animal feed (Otoo *et al*., 2001). Though the leaves are very rich in minerals and vitamins, the roots are more widely consumed in Ghana where the crop is particularly important in the Central, Volta and Upper East regions.

A variety may be defined as a taxonomic unit created and maintained by man, the first essential being that it should have an individuality which can be reproduced over a number of years, and secondly that it should be distinguishable by inherited morphological or physiological characters from other varieties. In Ghana, six white-fleshed and two orange-fleshed sweetpotato varieties have been released and typically yield 8-10 tons/ha, reaching 15-30 tons/ha at the farm level (Akoroda, 2009). These eight varieties were released by CSIR-Crops Research Institute in 1998 and 2005. Farmers grow a number of land race varieties as well, since adoption of released varieties has not been uniform and overwhelming.

1.2 PROBLEM STATEMENT

In Ghana and other parts of Sub-Saharan Africa (SSA), the same variety may be called different names in different places, complicating management of germplasm. Officially released sweetpotato varieties in Ghana are all selections from exotic introductions. Following release, no effort has been made to maintain true-to-type virus-tested foundation seed stocks. Virus-tested variety is generated from tissue culture plants that

have been tested for known viruses and found to be apparently free of these viruses. In developing countries like Ghana, over 80% of crops are sown from seed stocks selected and saved by farmers or exchanged and traded locally (Almekinders and Louwaars, 1999). The access to and maintenance of quality planting materials is a challenge for small holder farmers and researchers as well.

The sweetpotato virus disease complex (SPVD) is, by far, the most destructive viral disease in Africa (up to 50% in East Africa) and perhaps worldwide (Carey *et al*., 1999). Sweetpotato is vegetatively propagated from vine cuttings or roots. Sexual seeds are also used for its improvement, but only for development of new varieties. Farmers often take vines for propagation from their own fields year after year. In tropical regions including Ghana, the sweetpotato crop is grown in both major and minor cropping seasons. Vine cuttings from mature crops are used to plant new crops (Valverde *et al*., 2007). Consequently if virus diseases are present in the field they will be definitely transmitted with the propagation material to the newly planted field. In the USA, yield and quality of virus-tested Beauregard and Hernandez sweetpotato varieties decreased significantly and gradually with time (Bryan *et al*., 2003). Clark *et al*. (2002) and Villordon and La Bonte (1996) attributed decline in yield and quality of sweet potato to the accumulation of mutations and viruses. Mutation has the potential of altering the characteristics of a cultivar with time. It involves the gradual decline of "fitness" of the cultivars, resulting in a significant reduction of yield, poor resistance to pathogens and insect pests and other undesirable traits (La Bonte *et al*., 2001).

1.3 JUSTIFICATION

Sweetpotato cultivars are generally distinguished on the basis of morphological traits and have a wide variability of botanical characteristics. Clonal plants such as

sweetpotato produce ramets (genetically identical offspring) that have the potential to become independent of the parent plant. (Hosaka *et al*., 2005; Araki *et al*., 2009).

The use of descriptors in the characterization of sweetpotato is very necessary because they generally correspond to characteristics whose expressions are easy to measure, record or evaluate. They therefore permit relatively easy discrimination between phenotypes. Descriptors related to phenotypic characters mostly correspond to the morphological description of the plant and its architecture. (CIAT, 2007). Phenotypic characterization has been used for identification of duplicates, studies of genetic diversity patterns and correlation with characteristics of agronomic importance among other uses (CIAT, 2007). It is an important first step in the assessment of sweetpotato and it is done by assessing variation in vine, leaf, flower and storage root characteristics (CIP *et al*., 1991). Morphological and agronomic characters coupled with reaction to pests, diseases and other stresses have been used to characterize sweet potato. However, phenotypic characterization has certain limitations due to morphological plasticity (Prakash and He, 1996). It is therefore necessary to complement morphological characterization with molecular characterization.

Advances in molecular biology, principally in the development of the polymerase chain reaction (PCR) have resulted in powerful techniques which can be used for the screening, characterization and evaluation of genetic diversity. DNA fingerprinting has become an important tool for cultivar identification in plant breeding and for germplasm management. Microsatellite or simple sequence repeat (SSR) markers exhibit high levels of polymorphism (Tumwegamire *et al*., 2011) and several such markers have been developed for sweetpotato (Buteler *et al*., 1999; Hu *et al*., 2004).

Most of the local landraces and some of the introduced material in SSA have degenerated because of sweetpotato virus disease (Low *et al*., 2009) which means they have declined in yield potential. In countries where care is taken to provide pathogen-tested planting material, yields increase markedly up to seven times or more (Thottappily, 2009). Clean material can be obtained from original source or by cleaning up local material. The advantage of cleaning local material is that it is likely to be true-to-type. However, specialized equipment is required for thermotherapy and it is time consuming. This emphasize the need of introducing virus-tested putative source material of Ghanaian released varieties to determine if this could be a rapidly achievable alternative to cleaning up the local material, which is probably the preferred approach. Effective seed systems have several advantages including provision of different categories of farmers with planting material in sufficient quantities at the right time which is of appropriate physiological state, vigour and health. Planting materials provided are also of superior genotypes appropriate to farmers' purposes. Yield gains of 30%-60% can be obtained through the use of healthy planting material (Gibson *et al*., 2004; Clark and Hoy, 2006). In order to maintain superiority of genotypes and health of sweetpotato there needs to be capacity within seed systems for generation, dissemination and multiplication of new stock (Setimela *et al*., 2004).

1.4 OBJECTIVES

The main objective of this study was to compare the characteristics of released and elite sweetpotato varieties in Ghana with their original exotic source materials.

The specific objectives are

1. To compare Ghanaian released varieties with putative ramets using morphological descriptors and molecular markers
2. To compare putative ramets using yield and quality attributes

3. To assess the extent to which virus infection may affect the performance of better lines

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TAXONOMY AND ORIGIN OF SWEETPOTATO

Sweetpotato is a dicotyledonous root crop belonging to the morning glory family Convolvulaceae. The sweetpotato and the wild species closely related to it are classified in the family Convolvulaceae, genus Ipomoea, section Eriospermum (formerly Batatas), and series Batatas (Austin and Huamán, 1996). Linnaeus (1753) described the cultivated sweetpotato as *Convolvulus batatas*. In 1791, the botanist Lamarck described it as *Ipomoea batatas*. It is a hexaploid plant with 2n=6x=90 chromosomes. Although some plants morphologically quite similar to *I. batatas* with 2n=4x=60 chromosomes have been described and named, they are considered synonyms of this species (Austin, 1977). *Ipomoea batatas* is a self-incompatible species.

It is generally accepted that the sweetpotato is of American origin. Abundant evidence shows that sweepotato was spread widely through the migration routes of people in the New World tropics before the discovery of America (Austin, 1988). Based on the analysis of key morphological characters of sweetpotato and the wild *Ipomoea* species, Austin (1988) postulated that sweetpotato originated in the region between Yucatan Peninsula of Mexico and the Orinoco River in Venezuela. The highest diversity of sweetpotato was found in Central America using molecular markers (Zhang *et al*., 2000).

2.2 ECONOMIC IMPORTANCE AND DISTRIBUTION OF SWEETPOTATO

Roots and tubers, most notably cassava, sweetpotato, yam and potato are some of the most important primary crops. They play a critical role in the global food system,

particularly in the developing world, where they rank among the top 10 food crops (Phillips *et al*., 2004). Globally sweetpotato is the seventh most important food crop in the world in terms of production (Loebenstein, 2009). Sweetpotato is grown on about 8.2 million hectares producing about 102 million tons with 12.1 tons/ha as an average yield (FAOSTAT, 2010). Sweetpotato is one of the most widely grown root crops in Sub-Saharan Africa (Low *et al*., 2009) and it is particularly important in countries surrounding the Great Lakes in Eastern and Central Africa; Malawi, Angola, Mozambique and Madagascar in Southern Africa and Nigeria in West Africa (Woolfe, 1992). In Ghana, farmers plant 73,400 ha of sweetpotato yearly that comes after cassava and yam in order of importance (FAOSTAT, 2010).

Over 80% of the sweetpotato produced in SSA is consumed fresh by human beings. The remainder is used for animal feed. Awareness of sweetpotato as a healthy food crop is increasing, especially the orange-fleshed sweetpotato which is rich in provitamin A carotenoids (Woolfe, 1992).The roots are mainly starch and soluble carbohydrates, but the leaves and vines are high in amino acids, essential minerals and vitamins (Kenyon *et al*., 2006). Sweetpotato is a nutritious crop which contains a lot of minerals and vitamins. It is an excellent source of vitamin A and a good source of potassium. Vitamin C, vitamin B6, riboflavin, copper, pantothenic acid and folic acid are also contained in sweetpotato (Woolfe, 1992). In Ghana, cassava, cocoyam and yam have been the principal root and tuber crops over the years. As such, sweetpotato was neglected by research in the past, but with the inception of the National Root and Tuber Crops Improvement Project (NRTCIP) in 1988, some attention has been devoted to increasing the production of sweetpotato as a source of dietary energy, vitamins, minerals and proteins and its use as animal feed (FAO and IFAD, 2005). Although it is

cultivated mainly for the carbohydrate-rich tubers, the foliage has the potential for use as vegetable and animal feed (Otoo *et al.*, 2001).

2.3 BIOLOGY AND MORPHOLOGY

The complex interaction between environmental and genetic factors in sweetpotato is well documented. Previous reviews by Ravi and Indira (1999) reviewed source and sink relationship, photosynthesis, translocation and respiration as well as the effect of growth regulators and environmental factors relating to the physiology and yield of sweetpotato.

2.3.1 Growth Habit

Although sweetpotato is an herbaceous and perennial crop it is grown as an annual plant by vegetative propagation using either storage roots or stems cuttings. Its growth habit is mainly prostrate with a vine system that expands rapidly horizontally on the ground. The types of growth habit of sweetpotatoes are erect, semi erect, spreading or very spreading (CIP *et al*., 1991).

2.3.2 Storage root

Although sweetpotato shoot tips are consumed, the storage root is the main organ used for human consumption. The swollen root is generally called a 'storage root' (Hill *et al*., 1992) and by classical botanical definition is an enlarged true root (Kays *et al*., 1992). The initial sign of storage root formation is the accumulation of photosynthates consisting predominantly of starch (Chua and Kays, 1981). Storage root initiation in sweetpotato is reported to occur between the period 7 to 91 days after transplanting (DAT) and varies among cultivars. (Ravi and Indira, 1999). Villordon *et al*. (2009)

reported that the storage root initiation of two varieties (Beauregard and Georgia Jet) began to appear at 19 to 21 DAT respectively and differed in the magnitude and timing of the development. The yield of sweetpotato is highly variable. Differences in yield could be attributed to factors such as cultivar, propagating material, environment and soil (Kays, 1985; Ravi and Indira, 1999). The quantity of yield depends on the number of fibrous roots that will be induced to form storage roots. This subsequently results in either a high number (four to six uniform and high grade) or low number of roots that may be reduced to one very large storage root per plant or no marketable roots at all (Villordon *et al*., 2009). The shape and size of storage root can be between round and long irregular depending on the variety and environmental factors (Woolfe, 1992). Storage root skin ranges from white to dark purple and predominant flesh colour varies from white to orange and purple in various distributions. (Laurie and Niederwieser, 2004).

2.3.3 Stem

The sweetpotato stem is described as cylindrical. The length of the stem as well as the internodes depends on the growth habit of the cultivar and of the availability of water in the soil. Somda and Kays (1990) reported that stem length may range from 1 m to 6 m. Internode length may also range from a few centimetres up to more than 12 cm. Stem diameter can be thin or very thick and varies approximately between 4 to 12 mm (CIP *et al*., 1991).

Stem colour varies from green to totally pigmented with anthocyanins (red-purple colour) depending on the type of cultivar. Hairiness in the apical shoots, and in some cultivars also in the stems, varies from glabrous (without hairs) to very pubescent (CIP *et al*., 1991).

Sweetpotato plants produce three types of branching normally namely primary, secondary and tertiary at different periods of growth. The total number of branches varies between 3 and 20 among cultivars. Spacing, photoperiod, soil moisture and nutrient supply influence the branching intensity of sweetpotato (Somda and Kays, 1990).

2.3.4 Leaves and Petioles

Sweetpotato possess simple leaves which are spirally arranged on the stem in a pattern known as 2/5 phyllotaxis. This means that there are five leaves spirally arranged in two circles around the stem for any two leaves are located in the same vertical plane on the stem. In relation to cultivar type, the edge of the leave may be entire, toothed or lobed. The shape of the general outline of the leaf can be rounded, reniform (kidney shaped), cordate (heart-shaped), triangular, hastate, lobed and almost divided. Number of lobes generally range from 3 to 7 and can be easily determined by counting the veins that go from the junction of the petiole up to the edge of the leaf lamina. Petiole length varies widely with genotype and may range from approximately 10 to 40cm (CIP *et al*., 1991).

2.4 CHARACTERIZATION

Through characterization, variations that exist in a germplasm collection in terms of morphological and phenological characteristics of high heritability can be estimated. This means that the diversity in a germplasm collection is studied when characterization is done. Such variation may also include characteristics whose expression is little influenced by the environment. That is variability expressed by molecular markers. One important objective of germplasm characterization is to identify the accessions of a

germplasm collection so that they can be clearly distinguished or individualized (CIAT, 2007).

2.4.1 MORPHOLOGICAL CHARACTERIZATION

Phenotypic characterization in sweetpotato is done by assessing leaf, flower and storage root characteristics (CIP *et al*., 1991) and it has been traditionally used for identification of cultivars of this crop. Morphological characterization is important in the identification of duplicate accessions, detection of unique traits and also the structure of the population to be conserved, thus saving on storage space and simplifying selection by plant breeders (Reed *et al*., 2004). It has been used for various purposes including identification of duplicates, studies of genetic diversity patterns and correlation with characteristics of agronomic importance (CIAT, 2007).

Sweetpotato cultivars are generally distinguished on the basis of morphological traits and have a wide variability of botanical characteristics. The use of descriptors in the characterization of sweetpotato is very necessary. Descriptors are those characteristics by which germplasm can be known and its potential usefulness determined. Characterization descriptors permit relatively easy discrimination among phenotypes. Those related to phenotypic characters mostly correspond to the morphological description of the plant and its architecture (CIAT, 2007). Standard descriptor lists provide an international format thereby producing a universally understood language for plant genetic resource data (CIP *et al*., 1991).

Morphological characterization is an important first step in the assessment of sweetpotato diversity but has certain limitations due to morphological plasticity (Prakash and He, 1996) which is the tendency of a species to physically change

appearance in response to environment. One environmental factor which has been repeatedly identified as a key factor for the maintenance of genotypic diversity in plant populations is diseases. The structure, diversity and functioning of plant populations can be extremely affected through pathogens like viruses (Bradley *et al*., 2008).

High morphological diversity observed among the sweetpotato accessions may not be a conclusive indication of genetic diversity (Yada *et al*., 2010), as variations in environmental conditions such as soil types and fertility levels, light, temperature, and moisture regimes could still allow for different results to be obtained if morphological characterization is repeated in time and space (Morakinyo and Ajibade, 1998). Accordingly, they do not replace but complement phenotypic characterization and morpho-agronomic evaluation.

2.4.2 MOLECULAR CHARACTERIZATION

Due to changes in environment and sometimes mutation in sweetpotato, it is very necessary to complement morphological characterization with molecular characterization. The genome itself can be studied directly, using biochemical and molecular markers. These methodologies help locate genes of interest with greater accuracy but do not evaluate the effect of the environment on the expression of those genes (Westman and Kresovich, 1997).

