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CHARACTERIZATION OF SWEET POTATO (*Ipomoea batatas* Lam) ACCESSIONS AND THEIR RESISTANCE TO VIRAL DISEASES

LOVINNA YANKSON

2020

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CHARACTERIZATION OF SWEET POTATO ACCESSIONS AND THEIR RESISTANCE TO VIRAL DISEASES

BY

LOVINNA YANKSON

A thesis submitted to the Department of Molecular Biology and Biotechnology of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast in partial fulfillment of requirements for the award of Master of Philosophy Degree in Molecular Biology and Biotechnology

AUGUST, 2020

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature……………………..……… Date………………………….

Name: Lovinna Yankson

Supervisors' Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Supervisor's Signature…………………………… Date……………………….

Name: Prof. Aaron Tettey Asare

Co-Supervisor's Signature……………………… Date……………………….

Name: Prof. Elvis Asare-Bediako

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ABSTRACT

Sweet potato (*Ipomoea batatas* L.) production contributes significantly to food security worldwide especially in Africa; however, production is constrained by sweet potato viral diseases (SPVD). The use of resistant varieties remains the most effective and reliable method of managing the SPVD. This work sought to explore genetic diversity among 30 sweet potato accessions and to assess their resistance against SPVD in field trial. The field evaluation was based on the randomized complete block design with three replicates and the quantitative and qualitative data were subjected to analysis of variance and DNA PowerMarker. The resulting dendrogram from the morphological characterization separated the 30 accessions into 6 main clusters (A, B, C, D, E and F). Accessions such as CRI-Ogyefo, CRI-Apomuden, CRI-Ligri and CRI-Histarch were found to be closely related. The low mean gene diversity of 0.24 and PIC of 0.21 suggests narrow genetic base across the genome of the 30 sweet potato accessions. Incidence and severity of SPVD did not vary significantly ($p>0.05$) among the sweet potato accessions, although Faara and CRI-Histarch showed moderate symptoms of virus infection. Viral indexing following grafting unto *Ipomoea setosa* plant, using nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) detected only three (3) viruses, namely, *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato collusive virus* (SPCV) and *Sweet potato chlorotic stunt virus* (SPCSV). All the sweet potato accessions except Blue-blue 3 were infected by at least one of the three viruses detected. Thus, accession Blue-blue 3 is recommended for further screening for virus resistance since presence and absence of the virus may not necessarily mean it is immune to SPVD.

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DEDICATION

To my family

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
bp	Base pairs
CIAT	Centro Internacional de Agricultura Tropical (Spanish): International Center for Tropical Agriculture
CMV	Cucumber Mosaic Virus
CRIG	Crop Research Institute of Ghana
CSIR	Center for Scientific and Industrial Research
DNA	Deoxyribose nucleic acid
IBPGR	International Board for Plant Genetic Resources
IPGRI	International Plant Genetic Resources Institute
ISSR	Inter Simple Sequence Repeat
mL	milliliter
mm	millimeter
NCM-ELISA	Nitrocellulose Membrane-Enzyme Linked Immunosorbent Assay
PCR	Polymerase Chain Reaction
SPCaLV	Sweet Potato Caulimo-Like Virus
SPCFV	Sweet Potato Chlorotic Fleek Virus
SPCSV	Sweet potato chlorotic stunt virus
SPFMV	Sweet potato feathery mottle virus
SPLCV	Sweet Potato Leaf Curl Virus
SPMMV	Sweet Potato Mild Speckling Virus
SPVD	Sweet potato virus disease
SPVG	Sweet Potato Virus G

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SSR	Simple sequence repeats
SwPLV	Sweet Potato Latent Virus
USDA	United States Development Agency
WAP	Week After Planting
μL	microlitre

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CHAPTER ONE

INTRODUCTION

Background of Study

Sweet potato *(Ipomoea batatas* (L.) Lam) is ranked the 3rd most important root crop worldwide and the 4th in the developing world (Kays, 2005). 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). Almost 80% of all sweet potatoes are produced in Asia, particularly in China (70%). Korea currently produces approximately 330,000 tons from 22,000 ha, and its production is increasing every year.

It is an important food security crop, often crucial during famine due to its excellent drought tolerance. In some areas sweet potato can produce up to three harvests per year and it also provides more edible energy than all other food staples (Karyeija et al., 1998). In Africa, sweet potato plays a vital role in people's ability to sustain their families especially during periods of hardship. Sub-saharan Africa produces more than 7 million tons of sweet potato annually, about 5% of global production. Since the early 1960s, production in East and Central Africa has increased one-and-a-half times (to 25% of 1960 production). In Ghana, sweet potato 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). It has low demand for nutrients and is capable of providing reasonable yields by the rapid production of storage roots in seasons where other crops would fail (Kapinga & Carey, 2003; Mukhopadhyay et al., 2011).

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The crop from Central and South America but also has a secondary center of diversity in the southwest Pacific islands. Sweet potato was known to have been introduced to Africa by the Portuguese during the 16th and 17th century (Zhang et al., 2000). They are mostly grown in tropical temperate regions with low labour input and can produce high yield under marginal conditions (Otterdijk, 1999). This commodity is highly productive with low demand of input and labour and can also withstand recalcitrant growth conditions. Sweet potato is grown with altitudes ranging from 0-3000 m above sea level and ambient dry and night temperature of 15-33°C for optimum growth and root development (Troung et al., 2011). Temperature above 25°C is considered optimal for maximum growth. Excessive rainfall at early stage of establishment may aggravate weed problem resulting in low yield (Harrison & Jackson, 2011).

Sweet potato is produced by vegetative propagation using vine cuttings from product ion fields or from sprouted storage roots and it can be propagated by the use of seeds. Cultivars differ in their 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 spread of this crop has been very extensive and is now grown in tropical, subtropical and warmer temperate areas throughout the world (Steinbauer & Kushman, 1971). In Tanzania, Malawi, Mozambique, Zambia and Angola, sweet potato is an important food crop (Moyo et al ,1999). Uganda is the largest African producer and third largest producer in the whole world, growing approximately 2.2 million metric tons (Karyeija et

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al., 1998). Nearly 90% of the total African output comes from eastern and southern Africa (Ewell & Mutuura, 1991; Kanju, 2000; Karyeija et al., 2000.

It has been relied on as a source of calories in many circumstances, such as in Japan when typhoons have destroyed rice crops, during the depression of the 1930s in the United States, during famines in China in the 1960s, or when cassava was decimated by *East Africa cassava mosaic virus* in East Africa in the 1990s. Vines and/or storage roots can be used for direct human consumption or animal feed. Storage roots can be used for fermentation or as a source of starch for food processing or production of chemical stocks, including acids. Identification, characterization and evaluation of accessions are important for genetic improvement programmes and for detecting duplicates in germplasm banks. Collection, characterization and maintenance of local germplasm are the bases of varietal improvement (Mok & Schmiediche, 1998).

Morphological characterization has been used for various purposes including identification of duplicates, studies of genetic diversity patterns and correlation with characteristics of agronomic importance (CIAT, 1993). Sweet potato cultivars are generally distinguished on the basis of morphological traits and have a wide variability of botanical characteristics. Morphological and agronomic characters coupled with reaction to pests, diseases and other stresses have been used to characterize sweet potato. Phenotypic characterization of sweet potato is done by assessing variations in the vine, leaf, flower and storage root characteristics (Huaman, 1991) and it has been traditionally used for identification of sweet potato cultivars.

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Morphological characterization has limitations due to morphological plasticity and parallel evolution (Prakash & He, 1996) that is changes in an organism's behavior, morphology and physiology in response to a unique environment.

Problem Statement

Despite the economic importance of sweet potato and its adaptability in Ghana, the current crop yields are quite low due to both biotic and abiotic constraints. The damage caused by weevils and viruses are amongst the major constraints that result in more serious problems bringing massive losses worldwide. Viral diseases among sweet potato are widespread and can cause serious damage to the crop.

Since it is propagated vegetatively from vines, root slips or storage roots, it serves as an excellent medium of transfer of the viruses from one planting season to the next and these viruses interfere both directly and indirectly with the farmer's inability to realize the full potential of the crop. Sweet potato viral disease is widespread and regarded as a serious problem in Africa and can reduce yield drastically (Gibson et al. 1997). The consequences of viral infections are not only limited to reduction in crop yield but also undermine the ongoing efforts in genetic improvement of yield, quality and development of virus resistant cultivars.

Over 30 viruses infecting sweet potato, assigned to 9 families, have been identified: Bromoviridae (1 virus), Bunyaviridae (1), Caulimoviridae (3), Closteroviridae (1), Comoviridae (1), Flexiviridae (1), Geminiviridae (15), Luteoviridae (1) and Potyviridae (9). Half of them are recently described as DNA viruses belonging to the families Geminivirdae and Caulimoviridae.

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Most of these viruses are associated with symptomless infections in sweet potato and in some cases even in the indicator plant *Ipomoea setosa.*

Sweet Potato Virus Disease (SPVD) is mostly caused by co-infection of two or more of sweet potato viruses. The most drastic SPVD is the co-infection of Sweet potato feathery mottle virus (SPFMV; genus Potyvirus; Potyviridae) and Sweet potato chlorotic stunt virus (SPCSV; genus Crivirus; Closteroviridae) and it is the most devastating viral disease in sweet potato (Karyeija et al., 2000a; Mukasa et al.,2003.; Gibson & Aritua, 2002; Cuellar, et al., 2008). An infection by single virus strain causes little yield losses compared to co- or multiple-infections that cause the complex sweet potato virus disease (SPVD) (Ames de Icochea & Ames, 1997; Karyeija et al., 2000). In temperate zone production, the crop is generally affected by a complex of potyviruses and possibly other unknown viruses that typically cause yield reductions in the order of 20-40%.

The first report of SPVD in Africa was in 1930s from Congo Belge (now Democratic Republic of Congo) Sheffield, 1953), it was subsequently reported in Uganda, Kenya and Tanganyika (now part of Tanzania) (Hansford 1944, Sheffeild, 1953). Since then SPVD has been reported in Ghana (Clarke,), Nigeria (Schaefers & Temy, 1976), Cameroon (Ngeve & Bouwkamp, 1991), Madagascar, Zambia (Gibson, et al,. 1997), Togo, Liberia, Sierra Leone, Sao Tome, Ivory Coast (Thottappilly & Rossel, 1998), Benin and Gabon (Lenne, 1991).

SPFMV has serious impacts on sweet potato production and quality. It is transmitted by aphids (*Myzuz persicae)* and the infected tuberous are used for vegetative propagation. As a result, the disease spreads so fast and rapidly

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in the field and does not respond to pesticides. Shoot-apex culture has been used to overcome virus damage on sweet potato in Japan (Nagata, 1984), this method gives virus-free young potato, however it is very expensive and the sweet potato can still be infected even after cultivation. Research efforts to deal with this problem is often neglected in most of the developing countries including Ghana and our knowledge of sweet potato viruses its importance, distribution and control are rather limited.

Justification

Sweet potato virus disease (SPVD) severely affects sweet potato production (Gutierrez et al., 2003). Continuous use of susceptible varieties, absence of high yielding and early maturing and disease resistant varieties, and lack of effective control measures of SPVD contribute to low yields and disease build up and persistence. Generally, the traditional methods like the use of chemical and biological methods are not effective against viral diseases. Therefore, making use of resistant varieties remains the most effective and advantageous method. Developing resistant varieties through genetic recombination of local varieties and exotic ones with desirable genetic variations and attributes is helpful for breeding. This entails the use of complementary genetic analyses and selection of useful and desirable traits such as high yield and resistance to SPVD. Development of SPVD resistant varieties with desired phenotypic characteristics is a necessity tool in ensuring food security and incomes to small scale farmers.

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Objectives

Main Objective

The main objective was to characterize and screen sweet potato accessions for resistance to SPVD.

Specific Objectives

The specific objectives were to:

1. Characterize sweet potato accessions using morphological and molecular markers.
2. Assess tuber yield of sweet potato accessions.
3. Screen sweet potato accessions for resistance to sweet potato virus disease (SPVD).
4. Carry out serological detection of SPVD.

Significance of the Study

The information and the knowledge gathered from this research will help farmers and other stakeholders in the sweet potato production business to know which varieties of the sweet potato are high yielding, resistant to SPVD and which varieties are highly adaptable. Increase in farmers' field yield of sweet potato will contribute towards food security and poverty reduction. .

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CHAPTER TWO

LITERATURE REVIEW

Taxonomy, Origin and Distribution of Sweet Potato

"Ipomoea" was derived from the Greek words *ipos*, meaning "bind weed" and *homoios,* meaning "Ipomoea" in the direct translation meaning "resembling bindweed", suggesting for the twinning habit of sweet potato much like the bindweed.

It was first described in 1753 by Linnaeus as *Convolvulus batatas.* However, in 1791 Lamarck classified this species within the genus *Ipomoea* on the basis of the stigma shape and the surface of the pollen grains. The name was changed to *Ipomoea batats* (L.) Lam.

The species name *"batatas"* was originally the Taino name for sweet potato. Sweet potato has a chromosome number of 2n = 90. Since the basic chromosome number for the genus Ipomoea is 15, sweet potato is considered as hexaploid (2n = 6x = 90). The series Batatas consists of 13 species closely related to cultivated sweet potato (Huang & Sun, 2000). In the Batatas section there are 3 cytogenetic groups namely group A, B and X. Group A and X are self and cross compatible, group B is self-incompatible but cross-compatible, sweet potato belongs to group B, this means when self-pollinated they cannot produce viable seeds. This explains the high level of heterozygosity in sweet potato.

Based on analysis of morphological characters of sweet potato and the wild *Ipomoea* species, the centre of origin of *I. batatas* was thought to be somewhere between the Yucatan Pennisula of Mexico and the mouth of the

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Orinoco River in Venezuela (Austin, 1989). Sweet potato was domesticated in tropical America about 6000 BC and recorded in Polynesia, Hawaii and New Zealand naturally r by early seafarers in pre-Columbian times.

The Spanish introduced the crop to the Philippines in the 16th century from whence it spreads to other islands and Asian mainland by late 16th century. It was recorded in China, where it was promoted to mitigate drought during the Qing Dynasty (1644-1912). Portuguese seafarers introduced the crop into Western Mediterranean Europe, Africa, India and part of South-East Asia (O'Brien, 1972).

The spread of this crop has been very extensive and it now in tropical, subtropical temperate areas throughout the world (Steinbauer & Kushman, 1971). Trends in cultivation with respect to area from 1992 to 2011 has totally showed decrease in production in Asia from 6.4 to 3.4 million ha and increase in Africa from 1.2 to 3.1 million ha.

In African countries such as Tanzania, Malawi, Mozambique, Zambia and Angola, sweet potato is an important food crop (Moyo et al., 1999). Uganda is the largest African Producer and the third largest producer globally, by growing approximately 2.2 million tons (Karyejia et al., 1998). In Ghana, farmers plant 73,400 ha of sweet potato yearly, after cassava and yam in order of importance (FAOSTAT, 2010).

Sweet potato has short growing period and usually applied in crop rotation, helps in famine as reserve crop. Moreover, the crop can be cultivated in a range of agro-ecological zones because of its hardiness and flexible adaptability.

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Importance and Uses of Sweet Potato

Sweet potato is one of the traditional tuber crops adaptable to wide ecological range with relatively short growing season and of high yield potential even on infertile soil (Hahn, 1984). Hiroshi et al., (2000), reported that sweet potato leaf contained protein and crude fibre which are important for addressing protein deficiency diseases and colon diseases.

Thus, awareness of the high nutritional value of sweet potato especially the orange-fleshed sweet potato is increasing consumer demand for the crop among health-conscious consumers globally (USDA, 2015). Sweet potatoes traditionally grown in sub-Saharan Africa are white-fleshed varieties, which contain little or no beta-carotene (a precursor of Vitamin A).

In the developing countries, including Ghana, the storage root which is high in carbohydrates serve as one of the staple foods for millions of people. It does not only contain many nutrients, but it is also packed with medicinal benefits. Researches had shown that sweet potatoes contain anti-inflammatory, anti-diabetic, and anticancer properties. Storage roots of sweet potato have nutritional components like dietary fibres (pectin, cellulose, hemi-cellulose and lignin), proteins, vitamins (ü-Carotene, Vitamin B_1 and B_2, Vitamin C and Vitamin E), mineral contents (mainly K, Fe and Ca), energy and carbohydrates (Woolfe, 1992). Vitamin B_6 helps reduce the chemical homocysteine in our bodies. Homocysteine has been linked with degenerative diseases, including heart attacks.

