UNIVERSITY FOR DEVELOPMENT STUDIES

EVALUATION OF SOURCES OF PLANTING MATERIAL OF SWEETPOTATO (*Ipomoea batatas*, L) VARIETIES ON FIELD PERFORMANCE AND VIRAL LOAD

SUMAILA MOHAMMED

UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

FACULTY OF AGRICULTURE

DEPARTMENT OF AGRONOMY

EVALUATION OF SOURCES OF PLANTING MATERIAL OF SWEETPOTATO (*Ipomeoa batatas*, L) VARIETIES ON FIELD PERFORMANCE AND VIRAL LOAD

BY

SUMAILA MOHAMMED

(UDS/MCS/0028/15)

THESIS SUBMITTED TO THE DEPARTMENT OF AGRONOMY, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CROP SCIENCE

MAY, 2020

DECLARATION

Student

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere. References made therein are duly acknowledged.

Candidate's
Signature……Date……

Name**: SUMAILA MOHAMMED**

Supervisors'

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

Supervisor's Signature…….... Date……

Name: **DR. RAPHAEL ADU- GYAMFI**

Co-Supervisor Signature……Date……

DR. EWARD CAREY TED

ABSTRACT

Sweetpotato is an important crop for food security in many developing countries. Studies have shown virus diseases threat to the crop consistently and yet they remain the most difficult diseases to manage. The contributing factors to yield reductions in sweetpotato are insects and disease with viruses being the major cause of yield reduction. There are at least 15 well known characterized viruses. The aim of this reseach is to evaluate the field performance of three sources of planting material of some varieties and their virus status. The sources of planting material were *in vitro* generated platelets, Farmer's own materials and Field symptomless materials. Planting materials of four sweetpotato varieties, Apomuden, Bohye, Ligri and Dadanyuie were used. The trial was laid out in split plot design with the sources of planting material allocated to main plots and the varieties to sub-plots. The *in vitro* indexed plantlets were obtained from Biotechnology laboratory of Crop Research Institute, Fumesua. The Other two sources were obtained from International Potato Center CIP, Tamale. Nitrocellulose Membranes Enzyme Linked Immunosorbent Assay (NCM-ELISA) kits were employed for the detection of 10 sweetpotato viruses. After the serological reactions, SPFMV, SPMMV, SPMSV, SPCFV, SPCSV, and CMV indicated significant presence among the different sources of planting materials whilst SPC-6V virus, SPVG, SPCaLV and SPLV were negative. Farmer source of planting material recorded the highest virus symptoms. Apomuden and Bohye varieties recorded the highest virus score in the first and second virus symptom observational score respectively. Tissue culture *in vitro* materials recorded highest chlorophyll content. Vine yield was highest among the *in vitro* source of material. *in* vitro plantlets showed significant higher tuber root yield and other yield parameters than other sources of planting material. In the absence of *in vitro* generated material farmers will be advised to use field materials that have been selected mindful of virus symptoms.

ACKNOWLEDGMENTS

First of all, I am grateful to the God Almighty ALLAH for the wisdom, guidance strength, good health and willpower he gave me throughout my studies period.

I am highly indebted and grateful to my supervisors, Dr. Raphael Adu -Gyamfi and Dr. Eward Carey Ted for their constructive criticisms, invaluable advice and suggestions which ensure the full success and realization of this project work.

My special thanks goes to Dr. Edward Carey Ted for his financial support as well physical support during the project. I am grateful to you sir.

Also my special thanks goes to all CIP Staff Nyankpala – SARI for their field guidance especially Mr. Daniel Akansaki for his invaluable field guidance.

DEDICATION

This thesis is dedicated to my lovely mother, Hajia Ayi Issahaku Haruna and my lovely wife, Osman Huseinatu and I cannot forget my lovely boys, Ihsanuallah and Muhusin and all my family members who supported me one way or the other during my studies.

LIST OF ACRONYMS

ANOVA	Analysis of variance
CMV	Cucumber mosaic virus
CRI	Crop Research Institute
SPCaLV	Sweetpotato caulimo like virus
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization statistics
Ha	Hectare
kg	Kilogram
M	Meter
Min	Minute.
Mm	Millimeter
MOFA	Ministry of Food and Agriculture
NCM	Nitrocellulose membrane
ELISA	Enzyme Linked Immunosorbent Assay
PCR	Polymerase Chain Reaction.
PT	Pathogen Tested or virus-free plants.
SARI	Savannah Agricultural Research Institute
SED	Standard Error of Difference
SEM	standard error of means
SPC6V	C-6 a new flexuous virus
SPCFV	Sweetpotato chlorotic fleck virus

SPCSV	Sweetpotato chlorotic stunt virus
SPFMV	Sweetpotato feathery mottle virus.
SPLCV	Sweetpotato leaf curl virus
SPLV	Sweetpotato latent virus
SPMMV	Sweetpotato mottle virus.
SPMSV	Sweetpotato mild speckling virus,
SPVD	Sweetpotato viral disease
SPVG	sweetpotato virus G.
SSA	sub- Saharan Africa
TTBS	Tween-Tris buffered saline
TBS	Tris buffered saline

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **page**

LIST OF PLATES

Plate **Page**

CHAPTER ONE

1.0 INTRODUCTION

Sweetpotato is among the most essential root crops in the world and yearly production tonnage is over 133 million worldwide (Waramboi *et al.*, 2011). In tropical countries, sweetpotato is an unusually important crop (Smith *et al.*, 2009). It is cultivated in over 100 developing countries and ranks among the five most important food crops in over 50 of those countries including Ghana (FAOSTAT, 2012). Sweetpotato is essential, multipurpose but underutilized crop food and that in terms of total production ranks seventh amongst the world food crop (FAOSTAT, 2008). Sweetpotato is considered as active growing crop, with fast root formation and development which enhanced greater survival rate of the seedlings and great expected output (Alemu, 2004). The crop has the ability to yield well in Ghana and it can be utilized in many dietary dishes preparations in place of other staples food crops (Ellis *et al*., 2001). It is one of the main food, feed and industrial raw material, with worldwide over-all output is projected to be about 80 % (Islam, 2006). It is regarded as good food for human health because of its beneficial protein composition as well as high content of minerals, vitamins and antioxidants (Islam, 2006; USDA, 2007). Reported by Islam (2006) that sweetpotato leaves extracts contain antimutagenic which is a radical free, anticancer hunting agents, and antibacterial agents. leaves of sweetpotato therefore, is a real main source of antioxidant. Root of sweetpotato is high in nutrients such as pantothenic vitamin A, B6, C, riboflavin, copper and folic acid (Abd El-Baky *et al*., 2009). Reported by Zhang *et al*. (2009) sweetpotato has numerous industrial uses, including medicinal drives such as use for treating ulcer, diabetes, hookworms and internal bleeding.

However, sweetpotato in Ghana only increase from 111,477in 2003 to 136,906 in 2014 (FAO, 2017) representing about 23 % increase as against 86 % increase in production in Africa. In 2013,

Ghana's sweetpotato yield was 13.7 t/ha whilast in Egypt and Ethiopia it was 32 and 35 t/ha respectively.

The crop production is significantly limited due to pathogens mostly viruses, fungi and bacteria that can cause about a yield decrease of up to 98 % (Kapinga *et al*., 2007). Mostly fungi, viruses, and bacteria are plant pathogens that are mostly accountable for decreasing yield by causing economic losses in the crop more especially viruses (Zhang *et al*., 2009). The crop yield has been on drastic reduction in areas of cultivation due to systematic infection of virus (Karyeija *et al*., 2000; Okonkwo, 2002). Pozzer *et al*. (1995) also reported that, when sweetpotato virus index materials derived from meristems of heat - treated plants were compared to yields from cuttings taken from farmers' fields yield increases up to118 %.

1.1 Problem statement

Sweetpotato has the ability to contribute much more to eliminate hunger and address the problem of malnutrition and address problems of food insecurity in Sub-Saharan Africa including Ghana (Abidin, 2014).

However, the yield reduction in production areas has been a major challenge. Sweetpotato yield has been on drastic reduction in the country and other areas of cultivation due to viral infection for example Sweetpotato Feathery Mottle Virus (SPFMV) and other sweetpotato viral diseases which cause serious yield losses (Karyeija *et al*., 2000; Okonkwo, 2002). Furthermore, production of pathogen-free planting materials and disease information on viral yield is minute in West African sub region including Ghana where the crop is cultivated due to fact that farmers are not practicing commercial farming but piece-meal harvesting which makes it very difficult to quantify yield

potential of the crop (JRT, 2000)). According to Moyer and Salazar (1989) sweetpotato feathery mottle virus (SPFMV) is most destructive virus which causes infection across all over the world.

Reported by Salazar and Fuentes (2000) yield defect due to sweetpotato viral diseases are roughly to between 15 to 48 % in China, 34 to 97 % in Egypt is about 50 % or estimated more in Israel (Milgram *et al*., 1996) and 80 to 98 % in East Africa (Mwanga *et al*., 2002; Wambugu, 2003). Virtually any sweetpotatoes growing from non-pathogen clean materials source will contained at least one virus in them (Kapinga *et al*., 2007). Problem of viral disease is measured as one of the most severe cause of harvest loss and cultivar deterioration. Viruses buildup and other pathogens occurs in sweetpotato due to the propagation method, root-to-sprout in the commercial production. In most cultivation areas including Africa the easiest way of preserving the sweetpotato seeds by farmers for other seasons is by 'seed' roots due to that it became highly pathogenic infected with a lot of viruses and produces virus-infected vines during the growing season. Continuous use of non-pathogen tested planting materials may lead to drastic deterioration of the sweetpotato cultivar. Viruses in sweetpotato are the main cause in the deterioration in the crop production worldwide (Clark and Huang, 2006; Bryan *et al*., 2003; Wang and Valkonen, 2009). Viruses is the main problems of sweetpotato industrialization, most especially for subsistence farmers and result on food insecurity in developing countries (Loebenstein *et al*., 2003; Tairo *et al*., 2005). About twenty-two common types different strains of viruses reported to have caused destruction to sweetpotatoes universal (Salazar and Fuentes, 2000; O'Sullivan *et al*., 2005; Tairo *et al*., 2005; Leea and Pitrat 2007). Also, some of the virus's types have insect vectors that keeps on increasing the

rate virus infection as well as virus buildup over successive growing season so far there is favorable conditions for breeding and growth (Lee and Pitrat 2007).

1.2 Justification

Although the sweetpotato has the attribute of withstand adverse weather conditions such as drought and poor soil nutrition better than most crops during growth, there are pests and diseases that affects its performance. The disadvantage of producing sweetpotato by planting seed root (vegetative propagation) is reducing yield abilities of the cultivar. Therefore, there is the need to have new methods of preserving sweetpotato seed roots, including clean healthy and reliable seed pieces as well as *in vitro* tissue culture method (Villordon and LaBonte, 2003). Due the fact that, there is no reliable, viral resistant and higher yielding varieties makes *in vitro* cleaned plantings material the only way forward and best method to intensify yield and to maintain the production areas. Studies have demonstrated some benefits of using pathogen clean planting material such as higher yielding and quality compared to traditional growers non-tested material (Carey *et al*., 1999; Zhang and Salazar, 2000; Carroll *et al*., 2004).

However, as stated by Milgram *et al*. (1996) ; Clark and Hoy, (2006) some studies have contradicted that, virus free planting materials yield performances are the same or have no beneficial effects on storage roots and vines yields, and Gutierrez *et al*. (2003) whilst other reports have revealed that, SPFMV- virus especially infected plants performing better yield than the *in vitro* tissue culture controls and others studies have revealed yield reduction of up to 46 % of the infected plants (Gibson *et al*., 1998; Mukasa, 2004). Due to this controversy, there is a need to further investigate the performance of *in vitro* tissue culture clean planting materials on yield of sweetpotato cultivars. Continued use of non-pathogen tested symptomless vine cuttings by Ghanaian farmers could also be a reason why the expected performances of the crop have not been met. The study seeks to assess the concert of *in vitro* tissue culture clean healthy pathogen tested planting materials and other different non-pathogen tested sources of planting materials on yield in the savanna agro ecological zone in Ghana

1.3 Objectives

The main objective of the study was to determine the impact of different source of planting materials on yield of storage root.

The specific objectives were to determine:

1. the virus load on different of planting materials of sweetpotato.
2. the effect of virus on chlorophyll content.
3. how the health status of planting materials affects yield components of sweetpotato.
4. how the health status of sweetpotato varieties affect yield components.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and botany of sweetpotato

It is belief Spanish explorers found sweetpotato from New World and was sent to Philippines, Spain, and India, in the 15th and 16th centuries. Flowers can either be purple or white, and sweetpotato leaves can either be purple or green depends on the variety. Flesh can be white, cream, yellow, purple, light orange or orange, (Woolfe, 1992; Bovell-Benjamin, 2007). Sweetpotato cultivar was first name as *Convolvulus batatas* by Unnaeus and classified in 1753.

However, in 1791 Lamarck describe on the stigma shape basis this crop species within the genus *Ipomoea* and the surface of the pollen grains. Hence forth, the name changed to *Ipomoea batatas* (L) Lam. The cultivated species *I. batatas* includes plants that have high morphology variability. More than thousands of sweetpotato cultivars have been selected and cultivated in the Latin America sub zone since earliest times. At this current time, it is the crop is grown all over the tropics and sub-tropical zones. The sweetpotato is perennial, an herbaceous plant. But, it is vegetatively cultivated as a yearly plant by using either stem cuttings or storage roots as planting material. Its growth habit is mostly prostrate with a vine system that grows rapidly horizontally on the ground. The types of growth habit of sweetpotatoes are semi-erect, erect, very spreading, and spreading (Bovell-Benjamin, 2007).

2.2 Sweetpotato production

China is one of oldest countries seriously producing sweetpotatoes of 113.6 metric tonnage in 2004 that takes about 88.9 % of the world production (FAO, 2004). Recently (2012 – 2014 mean), annually, there are about 24.2 million tonnage produce of sweetpotato in Sub Saharan Africa (FAOSTAT, 2001); more than half (54 %) is produced in East Africa, 16 % in Southern African 21 % in West Africa; and about 9 % in Middle Africa. The area used for sweetpotato has increased much more and faster than maize and other cereals over the past 20 years (Low *et al*., 2008).

Sweetpotatoes has the capacity to grow faster than most cereals in tropical, subtropical, and temperate areas with Orange, white, and cream the most commonly grown and eaten (BovellBenjamin, 2007). Sweetpotato is ranked seventh most important food crop (for humans as well as animals) in the world with a projected yield of 135 million metric tonnages every year and currently grown in not less100 countries.

Sweetpotato is a tropical perennial crop, but mostly treated as an annual, the crop can also be cultivated in temperate climatic zones (FAOSTAT, 2014). The plant is taken to be a warm season crop, although its wide adaptation is changing with different geographical zones. Sweetpotato ideals weather for growth are temperatures at or above 24 ° C, well spread rainfall of 750 -1000 mm and slightly well drained sandy soil of pH between 5.6 - 6.6 as it cannot withstand highly waterlogging. In areas with tropical conditions, it flowers readily but the plant usually set few viable seeds because of the high temperatures.

However, most sweetpotato genotypes do not easily flower, others are sterile flower and most are self-incompatible. Both the leaves and, most especially, the tuberous roots are edible (Woolfe, 1992; Bovell-Benjamin, 2007). Sweetpotato is now worldwide distribution. In parts of Africa, Asia, and the Pacific, sweetpotatoes are an important staple crop (Woolfe, 1992; Bovell-Benjamin, 2007). Sweetpotato is an essential dietary crop in many areas of SSA, Where the crop is cultivated on about 2.1 million hectares (FAO, 2004), with an estimated production of 9.9 million tons (CIP, 1999). In Uganda, it is positioned as the second most essential food crop right after cassava in the eastern part and second to banana (*Musa sp*.) in the central and western parts of the country (Mukasa *et al*., 2003).

2.3 Source of sweetpotato planting materials

Vine slips of sweetpotato are better materials for planting in tropical areas than vines sprout from tubers for many important reasons Islam *et al*. (2002) it traditionally and conventionally cultivated by storage roots, seedlings or vine cuttings fragments. The age of the plant is an important factor in the recovery and survival of cuttings, especially in ecosystems where the length of the vegetative period is limited. Several factors influence the choice of plants from which to take cuttings assuming a shortage of planting materials does not mean that all plants have to be used. These include the degree of damage that may be caused to the crop if a nursery is not involved, the ease with which the cutting operation can be carried out, the plant's performance level and its health status.

In most of times the upper parts of the plant are used as planting materials. They can access easily since no rooting will have taken place there or roots little, and they are generally considered to perform better. A study by Degras, (2003) of two cultivars trial from August to February in Guadeloupe shown that, vine cuttings taken from the middle portion of the third stem gave better yields than cuttings taken from the top of the first two stems. The lower portions cutting from the first stem also did better than the upper one under these conditions.

Nevertheless, when all responses are compared, upper cuttings are definitely the ones to use. There have been a lot of trials to improve performance of sweetpotato through modifications in cutting characteristics. Several source of planting materials can be put into use to produce the crop vegetatively by vine slips or storage roots.

However, in areas where production cannot be carried on continuously and vines are unavailable for planting, root sprouts and storage root pieces are used for propagation. Strong healthy storage

roots from the passed cropping season are sprouted and used as vegetative propagated planting materials.

There are controversies regarding the vine cuttings length that is optimum. Onwueme (1991) indicated that tuber yield tend to increase with increase in the length of the vine cuttings used, and recommended a length of about 30 cm. Cuttings longer than 30 cm is not more useful, while much cuttings that are shorter established poorly, and result poorer yield. Gibson *et al* (1998) also stated between 20 - 40 cm long vine cuttings should be used for better storage root yield and that yield of 20 to 25 cm cutting slips results in poorer, as compared 40 to 45 cm slips produce better total storage root. Chen (2012) stated that, horizontal planting improved sweetpotato yield. In the USA and other zones with mild temperate situation where sweetpotato is cultivated, growers save seed roots from each cropping season. In early spring, roots seed are planted in beds, and sprouts from adventitious bedded roots are cut and cultivated for sweetpotato production. (Wilson and Averre, 1989). This method of vegetative propagation is disadvantageous due to virus accumulation. Adventitious sprouts of sweetpotato storage roots, which are used as planting materials for subsequent crops, are most variable phenotypically than planting materials derived from nodal stem cuttings of sweetpotato, and this may result to drastic reduction in root yield and

sweetpotato root quality (Villordon and La Bonte, 1995; 1996). Likewise, adventitious sprouts of sweetpotato infected with virus and nodal cuttings infected with virus drastic in yield reduction than nonsymptomatic sweetpotato planting materials that were virus tested free of known viruses. Sweetpotato feathery mottle virus (SPFMV) is one the serious viruses that have been found to cause drastic reduction in sweetpotato yield performance (Pozzer *et al*., 1995). Viral disease of sweetpotato [a synergy interface between Sweetpotato chlorotic stunt virus (SPCSV) and SPFMV] (Ngeve, 1991), an interaction effects of SPFMV and Sweetpotato sunken vein virus (SPCSV) (Milgram *et al*., 1996), and a combination of SPFMV and Sweetpotato latent virus (Ngeve, 1991) are also noted to cause drastic yield reduction. The disadvantage of producing sweetpotato by planting seed root is losing yielding capacity of the cultivar (Milgram *et al*., 1996).

Therefore, there is the need to have new methods in maintaining sweetpotato seed system roots, including clean and reliable seed pieces as well as *in vitro* technique have been studied (Villordon *et a*l., 2003).