Advances in molecular biology, principally in the development of the polymerase chain reaction (PCR) for amplifying DNA, DNA sequencing and data analysis, have resulted in powerful techniques which can be used for screening, characterization and evaluation of genetic diversity. DNA fingerprinting has become an important tool for cultivar identification in plant breeding and for germplasm management. A number of

different molecular assays have been applied in sweetpotato including Random Amplified Polymorphic DNA (Zhang *et al*., 1998), Inter Simple Sequence Repeat (Hu *et al.*, 2003), Simple Sequence Repeat (Hu *et al*., 2004), Amplified Fragment Length Polymorphism (Zhang *et al*., 2004), Selective Amplification of Microsatellite Polymorphic Loci (Tseng *et al*., 2002), and DNA Amplification Fingerprinting (He *et al*., 1995).

2.4.2.1 SIMPLE SEQUENCE REPEATS (SSRs)

Simple sequence repeats (SSRs), also called microsatellites, are small tandemly repeated sequences (1-6 bp) that are widely dispersed in eukaryotic genomes. Comparative studies in plants have shown that SSR markers, which are single locus markers with multiple alleles, are more variable than other markers and provide an effective means for discriminating between genotypes (Powell *et al*., 1996). Microsatellites or simple sequence repeats have been used in forensic studies, cultivar identification, cultivar percentage assessment, genetic diversity analysis, evolutionary studies, construction of molecular maps and to obtain patents and property rights for plant varieties (Gupta *et al*., 1999; Buteler *et al.*, 2002). SSR markers exhibit high levels of polymorphism and several such markers have been developed for sweetpotato (Buteler *et al*., 1999; Hu *et al*., 2004). Tumwegamire *et al.* (2011) reported high levels of polymorphism when they studied the genetic diversity in white and orange-fleshed sweetpotato farmer varieties from East Africa. The results obtained from that studies confirmed the extraordinary discriminatory capacity of SSR markers. Buteler *et al.* (2002) reported only a few useful microsatellite loci have been identified for sweet potato at present. These markers have been used in the assessment of genetic diversity and DNA fingerprinting (Zhang *et al*., 2001) among other uses such as genetic inheritance analysis (Buteler *et al*., 1999).

SSR markers exhibited remarkable discriminatory power and are therefore suitable for genetic diversity analysis in sweetpotato (Karuri *et al*., 2010). Simple sequence repeats deliver more information per unit assay than any other markers because of their high levels of allelic variation and their co-dominant character. Rakoczy-Trojanowska and Bolibok (2004) reported that SSRs are considered to be the most efficient markers for genetic diversity studies in many plants. EST-SSR is useful for qualitative and quantitative trait mapping, marker-assisted selection, evolution and genetic diversity studies in sweetpotato (Wang *et al*., 2011).

2.5 SWEETPOTATO PRODUCTION

2.5.1 Climate and Soil

Sweetpotato does best in tropical and subtropical climates. It requires an average temperature of 20-22 ^{0}C during its relatively short vegetative period of 3.5 to 5 months. Rainfall amount of 500-1250 mm is required in West Africa. Very high rainfall leads to excessive vine development (Obigbesan, 2009). It is sensitive to low temperature and grows favourably under well-aerated and moderate to slightly acidic sandy to sandy loam soil. Sweetpotato has the ability to tolerate harsh soil and climatic conditions and yet give satisfactory yield (van den Berg and Laurie, 2004). Heavy, poorly aerated soils prevent satisfactory development of storage roots resulting in poor shapes. Heavy soils also give low yields and render harvesting difficult. Optimum pH for sweetpotato is 5.8-6.0 and the crop can be cultivated even in high elevations as high as 1500 m above sea level (Obigbesan, 2009).

2.5.2 Propagation

Sweetpotato is propagated asexually from vine cuttings or sexually from seed (Woolfe, 1992), but the latter is done only by breeding programs. Propagation of sweetpotato is done by vegetative propagation using one of the following methods: sprouting of whole storage roots (sprouts are then used as planting materials), and use of stem or vine cuttings from plants used for production or from multiplication plots. In the latter method green vines of approximately 30 cm length with at least three leaf nodes are planted into the soil (Obigbesan, 2009). Sweetpotato is most commonly grown on mounds or ridges, and occasionally on raised beds, or on the flat. Deep cultivation enhances root growth and bulking of the sweetpotato roots. Mounds and ridges promote adequate drainage and ease of harvesting (Low *et al*., 2009).

2.6 PRODUCTION CONSTRAINTS

According to (Low *et al*., 2009), there are five major constraints to improved productivity and incomes from sweetpotato among the smallholder sector in Sub-Saharan Africa with Ghana inclusive. These are

(a) The lack of timely access to virus and pest-free planting material

(b) Lack of improved varieties adapted to local environments

(c) Damage due to the sweetpotato weevils, particularly in drier production areas

(d) Insufficient knowledge and use of better agronomic practices, and

(e) Lack of markets

2.6.1 SWEETPOTATO VIRUS DISEASE

Virus diseases are important constraints for sweetpotato production: more than half of the yield losses have been attributed to virus diseases (Karyeija *et al*., 2000; Ngeve and Bouwkamp, 1991). The sweetpotato virus disease complex (SPVD) caused by mixed infection of sweetpotato feathery mottle virus (SPFMV) and sweetpotato chlorotic stunt virus (SPCSV) is, by far, the most destructive viral disease in Africa (up to 50% in East Africa) and perhaps worldwide (Carey *et al*., 1999). In many cases, infection of sweetpotato by two or more different viruses causes greater damage than does infection by each of the viruses separately. This synergism has been documented for sweetpotato virus disease (Gibson *et al*., 1998; Karyeija *et al*., 2000). According to Karyeija *et al*. (1998) SPVD has been reported in a number of African countries including Ghana among other African countries like Nigeria, Rwanda, Uganda, Kenya and Tanzania.

In tropical regions like Ghana, the sweetpotato can be grown all year round. Vine cuttings from mature crops are used to plant new crops (Valverde *et al*., 2007). Since sweetpotato is a vegetatively propagated crop it is subject to accumulation of systemic pathogens in propagating materials (Bryan *et al*., 2003; Clark *et al*., 2002). Viral diseases occur wherever sweetpotato is cultivated. Because it is a vegetatively propagated crop, accumulation and perpetuation of viruses can become a major constraint for production. (Clark and Moyer, 1988).

2.6.2 SYMPTOMS OF SWEEPOTATO VIRUS INFECTION

Symptoms of the sweetpotato virus disease include stunted growth, leaf reduction and deformation, leaf curl/ roll up, leaf necrosis, chlorotic spots, necrotic spots, purple rings and can reduce yields of infected plants by over 90% (Gutierrez *et al*., 2003). Gibson *et al*. (1998) reported sweetpotato virus disease (SPVD) is the name commonly used in

Africa to describe a range of severe symptoms on sweetpotato generally attributed to virus infection. Symptoms vary with plant genotype but typically include stunted plants with small leaves, the latter often also being distorted, narrow (strap-like) and crinkled, with a chlorotic mosaic and/or vein-clearing, giving affected plants an overall pale appearance (Gibson *et al*., 1998). All these result into reduction in yield and quality. There are no reports of immune cultivars to sweetpotato viral infection (Gasura and Mukasa, 2010). SPVD is widespread and regarded as a serious problem in Africa, affected plants commonly yielding less than half that of symptomless ones (Mukiibi, 1977; Hahn, 1979).

Virus diseases often cause reduction in yield and quality of storage roots. Clark *et al.* (2002), and Villordon and La Bonte (1995) attributed decline in yield and quality of sweetpotato to the accumulation of mutations and viruses. Lewthwaite *et al*. (2011) reported in New Zealand that root yield of sweetpotato cultivars grown successively with vine cuttings from the field over a number of growing seasons appear to deteriorate in root yield and quality. Sweetpotato cultivars gradually decline in performance over years after they are released, and are often replaced within 20 years (Clark *et al*., 2002). The viruses that cause decline have not been fully determined and may vary from one part of the world to another (Valverde *et al*., 2007).

2.6.3 CONTROL OF SWEETPOTATO VIRUS DISEASES

Attempts at controlling sweetpotato viruses are relatively recent. They generally involve either use of resistant cultivars or ‘clean seed’ programs. A manifestation of some form of resistance (disease recovery phenomenon) has been reported in many vegetatively propagated crops like sweetpotato although there no reports of immune cultivars (Gasura and Mukasa, 2010). The relative merits of these two approaches are viewed

quite differently in various countries with different production systems (Valverde *et al*., 2007).

2.6.3.1 PATHOGEN-TESTED PLANTING MATERIAL

It is considered that the most effective method of minimizing sweetpotato viral disease is through the use of virus-tested propagation material derived from meristem shoot tip culture (Loebenstein *et al*., 2009). However, this method has not been extensively practised in Ghana. Combinations of heat treatment of plants followed by the culturing of plant meristems have proved the most successful method of eradicating viruses from plants. Pathogen-tested seed is generated from tissue culture plants that have been tested for known viruses and found to be apparently free of these viruses. Since it is not possible to prove the absence of all viruses, it is also called virus-tested not virus-free. Research institutes and a large number of scientists globally over the last 20 years have been involved in producing pathogen-tested tissue cultures of both agricultural and horticultural crops (Loebenstein *et al*., 2009). For vegetatively propagated crop such as sweetpotato, it is well known that the use of pathogen-tested propagation material provides the grower with a high-health crop of superior quality and a likely superior yield (Wang and Hu, 1980).

Most of the local landraces and some of the introduced material are degenerated because of sweetpotato virus disease (Low *et al*., 2009). Yield gains of 30%-60% can be obtained through use of healthy planting material in some cultivars (Clark and Hoy, 2006; Gibson *et al*., 2004). In countries where care is taken to provide pathogen-tested planting material as amongst others in US and Israel, yields increase markedly up to seven times or more (Thottappily, 2009). Fuglie *et al*. (1999) studied the impact of the "virus-free" production system in Shandong province, China. Cultivation of pathogen-

tested cultivars significantly increased yields ranging from 10.3% to 101.9% with an average increase of 37.9%. The increased effect on yield was much more markedly with old cultivars than with new ones. Marketable yield (tubers > 100 g) was increased by 22.2% when pathogen-tested plants were used as compared with virus-infected ones (Fuglie *et al*., 1999). Okpul *et al*. (2011) reported responses to viral infection can be considered as cultivar specific. This was demonstrated by a recent Australian study in which virus-tested cultured and field derived plants were compared. Beauregard showed the greatest gain with a 148% increase in total storage root yield when grown from tissue cultured plants, whilst 'Wanum' showed the greatest loss, decreasing in yield by 23% under the same experimental conditions.

2.7 SUSTAINABLE SEED SYSTEM

Improved seed systems have a proven track record in raising productivity of clonal crops such as sweetpotato through the provision of quality planting material and through the efficient dissemination of improved varieties. In Sub-Saharan African countries like Ghana, the same variety is called different names in different places which make management of these varieties very much complicated. A sustainable seed system has several advantages such as ensuring that growers have ready access to adequate quantities of planting material of the varieties they are interested in and at the time they are ready to plant. Setimela *et al*. (2004) reported in order to maintain superiority of genotypes and, in some cases, health, there needs to be capacity within seed systems for generation, dissemination and multiplication of new stock, new cultivars and or pathogen-free material.

2.8 RELEASED VARIETIES OF SWEETPOTATO IN GHANA

A variety may be defined as a taxonomic unit created and maintained by man, the first essential being that it should have an individuality which can be reproduced over a number of years, and secondly that it should be distinguishable by inherited morphological or physiological characters from other varieties (Bishaw and van Gastel, 2009). The new variety released must be distinct, uniform and stable. Variety release procedure is a collective term that refers to the released type, the attached terms and conditions, the protocols and administrative procedures used in releasing a new variety for seed production and distribution (Delouche and Goma'a, 1999).

Eight varieties of sweetpotato have been released by the CSIR-Crops Research Institute and one released by KNUST. Okumkom, Sauti, Faara and Santom Pona were released in 1998. Mean fresh tuber yield of these varieties across different locations 4 MAP are presented in Table 2 (CSIR-CRI, 1998). In 2005, Hi-Starch, Ogyefo, Otoo and Apomuden were also released. These are high yielding, resistant to pests and diseases and good for food and industrial products. Apomuden recorded the highest mean fresh tuber yields followed by Otoo, Ogyefo and High-Starch (Table 3), (CSIR-CRI, 2005). Officially released varieties in Ghana are all selections from exotic introductions.

TABLE 1. INFORMATION ON SWEETPOTATO VARIETIES OFFICIALLY RELEASED IN GHANA

Name	Year Released	Original Name	Origin
Okumkom	1998	TIS 8266	IITA
Sauti	1998	Tanzania, Kenya	Malawi
Faara	1998	TIS 3017	IITA
Santom Pona	1998	TIS 84/0320	IITA
Hi-Starch	2005	Hi-Starch	Japan
Ogyefo	2005	Mugande	Rwanda via CIP
Otoo	2005	Mogamba	Burundi via CIP
Apomuden	2005	Kamala Sundari	Bangladesh via CIP
Tek Santom	2003	Tib 2	IITA

Source: CSIR-CRI, Ghana

TABLE 2. CHARACTERISTICS AND YIELD OF SWEETPOTATO VARIETIES OFFICIALLY RELEASED IN GHANA IN 1998

Characteristics	Okumkom (TIS 8266)	Santom pona (TIS 84/0320)	Faara (TIS 3017)	Sauti (Kenya)
Total vine length (cm)	547	594	721.2	732.1
Vine apex colour	Green	Green	Green	Green
Colour (young leaves)	Green	Green	Green	Green
Leaf petiole colour	Green	Green	Green purple	Green
Leaf vein colour (abaxial view)	Green	Light green	Green purple	Green
Leaf shape	Cordate	Cordate	Angulatus	Palmatus
Tuber skin colour	Light purple	Cream	Deep purple	Cream
Tuber shape	Round, elliptic (mixture)	Long elliptic, round, irregular (mixture)	Long (elliptic)	Long irregular or curved
Tuber flesh colour	White	Cream or yellow	Cream	Yellow
Fresh tuber yield (t/ha) 1995-1997				
Forest	19.9	16.7	16.9	15.4
Transition	12.0	10.2	9.3	8.5
Mean (across different locations)	13.1	14.4	15.1	13.1
Dry matter (%) of tuber (1995,996)	32.6			
Forest	32.7	31.9	34.4	36.2
Transition	30.3	35.3	36.5	38.3

Source: CSIR-CRI, Ghana; Otoo *et al.* (2000)

TABLE 3. CHARACTERISTICS AND YIELD OF SWEETPOTATO VARIETIES OFFICIALLY RELEASED IN GHANA IN 2005

CHARACTERISTICS	Apomuden (Kamala Sundari)	Otoo (Mogamba)	Hi-Starch	Ogyefo (Mugande)
Total vine length (cm)	224.5	92	163	106
Vine apex colour	Purple	Green	Green	Purplish green
Colour (young leaves)	Purple	Green	Greem	Purple
Leaf petiole colour	Green	Green	Green	Green
Leaf vein colour (abaxial view)	Green	Purple	Green	Green
Leaf shape	Cordate	Triangular	Triangular	Cordate
Tuber skin colour	Reddish brown	Cream	Creamy brown	Ruby
Tuber shape	Obovate	Long-elliptic	Elliptic	Long elliptic
Tuber flesh colour	Reddish orange	Light orange	Cream	White
Fresh tuber yield (t/ha) 2001, 2002				
Forest (Fumesua)	48.9	30.7	14.7	25.9
Transition (Wenchi)	22.1	16.6	17.0	9.9
Mean (Across 5 locations)	22.6	15.4	6.0	11.3
Marketable fresh tuber yields (t/ha)				
Forest (Fumesua)	45.7	28.5	26.7	25.4
Transition (Wenchi)	21.6	15.6	12.5	8.9
Mean (Across 5 locations)	19.9	13.8	4.9	10.4
DM (%) of tuber	21.9	32.2	47.0	40.1

Source: CSIR-CRI, Ghana

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 LOCATION

Field work was carried out in two locations, Fumesua and Ejura in the Forest and Transition agro ecological zones (AEZ) of Ghana respectively. Laboratory work was carried out at the Sweetpotato Quality and Nutrition, and Molecular Biology Laboratories for post-harvest and molecular studies respectively at CSIR-Crops Research Institute, Fumesua.