Sweet potato varieties with high beta-carotene content (orange-fleshed ones) represent the least expensive source of dietary vitamins. Vitamin A deficiency in sub-Saharan Africa is a serious public health problem in central,

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eastern and southern Africa affecting young children (6 months to 6 years of age) and pregnant women (Ewell & Mutuura, 1991; Carey et al., 1999; Simwambana et al., 1999; Owour, 2000). Eyes are adversely affected due to vitamin A deficiency causing a disease called xerophthalmia (Ewell & Mutuura, 1991; Carey et al, 1999; Simwambana et al., 1999; Owour, 2000). As an excellent source of vitamin A, the orange fleshed sweet potato (OFSP) has the potential to address vitamin A deficiency. Recent varieties of OFSP released in Ghana has increased levels of beta-carotene which is a precursor to Vitamin A in the body. Carotenoids help strengthen the eyesight and boost the immunity to disease, they are powerful antioxidants that help ward off cancer and protect against the effects of aging. Studies at Harvard University indicates that more than 124,000 people showed a 32-percentage reduction in risk of lung cancer in people who consumed a variety of carotenoids-rich food as part of their regular diet.

Sweet potatoes also provide vitamin C (ascorbic acid, 35 mg/100 gfw) and vitamin D whereas other cereal-based foods have none (Ewell & Mutuura, 1991). Apart from vitamin C warding off cold and flu, it also plays a major role in bone and tooth formation, digestion and blood cell formation. It also helps improve wound healing, produces collagen which help maintain the youthful elasticity of the skin and protect the body against cancerous toxins. Vitamin D is very crucial for immune system and general health of the body. It also plays an important role in our energy levels, mood, and helps to build healthy bones, heart, nerves, skin, and teeth, and it supports the thyroid gland.

The leaves of sweet potato including shoot tips and petioles have nutritive values comparable to common dark-green leafy vegetables (Ishida et

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al, 2000; Bovel-Benjamin, 2007). It is also a good source of protein, containing 2.7-3.4 g/100 g of raw fresh leaves and are an important vegetable for most rural households in Malawi and other African countries (Ewell and Mutuura, 1991; Moyo et al., 1999; Kanju, 2000) and are also used for animal feed (Steinbauer & Kushrnan, 1971).

Botany and Morphology

Sweet potato is vegetatively propagated using either the storage tubers, or stem cuttings, but in rare occasions the seed can be used. Its growth habit is predominantly prostrate with a vine system that expands rapidly horizontally on the ground. The root system consists of fibrous roots and the storage roots. Fibrous roots absorb nutrients, water and they also give anchorage to the plant. Storage roots which are the lateral roots store photosynthetic products.

When the root system is obtained by vegetative propagation through tuber, adventitious roots arise and then develop into primary fibrous root which branch into lateral roots. Research had shown that the root can penetrate the soil to a depth of over 2 m depending on soil conditions (Onwueme 1978, Kays 1985). The lateral roots are subdivided into primary, secondary and tertiary roots. Primary root develops from the radicle which in turn produces many secondary and tertiary branches, thus forming a system of roots going through the soil in different directions.

In the early development of young adventitious roots, they separate into 2 classes of roots namely, thin and thick roots. Under adverse conditions thick roots give rise to string roots (primary fibrous roots) and pencil roots depending on the primary cambial activity and the amount of lignification of the cells of the stele (Hahn & Hozyo, 1984; Du Plooy, 1989). Pencil roots are

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between 5 and 15 nm in diameter, they are adventitious roots emerging from the subterranean node of the cutting when the conditions do not favour formation of storage roots. This results in partial lignification and uniform thickening of the entire root (Wilson and Lowe, 1973).

Other roots with no lignification are fleshy, swollen and thickened a lot. They develop into the storage roots. Roots from true seeds form a typical root with a central axle with lateral branches. The central axle later functions as storage root. The initial sign of storage root formation is the accumulation of photosynthates consisting predominantly of starch. It occurs between 7-91 days after transplanting and varies among cultivars (Ravi & Indira, 1999; Villordon et al., 2009).

The storage roots arise from pentarch or hexarch thick young roots if the cell between the protoxylem points and the central metaxylem cell do not become lignified or if only a slight proportion of these cells are lignified, (Ravi & Indira, 1996). Storage root growth depends on the sink strength, the potential of leaves to export photosynthates and photosynthetic efficiency of the leaves.

The high productivity of sweet potato is due to the sink potential of the storage root (Hozyo et al., 1971; Hozyo, 1997). The yield of sweet potato is highly variable and dependent on factors such as variety, propagating materials, environmental and soil conditions, (Kay, 1985). The quantity of yield depends on the number of fibrous roots that will be induced to form storage root. High yielding varieties or cultivars have a high bulking rate over a long period whereas cultivars with intermediate and low storage root yield have a high bulking rate for short duration or low bulking rate for longer

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duration. In late bulking accessions high bulking rate for short duration may also increase in storage root yield (Wilson, 1982). Average storage root yield of sweet potato varies between 10 to 28 t ha^{-1} (CTCRI, 2006).

Accessions may be classified into short duration or early maturing (12-17 weeks), medium duration (17-21 weeks) and long or late maturing (≥ 21 weeks) types, (Yanfu et al., 1989). The formation of a storage root can be in clusters around the stems. If the root stalk that joins the root to the stem is absent or is very short, it forms a closed cluster. If the stalk is long, it forms an open cluster. In some other cultivars the storage is formed at a considerable distance from the stem, therefore the storage root formation is dispersed or very disperse.

The surface of surface roots is usually smooth but some cultivars show some abnormalities in their appearance. Some appear as alligator-like skin, longitudinal grooves or prominent veins. On the surface of some cultivar lenticels can be located due to excess water in the soil. The shape and outline of the storage root can be between round, round-elliptic, obovate, oblong, long oblong, long elliptic or long irregular depending on the variety and environmental factors (Woolfe, 1992). Predominantly the storage root skin color ranges from white to yellow to dark purple. The root flesh colour ranges from white to pale orange to flesh that are strongly pigmented with anthocyanins.

Sweet potato has long thin stems that can produce roots at the nodes as it trails along the soil surface. The stem of a sweet potato is cylindrical in shape and the length of the internodes depends on growth habit and environmental conditions such as availability of water in the soil.

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Based on the length sweet potato plant can be classified as erect, bushy, intermediate or spreading, (Yen, 1974; Kays, 1985). Erect cultivars have height approximately 1 m long while spreading cultivars can reach more than 6 m long. Some stems have twinning characteristics. Internode length can vary from short to very long (few centimeters to more than 12 cm). Planting density has an effect on the internode length as well as on vine length (Somda & Kays, 1990a). Stem diameter of sweet potatoes can be thin or very thick and varies approximately between 4 to 12 cm (CIP ,1991).

Branching, number and length of the vines are cultivar dependent and varies from one cultivar to another. Sweet potato plant produces 3 types of branches and varies between 3 and 20 among cultivars. Environmental conditions such as soil moisture and other conditions such as photoperiod, plant spacing and nutrient supply influence the branching intensity in sweet potato plant (Somda & Kays, 1990a; Sasaki et al, 1993).

Depending on the type of accession, stem color varies from green purple to totally pigmented with anthocyanins. Hairiness of the stem varies from glabrous (without hairs) to very pubescent (CIP et al., 1991). Sweet potato plant has simple leaves which are spirally arranged and they alternate on the stem in a pattern known as 2/5 phyllotaxis (there are 5 leaves spirally arranged in 2 circles around a stem of any two leaves located in the same vertical plane on the stem). The total number of leaves per plant varies from 60 to 300 (Somda et al., 1991) and this is dependent on plant density and irrigation (Nair and Nair, 1995).

The lamina is extremely variable in size and shape even on leaves on the same plant. The edge of the leaf lamina can be entire, toothed or lobed

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depending on the type of cultivar. The base of the leaf lamina has two lobes that can be straight or rounded. The general shape outline of sweet potato leaves can be rounded, reinform (kidney shaped), cordate (heart shaped), triangular, hastate (trilobular and spear-shaped with the two basal lobes being divergent), lobed and almost divided. Lobes of leaves differ in the degree of the cut, varying from superficial to deeply lobed. The number of lobes range from 3 to 7 and can easily be determined by counting the vines that go from the junction of the petiole up to edge of the leaf lamina.

Leaf colour can be green-yellowish, green or purple pigmented in part or the entire leaf blade. Some accessions show purple young leaves and green leaves when matured. The leaves have glandular hairs and are more numerous in the lower surface of the leaf. Leaf size and hairiness of an accession are dependent on the accession and environmental conditions. The leaf vines are palmated and their color can be green to partially or totally pigmented with anthocyanins which is useful in differentiating cultivars.

Petiole length varies widely with accession and ranges from long to very long from 10 to 40 cm (CIP 1991). The petiole can grow in a curved or twisted manner so as to expose the lamina to maximum light. Petiole can be green or with purple pigmentation at the junction with the lamina and/or with the stem or throughout the petiole. On both sides of the insertion with the lamina there are small nectaries.

Sweet potato cultivars differ in their ability to flower, some cultivars do not flower others produce few flowers and others flower profusely. The flowers are born solitarily or on cymose inflorescences that grow vertically upward from the leaf axis. The flower is bisexual besides the calyx and

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corolla; they have stamens that are the male organs or androecium and pistil that is the female organ or gyanecium. Calyx consists of 5 sepals; 2 outer and 3 inner that are attached to the floral axle after the petals dry up and fall.

Corolla consists of 5 united petals that are fused forming a funnel, generally with lilac or purple throat (inside of the tube). Some cultivars produce white flowers. Androecium consists of 5 stamens with filaments that are covered with glandular hairs and that are partly fused to the corolla. The length of the filaments varies in relation to the position of the stigma. The anthers are whitish yellow or pink, with a longitudinal dehiscence. The pollen grains are spherical with the surface covered with very small glandular hairs.

The gynoecium consists of a pistil with superior ovary, 2 carpels and 2 locules that contain one or two ovules. The style is relatively short and ends in a broad stigma that is divided into 2 lobes that are covered with glandular hairs. At the base of the ovary, there are basal yellow glands that contain insect-attracting nectar. The stigma is receptive in the morning. Each flower opens before dawn on a particular day, stays open for few hours, then closes and wilts on the same day. The length of time that a flower stays open is dependent on the weather; it stays longer if the weather is cold and cloudy.

Fruits and Seeds

Sweet potato fruit is a capsule which is 5 to 8 mm in diameter, spherical with terminal tip and can be pubescent or glabrous. Each capsule contains one to four seeds that are slightly flattened on one side and covers on the other. Micropyle is located in a hollow or the flattened side. Endosperm is present in the seed in addition to cotyledons. Seed shape can be irregular, slightly angular or rounded. The color ranges from brown to black and the size

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approximately ranges from 3mm long. The embryo and endosperm are protected by a very hard and impermeable testa.

Seed germination is very difficult and requires scarification by mechanical abrasion by clipping the testa or by chemical treatment that is with concentrated sulphuric acid for about 15 minutes (Purseglove, 1984; Onwueme, 1978). Germination of scarified seeds occurs in 1-2 days. There is elongation of the radical and development of the radicle to primary root system. Germination is epigeal, since the cotyledon is carried above soil level (Onwueme, 1978).

Characterization of Sweet Potato

Plant genetic resources are the most valuable and essential raw material in meeting the current and future needs of crop improvement programmes to satisfy the demand of an increasing human population (Ntare et al, 2006). The characterization of germplasm diversity and the genetic relationships among cultivars, genotypes and breeding lines are critical in crop improvement programmes. The main objective of germplasm characterization is to identify the accessions of a germplasm collection so that they can be clearly distinguished or individualized (CIAT, 2007).

The productivity of sweet potato is mainly dependent on the acquisition of accessions which possess desirable traits and development of high yielding varieties with desired attributes. Sweet potato has a very high genetic variability and thousands of accessions of sweet potato exist in germplasm collections. More than 8000 accessions and breeding lines of sweet potato (2n=6x=90), and nearly 26000 accessions of *Ipomoea* species are maintained in 83 gene banks world-wide (Rao et al, 1994). Since the plant is

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highly heterozygous, there is an extensive variability within the species which is available for exploitation by plant breeders.

Therefore, applying quantitative and qualitative approaches to exploiting its variability is of great importance. Through characterization, both morphological and genetic diversity can be estimated. Studies of genetic diversity are considered more reliable when molecular markers are utilised because they cover a large part of the genome and are not influenced by the environment (Goulao & Oliveira 2001).

Morphological Characterization

Morphological or phenotypic characterization in sweet potato is by assessing the various characteristics of the plant example leaf, flower and storage root (CIP et al 1991) and it is used generally for identification of cultivars of the crop. Morphological characterization has been used for various purposes such as identification of duplicates, genetic diversity patterns studies and also correlation with characteristics of agronomic importance (CIAT 1993). Plant breeders use the result of morphological characterization in simplification selection that is detection of unique traits and also the structure of the population to be conserved. This direct inexpensive, easy to use method is perceived as the strongest determinant of agronomic value and taxonomic classification of plants (Li et al., 2009). This tool bas been used in sweet potato to successfully analyze genetic diversity for germplasm conversation (Huaman, 1992; Karuri 2009).

The use of descriptors developed by CIP is very important and has been used to assess morphological variations in sweet potato collections. Descriptor in plant genetics is defined as attribute, characteristic or measurable

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trait that is observed in an accession of a genebank. Descriptors lists provides a standard scale that can be used in the conservation of germplasm especially by curators of genebanks.

Morphological characterization has certain limitations due to morphological plasticity and parallel evolution (Prakash & He 1996). Therefore, it is very important not to rely only on morphological characterization in assessing genetic diversity but combination of morphological characterization and molecular markers.

Molecular Characterization

In order to optimize the characterization efficiency, morphological characterization is coupled with molecular techniques to give accurate result. Molecular markers have increasingly been employed to investigate sweet potato genetic diversity for germplasm and genetic enhancement (Karuri et al., 2010; Cruz da Silva et al., 2013) and have produced more accurate data on genetic distances in a genotype. The use of molecular markers is environmentally independent.

Advances in molecular biology especially in the development of PCR for DNA amplification, DNA sequencing and data analysis have resulted in powerful techniques which can be used in characterization and genetic diversity studies. The most widely used molecular markers in sweet potato genetic analysis are Simple Sequence Repeats (SSRs), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP).

SSRs are considered to be the most efficient markers for genetic diversity in many plants (Rakoczy-Trojanowsky & Bolibok 2004) including

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sweet potato (Zhang et al, 2000). This is because of their high levels of allelic variations and their co-dominant characters which mean they deliver more information per unit assay than any other marker system.

Sweet Potato Production

Climatic requirements

Sweet potato is grown from 48°N to 40°S of the equator with altitude ranging from 0 to 3000 m above sea level (Low et al., 2009; Troung et al., 2011). The crop requires ambient day and night temperature of 15°C to 33°C for optimum growth and root development. Storage roots are sensitive to changes in soil temperature, depending on the stage of root development.

In terms of rainfall, sweet potato grows best with a well distributed annual rainfall of 600-1600 mm (Low et al., 2009). Excessive rainfall at early stage of establishment may aggravate weed problem and excessive vine development which will result in low yield (Obigbesan, 2009; Harrison & Jackson, 2011). The crop is extensively grown under rain-fed conditions and is relatively drought tolerant. Sweet potato also requires full sunlight; however, it can tolerate a 30-50% reduction of solar radiation (Troung et al., 2011). Air and soil temperature evidently regulate competition between shoots and storage root growth.

Soil requirement

A well-drained sandy loam is preferred and heavy clay soils should be avoided, as they can retard root development which can result in growth cracks and poor root shape. Lighter soils are easily washed away from the roots at harvest time (Watanabe et al., 1968). During wet or rainy season, it is

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recommended that green manure droppings with sterile forage sorghum should be thoroughly incorporated and decomposed in the soil. Good drainage is essential since the crop cannot withstand water logging. Where the water table is high, the crop is planted on mounds and ridges.

Propagation of sweet potato

Sweet potato is propagated asexually by vine cuttings or sexually from seeds (Woolfe 1992) but in commercial production the use of vine cuttings is preferred. In vegetative propagation either of these two methods are employed: sprouts from whole storage roots or stem or vine cuttings.

Sprouts are grown from plant stock selected for its appearance, free of diseases and off types. Healthy tubers of 20 to 50 g should be planted 3 cm deep (Ikemoto, 1971). The use of sprouts derived from tubers for direct planting of sweet potato is however, not recommended because it usually results in low yields compared to stem cuttings.

Vine cuttings are the usual method of propagating sweet potato. Tip cuttings of about 30 to 40 cm long with approximately 8 nodes are collected from the nursery beds or the last established planting (Obigbesan, 2009). Cuttings should be taken from crops that are old enough to provide material without excessive damage. The vine cuttings can be left under a moist cloth in a shade for a couple of days before planting, this promotes nodal rooting. At the recommended plant spacing, 330 cuttings are required for a 100 m row.