2.4 Economic importance of sweetpotato

Sweetpotato is part of the world's most essential, highly resistance but less utilized dietary crop grown purposely for its storage roots (Tortoe, 2010). It is among the most essential world food crop. It is rank as the seventh most important food crop worldwide in view of total production, thirteenth in value production and fifth rank among caloric providing diet (Tortoe, 2010).

Also, FAOSTAT (2008) reported that, Sweetpotato is one of the most worldwide essential crop, underexploited and multipurpose food crop that positioned seventh in the global crop performance. Lower agricultural input but highly yielding and rich with a lot of nutrients, mainly of starches, brand it one of the important edible foods for a lot of people, particularly in the developing countries. It is one fast maturing crop with a crop cycle that takes between three (for early

Maturing type) to four months for late maturing type (Anyaegbunam *et al*., 2008) and the early maturing type can grow three to four times in a year (Okonkwo, 2002). China is the number one in terms sweetpotato production in the world, followed by Uganda and Nigeria (FAO, 2004).

According to Kays (2005) sweetpotato is among most essential food crops worldwide, which is recorded as the third most useful root crop next to potato and cassava and is positioned seventh in global food crop production. The qualities of orange-fleshed is outstanding vitamin A source types make it an instant straight forward to eliminate vitamin A malnutrition problem in African continent (Woolfe, 1992).

2.4.1 Sweetpotato as food and source of vitamin A

Sweetpotato classification is associated to 15 wild *Ipomoea* species, but *batatas* species is the only crop species that produces useful edible roots (Diaz *et al*., 1992). Abidin (2004) stated that, the immature fresh leaves and vine parts of sweetpotato plants are broadly eaten as vegetables in most West African sub regions (eg. Guinea, Sierra Leone and Liberia), and north eastern Uganda as well as East Africa. The crop is e rich in production of vitamins, carbohydrates, proteins as well income generation per unit area of land and time (Magagula *et al*., 2010).

Sweetpotato provide the highest photosynthetic fixation ability per unit area than any crop that can be cultivated in temperate climate (JRT, 2000). Reported by Woolfe (1992) that sweetpotato has the ability of producing up to 30 % more carbohydrates yield per unit area than corn. Indeed, sweetpotato produces more edible energy per hectare per day than any other major food crop, and the enormous potential of this crop as a food and carbohydrate source is widely recognized. Tairo *et al*. (2005) stated that sweetpotato is known for its high-grade of carbohydrate for starches food

and pharmaceutical industries. Sweetpotatoes are healthy food with little amount of fat and protein, but high in starch. Tubers and leaves are good sources of antioxidants fiber, zinc, potassium, sodium, manganese, calcium, magnesium, iron, and vitamin C (Antia *et al*., 2006). Sweetpotatoes orange-fleshed (OFSP) are also very healthy sources of vitamin A (VA) (Hagenimana *et al*., 1999; Tairo *et al*., 2005). Due to greater amount of vitamin A precursor concentration and good harvests, of orange flesh sweetpotatoes, numerous small-scale attempts have been made to intensify Vitamin A status (van Jaarsveld *et al*., 2005). Sweetpotatoes vary in colour and carotenoid concentration. The beta carotene is main precursors for Vitamin A-forming carotenoid in sweetpotatoes (ARS, USDA, 2010), though minute amount of alpha-carotene and beta cryptoxanthin can be seen in some varieties. The quantity of beta-carotene is highly dependent on the variety of sweetpotato (Hangenimana *et al.,* 1999). Tairo *et al.* (2005) vitamin precursors in the body are less effective. Isotopic dilution test of beta-carotene transformation in healthy well-fed subjects show adjustable transformation ratios, with some healthy volunteers forming negligible amounts of vitamin A. Research by van Jaarsveld *et al.* (2005) stated that, carotenoid alteration ratio in the body is predicted to be 6-µg beta-carotenoid: 1-µg vitamin A or 12-µg beta-carotene: 1-µg vitamin A. The aim for the relatively meager transformation of beta-carotene to vitamin A is due many different factors.

Though, one important aim is that carotene are sickly absorbed from a lot of diets. Carotene captivation is actually variable and rely on the carotenoid, its diet matrix, and the different. Beta carotene is better captivated from orange Coloured vegetable and fruits than from green vegetables. Beta-carotene absorption is much better when fed with oil (Huang *et al*., 2000) than without. One human study has estimated that the Vitamin A (VA) equivalency of the carotenoids found in sweetpotato. The vitamin A equivalency of beta carotene nourished to Bangladeshi men with

reasonable vitamin A stores is estimated to be 13.4 - µg beta-carotene to 1-µg retinol (Tanumihardjo, 2006).

Nevertheless, people and animal models with low vitamin A status appear to transform a greater proportion of beta-carotene to Vitamin A (Porter-Dosti *et al*., 2006; Tanumihardjo 2009). E.g., the translation ratio of beta-carotene to vitamin A is poorly fed Filipino child varied in reverse with vitamine A position. The transformation proportion in vitamin A exhausted gerbils fed with maize meal is 3-µg beta-carotene to 1-µg retinol (Tanumihardjo and Howe, 2006).

2.4.2 Utilization of sweetpotato

Sweetpotato storage roots and vines can be process in to animal feed supplements and also be feed raw to animals. Various process product such as flour, drinks, total candy and pastas are manufactured in native industries (CIP, 1987). Sweetpotato is a multipurpose plant crop because the top part of the crop can be fed to animal or as vegetable, and storage root are eatable by humans (CIP, 1987).

Even though the greeneries and shoots are also eatable, the highly calories root tuber are usually the most essentials produce. In few tropical zones, they are an essential diet-crop. The utilization of the sweetpotato storage roots, stems and greeneries can freely be consumed by animal such pigs, goats, cattle, and even poultry and fish as well when they are processed into eatable forms like hay or silage (CIP, 1987). Most people often consume the immature leave that are less fibrous as a salad green or vegetable. Products of sweetpotato that are been processed include: a puree, frozen patties, pie fillings it is also used for that purpose, sauces (for example tomato sauce

manufacturing), in fruit-flavored sweetpotato jams and baby foods example with orange, mango, guava, and pineapple (CIP, 1987).

It is also a very major ingredient for starch production operation in China. In develop countries such as United states, complete, halved, chunks or puréed sweetpotatoes are preserved in cans. Cold: French fries, Cubes, slices, mash, halves, quarters and whole roots can be cold. Sweetmeat: sweets and candies, and sweetie-coated or salty crisps for snack foods are made from it. Mashed sweetpotato is recycled as a component for baking foodstuffs and desserts as an auxiliary for extra luxurious ingredient of ice cream, tarts, and 20 % supplement Sweetpotato can be process into flour which can be used as in place of wheat flour in the backing industries for baking bread, biscuits or cakes. It can also use as a substrate for alcoholic drinks and for making pasta and other beverages (Edmond *et al*., 1971). The industrial use of sweetpotato tubers includes highly calories starch extraction and the production, acetic acid, yeast and hard drinks. (González *et al*., 1999; Zuraida, 2003).

2.4.3 Sweetpotato nutritional value

Nutritious worth on a world scale, sweetpotato delivers appreciable amounts of carbohydrates equated to other dietary staffs. The essential amino acid amount is a bit inferior compare to Irish potato and crops produce grains, better still contribute appreciable quantities of amino acids. Sweetpotato has a less glycerol amounts that is good for human health. It has a lot of nutrients values such Hi-starch variety contains more starches, dietary fibra, vitamin A rich in complex carbohydrates, (a beta carotene equivalent nutrient), vitamin B6 and vitamin C. yellow, Pink and green cultivars are high in carotene, the vitamin A precursor. Sweetpotato is regarded as good for

human health because of its useful essential amino acid components as well as high content of vitamins, antioxidants and minerals, (USDA, 2007; Islam, 2006; Tumwegamire *et al.*, 2011). Sweetpotato cultivars that are dim orange flesh colour contain better beta carotene than lighter colored flesh and their cultivation be encouraged to increased Africa, where vitamin A precursor is need a tremendously essential to combat white blindness health problems. The word "sweet," does not prevent diabetic patients to consume the varieties because certain varieties have much lower sugar that cannot affect them as some current studies shows that to sweetpotato helps in stabilization of blood sugar level to inferior insulin resistance (CIP, 1987). Studies by Scott and Maldonado (1999); (Ojeniyi and Tewe (2001) and Kapinga (2007) opined that 100 g of sweetpotato can be enough source beta-carotene to provide from 0 to 100 % of the optional daily vitamin A requirement (350 ug) / day for young as well as infants' children.

However, some vitamin A precursor cannot be transformed and utilized by the body, this quantity can easily be supplied by just 100 g of sweetpotato orange-fleshed so this converts to about 2400 ug of vitamin A. The quantity of orange flesh weightiness needed to provide a daily needs of vitamin A is much small (Carey *et al.*, 1999). nutrient value of sweetpotato storage root in terms of glucose amount and therefore good energy source. Earlier studies showed that, (Hiroshi *et al.*, 2000) sweetpotato leaves have crude fiber and protein which are very essential for combating problems with protein deficiency and ears blindness.

Though, it is having the danger inhibitors trypsin, anti-nutritional factor but they do not survive after cooking and are have no effects on cooked roots (CIP, 1987; Woolfe, 1992). Other studies also revealed that both sweetpotato tuber and leaf contain micro nutrients that are essential for healthy body but there is certain amount of anti-nutrients, such as phytate, oxalate and tannin (Osagle, 1998; Fleming, 1981 and Udoession and Ifon, 1990). These anti-nutrients factors have

the ability to alter digestion system and prevent nutrients absorption into the blood streams. Therefore, the nutrient content of sweetpotato at raw as well at cooked state must be known.

Table 1: Nutrient content percentage of sweetpotato toasted granule per 100gm

Sweetpotato nutrients	Protein %	Fat %	Fiber %	Sugar %
Sweetpotato Toasted granules	1.76	0.25	6.65	0.30
Boiled Sweetpotato	1.65	1.0	1.8	5.70
Raw Sweetpotato	1.65	0	4.0	6.00

Source: CIP (2009) cooking demonstration

2.5 Constraints of sweetpotato production

Sweetpotato is propagated vegetative and are therefore prone to systemic virus accumulation and other pathogens in the propagating material. Clark *et al*. (2002) and Bryan *et al*. (2003) and Bryan *et al*. (2003) also stated that, cultivar deteriorate, a situation in which quality and yield characteristics, or both decline within a successive period of time after releasing newly variety, has developed a serious concern. There are much more confidence that virus buildup in sweetpotato are core reason of frequency deteriorations and mutations increasing rate as well as other pathogens in the planting stock (Bryan *et al*., 2003). According to CIP, (1998) sweetpotato production does not meet the high population demand and there is the need to increase production.

Farmers experience significant yield reduction and the yield levels are 20 % of the crop's potential (50 tonnes/ha) observed under experimental conditions (Ndolo *et al*., 1997; Qaim, 1999), so there is ample chance to boost yields. Difficulty in enhancing sweetpotato production includes, lack of good planting stocks in some dry areas, lack of prospective market for the crop due to unappropriated transport systems, poor soil productiveness, low practices of agronomy and low usage of high yielding varieties (Moyer and Salazar, 1989; Wambugu, 1991; Carey *et al*., 1996; Ateka *et al.*, 2007).

The disadvantage of vegetative propagation method is the source of viruses acquired and mutations by sweetpotato crop throughout the preceding growing season will be present in the storage roots, and consequently, in the adventitious sprouts produced. Adventitious shoots of sweetpotato storage roots, which are used as planting materials for subsequent cropping, the sweetpotato is highly variable phenotypically than other planting materials sourced from nodal cuttings of the stem of sweetpotato, and this may contribute to decreases in yield and root quality of sweetpotato. Also, it stated by Clark and Moyer, (1988); Moyer and Salazar, (1989); Njuguna *et al*. (1990) and Ames *et al*. (1996) that, a wide range of pathogens the attacked sweetpotato crop, these include viruses, bacteria, nematodes and fungi.

Similarly, adventitious shoots of diseased sweetpotato planting materials with virus produce less than and nodal cuttings infected with virus that are virus-indexed nonsymptomatic sweetpotato planting materials (i.e., verified free of known viruses) (Villordon and La Bonte, 1995; 1996; Collins, 1994). Sweetpotato viruses that have been established to decrease yield in sweetpotato is including sweetpotato feathery mottle virus (SPFMV) (Pozzer *et al*. 1995). SPFMV is the most spreading virus infecting sweetpotato in many areas and it is can be found nearly everywhere where sweetpotatoes are cultivated worldwide (Clark and Moyer, 1988). It is non-persistently spread by aphids (e.g., *Myzus persicae* and *Aphis gossypii*). Leaves indications include chlorosis on veins

and feathering, and spots chlorotic with purple borders that can be seen mostly on older leaves of the sweetpotato plant. Storage root indications may include russet crack, inner cork, form malformations, and superficial discoloration liable on the cultivar and virus strain existent (Moyer and Salazar, 1989) The main limitations to production of sweetpotato include continuous usage of low producing and late maturing local cultivars, post-harvest yield losses, diseases and pests and inadequate planting material. The most important disease in Kenya and other areas of sweetpotato production is the sweetpotato virus disease (SPVD) (Wambugu, 1991).

In addition, there is poor institutional support and recognition and inadequate research capacity in most African countries. The sweetpotato virus disease (SPVD) and the sweetpotato weevils are the main biotic constraints. Sweetpotato is constrained by numerous factors such as disease and insect, moisture stress (drought), decline in soil fertility, poor crop management practices, lack of access to disease free vines of improved varieties are the major problems. Among other factors contributing to yield reductions in sweetpotato, insects and diseases are the major, resulting in yield reduction as high as 98 % (Kapinga *et al*., 2007, Gurmu, *et al*., 2015). Plant diseases caused by viruses, fungi and bacteria are responsible for the escalated economic losses of sweetpotato worldwide.

Amongst the disease causing pathogens, viral infectious diseases are the core restrictions of sweetpotato production and productivity (Njeru *et al.*, 2004). Usually virus symptoms appear on leaves due to numerous contaminations in the field with the most frequent met mixture exist between sweetpotato chlorotic stunt virus (SPCSV) and sweetpotato feathery mottle viruses (SPFMV). This double infection is core reason why sweetpotato viral disease (SPVD) is serious

been clearly stated to be the main viral disease in Africa and the world at large treat to sweetpotato production (Mukasa *et al*., 2003).

2.6 Pest and virus insects vector of sweetpotato

The aphids are the most important vector of one or more viruses that are causing damage to cucurbit crops (Eastop, 1983). These viruses are usually spread by aphid's insects in a nonpersistent means (Nameth *et al*., 1986). Whereas, the whitefly of sweetpotato, *Bemisia tabaci Gennadius*, (Byrne *et al*., 1990) is one the essential agricultural crop pest as well as horticultural crops across all the world, causing serious damage indirectly by the excretion of honeydew and directly through feeding and as causative agent of virus diseases (Cohen, 1990). Aphids feed on plant sap by piecing and sucking, and then transfer viruses.

The mostly aphid transferred virus from sweetpotato plant to sweetpotato plant in an open farm. The most common virus transferred by aphids is sweetpotato feathery mottle virus. The aphids with winged can move to long a distances travel and introduce viruses into new area that are not affected. The wide host range insect, *A. gossypii* sucked plant sap from many plants including cotton, cucur-bits, and many legumes. Aphids are said to be a cosmopolitan, their core effects impact on sweetpotato is as vectors of viruses. Whitefly types insects can root necrosis and yellowish damage of the diseased sweetpotato leaves. Spreading of viral infection is mostly by insects (Loebenstein *et al*., 2003; Byamukama *et al*., 2004; Valverde *et al*., 2004). Souto *et al*. (2003) stated that the virus is widespread because is being mechanically transferred by aphids Myzus persicae and *Aphis gossypii* in a nonpersistent manner.

2.7 Sweetpotato virus and their complexes effects

There are several problems that have not been resolved in the classification of sweetpotato viruses. Numerous viruses related with particular diseases or, indications for example interior cork, have never been characterized or isolated. Virtually few viruses have been characterized and termed, but because the isolates are no longer available for proper evaluation it become extremely difficult if not impossible to characterize it (Karyeija *et al*., 2000). Thus, determine their relatedness is impossible to compare to other sweetpotato viruses.

Even though, sweetpotato feathery mottle virus (SPFMV) is not uncommon in places wherever there is cultivation of sweetpotatoes, and thus obvious that there are numerous specific phylogenetic bunches within this nomenspecies (Kreuze *et al*., 2000), and therefore, proposed that more or less of these distinct species like strain C, should be treated like cluster Tairo *et al*. (2005) in some cases, these also correlate with certain essential biotic properties. Souto *et al*. (2003); Kokkinos and Clark, (2006) as stated for example, the russet crack strain of sweetpotato feathery mottle virus (SPFMV) synergistically interrelates with sweetpotato leaf curl virus (SPCSV) to tempt typical sweetpotato viral disease (SPVD) indications, but the common strain of SPFMV does not.

2.7.1 Some classification of sweetpotato virus and their effects

The finest way to minimize this viral diseases, as well as other viral problems of sweetpotato is by using pathogen free planting materials after virus-tested sourced plants. Sweetpotato virus G Genus Potyvirus (SPVG) is reported to be the most widely spread in Egypt as well as China (Colinet *et al*., 1994, 1998), and (IsHak *et al*., 2003), the USA (Souto *et al*., 2003; Kokkinos and Clark, 2006), Peru (Untiveros *et al*., 2007), Spain (Trenado *et al*., 2007), Tanzania (Kapinga and Ndunguru, 2007), Peru (Untiveros *et al*., 2007), Spain (Trenado *et al*., 2007), Japan, Ethiopia,

Nigeria, and Barbados. Currently, the virus is also found in certain zones of the Pacific Ocean and their molecular characterization is being determine and compared to other isolates (Kreuze *et al*., 2000). SPVG causes mottling in *I. nil* and chlorotic spotting in *I. setosa* and *I. tricolor* (Souto *et al*., 2003). Cylindrical inclusions bodies, which consisted of pinwheels and scrolls, were observed in the cytoplasm of epidermal, mesophyll, and vascular cells of infected *I. nil* and *I. setosa* (Souto *et al*., 2003). A semi sequence of SPVG (X76944) has been obtained from RT-PCR, showing an identity of around 70 % and 80 % in the amino acid sequence between the conserved and complete core of the coat protein of SPFMV, respectively (Colinet *et al*., 1998). Whilst that of SPFMV has 316 amino acids the SPVG coat protein has 355 amino acids (Colinet *et al*., 1994). Comparison with coat protein sequences of known potyviruses indicates that SPVG is a member of the genus Potyvirus. Most strains of SPVG are likely to come from China (Colinet *et al*., 1998). SPVG-CH2 mostly contain about 89.2 % and 90.6 % amino acid sequence identities with SPVG-CH in the NCP and the N-terminal region of the coat protein core (N-CP core), respectively. Sweetpotato virus 2 tentative member Genus Potyvirus (SPV2).