3.2 FIELD WORK

3.2.1 GENOTYPES

Twenty genotypes were used in the field work. These included nine released varieties, 10 pathogen-tested introduced varieties and advanced genotypes of CSIR-CRI Sweetpotato breeding programme (so-called mega clones) and one local clone. The nine released varieties (CSIR-CRI 1998, 2005) were Okumkom, Sauti, Faara, Santom Pona, Hi-Starch, Ogyefo, Otoo, Apomuden, all released by CSIR-Crops Research Institute ,and Tek Santom released by KNUST. TIS 8266 (CIP 440070; PI0134), TIS 3017 (CIP 440064; PI0130), Tanzania (CIP 440166; PI0101), Kenya (PI0047) and Mogamba (CIP 440034; PI0058) constituted the pathogen-tested introduced varieties. TIS 8266 and TIS 3017 were received from CIP Headquaters, Peru whiles Tanzania, Kenya and Mogamba were received from CIP-Kenya. The five mega-clones were Mohc, Ningshu 1, Cemsa 74-228, Kemb 37 and 199062.1. The local clone used was Fiaso Local Red.

TABLE 4. PUTATIVE RAMETS FOR FIELD WORK

Putative clone	Genotype	PI No.	Year of field introduction
1	Otoo		2001
	Mogamba	0058	2011
	Mohc		2008
2	Sauti		1995
	Kenya	0047	2011
	Tanzania	0101	2011
3	Okumkom		1995
	TIS 8266	0134	2010
4	Faara		1995
	TIS 3017	0130	2010

Genotypes without PI No were introduced prior to implementation of the PI numbering system

3.2.2 LAND PREPARATION AND PLANTING

At each site, the trial was carried out on a total plot size of 246.4 m^2. The land used for the trial was cleared and ploughed. Ridges were made manually using hoes. Planting materials of cultivars and introduced virus-tested materials of the genotypes were field multiplied at Fumesua, before planting in replicated trials at 2 locations during the 2011 major growing season. Planting was done on 14th and 25th July, 2011 at Fumesua and Ejura respectively. For each genotype, 16 vines per plot were sown in two rows on ridges, spaced at 1m between and 0.3 m within rows. Each vine cutting with a length of about 30 cm was inserted at a slant with two-thirds buried below the soil surface. One genotype (SPK 004) was grown at the edges of the trial to serve as a border. The trials were weeded as needed. The trials were rain-fed.

3.2.3 EVALUATION OF MORPHOLOGICAL TRAITS

Twenty morphological traits for the sweetpotato descriptors were scored using a scale of zero to nine (CIP/AVRDC/IBPGR, 1991) at 90-120 days after planting (DAP). These traits can be grouped into foliar morphology (90-100 DAP) and storage root (120 DAP) descriptors.

Foliar morphology traits included

1. plant type (PTP)
2. vine internode length (VNL
3. vine internode diameter (VID)
4. predominant vine colour (PVC)
5. secondary vine colour (SVC)
6. vine tip pubescence (VTP)
7. general outline of the leaf (GOL)
8. leaf lobes type (LLT)
9. leaf lobe number (LLN)
10. shape of central leaf lobe (SCL)
11. mature leaf size (MLS)
12. abaxial leaf vein pigmentation (ALP)
13. mature leaf colour (MLC)
14. immature leaf colour (ILC)
15. petiole length (PTL)
16. petiole pigmentation (PPT)

Storage root traits included

17. storage root shape (SRS)
18. predominant skin colour (PSC)
19. intensity of predominant skin colour (IPC)
20. predominant flesh colour (PFC).

Samples of six plants, three from each row per plot were scored for the foliar morphology characters. With respect to the storage root characters five tubers were selected as samples and scored. Vine internode length and mature leaf size were

measured using 30 cm ruler. MLS was measured as length from the basal lobes to the tip of the leaves. Length of main vine (plant type) and vine internode diameter were measured with a measuring tape and vernier calipers respectively. For the qualitative attributes, visual observations were made. Traits relating to length or size were scored as the average value of measurements made on the sample plants. Vine and leaf characters were recorded as the average expression of the character observed in a section of the main stem located in the middle portion of several main stems.

3.2.4 EVALUATION OF VIRUS INCIDENCE AND SEVERITY

Severity of sweetpotato virus symptoms was scored for each cultivar every two weeks from 6 weeks after planting (WAP) through fourteen WAP using a 5-point severity rating scale Hahn (1979) where: 1 = no visible symptoms; 2 = mild symptoms; 3 = moderate symptoms; 4 = severe symptoms; and 5 = very severe symptoms. In each plot, the number of plants exhibiting each severity rating was counted, and a weighted mean severity value based on incidence was derived. This value was derived by multiplying number of plants scored at each severity level by the severity score, and dividing the sum of these values by the total number of plants per plot. Line graphs were drawn using mean weighted severity values against time from 6 WAP to 14 WAP which demonstrated graphic presentation of performance of released varieties and putative ramets.

3.2.5 HARVESTING

Harvesting was done 120 DAP. Plots were harvested by uprooting the centre six plants of each row, leaving a plant at both ends, which served as borders. Vines were first cut

with cutlasses and the storage roots were uprooted with hoes. Data was taken on the following parameters

- Weight of commercial roots (CRW) (roots weighing > 100g)
- Weight of non-commercial roots (NCRW) (roots weighing ≤ 100g)

  Commercial yield (tons/ha) for each plot was calculated as CRW*10/plot area (m^2)

  Non commercial yield (tons/ha) for each plot was calculated as NCRW*10/plot area (m^2)

  Total yield (tons/ha) for each plot was calculated as Commercial yield + Non commercial yield (Riis-Jacobson, 2011).

3.2.6 EVALUATION OF QUALITY TRAITS

Near Infrared Reflectance Spectroscopy (NIRS) method (Shenk and Westerhaus, 1993) was used. Harvested roots packed into labelled plastic polythene bags were sent to the laboratory. The roots were put together into a composite pile and samples of five roots were taken for analysis. Samples were sorted based on the size of roots. The roots were washed with running tap water to remove soil particles and debris. They were allowed to dry and packed into brown paper bags which were labeled with respect to plot numbers and names of genotypes.

The roots arranged in the brown paper bags were lined up according to genotypes with three replications. That is harvested roots from a particular genotype from different replications were put together. Samples were prepared by cutting each root lengthwise into four sections with kitchen knives. Two opposite sections of each of the sectioned roots were taken to prepare samples of approximately 50 g by slicing them using a stainless steel kitchen vegetable slicer. This was placed in transparent polythene bags,

weighed, and frozen. The samples were then crushed to break into small particles and lyophilized using YK-118 Vacuum Freeze Dryer (True Ten Industrial Company Limited Taichung, Taiwan). Dried samples were weighed, milled into flour in a stainless steel mill (3383-L70, Thomas Scientific, Dayton Electric Manufacturing Company Limited, Niles, IL 60714, USA) to pass a 200 mesh screen. The milled samples were stored in sealed transparent bags which were duly sealed. The samples were then scanned to determine protein, minerals and carbohydrate components using XDS Rapid Content Analyzer (Hoganae, Sweden).

The following parameters were determined

1. Dry matter (dry weight/fresh weight × 100)
2. Protein %
3. Iron (Fe) ppm
4. Zinc (Zn) ppm
5. Starch %
6. Fructose %
7. Glucose %
8. Sucrose %

3.3 MOLECULAR WORK

3.3.1 GENOTYPES

Thirty four (34) genotypes were used in the molecular studies. These genotypes comprised released varieties, introduced varieties and one local accession. Some of the introduced genotypes were putatively the same varieties which had been introduced

more than once, but have been given different plant introduction numbers (PI Nos.) with each introduction.

TABLE 5. GERMPLASM FOR MOLECULAR STUDIES

Laboratory Code	PI No	Genotype	Year of field introduction
1		Ogyefo	2001
2		Otoo	2001
3		Sauti	1995
4		199062.1	2008
5		Faara	1995
6		Hi-Starch	2001
7		Okumkom	1995
8		Cemsa 74-228	2001
9		SantomPona	1995
10	0503	NingShu 1 (2)	2011
11		Kemb 37	2001
12		Mohc	2008
13		Apomuden	2001
14		TekSantom	2001

Genotypes without PI No were introduced prior implementation of the PI numbering system. (1) = Elite variety introduced first (2) = Elite variety with same name introduced second

TABLE 5 (CONT'D). GERMPLASM FOR MOLECULAR STUDIES

Laboratory Code	PI No	Genotype	Year of field introduction
15		Fiaso local red	Local
16	0134	TIS 8266 (CIP 440070)	2010
17	0047	Kenya	2011
18	0130	TIS 3017 (CIP 440064)	2010
19	0101	Tanzania (CIP 440166)	2011
20	0058	Mogamba (CIP 440034)	2011
21	0374	Mugande (UG 118)	2011
22	0387	Mugande (PE 148)	2011
23	0456	Mugande (RW 360)	2011
24	0461	Kamala Sundari (BG 402)	2011
25	0454	Kemb 10 (KN 352)	2011
26	0496	Kemb 10 (UG 191)	2011
27	0498	440169-Kemb 10 (PE 143)	2011
28	0497	Kemb 10 (UG 061)	2011
29	0501	Kemb 37 (KN 349)	2011
30		Ningshu 1 (1)	2008
31	0384	199062.1 (TZ 596)	2011
32	0499	199062.1 (CP 449)	2011
33	0148	Tib 2 (CIP 440096	2010
34	0334	Mohc (LM 483)	2011

Genotypes without PI No were introduced prior to implementation of the PI numbering system. (1) = Elite variety introduced first (2) = Elite variety with same name introduced second

3.3.2 DNA EXTRACTION AND PURIFICATION

The 34 genotypes were established at the screen house. Young-tender leaves were harvested when they were 14 days old for DNA extraction. Genomic DNA was isolated as described by Egnin *et al.* (1998) and modified by the Council for Scientific and Industrial Research - Crops Research Institute (CSIR-CRI) laboratory. 0.2 g of tender sweetpotato leaves were crushed in liquid nitrogen and 800 µl of lysis buffer (50 mM Tris HCl (pH 8.0), 300 mM NaCl, 20 mM EDTA, 20% PVP, 1.5% sarkocine and 0.1 g/L $Na_2S_2O_5$) was used to lyse nuclear membranes. Protein contaminates from the cell lysate were removed using 400 µl of 5 M potassium acetate (instead of 800 µl of phenol chloroform isoamyl alcohol as used by Egnin *et al*., 1998) and the samples centrifuged at 1300 rpm for 15 min. RNA was removed by adding 4 µl RNase A (10 mg/ml), and incubating at 37°C for 30 min. DNA was precipitated using 700 µl of ice-cold isopropanol and centrifuged at 13000 rpm for 15 minutes. 80% ethanol was used to wash DNA and centrifuged at 13000 rpm for five minutes. Ethanol was discarded and DNA pellets were air-dried at room temperature. DNA pellets were dissolved in 1 X TE (Tris-ethylenediaminetetracetic acid) buffer after which quality of DNA was determined on 0.8% agrose gel.

3.3.3 SIMPLE SEQUENCE REPEAT (SSR) AMPLIFICATION

The reaction was carried out in Multigene Gradient thermal cycler (Labnet International Inc., California, USA) with heated lid to reduce evaporation. The DNA from the 34 cultivars were fingerprinted using SSR markers in a 10 µl PCR (Polymerase Chain Reaction) reaction mixture containing 1.0 µl of buffer (10X), 0.9 µl of $MgCl_2$ (25 mM), 0.4 µl of dNTPs (Deoxynucleotide Triphosphates) (10 mM), 1 µl of both forward and reverse primer (10 µM), 0.125 µl Taq polymerase (5 U), 3.0 µl of genomic DNA template all together with 2.575 µl of nuclease free PCR water.

Five informative SSR markers (Table 5) previously selected from 26 pairs of SSR primers confirmed for sweetpotato (Buteler *et al*., 1999) were used for polymerase chain reactions (PCR). The amplification conditions were set up as 94 ^{o}C for initial denaturation for 4 minutes, annealing at between 56.0 and 62.0 ^{o}C (depending on the annealing temperature of the primer as per Table 5); polymerization at 72 ^{o}C for 1 minute; with step 2 repeated 30 times, and a final extension at 72^{o}C for 7 minutes.

TABLE 6. SSR MARKERS USED TO CHARACTERIZE SWEETPOTATO GENOTYPES.

Name of primer	Forward primers	Reverse primers	Temperature ^{0}C
IB-S11	5^{1}-CCCTGCGAAATCGAAATCT-3^{1}	5^{1}-GGACTTCCTCTGCCTTGTTG-3^{1}	58
IB-S17	CAGAAGAGTACGTTGCTCAG	GCACAGTTCTCCATCCTT	58
J10A	TCAACCACTTTCATTCACTCC	GTAATTCCACCTTGCGAAGC	58
IBC5	CCACAAAAATCCCAGTCAACA	AGTGGTCGTCGACGTAGGTT	62
IBC12	TCTGAGCTTCTCAAACATGAAA	TGAGAATTCCTGGCAACCAT	56

3.3.4 PAGE AND SILVER NITRATE STAINING

PCR products were separated using PAGE (polyacrylamide gel electrophoresis). The amplified DNA fragments were separated on 6% polyacrylamide gel at 200 v for 30-45 min in TBE (Tris-borate-ethylenediaminetetracetic acid) (1X) using a mini-protean 3 cell electrophoretic apparatus (MS Major Science, UK). A 100 bp DNA marker (gene rule) was used as a standard and the DNA amplified fragments were visualized in the gel by silver nitrate staining. The slides were placed in plastic bowls. Glacial acetic acid was added for 10 minutes to remove slides from gel. Nitric acid (1.5%) was added for 5 minutes and silver nitrate for 15 minutes to stain the amplified bands. The gels were

gently shaken in the bowls for the period the reagents were added. After each reagent was added, the gels were gently washed with distilled water before another reagent was added. A developer (containing Na_2CO_3, 37% formaldehyde and sodium thiosulphate) was added to visualize the bands. The developing of the bands was stopped by adding glacial acetic acid. The gel was stored in distilled water. PCR products were scored for presence (1) or absence (0) of bands.

3.3.5 EXPERIMENTAL DESIGN AND DATA ANALYSIS

The field trial was laid in a randomized complete block design with three replications. The GenStat Discovery Edition (Version 4, VSN International Limited, UK.) was used for data analysis. Cluster analysis was done using unweighted pair-group average method based on Jaccard's coefficient similarity matrix for both molecular and foliar morphology phenotypic data. The similarity matrices were used to generate dendrograms. Pre-harvest, harvest and post-harvest data were subjected to Analysis of variance (ANOVA). LSD (0.05) was used to separate treatment means.

Restricted Maximum Likelihood (REML) variance components analysis was also used to analyze data for weighted severity virus score which was derived from incidence and severity of virus symptoms taken every two weeks till the fourteenth week after planting (WAP). Since data in a repeated measure are dependent and correlated, REML variance components analysis provides an effective analysis for repeated measurements. It involves the use of mixed models approach to test the significance of evaluated date, genotype and the interaction between evaluated date and genotype. Treatment means were separated using lsd (0.05) to separate treatment means where differences were significant ($p < 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 GENETIC RELATIONS OF VARIETIES BASED ON PHENOTYPIC CHARACTERS

A dendrogram based on foliar morphological phenotypic characterization of 20 genotypes in the Forest agroecological is presented in Fig 1. Similarity coefficients ranged from 0.65 to 1. Tanzania and Kenya were identical in all the 16 foliar morphology characters. These two genotypes showed similarity with the released variety Sauti at a similarity level of 0.97. Comparably, Mogamba and Mohc clustered together at a similarity level of 0.99 which were similar with the released variety Otoo at a similarity level of 0.98.