Sweet potato is grown on raised beds or mounds. This provides the developing roots with loose, friable soil to expand to their potential size and shape. It also provides adequate drainage and allows easy harvesting with

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a mechanical digger. Mounds should be about 30 cm high and 40 cm wide at the base, and 1.5 to 2 m apart from each other. Planting time is mainly determined by the climate of the location. Sweet potatoes are normally damaged by light frost and the plants require high temperatures for a period of 4-5 months of yield.

In Ghana and most African countries sweet potato is planted from January to March. Cold spells during wet or rainy seasons can adversely affetct root development. In very hot areas, planting should be avoided from November to middle of February as storage root formation is reduced at high temperature. Vine cuttings are generally planted vertically at an angle or horizontally at the surface with 3 to 4 nodes in the soil. Chen et al. (1982) reported on the modification of a mechanical planter that permits sweet potato cuttings to be planted horizontally, thus resulting in greater yields. The placement of the vine or sprout is done by hand. Cultivars with trailing stems are planted wide apart than those with semi trailing stems. As plant population per hectare increases the number of tubers or storage roots per plant decreases, the mean weight per tuber decreases. Sweet potato is best planted early in the season so that the early rainy season can be utilized.

The recommended fertilizer rate for sweet potato production is based on crop removal figures. Research has shown that Nitrogen, Phosphorus and Potassium (NPK) when used in their right quantities in conjunction with yearly soil nutrients testing and monitoring petiole sap nutrients can produce high yields. The fertilizer is applied at the base and is divided into 2 dressings at 4 to 6 weeks and at 10 to 12 weeks after planting. Calcium can be supplied in the form of lime or dolomite to adjust the soil pH.

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Constraints of sweet potato production.

Sweet potato farmers have numerous challenges that affect production. These include crop diseases, insect infestation, weather conditions, high input costs, low market prices, labour shortage and storage constraints. These abiotic, biotic and socio-economic challenges affect sustainability of the crop production.

Abiotic constraints

Farmers and growers suffer more than 25% yield losses due to extreme abiotic conditions such as high temperature, frost and floods. In Africa abiotic constraints related to sweet potato production are primarily temperature and precipitation related events (Franke et al., 2013). Sweet potato in various regions suffers from low temperature in the early planting season thus damaging crops after emergence. Heat wave during growing season and at the time of bulking may lead to temporarily reduced or halted growth as well as reduced quality of the tuber (UNECE, 2014).

Petiole sap nutrient monitoring is advisable so that the desired nutrient levels for different growth phases be checked. Any trace element deficiency would be detected by regular petiole testing. The recommended rates of side dressing fertilization should be calculated based on crop area (e.g. 20 rows, 50 m long at 2 m spacing =2000 m^2 or 0.2 ha). If using drip method the fertilizer can either be injected or applied on the soil or irrigated into the soil.

Biotic constraints

Sweet potato production is greatly affected by several biotic constraints such as bacteria, fungal and viral diseases, insect pest and weeds

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(Ndunguru et al., 2009). In the early 1946-1950's viral diseases were recognized as causing deterioration in the quality and yield of sweet potato. Sweet potato viral diseases (SPVD) are caused by the dual infection and synergistic interaction of sweet potato chlorotic stunt virus and sweet potato feathery mottle virus (Mukasa et al., 2006).). SPVD is one of the devastating diseases causing in plant growth, root yields and quality.

The damage caused by SPVD ranges from 50 to 98%. Yield losses due to viral diseases was estimated to be 15-48% in China, 34-97% in Egypt (Salazar & Fuentes, 2000), 50% or more in Israel (Milgram et al., 1996.) and 80-98% in East Africa (Mwanga et al., 2002; Wambugu, 2003). A suspected viral disease of sweet potato was first reported in 1944 in South Carolina (Steinbauer & Kushman, 1971). Currently there are more than 14 viral diseases of sweet potato that have been reported (Brunt, Crabtree, Dallwitz, Gibbs & Watson.,1996; Di Feo et al., 2000).

Sweet potato weevils *Cylas* spp is another major constraint in sweet potato production (Kapinga & Carey 2003). The weevils feed on vines and storage roots thereby reducing the quality and yield of the crop. Infestation levels are highest under dry conditions due to many cracks which appear when the soil dries (Muyinza et al., 2007).

Many genera of plant parasitic nematodes are associated with sweet potato production in the field but only three are important. The three are the sting nematodes (*Belonolaimus gracilis),* the root lesion nematode (*Pratylenchus*) and the root knot nematode (*Meloidogyne*) which is the widest spread of the three. Nematodes attack causes poor growth, low yield and

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cracked tubers. A nematicide (aldicarb 350/100 m planting furrow) has been found to be an effective chemical control method.

Other biotic constraints like millipedes, Alternaria leaf spot, stem blight, black rot, Fusarium rot, bacterial rot, other nematodes and vertebrate pest such as rats are also a threat to sweet potato production (Kapinga et al., 1995; Fugile, 2007). The levels of damages due to diseases and pests depend on the causal agents, intensity of infestation, variety and prevailing environmental conditions (Thottappilly & Loebenestein, 2009).

Sweet Potato Virus Diseases (SPVD)

Among biotic constraints, virus diseases are the most devastating disease-causing reduction in plant growth, root yield and quality. Compared to viruses of other agriculturally important crops, that of sweet potato has been poorly studied. The yield losses have significant negative impact on food security and income for farmers and growers.

The physiology, metabolic activity or even anatomy of the plant is affected by viruses and any of these changes may affect the ease with which the host will respond to viruses' activity (Hull, 2002). Viruses affecting sweet potato can be spread by the use of foliar cuttings taken from infected plants. Depending on cultivar, infecting virus, stage of infection and whether the crop is infected with a single or multiple virus, viral diseases may cause up to 100% yield loss (Ngeve & Bouwkamp, 1991).

An infection by single virus strain causes little yield loss compared to co-or multiple-infections, and these cause the complex sweet potato virus disease (SPVD) (Ames de Icochea & Ames, 1997; Karyeija, Kreuze, Gibson, & Valkonen, 2000).). Although SPVD was first reported in the scientific

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literature in the 1940s, it was not until 30 years later that the cause was known. Over 30 viruses infecting sweet potato, assigned to 9 families, have been identified: Bromoviridae (1), Bunyaviridae (1), Caulimoviridae (3), Closterovi ridae (1), Comoviridae (1), Flexiviridae (1), Geminiviridae (15), Luteoviridae (1), and Potyviridae (9).

Sweet potato feathery mottle virus (SPFMV) is the most common sweet potato virus around the world (Moyer & Salazar, 1989). SPFMV genomes constitute a positive sense single stranded linear ssRNA. They gain entry into host cell via the stylets of several aphid species. SPFMV has a narrow host range that is limited to plants of the family *Convolvulaceae* and some strains have been reported to infect *Nicotiana benthamaiana* and *Chenopodium amaranticolor* (Moyer, Cali, B.B., Kennedy & Abdou-Ghadir, 1980).

Diagnosis of SPFMV can routinely be carried out by indexing on a sensitive indicator host *I. setosa* , serological tests with available antibodies from CIP and further confirmation by RT-PCR. Sweet potato chlorotic stunt virus (SPCSV) is a member of the genus *Crinivirus*. It is an agriculturally significant pathogen of sweet potato transmitted by whiteflies (*Bemisia tabaci*) in a semi-persistent manner (Sim, Valvaerde & Clark, 2000). SPCSV has a limited host range including mainly the genus Ipomoea and some species of *Nicotiana* and *Amaranthus palmeri* (Cohen, Frank, Vetten, Lesemann, & Loebenstein, 1992.).

Sweet potato leaf curl virus (SPLCV), a *Begomovirus* belonging to the family *Geminiviridae* has recently been reported in sub-Saharan Africa, although only in Kenya (Miano, Labonte, & Clark. 2008). However, SPLCV

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has a worldwide distribution including USA, china, Japan, and Spain (Loktrakul, Valverde, Clark, & De La Torre, 1998; Lozano, Trenado, Valverde, & Navas-Castillo, 2009).

Members of the *Geminiviridae* family have particles that resemble paired spheres containing single-stranded (ss) DNA either as a single component for monopartite viruses or as two components. Begomovirus is the largest genus, containing viruses transmitted by whitefly, particularly *Bemisia tabaci,* and infecting dicotyledonous plants (Fauquet et al., 2003; Lotrakul, Valverde, & Clark, 2003).

Besides SPLCV, Ipomoea crinkle leaf curl virus (ICLCV) (Cohen et al., 1997), sweet potato leaf curl Georgia virus (SPLCGV) and Ipomoea yellow vein virus (IYVV) (Banks et al., 1999) have been reported from Spain.

The most severe disease in sweet potato is caused by the dual infection and synergistic interaction of sweet potato chlorotic stunt virus (SPCSV) family and sweet potato feathery mottle virus (SPFMV) (Karyeija, Gibson & Valkonen, 1998; Kreuze et al., 2009; Untiveros, Fuentes & Kreuze, 2008). SPVD is, by far, the most destructive viral disease in Africa (up to 50% in East Africa) and perhaps worldwide (Carey et al., 1999). SPVD has been reported in a number of African countries including Ghana among other countries like Nigeria, Rwanda, Uganda, Kenya and Tanzania.

Synergism has been observed in SPCSV and the possibly whitefly-transmitted sweet potato mild mottle virus (SPMMV) (Gutierrez, Fuentes, Salazar 2003; Hahn, 1979).

Among the well-recognized sweet potato viruses Sweet Potato Mottle virus (SPFMV, potyvirus), has a pervasive distribution, whiles others are

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localized to one or more geographic areas (Moyer and Salazar 1989; Mukasa 2004). Ubiquitous nature of the strains of SPFMV hinders the identification of other viruses. SPFMV is transmitted by a wide range of aphid species (eiliana) and is spread mainly by winged adults even of species that do not colonize sweet potato flying from plant to plant.

Since it is the spread of SPCSV by white flies that synergizes SPFMV. Whiteflies are also usually the driving force behind the spread of SPVD.

Sweet potato mild speckling virus (SPMSV), sweet potato virus G (SPVG) and sweet potato latent virus (SPLV) have also been reported to affect sweet potato (Feng et al., 2000; Ndunguru & Kapinga, 2007). These viruses not only adversely affect sweet potato yields and quality but also decrease plant resistance to insect pests (Feng, Yifu, & Pinbo 2000).; Bryan, Schultheis, Pesic-Vanesbroeck, & Yencho 2003.; Yang, 2010).

Detection of viruses

The detection and identification of sweet potato viruses remains a difficult procedure and this is complicated by frequent occurrence of mixed infections and synergistic complexes (Tairo, Kullaya & Valkonen 2004).

Virus detection methods like visual observation, serological detection (enzyme linked immunosorbent assay (ELISA)), biological indexing, polymerase chain reaction (PCR) and its variant reverse-transcription polymerase chain reaction (RT-PCR) have been used for virus indexing in sweet potato (Ghosh & Aglave, 2007). Of all the detection methods, PCR with its variant RT-PCR is the most sensitive and reliable (Opiyo et al., 2010). Generally, these methods are used regardless of their limited reliability and sensitivity.

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Virus symptoms are often difficult to distinguish and expression often varies with virus strain, host cultivar, age of plants, abiotic factors, multiple infections and synergistic reactions. Nutritional deficiencies also cause symptoms that could be mistaken for those caused by virus infection.

After few weeks of planting, the plants can be indexed for virus infection. This is done using visual observation of disease symptoms on young fresh leaves and estimating disease incidence (DI) using the formula described by Njock and Ndip (2007):

$$\text{Disease Incidence } (\%) = 100 \left\{ \left(\sum_{s=2}^{5} [\ Xs\ ] \right) / \left(\sum_{s=1}^{5} [\ Xs\ ] \right) \right\}$$

Where X- number of plants given the score and s-severity class (1-5)

Visual symptoms include vein-clearing, chlorosis, leaf cupping upward and downward, leaf curling and/or rolling, mottling, rugosity, stunting, leaf deformities, necrosis, inability to flower, poor root development, poor uptake of water and nutrients, reduced canopy growth, necrosis, misshapen roots, low yields and in some extreme severe infections, plant death.

Biological indexing of sweet potato can be done by employing susceptible indicator host plants, which involves grafting of sweet potato shoots on to indicator host *I. setosa* (Moyer & Salazar, 1989). The indicator plant shows symptoms which may be difficult to detect in original sweet potato plants due to low titers of virus. The identification of the virus strain requires further confirmation by assays based on serology and/or reverse transcription Polymerase Chain Reaction (RT-PCR). This method is useful for viruses that cannot be mechanically transmitted (Walkey, 1991).

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There are a variety of serological tests available to detect viruses. These are based on a reaction between the proteins in the pathogen and an antibody produced against them. Nitrocellulose membrane-enzyme linked immunosorbent assay (NCM-ELISA) is the main serology method currently used to detect viruses in sweet potato. ELISA has become the preferred test because of its simplicity, adaptability, sensitivity and accuracy. There are obstacles inhibiting the effectiveness of ELISA method, which are, low concentration and irregular distribution of viruses in sweet potato (Esbenshade & Moyer, 1982).

Another factor is the presence of phenolics, latex and inhibitors in sweet potato tissue that adversely affect tests. The accuracy of serology may also be hampered by multiple infections of different viral strains because the specificity of antibodies is lost due to genetic variability of viral strains during synergism (Tairo, Jones & Valkonen, 2006). Hence, subsequent confirmatory tests are needed to validate the results.

It is required that infected material is first grafted on *Ipomoea setosa*, as the virus concentration will be higher in the indicator species and the ELISA will not be affected by inhibitors present in sweet potato sap. Polyclonal antibodies specific to the virus, as well as NCM strips spotted with sap from virus-positive and non-infected controls are used. The development of a purple colour on the sample spots confirms virus positive sample (Gutierrez, Fuentes & Salazar, 2003).

Molecular techniques like PCR and its variant reverse transcription (RT-PCR) has provided information on many new and uncharacterized viruses that can cause infections in sweet potato (Gibbs & Mackenzie, 1997). It uses

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synthetic nucleic acid probes on the in-vitro amplification of the specific nucleic acid sequences. These technologies increase the amount of genetic material in a sample that initially contains very small amounts, to a level that it can be detected. The products are then separated by placing them on an agarose gel and subjecting this to an electric current. Although PCR and RT-PCR are very sensitive methods for virus detection, it is recommended that infected material is grafted on *I. setosa* as the virus concentration will be significantly higher in the indicator species.

Management of sweet potato viruses

There is no effective and complete control method against the disease to date, however managing viruses infecting sweet potato will require knowledge on aetiology and ecology of the viruses. The control of these viruses still remains difficult in subsistence farming. Efforts to control the spread of the viruses by the control of the vectors have been unsuccessful.

Traditional cultivation methods such as exchanging of planting materials among farmers promote spread and perpetuation of viruses infecting planting material (Karyeija, Gibson & Valkonen ,1998a). Both chemical and biological control methods are not so effective against viral diseases (Garcia-Arenal & McDonald, 2003; Maule, Caranta, & Boulton, 2007). There are both conventional and modern control methods in managing of virus infection, but few have been identified as effective; these are selection of SPVD resistant cultivars, pathogen derived resistance method, use of disease or virus free planting materials and also removal of all infected plants.

Firstly, the selection of SPVD resistant accessions. Currently the focus of much research in sub-Sahara Africa is the breeding of virus resistant

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cultivars. This is however hindered by the diversity of the sweet potato viruses, for example sweet potato varieties may be resistant to SPFMV, which may be susceptible to other viruses or possible multiple infections. There is therefore the need to breed resistant cultivars with no or little tendencies of viral infections. Breeding for resistance to single viruses has been accomplished.The resistant variety should be high yielding and possess tolerance to other agronomic restraints that contribute to yield decline such as nematodes and weevils. In West Africa SPVD-resistant cultivars have been selected by field exposure and graft-inoculation of storage roots (Hahn, Terry & Leuschner, 1981). In Uganda cultivars to SPFMV have been identified (Aritua, Adipala, Carey & Gibson, 1998a). Also, in Kenya, a single variety showing tolerance to single virus infections by SPFMV, SPMMV and SPCSV have been identified (Njeru et al., 2004).

In general, resistant varieties are rarely available in addition to being low yielders and late maturing (Abidin et al., 2005), therefore it is important to improve virus resistance through development and deployment of SPVD resistant and high yielding varieties. Phytosanitation involves the eradication of sources of infection and inoculum. Farmers usually select stem cuttings relatively unaffected by SPVD as parents of the next crop (Bashaasha, Mwanga, Ocitti p'Obwaya, & Ewell, 1995; Kapinga, Ewell, Jeremiah & Kileo, 1995) and regard this as a main control method of SPVD. Phytosanitary measures like quarantine, good sanitation practices and use of virus free vegetative propagules have been reported to decrease the population of vectors responsible for spreading SPVD. A resistant cultivar with consumer appeal like good taste and consistency is an advantage.