Sweetpotato virus II, *Ipomoea* vein mosaic virus and sweetpotato virus Y (Moyer *et al*., 1989; Souto *et al*., 2003; Ateka *et al*., 2007). In Taiwan that virus is first identified in sweetpotato plants (Rossel and Thottappilly, 1988), and then also isolates obtained from sweetpotato clones from China, Portugal, South Africa, China, USA (Souto *et al*., 2003), Spain (Trenado *et al*., 2007), Australia (Tairo *et al*., 2006), and Peru (Untiveros *et al*., 2007). In length, SPV2 has 850 nm, induces cytoplasmic cylindrically filamentous particles inclusions consisting of pinwheels and scrolls (Souto *et al*., 2003; Ateka *et al*., 2007). The virus nonpersistent transmitted by *M. persicae* and mechanically transmitted to several species of genera *Chenopodium*, *Datura*, *Nicotiana*, and *Ipomoea*. SPV2 causes leaf distortion and vein clearing on *N. benthamian*, chlorotic local lesions

on *Chenpodium spp*., vein mosaic on *I. nil, I. setosa* and *I. tricolor* (Souto *et al*., 2003; Ateka *et al*., 2007).

2.7.2 Effects of virus on sweetpotato root yield

It reported from earlier research that, In Ethiopia, sweetpotato root performance is drastically declining due to the fact that there is synergistic infection of SPFMV and SPCSV which caused about 37 % reduction (Tesfaye *et al*., 2013). Mukasa *et al*. (2003) also revealed that, declining of root yield in Africa due to virus infection can reach 98 %. Sweetpotato being one of the crop that is propagated vegetatively, it is prone to pathogens such as viruses, bacteria and fungi that can spread and persist over successive crop cycles (Bryan *et al*., 2003). Crop plants that are affected by SPVD are easily seen by farmers due to the symptoms being severe and can be prevented or control by different combination of cultural practices such as removal and not selecting (Positive and negative selection approach) them as planting material for the next cropping season (Aritua *et al*., 1999).

Also, it is reported in other countries that the effect of SPFMV on performance of sweetpotato crop are controversial. Some earlier reported virtually zero effects on yield performance of marketable storage roots and vines in comparison with healthy plants (Milgram *et al*., 1996; Clark and Hoy, 2006), whilst others reported that, SPFMV infected sweetpotato cultivars producing even better yield than the control healthy once (Gutierrez *et al*., 2003), and the others studies revealed yield losses of up to 46 % (Mukasa, 2004). Sweetpotatoes are propagated vegetatively from root slips (sprouts), vines, or tubers, and local farmers usually taking their seeds from previous field.

So, if pathogens causing diseases are in the field they will surely be transferred from the planting materials to the newly planted field, often resulting in a drastic decrease in yields.

However, different areas yields vary greatly or even fields in the same location. Thus, the mean yield in African countries is about 7.02 tons/ha, with yields of 4.4, 9.4, 2.5 and 3.2 ton/ha in Uganda, Kenya, Sierra Leone and Nigeria respectively. The yields in Asia are significantly higher, mean yield of 12.41 tons/ha. Japan, Korea, China, and Israel have the highest yields with about 25.8, 16.4, 21.6, and 44.4 tons/ha respectively. It reported that in South America the mean yield is 10.74 tons/ha, with, Uruguay, Argentina and Peru in the lead with, 16.35, 13.68 and 17.2 tons /ha, respectively. In USA the average yield of sweetpotato is 20.1 tons/ha (FAOSTAT, 2007). These differences in yields are mainly due to variation in quality of the planting materials, often taken from the previous season of farmer's fields. Often these fields are usually infected with several viruses, thereby increasing the effect on yields. In China, on average, losses of over 20 % due to sweetpotato virus diseases are observed (Gao *et al*., 2000), mainly due to sweetpotato latent virus (SPLV) and sweetpotato feathery mottle virus (SPFMV). In countries such as USA and Israel, yields increase markedly, up to 7 times more because care is taken to provide virus-free planting material. In some countries, such as Kenya, Tanzania and Uganda, virus diseases are a main problem for sweetpotato production (FAOSTAT, 2007).

2.7.3 Sweetpotato virus effects on quality of storage root

According to Zhang and Salazar, (2000) the storage roots quality of sweetpotato is mostly affected by virus by either changing skin color or shape. Also, virus affects leave and tuber root quality by changing in the skin pigment and shape of storage roots (Averre *et al*., 1993; Schultheis *et al*.,1994 and Bryan, 2002;). Yield losses exceeding 70 % have been connected to the synergistic effect of SPFMV and SPCSV in Uganda (Gibson *et al*., 1998; Gibson and Aritua, 2002). Among the 11 well-characterized sweetpotato viruses, sweetpotato feathery mottle virus (SPFMV; Potyvirus) has a serious damage, while the others are geographically spread to one or more areas (Moyer and

Salazar, 1989; Mukasa, 2004). Earlier studies revealed the important benefits of virus free planting materials in terms of yield performance and storage root quality compared to non-tested material plantings from traditional farmers (Carey *et al*., 1999; Zhang and Salazar, 2000; Carroll *et al*., 2004).

Furthermore, high tendency of reinfection of healthy planting materials makes it essential for constant use of certified, virus-tested seed roots or cuttings (Ling *et al*., 2010). A comparative study of sweetpotato planting material derived from *in vitro* tissue culture technique, virus tested mericlones propagated in the screen house through nodal cuttings and vines sprouts from root adventitious have been studied (Bryan, 2002 and Bryan *et al*., 2003). This study has revealed a decrease in yield and quality of storage root after the first cycle of adventitious sprouts propagation. Furthermore, yield performance and root quality keeps on decreasing slowly. The magnificent decrease in yield performance and root quality after the first cycle adventitious sprouts propagation could be due to pathogen and virus infection and/or the increased variability due to mutation and virus accumulation in adventitious propagules (Clark *et al*., 2002). The potential position of begomo viruses is indicated in part by the study of Clark and Hoy (2006) in which they revealed that yields performance of 'Beauregard' sweetpotato reduced by 25 - 30 % by sweetpotato leaf curl virus (SPLCV) even though, the fact that no symptoms were seen on any part of the plant.

Although certain farmers might think that, selecting symptomless vine cuttings as propagating materials from cultivars that are resistant to viral disease (Gibson *et al*., 1998), and/or that uprooting and burning symptomatic plants (rogueing) from areas of production might be an efficient ways of controlling sweetpotato virus disease (SPVD) (Gibson *et al*., 2004). Deterioration in yield and quality root in sweetpotato has been due to the compilation of viruses and causing mutagens alteration of the planting materials (Clark *et al*., 2002; Dangler, 1994; Villordon and La Bonte, 1995, 1996).

2.8 Ways of sweetpotato virus transmission

There are several ways of virus transmission which can be mechanical or by grafting, by vector such as whiteflies and aphids or by through seed or a combination of both modes mention (CadenaHinojosa and Canpbell, 1981). Viral etiology diseases of sweetpotatoes have been shown to be insect, graft and mechanical transmissible or combination of the above modes (Clark and Moyer, 1988).

However, some viruses' mode of spreading has not been understood clearly due to the way of some viruses share the same vectors and others occur in more than one serotype or strain (Carey *et al*., 1996). Also (C-6) C-6 a new flexuous virus a new flexuous virus C6, SPCaLV, SPLV, SPMSV and sweetpotato chlorotic fleck virus (SPCFV) are yet to be determine whether there are transmitted by any vector, while SPFMV and CMV have been known to be transmitted by aphids and SPLCV, SPCSV and SPMMV are transmitted by whiteflies (Clark and Moyer, 1988; Carey *et al*., 1996). Mostly, infected vines and insect vector such as whiteflies are the two main source of virus transmission and perpetuation (Clark and Moyer, 1988). SPCSV is mostly limited to plant sap and is transmitted by *Bemisia tabaci* the whitefly in a semi-persistent manner (Karyeija *et al*., 2000). It can transmit or acquire SPCSV within a period of 1 hour or less and can remain viruliferous for 24 -28 hours (Larsen *et al*., 1991). SPCSV transmission in Africa can either be the cassava specific biotype of *B. tabaci* (Burban *et al*., 1992) or *B. tabaci* naturally colonizing sweetpotato (Cohen *et al*., 1992; Gibson *et al*., 1998). Also it has been revealed that *B. afer* is on sweetpotato in Africa (Legg *et al*., 1994) but test of its ability to transmit SPCSV is not yet understood and not have been reported. Although it less effective the second genus of whiteflies thus banded winged whitefly, *T. abutilonea* can also transmit SPCSV (Gibson and Aritua, 2002). It is reported that virus can be mechanically transmitted either by contact between plants or

inoculation. It can also be transmitted by grafting (Brunt *et al*., 1996). Due to the fact that virus infects sweetpotato plants systemically, it is disseminated in infected vegetative cuttings used as propagules. Plants grown from such propagules are main infection sources of crops. SPFMV is transmitted in a non-persistent manner (Ames *et al*., 1996) and most efficiently by the potato aphid (*Myzus persicae*), the 14 aphid of cotton (*Aphis Gossipii*) and less efficiently by the groundnut aphid (*A. craccivora*) and *Lipaphis erusioni* (Wanbugu 1991).

Also, viruses are transmissible by stem and tuber core grafting, but the probability of seed transmission is low (Ames *et al*., 1996). It has been transmitted to *l.nil* by sap inoculation from symptomatic tissue (Moyer and Cali, 1985). SPFMV is perpetuated from one cropping cycle to the next through planting materials (vines) which facilitates its movement and multiplication, since it can also have transmitted through vegetative propagation. SPMMV is transmitted by whiteflies (*Bemisia tabaci*) and biotypes of *Bemisia tabaci* may differ in their transmission ability (Wanbugu 1991). The virus can be easily transmitted mechanically to susceptible sweetpotato clones and test plants in the *Solanaceae* and *Convolvulaceae* families. It is also transmitted by grafting (Wanbugu 1991).

2.9 Magnitude of yield decline due to sweetpotato virus

Some crop species transmit virus from seasonal to seasonal because planting materials are generated from previous plants. An example of such crop species are sweetpotato, potato and cooking bananas that safeguard food safety in many parts of the world are propagated vegetatively and are therefore particularly disposed to damages caused by viruses (Hadidi *et al*., 1998; Loebenstein *et al*., 2001; Loebenstein and Thottapilly, 2003). China is the highest producer of

sweetpotato (82,474,410 tonnes) and making Asia the leading producer of sweetpotato in the world (FAO, 2012).

In Africa, Nigeria (2,883,408 tonnes) ranked first after Uganda while Ghana produces 90,000 tonnes on 65,000 ha area (FAOSTAT, 2006). In Ghana, sweetpotato is produced exclusively by peasant farmers. Consequently, the possible support of this crop toward food security in Ghana is underestimated as there is a huge gap between potential yield and the yield of peasant farmers (CRI, 2002). In the tropical countries, SPVD is reported to be the most destructive disease of sweetpotato (Gibson *et al*., 1998; Carey *et at*., 1999). It causes huge decrease in both storage root performance vine yield of the sweetpotato plant, generally reducing yield by 50 to > 90 % (Gibson *et al*., 1998). Virus diseases are found to be most essential constraints for sweetpotato (*Ipomoea batatas* (L.) Lam.) Production, more than half of the yield reduction is reported to be due to sweetpotato virus (Gibson *et al*., 1999, Ngev, 1991).

Studies have revealed that, over 20 or more viruses infect sweetpotato and out of that only very few have been recognized and characterized (Brunt *et al*., 1996, Mayer *et al*., 1989). Yield reduction that is attributed to sweetpotato virus is ranged between 15 – 48 % in china and 34 –97 % in Egypt (Salazar and Fuentes, 2000), 50 % or more in Israel (Milgram *et al*., 1996) and 80 – 98 % in East Africa (Mwanga *et al*., 2002). It has been reported that the most prevailing sweetpotato virus worldwide is the sweetpotato feathery mottle virus Genus Potyvirus (SPFMV). In Japan and USA certain isolates brought much economic destruction by causing cracks induction in some varieties. In Africa, SPFMV causes a severe sweetpotato virus disease (SPVD) in a complex infection with the sweetpotato sunken vein virus (SPSVV). Most sweetpotato cultivars infected by SPFMV alones however only cause mild circulars pots on their leaves or some light green patterns along veins (Mwanga *et al*., 2002).

However, when SPFMV infected sweetpotato plant along with the whitefly-transmitted SPCSV stunting of the plants, feathery vein clearing and yellowing of the plants are observed. It has been revealed that, in a controlled experiment, SPFMV-infected sweetpotato did not show any differences in yield performance when compare with the *in vitro* cleaned plant, but the complex infection with SPCSV cause reduction up to 50 % or even more (Gutierrez *et al*., 2003; Milgram *et al*., 1996). Also individual effects of SPFMV or SPCSV on yields performance are reported to be negligible or close to 30 % losses, but in infection complex with SPFMV or other viruses yield losses of 50 % and more are observed (Milgram *et al*., 1996; Gutierrez *et al*., 2003; Untiveros *et al*., 2007). SPCSV and/or SPSVV are transmitted by different strains of whitefly *Bemisia tabaci* biotype B, *Trialeurodes abutilonea*, and *B. afer* (Valverde *et al*., 2004 and Gamarra *et al*., 2008).

2.10 Source of sweetpotato virus

Viral disease is said to be the main problem of sweetpotato production. Due to the fact that sweetpotato is a vegetative propagated plant, it is prone to viruses' perpetuation and accumulation which leads to reduction in yield performance (Loebenstein *et al*., 2004). Virus diseases usually cause deterioration in yield performance and storage roots quality (Clark and Moyer 1989). In US, yield reduction is recorded to be between 30 – 50 % in farmers' fields due to virus but drastic reduction of 80 - 90 % have been recorded (Clark and Hoy, 2006). The most essential sources of sweetpotato virus infection is the source of planting materials. Uncleaned planting materials such as infected vine cuttings or even infected roots used for planting materials from the previous season.

Although cleaned planting materials can be easily reinfected by some aphid or whiteflies virus transmitters (Moyer and Salazar 1989; Valverde *et al*., 2004). Mostly sweetpotato varieties that

are released for about 20 years ago usually deteriorates slowly in yields due systematic accumulation of virus (Clark *et al*., 2002). The degradation of cultivars over time after release is due to accumulation of virus and other pathogens. The sweetpotato virus that brings about deterioration are all not determined. In Africa, SPCSV is mostly in synergy with other virus such as sweetpotato feathery mottle virus (SPFMV, genus Potyvirus) and the sweetpotato virus disease (SPVD) causes quick deterioration of cultivars (Gibson *et al*., 1999; Karyeija *et al*., 2000).

In most of the mild temperate climates countries such as United States where sweetpotato is cultivated, producers save seed roots from each crop in every cropping season. In early spring, root seeds are planted in beds, and newly sprouts from bedded roots are cut and transplanted to the field to produce the sweetpotato crop (Wilson and Averre, 1989). This method of propagation is not advantageous due to virus accumulation and mutagens alteration acquired by sweetpotato plants from the previous growing season will be present in the storage roots, and consequently, in the adventitious sprouts produced. Sprouts of the adventitious root of sweetpotato, which are used as sweetpotato seeds have higher variability phenotypically than nodal stem cuttings seed material of sweetpotato, and this may attribute to the deterioration in yield and root quality of sweetpotato (Wang and Valkonen, 2008).

2.11 Methods of controlling sweetpotato virus

In this recent time the most effective way of controlling viral diseases is by providing farmer or grower with plantlets that are virus free. Such propagated materials can be obtained from meristems shoot tip cultures, also in combination with cryotherapy (Wang and Valkonen, 2008). Such propagated materials are generated in Israel and in the Shandong province of China (Gao *et al.,* 2000). Due to the fact that pathogen tested planting materials are used in Israel, sweetpotato yield performance increased up to 100 %, whilst in China increment is ranging between 22 – 92 %

because of the usage of virus free planting materials. The use of chemical control of virus have been reported to be costly and not even effective way of controlling the virus (Wang and Valkonen, 2008).

However, the effectiveness of controlling sweetpotato feathery mottle virus to control Russet crack disease with the use of a mild strain was reported to be effective (Mwanga *et al*., 2002). Using pathogen tested planting materials is expensive so in Israel the use of certified material is very common practice, whilst China extended the use of virus free planting material. However, in African countries production of virus free planting materials are usually in very limited quantities. Also the used of some cultural practice can be an effective measure to control viral diseases. For examples, of such cultural practices include destroying (rogueing) of diseased plants selection of disease-free planting material, and wild *Ipomoea spp*, especially in young crops, isolating new crops (15 – 20 m far) from old diseased crops, destroying crop residues, and protecting crops with barriers or intercropping with maize (Gibson and Aritua, 2002).

Breeding to produce virus resistant cultivars could also be the future answer and such breeding agenda are in operation in Uganda, combining SPVD resistance with desirable agronomic traits such as yield, earliness and acceptable culinary quality (Karyeija *et al*., 2000; Mwanga *et al*., 2002). Progress has been made and several viral resistant cultivars have been released (Karyeija *et al*., 2000). Several trials have been performed on the viral resistant cultivar released at different place to confirm the cultivar will retain resistance. Thus, many of the clonal cultivars that were resistant to SPFMV in CIP's tests were found not to be resistant, when Israeli and Ugandan isolates were tested (Karyeija *et al*., 1998).

Several programs have been used as an attempts to manage the most difficult problem in sweetpotato production, the viruses are relatively recent, and mainly involve either use of 'clean seed' or resistant cultivar or programs. Resistance is an attractive option for disease management

as it generally does not require significant expenditures by the farmer. In the case of sweetpotato, there have likely been unpremeditated gains in progress of virus resistance as growers and breeders both want to selected for good yield, and/or mild symptoms, in plantings that are exposed to natural virus infection, but this has not been recorded. There have also been great achievements in producing cultivars that are resilient to certain symptoms development, such as internal cork or russet crack, although there is no much of information on the etiology of these disorders (Clark and Moyer, 1988).

Even though, efforts embattled at true combat to specific viruses or virus complexes are relatively recent. Earlier efforts to grow virus opposed sweetpotato is focused on SPFMV due to the fact that it spread worldwide. Cultivars that have been labeled as resistant to SPFMV in different countries and used as graft immunization techniques to study resilience to SPFMV (Owour, 2000). They found important general combining abilities but no major specific combining abilities and suggested that additive gene action is important in resistance to SPFMV. Moreover, cultivars described as resistant to SPFMV in areas such Peru are found not to be resistance in areas like East Africa (Mwanga *et al*., 1991; Gibson *et al*., 1998; Karyeija *et al*., 1998; Mwanga *et al*., 2002). Further studies have showed that there is not only a diversity of potyviruses infecting sweetpotato worldwide, but considerable diversity within what has been called SPFMV (Kreuze *et al*., 2000), which greatly complicates efforts at developing resistance. There are also different serotypes of SPCSV, and sweetpotato varieties found to be resistant to SPVD in Nigeria, where the West African serotype of SPCSV predominates is vulnerable in Uganda where the East African serotype is principal (Mwanga *et al*., 1991; Alicai *et al*., 1999; Carey *et al*., 1999). There can also be substantial assortment of viruses within a location, as has been establish for SPFMV, SPCSV, and SPMMV (Mukasa *et al*., 2003; Tairo *et al*. 2005). Tairo *et al*. (2005) deliberate the consequences of variability of sweetpotato viruses for efforts to breed for resistance.

The essential point is that it is vital to assure that resistance is satisfactorily full to give protection from local strains. Furthermore, Karyeija *et al*. (2000) confirmed that infection with SPCSV overcomes resistance to SPFMV. In East Africa, many growers produce landraces that have been shared from time to time. Even though, SPVD is a factor that is limiting sweetpotato cultivation in sub-Saharan Africa (SS. A), the impact of the disease is mitigated by use of resistant cultivars and landraces selected from local germplasm (Karyeija *et al*., 1998). A fascinating idea for managing SPFMV involves the introduction of a rice cysteine-inhibitor gene. This gene hinders the proteolysis of the viral poly protein, thereby inquisitive with viral duplication. Amended resilience to SPFMV was detected in 18 of the 25 transgenic lines after difficult vaccination with the russet crack strain of SPFMV (Cipriani *et al*., 2001).