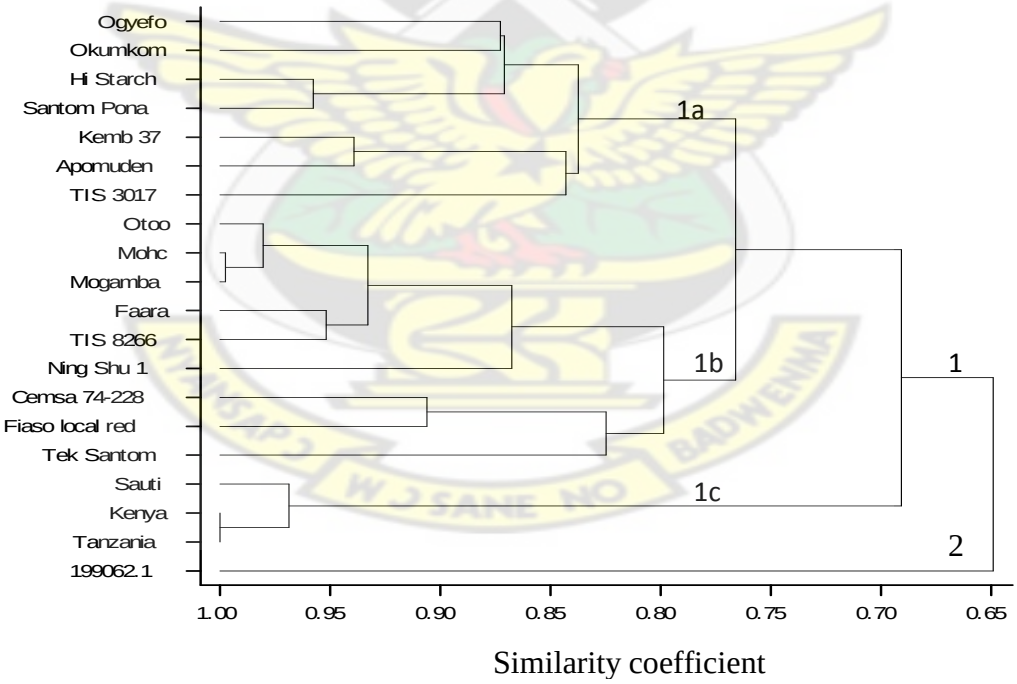

Fig. 1 Dendrogram of 16 phenotypic foliar morphology characters of 20 genotypes in the Forest AEZ of Ghana based on Jaccard's coefficient of similarity index using UPGMA. Two main clusters were identified with three subgroups

Okumkom and TIS 8266 as well as Faara and TIS 3017 which are putative ramets were much less closely related on the basis of morphological descriptors. The genotype 199062.1 formed one cluster and was different from all other genotypes (Table 7)

TABLE 7. DENDROGRAM GROUPS (0.65) AND SUB-GROUPS (0.80) BASED ON MORPHOLOGICAL DESCRIPTORS IN THE FOREST AEZ

Cluster	Sub-cluster	Genotypes
1	1a	Ogyefo, Okumkom, Hi Starch. Santom Pona, Kemb 37, Apomuden, TIS 3017
	1b	Otoo, Mohc, Mogamba, Faara,TIS 8266, Ning Shu 1, Cemsa 74-228, Fiaso local red, Tek Santom.
	1c	Sauti, Kenya, Tanzania
2		199062.1

Similar results were obtained when the characterization was repeated at Ejura in the Transition AEZ. Similarity coefficient ranged from 0.65 to 1. The introduced genotypes Kenya and Tanzania had a similarity coefficient of 1 (Fig. 2). Tanzania and Kenya were identical and was similar to Sauti just like Mogamba, Mohc and Otoo showed similarities. Again, the putative ramets TIS 8266 and Okumkom were found in different sub-clusters similarly to TIS 3017 and Faara. There were slight changes in groupings (Fig. 2) because of differences in characters based on quantitative measurements such as main vine length and mature leaf size (Table 8).

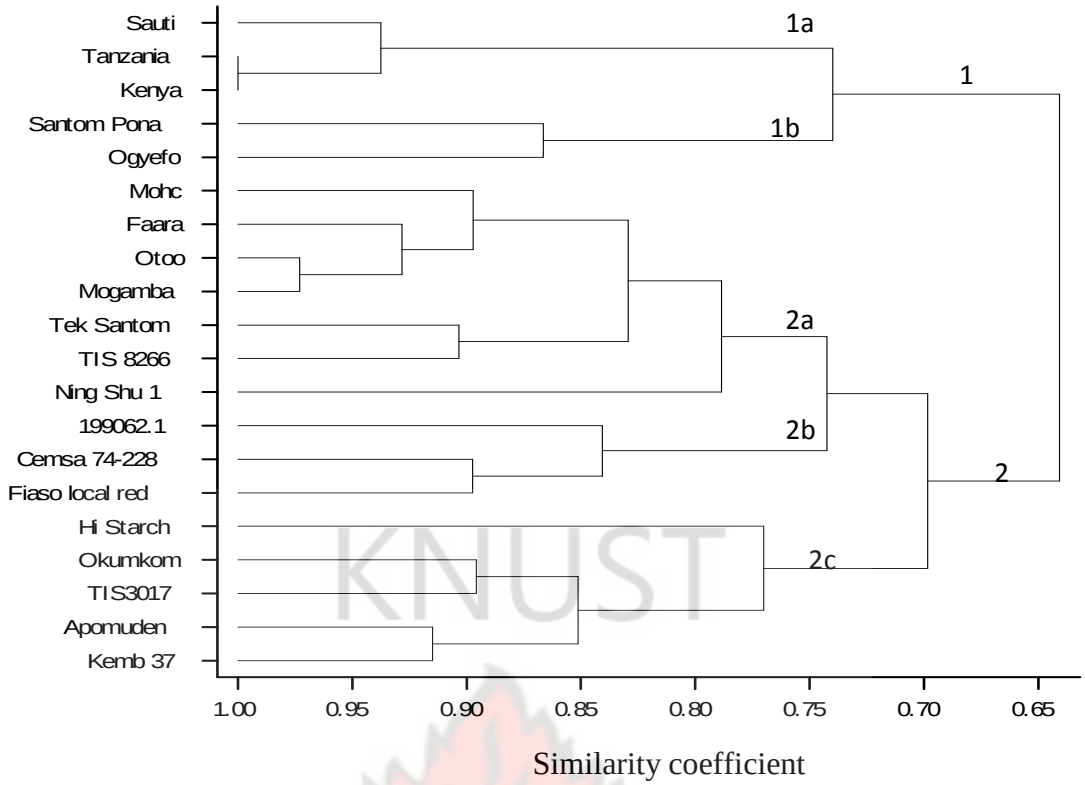

Fig. 2 Dendrogram of 16 phenotypic foliar morphology characters of 20 genotypes in the Transition AEZ of Ghana based on Jaccard's coefficient of similarity index using UPGMA. Two main clusters were identified with five subgroups

TABLE 8. PHENOTYPIC DESCRIPTORS OF SWEETPOTATO GENOTYPES HYPOTHESIZED TO BE RAMETS OF THE SAME CLONE

Putative Clone	Genotype	plant type	vine internode length	vine internode diameter	predominnt vine colour	secondary vine colour	vine tip pubescence	general outline of leaf	leaf lobe type	leaf lobe no.	shape of central leaf lobe
1	Otoo	erect	short	intermediate	Green with many purple spots	Purple nodes	sparse	Triangular	very slight	1	Toothed
	Mogamba	Semi-erect	short	intermediate	Green with many purple spots	Purple nodes	sparse	Triangular	slight	3	Triangular
	Mohc	Semi-erect	short	intermediate	Green with many purple spots	Purple nodes	sparse	Triangular	slight	3	Triangular
2	Sauti	semi -erect	intermediate	Intermediate	mostly dark purple	green nodes	Absent	Lobed	deep	5	Elliptic
	Kenya	semi -erect	Intermediate	Thin	mostly dark purple	green nodes	Absent	Lobed	deep	5	Elliptic
	Tanzania	semi -erect	Intermediate	Thin	mostly dark purple	green nodes	Absent	Lobed	deep	5	Elliptic
3	Okumkom	extremely spreading	Intermediate	very thin	mostly purple	green with few purple spots	Heavy	Triangular	very slight	3	Triangular
	TIS 8266	semi-erect	intermediate	Thin	mostly purple	mostly purple	Sparse	Triangular	very slight	3	Triangular
4	Faara	semi-erect	short	Thin	mostly dark purple	mostly purple	Sparse	Triangular	very slight	1	Triangular
	TIS 3017	semi-erect	intermediate	Thin	mostly dark purple	green nodes	heavy	Lobed	moderate	5	Triangular

TABLE 8 (CONT'D). PHENOTYPIC DESCRIPTORS OF SWEETPOTATO GENOTYPES HYPOTHESIZED TO BE RAMETS OF THE SAME CLONE

Putative clone	Genotype	Mature leaf size	Abaxial leaf vein pigmentation	Mature leaf colour	Immature leaf colour	Petiole length	Petiole pigmentatio	Storage root shape	Predominant skin colour	Intensity of predominant skin colour	Predominant flesh colour
1	Otoo	medium	All veins mostly or totally purple	Green	Green	Short	Green with purple at both ends	Long elliptic	cream	intermediate	Pale orange
	Mogamba	medium	All veins mostly or totally purple	Green	Green	Short	Green with purple at both ends	Long elliptic	cream	intermediate	Pale orange
	Mohc	medium	All veins mostly or totally purple	Green	Green	Short	Green with purple at both ends	Long elliptic	cream	Dark	Pale orange
2	Sauti	Large	Green	Green	Green with purple edge	Short	green	elliptic	cream	Dark	Dark yellow
	Kenya	Large	Green	Green	Green with purple edge	intermediate	green	Long elliptic	cream	intermediate	Dark yellow
	Tanzania	Large	Green	Green	Green with purple edge	intermediate	green	Long elliptic	cream	intermediate	Dark yellow
3	Okumkom	medium	Green	Green with purple edge	Green with purple edge	Short	green	Round elliptic	Dark purple	Pale	Cream
	TIS 8266	Medium	All veins most or totally purple	green	Green	Short	Green with purple stripes	Ovate	Cream	intermediate	Orange
4	Faara	medium	All veins most or totally purple	Green with purple edge	Green with purple edge	Very short	Totally or mostly purple	Elliptic	Dark purple	Dark	Dark yellow
	TIS 3017	medium	Green	Green with purple edge	Green with purple edge	Very short	green				

Minor differences in morphological traits were observed between two pairs of ramets while the other two pairs differed in 50% or more of the characters scored phenotypically (Table 8). Otoo and Mogamba were identical in all traits but differed in plant type and leaf lobe characters including leaf lobe type and number and shape of central leaf lobe. Mohc and Mogamba had all characters the same with shape of central leaf lobe as the only difference. Kenya and Tanzania were duplicates. Sauti and Kenya were indistinguishable in all characters. However, differences were observed in vine internode diameter, petiole length, storage root shape, intensity of predominant skin colour. Faara and TIS 3017 shared 50% of the observed characters in common while Okumkom and TIS 8266 were identical in less than 50% characters (Table 8). Faara and TIS 3017 differed in vine internode length, secondary vine colour, vine tip pubescence, general outline of leaf, leaf lobe characters, abaxial leaf vein and petiole pigmentation. Okumkom and TIS 8266 distinguished themselves in characters like plant type, vine internode diameter, secondary vine colour, vine tip pubescence, abaxial leaf vein pigmentation, mature and immature leaf colour, petiole pigmentation, storage root shape, predominant skin colour and intensity as well as predominant flesh colour (Table 8).

4.2 GENETIC RELATIONS OF GENOTPES BASED ON MOLECULAR MARKERS

The five SSR markers used to discriminate the released, elite and introduced genotypes produced a total of 210 polymorphic bands at 66 loci with a mean of 3.2 alleles per locus. All markers were polymorphic. Plate 1 presents result for IB-S17 SSR marker with the mean number of alleles ranging from one to eight per SSR marker locus. The PCR products ranged between 50 bp to 500 bp in size.

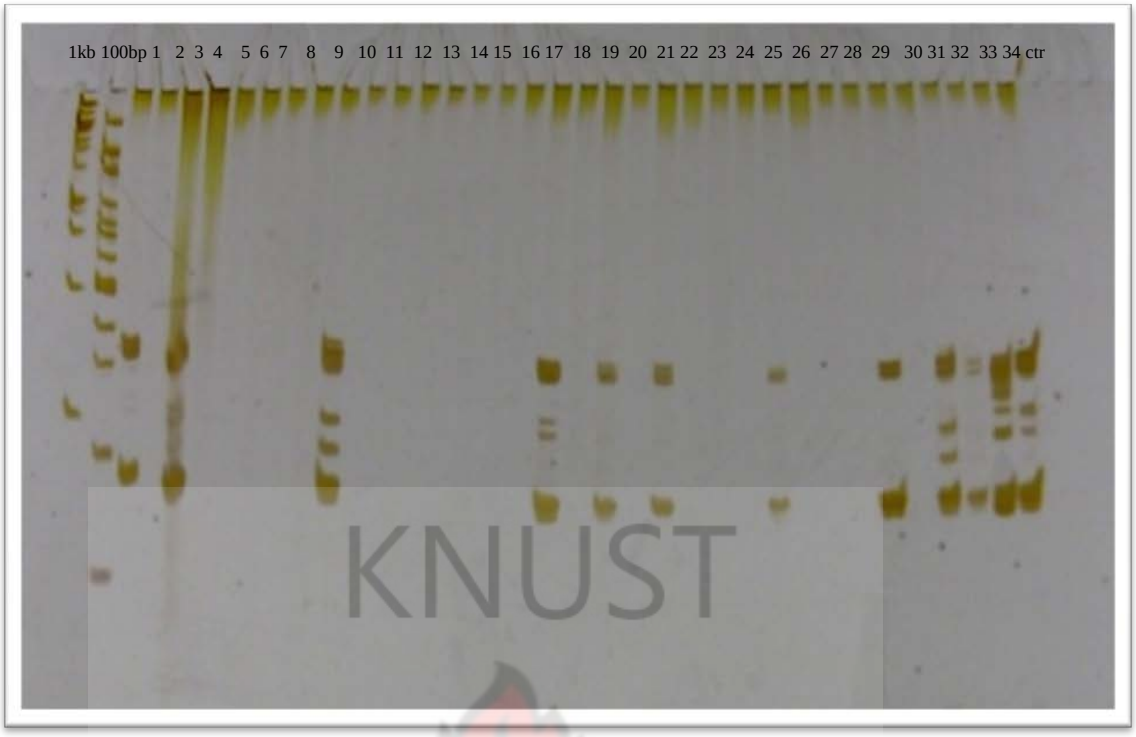

Plate 1. IB-S17 SSR marker showing polymorphic bands

A dendrogram based on molecular characterization of 34 genotypes is presented in Fig 3. Genetic similarity ranged from 0.58 to 1. The five SSR markers could not effectively discriminate between putative ramets unlike the morphological descriptors used in this study. About one-third of the total number of genotypes used in the study which are not known ramets were found to be identical with a similarity coefficient of 1.