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Secondly, the pathogen-testing (PT) or pathogen testing derived materials. This method does not only alleviate the problem of progressive yield decline but also has the benefit of providing growers and farmers with increase yield opportunities and economic benefits in a more rapid timeframe. This solution involves removal of viruses from locally infected preferred cultivars. This strategy has proven to be a highly effective and rapid solution to reduce the spread of SPVD in major sweet potato producing countries such as China (Fuglie, Zhang, Salazar & Walker, 1999) and South Africa have had an outstanding success with the use of pathogen-testing (PT) planting materials. Scientists use virus removal technologies such as meristem extraction with or without thermotherapy along with tissue culture technology to remove viruses from sweet potato plants.

Furthermore, the use of virus free planting material to combat the decline in crop yield due to virus infection is to encourage the use of virus free planting materials by farmers and growers. The use of virus-free and certified planting materials is likely to reduce the effects of SPVD (Thottappilly & Loebenstein, 2009). Another method is the removal of all infected plants. During crop growth plants showing SPVD symptoms should be removed especially during the first month after planting. The early removal of infected plants can reduce the spread of the viruses to the other plants.

Roguing involves the removal and destruction of plant as soon as they become infected and this therefore reduce the development of virus sources (Dent 1995). The destruction of surviving crops in old and sometimes abandoned fields conceivably reduces sources of infection and virus incidence.

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However, it is known that virus resistance only slows down but does not prevent virus spread in vegetatively propagated crops.

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CHAPTER THREE

MATERIALS AND METHODS

Location

Field work was carried out at the Teaching and Research Farm of School of Agriculture, University of Cape Coast. The location of the experimental site (5.1036°N, 1.2825° W) falls within the Coastal Savanna agro-ecological zone of Ghana. The site has a semi-arid to dry climate with a mean average temperature ranging from 23°C to 26°C during the rainy season. The experiment was conducted in three (3) seasons between the years July 2016 to November 2018. Cape Coast is a coastal savannah agro-ecological zone hence experiences a bimodal rainfall. The major season begins from June with the maximum rainfall from July to mid-August whereas the minor seasons begin in September to November, with maximum in October (Addo-Quaye et al., 2011)

DNA analyses were also carried out in the Molecular Biology and Biotechnology laboratory of the University of Cape Coast, Cape Coast, Cordoba Plant Physiology Laboratory, Universidad de Cordoba, Cordoba, Spain and Virology Laboratory of Crop Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR), Fumesua, Kumasi, Ghana

Plant materials (Sweet potato accessions)

Thirty (30) sweet potato accessions were collected from CSIR-CRI, Plant Genetic Resources Research Institute (PGRRI), Bunso and local farms in

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and around Cape Coast Metropolis. The accessions and their sources are presented in Table 1.

Table 1: Sources of sweet potato accessions used for the study

Accessions	Source
Faara	CSIR-CRI
Sauti	CSIR-CRI
Santom pona	CSIR-CRI
CRI-Ogyefo	CSIR-CRI
CRI-Apomuden	CSIR-CRI
CRI-Otoo	CSIR-CRI
CRI-Ligri	CSIR-CRI
Purple	Cape Coast
Blue-blue 1	Cape Coast
Blue-blue 2	Cape Coast
Blue-blue 3	Cape Coast
CSP1	CSIR-CRI
CSP 2	CSIR-CRI
Tech santom	CSIR-CRI
Ex/Bunso/04	Bunso
CRI-Dadanyuie	CSIR-CRI
CRI-Bohye	CSIR-CRI
CRI-Histarch	CSIR-CRI
Apomuden local	Cape Coast
Ex/Bunso/01	Bunso
Bot/03/030	Bunso
Tag/03/019	Bunso
Ex/Bunso/02	Bunso
Ok/03/014	Bunso
Dos/03/017	Bunso
CRI-Patron	CSIR-CRI
Ex/Bunso/03	Bunso
Ex/Bunso/05	Bunso

Field Layout and Experimental Design

The land was cleared, ploughed and harrowed. The experimental layout was based on the Randomized Complete Block Design (RCBD) with three (3) replicates with each consisting of thirty (30) plots. Plots were

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prepared in the form of ridges, each measuring 4.5 m by 1.5 m with 1 m between ridges. For each accession, averagely 20 cuttings were planted at a distance of 0.4 m.

About 0.3 m of each vine cutting was inserted at a slant with two-thirds buried below the soil surface. Cultural practices such as irrigation, wedding and fertilizer application were done on the field to ensure the growth of the plants. Weeding was done at 4 weeks intervals using a hoe. Watering was done using sprinkler irrigation periodically.

Evaluation of Morphological Traits (Morphological characterization of sweet potato accessions)

Morphological characterization of the above and below ground parts was conducted (CIP, AVRD, IBPGR., 1991) 2 to 4 months after planting. A total of 20 characters (14 aerial and 6 storage root characters) were evaluated for each genotype.

Foliar or aerial descriptors that were qualitatively and quantitatively scored included twinning, vine internode length (VNL), growth cover (GC), predominant vine colour (PVC), growth habit (GH), vine tip pubescence (VTP), general outline of the leaf (GOL), mature leaf colour (MLC), leaf lobe number (LLN), shape of central leaf lobe (SCL), mature leaf colour (MLC), immature leaf colour (ILC), petiole length (PTL), and petiole pigmentation (PPT).

Storage root traits included storage root shape (SRS), storage root weight (SRW), bulk weight (BW), storage root skin and flesh (SRSF), storage root cortex (SRC) and number of storage root (SR). Key for scoring morphological traits are presented in Table 2.

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Table 2: Key for scoring morphological characters of sweet potato.

Characteristics	Code	Score
Twining	T	0- Non twining, 3-Slightly twining, 5-Moderately twining, 7-Twining, 9-Very twining.
Growth Habit	G.H	3- Erect (<75cm), 5-Semi-erect (75-150 cm), 7-Spreading (151-250 cm), 9-Extremely spreading (>250 cm).
Ground Cover	G.C	3- Low (<50%), 5- Medium (50-74%), 7- High (75-90%), 9-Total (>90%).
Vine Internode Length	V.I.L	1-Very short (<3 cm),3-Short (3-5 cm), 5-Intermediate (6-9 cm), 7-Long (10-12 cm), 9-Very long (>12 cm)
Predominant Vine Colour	P.V.C	1-Green, 2-Green with few purple spots, 3-Green with many purple spots 4-Green with many dark purple spots, 5-Mostly purple, 6- Mostly dark purple 7-Totally purple, 8-Totally dark purple
Secondary Vine Colour	S.V.C	Same as P.V.C
Vine Lobe Pubescence	V.L.P	0-Absent, 3- Sparse, 5-Moderate, 7- Dense
General Leaf Outline	G.L.O	1-Rounded, 2- Reniform (kidney-shaped), 3-Cordate (heart-shaped), 4-Triangular 5-Hastate (trilobular and spear-shaped with the basal lobes more or less divergent) 6-Lobed, 7-Almost divided
Leaf Lobe Number	L.L.N	Sweet potatos normally have the leaf lobe number to be odd number: 1-, 3-, 5-, 7-, and 9-.
Central Leaf Lobe Shape	C. L. L. S.	0-Absent, 1-Toothed, 2-Triangular, 3-Semi-circular, 4-Semi-elliptic, 5-Elliptic, 6-Lanceolate, 7-Oblanceolate, 8-Linear (broad), 9-Linear (narrow).
Mature Leaf Colour	M.L.C	1-Yellow-green, 2-Green, 3-Green with purple edge, 4-Greyish-green (due to dense pubescence), 5-Green with purple veins on upper surface, 6-Slightly purple, 7-Mostly purple, 8-Green upper surface, purple lower surface, 9-Purple on both surfaces,
Immature Leaf Colour	Im. L.C.	1-Yellow-green, 2-Green, 3-Green with purple edge, 4-Greyish-green (due to dense pubescence), 5-Green with purple veins on upper surface, 6-Slightly purple, 7-Mostly purple, 8-Green upper surface, purple

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Table 2 Cont'D

		lower surface, 9-Purple on both surfaces.
Petiole Length	P.L	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).
Petiole Pigmentation	P.P.	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, some others green, 9-Totally or mostly purple.
Storage Root Weight	S. R.W.	
Bulk Weight	B. W	
Storage Root Cortex thickness	S. R.C.T	1- very thin <1mm, 3-thin 1-2mm, 5-intermediate 2-3mm, 7-thick 3-4mm, 9-very thick>4mm
Storage root shape	S.R.S	1-round, 2-round elliptic, 3-elliptic, 4-ovate, 5-obovate, 6-oblong, 7-long oblong, 8-long elliptic, 9-long irregular or curved.
Storage root skin/ flesh colour	S.R.S.C	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.
Number of storage root	N.S.R.	Average of ten (10) plants.

Source: CIP, AVRDC, IBPGR (1991)

Molecular Characterization of Sweet Potato Accessions

DNA extraction

Total genomic DNA from freshly harvested leaves was extracted using Qiagen Quick-Start Protocol DNeasy® Plant Mini Kit manufactured in Hilden, Germany with some modifications.

The leaf samples were disrupted using mortar and pestle. Buffer AP1 (400 µL) and 4 µL RNase A are added to 50 mg of the disrupted leaf samples, they were vortexed and incubated for 10 minutes at 65°C. One hundred and

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thirty microliters (130 µL lysate) Buffer P3 was added, mixed and incubated for 5 minutes on ice. The lysate was centrifuged for 5 minutes at 14,000 rpm. It was then pipetted into QIA shredder spin column placed in a 2 ml collection tube and centrifuged again for 2 minutes.

The flow-through was transferred into new tube without disturbing the pellet and 1.5 volume of Buffer AW1 was added and mixed by pipetting. Six hundred and fifty (650 µl) of the mixture was transferred into DNeasy Mini spin column placed in a 2 ml collection tube. It was centrifuged for 1 minute at 8000 rpm. The flow-through was discarded and the step was repeated with the remaining sample.

The spin column was placed in 2 ml collection tube, 500 µL Buffer AW2 was added and centrifuged for 1 minute at 8000 rpm, flow-through was then discarded. Another 500 µL Buffer AW2 was added and centrifuged for 2 minutes at 14000 rpm. The spin column was carefully removed from the collection tube so that the column does not come into contact with the flow-through. The spin column was transferred to a new 1.5 mL microcentrifuge tube and 100 µL buffer AE was added for elution. This was incubated for 5 minutes at room temperature and centrifuged for 1 minute at 8000 rpm.

Primer dilution

Twenty-one (21) Simple Sequence Repeats (SSR) primers were bought by the Department of Molecular Biology and Biotechnology of the University of Cape Coast from Biommer. The primers were spun with a short spin using a centrifuge before the tubes were opened; this was to ensure that any dislodged pellet caused by the shipping would settle to the bottom of the tube.

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A master stock (100 µM) of the primers were prepared using the formula 100 µM x n moles lyophilized primer.

The master stock primers suspended in molecular grade water (H_2O), were allowed to sit at room temperature for 10 minutes and well before they were used for preparing working concentration. The primer master stocks were diluted with molecular grade H_2O in the ratio 1:10 to form the working solution (10 µM). The working solutions were stored at 4^oC.

Primer screening

A total of twenty-one (21) SSR primers were screened and optimized for polymorphism assessment by varying the annealing temperatures within the range of 50 to 63 ^oC using Bulk Segregant Analysis method (Michelmore et al.,1991). Seven primers that showed good and clear polymorphism with the PCR products were selected and used for this study.

Polymerase Chain Reaction (PCR) analysis

Seven (7) informative SSR markers previously selected from 20 pairs of SSR primers confirmed for Sweet potato (Buteler et al., 1999) were used for Polymerase Chain Reaction (PCR).

AccuPower Taq PCR Premix containing *Taq* DNA polymerase, dNTPs, reaction buffer, tracking dye and patented stabilizer obtained from Bioneer, California, USA was used for this work. Eight and a half microlitres (8.5 µL) of molecular grade H_2O, 0.5 µL each of the reserve and the forward primer and 0.5 µL of eluded DNA were added to the AccuPower reaction mix tube.

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Each SSR primer was used to run the set of DNA samples extracted from the 30 sweet potato genotypes. PCR amplifications were carried out in BIO RAD T100™ Thermal-Cycler (Applied Biosystems). PCR amplification conditions used involved denaturing at 95° C for 3 minutes, annealing at temperatures varying for each primer for 1 minute and extension at 72°C for 1 minute. This cycle was repeated 34 times and final extension at 72°C for 5 minutes.

The PCR products were electrophoresed on 2% Agarose gel (w/v) at a constant voltage of 90 volts for 45 minutes and autoradiograped.

Gel electrophoresis

The 2% Agarose was cast in a tray with barriers, and 15 well-forming combs were inserted to create wells. A 100 mL of the Agarose gel was prepared by dissolving 2 g of the Agarose in 100 mL of x1 TAE buffer. The mixture was stained with 3.0 µL of ethidium bromide, it was then poured into the tank and distributed across the whole surface, removing bubbles. The mixture was allowed to solidify. The whole assembly was transferred into electrophoresis tank and the comb was removed; the assembly was submerged in X1 TAE buffer.

The PCR products were loaded into the wells. During loading, care was taken to avoid puncturing the agarose gel. The lid of the electrophoresis tank was then fixed. The PCR products were resolved for 45 minutes at 120 mA and 90 V and were visualized on an UV transilluminator.

The bands of the gels were photo documented with a digital camera. The size of the DNA bands in base pairs were determined using 100 bp DNA standard ladder obtained from Bioneer.

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Evaluation of Virus Incidence and Severity

The evaluation of the disease incidence and severity were done during the second planting season (July to October, 2017).

At 6 weeks after planting (WAP), severity of sweet potato virus symptoms was scored for each cultivar every two weeks for 4 consecutive times using 5-point severity rating scale (Hahn, 1979) where;

0 = no symptoms

1 = mild symptoms in few plants

2= apparent symptoms on more than 50% leaves

3= severe symptoms on more than 70% leaves and

4= very severe symptoms on more than 90% leaves

Disease Incidence (DI) was estimated using the method described by Njock and Ndip (2007) as: Disease Incidence (%) = $100 \{(\Sigma_{s=2}^{5} [Xs]) / (\Sigma_{s=1}^{5} [Xs])\}$

Where X- number of plants given the score and s-severity class (1-5)

Ten (10) plants per accession were randomly assessed.

Yield evaluation

Harvesting of the sweet potato was done 4 months after planting. Yield parameters were evaluated for all the three planting seasons. Parameters such as storage root weight, bulk weight and number of storage root were evaluated per accession. Storage root weight and bulk weight per accession were measured using electronic balance and recorded in grams. Number of the storage root was counted manually.

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Biological Indexing or Graft Inoculation

The biological indexing was done at CSIR-Crop Research Institute (CSIR-CRI), Fumesua, Kumasi. The seeds of *Ipomoea setosa* were provided by CSIR-CRI, Fumesua. Thirty (30) sweet potato accessions were collected from the field and then grafted onto 4-week-old *I. setosa* test plants under greenhouse conditions. The *I. setosa* plants were raised from seeds which were scarified. The scarification was done by soaking in 95% concentrated sulphuric acid for an hour and rinsed thrice in distilled water. The scarified seeds were then placed on wet paper-towel in a petri dish and allowed to germinate. The seedlings were then transferred into pots.

After 4 weeks, the seedlings were cut back and used as rootstocks. A wedge was created on the stem using disposable blade. The scion was grafted onto the *I. setosa* and the union wrapped with paraffilm to prevent desiccation. Each grafted plant was covered with a transparent polyethylene bag, pre-wet with water to create humid atmosphere and they were placed in an insect proof screen house. The bag was removed after 3 days.

A control setup was made using healthy *I. setosa* as scion and grafted onto another *I. setosa* root stock. The grafted plants were observed weekly for symptoms over 4 weeks. The plants were then tested for viruses using NCM-ELISA.

Serological detection of Sweet Potato Viruses using Nitrocellulose membrane-enzyme linked immunosorbent assay (NCM-ELISA)

NCM-ELISA is an immune-enzymatic test that uses nitrocellulose membrane instead of the polystyrene microtitration plates as a support for the reagents used in the serological reaction.

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The NCM-ELISA was used to test for sweet potato viruses. This work was done in conjunction with the Virology Department of CSIR-CRI, Fumesua. An NCM-ELISA kit for detecting 10 viruses was provided by the International Potato Centre (CIP) Lima, Peru.