However, when stem cuttings are arranged from the accepting transgenic plants and grafted with healthy *I. setosa* scions, virus symptoms appeared on the scions. Speciously, the mutagen sweetpotato plants still contained some virus. When non-indigenous cultivars, such as highyielding North American varieties are grown in SSA, most plants quickly develop SPVD signs and their yield is radically reduced (Aritua *et al*., 2000). By contrast, plantings of locally adapted varieties normally have a lower proportion of plants with SPVD indicators (25-30 % reported by Aritua *et al*., 1998), the symptoms appear later, plants may recover from SPVD, and the yield reduction attributable to SPVD is not as great. Sweetpotato is an in determinant plant without a defined physiological maturity, and as such, storage roots may continue to enlarge for a long time. Regrettably, many of these varieties are low yielding (Gibson *et al*., 2000), producing a satisfactory yield only after very long production periods, and it has been said that 'Extensive' use of less fruitful SPVD-resilient development of SPVD-resistant landraces with improved yielding ability.

There thus remains a need for improved SPVD resilient genotypes that produce acceptable yields in a smaller period, joint with other desired features. Landraces may be the most damaging extent of SPVD (Gibson *et al.*, 2004).

2.11.1 Thermotherapy as a method of control sweetpotato virus

Thermotherapy treatment is a process of putting plants, or more frequently a part of the plants, in a temperature condition range within 35 °C and 54 °C, and maintaining physiological tolerance limits of the plant, for a recommended period. Practically, the recommended temperature represents the optimal condition between virus deterioration and plant survival, considering that plants have higher thermal threshold than virus. Sensitivity of plant to heat is lower than some virus and that thermal treatment will destroy the virus in the plant sap whilst the plant cell still remains healthy due to the fact that plant tissues can be more easily be reversed than viral damage (Spiegel *et al*., 1993). Thermal treatment can be applied to cause phonotypical alterations for example double nodes and modified leaf shape (Koruza and Jelaska, 1993). Also it is reported that, specific effects were found in grapevine, such as an increment in grape quality (Mannini *et al*., 1996) or in phenolic concentration in leaves and berries (Guidoni *et al*., 1997).

2.11.2 Resistant varieties as a method of virus control

Approaches for controlling disease such as traditional measures, phytosanitary methods, control of vectors and arrangement of genetic resistant to avert or limit the extent of damage have been suggested. Among these, use of disease resistant cultivar is an ultimate choice in terms of efficiency and sustainability for controlling any plant disease in universal and sweetpotato virus disease (SPVD) in specific (Maule *et al*., 2007). The use of virus resistant sweetpotato cultivars to

reduce the effect of SPVD under farmer's field has been recorded by Miano *et al*. (2008). Breeding of SPFMV resistant plants is initiated by CIP (Owour, 2000). Several clones that were found to be resistant to SPFMV in CIP's tests were found not to resistant when exposed to Israeli and Ugandan isolates (Karyeija *et al*., 1998). Actually, worry diversity requires that breeding and selection have to be done in various locations. On the other hand, a substantial number of African sweetpotato landraces have resistance to this virus (Carey *et al*., 1997). Another approach was the development of transgenic sweetpotatoes with coat mediated (CP) fighter to SPFMV (Okada *et al*., 2001). Also, CP-mediated introduced resistance into several African genotypes and cultivar CPT-560 was evaluated in Kenya in a cooperative project between Monsanto Co., USA and Kenya Agriculture Research Institute (KARI) (Qaim, 1999).

However, these transgenic lines were not resistant to the 'complex' infection with SPCSV, causing the SPVD (Wambugu, 2004). Transformation of sweetpotato plants with viral coat protein genes may impart a coat protein-mediated resistance to SPVD. In 2001, twelve lines of sweetpotato variety CPT 560 transformed with the SPFMV coat protein gene were field tested under controlled conditions in four important sweetpotato growing agro-ecologies in Kenya. Based on virus resistance, yields of storage roots and vines, four transgenic lines were selected and evaluated further in 2002. Results from these field trials did not provide an adequate level of SPVD resistance as expected based on visual assessment of symptoms and tissue printing bioassays. Possible explanations for this unsatisfactory level of protection may be due to synergistic effects of other sweetpotato viruses in the environments where the lines were tested. Secondly it is possible that the first generation gene constructs were not efficient against the Kenyan strains of the virus since they were developed based on American strains of the virus. The second generations of transgenic plants are currently being developed at the Kenya Agricultural Research Institute (KARI) Biotechnology center and are at various stages of development. These have improved gene

constructs developed using coat protein mediated resistance (CP-MR) for SPFMV control is a form of genetically engineered resistance, whereby the expression of viral coat protein genes in the transgenic plants induces a cross "protection-like'' phenomenon that confers resistance to the virus from which the gene was initially derived as well as to related viral strains (Beetham and Mason, 1992). It is still largely unclear what the molecular mechanisms of CP–MR are although some theories have been proposed. Monsanto donated the gene constructs and initial research on genetic transformation of six Kenyan sweetpotato varieties against SPFMV was done at Monsanto laboratories in the US. At the initial stages of the project, only one of the six sweetpotato varieties, CPT 560, was readily transformed with the donated constructs (Gibbons, 2000). The actual transfer of the transgenic sweetpotato technology from Monsanto to KARI took place in the year 2000.

2.12 Impact of *in vitro* tissue culture on sweetpotato storage root yield

The first appropriate steps of accurate mechanism of viral diseases management is strategic indexing. Due the fact that sweetpotato is vegetative propagated plant (Lepoivre, 1998) meristematic propagation has many returns such as of disease-free planting materials production in huge numbers therefore permits rapid distribution of disease free and better quality planting materials within and among countries, as the materials are readily qualified as healthy (FAO, 2003) and produces consistently hence, they are highly marketable (Vuylsteke and Talengera, 1998) In Brazil, yield increment of 118% are been experienced when cleaned diseased free planting materials derived from meristems of heat treated plants are compared to yields from planting materials taken from fields (Pozzer *et al*., 1995). This is an exceptional finding where SPFMV alone noticeably reduced yields, and it could be that another virus could have been existent.

Furthermore, higher yielding capabilities of virus free plantlets makes the need to use certified seed an endless option, the diseased free planting materials (Ling *et al*., 2010). Plant meristem culture is a unique technique to remove disease pathogens from planting materials. Even without visible symptoms, infected plants exhibit reduced growth and yield performance reduction, and could spread the disease to non-target varieties. SPVD epidemics have been, in many cases, associated with the disappearance of a former elite cultivar (Gibson *et al.*, 1998). Yield loss due to viral diseases was estimated to be 15–48 % in China, 34–97 % in Egypt (Fuentes and Salazar 2000), 50 % or more in Israel (Milgram *et al.*, 1996) and 80–98 % in East Africa (Mwanga *et al*., 2002; Wambugu, 2004). Quality was also affected by alterations in the shape and skin color of sweetpotato storage. Plant meristem culture is a unique technique to free away various pathogens including viruses, viroides, mycoplasma, bacteria and fungi (Bhojwani and Razdan, 1996). Meristems are frequently devoid of systemic pathogen due to the absence of differentiated conducting tissues. In addition, the use of planting material derived from pre-existing meristems has been proposed to reduce the amount of variation among the propagules and to retain genetic integrity (Villordon and LaBonte, 1996). Therefore, its application may help to slow down the process of cultivar decline due to accumulation of viruses and mutations. Reports have been published on successful meristem culture and virus indexing in sweetpotato and other crops over two decades (Alam *et al*., 2004). Nevertheless, sweetpotato improvement through virus-indexed mericlones is important for unlocking yield potential of diversified elite genotypes grown under various agro-ecological zones and cultural practices by using disease-free and uniform propagules. However, sweetpotato has a very wide genetic base and highly heterogeneous tissue culture response. Therefore, improvement of diverse elite cultivars grown under various agro ecological zones and traditional measures through virus indexed and uniform mericlones is important for revealing yield potential. Virus detection is a routine work for virus-free planting material

production and safe undertaking of germplasm. Serology or other molecular analyses are expensive for many developing countries. Ipomoea setosa is a nearly universal sensitive indicator plant for sweetpotato viruses, which is used for graft conducted virus revealing. Recent international policies manuscript show that graft indexing effectively disclosed most sweetpotato viruses (Moyer *et al*., 1989; Laurie *et al*., 2000; Loebenstein *et al*., 2003; Mukasa *et al*., 2003). The use of meristem tissue propagation methods, which eradicates viruses through meristematic tissue of tip, has been combined into a number of state sweetpotato seed certification programs to produce high excellence virus tested seed standard for growers (Dangler, 1994). An explanation which makes it possible to yield larger numbers of equal, healthy plants is meristem culture. Sweetpotato can be propagated by stimulating the increasing of apical and Axillary buds' development, by somatic embryogenesis and by means of adventitious buds (Gosukonda *et al*.,

1995; González *et al*., 1999; Mukherjee, 2002). The methods which are paramount suitable for the growing of plants for profitable plantations are those which use stem with buds. When such methods are used, the shoots develop from meristems and somaclonal variation is sporadic (Larkin and Scowcroft, 1981). According to results, the yields of meristem cultured sweetpotatoes are higher (1.8 tons per hectare) than outmoded sweetpotato cultivars (0.5 tons per hectare). The yield levels for meristem tissue cultured sweetpotatoes are smaller than the Zimbabwean national mean yield of 6 tons per hectare. These results also vary from those of Moyo *et al*. (2004), who found that sweetpotato yields ranged from 10.2 to 14.0 tons per ha in Nkhata Bay of Malawi. The variations in discoveries could be ascribed to the fact that most smallholder farmers grow sweetpotatoes under dry land conditions. However, the ability of tissue cultured cultivars to increase output are confirmed in Peru by Fonseca *et al.* (2003), who showed that tissue cultured varieties had various advantages, such as higher yields. Additionally, the National Agricultural Research Institute in the Valley of Peru introduced meristem cultured cultivars and farmers desired the worthy and profitable value of it. Smallholder farmers rarely apply chemical manures in their sweetpotato cultivation (Moya, 2004).

2.13 Growing under ambient temperature

Sweetpotato cuttings that are generated is adaptable to different conditions depending on environmental. Soil and air mean temperatures are the most underrated possibly cause of transplant shock and meager formation of storage root in the sweetpotato (Belehu, 2003).

Mostly in high atmospheric ambient temperature without satisfactory cooling from extra water source can cause a theatrical impact on adventitious root formation and development leading to

low general yield (Coleman *et al*., 2003). High ambient temperatures are inhibitory to storage root formation (Sato, 1981).

This crop although, is domesticated in the sub-tropical, tropical and warm temperate areas of the world, it is essentially a warm adapted crop (Onwueme, 1978; Bourke, 1989). The thermal optimal is further than 24°C with the vacillating temperature between 24 to 28°C most appropriate for early root and shoot formations and development (Belehu, 2003).

2.14 Temperature effect

Sensitivity of sweetpotato to low temperatures is much more serious. Because the plant is a tropical crop, it might not survive at temperatures lower than 12 °C (Belehu, 2003); at 15 °C, he revealed that plants were able to survive but growth was very poor. The plant growth rate increased with increasing temperature up to 35 °C, but the crop development was severely suppressed when the temperature reached 38 °C. Ravi and Indira (1999) established that less than 15 °C, the storage root development was suppressed whereas air temperatures above 30 °C increased oxidase activity of indole acetic acid which caused drop in storage root formation and growth.

Villordon *et al*, (2011; 2010) emphasized that soil temperatures at 30 °C and greater in the upper 10 cm of the soil profile during the root establishment and storage root initiation phases reduced adventitious root counts and storage root yields. Research by Pardales *et al*, (1999) has reinforced this and has showed that root area temperatures of 40 °C or higher have greater effects on length and formation in adventitious roots. This may also have a more impact on the deeper nodes at lower soil temperatures.

In developed countries, sweetpotato is commercially grown as a highly valuable vegetable under thorough managed production schemes. Therefore, farmers are forced to produce greater yields and unvarying storage roots which are attractive for markets, whilst under subsistence farming

food security is a main priority. Even though, storage root yields are unpredictable from plant to plant. Some plants have few or no storage roots while others yield more marketable roots. Storage root yield is dependent on both the number and size of the roots being produced.

According to Wilson (1992), the number of storage roots that a plant will bear is strongminded very early in the cropping cycle. Bourke (1991) has used a sigmoid curve shape of the storage root growth in Papua New Guinea with a very slow growth at an early stage.

Among promulgation material other factors that cause wide inconsistency in yield of sweetpotato include, variety, soil factors and environment (Bourke 1991). The interaction among environmental and genetically factors influences leaf size and formation, abscission, leaf photosynthesis, dry matter production and partitioning, storage root formation and development (Ravi and Indira, 1999).

Soil as well as air temperature remain the two most influential factors in the formation and development of sweetpotato storage roots (Ravi and Indira, 1999). A study by Bourke (1991) showed that plants grown with less than 2.5 % oxygen in the root zone produced more fibrous roots than plants grown with 21 % oxygen in the root zone with only 10.9 % fibrous roots. This has also been supported by Wilson (1992), who discovered that dry and compact soil cause serious disadvantage to storage root growth and development. Under field conditions, high soil temperature leads to transplanting shock and poor storage root development. Extreme high temperature without adequate cooling from irrigation can cause leaf loss and can have an intense impact on adventitious root progress (Coleman *et al*., 2003). Research by Pardales *et al*, (1999) has specified that root zone temperatures of 40 °C or even higher have greater effect on length and development in adventitious roots. This may also have higher impact on the deeper nodes at lesser soil temperatures.

Whilst great soil temperature will remain a main problem in tropical weather, farmers may need to consider optimizing soil temperature to improve sweetpotato storage root formation and development. The overview of plasticulture worldwide has proved fruitful in vegetable crops and the sweetpotato industry. Coloured plastic mulches are used extensively in commercial sweetpotato production, other mulch types are locally available to manipulate high soil temperature regimes. Great soil temperature has been associated in initial root establishment which affirms that although sweetpotato is a warm loving crop, extremely high soil temperature is harmful to its yield.

Trials conducted by Coleman *et al*. (2006) recorded soil temperature at various depths but data collected was not analyzed due to unreplicated samples.

In another experiment under a modified controlled temperature, it is evident that the information was limited due to a shorter growing season. The evidence gathered, therefore, will help to address the increasing soil temperature under field conditions in the tropics. Given that the crucial stage of growth of sweetpotato is between 1 to 7 days after planting, options for use of mulch are very crucial (Coleman *et al*., 2006).

2.15 Propagation

Sweetpotato usually treated like an annual crop but it is a perennial plant. It is traditionally planted by vegetative propagation. The vine cuttings or portion of stem are from both terminal, the growing tip and basal- sections of the runners. The slips or sprouts produced from storage roots prove outstanding material for planting for commercial farmers worldwide (Khan *et al*., 2008).

Vine cutting for field planting is selected from vigorous growing, healthy plants. Mature plants are considered less active and should never be selected as propagule materials. This may not be avoidable where there is no specialized system to produce planting material. This is a popular

practice for sweetpotato farmers in the developing countries. In the Melanesian society planting materials are accessed from old plants or other growers as well as from self-seeded plants as reported by Tjintokohadi *et al*. (2007) in Solomon Islands. In PNG, the vegetative planting material is collected from established gardens that are 8 – 12 months old (ACIAR, 2009). In this way, the risk of spread of diseases and pests to new location is increased.

Commercial practices: Storage roots have been the main source of diet. It is now commercialized in temperate regions and there is important propagation material worldwide (Khan *et al*., 2008).

Supply of large volumes of propagule materials from storage roots provides the best alternative for large to small scale operators throughout the world. In the USA where this practice has been adopted since the early 1970s, growers' cost, diseases and pest risks have reduced (Lebot, 2010).

Storage roots have a number of advantages over cuttings: these include, the longer period of storage and, root selection can be from shapes and sizes which better maintain genetic integrity thereby maximizing the subsequent crop's potential yield. Slip cuttings from storage roots may be produced from a maximum of six harvests depending upon cultivar, root size and vigour (age) of the bedding storage roots (North Carolina Sweetpotato Commission, 2011).

Selection of storage roots that are true-to-type and free of disease is important to raise health sprouts. Root size may vary from field selection with the smallest size reaching 20 mm to 39 mm

in diameter; however, the North Carolina Sweetpotato Commission report (2011) suggested that small roots produce a similar numbers of sprouts to large roots; each root can produce up to 15 plants, with as many as six sprouts growing on each root at one time.

Normally storage roots are treated immediately after harvest to heal possible injuries from harvest and minimize storage losses. The roots are kept away from excessively moist conditions as the curing process allows wounds to recover most rapidly at 26 °C to 32 °C. Relative humidity of 85% to 90% with sufficient ventilation is necessary. Wounds and bruises heal and a protective cork layer develops over the entire root surface. The cork layer and suberin act as a barrier to decay causing organisms and to moisture loss during storage (Hall, 1994; Motes and Criswell, 2012). Curing may last one to three weeks but a longer more extended period would reduce sprout emergence, enhance shrinkage, and shorten storage life in non- refrigerated facilities (Steinbauer and Kushman, 1971). Presprouting enhances early shoot harvesting, vigour and produces 2 to 3 times as many plants. Fertilizer application, use of mulch and irrigation encourages early sprouting. Normally shoots begin to appear within 4 -6 weeks after sowing, and harvesting of sprout is done when the shoots are 250 to 400 mm long. Second pruning can be earlier than 15 days after the first. Presprouting for a period of 7 - 10 days increased sprout emergence (Hall,
1993). Yield has been significantly preserved using slips produced from roots (Clark *et al*., 2002).

In the USA and other mild temperate climatic regions, farmers saved roots from each crop for future planting during the off-season. In Australia during the previous decade, storage roots have exchanged stem cuttings for farmers (E. Coleman, 2011).

2.16 Soil type

Sweetpotato is able to dwell on a wide range of soil types, from light sandy loams to medium clays. It is grown on all major landforms in the Pacific regions including Australia except those subject to long-term inundation (swamps) (Bourke, 2009). Soil fertility is preserved by natural fallows. This practiced is very commonly but with the tradition of slash and burn, the soil is quickly degraded and becomes less fertile. Crop rotation with the inclusion of other crops such as legume (eg; groundnuts), may not adequately supply satisfactory nutrients for the next planting. Bourke (2009) established the nitrogen fixing tree (Casuarina oligodon), green manuring, coffee cherry pulp and chicken manure help enhance soil fertility in sweetpotato fields.

2.17 Transplanting

Sprouts are normally planted vertically with the base 5 to 10 cm below the soil surface. Stem cuttings are usually planted vertically, V-shape and horizontally (flat) with 3 to 4 nodes or almost two-thirds of the stem cutting length under the soil surface (Onwueme, 1978; Lebot, 2009). Planting horizontal to a depth of 10 -15 cm with longer stem cuttings of 30 cm optimizes marketable storage root numbers (Holwerda and Ekanayake, 1991; Coleman *et al*., 2006).