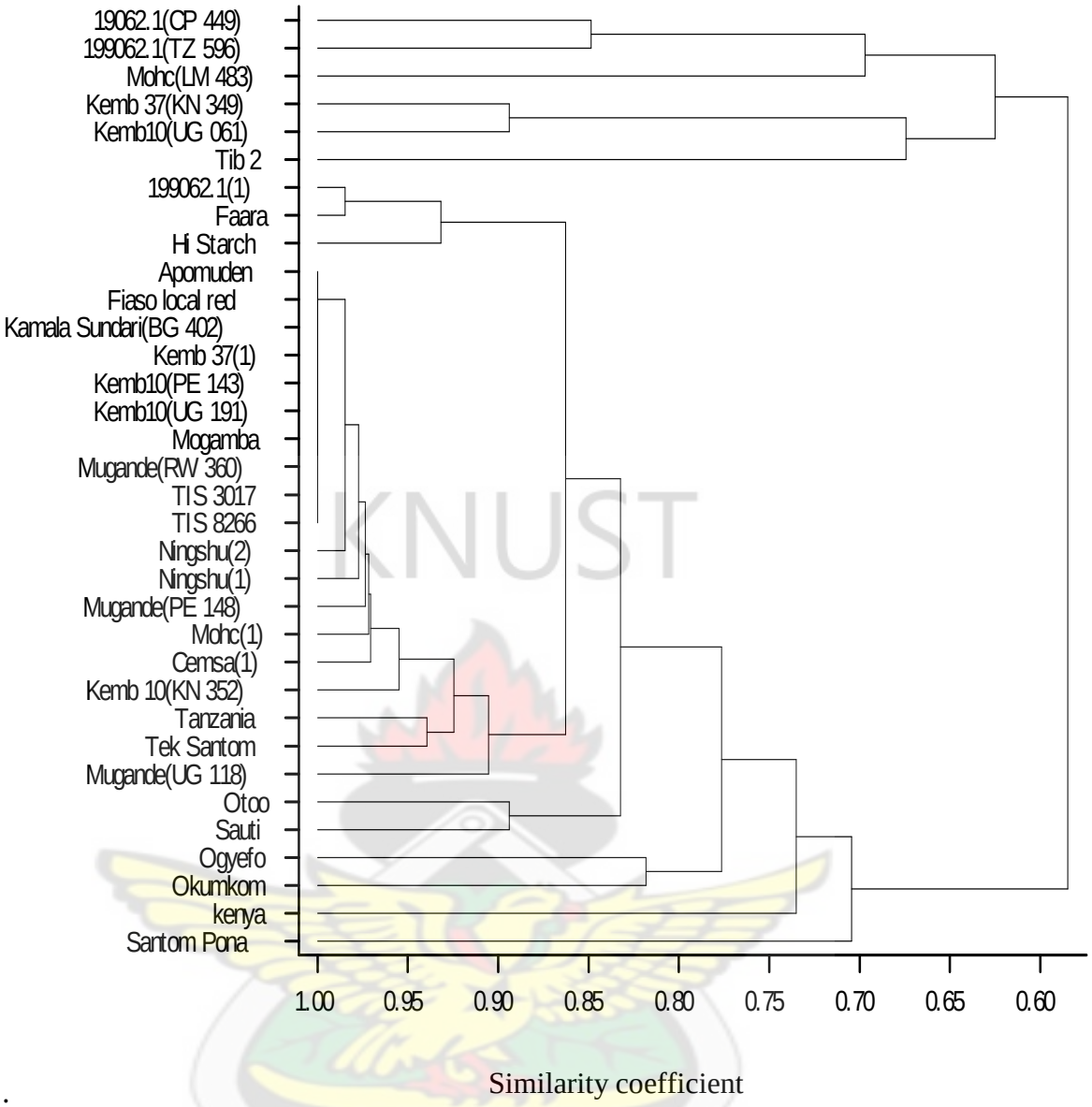

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Fig. 3 Dendrogram of five SSR markers of 34 genotypes based on Jaccard's coefficient of similarity index using UPGMA.

4.3 EVALUATION OF QUALITY TRAITS

Analysis of variance (ANOVA) revealed significant differences between putative ramets at the two locations (Table 9).

TABLE 9. COMPARISON OF QUALITY TRAITS BETWEEN PUTATIVE RAMETS IN THE FOREST AEZ IN THE 2011 MAJOR GROWING SEASON

Putative clone	Genotype	Dry Matter % fresh root	Iron ppm	Zinc ppm	Protein	Starch	Fructose	Glucose	Sucrose
						% dm			
1	Otoo	37.60	1.57	0.83	4.63	70.47	0.57	1.67	9.63
	Mogamba	34.93	1.63	0.83	4.67	70.33	0.43	1.80	13.70
	Mohc	38.33	1.60	0.90	4.80	71.13	0.40	1.67	12.97
2	Sauti	40.60	1.50	1.03	4.80	71.23	0.37	1.93	6.93
	Kenya	39.17	1.57	1.07	5.30	70.73	0.27	1.63	7.60
	Tanzania	40.77	1.57	1.07	5.03	71.10	0.33	1.70	7.50
3	Okumkom	37.13	1.67	0.93	4.70	68.17	2.10	3.77	8.17
	TIS 8266	32.73	1.87	1.27	5.93	67.13	1.37	2.70	9.33
	lsd (5%)	2.86	0.26	0.13	0.96	2.41	0.57	0.70	3.47
	CV (%)	4.50	8.90	7.30	11.70	2.10	44.30	19.30	22.60

The released variety Okumkom was significantly different from its original source material in more than 50% of the quality traits evaluated. These differences were manifested in percentage dry matter in fresh root as well as percentage protein, zinc and

fructose contents in dry matter. Otoo was identical to Mogamba, its original source material in all quality traits with significant difference in only percentage sucrose content in dry matter. Mogamba and Mohc exhibited significant difference in dry matter contents (Table 9). TIS 3017 was not evaluated because it did not produce marketable yield which was needed for NIRS analysis.

TABLE 10. COMPARISON OF QUALITY TRAITS BETWEEN PUTATIVE RAMETS IN THE TRANSITION AEZ IN THE 2011 MAJOR GROWING SEASON

Putative clone	Genotype	Dry Matter % fresh root	Iron ppm	Zinc ppm	Protein	Starch	Fructose % dm	Glucose	Sucrose
1	Otoo	36.21	1.57	0.95	4.58	69.91	0.37	1.61	10.57
	Mogamba	32.16	1.66	0.92	4.52	66.65	0.81	2.07	13.12
	Mohc	36.26	1.56	0.83	4.39	69.05	0.53	1.45	11.86
2	Sauti	38.78	1.37	1.05	4.56	69.18	0.85	2.43	7.77
	Kenya	36.90	1.52	1.14	5.38	69.94	0.50	2.14	6.74
	Tanzania	39.84	1.57	0.93	4.08	68.32	0.76	2.04	8.67
3	Okumkom	35.58	1.46	0.89	3.56	68.43	0.98	2.14	10.66
	TIS 8266	33.27	1.99	1.40	6.83	67.22	0.97	2.06	8.98
	lsd (5%)	3.44	0.28	0.19	1.14	3.16	0.81	1.04	2.98
	CV (%)	5.60	10.40	10.80	14.70	2.70	73.30	31.50	19.4

In the Transition AEZ, ANOVA revealed significant differences in percentage protein and mineral (Fe and Zn) content in dry matter between Okumkom and its putative ramet TIS 8266. Otoo and Mogamba exhibited significant differences in dry matter content just like Mohc and Mogamba. Protein content in dry matter significantly differed in Tanzania and Kenya (Table 10)

4.4 SEVERITY OF VIRUS SYMPTOMS

Restricted Maximum Likelihood (REML) variance component analysis disclosed significant differences of weighted severity between genotypes at the two locations. Mogamba and TIS 8266 recorded the lowest and the highest virus severity symptoms in both the Forest and Transition AEZ respectively (Table11).

TABLE 11. COMPARISON OF MEAN WEIGHTED SEVERITY OF VIRUS SYMPTOMS OF PUTATIVE RAMETS AT TWO LOCATIONS IN THE 2011 GROWING SEASON

Putative clone	Genotype	Year of field introduction	Forest	Transition
			Weighted severity (1-5)	
1	Otoo	2001	1.98	1.91
	Mogamba	2011	1.84	1.86
	Mohc	2008	1.95	1.92
2	Sauti	1995	2.10	2.05
	Kenya	2011	2.33	2.15
	Tanzania	2011	2.17	2.40
3	Okumkom	1995	2.43	2.19
	TIS 8266	2010	2.76	2.40
4	Faara	1995	2.15	1.93
	TIS 3017	2010	2.65	2.12
	lsd (5%)		0.19	0.19

Severity of virus symptoms was lower in the released varieties Okumkom, Faara and Sauti than putative ramets at the two locations. These differences were significant between Okumkom and TIS 8266 at both locations whiles between Faara and TIS 3017 as well as Sauti and Kenya, significant differences were observed in the Forest AEZ. There was significant difference between Sauti and Tanzania in the Transition AEZ. There were no significant differences among Otoo, Mohc and Mogamba at the two locations (Table 11).

4.5 PROGRESSION OF VIRAL INFECTION IN PUTATIVE RAMETS FROM 6 WAP TO 14WAP IN AEZ

Trends of severity of virus symptoms for Ghanaian released sweetpotato varieties compared with putative ramets is presented in (Fig 4-7). On the average, the lowest severity for most of the genotypes was recorded at the early stages of growth (8 WAP) and increased with time reaching its peak in the latter stages (12 WAP) and either decreased or remained stable at 16 WAP. Weighted severity increased with age of ramets because of the multiplicative effect of virus infection with time.

Mogamba was less susceptible to severity of virus symptoms than putative ramets at the two locations from 8 WAP to 14 WAP (Fig 4)

Forest **Transition**

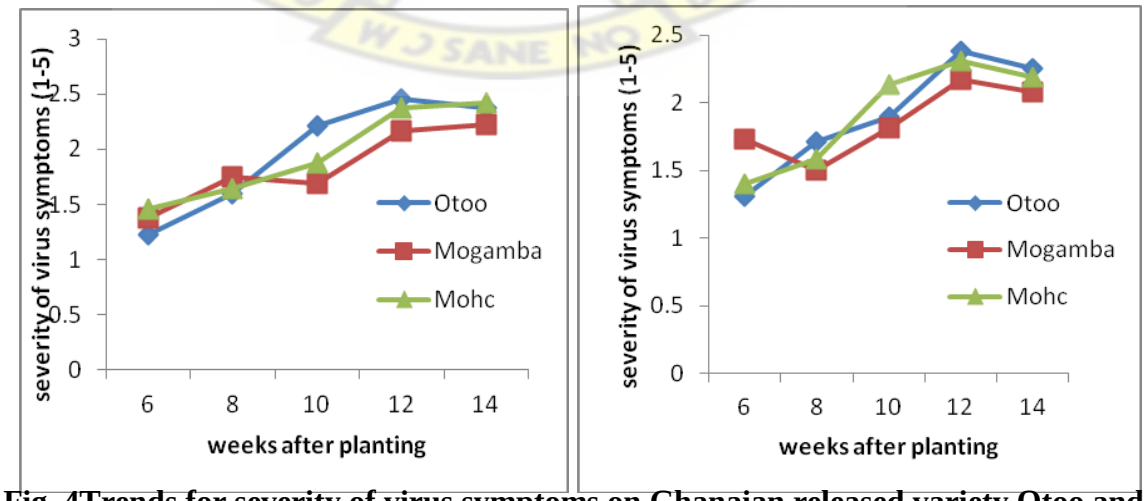

Fig. 4Trends for severity of virus symptoms on Ghanaian released variety Otoo and putative ramets Mohc and Mogamba at two locations

Sauti was relatively less susceptible to virus symptoms than putative ramets (Fig 5)

Forest **Transition**

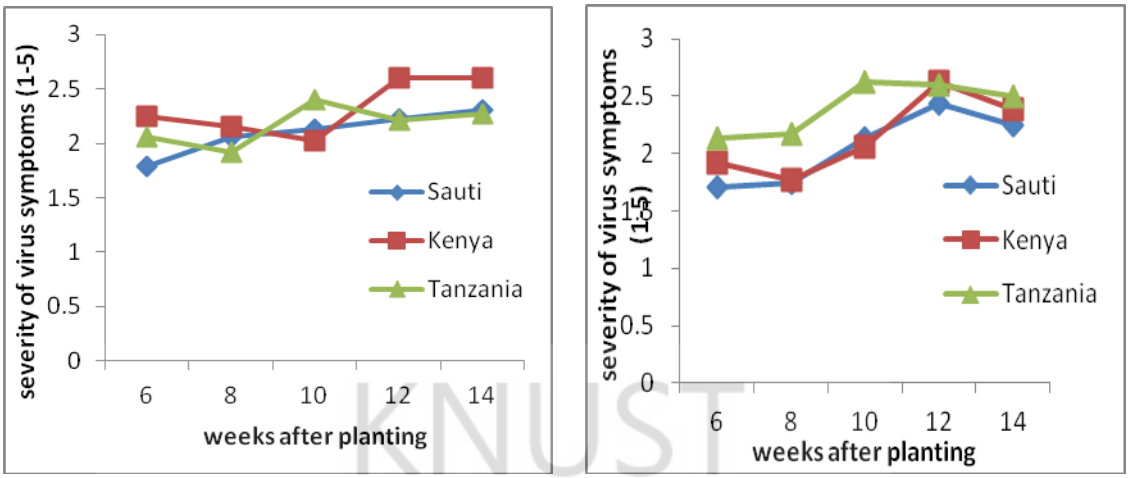

Fig. 5 Trends for severity of virus symptoms on Ghanaian released variety Sauti and putative ramets Tanzania and Sauti at two locations

TIS 3017 was more highly susceptible to virus symptoms than Faara (Fig 6)

Forest **Transition**

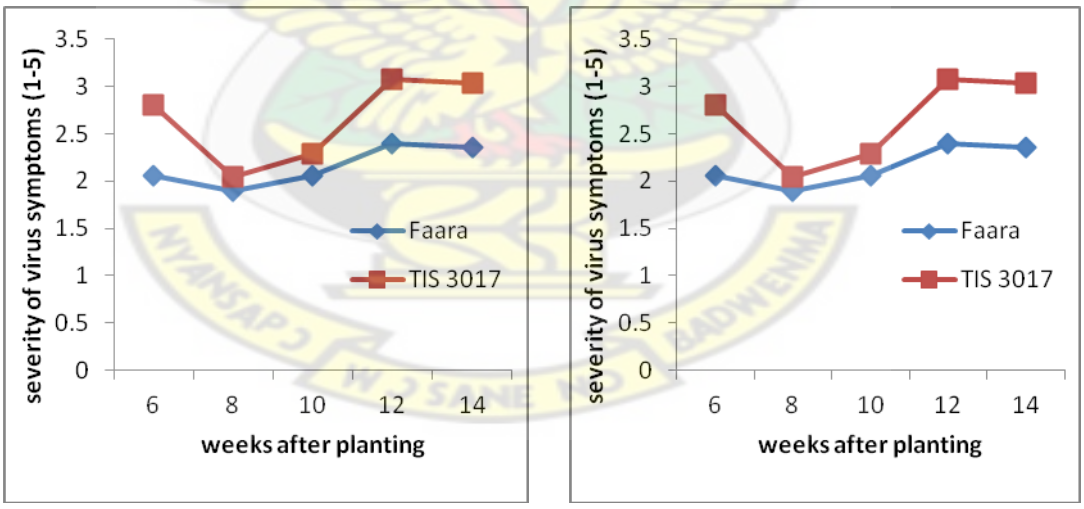

Fig. 6 Trends for severity of virus symptoms on Ghanaian released variety Faara and TIS 3017

TIS 8266 was more higly susceptible to virus symptoms than putative ramet Okumkom.