Three leaves, one each from the top, middle and bottom parts of sweet potato vines, were selected from symptomatic and symptomless plants of each sweet potato accession. 90 plant leaves, 6 per accession were used. For each sample, a leaf area measuring 1 cm in diameter contained in a plastic bag was cut using a test tube. The leaf disks were ground with 3 ml of extraction buffer (Tris-buffered saline (TBS) pH 7.5 containing 0.2% sodium sulphite], they were then assayed for the presence of viruses as described by Gibb and Padovan (1993).

Only eighteen (18) out of the thirty (30) varieties were successfully grafted. They are as follows: CRI-Otoo, CRI-Bohye, CRI-Ligri, CRI-Histarch, CRI-Apomuden, OK/03/018, CRI-Ogyefo, Ex-Bunso/05, CRI-Patron, Ex-Bunso/09, CRI-Apomuden 2, Dos/03/017, OK/03/014, Blue-blue 3, Okumkom, Ex-Bunso/1, Santom-Pona and Purple Mepeasem.

These eighteen samples were tested for the presence or absence of the ten sweet potato viruses using the NCM-ELISA method.

Data Analysis

Data on the qualitative parameters (figure 5) were analyzed with Analysis of variance (ANOVA) using Genstat software version 12.1 (Nelder.,2017) and a dendrogram to show genetic relatedness was generated using Minitab v18. Molecular analysis of the 7 SSR markers were generated using PowerMarker version 3.5 and Molecular Evolutionary Genetics

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Analysis version 4 (MEGA4 (Tamura, Dudley, Nei & Kumar, 2007)) to determine genetic relatedness among the sweet potato accessions. Generalized Linear Models (Turner., 2008) statistical tool was used to analyze the viral disease incidence and the severity scores.

Data on yield parameters were subjected to one-way analysis of variance (ANOVA) to determine significant differences among the 30 sweet potato accessions. The means were separated by the least significant difference (LSD) method at 5% level of probability using GenStat Discovery version 4(VSN International). They were than analyzed using Correlation Analysis to establish the relationship between disease incidence and yield.

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CHAPTER FOUR

RESULTS

Morphological Characterization

The 20 descriptors evaluated suggest that variations existed among the sweet potato accessions (Table 3).

Qualitative traits evaluation

Twining ability was observed among the sweet potato accessions; 47 % of the sweet potato accessions had twinning ability, 40% moderately twinning and 13% did not possess this ability.

Growth Habit is basically the length of the main vines. Seventy three percent (73%) of the 30 accessions of sweet potato had the vines spreading and the others were erect and semi-erect. The estimated percentage ground cover indicated that 57 % of the sweet potato accessions recorded high (75-90%) ground cover, 27% had medium (50-74%), the rest had 10% and 6.6% total or entire (>90%) and low (<50%) ground cover respectively.

About 97% of the sweet potato accessions had green matured leaf colour and 3% green with purple veins on upper surface. However, for immature leaf color; 70 % of the accessions were green, 17 % were green with purple edged and 6 % were green with purple veins on upper surface and the others mostly purple. The predominant vine colours were mostly green with few purple spots (50%) and others were entirely green.

For vine tip pubescence (the degree of hairiness of immature leaves recorded at the apex of the vines), 60% of the sweet potato genotypes did not

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record any hairiness, 27% were sparsely and 7% were recorded for moderately and dense hairiness.

Leaf general outline (Figure 1) was such that majority of the sweet potato accessions were generally triangular, lobed, cordate (heart-shaped) and hastate recorded 73%, 17%, 7% and 3% respectively.

Shape of central leaf lobe (figure 3) - 60% of the sweet potato investigated had triangular shape of central leaf lobe, 30 % semi-circular and 10% lanceolate.

Petiole pigmentation varied such that 43% had green with purple near leaf, 40% had green, 10% had green with purple near stem and 7% had green with purple at both ends were observed.

Storage root shape (Figure 2) of the sweet potato accessions recorded 44.4% as round elliptic, 22.2% as elliptic, 16.6% as round elliptic and 16.7% as long elliptic.

The predominant storage roots skin and flesh colour (Figure 4) was cream recording 55%, 25% were dark orange, 10% were pale yellow, 5% were pale orange and 5% were strongly pigmented with anthocyanin.

Quantitative traits evaluation

The predominant numbers of lateral and central leaf lobes were observed on the leaves located in the middle section of the vine. The numbers of lobes recorded were 1, 3 and 5 and the percentage recordings 7%, 73% and 20% respectively.

Storage root cortex thickness (Table 5) of 308 cm was the highest recorded for CRI-Ogyefo and the least which was 11cm was recorded for Dos/03/017. Petiole length (Table 5) was highest in Dos/03/017(23.3 cm) and

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least (4.6 cm) in CRI-Dadanyuie. The average length recorded was between 14.0 cm and 18.0 cm.

A: Lobed shape

B: Triangular shape

*Figure 1:*Variations in the general leaf outlines

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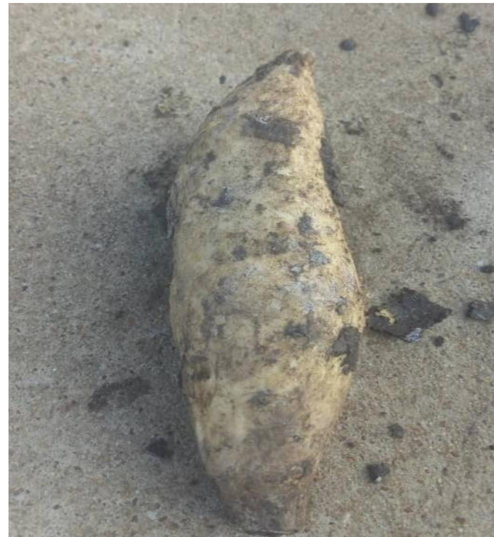
A: Round

B: Long elliptic



C: Ovate

Figure 2: Storage root shapes of sweet potato accessions

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A: Triangular

B: Toothed

Figure 3: Leaf shapes of sweet potato accessions.

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A: Purple root skin colour

B: Cream root skin colour

C: Flesh colour of the sweet potato accessions

Figure 4: Storage root skin and flesh color.

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Table 3: Morphological characters (qualitative) observed in the sweet potato accessions

Accessions	Twining	Growth habit	Ground cover	Vine internode Length	Predominant vine color	Vine tip pubescence	General leaf outline
Faara	0	3	5	Very short	2	0	Triangular
Sauti	0	5	5	Short	1	0	Lobed
Santom pona	0	7	3	Short	1	0	Cordate
CRI –Ogyefo	9	7	7	Short	2	0	Triangular
CRI – Apomuden	9	7	5	Short	4	0	Triangular
CRI – Otoo	5	7	7	Short	1	0	Triangular
CRI – Ligri	5	7	5	Short	2	0	Triangular
Purple mepeasem	5	7	7	Short	1	0	Lobed
Blue-blue 1	9	7	7	Very short	2	3	Hastate
Blue-blue 2	5	7	7	Very short	1	0	Triangular
Blue-blue 3	5	7	7	Very short	2	0	Lobed
CSP 1	5	7	7	Short	2	3	Lobed
CSP 2	5	7	7	Short	3	7	Triangular
Tech santom	9	7	7	Short	2	3	Triangular
Okumkom	9	7	5	Intermediate	2	0	Triangular
Ex- Bunso-04	9	7	7	Intermediate	1	3	Triangular
CRI- Dadanyuie	5	5	3	Very short	2	0	Triangular
CRI-Bohye	0	5	5	Very short	1	0	Triangular
CRI-Histarch	9	7	5	Short	2	0	Lobed
Apomuden local	0	7	7	Short	2	0	Triangular
Ex-Bunso/01	9	7	7	intermediate	1	0	Cordate
Bot/03/030	9	7	7	intermediate	2	3	Triangular
Tag/03/019	5	7	7	Short	1	3	Triangular
Ex-Bunso/02	5	7	7	Short	2	7	Triangular
Ok/03/018	9	7	7	Short	2	3	Triangular
Ok/03/014	9	9	9	intermediate	3	7	Triangular
Dos/03/017	9	7	7	intermediate	1	0	Triangular
CRI-Patron	5	5	5	Very short	2	0	Triangular
Ex-Bunso/03	5	9	9	Short	1	7	Triangular
Ex-Bunso/05	9	9	9	Short	1	7	Trianguar

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Table 4: Morphological characters (qualitative) observed in the sweet potato accessions cont'd

Accessions	Leaf lobe number	Central leaf lobe shape	Mature leaf size	Mature leaf color	Immature leaf color	Petiole length	Petiole pigmentation
Faara	3	3	Medium	2	2	short	3
Sauti	5	6	Medium	2	2	short	1
Santom pona	1	3	Medium	2	2	Very short	1
CRI –Ogyefo	5	3	Medium	2	2	short	1
CRI – Apomuden	3	3	Medium	2	7	short	3
CRI – Otoo	3	2	Medium	2	2	short	1
CRI – Ligri	5	6	Large	2	3	short	3
Purple Mepeasem	3	2	Medium	2	2	short	3
Blue-blue 1	3	2	Medium	2	2	short	3
Blue-blue 2	5	2	Large	2	2	short	2
Blue-blue 3	5	2	Medium	2	3	short	2
CSP 1	3	2	Medium	2	2	short	3
CSP 2	3	2	Medium	2	2	short	3
Tech santom	3	3	Medium	2	7	short	3
Okumkom	3	3	Medium	2	2	short	1
Ex- Bunso-04	3	3	Small	2	5	short	1
CRI- Dadanyuie	3	3	Medium	5	5	Very short	4
CRI-Bohye	5	6	Medium	2	2	short	1
CRI-Histarch	3	3	Medium	2	2	short	1
Apomuden local	1	2	Small	2	3	short	1
Ex-Bunso/01	3	2	Medium	2	2	short	2
Bot/03/030	3	2	Medium	2	2	short	3
Tag/03/019	3	2	Medium	2	2	short	3
Ex-Bunso/02	3	2	Medium	2	2	short	3
Ok/03/018	3	2	Medium	2	2	short	3
Ok/03/014	3	2	Medium	2	2	short	3
Dos/03/017	3	2	Medium	2	2	intermediate	1
CRI-Patron	3	2	Medium	2	3	short	4
Ex-Bunso/03	3	2	Medium	2	2	short	1
Ex-Bunso/05	3	2	Medium	2	3	intermediate	1

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Table 5: Morphological characters (quantitative) observed in the sweet potato accession

Accessions	Storage root weight (kg)	Storage root cortex thickness (cm)	Storage root shape	Storage root skin & flesh color	Numb er of storage root
Purple Mepeasem	0.038	21.8	2	8	5
CSP 2	0.301	21.4	2	2	1
CRI – Otoo	0.497	22.6	8	2	1
Apomuden(Local)	0.386	21.3	8	4	5
Blue Blue 3	0.386	21.9	2	2	5
Santom Pona	0.324	22.5	2	2	3
CRI – Ogyefo	0.804	308	4	8	1
CRI – Bohye	0.37	24.9	1	2	3
Sauti	0.199	15	8	2	1
CRI – Apomuden	0.895	34.6	4	4	1
Bot/03/030	0.457	26	3	8	1
Okumkom	0.647	35.6	1	8	3
CRI – Patron	0.312	20.7	2	2	4
Blue Blue 1	0.496	18.8	3	2	5
Tech Santom	0.288	18.5	2	2	4
CSP 1	0.479	24.4	2	2	4
Blue Blue 2	0.629	26.7	2	2	4
CRI – Ligri	0.858	39.4	1	6	5
DOS/03/017	0.065	11	3	8	3
EX - Bunso 01	0.049	10.2	3	9	5
CRI-Dadanyuie	0	0	0	0	0
Ex/Bunso-05	0	0	0	0	0
CRI-Histarch	0	0	0	0	0
OK/03/013	0	0	0	0	0
Ex-Bunso/04	0	0	0	0	0
Ex-Bunso/02	0	0	0	0	0
Ex-Bunso/03	0	0	0	0	0
Tag/03/019	0	0	0	0	0
Ok/03/014	0	0	0	0	0
Faara	0	0	0	0	0

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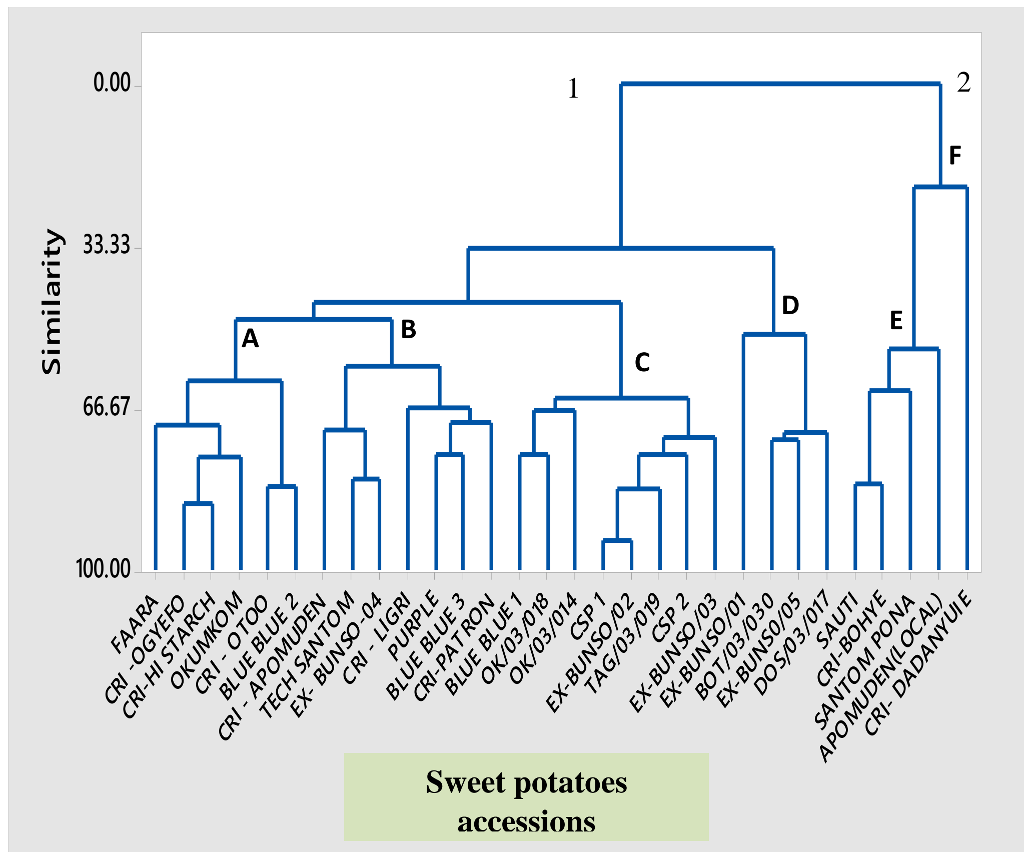

Figure 5: Dendrogram showing relationship among 30 sweet potato accessions

Cluster analysis

The results of cluster analysis constructed using Neils method based on the 14 qualitative morphological parameters are presented in Figure 5. The sweet potato accessions were grouped into 2 major clusters. Cluster 1 consists of 4 subclusters (A, B, C and D), and cluster 2 consist of 2 subclusters (E and F). The dendrogram suggests that variations exist among the sweet potato accessions. The morphological traits including, twining, growth habit, ground cover, vine internode length, predominant vine colour, general leaf outline, leaf lobe number, petiole length, petiole pigmentation, mature and immature leaf colour discriminated the 30 sweet potato accessions into two main clusters and 6 sub-clusters (A, B,C,D, E and F) at 50% similarity level. Cluster 'D'

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shows a high propensity of sweet potato accessions coming from the same source (Plant Genetic Resource Research Institute PGRRI).

Molecular Characterization

Out of the 21 SSR primer pairs used to assess genetic diversity among the 30 sweet potato accessions, only 7 showed polymorphism across the genome and therefore were used for the analysis. Table 6 shows the primers and their annealing temperatures used for the analysis.

The polymorphic SSR primers selected for the analysis generated a total of 70 scorable fragments.

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Table 6: SSR Primers used and their annealing temperatures

Primer	Forward	Reverse	Annealing temperature (°C)
SSR09	AAGTTAATCTAAGGTGGCGGGG	CGTCGATTCCAGTCTAATCCAATCC	63
SSR07	TTTTCAACGACAAGCCTCTTGC	TCAAAGGTCCGCATGGAAATC	58
IBC5	CCACAAAAATCCCAGTCAACA	AGTGGTCGTCGACGTAGGTT	63
690524	AAGGAAGGGCTAGTGGAGAAGGTC	CAAGGCAACAAATACACACACACG	63
IBR16	GACTTCCTTGGTGTAGTTGC	AGGGTTAAGCGGGAGACT	58
IBR19	GGCTAGTGGAGAAGGTCAA	AGAAGTAGAACTCCGTCACC	58
IB242	GCGGAACGGACGAGAAAA	ATGGCAGAGTGAAAATGGAACA	50

Source; Buteler et al., 1999, Karuri et al 2010, Gwandu et al 1995, Acheampong 2012

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The dendogram (Figure 6) illustrates the combined data obtained from the 7 polymorphic primers which grouped the genome of the thirty (30) sweet potato accessions into two major clusters, with similarity coefficients that ranged from 0 to 20%. Cluster 1 only consist of one sweet potato accession CSP1, while the other remaining 29 sweet potato accessions made up cluster 2.