Sweetpotato cuttings can be held 48 – 72 hours after cutting to promote root initiation prior to planting, hence, reduce transplanting shock. The leaves should be removed leaving only a few at the tip and the vines wrapped in a wet sack or cloth. They should be kept in a cool, moist and shady place. This technique may also encourage root progression for ease of plant growth and establishment but may also have a damaging effect on yield if the newly developed roots are damaged during the planting process (Coleman, 2006). The crops are usually planted in mounds, ridges or rows with a plant spacing of 20-40 cm but, according to Lebot (2009), cultivars with

wide canopies and sprawling stems are planted at wider spacing than cultivars with gathering type or less disperse canopies.

For earlier harvesting a plant spacing of 30 cm was considered optimum and gave greatest economic achievement (Coleman *et al*., 2006). However, plant density affects yield as well as yield percentage in various size grades.

For a higher plant density, canopy development is rapid with potentially greater yield. Farmers who are into commercialization in Australia transplant as many as 45,000 plants per hectare (E Coleman, 2011). Commercial farmers in China, transplant 40,000 to 50,000 plants per hectare (Bouwkamp, 1985). The duration from planting to harvesting varies depending on factors associated with local conditions and practices. Lebot (2009) established that cuttings from the middle and base of the plant can be used but may produce lower yields.

2.18 Irrigation

Farmers in Africa as well as Ghana hardly practice Irrigation in sweetpotato cultivation. The crop survives and produces economic yields on natural rainfall at planting and during growth stages. In the case of excess water and flooding, the use of mounds or ridges prevents plants from being waterlogged. Soil moisture at planting is critical for the initial establishment and development of adventitious roots within the first 3 to 7 days after planting (Villordon *et al*., 2011). Belehu (2003) established that better root growth was achieved between 12 and 20 days after planting from cuttings planted at 80 % of soil field capacity. Gomes and Carr (2003) investigated the effect of water availability and vine harvesting productivity and suggested that between 360 and 800 mm was required for sweetpotato. Excess water is detrimental to growth due to poor aeration. This has been further confirmed by Coleman *et al.* (2006) who established trickle and/or overhead sprinkler

application to be an effective means to irrigate within the first 7 days for early plant establishment and storage root bulking.

2.19 Harvesting

The harvesting time of sweetpotato is very much dependent on cultivar and environmental weather conditions. Sweetpotato is ready for harvesting between 4 and 8 months as compared to other root crops, but most cultivars are harvested within 4 to 5 months after planting (Lebot, 2009; Onwueme, 1978). In the tropics, sweetpotato cultivars usually takes 140 to 180 days to mature compared to 80 or 120 days after planting in temperate regions (Khan *et al*., 2008), this could also be due to the high performing, early maturing varieties generally planted in temperate regions.

According to Lebot (2009), in the tropics, most varieties are harvested as the roots reach marketable size. Lebot (2009) showed that immature plants produce mostly green leaves while yellowing of mature leaves shows maturity of storage roots. This can also be observed from the storage root sap that rapidly turns black and sticky when dry and the rejuvenation of new shoots from the storage roots if harvesting is delayed. Injury to the roots by insects including sweetpotato weevil, (*Cylas spp*), diseases and rat may also increase crop losses if storage root are left too long in the field.

In commercial farming systems, a rotary or flail-type mower is used to remove vines at the base before a double mouldboard plough or modified potato harvester is used to bring roots to the surface. Where sweetpotato is grown mainly for home consumption, staggered harvesting is the normal practice (Lebot, 2009). This allows for the smaller roots to also be harvested later.

2.20 Storage

Matured sweetpotato skin is very delicate and easily damaged: therapeutic sweetpotatoes hardens and seals the skin and therefore reduce future infections, reduces desiccation as well as increases visual appeal. In temperate conditions the harvested roots can be stored for a longer period (Boyette, 2009). This process permits healing of cuts, bruises and skinning on storage roots. A curing period may last 3- 7 days under 30 – 32 °C and 85 - 90 % relative humidity (Boyette, 2009).

Temperatures above 16 °C and high humidity encourage sprouting, pithiness and poor appearance (Lebot, 2009; Padda and Picha, 2008). The temperatures below 12 °C can cause chilling injury which will result in weight loss, internal breakdown, off flavours, and rot. It also results in uneven cooking of roots that can have unpleasant texture. Storage roots that are properly cured and are free from disease or other physiological problems will store for as long as 13 months and remain marketable under these conditions.

2.21 Sweetpotato virus

2.21.1 Major virus diseases in sweetpotato

Sweetpotato viruses are among some of the overwhelming diseases in the world. At least twentytwo viruses are known to be pathogens of sweetpotato of which more than ten types are recognized to cause damage to the sweetpotato industry worldwide (Ling *et al*., 2010; Wang *et al*., 2010). The economic losses due to these virus diseases are significant. There are several major viral diseases in sweetpotato but only a few have been studied and identified (Kokkinos and Clark, 2006).

The presence of sweetpotato feathery mottle virus (SPFMV) has been reported in Australia as early as 1967 (Smith *et al*., 1967), but was sighted in 1993 and 1994 (Hadidi *et al*., 1998). In China

for example, only three virus diseases were accountable for yield losses of 20 – 30 % in sweetpotato regions between 1988 and 1991 (Valverde *et al*., 2008). Only six viruses have been reported in Australia (Jones and Dwyer, 2007; Hughes and Dennien, 2013). Similar viruses have also been seen in Solomon Islands and Papua New Guinea (Tairo *et al*., 2006). Sweetpotato virus 2 (SPV- 2) (Tairo *et al*., 2006), sweetpotato chlorotic fleck virus (SPCFV), (Jones and Dwyer, 2007), and sweetpotato caulimo-like virus (SPCaLV) (Brunt *et al*., 1990). Sweetpotato virus G (SPVG), Begomovirus and various virus complexes involve these viruses (Hughes and Dennien, 2013). The cultural practice of vegetative propagation provides an efficient way for viruses to be perpetuated and disseminated between cropping seasons or growing areas (Salazar and Fuentes, 2001).

The symptoms of sweetpotato virus diseases are: chlorotic spots, sometimes surrounded by purple areas on leaves, mottled, curled, wrinkled and yellow leaves and split storage roots (Valverde *et al*., 2008).

Various methods of eliminating virus disease of sweetpotato have been developed. These include meristem-tip culture and thermotherapy (El-Far and Ashoub, 2009; Valverde *et al*., 2008; Wang *et al*., 2010) and the use of more enlightened technology. Thus, the use of virus- tested plants can improve cultivar productivity by as much as 81 – 224 % (Clark and Hoy, 2006). However, the yield of virus-tested clones normally decreases progressively with successive plantings seasons as a result of re-infection by viruses. Hence, an effective production and operation system is needed to frequently replenish planting stock (Feng *et al*., 2000).

2.21.2 Sweetpotato Feathery Mottle Virus (SPFMV) Disease

Due to the existence of many different strains and symptoms induced by these strains differ, many names have been used to describe the virus. The names include internal cork virus, sweetpotato leafspot virus, sweetpotato ringspot virus, sweetpotato virus A, and russet crack virus (Moyer and Salazar, 1989). SPFMV was first categorized and described in 1978 but until 1998 SPFMV was the only virus reported in the USA (Moyer and Kennedy, 1978). Sweetpotato feathery mottle virus (SPFMV), infects sweetpotatoes worldwide (Feng *et al.*, 2000) and is the most common virus found in the United States. It is a member of the Potyvirus genus and Potyviridae family.

The most distinctive symptom of the virus, irrespective of strain, present is the cholorotic feathering of the leaf midrib and, in some genotypes, the expression of chlorotic spots with purple rings (Moyer and Salazer, 1989).

SPFMV is aphid and graft-transmissible and often found in mixed infections which often shelter the presence of other viruses in sweetpotato, especially those belonging to the same family, such as sweetpotato virus G (SPVG) and Ipomoea vein mosaic virus (IVMV), making the effort to detect or isolate them specifically very difficult (Souto, *et al.*, 2003). This has caused much confusion in earlier work, since findings were merely based on symptoms, host range and transmission (Moyer and Salazer 1989).

Identifying SPFMV, like other sweetpotato viruses is a difficult activity. There has been new technology advanced over the years to detect the presence of viruses on sweetpotato crops but the more traditional method was the use of indicator plants, *I. setosa* and *I. nil spp*. Kokkinos and Clark (2006) have developed a real-time polymerase chain reaction (PCR) assays to detect and quantify single and complex reactions of the potyviruses. It has been useful to detect SPFMV and other

symptomless viruses in plant tissues due to inhibitors in sweetpotato that interfere with other types of assays, or low virus titers, or both.

Serological reactions may be predisposed by inhibitors in sweetpotato such as latex, polyphenols, and polysaccharides, and may explain the difficulty of detecting these viruses in symptomless tissues by enzyme-linked immunosorbent assay (ELISA). However, the inherent difficulty in isolating and detecting SPFMV as well as other viruses directly from sweetpotato is reflected in the fact that the majority of studies used indicator plants such as *I. setosa*, *I. nil*, and others for the indirect isolation of these viruses. Even though, at 3 weeks after inoculation, the titres of SPFMV were not different among the three hosts, the titre levels of potyviruses in *I. setosa and I. nil* were clearly and consistently above the threshold of detection; whereas, in sweetpotato plants, they often were near or below the threshold (Kokkinos and Clark, 2006.).

2.21.3 Sweetpotato chlorotic stunt virus (SPCSV) disease

Sweetpotato chlorotic stunt virus (SPCSV), a member of the crinivirus genus, is transmitted by adult whitefly, *Bemisia tabaci* (Gennadius) and Trialeurodes abutilonea. It combines with sweetpotato feathery mottle virus (SPFMV), as components of sweetpotato virus disease (SPVD), a devastating disease originally described in Africa (Schaefers and Terry, 1976) It can synergistically interact with other important viruses including sweetpotato mottle virus (SPMMV), sweetpotato virus G (SPVG), and cucumber mosaic virus (CMV) (IsHak *et al*., 2003; Tairo *et al*., 2005). SPVD can cause yield reduction as high as 50 %. Milgram *et al.* (1996) observed that whilst there was no significant effect of combined SPFMV and SPCSV on yield, a 30 % yield reduction was observed for SPCSV in the second year. Gutierrez *et al*. (2003) found a similar result in Peru; that SPFMV alone did not significantly affect yield, but in combination with SPCSV a significant yield reduction occurred. In North Carolina (USA), two isolates were obtained in 2001 and 2003,

respectively, from plants of cv. Beauregard exhibiting symptoms typical of SPVD, including stunting, leaf narrowing and distortion, vein clearing, purpling or yellowing, and chlorotic mosaic. Samples extracted from symptomatic plants tested positive for SPCSV which was shown to combine well with SPFMV.

2.21.4 Sweetpotato leaf curl virus (SPLCV) disease

Sweetpotato leaf curl virus (SPLCV-US) was first found in ornamental sweetpotato and some breeding lines but not in sweetpotato grown for commercial vegetable production. Sweetpotato leaf curl virus (SPLCV) has been shown to have increased significantly in many parts of the world.

In the USA this increase was due to the use of infected propagating material and increasing population of its vector (Ling *et al*., 2010). The spread of SPLCV can be very rapid in response to increasing whitefly population. Hence, management of the whitefly population should be a critical element in control of this virus.

Sweetpotato leaf curl virus is also a whitefly- transmitted disease, which belongs to the genus Begomovirus. This disease has been reported worldwide and has been found in Italy, Spain Japan, China, Taiwan, Korea, Kenya and USA (Trendo *et al*., 2007; Ibarra-Jimenez *et al.* 2012).

SPLCV does not induce indications in the foliage of most genotypes but present curling of leaves may be observed in warm environments or it may require the presence of other viruses to happen (Trendo *et al*., 2007). (SPLCV can be detected using complex techniques such as real-time polymerase chain reaction (RT-PCR) as antibody detection and rolling circle intensification (RCA) using the bacteriophage. The simplest technique is biological indexing using vulnerable indicator plant such as *I. setosa* (Clark and Hoy, 2006; Trendo *et al*., 2007).

2.21.5 Sweetpotato caulimo-like virus (SPCaLV)

Sweetpotato caulimo-like virus (SPCaLV) is a distinct member of the genus Cavemovirus (family Caulimoviridae) (Trendo *et al*., 2007). SPCaLV was first detected in sweetpotato from Puerto Rico. It was detected in a complex with other viruses (including sweetpotato feathery mottle virus (SPFMV) from the South Pacific region including Tonga, Papua New Guinea, New Zealand, Solomon Islands, and Australia) (Pearson and Grisoni 2002; Rannali *et al*., 2008; Salazar and Fuentes 2000; Tairo *et al*., 2006), and Madeira, Kenya, Uganda, and USA (Mukasa *et al*., 2003). Previous attempts to characterize SPCaLV failed because of its complexity compared to other members of the Caulimoviridae. Unlike other viruses, it can be directly detected in sweetpotato, thus saving time during routine virus indexing using a quick DNA extraction protocol and PCR primers or use of NCM- ELISA (De Souza and Cuellar, 2011). Sweetpotato plants infected with SPCaLV usually show no distinct viral symptoms.

Indexing produces faint chlorotic spots or tiny areas of vein clearing which may develop into general chlorosis, wilting and premature death of leaves. SPCaLV is not transmitted by aphids, mechanical means, seed or by contact between plants. Its vector is unknown. The impacts on yield are unknown (Riis-Jacobsen, 2011).

2.21.6 Global production

Internationally sweetpotato is deteriorating in area grown. There was a highest decline from about 13.4 million hectares in 1961 to 8.9 million hectares in 2006. The decline was at 1 % in the first ten years to 1970 and then 2 % per annum in the later years. The decline was more predominantly seen in Asia and Latin America. This was mainly due to the decrease of the production area by approximately 44.4%. However, the effect of this significant decline was equipoise by the growth in sweetpotato area in Africa by 3 % over the same period (Srinivas 2009).

However, yields internationally increased for the same period above, except during 2001 to 2006, where a significant 1 % decline per annum was documented. On average, sweetpotato yield per hectare has doubled which counterweights the effect of decline in sweetpotato area of production. Over the past thirty years Latin America and African continents continue to show a decline in productivity from less than 0.4% per year (Srinivas, 2009).

FAO statistics, (2008) indicated that over 82 % of the world's production is in Asia. With the decline in yields across Asia, only four of the eleven leading sweetpotato production countries are in Asia; eleven countries account for more than 95 % of the increase in sweetpotato production in developing countries over the past four decades. Over the past thirty years, sweetpotato production has fallen sharply in Bangladesh, Philippines, Latin America, Sub -Saharan Africa and other industrialized countries.

Asia which saw China is the single largest producer of sweetpotato in the world, accounted for 70 % of total area under sweetpotato in cultivation in the world, with an output of about 100 million metric tonnes annually. The decline in Asia was also due to China setting a lower priority on sweetpotato production being focused on cereals and other industrial crops that provided them with better productivity. However, there was a general increase in processing and utilization of sweetpotato for animal feed and industrial starch (Fuglie 2007).

2.21.7 Method of detection and virus indexing

The decline of yield and quality of sweetpotato production in commercial sectors has resulted in development of techniques and methodology for virus elimination processes.

The identification of a virus typically requires the application of physical, biological, serological and molecular methods. Traditional generic methods for identifying and characterizing sweetpotato virus diseases include the use of electron microscopy or indicator plants as bioassays.

These methods are limited in their scope and only permit partial classification of viral agents. Recently technologies such as diagnostic microarrays and mass spectrometry have been proposed as generic tools for identifying viruses (Gruden *et al*., 2008), although all require some prior knowledge of the agents to be identified.

2.21.7.1 Serological detection

Antisera to SPFMV, SPLV, SPMMV and SPCLV are available from the ELISA kits. A high titre for SPFMV antiserum and monoclonal antibody is made available to detect SPFMV (Magagula *et al*.,2010). Serological screening methods such as nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) and dot enzyme-linked immunosorbent assay (Dot- ELISA) are commonly used. Three leaves are sampled from the upper, middle and lower portion of the grafted *Ipomoea spp* to be tested by pooling three discs about one centimeter (1 cm) in diameter per leaf and grinding them in a polyvinyl bag in ELISA extraction buffer. The polyclonal antibody for the different viruses is visually discovered on the membrane sample spot after adding of substrate. Records of positive reaction gives purple colour following the process as described outline in International Potato Centre (CIP) Dennien *et al*., (2013).

2.21.7.2 Visual investigation

This is a simple method which requires good eye sight to distinguish healthy plants from infected plants. There are differences between symptomless and diseased vines. The healthy plants generally exhibit fast growth, and dark green leaves but diseased crops are slow to grow with small

leaves which may show the following; mosaic, vein clearing, chlorosis, curling or wrinkling, flowering or less obvious, russet feathery mosaic and ring spots on the old leaves.

Although diseased plants can be rejected easily, some plants latently infected by sweetpotato latent virus (SPLV) or sweetpotato symptomless virus (SPSV) may escape visual selection. Therefore, visual investigation method is not totally effective in eliminating all infected plants (Feng *et al*., 2000).

2.21.7.3 Electron microscopy

Electron microscopy now associates well with immunosorbent electron microscope (ISEM) to detect viruses. These methods were being used to detect SPFMV and SPLV including tomato mosaic virus (TMV) by Yang *et al*. (1998). This method requires a fine preparation of sap to be observed under an electron microscope (EM). It requires correct sampling of suspected diseased plants. The virus may not be revealed under the microscope if sampling is done on plant tissue with uneven disseminations and low concentrations of the virus. EM is merely used to classify and study viruses and not for routine diagnosis as the equipment is expensive and requires biochemical reagents.

2.21.7.4 Indicator plants

The stem of the suspected disease plant is cut into two sections. The upper section is grafted onto the tip, while the lower section becomes the scion grafted on to a plant of *I. setosa*. *Ipomoea setosa spp* is a convenient indicator plant for detection of sweetpotato viruses. There are other *Ipomoea* (indicator) plant spp (Cohen *et al*., 1988) but *Ipomoea nil* (L.) Roth and *Chenopodium quinoa* are useful indicator plants. Depending on the virus type, after 14 days, the apparent symptoms such as

mosaic, curled or wrinkled leaves or vein clearing appear on the plant of *I. setosa* is used widely to detect virus-free plantlets in a screen house or field.

Although it is simple and suitable to index potential virus-free sweetpotato samples which show limited symptoms, it is still difficult to differentiate sweetpotato viruses and it is time consuming. When grafting is combined with serological detection method, the detection efficiency is improved (Feng *et al*., 2000; Lotrakul *et al*., 1998).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study area

The study was conducted at Botanga irrigation fields, located within the Kumbungu District of Northern region of Ghana from September – December 2017. Botanga is located on latitude 009° 25' 41" N, longitude 000° 58' 42" W and altitude 183 m above sea level. The area has unimodal rainfall pattern of dissemination which starts from April and end in October. It has a mean annual rainfall of about 1046 mm. The mean temperature of the locality is 28.3 ^{o}C. The soil type of the study locality has been categorized under Nyankpala series. These soils are normally dark brown in colour, moderately drained sandy loam. The undergrowth is made up of short deciduous fire impervious trees which do not form a close covering and the ground flora is made up of diverse species of grasses (SARI, 2004).

3.2 Source of planting material and sampling method

Three sources of planting materials were used namely; healthy tested pathogen free vines (*In vitro*) laboratory generated cleaned source, apparently 'clean' Field generated materials (symptomless) planting materials and Farmer's materials source (supposedly infected).