Forest **Transition**

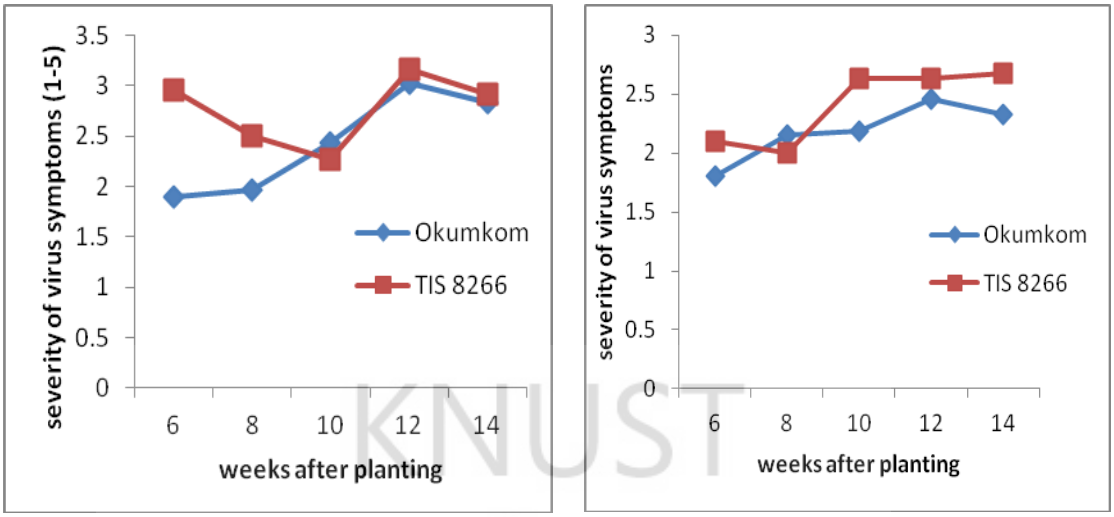

Fig .7 Trends for severity of virus symptoms on Ghanaian released variety Okumkom and TIS 8266

4.6 EVALUATION OF YIELD

Total yield varied between 0 yield and 20 tons/ha and 0.14 and 21.20 tons/ha in the Forest and Transition AEZ respectively among cultivars. Mogamba recorded the highest yield in the Forest while Santom Pona recorded the highest in the Transition AEZ. TIS 3017 was the genotype with the lowest yield at the two locations.

TABLE 12. COMPARISON OF TOTAL AND COMMERCIAL YIELD BETWEEN PUTATIVE RAMETS AT TWO LOCATIONS IN THE 2011 MAJOR GROWING SEASON

			Forest		Transition	
Putative clone	Genotype	Year of field introduction	Total yield	Commercial yield	Total yield	Commercial yield
1	Otoo	2001	12.31	8.70	16.39	11.44
	Mogamba	2011	20.00	18.43	20.83	14.07
	Mohc	2008	11.76	8.24	15.56	8.36
2	Sauti	1995	7.59	5.93	3.01	1.39
	Kenya	2011	12.69	11.48	5.05	2.92
	Tanzania	2011	9.17	7.31	1.39	0.46
3	Okumkom	1995	7.87	5.19	10.19	8.10
	TIS 8266	2010	2.69	1.76	4.40	2.50
4	Faara	1995	5.09	3.61	5.19	3.06
	TIS 3017	2010			0.14	
	lsd (5%)		4.56	4.01	10.43	8.92
	CV %		34.00	38.00	61.40	78.00

Significantly, higher yields were observed in the Forest AEZ for two recently introduced genotypes. Mogamba produced significant higher total and commercial yield than Otoo and Mohc. Comparably, total and commercial yield was significantly greater in Kenya than Sauti its putative ramet. However, the released variety Okumkom recorded significant greater total yield than TIS 8266 (Table 12).

CHAPTEER FIVE

5.0 DISCUSSION

5.1 GENETIC RELATIONS AMONG PUTATIVE RAMETS

Phenotypic characterization revealed a similarity coefficient of 1.00. The genotypes with a similarity coefficient of 1 were considered duplicate genotypes. The presence of duplicates could be due to the movement of planting materials across two locations as well as differences in languages and ethnic groups (Yada *et al*., 2010). Duplicates are identical genotypes which have different names at different places due to language differences. Characterization descriptors permit relatively easy discrimination among phenotypes and have been used for identification of duplicates (CIAT, 2007).

The released varieties Sauti and Otoo were very similar to their original source materials. Sauti which was introduced to Ghana in 1995 was very much similar to Tanzania and Kenya introduced in 2011 based on phenotypic characterization. Similarly, Otoo introduced in 2001 showed very high level of similarity to Mogamba introduced in 2011 and Mohc introduced in 2008. However, subtle differences were observed in vine internode diameter and petiole length which could perhaps be attributed to mutation and diseases. Frequent mutations take place in sweetpotato resulting in numerous new genotypes which presents a challenge for germplasm conservation. According to Villordon and La Bonte (1996), sweetpotato clones naturally mutate for traits like root and skin color, and leaf and vine characteristics with time. No differences were also observed in all the quality characteristics evaluated. According to Woolfe (1992), mean dry matter varies with cultivars, location and other factors. Tanzania and Kenya which were found to be duplicates did not show significant variation in dry matter content. In the case of Otoo, Mogamba and Mohc differences

were observed in plant type (Table 8). Ramets are genetically identical offsprings from clonally propagated crops which have the potential of becoming independent of the parent plant (Araki *et al*., 2009; Hosaka *et al*., 2005). Differences in phenotype of identical genotypes in a clonal population could be attributed to two main sources of variation: genetic and environment. The occurrence of non growth factors such as diseases and pests like SPVD could impose different intensities of environmental stress on plants which has the potential of causing change in the expression of some traits (Acquaah, 2007) such as length of main vine and foliage colour.

Faara (originally introduced as TIS 3017 in 1995) and recently introduced TIS 3017 from CIP Headquarters as well as Okumkom (introduced originally as TIS 8266) and recently introduced TIS 8266 did not show much similarity with respect to most of the traits evaluated. The differences observed between these two pairs of putative ramets could be attributed to virus status, degeneration in culture or mislabeling. Pathogens like viruses can profoundly affect the structure, diversity and functioning of plant populations (Bradley *et al*., 2008). Diseases and pests could also account for phenotypic differences as discussed earlier. Tissue culture techniques were employed in generation and maintenance of TIS 3017 and TIS 8266 which were virus-tested genotypes. Potential result of long term tissue culture maintenance could induce somaclonal variation which is common in sweetpotato (Anwar *et al.*, 2010) and could be assessed by observation of phenotype or direct DNA evaluation of plants (Smy' kal *et al.*, 2007). These highlights the need for an *in situ* or at least an *ex silico* aspect to germplasm conservation and foundation seed maintenance and has implications for CIP's global germplasm strategy. Okumkom and TIS 8266 just like Faara and TIS 3017 which were distantly related seem to show another problem with gene banks because of wrong labelling which could result in the danger of mix-ups. Sweetpotato breeders encounter

unexpected results due to mislabeling of genotypes which leads to mix-ups (Grüneberg *et al*., 2010**).** Mislabelling in genotypes with numbers as its name is rampant because the numbers could easily be mixed up to represent another genotype which might not be the case. TIS 8266 was distantly related to Okumkom probably because TIS 8266 which bears the CIP number 440070 probably suffered the problem of mislabeling in the gene bank. This is also true for TIS 3017 which has the CIP number 440064.This emphasizes the need to confirm and preserve the identity of genotypes during the process of multiplication and evaluation (Grüneberg *et al.,* 2010**).**

The five SSR markers used in the study could not effectively discriminate between different sets of putative ramets. Ten genotypes were found to be duplicates. Apomuden and Kamala Sundari which are known ramets (CSIR-CRI, 2005) were found to be indistinguishable from Kemb 10 (PE 143) and Kemb 10 (UG 191) which is a different set of putative clone. Phenotypic studies revealed that 199062.1 introduced in 2008 was the most distinct genotype (Fig 1) from all other varieties. However, SSR markers used in this present molecular study revealed that this genotype is very similar to the Ghanaian released variety Faara at a similarity level of 0.98. This presupposes that the five SSR markers selected from 26 SSR markers reported to be informative markers for sweetpotato did not work perfectly in this study. Limited number of SSR markers and null amplifications of some of the genotypes were the major reasons which accounted for this. According to Mohammadi and Prasanna (2003), one specific problem often encountered during analysis of genetic relationships in crop plants by molecular markers, particularly SSR markers, is the failure of some genotypes to show amplification for some SSR primers.

5.2 SEVERITY OF VIRUS SYMPTOMS

Sweetpotato viral infection symptoms were observed in almost all the varieties tested at different times at the different locations. There are no reports of immune cultivars to sweetpotato viral infection (Gasura and Mukasa, 2010). Mogamba was the genotype with the least progression of viral infection with mild symptoms from 8 WAP to 12 WAP at both locations. This genotype expressed apparently no symptoms at 6 WAP in the Forest AEZ (Fig 4). Severity of virus symptoms was rapid in TIS 8266 as it expressed the highest mean weighted severity at both locations. Generally, moderate to mild symptoms were observed among putative ramets. Gruneberg *et al*. (2009) reported that the sweetpotato virus (SPVD) pressure in the Forest and Savanna regions of Ghana is moderate.

5.3 EVALUATION OF YIELD

TIS 3017 was the genotype with the lowest total yield across the two locations and did not produce marketable yields. This could be attributed to decline of yield ability in tissue culture or virus infection. According to Villordon *et al*. (2009) the quantity of yield depends on the number of fibrous roots that will be induced to form storage roots results in high number of yield or no commercial roots at all. The recently introduced virus-tested genotypes Mogamba and Kenya produced significantly higher yields than corresponding released varieties Otoo and Sauti which have undergone about 10-15 years of clonal generations since their introduction in Ghana. Fuglie *et al*. (1999) studied the impact of the “virus-free” production system in Shandong province, China and observed increment in commercial yield (tubers > 100 g) by 22.2% when virus-plants were used as compared with virus-infected ones. Otoo and Mohc recorded relatively significantly lower yields than their ramets probably because of invisible asymptomtic virus infection (Gasura and Mukasa, 2010). van Molken and Stuefer

(2011) revealed that there was a reduction in the biomass of roots by 28% in white clover ramets which was caused by white clover virus infection. Similar scenario was observed in the varieties Kenya, and Sauti in which the yields of Sauti was significantly lower.

Okumkom introduced about 14 years ago recorded significant higher yield than putative ramet TIS 8266 introduced in 2010 probably because of severity of virus symptoms which was higher in TIS 8266. Most introduced genotypes are degenerated because of SPVD (Gibson *et al*., 2004) and reduce yields of infected plants by over 90% (Gutierrez *et al*., 2003).

There was a strong negative correlation (-0.87) between total yield and mean weighted severity of cultivars in the Forest AEZ. This means that as severity of virus symptoms increased, yield decreased considerably. Mogamba which is the genotype with the lowest weighted virus severity produced higher yields in each location than all other genotypes at both locations. TIS 8266 and TIS 3017 had significantly higher weighted severity scores than putative ramets and consequently recorded lower yields.

CHAPTER SIX

6.1 CONCLUSION

The studies have revealed that the Ghanaian released variety Otoo was closely related to the recently introduced variety, Mogamba, its original source material and Mohc the elite variety. Similarly, Sauti, also a Ghanaian released variety, was closely related to the recently introduced varieties Tanzania and Kenya. Conversely, Faara and Okumkom were not closely related with putative ramets TIS 3017 and TIS 8266 respectively.

Kenya and Mogamba yielded higher than putative ramets. The use of virus-tested planting material of Kenya and Mogamba could be used to improve yield as a source of clean planting material. In contrast, the recently introduced virus-tested genotypes TIS 3017 and TIS 8266 produced lower yields than putative ramets, Faara and Okumkom respectively. These two varieties should be cleaned locally using *in vitro* tissue culture techniques.

Mogamba was the genotype with the lowest disease progression based on mean weighted severity at the two locations with mild viral symptoms and recorded the highest yield among all other recently introduced genotypes.

6.2 RECOMMENDATION

Further studies should be conducted in molecular work using all the 26 SSR markers confirmed for sweetpotato and EST SSRs since the five markers reported to be informative were few in number and could not effectively discriminate between putative set of clones.

More attention should be given to maintenance of seed quality and virus-free foundation seed stocks and continuous selection for trueness to type. Clean up should be initiated of all varieties. The health status of the recently introduced virus-tested genotypes were not tested at the start of the trial, which means that comparisons made were not conclusive and therefore there is the need for further research.

REFERENCES

Acquaah G. (2007). Principles of Plant Genetics and Breeding. Blackwell Publishing Ltd 74-87pp.

Akoroda M. (2009). Sweetpotato in West Africa. In: G. Loebenstein and G. Thottappilly (eds), The Sweetpotato. Springer Science+Business Media B.V. 2009. 441-468pp.

Almekinders, C.J.M. and Louwaars, N.P. (1999). Farmers' seed production: New approaches and practices. London, Intermediate Technology Publications. 291p.

Anwar N., Kikuchi A., and Watanabe K.N. (2010). Assessment of somaclonal variation for salinity tolerance in sweet potato regenerated plants. *African Journal of Biotechnology* . 9(43) 7256-7265.

Araki K., Shimatani K., and Ohara M., (2009). Dynamics of distribution and performance of ramets constructing genets: a demographic–genetic study in a clonal plant, *Convallaria Keiskei Annals of Botany* doi:10.1093/aob/mcp092 1-7pp.

Austin D.F. 1977. Hybrid polyploids in *Ipomoea* section Batatas. *J. Hered*. 68:259-260.

Austin D.F. 1988. The taxonomy, evolution and genetic diversity of sweet potatoes and related wild species. 27-59pp. In: Exploration, maintenance and utilization of sweetpotato genetic resources. Report of the First Sweetpotato Planning Conference 1987. International Potato Center, Lima, Peru.

Austin, D.F. and Huamán Z. (1996). A synopsis of *Ipomoea* (Convolvulaceae). In: *The Americas Taxon*. 45:3–38.

Bishaw Z., and Van Gastel J.G. (2009). Variety release and policy options. In: Plant breeding and farmer participation 565-588 pp. In: Ceccarelli S., Guimaraes E.P., and Weltzien E (eds) Food and Agricultural Organization Rome.

Bradley D.J., Gilbert G.S., Martiny J.B.H. (2008). Pathogens promote plant diversity through a compensatory response. *Ecology Letters* 11: 461–469.

Bryan A.D., J.R. Scultheis, Pesic-VanEsbroeck Z., Yencho G.C., (2003). Cultivar decline in sweetpotato: II. Impact of virus infection on yield and storage root quality of 'Beauregard' and 'Hernandez'. *J. Amer. Soc. Hort. Sci*. 128 (6): 856-863.

Buteler, M.I., Jarret R.L., and LaBonte D.R. (1999). Sequence characterization of microsatellites in diploid and polyploid Ipomoea. *Theor. Appl Genet*. 99:123-132.

Buteler, M.I., LaBonte D.R., Jarret R.L., and Macchiavelli R.E. (2002). Microsatellite-based paternity analysis in polyploid sweetpotato. *J. Am. Soc. Hort Sci* 127:392-396.

Carey, E. E., Gibson, R. W., Fuentes, S.,Machmud, M., Mwanga, R. O. M., Turyamureeba,G., Zhang, L., Ma, D., Abo El-Abbas, F., El-Bedewy, R., and Salazar, L. F.(1999). The causes and control of virus diseases of sweetpotato in developing countries: Is sweetpotato virus disease the main problem?. Pages 241-248 in: Impact on a Changing World. CIP Program Report 1997–98. CIP, Lima, Peru.

Chua, L.K. and Kays S.J. (1981). Effect of soil oxygen concentration on sweet potato storage root induction and development. *Hort Science 16* :71-73.

CIAT (2007). Germplasm characterization. 171-184 pp. In: Pineda B. and Hidalgo R.. Multi-Institutional distance learning course on the ex-situ conservation of plant genetic resources. (eds).

CIP, AVRDC, IBGR, Huaman, Z. (ed) 1991. *Descriptors for sweetpotato*. Rome, Italy. 43-64 pp

Clark, C. A., and Hoy, M. W. (2006). Effects of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. *Plant Dis*. 90:83-88.

Clark, C.A., Valverde, R.A., Fuentes, S., Salazar, L.F., Moyer J.W. (2002). Research for improvement management of sweetpotato pests and diseases 103-112 pp. *In*: T. Ames (eds). *Proceedings of the first international conference on sweetpotato food and health for the future. Acta Hort* 583.

Clark, C. A., and Moyer, J. W. (1988). Compendium of Sweet Potato Diseases. The American Phytopathological Society, St. Paul, MN.