Allele frequency ranged from 0.8000 (IB-R16) to 0.8880 (SSR07) with a mean of 0.8600. Polymorphic information content (PIC) which are used to assess the allele diversity at a specific locus varied from 0.1773 (SSR07) to 0.2688 (IB-R16) with mean of 0.2084. Gene diversity ranged from 0.197 in primer SSR-07 to 0.3200 in primer IB-R16 with the average of 0.2381 as shown in Table 7.

PCR products ranged between 75 bp and 300 bp in size. The 7 SSR markers could not effectively discriminate some of the sweet potato accessions.

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Table 7: Allele frequency, gene diversity and polymorphic information content from 30 sweet potato accessions based on 7 SSR markers

Primer name	Major Allele Frequency	Sample Size	Number of Observations.	Number of Allele	Gene Diversity	PIC
SSR09	0.8334	30.0000	30.000	2.0000	0.27555	0.2366
SSR07	0.8889	30.0000	30.000	2.0000	0.1970	0.17733
IBC5	0.8334	30.0000	30.000	2.0000	0.2756	0.2366
690524	0.8333	30.0000	30.000	2.0000	0.2778	0.2392
IBR16	0.8000	30.0000	30.000	2.0000	0.3200	0.2688
IBR19	0.8778	30.0000	30.000	2.0000	0.2126	0.1900
IB242	0.8778	30.0000	30.00	2.0000	0.2140	0.1910
Mean	0.8600	30.0000	30.000	2.0000	0.2381	0.2084

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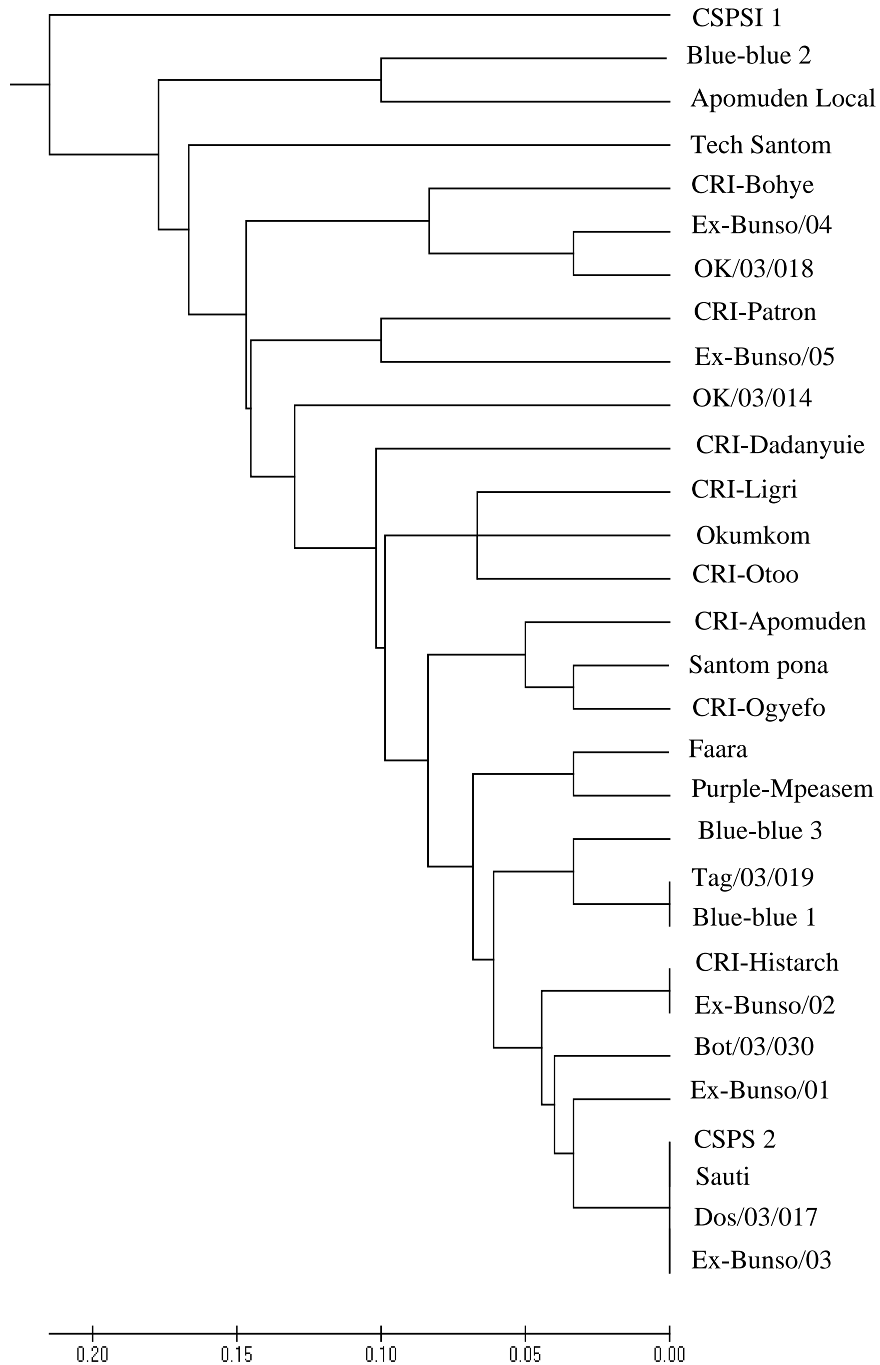

Figure 6: A dendrogram illustrating the relatedness among 30 sweet potato accessions generated using seven polymorphic SSR markers and the sequential clustering algorithm (UPGMA) based on genetic similarity

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Evaluation of Yield, Virus Disease Incidence and Disease Severity score

Yield evaluation

Total mean yield varied between 148.1 kg/ha and 27259.3 kg/ha among the sweet potato accessions across three (3) seasons, with accession Ex-Bunso/04 recording the least and accession CRI-Apomuden recording the highest.

In season 1 (Figure 7) almost 50% of the accessions did not form any storage root examples are Faara, Sauti, Blue-blue 3, CSP 1 and Purple Mpeasem did not produce any marketable yield. However, accessions such as CRI-Apomuden, Okumkom, Apomuden local and CRI-Ligri had very storage root yield, recording 38666 kg/ha, 25185 kg/ha, 25037 kg/ha and 20444 kg/ha respectively.

In season 2 (Figure 8) very low yielding accessions in season 1 recorded high yield. For example, is Purple Mpeasem which did not record any yield at all in season 1 recorded the highest with 41481.5 kg/ha. Also, some accessions which recorded good yield in season 1 could not produce any marketable yield in season 2. Accession Tag/03/019 recorded 18666.7 kg/ha in season 1 did not record any yield in season 2. Accessions such as Ok/03/014, Dos/03/017, CRI-Histarch and Ex-Bunso/04 did not record any yield at all.

In season 3 (Figure 9), CRI-Apomuden, Apomuden local and Purple Mpeasem recorded 22666.7 kg/ha each as the highest yield of the storage root, whereas accessions Ex-Bunso/01 and Ex-Bunso/04 recorded the least of 444.4 kg/ha.

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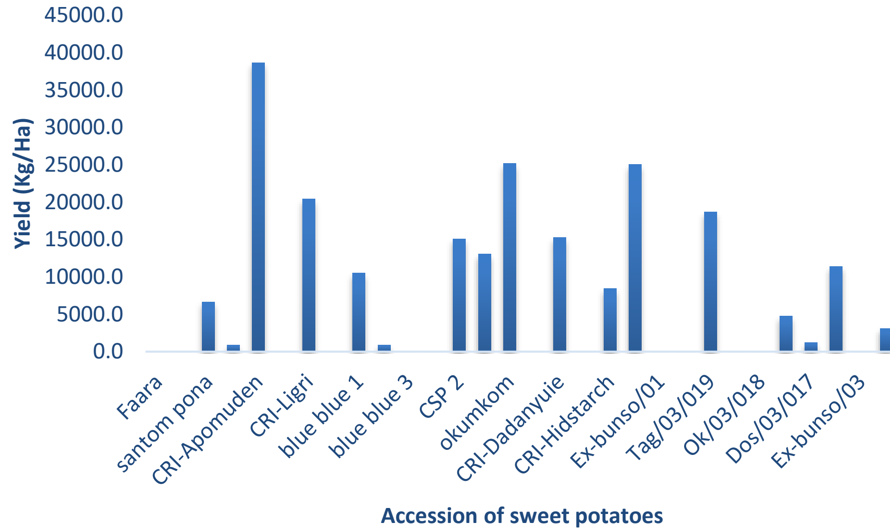

Figure 7: The yield of sweet potatoes in the 1st season (July-October 2016)

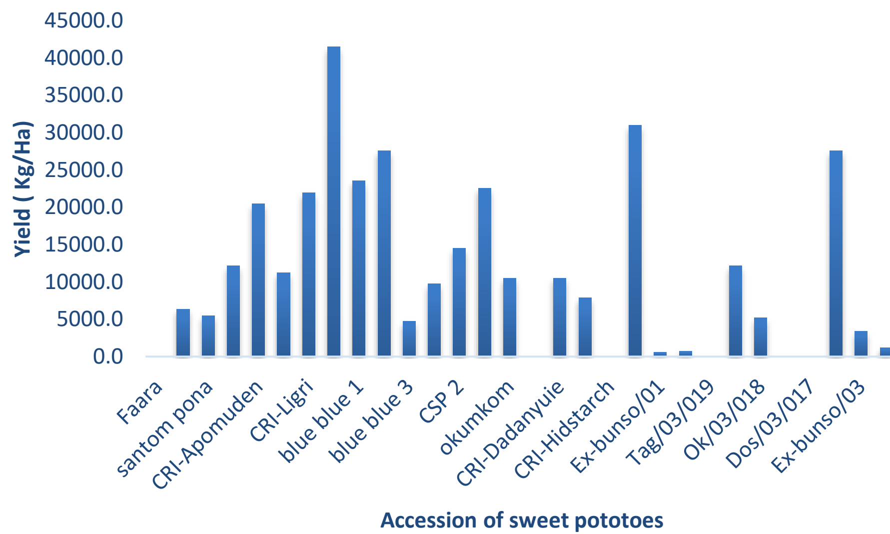

Figure 8: The yield of sweet potatoes in the 2nd season (June –October 2017)

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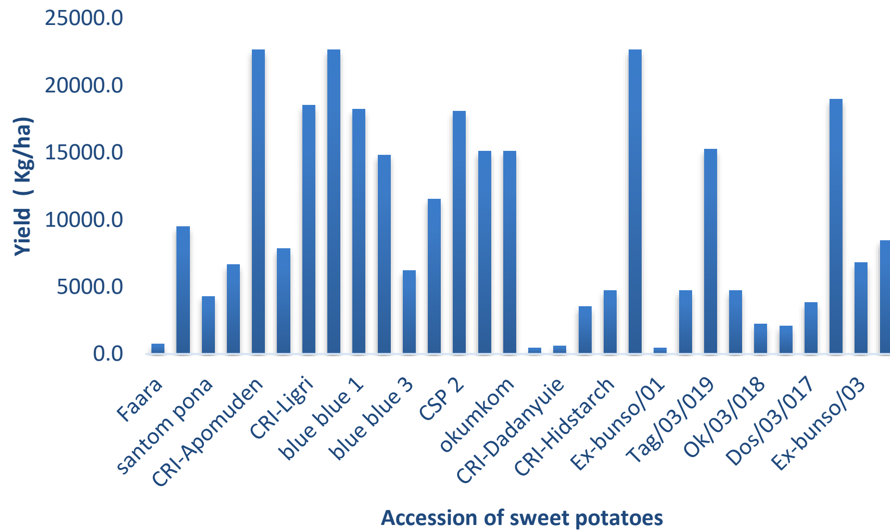

Figure 9: The yield of sweet potatoes in the 3rd season (July –November 2018)

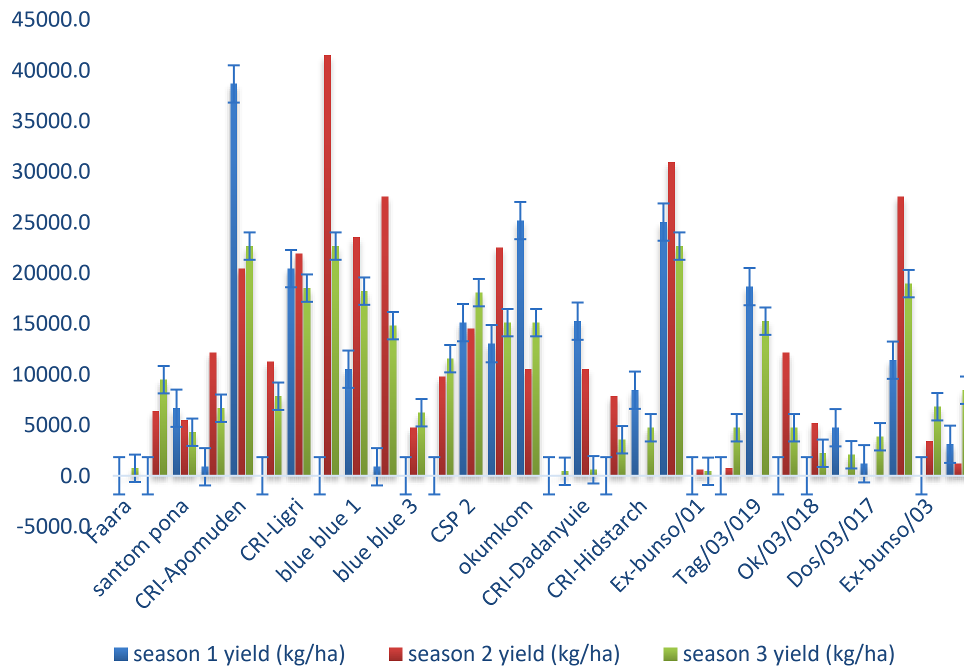

Figure 10: The yield of Sweet potatoes across the three planting seasons.

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Virus disease Incidence (DI) on sweet potato accessions on the field.

Viral disease incidence (Table 10), varied from 0 to 90% indicating no to high disease incidence. At 6 WAP, Ok/03/014 and CRI-Patron recorded 26.67% indicating mild virus infection across the accessions; Faara did not record any viral disease symptoms. At the 8 WAP Dos/03/017 and Cri-Otoo recorded 56.7% each, indicating moderate incidence. Ex-Bunso/02 recorded the least incidence with 30% at the week under study.

At the 10 WAP after planting Cri-Histarch had 70% viral disease incidence indicating severe incidence; majority of the accessions had moderate incidence averagely recording 56%. At 12WAP week Ex-Bunso and Cri-Ligri each recorded 90% DI, indicating very severe.

In spite of these varied observations, the mean DI of 50.3% was recorded for all accessions during the second (2nd) season of planting. Sweet potato virus disease symptoms were less variable. Generally, they comprised of leaf yellowing and at times generalized mosaic and or plant stunting. Figure 11 shows the various disease symptoms observed on the sweet potato accessions.