Healthy tested vines (*In vitro* tissue culture source) were generated from the Biotechnology laboratory complex of Plant Tissue Culture of the Crop Research Institute (CRI), Fumesua. The meristem of sweetpotato were used to generate *in vitro* virus free plantlets by using tissue culture cleaning techniques and materials were tested by grafting on *Ipomeas setosa* which is very susceptible to virus to ensure that those supposedly cleaned materials were really clean before planting. The major steps that were involved in the *in vitro* virus cleaning process were the used of heat therapy chamber to reduce the virus load if any from viral symptomatic vines cuttings taking from multiplication fields, and this also enhanced sprouting of nodal cuttings to obtain

partially clean meristems. The partially clean meristems were excited in the tissue culture laboratory with the help of microscope and then cultured on MS, Murashige and Skoog media (1962) for about three months and then weaning and hardening process were followed to obtained vigorous plantlets for the virus health status trial. The use of *Ipomeas setosa* for virus test indexing has been reported by Gibson *et al.* (1998).

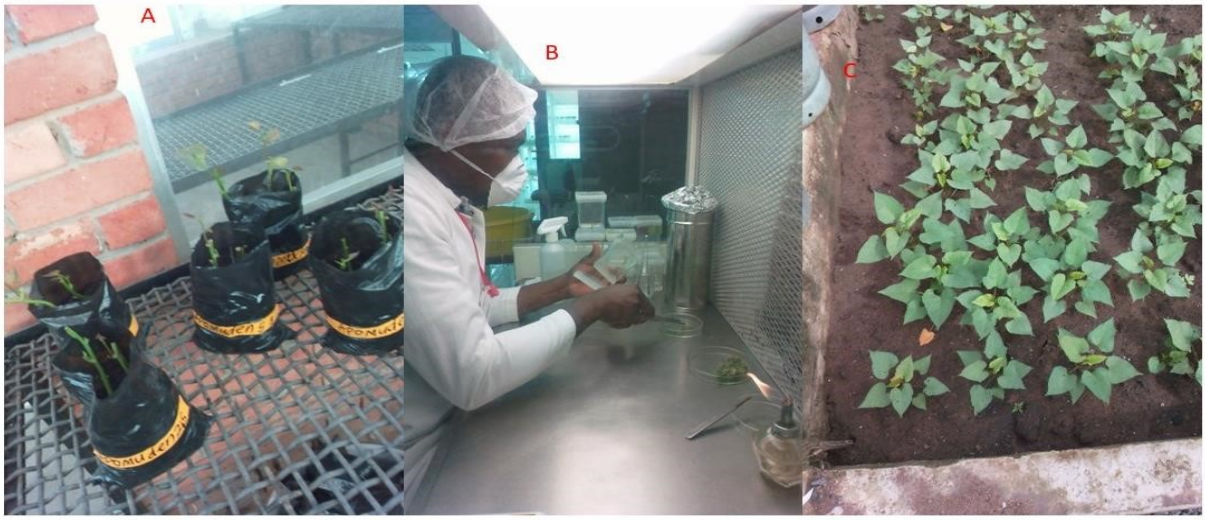


Plate 1: In vitro Laboratory generated planting material. (A) Cuttings grown on plastic bags and exposed to hot temperature therapy (B) Apical shoot cutting (C) Plantlets generated from meristem being weaned.

In the second material source apparently healthy looking and symptomless materials were selected using positive selection approach on International Potato Center multiplication fields at Savanna Agricultural Research Institute field, Nyankpala. Such materials were cleaned first before they were introduced to field for about four to five years earlier. The field derived planting materials were obtained using the "seemingly" cleaned healthy plantlets, which was not virus tested planting materials at the time this trial was to be conducted. Apparently 'clean' healthy field-derived planting materials were produced by rogueing plants showing virus symptoms (Negative Selection) on the field and leaving the healthy ones. These apparently healthy ones were used as

the second materials source. Even though unhealthy plants can be excluded easily, some plants latently diseased by sweetpotato latent virus (SPLV) or certain sweetpotato viruses without symptoms of virus may escape visual selection. Consequently, visual method of examination is not totally efficient in discarding viral diseased crop (Feng *et al*., 2000).

The third planting material source called Farmer source were materials that were taken from the open fields of vine multiplier farmers which were given to them for about five years ago. These materials include retools plants from sprouting from roots and survival vine that were not intentionally selected. These materials have not been passed through *in vitro* tissue culture cleaning process for the past four to five years which were considered to be viral infected materials. In each source of planting materials, four varieties, namely Apomuden Ligri Bohye and Dadanyuie were selected for the field experiments.

3.3 Experimental set up and design

The experimental design that was used to conduct the trial was split plot where the source of planting materials represented the main plots and the varieties represented sub plots. The treatments were replicated three times. A sub-plot size of 4 × 5 m was used for planting 17 cuttings per row for 4 rows. The lengths of the cuttings were between 25 cm to 30 cm with four nodes each.

Cutting were planted two nodes in the soil for rooting and two nodes outside for sprouting. Plantlets were spaced at 1 m between rows and 0.30 m within plants. The experiments were conducted during the dry season under irrigation in order to reduce cross infection by the virus insect vectors among the different sources of planting materials. Again, in order to minimize cross infection among different sources planting materials, a 10 m alleys were created in between main plots and

Maize (Zea mays, L) were planted in the alleys. Cultural practice such as vine lifting, weeding, and reshaping were carried out. Harvesting was done at 120 days after planting (DAP).

3.4 Data collection on virus symptoms and performance scores

Sweetpotato plants were checked for disease/viral symptoms progress and severity symptoms observation in accordance with the International potato center, (CIP) protocol. Monitoring virus symptoms in the field was an important aspect of the trial to give details of infections which could affect the storage root yield. Infections in some varieties were difficult to detect as some virus were transient, mild, or may not appear at all on sweetpotato foliage. However, NCM-ELISA test was conducted to confirm the presence of viral symptoms and those viruses which were not induced on the foliage as well as virus that were mild and could not be detected by visual observation. The virus symptoms monitoring started at four weeks after planting and after full plant establishment. The first set of viral scores data (Virus 1) were taken on the fourth week after planting for four weeks and average were taken. Second phase of viral scores data (Virus 2) were collected for four weeks starting on eighth week after planting.

A severity viral symptoms scores of 1–9 were used (CIP, 1999), where

- 9 = Severe virus symptoms in all plants per plot
- 8 = Clear virus symptoms at all plants per plot
- 7 = Clear virus symptoms at 67 to 99 % of plants per plot (2/3 to almost all), (not stunted),
- 6 = Clear virus symptoms at 34 to 66 % of plants per plot (more than 1/3 less than 2/3),
- 5 = Clear virus symptoms at 16 to 33 % of plant per plot,

- 4 = Clear virus symptoms at 6 to 15 % of plants per plot,
- 3 = Clear virus symptoms > 5 % of plants per plot, 2 = unclear virus symptoms;
- 1 = Plants showing no symptoms;

Source (CIP, 1999).

Opti-Science Cc4-200 Chlorophometer SPAD readings were taken within the first month and was repeated two more times in the third and four months before harvesting and average was taken.

3.5 NCM-ELISA: virus detection test

During the period of the experiment, vine with leaves samples were taken from each plot to evaluate virus load and virus type present in each source of planting materials and varieties. This was done by using an immuno-enzymatic virus reaction, NCM-ELISA which involved the use of nitrocellulose membranes instead of the polystyrene micro titration plates as a support for the reagents used in the serological reaction. It also has another very essential advantage over the others. For example, the samples can be dotted onto nitrocellulose membrane and stored for numerous weeks before use, they can even be transferred to other laboratory for completion.

The steps involved in NCM-ELISA virus detection were mostly done under room conditions:

- Very minute amount (2 to 30 µl) of the sample (plant sap) were blotted and dried.
- The portion that were not utilized by the samples were blocked with blocking solution.
- Specific antibodies (virus antibody 1) were used to react the virus particles.
- Then virus specific antibodies were detected by means of an appropriate substrate using the enzyme labeled antibodies (virus antibody 2).

3.6 Technique- Sample preparation for virus detection test (Virology test)

Negative selection approach was used in the field to select those plants showing viral symptoms. Vines cuttings with leaves were collected from each plot in to a brown labelled envelopes bags. The envelopes were kept on ice in the field and then conveyed to the laboratory with the help of ice chest on the same day. The next day, the virus detection test process was then started. At the laboratory the samples were kept in a fridge at 4 ^{o}C to keep them fresh overnight. A multiple composite sampling was done from each plant materials to be examined per plot, thus taken one leaf from bottom, middle part and one leaf from the top levels. All the three leaves were arranged together in a sample bag. Approximately 1 cm in diameter test tube was used to gently press the three leaves sampled to cut three discs from each of the three leaves in the sample bag. The rest of the leaves were removed from the sample bag leaving the three leaf discs. The leaf disc ground with 3 ml of extraction buffer was added for extraction, thus 1 ml of extraction buffer per leaf disc. The tissues were ground fully by using piece of round wood. Final sap dilution was approximately 1/50 (lower dilutions could give nonspecific reaction or interfere with the final developed reaction because of higher concentration of polysaccharide components in sap). The bags were in a standing position for 30-45 minutes at room conditions until the plant sap phased out (this was achieved by placing the bags in a large beaker).

3.7 Sample application to nitrocellulose membrane

The nitrocellulose membranes were cut into 10 pieces of the sizes needed for the detection of 10 different viruses. The membranes were identified by writing the name of the virus (or the number coding each virus) on the top. The membranes pre-wet in TBS for at least 5 minutes prior to use. Meanwhile the dot blotting apparatus was connected to a vacuum pump. The pre-wet piece of Whitman's paper was placed over the dot blot manifold and pre-wet nitrocellulose membrane was

also placed on over the filter paper. A piece of parafilm was used to block the remaining area of the manifold not covered by the nitrocellulose membrane and carefully applied to a vacuum (200 to 230 mm of mercury) by turning the pump on. A 30 µl sample (plant sap) was pipetted into each well formed on the nitrocellulose membrane by the vacuum. Care was taking not to pipette plant tissue. Using a clean tip for each sample, the process was repeated until all the 36 samples were spotted. The nitrocellulose membranes were removed from the gadget and were conveyed onto a well dry filter paper piece and was allowed to dry for about 15 - 30 minutes.

3.8 Serological test process

The dry membranes of nitrocellulose were immersed in a blocking solution (TBS + 2 % milk + 2% TRITON X-100) for 1 hour at room conditions with gentle shaking (50 rpm). The first antibody 1 (virus specific antibody) and TBS plus 2 % of milk was further added and gestated overnight at room conditions with gentle shaking (50 rpm). The next day, the nitrocellulose tissues were cleaned in TTBS for 3 minute by washing for three times each with very fast shaking (100 rpm). The second antibody, GAR (Goat anti-rabbit) was added in TBS (Tris Base) plus 2 % of milk and then incubated for 1 hour at room conditions with gently shaking (50 rpm). Then the tissue nitro membranes were then washed again in TTBS (TBS 2,000 ml Tween-20 1.0 ml) (0.05 %) four times for three minutes with very fast shaking (100 rpm). The tissue nitro membranes were incubated for about 30 minutes. In the case of SPCSV tissue was incubated for 1.5 h in a substrate mixture (20 mg N, N-dimethylformamide 1.2 ml) at room conditions with gentle shaking (50 rpm) for colour formation process. The coloring process was stopped by disposing the substrate mixture which enhanced colour formation and then nitrocellulose membranes were dipped in distilled water to stop the reaction completely. The nitrocellulose membrane tissues were rinsed in flowing tap water for three times for about 3 minutes each. The tissues were allowed to dry before the

reactions data were recorded on the NCM ELISA recording sheet using a scale of 0-5 (CIP, 1999) based on the intensity of the coloration comparing with positive controls. Where zero (0) represented negative reactions and 1 to 5 represented positive reactions, with one being the least. Positive reactions were those showing different shades of purplish colour.

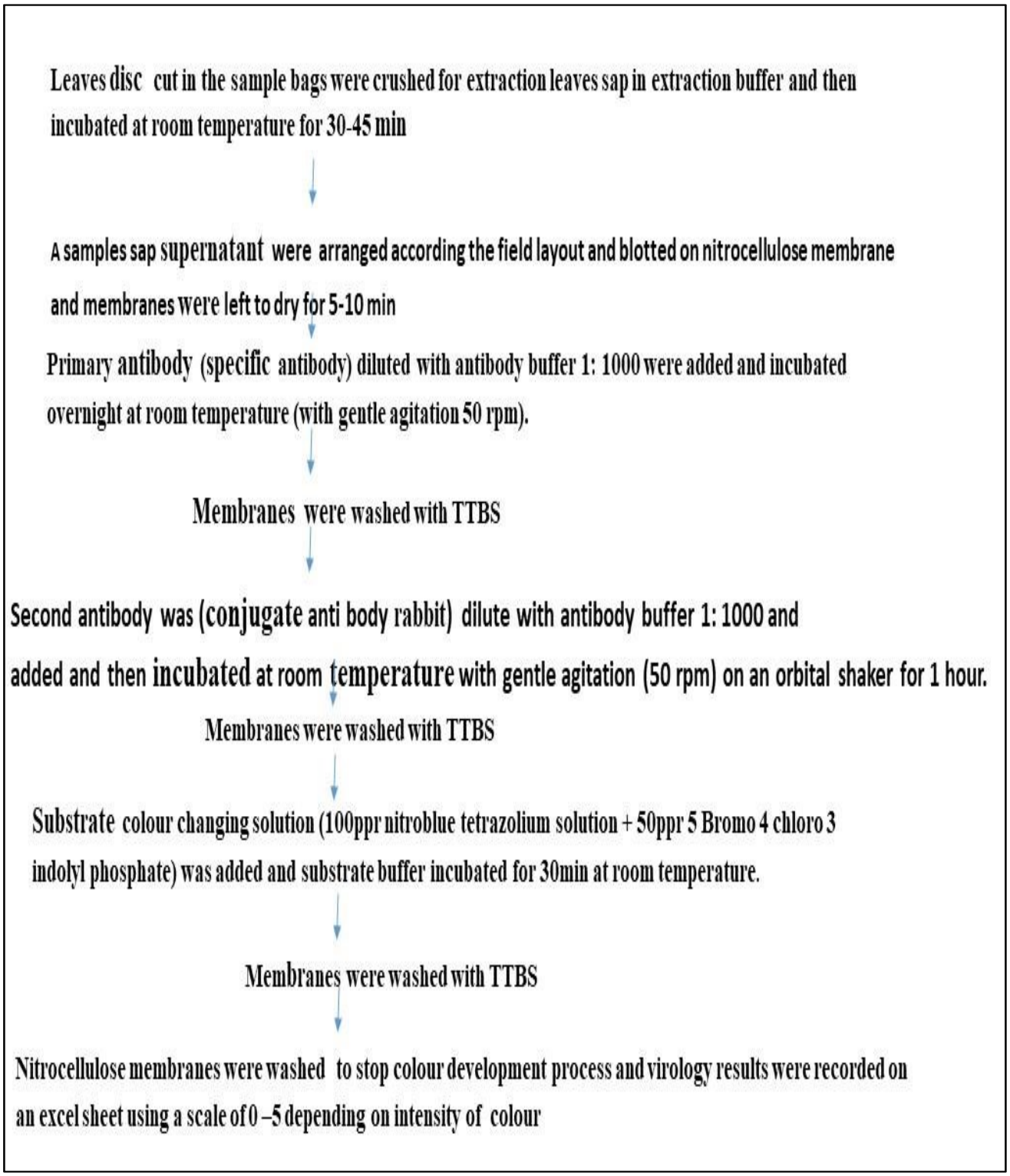


Figure 1: The steps involved with NCM- ELISA virus detection test

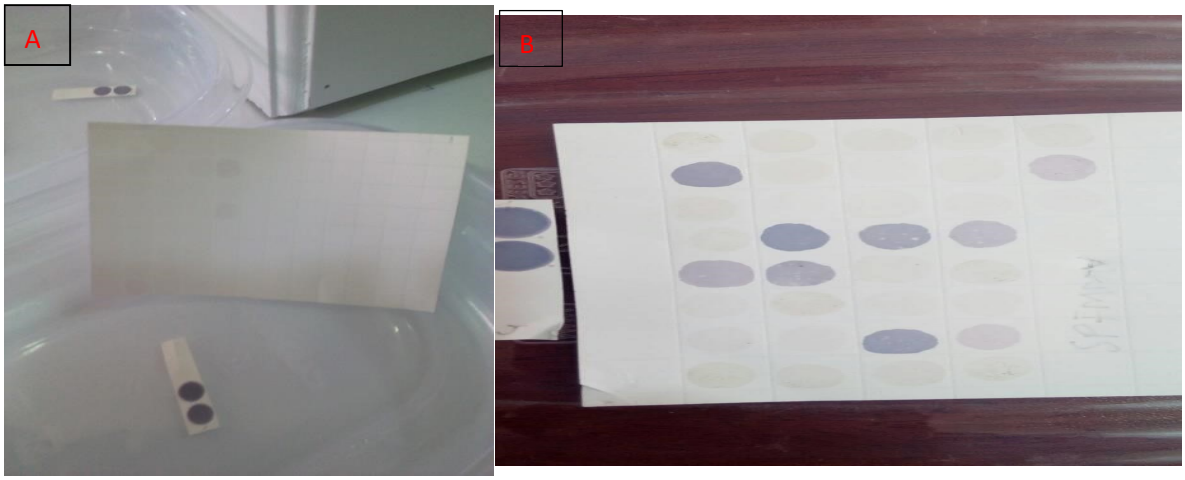


Plate 2: An Enzyme Linked Immunosorbent assay (ELISA) result and their respective positive control (A) container with Blotted membranes immersed in TTBS (B) container with Blotted membranes immersed in TBS

3.9 Harvesting yield data

Harvesting was done at 4 months after planting. Data collection was done for only two inner rows (net plot) of each plot harvested, leaving a plant each at the beginning and the end of the rows (Border plants), and giving a net plot of 9 m^2 area. At harvest, total storage root yield, marketable root yield, non-marketable root, total storage root number, marketable root number, nonmarketable root number and vine yield were determined. Biomass was calculated from the total root weight per plot (kg/plot) and the total vine weight per plot (kg/plot).