CSIR-CRI (1998). Second inspection of sweetpotato for varietal release. 1-36 pp.

CSIR-CRI (2005). Sweet potato genotypes proposed for release. 1-35 pp.

Delouche J., and Goma'a A.A. (1999). Policy procedures for release of new publicly developed crop varieties in Egypt. Agricultural Policy Reform Project, Report No 62. MALR, Cairo, Egypt. 66p.

Egnin M., Mora A., and Prakash C.S. (1998). Factors enhancing *Agrobacterium tumefaciens* – Mediated Gene Transfer in peanut (*Arachis hypogeal* L.). *In vitro Cellular Dev. Biol. Plant*, 34: 310-318.

FAO and IFAD (2005). A review of cassava in Africa with country case studies on Nigeria, Ghana, the United Republic of Tanzania, Uganda and Benin. *Proceedings of the validation forum on the global cassava development strategy.* Volume 2.

FAOSTAT (2010). Database of the Food and Agriculture Organization of the United Nations. Available online URL http://apps.fao.org. Date assessed: 3rd March, 2011.

Fuglie K.O., Zhang L., Salazar L.F., Walker T.S. (1999). Economic impact of virus-free sweet potato planting material in Shandong Province, China. *International Potato Center Program Report* for 1997-98, 249-254 pp.

Gasura E. and Mukasa S.B. (2010). Prevalence and implications of sweetpotato recovery from sweet potato virus disease in Uganda. *African Crop Science Journal*. 18 (4): 195 – 205.

Gibson, R.W., Mpembe, I., Alicai, T., Carey,E. E., Mwanga, R. O. M., Seal, S. E., and Vetten,H. J. (1998). Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathol*. 47:95-102.

Gibson R., Aritua V., Byamukama E., Mpembe I., Kayongo J. (2004) Control strategies for sweet potato virus disease in Africa. In: Thresh J.M, Jones R.A.C, Kuehne T. (Eds) *Virus Research* 100: 115-122.

Gruneberg W.J., Eyzaguine, R.., Espinosa, J., Mwanga, R.O.M., Andrade, M., Dapaah H., Tumwegamire, S., Agili, S., Ndingo-Chipungu, F.P., Attaluri, S., Kapinga R., Nguyen T., Kaiyung, X., Tjintokohadi, K., Carey, T., and Low J. (2010). Procedures for the evaluation and analysis of sweetpotato trials. 2nd version. 3-5pp.

Gruneberg, W.J., R. Mwanga, M. Andrade, and H. Dapaah. (2009). Sweetpotato Breeding. *In* M. Andrade, I. Barker, D. Cole, H. Dapaah, H. Elliott, S. Fuentes, W.J. Gruneberg, R. Kapinga, J. Kroschel, R. Labarta, B. Lemaga, C. Loechl, J. Low, J. Lynam, R. Mwanga, O. Ortiz, A. Oswald, & G. Thiele. Unleashing the potential of sweetpotato in Sub-Saharan Africa: current challenges and way forward. Nairobi, CIP-SSA.

Gupta, P.K., Varshnet R.K., Sharma P.C., Ramesh B. (1999). Molecular markers and their applications in wheat breeding. *Plant Breed* 118:369-390.

Gutierrez D.L, Fuentes S., Salazar L. (2003). Sweet potato virus disease (SPVD): distribution, incidence, and effect on sweet potato yield in Peru. *Plant Disease* 87, 297-302

Hahn S.K. (1979). Effects of viruses (SPVD) on growth and yield of sweet potato. *Experimental Agriculture* 15: 252–256.

He G., Prakash C.S. and Jarret R.L. (1995). Analysis of genetic diversity in a sweetpotato (*Ipomoea batatas*) germplasm collection using DNA amplification fingerprinting. *Genome* 38: 938-945.

Hill W.A., Bonsi C.K., Loretan, P.A. (1992). Sweet potato research: Current status and future needs xvii-xxv In: Hill W.A., Bonsi C.K., Loretan, P.A. (eds) Sweet potato technology for the 21st century. U.S.A. Alabama, Tuskegee University.

Hosaka N., Gómez S., Kachi N., Stuefer J.F., Whigham D.F. (2005).The Ecological Significance of Clonal Growth in the Understory Tree, Pawpaw (*Asimina triloba*) *Northeastern naturalist* 12(1):11–22.

Hu J.J., Nakatani M., Lalusin A.G., Kuranouchi T and Fujimura T (2003). Genetic analysis of sweetpotato and wild relatives using Inter-Simple Sequence Repeats (ISSRs). *Breeding Science.* 53: 297-304.

Hu J.J., Nakatani M., Lalusin A.G., Fujimura T. (2004). New microsatellite markers developed from reported *Ipomoea trifida* sequences and their application to sweetpotato and its related wild species. *Sci. Hort.* 102: 375-386.

Karuri, H.W., Ateka, E.M., Amata, R., Nyende, A.B., Muigai, A., Mwasame, E and Gichuki, S.T. (2010). Evaluating diversity among Kenyan sweet potato genotypes using morphological and SSR m arkers. *Int. J. Agric. Biol.*, 12: 33–38.

Karyeija R.F., Gibson, R.W., and Valkonen J.P.T. (1998). Resistance in sweetpotato virus disease (SPVD) in Wild East Africa *Ipomoea*. *Ann. appl. Biol* 133:39-44

Karyeija, R. F., Kreuze, J. F., Gibson, R. W.,and Valkonen, J. P. T. (2000). Synergistic interactionsof a potyvirus and a phloem-limited crinivirus in sweetpotato plants. *Virology* 269: 26-36.

Kays S.J. (1985). The physiology of yield in the sweetpotato pp 79-132 . *In*: Boukamp J.C. (ed) Sweetpotato products: A natural resource for the tropics. Brouwkamp J.C.(ed) CRC Press, Boca Raton, FL.

Kays S.J., Collins W.W., Bouwkamp, J.C. (1992). A response: the sweet potato is a root not a tuber. pp 307-313. *In*: Hill W.A., Bonsi C.K. and Loretan P.A. (eds) Sweet potato technology for the 21st century. U.S.A. Alabama, Tuskegee University.

Kenyon L., Anandajayasekeram P., and Ochieng C. (2006). A synthesis / lesson-learning study of the research carried out on root and tuber crops commissioned through the DFID RNRRS research programmes between 1995 and 2005. 1-41 pp.

La Bonte, D. R., VIllordon A.Q., and D. S. Fajardo. (2000). Mutations in sweetpotato. 1-9 In: Nakazawa, Y. and Ishiguro K. (eds) International workshop on sweetpotato cultivar decline study, proceedings, Kyushu National Agricultural Experimentatl Station, Miyakkonjo, Japan.

La Bonte D. R., Villordon A.Q., Fajardo D.S. (2001). Mutations in sweetpotato. 1-9 pp. In: 2000 International Workshop on Sweetpotato Cultivar Decline. Nakazawa Y., Ishiguro K (ed). Kyushu National Agricultural Experiment Station, Japan.

Laurie S.M., Niederwieser J.G., (2004). The sweet potato plant In: Niederwieser J.G. (ed.) *Guide to sweet potato production in South Africa* Vol 1: 7-14. Agricultural Research Council. South Africa, Pretoria.

Lewthwaite S.L., Fletcher P.J., Fletcher J.D., Triggs C.M., (2011). Cultivar decline in sweetpotato (*Ipomoea batatas*). *New Zealand Plant Protection* 64: 160-167.

Linnaeus, C. (1753). *Species plantarum*, exhibentes plantas rite cognitas, and genera relatas, cum differentis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, *secumdum systema sexual digestas* 2: 561-1200

Loebenstein G. (2009). Origin, distribution and economic importance. Origin, distribution and economic importance 9-12 pp In: Loebenstein G., and Thottappilly G. (eds), The Sweetpotato. Springer Science+Business Media B.V.

Loebenstein G., Thottappilly G., Fuentes S., Cohen J. (2009). Virus and phytoplasma diseases. 105-134 pp In: Loebenstein G., and Thottappilly G. (eds), The Sweetpotato. Springer Science+Business Media B.V.

Low J., Lynam J., Lemaga B., Crissman, C., Barker, I., Thiele G., Namanda S., Wheatley C., and Andrade M. (2009). Sweetpotato in Sub-Saharan Africa. 359-390 pp. In Loebenstein G., and Thottappilly G., (eds), The Sweetpotato. Springer Science+Business Media B.V.

Mohammadi S.A. and Prasanna B.M. (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. *Crop Sci*: 1235-1248.

Morakinyo, J.A., and S.R. Ajibade. (1998). Characterization of the segregants of an improved cowpea line IT84K-124-6. *Nigerian J. Sci.* 32:27–32.

Mukiibi J., (1977). Effects of mosaic on the yield of sweet potatoes. 169-170 pp. In: *Proceedings of the 4th International Society for Tropical Root Crops.* Cali, Columbia: CIAT.

Ngeve, J. M., and Bouwkamp, J. C. (1991). Effects of sweet potato virus disease (SPVD) on the yield of sweet potato genotypes in Cameroon. *Exp. Agric.*27:221-225.

Obigbesan G.O. (2009). Package for high quality sweetpotato production: Do's and don'ts. 6-7 pp In: Sweetpotato in Nigeria. In Akoroda M. and Egeonu I. (eds). *Proceedings of the first national sweetpotato conference held during 16-18 September, 2008 at the first Bank Hall, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria*.

Okpul T., Maltby J., Guaf E., Coleman E.A., Dennien S., Harding R.M., Dieters M.J., Godwin I.D. (2011). Cultivar-specific effects of pathogen testing on storage root yield of sweetpotato, *Ipomoea batatas*. *Annals of Applied Biology* 158: 288– 296.

Otoo, J.A., Osei C.K., Danso A.K., Asamoah O.N., Gyasi-Boakye S., Ansah, I.O.O, Bersae, J.N., Adjekum, A.A., Asante S.N.A., and DeGraft Johnson, A. (2000). Steps to good sweet potato production. RTIP, MFA and CSIR-Crops Research Institute, Kumasi, Ghana 16p.

Otoo J. A., Missah A. and Carson A.G. (2001). Evaluation of sweetpotato for early maturity across different agro-ecological zones in Ghana. *African Crop Science Journal.* 9 (1):25-31

Phillips, T.P., Taylor, D.S., Sanni, L. and Akoroda, M.O. 2004. A Cassava industrial revolution in Nigeria: The potential for a new industrial crop. IFAD/FAO/UN Rome 2004. 49 pp.

Powell W., Morgante M., Andre C., Hanafey M., Vogel MJ, Tingey S.V., Rafalski A (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellites) markers for germplasm analysis. *Mol. Breeding* 2: 225-235.

Prakash, C.S. and G. He, (1996). DNA marker-based genetic relatedness in United States sweet potato cultivars. *J. American Soc. Hort. Sci.* 121:1059–1062.

Rakoczy-Trojanowska, M. and Bolibok H., (2004). Characteristics and a comparison of three classes of microsatellite-based markers and their application in plants. *Cel. Mol. Biol. Let.* 9: 221–238.

Ravi, V. and Indira P. (1999). Crop physiology of sweet potato. *Hortic. Rev.* 23:277-339.

Reed B.M., Engelmann F., Dullo M.E., and Engells J.M.M. (eds.) (2004). *Technical guidelines for the mangagement of field and in vitro collections.* IPGRI handbook for genebanks No 7. International Plant Genetic Resource Institute. Rome, Italy.

Riis-Jacobsen J. (2011). Clone selector 2.0 users guide. sweetpotato 42p.

Setimela, P.S., Monyo, E. and Bänziger, M. (eds). 2004. Successful Community-Based Seed Production Strategies. CIMMYT, Mexico. D.F.

Shenk, J.S. and Westerhaus (1993). Analysis of agriculture and food products by near infrared spectroscopy. The Pennsylvania State University and Infrasoft International, State College, PA.

Smy´kal P, Valledor L, Rodrıguez R, Griga M (2007). Assessment of genetic and epigenetic stability in long-term *in vitro* culture of pea (*Pisum sativum* L.). *Plant Cell Rep*. 26: 1985-1998.

Somda S.C., and Kays S.J. (1990). Sweet potato canopy and Morphology: Leaf distribution. *Journal of the American Society for Horticultural Science* 115: 39-45.

Thottappily G. (2009). Introductory remarks. 3-8 pp. In: Loebenstein G., and Thottappilly G. (eds), The Sweetpotato. Springer Science+Business Media B.V.

Tseng Y.T., Lo H.F. and Hwang S.Y. (2002). Genotyping and assessment of genetic relationships in elite polycross breeding cultivars of sweet potato in Taiwan based on SAMPL polymorphisms. *Botanical Bulletin of Academia Sinica*. 43: 99-105.

Tumwegamire S.,Rubaihayo P.R., LaBonte D.R., Gruneberg W.J, Kapinga R., and Mwanga R.O.M. (2011). Genetic Diversity in White- and Orange-fleshed Sweetpotato Farmer Varieties from East Africa evaluated by Simple Sequence Repeat (SSR) Markers. *Crop Sci*. 51:1132–1142.

Valverde R.A., Clark C.A., Jari P. T. Valkonen J.P.T. (2007). Viruses and Virus Disease Complexes of Sweetpotato. *Plant Viruses* 1(1): 116-126.

van den Berg, A.A. and Laurie S.M. (2004). Cultivation. In Niederwieser J.G. (ed.) Guide to sweet potato production in South Africa 1: 15-26. Agricultural Research Council, Pretoria.

van Molken T., Stuefer J.F., (2011). The potential of plant viruses to promote genotypic diversity via genotype by environment interactions. *Annals of Botany* doi:10.1093/aob/mcr07. 1-7pp.

Villordon A.Q. and LaBonte D.R. (1995). Variation in randomly amplified DNA markers and storage root yield in 'Jewel' sweetpotato clones. *J. Amer. Soc. Hort. Sc.* 120: 734-740.

Villordon, A.Q. and La Bonte, D.R.(1996). Genetic variation among sweetpotatoes propgated through nodal and adventitious sprouts. *J. Amer. Soc. Hort. Sci*. 112:170-174.

Villordon A.Q., La Bonte D.R., Firon N., Kfir Y., Pressman E., Schwartz A. (2009). Characterization of adventitious root development in sweetpotato. *HortScience* 44 (3): 651-655.

Wang P.J. and Hu C.Y. (1980). Regeneration of virus-free plants in-vitro. 61-99 pp In: Fiecher A. (ed) Advances in biochemical engineering. Springer Verlag, Heidelberg.

Wang Z., Li J., Luo Z. ,Huang, L. Chen X.,Fang B., Li Y.,Chen J., and Zhang X.(2011) Characterization and development of EST-derived SSR markers in cultivated sweetpotato (*Ipomoea batatas) BMC Plant Biol.* 11:139.

Westman A.L., Kresovich S., (1997). Use of molecular techniques for description of plant genetic variation In: Callow J.A., Ford-Lloyd B.V., Newbury H.J., (eds). Biotechnology and plant genetic resource conservation and use. Biotechnology in agriculture series, no 19 CAB International, New York. CIAT publication no 360.

Woolfe, J.A. 1992. Sweet potato: An untapped food resource. Cambridge University Press, UK 1-363 pp.

Yada B., Tukamuhabwa P., Alajo A., Mwanga R.O.M. (2010). Morphological Characterization of Ugandan Sweetpotato Germplasm *J. Crop Sci.* 50:2364–2371.

Zhang D, Ghislain M, Huaman, Z., Golmirzaie A and Hijmans R (1998) RAPD variation in sweetpotato [Ipomoea batatas (L.) Lam.] cultivars from South America and Papua New Guinea. *Genetic Resources Crop Evolution.* 45: 271-277.

Zhang, D., Cervantes J., Huaman , Z., Carey E., and Ghislain, M. (2000). Assessing genetic diversity of sweet potato (*Ipomoea batatas* (L.) Lam.) from tropical America using AFLP. *Genet. Resour. Crop Evol.* 47*:659-665*.