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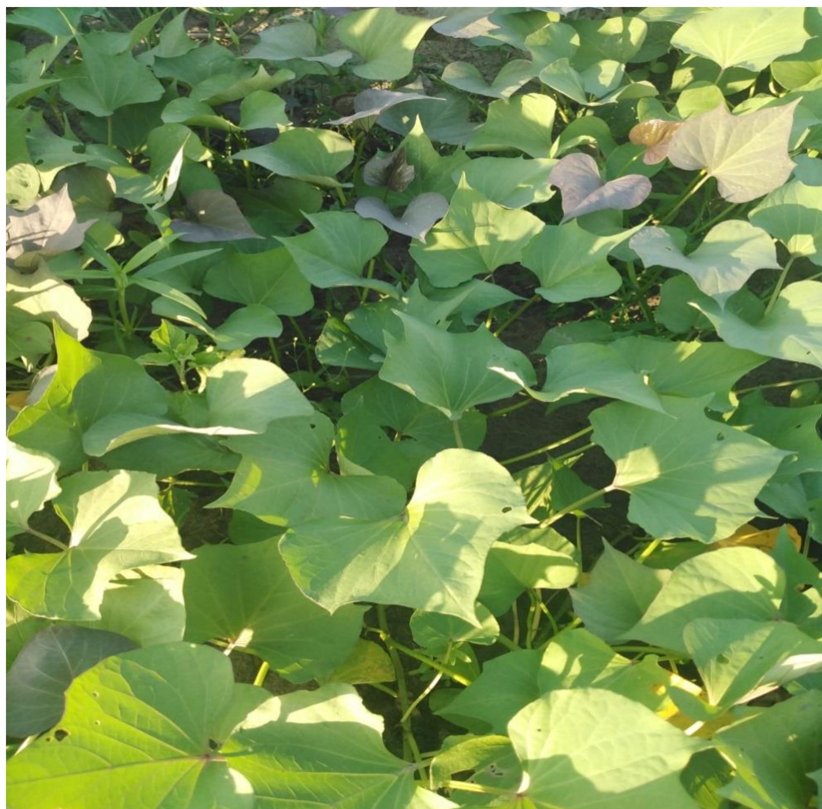

A

B

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C

Figure 11: Viral disease symtoms observed on the open field. **A** shows no symptoms, **B** shows severe purpling and blotches and **C** shows yellowing of the leaf and stunt growth

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Table 8: Disease incidence of viral disease recorded for 30 sweet potato accessions between 6WAP and 12WAP in percentages for 2nd season of planting

Accessions	Disease incidence in percentages			
	6 WAP	8 WAP	10 WAP	12 WAP
Faara	0	46.67	53.33	84.61
CRI-Apomuden	16.67	43.33	56.67	88
Okumkom	40	50	56.67	76
CRI-Patron	26.67	36.67	56.67	76
Tech santom	13.33	50	56.67	76.67
CRI-Dadanyuie	20	46.67	53.33	80
Blue-blue 3	16.67	46.67	60	70
Dos/03/017	16.67	56.67	66.67	73.33
CSP 1	16.67	43.33	56.67	66.67
Blue-blue 1	20	43.33	66.67	76.67
CSP 2	20	40	53.33	70
Ex-Bunso/01	16.67	40	63.33	70
Sauti	13.33	46.67	56.67	83.33
Apomuden local	16.67	46.67	63.33	76.67
Blue-blue 2	20	40	60	83.33
CRI-Histarch	23.33	40	70	86.67
Ex-Bunso/05	20	36.67	66.67	80
Ok/03/018	26.67	36.67	56.67	86.67
Ex-Bunso/04	23.33	46.67	60	83.33
Bot/03/030	23.33	43.33	60	83.33
CRI-Ogyefo	23.33	40	53.33	80
Purple Mepeasem	23.33	33.33	53.33	66.67
CRI-Otoo	20	56.67	60	86.67
Santom pona	23.33	40	60	70
Ex-Bunso/02	20	30	63.33	80
Ex-Bunso/03	23.33	36.67	50	80
Tag/03/019	26.67	46.67	53.33	83.33
CRI-Ligri	23.33	40	46.67	90
CRI-Bohye	23.33	43.33	60	90
Ok/03/014	26.67	46.67	50	80

Virus disease severity

At 6WAP there was no significant difference ($P > 0.05$) in disease incidence among the sweet potato accessions. Faara recorded 0.004(Table 11) which is the least meaning there was approximately no viral infection. Week 8 after planting was no different with the p-value of 0.997. Ok/03/014 recorded 0.538 as the least while CRI-Bohye and Dos/03/017 recording the highest with

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0.905 and 0.860 respectively. The disease increased with time as 12 WAP diseases were recorded high across the accessions (Table 11).

ANOVA did not show any significant difference in the mean symptom severity among the sweet potato genotypes in week 6 (s.e.d=0.1483, p=0.848), week 8 (s.e.d 0.2110, p=0.997) and week 10 (s.e.d=0.996, p=0.2819). However, at week 12, there was a significant difference among the sweet potato accessions (s.e.d=0.3856, p=0.023). Table 11 shows the disease severity of the various sweet potato accessions.

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Table 9: Mean disease severity of the sweet potato accessions for 2nd season

Accessions	Disease Severity			
	Week 6	Week 8	Week 10	Week 12
CSP 1	0.206	0.723	1.103	2.213
Apomuden local	0.275	0.585	1.034	2.304
Blue -blue 1	0.275	0.688	1.103	2.260
Blue-blue 2	0.309	0.585	1.068	2.144
Blue-blue 3	0.275	0.723	1.068	2.090
Bot/03/030	0.309	0.757	1.068	2.221
CRI-Apomuden	0.219	0.566	0.868	1.956
CRI-Bohye	0.337	0.905	1.203	3.176
CRI-Dadanyuie	0.240	0.723	1.068	2.271
CRI-Histarch	0.275	0.585	1.344	2.229
CRI-Ligri	0.275	0.654	1.034	2.762
CRI-Ogyefo	0.171	0.792	1.137	2.347
CRI-Otoo	0.240	0.998	0.999	2.378
CRI-Patron	0.275	0.654	1.241	2.049
CSPS 2	0.275	0.585	0.930	2.087
Dos/03/017	0.171	0.860	0.896	1.793
Ex-Bunso/01	0.275	0.688	0.965	2.084
Ex-Bunso/02	0.240	0.585	1.068	2.572
Ex-Bunso/03	0.263	0.634	1.299	3.103
Ex-Bunso/04	0.240	0.619	1.068	2.638
Ex-Bunso/05	0.240	0.619	1.172	2.394
Faara	0.004	0.638	0.870	2.104
Ok/03/014	0.337	0.538	1.136	2.995
Ok/03/018	0.27	0.688	1.068	2.477
Okumkom	0.504	0.771	0.903	2.209
Purple Mepeasem	0.344	0.757	1.034	2.407
Santom pona	0.412	0.723	1.137	2.414
Sauti	0.309	0.585	0.758	2.320
Tag/03/019	0.275	0.688	1.102	2.852
Tech-santom	0.171	0.723	1.034	2.138
p-values	**0.848**	**0.997**	**0.996**	**0.023**
s.e.d	**0.1483**	**0.2110**	**0.2819**	**0.3856**

Correlation of sweet potato accessions yield (2nd season) to sweet potato virus disease incidence

The correlation between yield (2nd season) and sweet potato virus diseases was negatively correlated and insignificant.

Pearson correlation of yield and Incidence = -0.120

P-Value = 0.528

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Identification of SPVD using Nitrocellulose membrane-enzyme linked immunosorbent assay (NCM-ELISA)

From the NCM-ELISA results, only three (3) viruses were detected, namely, *sweet potato feathery mottle virus* (SPFMV), *sweetpotato collusive virus (SPCV)* and *sweetpotato chlorotic stunt virus* (SPCSV) even though all the 10 viruses of sweetpotato were tested. Among the eighteen samples, Blue-blue 3 did not test positive for any of the viruses even though it exhibited some SPVD symptoms like vein clearing, necrotic spots and leaf reduction.

There were five single infections of SPFMV in Dos/03/017, OK/03/014, Ex-Bunso/01, Cri-Bohye and Santom-Pona and one single infection of SPCSV in CRI-Apomuden 2 (Table 12).

Co-infections of SPFMV and SPCV were also recorded in three sweet potato accessions (Table 12). Co- infections SPFMV and SPCSV were also seen in CRI-Otoo and OK/03/018. SPCV and SPCSV co-infections were found on CRI-Ligri, CRI-Ogyefo and Okumkom.

Mixed infections of all the three viruses that were detected were also recorded in CRI-Apomuden, CRI-Patron and Ex-Bunso/09. Depending on the type of the virus present, ELISA reaction gave a weak to very strong purple colour.

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Table 9: NCM-ELISA detection of SPVD among sweet potato accessions

Accessions	SPFMV	SPCV	SPCSV
CRI-Otoo	+	-	+
CRI-Bohye	+	-	-
CRI-Ligri	-	+	+
CRI-Histarch	-	+	-
CRI-Apomuden	+	+	+
Ok/03/018	+	-	+
CRI-Ogyefo	-	+	+
Ex-Bunso/05	-	+	-
CRI-Patron	+	+	+
Ex-Bunso/09	+	+	+
Apomuden Local	-	-	+
Dos/03/017	+	-	-
Ok/03/014	+	-	-
Blue-blue 3	-	-	-
Okumkom	-	+	+
Ex-Bunso/01	+	-	-
Santom Pona	+	-	-
Purple Mepeasem	-	+	-

+ denotes presence of virus; - denotes absence of virus

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CHAPTER FIVE

DISCUSSIONS

Morphological Characterization

The rational for management of plant genetic resources for conservation and breeding purposes involves characterization of germplasm collections. The identities of the 30 genotypes were established using morphological or phenotypic characteristics as described in the sweet potato descriptors by CIP, AVRDC, IBPGR (1991). Both qualitative and quantitative parameters studied in the research showed variations which were evident among the 30 accessions of sweet potato. Morphological characters were highly variable among the sweet potato accessions studied.

Descriptors such as storage root color, twining, vine tip pubescence, mature and immature leaf color showed variations among the sweet potato accessions. The high variability in morphological traits among the 30 sweet potato accessions are consistent with reports by Elameen et al.(2011), Yada et al. (2010), Vimilar & Hariprakash (2010), Tsegaye, Sastry, & Dechassa (2007) and Veasey et al. (2007) who recorded variations in vine internode length, PTY, GOL, LLT, LLN and SRA in sweet potato.

The clustering pattern of the 30 sweet potato accessions in relation to the phenotypic morphological descriptors are consistent. The presence of numerous intermediates in the storage root clearly reveals incomplete dominance as well as the occurrence of multiple alleles for this trait. Accessions CSP1 and Ex/Bunson-02 are so closely related with 96.5% similarity among them, followed by CRI-Ogyefo and CRI-Histarch with 84%

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similarity. Accessions Faara and CRI-Dadanyuie are the most dissimilar with 27% similarity.

Molecular Characterization

Conservation and sustainable use of genetic resources is essential to meet the demand for the future food security. Successful conservation of any given gene pool is largely dependent on understanding the diversity and its distribution in a given region (Zhang et al.,1999).

In this study the genetic diversity observed among the sweet potato was low to the extent that some sweet potato accessions could not be distinguished and appeared as duplications. The dengrogram (figure 6), grouped the sweet potato accessions into 2 major clusters at 19% co-efficient of dissimilarity. Accessions CSP 2, Sauti, Dos/03/017, Ex/Bunso/03 and accessions Tag/03/019, Blue- blue 1 are the most closely related accessions with 4% co-efficient of dissimilarity. They could be duplicates, presence of duplicates could be due to the movement of planting materials across locations as well as difference in languages and ethnic groups (Yada et al., 2010).). Duplicates are identical genotypes which have different names at different places due to language difference. The duplicates identified using the SSR markers may have the same or almost the same morphology.

Gene diversity shows the diversity among accessions of a plant, the higher the gene diversity the higher the diversity among the accessions. Primers SSR09, IBC5 and IBR16 each showed gene diversity of 0.3200. The reason for the low diversity could be attributed to narrow geographic zone of collection of the sweet potato accessions or narrow genetic base of the crop.

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Low PIC values (0.1773 – 0.2688) observed in this study with mean of 0.2084 indicate that there is high genetic similarity among the sweet potato accessions. The 7 SSR markers used in the study could not effectively discriminate between the genotypes. This presupposes that the 7 SSR markers selected from 24 SSR markers reported to be informative markers for sweet potato did not work perfectly in this study. Limited number of SSR markers and null amplification of some of the accessions could be the major reasons for the observation in the current study.

The weak correlation between the morphological based dendrogram and the SSR based was confirmed by the different duplicates identified. Earlier studies have reported low correlation between morphological and molecular markers in many crops (Koehler-Santos, DornellesAL & de Freitas 2003; Ferriol, Pico, Fernandez & Nuez 2004). The suggested reasons were that it could be a result of the independent nature of morphological and molecular variations.

Evaluation of Yield, Virus Disease Incidence and Severity

Yield

The mean yield across the three planting seasons ranged from 148.1 kg/ha and 27259.3 kg/ha.

In season 1 almost 50% of the accessions did not produce any storage root thus recording zero yield. There was a change in seasons 2 and 3 when majority of the accessions recorded yield. According to Villordon et al. (2009) the quantity of yield depends on the number of fibrous roots induced to form storage root which can result in high yield. There was high variability

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among the various sweet potato accessions, for instance the mean bulk weight across the three planting seasons, for accessions CRI-Apomuden, Apomuden local and Purple Mpeasem which recorded 27259 kg/ha, 26222 kg/ha and 21382.7 kg/ha respectively being the highest compared to Ex-Bunso/04 and Ex-Bunso/01 which recorded the least 148 kg/ha and 345.6 kg/ha respectively.

The majority of the accessions which recorded least numbers of storage roots have their original source from outside Cape Coast Metropolis. Their inability to produce any storage root over the three (3) planting seasons could be due to virus concentration (60-90%) at the planting location and possibly unfavorable environmental conditions at the planting location.

Virus disease incidence and severity

Sweet potato viral infection symptoms were observed in all the accessions at different time intervals after planting. There was no record of immune cultivars to sweet potato viral infections (Gasura & Mukasa, 2010). The virus disease symptoms observed were similar to those commonly known to occur in diseased sweet potato plants grown elsewhere (Tairo, Kullaya & Valkonen, (2004)). Major symptoms observed during the study were venial chlorosis, leaf mosaic, leaf mottling, leaf curling and purpling. These observations were in agreement with the observation made by Tairo et al. (2004).

At 6WAP the accession Faara recorded no viral symptoms in this study, whiles the accession Okumkom recorded the highest symptoms. The low incidence of disease in Faara but rather high DI in the same accession at 12WAP could propabably be due to insect feeding, virus multiplication or

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multiple virus infection as also indicated by earlier studies by Ateka et al, (2004): Karyeija, Kreuze, Gibson & Valkonen (2000). At 12WAP CRI-Ligri and CRI-Bohye recorded 90% infection associated with the SPVD symptoms, while CSPS 1 recorded the least of 66.6% of plant affected with SPVD.

The disease incidence increased from the 6th week to the 12th week with all accessions showing symptoms of virus except Faara which showed no symptoms at the 6th week. Most accessions which showed mild and moderate disease incidence increased to severe disease indicating that they were susceptible to virus infection with time. .

After the GLM predictions of incidence of viral diseases, the p-values over the weeks of study did not show any significant positive results, they were 0.551, 0.995, 0.990 and 0.735 for weeks 6, 8, 10 and 12 respectively.

Viral disease incidence varied from 0 to 90% across the sweet potato accessions. This could be due to several factors such as virus concentration in the planting materials and the genetic constitution of the individual accessions. The mean DI and the index of symptoms severity appear to have been influenced by differential symptom expression of the various accessions in earlier studies (Mukasa, Rubaihayo, & Valkonen, 2003). This research has indicated that disease symptoms expression is influenced by many factors including virus species or strain types, host response, type of infection (primary or secondary), plant age at infection, insect feeding and environmental conditions. The disease symptoms were sometimes absent on young leaves of accessions after the 12th week suggesting that these accessions were able to recover from the virus infection at some periods in their developmental stages. This can be due to the plant cell defense mechanisms

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against diseases known as hypersensitivity resistance and/or systematic acquired resistance.

This study did not identify accessions with complete immunity based on the visual assessment. On the whole, viral disease incidence and severity increased over the weeks under study, this could be due to temperature changes as it was getting closer to the dry season. Higher temperatures might have favored the rapid development of aphid vectors and hence increases the chances of transmitting viral diseases in the sweet potato genotypes.

Correlation of sweet potato yield (2nd season) to sweet potato disease incidence

It was evident during this study that there was variation in the disease incidence and severity among the sweet potato accessions. Constraints like biotic factors which include viruses interfere with physiological function(s) of the plant which leads to the different symptoms and these alterations cause a reduction in yield and quality of storage roots produced.

From the result of the Pearson Correlation between yield and disease incidence shows inverse correlation between yield and sweet potato disease incidence. Though as the yield increase virus disease incidence also increases, but it is insignificant because of the pvalue of 0.773.

Though the incidence of the viral disease increased as the plant aged in accession CRI-Apomuden, it had yield of 20444.4 kg/ha with incidence of 88%.

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Virus Detection Using Nitrocellulose membrane-enzyme linked immunosorbent assay (NCM-ELISA)

During the evaluation there was high incidence and severity of virus symptoms among the sweet potato accessions and this equally reflected in the serological test.

Out of the total 30 sweet potato accessions used for the study, 18 accessions became successful after grafting inoculation with the *I. setosa.* Sweet potato virus disease (SPVD) was observed in all the sweet potato accessions. Only three (3) viruses were detected, SPFMV, SPCV and SPCSV even though all the 10 viruses of sweet potato were tested for. In this study SPFMV was the most commonly detected during the study, confirming its widespread occurrence wherever sweet potato is grown (Loebenstein, Thottappilly, Fuentes & Cohen 2009; Ndunguru & Kapinga, 2007). The sweetpotato accessions infected with only SPFMV either did not show symptoms at all, or produced only mild symptoms of vein clearing and chlorotic spots. The low occurrence of SPCSV in this study contradicts reports of its wide distribution in West and East Africa (Ndunguru et al.,2009: Carey et al.,1999a). The low incidence may be explained by the fact that its vector is not so efficient to spread it, or that local accessions are genotypes having a natural resistance to it.