3.10 Data Analysis

The Data collected were subjected to general Analysis of Variance (ANOVA) using Genstats statistical tool (4^{th} Edition) and means were separated using least significant difference at the 0.05(5%) level. The serological score data and other score data were transformed before analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 Field symptomatic virus scores

4.1.1 First field virus score (Virus 1 scores)

The source of planting materials significantly influenced (*P = 0.018*) first field visual observation score (virus 1 scores). The Farmer's material recorded the greatest virus severity symptoms score followed by Field generated materials, while the least virus severity score was realized in tissue culture *in vitro* generated material. There was general decreasing trend of field visual viral symptoms score rating from Farmer's material to tissue culture *in vitro* material of all varieties (Figure 2). Virus 1 scores did not significantly influence by variety used. Among the varieties Ligri showed the least viral symptoms scores in all source of planting materials than the other varieties. Apomuden and the Dadanyuie showed the highest field virus symptomatic rating among Farmer's materials and Ligri recorded the least. Among the Field materials Apomuden showed the most serious viral scores and the Dadanyuie and Ligri recorded the least viral symptomatic scores. Among the *In vitro* laboratory cleaned generated materials Ligri showed the lowest viral symptomatic score and other varieties showed equivalent virus rating (Figure 2). However, there was no significant (*P = 0.156*) interaction effects between source of sweetpotato planting materials and varieties used

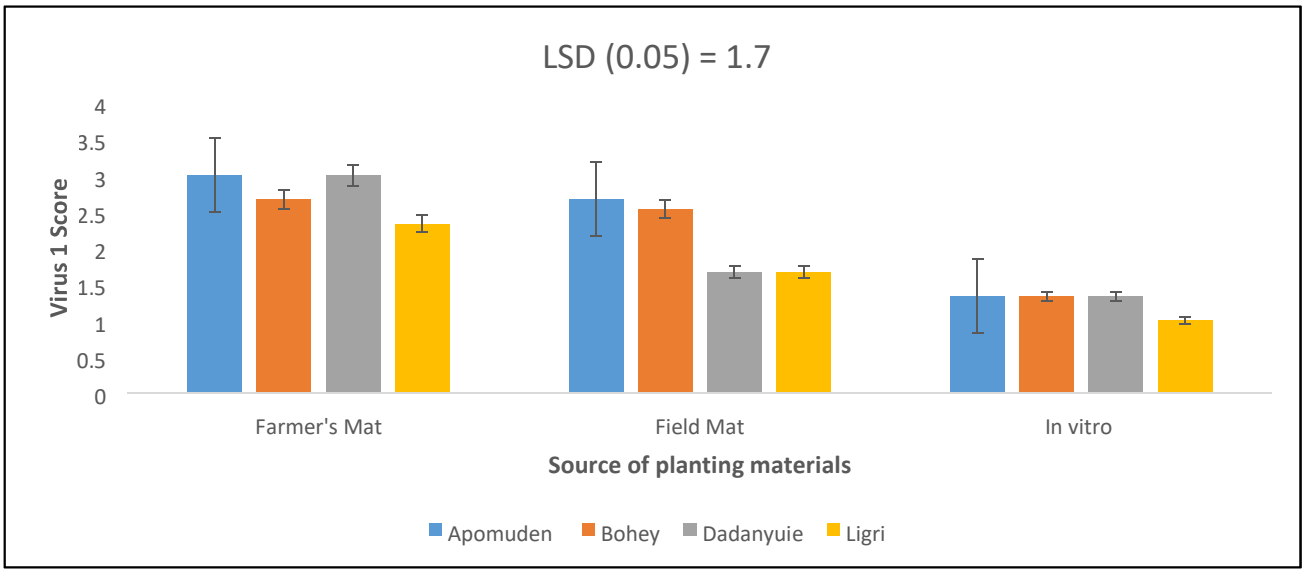


Figure 2: Effect of source of planting material and variety on field symptomatic virus scores; The data represent the average virus scores from 4 to 7 week; Bar represent Standard Error of Means (SEM).

4.1.2 Second field virus score (Virus 2 scores)

The second phase of viral symptoms rating (Virus 2) was taken on eighth week for four weeks and average was taken. There was a significant interaction effect ($P = 0.03$) of source of planting materials and sweetpotato variety on field visual observational viral scores (Figure 3).

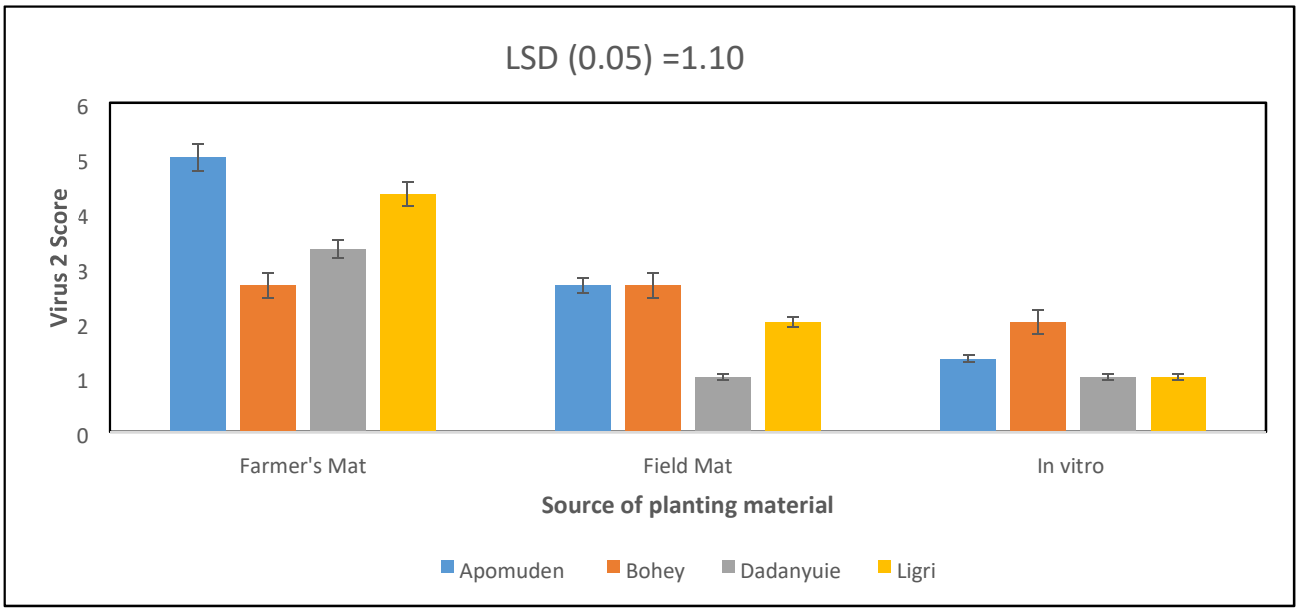


Figure 3: Interaction effect of source of planting material and variety on field symptomatic virus score; the data represent average virus score from week 8 to 11; Bar represent SEM.

Also, there was significant influenced (*P = 0.049*) of sweetpotato variety on field viral symptomatic visual observational scores. Apomuden and Ligri varieties had significantly higher viral score on the Farmer's planting material source. However, in the apparently 'clean' field and *in vitro* clean planting material sources, Bohye variety which recorded the least viral score in the Farmer source of planting material recorded higher viral score. Though the score was significantly low when compared with Farmer's source material. There was significant (*P= 0.048*) influence of source of planting materials used. In general, there were progressively increased of virus score of field visuals virus symptomatic observational scores from *in vitro* tissue culture generated materials to Farmer's material among the source of planting materials used (Figure 3).

Plate 3: Field visual viral symptomatic plant in farmer materials with chlorotic spots purple pigments

4.2 Serological virus test result

The serology test result revealed that, among all the ten known classified virus types that were looked, six of them were found to be significantly positive across all sources of planting materials (Table 2). *In vitro* generated planting material source were significantly low in viral load in all the six virus types. In four of the virus types namely SPCSV, SPFMV, SPSMV and CMV there were significant differences in viral load between Field and Farmer's planting material source thus the Farmer's material source recorded significantly higher viral load than the Field material. In the other two viruses type (SPFMV and SPCFV) there were no significant difference between Field and Farmer's sources. There was presence of very small amount of virus in the *in vitro* generated materials (Table 2).

Table 2: The NCM ELISA virus test showing virus types and their load in each planting materials source

Virus type	Source of planting material			Mean	P –value	LSD
	Farmer.	Field	*In vitro*			
SPCSV	2.58	1.75	0.25	1.58	*<0.001*	0.59
SPFMV	2.83	2.5	0.5	1.94	*0.005*	0.97
SPCFV	1.92	1.58	0.5	1.33	*0.013*	0.73
SPLV	0	0	0	0	*0*	0
SPMMV	3.08	1.92	0.5	1.83	*0.003*	0.86
SPMSV	2.417	1.583	0.25	1.417	*<0.001*	0.52
SPVG	0	0	0	0	*0*	0
SPC -6V	0	0	0	0	*0*	0
SPCaLV	0	0	0	0	*0*	0
CMV	2.167	1.417	0.25	1.678	*<0.001*	0.44
Mean	1.500	1.075	0.225	0.9775		

Also, the virology result indicated that, sweetpotato varieties had significant viral load. CMV, SPCSV and SPMSV indicated highly significantly difference at ($P \leq 0.001$) across variety whilst SPFMV, SPMMV and SPFMV differed significantly at ($P \leq 0.05$) across variety. However, four virus types amongst the ten viruses tested for indicated negative reaction across source of planting materials (Table 2) and varieties (Table 3).

Table 3: The NCM ELISA virus test showing virus types and their load in each variety

Virus type	Apomuden	Bohye	Dadanyuie	Ligri	Mean value	P-value	LSD
SPCSV	2.22	1.11	1.22	1.56	1.53	*< .001*	0.67
SPMMV	2.67	1.78	1.33	1.76	1.83	*0.007*	0.65
SPMSV	2.351	1.732	0.350	1.243	1.316	*<.001*	0.48
SPCFV	2.89	2.11	1.11	1.67	1.96	*0.005*	0.67
SPLV	0	0	0	0	0	0	0
SPFMV	2.11	1.22	1.00	1.00	1.33	*0.003*	0.61
SPVG	0	0	0	0	0	0	0
SPC6V	0	0	0	0	0	0	0
SPCaLV	0	0	0	0	0	0	0
CMV	2.00	1.111	0.667	1.333	1.278	*<0.001*	0.50
Mean	1.4197	0.8914	0.5577	0.977			

Plate 4: An Enzyme Linked Immunosorbent assay (ELISA) results showing (A) high positive reactions of different virus type and mild reactions (B) mild positive reactions of different viruses types and negative reactions.

4.3 Chlorophyll content (SPAD values)

The sweetpotato source of planting materials was highly significantly influenced (*P = 0.001*) by chlorophyll content (Opti-Science Cc4-200 Chlorophometer SPAD). The *In vitro* generated material recorded higher SPAD meter value more than Field generated planting materials and Farmer's materials by 43.73 % and 31.40% lower SPAD values than tissue culture *in vitro* and Field materials respectively. Thus chlorophyll content decreases from tissue culture laboratory cleaned materials to Farmer's materials (pre- supposedly infected materials) (Figure 4).

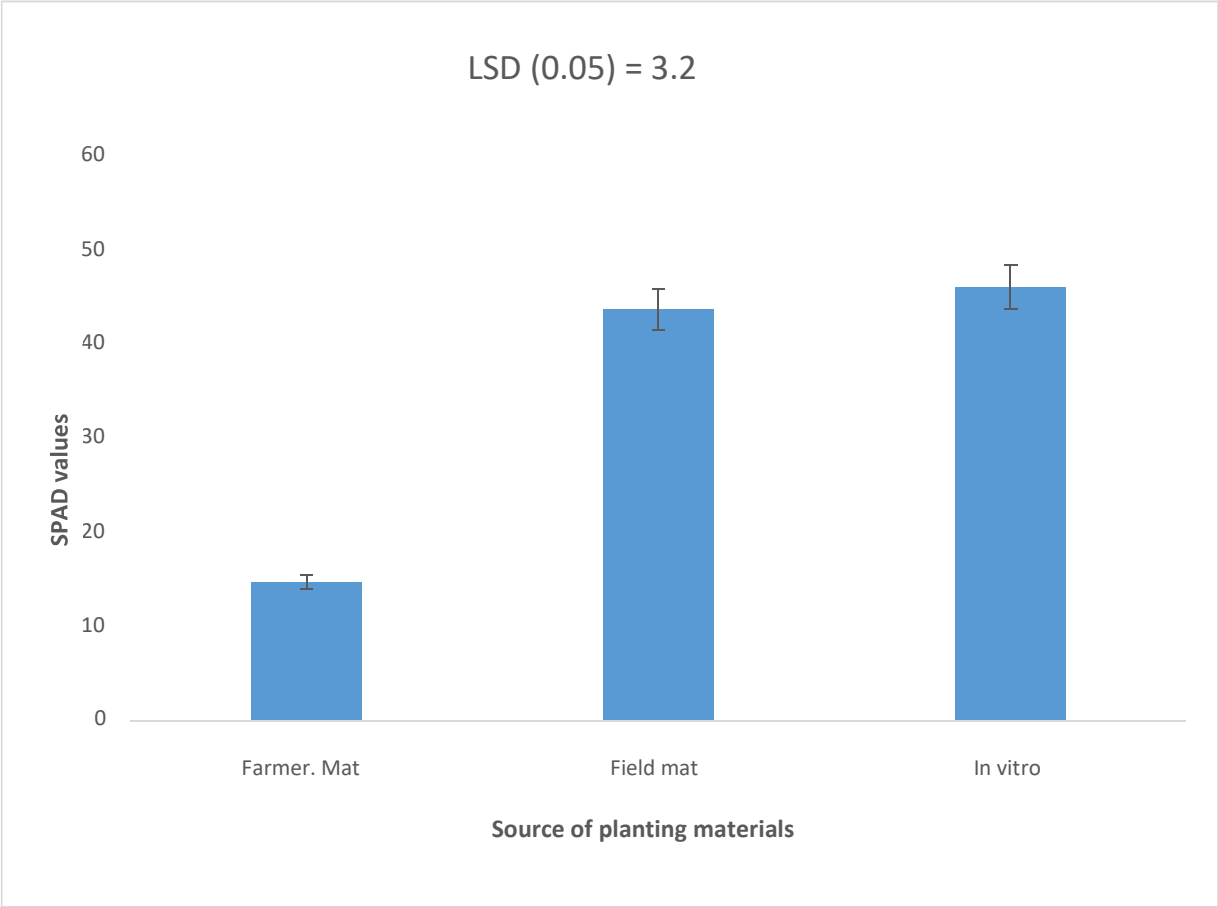


Figure 4: Effect of source of planting material on leaf chlorophyll content. Error bars represent SEM.

Also, there was significant effect of variety of different sources of sweetpotato planting on chlorophyll content (*P = 0.005*). Among the varieties, Dadanyuie recorded the highest SPAD meter value followed by Bohye and Apomuden recorded the least SPAD value (Figure 5).

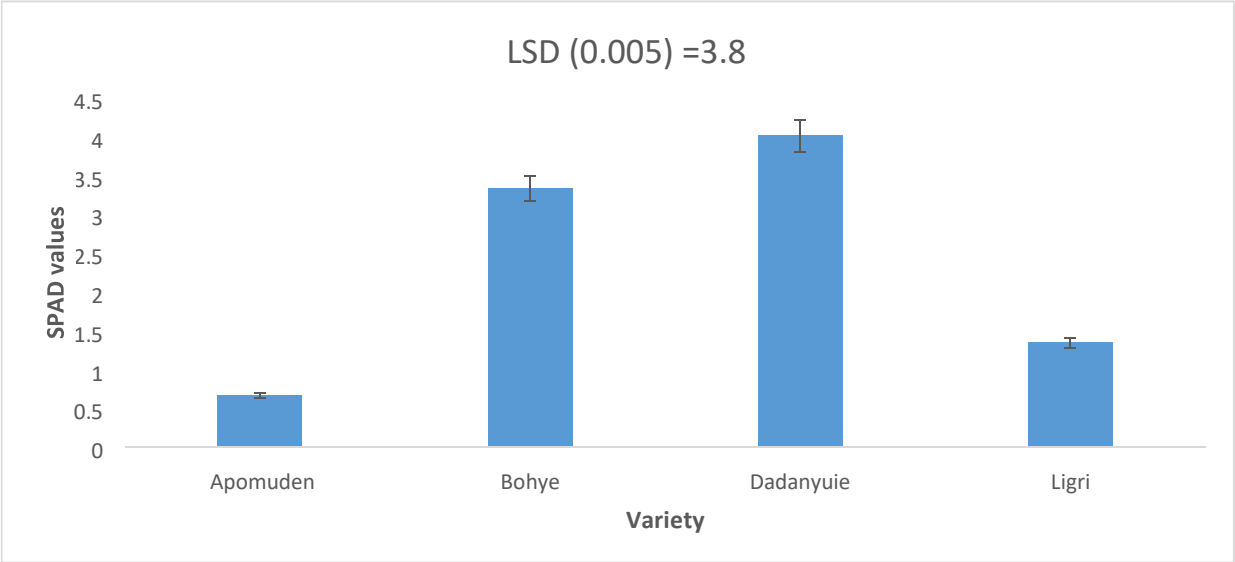


Figure 5: Effect of variety on sweetpotato leaf chlorophyll content; Bars represented standard error of means (SEM)

However, there was no significant interaction effects between source of planting materials and the varieties, but there was different between two sources when compared. All the varieties under Farmer's material source showed similar values except Dadanyuie which recorded significant higher value (Figure 6). Under field planting materials, Apomuden and Bohye showed similar chlorophyll content. Also, under *in vitro* tissue culture cleaned materials Bohye and Ligri varieties recorded low chlorophyll and Apomuden and Dadanyuie recorded the highest chlorophyll content. It was also observed that, among all source of planting materials Dadanyuie differed significantly higher than all the other varieties (Figure 6).

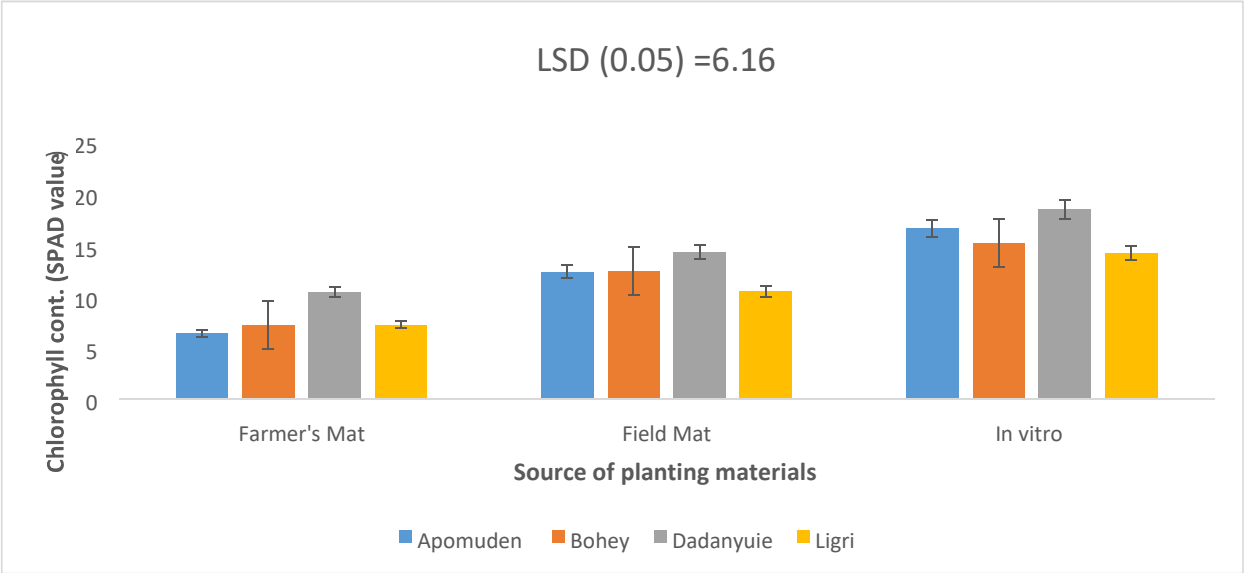


Figure 6: Effect of source of planting materials on leave chlorophyll content; Bars represented SEM.

4.4 Vine yield

There was highly significant difference (*P = 0.001*) in vine yield among the source of planting materials. The highest vine yield was observed *in vitro* virus clean plantlets (Figure 7).