Zhang, D., Huaman Z, Rodriguez F, and Ghislain M.,. (2001). Identifying duplicates in sweetpotato [*Ipomoea batatas* (L.) Lam] varieties using RAPD. *Acta Horticulturae* 546:535–541.

Zhang, D., Rossel G, Kriegner A., and Hijman R.(2004). AFLP assessment of diversity in sweetpotato from Latin America and the Pacific region: Its implications on the dispersal of the crop. *Genet. Resour. Crop Evol.* 51: 115 – 120.

APPENDICES

APPENDIX 1 DESCRIPTORS FOR SWEETPOTATO (CIP/AVRDC/IBPGR)

Character	Score/Interpertation
Plant type	Determined by the length of the main vines 3 =Erect (<75 cm) 5 =Semi-compact (75 – 150 cm) 7= Spreading (151 – 250 cm) 9= Extremely spreading (>250 cm)
VineiInternode length	1= Very thin (< 4mm) 3= Thin (4 - 6 mm) 5= Intermediate (7 – 9 mm) 7= Thick (10 – 12 mm) 9= Very thick (>12 mm)
Vine internode diameter	1= Very short (<3 cm) 3= Short (3 – 5 cm) 5= Intermediate (6 – 9 cm) 7= Lon= (10 –12 cm) 9= Very long (> 12 cm)
Predominant vine colour	1= Green 3 =Green with few purple spots 4= Green with many purple spots 5= Green with many dark purple spots 6= Mostly purple7 =Mostly dark purple 8 =Totally purple 9 =Totally dark purple
Secondary vine colour	0= Absent 1= Green base 2= Green tip 3= Green nodes 4 =Purple base 5 =Purple tip 6= Purple nodes
Vine tip pubescence	0= None 3 =Sparse 5= Moderate 7 =Heavy 9= Very heavy
General outline of leaf	1 =Rounded 2 =Reniform (kidney-shaped) 3 =Cordate (heart-shaped) 4 =Triangular 5 =Hastate (Trilobular, spear-shaped, with the basal lobes more or less divergent) 6= Lobed 7 Almost divided
Type of leaf lobes	0 No lateral lobes (entire) 1 Very slight (teeth) 3 Slight 5 Moderate 7 Deep 9 Very deep
Number of leaf lobes	Generally sweetpotatoes have 1, 3, 5, 7 or 9 leafs lobes.
Shape of central leaf lobe	0= Absent 1= Teeth 2= Triangular 3= Semi-circular 4= Semi-elliptic 5= Elliptic 6=Lanceolate 7= Oblanceolate 8= Linear (broad) 9= Linear (narrow
Mature leaf size	3= Small (<8 cm) 5= Medium (8 – 15 cm) 7 =Large (16 – 25 cm) 9 =Very large (>25 cm)

APPENDIX 1 CONT'D

Character	Score/ Interpretation
Abaxial leaf pigmentation	1 =Yellow 2=Green 3= Purple spot at 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 totally purple 9= Lower surface and veins totally purple
Mature leaf colour	1 =Yellow-green 2= Green3= Green with purple edge 4= Greyish (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
Immature leaf colour	1 Yellow-green 2 Green 3 Green with purple edge 4 Greyish (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
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
Petiole length	1= Very short (<10 cm) 3= Short (10 – 20 cm) 5= Intermediate (21 – 30 cm) 7 Long= (31 – 40 cm) 9= Very long (>40 cm)
Storage root shape	1= Round 2= Round elliptic 3= Elliptic 4 =Obovate 5= Ovate 6 =Oblong 7= Long oblong 8=Long elliptic 9 =Long irregular or curved.
Predominant Skin colour	1= White 2= Cream 3= Yellow 4= Orange 5= Brownish orange 6= Pink 7= Red 8= Purplered 9= Dark purple
Intensity of predominant skin colour	1= Pale 2= Intermediate 3= Dark
Predominant flesh colour	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

APENDIX 2A PHENOTYPIC CHARACTER SCORES IN THE FOREST AEZ

Genotype/Character	PTP	VIL	VID	PVC	SVC	VTP	GOL	LLT	LLN	SCL
Ogyefo	5	5	5	7	3	5	4	1	1	1
Otoo	3	3	5	4	6	3	4	1	1	1
Sauti	5	5	5	7	3	0	6	7	5	5
199062.1	3	1	3	1	6	0	6	7	7	6
Faara	5	3	3	7	6	3	4	1	1	2
Hi Starch	5	3	3	1	5	5	4	3	3	2
Okumkom	9	5	1	6	3	7	4	1	3	2
Cemsa 74-228	7	5	3	4	6	3	6	7	5	6
Santom Pona	5	3	3	3	3	3	4	0	1	1
Ning Shu 1	5	3	3	3	6	0	4	1	9	1
Kemb 37	7	5	3	6	1	3	5	5	3	2
Mohc	5	3	5	4	6	3	4	3	3	2
Apomuden	5	5	3	6	1	0	4	1	1	1
Tek Santom	9	5	3	6	6	7	4	3	3	2
Fiaso local red	5	5	3	6	6	0	6	5	5	2
TIS 8266	5	5	3	6	6	3	4	1	3	2
Kenya	5	5	3	7	3	0	6	7	5	5
TIS 3017	5	5	3	7	3	7	6	5	5	2
Tanzania	5	5	3	7	3	0	6	7	5	5
Mogamba	5	3	5	4	6	3	4	3	3	2

APENDIX 2A CONT'D PHENOTYPIC CHARACTER SCORES IN THE FOREST AEZ

Genotype/Character	MLS	ALP	MLC	ILC	PPT	PTL	SRS	PSC	IPC	PFC
Ogyefo	5	3	3	2	5	3	3	9	1	1
Otoo	5	8	2	2	3	4	8	2	2	6
Sauti	7	2	2	3	3	1	3	2	3	5
199062.1	5	8	1	3	3	4	2	4	1	6
Faara	5	8	3	3	3	9	3	9	3	5
Hi Starch	5	2	2	3	3	1	3	4	2	4
Okumkom	5	2	3	3	3	1	2	9	1	2
Cemsa 74-228	5	8	3	6	3	4	2	4	1	2
Santom Pona	5	2	2	2	3	1	1	5	1	4
Ning Shu 1	5	5	2	3	1	4	3	5	2	4
Kemb 37	5	2	3	7	3	1	3	9	2	2
Mohc	5	8	2	3	3	4	8	2	3	6
Apomuden	5	2	3	7	3	1	3	4	3	7
Tek Santom	5	8	3	6	3	6	9	9	2	7
Fiaso local red	5	8	3	3	3	5	3	8	1	2
TIS 8266	5	8	2	2	3	6	4	2	2	4
Kenya	7	2	2	3	5	1	9	2	2	5
TIS 3017	5	2	3	3	3	1	*	*	*	*
Tanzania	7	2	2	3	5	1	9	2	2	5
Mogamba	5	8	2	2	3	4	8	2	2	6

* Missing data as a result of no yield

APENDIX 2B PHENOTYPIC CHARACTER SCORES IN THE TRANSITION AEZ

Genotype/Character	PTP	VIL	VID	PVC	SVC	VTP	GOL	LLT	LLN	SCL
Sauti	7	7	5	7	3	0	6	7	5	5
Mohc	7	5	5	4	6	3	4	3	3	2
Tanzania	7	5	5	7	3	0	6	7	5	5
Faara	7	5	5	7	6	3	4	1	1	2
Hi Starch	9	7	3	1	5	5	4	3	3	2
199062.1	7	5	3	1	6	0	6	7	7	6
Santom Pona	7	5	5	3	3	3	4	0	1	1
Okumkom	9	5	3	6	3	7	4	1	3	2
Ogyefo	7	7	5	7	3	5	4	1	1	1
Apomuden	7	5	3	6	1	0	4	1	1	1
Otoo	7	5	5	4	6	3	4	1	1	1
Kenya	7	5	5	7	3	0	6	7	5	5
Tek Santom	9	5	3	6	6	7	4	3	3	2
Cemsa 74-228	9	5	3	4	6	3	6	7	5	6
Kemb 37	9	5	3	6	1	3	5	5	3	2
Fiaso local red	7	5	3	6	6	0	6	5	5	2
TIS 8266	9	5	3	6	6	3	4	1	3	2
TIS 3017	7	5	3	7	3	7	6	5	5	2
Ningshu 1	5	5	3	3	6	0	4	1	9	1
Mogamba	5	5	5	4	6	3	4	3	3	2

APENDIX 2B CONT'D PHENOTYPIC CHARACTER SCORES IN THE TRANSISTION AEZ

Genotype/Character	MLS	ALP	MLC	ILC	PPT	PTL	SRS	PSC	IPC	PFC
Sauti	7	2	2	3	5	1	3	2	1	5
Mohc	7	8	2	3	5	4	8	2	3	6
Tanzania	7	2	2	3	5	1	9	2	2	5
Faara	5	8	3	3	5	9	3	9	3	4
Hi Starch	5	2	2	3	5	1	3	4	2	4
199062.1	5	8	1	3	5	4	2	4	1	6
Santom Pona	7	2	2	2	5	1	1	5	1	4
Okumkom	5	2	3	3	3	1	2	9	1	2
Ogyefo	7	3	3	2	7	3	3	9	1	1
Apomuden	5	2	3	7	5	1	3	4	3	7
Otoo	5	8	2	2	5	4	8	2	2	6
Kenya	7	2	2	3	5	1	9	2	2	5
Tek Santom	5	8	3	6	5	6	9	5	2	7
Cemsa 74-228	5	8	3	6	3	4	2	4	1	2
Kemb 37	5	2	3	7	3	1	3	9	2	2
Fiaso local red	5	8	3	3	3	5	3	8	1	2
TIS 8266	5	8	2	2	3	6	4	2	2	4
TIS 3017	5	2	3	3	3	1	*	*	*	*
Ningshu 1	5	5	2	3	3	4	3	5	2	4
Mogamba	5	8	2	2	5	4	8	2	2	6

* Missing data as a result of no yield

APPENDIX 3A REML variance components analysis for weighted severity (Forest AEZ)

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Genotype_1	254.46	19	13.39	200.0	<0.001
weeks	282.04	4	70.51	200.0	<0.001
Genotype_1.weeks	160.40	76	2.11	200.0	<0.001
Genotype_1.weeks	160.40	76	2.11	200.0	<0.001

APPENDIX 3B REML variance components analysis for weighted severity (Transition AEZ)

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Genotype_1	233.81	19	12.31	200.0	<0.001
weeks	264.82	4	66.21	200.0	<0.001
Genotype_1.weeks	69.37	76	0.91	200.0	0.672
Genotype_1.weeks	69.37	76	0.91	200.0	0.672

ANALYSIS OF VARIANCE

APPENDIX 4A Variate: Dry_matter (Fumesua)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		15.497	7.748	2.60	
Blk.*Units* stratum						
Genotype	17	(2)	1075.785	63.281	21.26	<.001
Residual	34	(4)	101.184	2.976		
Total	53	(6)	1190.932			

APPENDIX 4B Variate: Dry_matter

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		57.721	28.860	6.76	
Blk.*Units* stratum						
Genotype	18	(1)	1200.545	66.697	15.63	<.001
Residual	32	(6)	136.567	4.268		
Total	52	(7)	1355.650			

APPENDIX 5A Variate: Protein

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		1.1290	0.5645	1.69	
Blk.*Units* stratum						
Genotype	17	(2)	20.0734	1.1808	3.53	<.001
Residual	33	(5)	11.0355	0.3344		
Total	52	(7)	31.5981			

APPENDIX 5B Variate: Protein

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.

Blk stratum	2		8.6834	4.3417	9.30	
Blk.*Units* stratum						
Genotype	18	(1)	40.9571	2.2754	4.87	<.001
Residual	32	(6)	14.9368	0.4668		
Total	52	(7)	61.6144			

APPENDIX 6A Variate: Fe

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		0.09226	0.04613	1.93	
Blk.*Units* stratum						
Genotype	17	(2)	3.20491	0.18852	7.88	<.001
Residual	33	(5)	0.78995	0.02394		
Total	52	(7)	4.06189			

APPENDIX 6B Variate: Fe

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		0.39186	0.19593	6.90	
Blk.*Units* stratum						
Genotype	18	(1)	3.29996	0.18333	6.45	<.001
Residual	32	(6)	0.90902	0.02841		
Total	52	(7)	4.52813			

APPENDIX 7A Variate: Zn

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		0.030725	0.015363	2.54	
Blk.*Units* stratum						
Genotype	17	(2)	1.205511	0.070912	11.74	<.001
Residual	33	(5)	0.199363	0.006041		
Total	52	(7)	1.428679			

APPENDIX 7B Variate: Zn

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		0.17609	0.08805	7.00	
Blk.*Units* stratum						
Genotype	18	(1)	2.05393	0.11411	9.08	<.001
Residual	32	(6)	0.40222	0.01257		
Total	52	(7)	2.54047			

APPENDIX 8A Variate: Starch

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		9.720	4.860	2.32	
Blk.*Units* stratum						
Genotype	17	(2)	944.411	55.554	26.50	<.001
Residual	33	(5)	69.192	2.097		

Variate8B: Starch

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		3.583	1.792	0.50	
Blk.*Units* stratum						
Genotype	18	(1)	913.829	50.768	14.09	<.001
Residual	32	(6)	115.339	3.604		
Total	52	(7)	1026.767			

APPENDIX 9A Variate: Fructose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		0.0902	0.0451	0.38	
Blk.*Units* stratum						
Genotype	17	(2)	26.6405	1.5671	13.33	<.001
Residual	33	(5)	3.8808	0.1176		
Total	52	(7)	30.5472			

APPENDIX 9B Variate: Fructose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		0.4043	0.2022	0.86	
Blk.*Units* stratum						
Genotype	18	(1)	17.0413	0.9467	4.01	<.001
Residual	32	(6)	7.5601	0.2363		
Total	52	(7)	24.9039			

APPENDIX 10A Variate: Glucose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		0.6796	0.3398	1.90	
Blk.*Units* stratum						
Genotype	17	(2)	42.2706	2.4865	13.88	<.001
Residual	33	(5)	5.9120	0.1792		
Total	52	(7)	48.2770			

APPENDIX 10B Variate: Glucose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		0.6938	0.3469	0.88	
Blk.*Units* stratum						
Genotype	18	(1)	30.7285	1.7071	4.33	<.001
Residual	32	(6)	12.6205	0.3944		
Total	52	(7)	43.9257			

APPENDIX 11A Variate: Sucrose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		31.162	15.581	3.57	
Blk.*Units* stratum						
Genotype	17	(2)	431.664	25.392	5.82	<.001
Residual	33	(5)	144.064	4.366		
Total	52	(7)	602.391			

APPENDIX 11B Variate: Sucrose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Blk stratum	2		1.338	0.669	0.21	
Blk.*Units* stratum						
Genotype	18	(1)	414.985	23.055	7.19	<.001
Residual	32	(6)	102.629	3.207		
Total	52	(7)	517.987			

12 APPENDIX AVariate: total_yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	14.868	7.434	0.97	
Rep.*Units* stratum					
Genotype	19	1205.076	63.425	8.31	<.001
Residual	38	289.903	7.629		
Total	59	1509.			

APPENDIX 12B Variate: total_yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	419.53	209.76	5.26	
Rep.*Units* stratum					
Genotype	19	2579.32	135.75	3.41	<.001
Residual	38	1514.81	39.86		
Total	59	4513.65			

APPENDIX 13A Variate: commercial_yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	25.823	12.911	2.19	
Rep.*Units* stratum					
Genotype	19	962.482	50.657	8.61	<.001
Residual	38	223.702	5.887		
Total	59	1212.006			

APPENDIX 13B Variate: commercial_yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	276.77	138.39	4.75	
Rep.*Units* stratum					
Genotype	19	1556.51	81.92	2.81	0.003
Residual	38	1106.06	29.11		
Total	59	2939.34			