There were co-infections of SPFMV and SPCV, SPFMV and SPCSV and co-infection of SPCV and SPCSV. Mixed infections of all the three viruses were detected in CRI-Apomuden, CRI-Patron and Ex-Bunso/09. The lack of detection of other viruses such as SPLV and SPC6V which are widely distributed, by NCM from sweet potato plants or grafted *I. setosa* could be due

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to their absence on tested accessions or low concentrations in the leaf samples and/or because they are serologically distinct virus strains (Tairo, Jones & Valkonen, 2006: Mukasa, Rubaihayo & Valkonen,2003).

Symptomless infection of sweet potato plants has been reported (Clark & Hoy, 2006; Loktrakul, Valverde, Clark & De La Torre1998). Since antibodies to detect SPLCV are not available, the virus has primarily been detected by PCR (Li, Salih & Hurtt, 2004). Even though this study did not identify genotypes with complete immunity based on visual assessment, Blue-blue 3 did not record any viral infection in the serological test

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CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

Sweet potato is a vital stable food crop for many communities in the developing world. Unfortunately, the crop is underexploited compared to other crops despite its contributions. Virus diseases have been identified as one of the major constraints to sweet potato production. Characterization of the sweet potato virus diseases is the first step to identifying resistant genotypes.

There was some evidence of genetic variability among the 30 accessions of sweet potato studied based on the agro-morphological characters evaluated. However, accessions Ex/Bunso-02 and CSP1 are so closely related in morphology and genetic makeup. Total root yield of the sweet potato accessions, CRI-Apomuden, Apomuden local and Purple Mpeasem were the highest.

There was weak correlation between the sweet potato accessions yield and disease incidence. The viral disease incidence had no significant effect on the sweet potato yield.

Using symptomatology, biological indexing, nitrocellulose membrane ELISA (NCM-ELISA), the sweet potato accessions were assessed for disease incidence and symptom severity. Symptom expression varied among the accessions from mild to severe. At week 6 after planting all accessions except Faara showed a degree of viral symptoms. The viral symptoms increased over the weeks after planting, with the most severe symptoms being expressed at week 12 after planting. Mean disease incidence varied significantly ($p < 0.05$)

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among the sweet potato accessions, ranging from 10% to 90% with an average of 50.6%. Using NCM-ELISA, out of the 30 sweet potato accessions only 18 were successfully indexed. Three (3) viruses were detected, namely, *sweet potato feathery mottle virus* (SPFMV), *sweet potato collusive virus* (SPCV) and *sweet potato chlorotic stunt virus* (SPCSV). out of the successfully indexed sweet potato accessions 20% tested positive to SPFMV single infection, 12% to SPCV single, 4% positive to single infection of SPCSV, There were 20% co-infections of SPFMV and SPCSV, 24% to co-infections of SPFMV and SPCV, 20% of the accessions tested positive to co-infections of SPCV and SPCSV, 8% of the successfully indexed sweet potato accessions tested positive to co-infections of SPFMV and SPCV, and 12% tested positive to all the three viruses present. One accession that is Blue –blue 3 did not test positive to any of the viruses present though it showed severe symptoms on the field.

This work emphasizes the importance of selecting resistant cultivars as planting materials and the enforcement of quarantine measures to minimize the introduction of viruses into Ghana and their subsequent spread.

Conclusions

Phenotypic variations among the sweet potato accessions suggest little allelic variations within the genome of sweet potato accessions.The cluster analysis showed characters contributing differently to the total phenotypic variability. Key component traits contributing to total root yield include number of storage root, bulk weight and storage root weight. Based on both morphological and molecular dendrograms (figures 5 and 6), accessions such

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as CSP1, Ex/Bunso/02, Sauti, CSP2 are so closely related thus indicating higher level of similarity

The low mean gene diversity of 0.2381 and PIC of 0.2084 suggests narrow genetic base across the genomes of the 30 sweet potato accessions. In all CSPS 2, Dos/03/017, Ex-Bunso/03 and Sauti could not be distinguished based on the 7 SSR markers.

In this study, viral diseases symptoms observed using visual examination showed that all the sweet potato genotypes, independent of their origin expressed virus associated symptoms indicating susceptibility to SPVD. The use of the serological test, NCM-ELISA was found to be an effective tool for virus detection as it revealed 3 viruses (SPFMV, SPCV and SPCSV) infecting the sweet potato accessions. Co-infections of SPFMV and SPCV, SPFMV and SPCSV and SPCV and SPCSV were observed. Mixed infections of all the three viruses were detected. No virus was detected in association with Blue-blue 3.

Recommendations

1. Information on distribution and importance of sweet potato viruses across the country will help sweet potato breeders and policy makers to determine the appropriate strategies for disease control through breeding and development of seed systems.
2. Gene Banks should give more attention in the maintenance of seed quality and virus-free foundation seed stocks and continuous selection of resistant varieties.

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3. Based on the NCM-ELISA as response to virus infection, genotype Blue-blue 3 is recommended sweet potato breeders for use in breeding work since no virus was detected in it.
4. Department of Molecular Biology and Biotechnology should carry out genetic diversity studies on the three viruses detected to ascertain their strains.
5. Based on this study, it is recommended to the farmers and Agric extension officers that the varieties Purple Mpeasem, CRI-Apomuden, and Apomuden Local should be grown more since they had high yield. This will in turn help solve future food insecurity.
6. Further studies should be considered in molecular work using more SSR markers confirmed for sweet potato, since the 7 SSR markers could not completely discriminate all the 30 sweet potato accessions in the current study.

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APPENDICES

APPENDIX 1: AGRO MORPHOLOGICAL CHARACTERS USED FOR EVALUATING THE 30 SWEET POTATO ACCESSIONS

Plant organ	**Characters scored**
Vine	Vine internode length (VIL), predominant vine colour (PVC), twining, ground cover, growth habit, vine tip pubescence.
Leaf	General outline of leaf (GOL), leaf lobe type (LLT), leaf lobe number (LLN), shape of central leaf (SCL), mature leaf size (MLS), mature leaf colour (MLC), immature leaf colour (ILC), petiole pigmentation (PP), and petiole length (PL).
Storage root	storage root shape (SRS), predominant skin colour (PSC), predominant root flesh colour (PFC),
Agronomic traits	Root shape (RS), bulk weight of roots (WLR), Root cortex thickness.

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APPENDIX 2: PARAMETERS FOR BOTH QUALITATIVE AND QUANTITATIVE ANALYSES.

QUANTITATIVE PARAMETERS	QUALITATIVE PARAMETERS
Vine internode length	Twinning
Number of tubers per node	Storage root shape
Leaf lobe number	Growth habit
Mature leaf size-leaf length	Ground cover
Petiole length	Predominant vine colour
Weight per plant	Vine tip pubescence
Tuber weight per plot-bulk weight	General leaf outline
	Central leaf shape
	Mature leaf colour
	Immature leaf colour
	Petiole pigmentation
	Predominant skin colour
	Predominant flesh color

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APPENDIX 3: ANALYSIS OF VARIANCE(ANOVA)

Single factor ANOVA on some selected quantitative data

ANOVA						
Source of Variation	***SS***	***Df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	6642.199	4	1660.55	194.7638	3.02E-57	2.434065
Within Groups	1236.265	145	8.525968			
Total	7878.465	149				

Single factor ANOVA on root characters

ANOVA						
Source of Variation	***SS***	***Df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	844945.5	7	120706.5	6.32185	0.000117	2.323171
Within Groups	591899.7	31	19093.54			
Total	1436845	38				

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APPENDIX 4: PCR WITH THE VARIOUS AMPLICONS

	SSR09		SSR07			IBC5		690524	IBR16	IBR19			IB242		
Accession	300BP	250BP	175BP	200BP	225BP	75BP	100BP	100BP	125BP	225BP	300BP	125BP	200BP	225BP	300BP
Faara	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Sauti	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Santom pona	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
CRI-Ogyefo	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
CRI-Apomuden	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
CRI-Otoo	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
CRI-Ligri	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Purple	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Blue-blue 1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Blue-blue 2	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Blue-blue 3	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
CSPS 1	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0
CSPS 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tech-Santom	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1
Okumkom	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
EX-Bunso/04	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0
CRI-Dadanyuie	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
CRI-Bohye	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0
CRI-Histarch	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Apomuden (local)	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1
Ex-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Bunson/01															
Bot/03/030	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Tag/03/019	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Ex-Bunso/02	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Ok/03/018	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0
Ok/03/014	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Dos/03/017	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CRI-Patron	0	0	0	0	0	1	1	0	0	1	0	0	0	1	0
Ex-Bunso/03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ex-Bunso/05	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0

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APPENDIX 5: DATA ANALYSIS OF DISEASE INCIDENCE AND SEVERITY

Generalized Linear Models (binomial family) statistical tool was used to analyze the viral incidence data.

GLM predictions of incidence of viral diseases from 6WAP to 12WAP.

6WAP			
ACCESSION	Prediction	s.e.	Predicted mean percentage incidence*
CSPS 1	-1.609	0.49	0.024604
APOMUDEN-LOCAL	-1.609	0.49	0.024604
BLUE-BLUE 1	-1.386	0.46	0.041115
BLUE-BLUE 2	-1.386	0.46	0.041115
BLUE-BLUE 3	-1.609	0.49	0.024604
BOT/03/030	-1.19	0.43	0.064565
CRI-APOMUDEN	-1.609	0.49	0.024604
CRI-BOHYE	-1.19	0.43	0.064565
CRI-DADANYUIE	-1.386	0.46	0.041115
CRI-LIGRI	-1.19	0.43	0.064565
CRI-OGYEFO	-1.19	0.43	0.064565
CRI-OTOO	-1.386	0.46	0.041115
CRI-PATRON	-1.012	0.41	0.097275
CSPS 2	-1.386	0.46	0.041115
DOS/03/017	-1.609	0.49	0.024604
EX-BUNSO/01	-1.609	0.49	0.024604
EX-BUNSO/02	-1.386	0.46	0.041115
EX-BUNSO/03	-1.19	0.43	0.064565
EX-BUNSO/04	-1.19	0.43	0.064565
EX-BUNSO/05	-1.386	0.46	0.041115
FAARA	-19.137	1584.01	7.29E-20

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HI-STARCH	-1.19	0.43	0.064565
OK/03/014	-1.012	0.41	0.097275
OK/03/018	-1.012	0.41	0.097275
OKUMKOM	-0.405	0.37	0.39355
PURPLE MPEASEM	-1.19	0.43	0.064565
SANTOM PONA	-1.19	0.43	0.064565
SAUTI	-1.872	0.54	0.013428
TAG/03/019	-1.012	0.41	0.097275
TECH-SANTOM	-1.872	0.54	0.013428
Deviance ratio	0.94		
Approximate chi *P value*	0.551		

* Antilog of mean GLM prediction

8WAP			
ACCESSION	Prediction	s.e.	Predicted mean percentage incidence*
CSPS 1	-0.2683	0.3684	0.539138
APOMUDEN-LOCAL	-0.1335	0.366	0.73536
BLUE-BLUE 1	-0.2683	0.3684	0.539138
BLUE-BLUE 2	-0.4055	0.3727	0.393097
BLUE-BLUE 3	-0.1335	0.366	0.73536
BOT/03/030	-0.2683	0.3684	0.539138
CRI-APOMUDEN	-0.2683	0.3684	0.539138
CRI-BOHYE	-0.2683	0.3684	0.539138
CRI-DADANYUIE	-0.1335	0.366	0.73536
CRI-LIGRI	-0.4055	0.3727	0.393097
CRI-OGYEFO	-0.4055	0.3727	0.393097
CRI-OTOO	0.2683	0.3684	1.854812
CRI-PATRON	-0.5465	0.3788	0.284119
CSPS 2	-0.4055	0.3727	0.393097

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DOS/03/017	0.2683	0.3684	1.854812
EX-BUNSO/01	-0.4055	0.3727	0.393097
EX-BUNSO/02	-0.8473	0.3984	0.142135
EX-BUNSO/03	-0.5465	0.3788	0.284119
EX-BUNSO/04	-0.1335	0.366	0.73536
EX-BUNSO/05	-0.5465	0.3788	0.284119
FAARA	-0.1335	0.366	0.73536
HI-STARCH	-0.4055	0.3727	0.393097
OK/03/014	-0.1335	0.366	0.73536
OK/03/018	-0.5465	0.3788	0.284119
OKUMKOM	0	0.3651	1
PURPLE MPEASEM	-0.6931	0.3873	0.202722
SANTOM PONA	-0.4055	0.3727	0.393097
SAUTI	-0.1335	0.366	0.73536
TAG/03/019	-0.1335	0.366	0.73536
TECH-SANTOM	0	0.3651	1
Deviance	0.45		
Approximate pvalue	0.995		

10 WAP			
ACCESSION	Prediction	s.e.	Predicted mean percentage incidence*
CSPS 1	0.2683	0.3684	1.854812
APOMUDEN-LOCAL	0.5465	0.3789	3.519654
BLUE-BLUE 1	0.6931	0.3873	4.932874
BLUE-BLUE 2	0.4055	0.3727	2.5439
BLUE-BLUE 3	0.4055	0.3727	2.5439
BOT/03/030	0.4055	0.3727	2.5439
CRI-APOMUDEN	0.2683	0.3684	1.854812
CRI-BOHYE	0	0.3651	1
CRI-DADANYUIE	0.1335	0.366	1.359878
CRI-LIGRI	-0.1335	0.366	0.73536

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CRI-OGYEFO	0.1335	0.366	1.359878
CRI-OTOO	0.4055	0.3727	2.5439
CRI-PATRON	0.2683	0.3684	1.854812
CSPS 2	0.1335	0.366	1.359878
DOS/03/017	0.6931	0.3873	4.932874
EX-BUNSO/01	0.5465	0.3789	3.519654
EX-BUNSO/02	0.5465	0.3789	3.519654
EX-BUNSO/03	0	0.3651	1
EX-BUNSO/04	0.4055	0.3727	2.5439
EX-BUNSO/05	0.6931	0.3873	4.932874
FAARA	0.1335	0.366	1.359878
HI-STARCH	0.8473	0.3984	7.035582
OK/03/014	0	0.3651	1
OK/03/018	0.2683	0.3684	1.854812
OKUMKOM	0.2683	0.3684	1.854812
PURPLE MPEASEM	0.1335	0.366	1.359878
SANTOM PONA	0.4055	0.3727	2.5439
SAUTI	0.2683	0.3684	1.854812
TAG/03/019	0.1335	0.366	1.359878
TECH-SANTOM	0.2683	0.3684	1.854812
Deviance	**0.40**		
Approximate p-value	**0.990**		

12WAP			
ACCESSION	Prediction	s.e.	Predicted mean percentage incidence*
CSPS 1	0.693	0.3873	4.931738
APOMUDEN-LOCAL	1.19	0.4317	15.48817
BLUE-BLUE 1	1.19	0.4317	15.48817
BLUE-BLUE 2	1.609	0.4899	40.64433
BLUE-BLUE 3	0.847	0.3984	7.030723
BOT/03/030	1.609	0.4899	40.64433
CRI-APOMUDEN	1.992	0.6154	98.17479
CRI-BOHYE	1.197	0.6085	57.3983
CRI-DADANYUIE	1.386	0.4564	24.32204
CRI-LIGRI	11.197	0.6085	57.3983

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CRI-OGYEFO	1.386	0.4564	24.32204
CRI-OTOO	1.872	0.5371	74.4732
CRI-PATRON	1.153	0.4683	14.22329
CSPS 2	0.847	0.3984	7.030723
DOS/03/017	1.012	0.4129	10.28016
EX-BUNSO/01	0.847	0.3984	7.030723
EX-BUNSO/02	1.386	0.4564	24.32204
EX-BUNSO/03	1.386	0.4564	24.32204
EX-BUNSO/04	1.609	0.4899	40.64433
EX-BUNSO/05	1.386	0.4564	24.32204
FAARA	1.705	0.5435	50.69907
HI-STARCH	1.872	0.5371	74.4732
OK/03/014	1.386	0.4564	24.32204
OK/03/018	1.872	0.5371	74.4732
OKUMKOM	`1.153	0.4683	14.22329
PURPLE MPEASEM	0.693	0.3873	4.931738
SANTOM PONA	0.847	0.3984	7.030723
SAUTI	1.609	0.4899	40.64433
TAG/03/019	1.609	0.4899	40.64433
TECH-SANTOM	1.19	0.4317	15.48817
Deviance	**0.82**		
Approximate p-value	**0.735**		

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