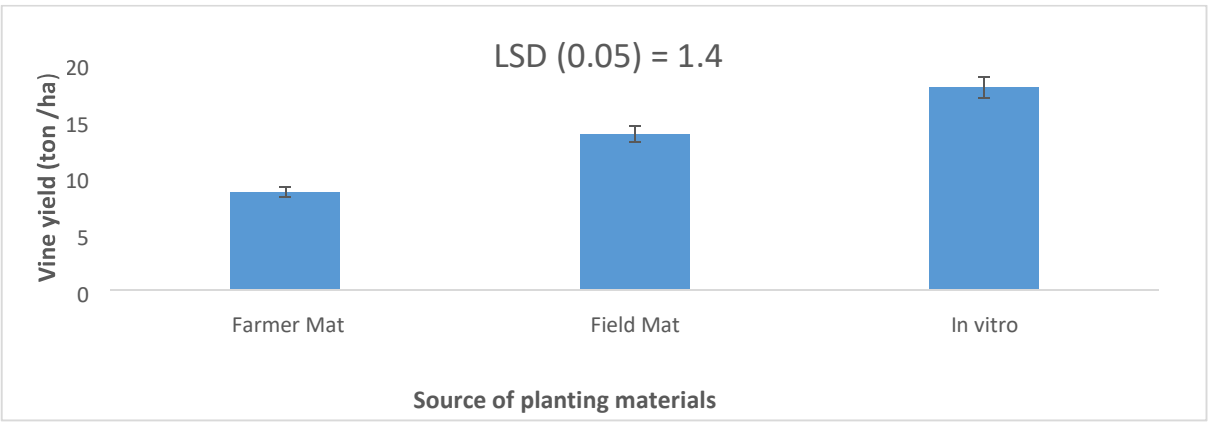


Figure 7: Effect of sweetpotato source of planting materials on vine yield; Bars represented SEM.

Thus *in vitro* tissue culture generated planting materials recorded a relatively higher vine yield followed closely by field generated planting materials and Farmer's planting materials recorded the least vine yield. Vine yield by *in vitro* planting materials source was 22.8 % and 51.3 % higher than Field and Farmer's source of planting materials respectively (Figure 7).

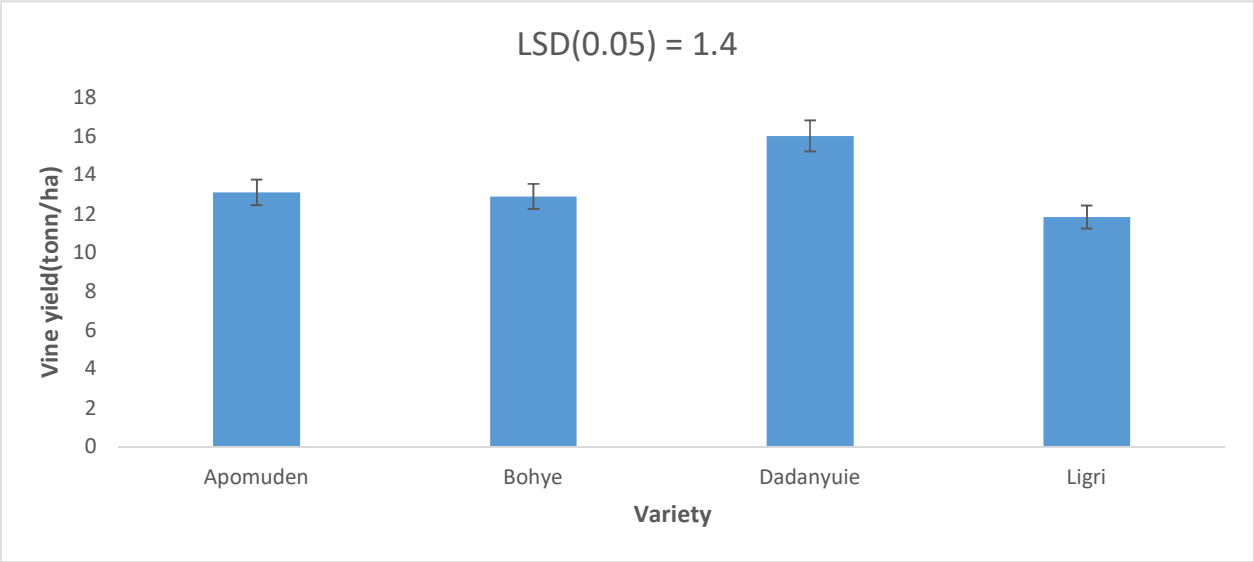


Figure 8: Effect of sweetpotato variety on vine yield of sweetpotato; Bars represented SEM.

In all the three planting materials sources, Dadanyuie variety produced the highest vine yield (Figure 8). The *in vitro* planting material source produced higher vine biomass than Field apparently "clean" generated and Farmer's materials, thus there was gradually reduction in vine yield from tissue culture planting materials (pathogen tested plantlets) to Farmer's planting materials (4 to 5 years after pathogen cleaning testing) (Figure 9).

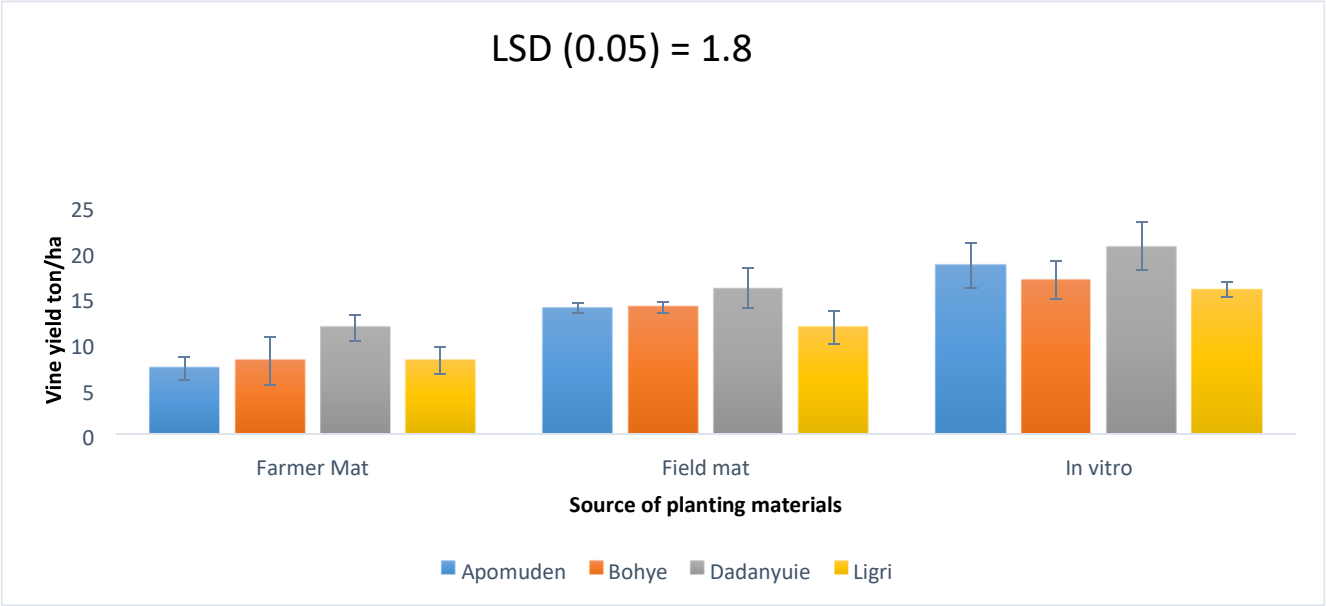


Figure 9: Interaction effect of source of planting materials and variety on vine yield. Bars represented SEM.

There was highly significant (*P = 0.001*) influence of sweetpotato varieties on vine yield. Among all the varieties, Dadanyuie produced the greatest vine yield across all source of planting materials and Farmer's Apomuden produced the lowest vine yield in father's materials. However, Apomuden produce average vine in Field generated materials and *in vitro* generated materials field (Figure 9).

4.5 Root Yield

The source of planting materials significantly influenced (*P = 0.026*) the root yield (Figure 10). The *in vitro* recorded the highest root yield of 19.74 ton/ha followed by Field generated planting materials of 16.08 ton/ha and Farmer planting materials gave the least yield of 10.34 ton/ha. Root yield was higher *in vitro* generated platelets and was least in Farmer's materials.

Thus yield improvement of 18.5 % and 47.6 % were recorded by the *in vitro* over the Farmer's materials and Field generated materials respectively (Figure 10).

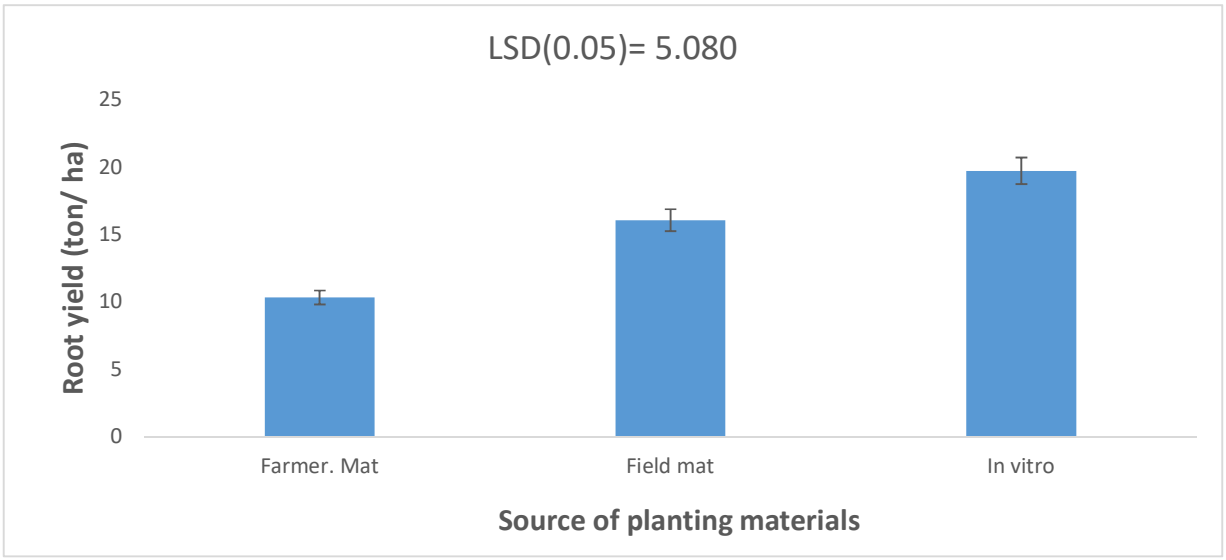


Figure 10: Effect of source of planting material on root yield of sweetpotato varieties.

The effects of source of planting materials significantly (*P = 0.026*) influenced the total root yield (Figure 10). Variety also have significant effects on root yield (*P = 0.048*) (Figure 10). *In vitro* Dadanyuie variety produced the highest root yield (23.83 ton/ha) and was not significantly different from Apomuden and Ligri, but followed closely by Apomuden and Bohye performed least (Figure 11).

Similarly, among all the Field generated materials, Dadanyuie yield significantly higher (*P = 0.014*) Bohye but was not significantly different from Apomuden and Ligri. Bohye, that was observed to perform worst amongst *in vitro* generated materials, yield better than all other varieties of Farmer's generated planting materials but the yield was not significantly different.

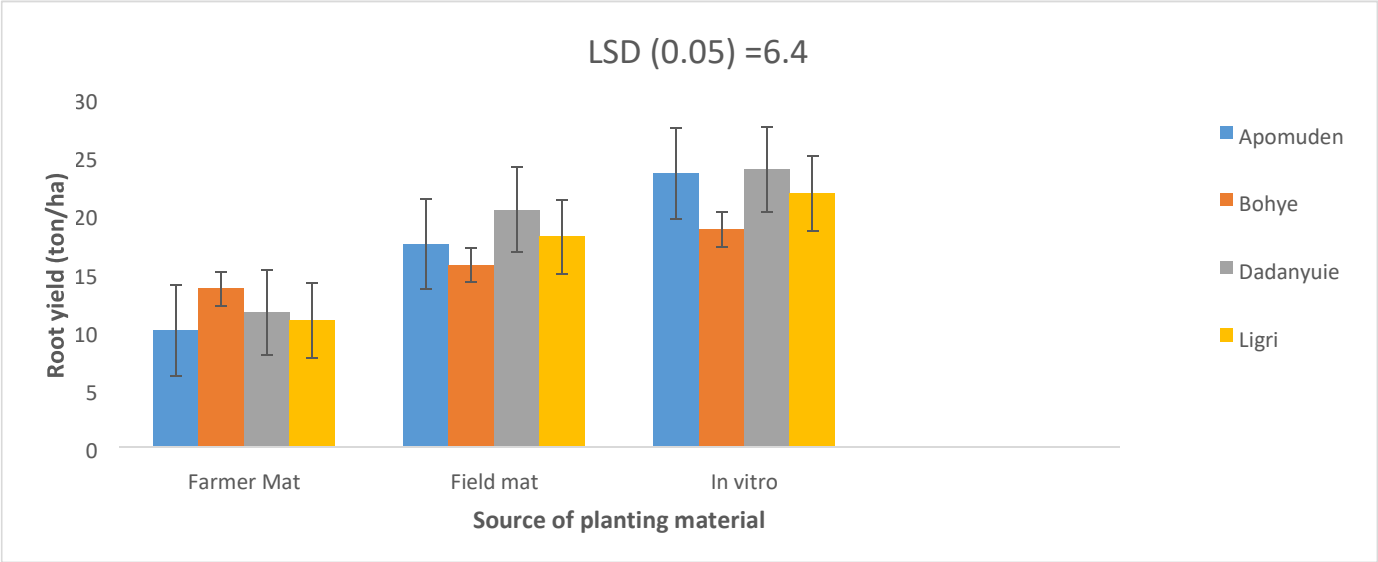


Figure 11: Interaction effect of source of planting materials and variety on total root yield. Bar with represents SEM.

4.6 Marketable and Non- marketable yield

There was significant *(P = 0.014*) difference in marketable root yield among the different sources of planting materials (Figure 11). Marketable root yield was higher in *in vitro* generated plantlets and was least in Farmer's materials. Thus *in vitro* generated platelets produced more marketable root yield of 11.23 % and 17.33 % better than Farmer's materials and Field materials respectively. Although varieties used showed similar performance, variety Dadanyuie produces highest of 15.58 ton/ha better and Bohye varieties performed worst of 13.99 ton/ha in marketable root yield. However, marketable root yield did not show significant interaction effect between source of planting material and variety used (Figure 11). Even though, Non-marketable root did not show any significant *(P = 0.226)* difference among the different sources of planting materials but there was significant *(P = 0.005)* interaction effects between source of planting materials and variety used. Amongst varieties used *in vitro* cleaned planting material generated, produced higher marketable root yield than uncleaned generated planting materials from Field and Farmer's. The tissue culture *in vitro* cleaned planting materials Dadanyuie recorded the highest value (23.82)

followed closely by Apomuden and Ligri recorded the worst (Figure 11). In terms of the Field materials Ligri recorded the highest value followed closely by Apomuden and Bohye recorded the lowest non- marketable root yield. However, in terms of farmers' generated materials, Bohye recorded the highest followed by Dadanyuie and Apomuden recorded the worst (Figure 11).

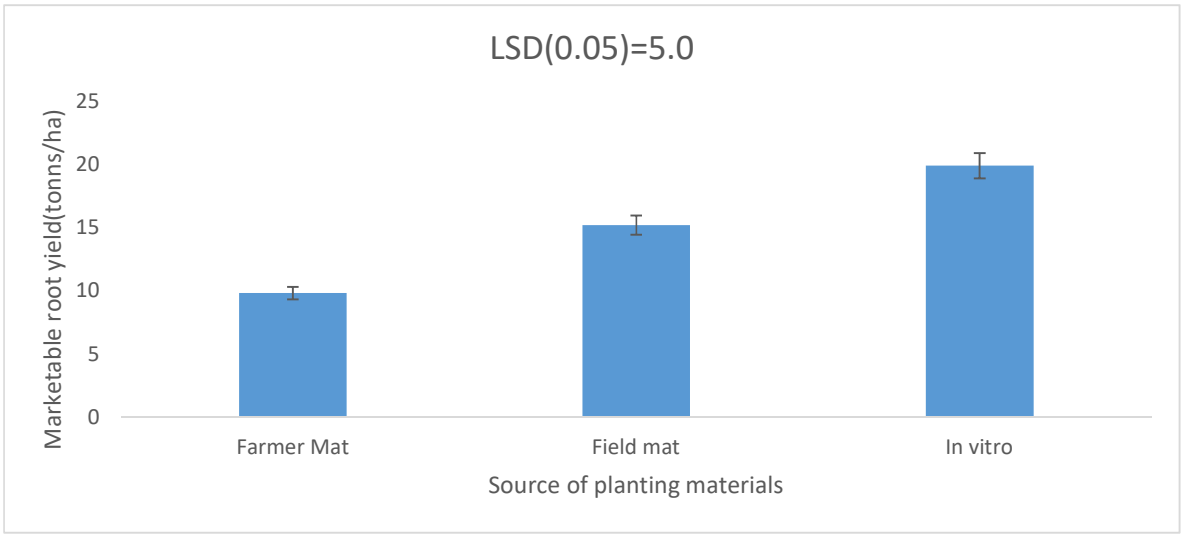


Figure 12: Effects of source of planting materials on Marketable root yield. Bars represented SEM.

4.7 Number of Marketable Root

The marketable root number did show significant difference (*P = 0.010*) among the different sources of planting materials used. The tissue culture *in vitro* generated planting materials recorded the highest mean value and Farmer's generated materials recorded the lowest mean value

(Table 4). However, number of marketable root did not show significant (*P = 0.192*) difference among the varieties used (Table 4). The interaction between the planting material source and the variety was significantly different (*P= 0.030*). Across the varieties *in vitro* planting material produced significantly higher marketable roots number than the other two sources (Table 4). In the variety, Dadanyuie *in vitro* planting material produced about 58 % marketable root yield more than the Farmer source of planting material. Among the varieties, Apomuden recorded the highest

mean value (27.33) followed closely by Ligri (26.89) and Bohye recorded the lowest mean value (25.44).

Table 4: Effect of sources of planting materials on Marketable Root number

Source of Planting material (S)	Varieties (V)				Source
	Apomuden	Bohye	Dadanyuie	Ligri	Mean
Farmers	15	20	17	16	17
Field	28	25	28	28	27
In vitro.	39	31	41	35	36
Variety Mean	27.3	25.3	29	26	

LSD (0.05): Planting material source (S) = 8.95; Variety (V) =3.29; Interaction (SxV) = 9.05

4.8 Number non-Marketable Root

Source of planting materials did not significantly ($P < 0.05$) affect number of non- marketable roots number produced. However, main effect of variety did show significant effects ($P < 0.041$) on number of non- marketable roots produced. In general, Bohye recorded the highest nonmarketable root mean value and Apomuden recorded the lowest (Table 5).

Table 5: Effects of source of planting material on non-marketable root number

Source of Planting material (S)	Varieties (V)				Source
	Apomuden	Bohye	Dadanyuie	Ligri	Mean
Farmers	26	26	31	24	35.6
Field	27	31	67	40	55
in vitro.	35	31	46	23	45
Variety Mean	29.3	29.30	48	29	

SD (0.05): Planting material source (S) = 24.82; Variety (V) =13.37; Interaction (SxV)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effects of source of sweetpotato planting material and variety on field virus scores and virus load

The different source of planting materials and varieties affected the field virus severity symptoms rating. Field visual observation on sweetpotato leaves showed different degree of symptoms which were suspected to be viral infection. Among these virus symptoms observed were chlorotic spot bordered by purple pigment, vein discoloration, and leaves curls, slightly orange leaves, yellowing of upper and middle leaves, yellow veins, and stunted growth. These symptoms observed agree with earlier assessing report by Gibson *et al*. (1998) which stated that SPCSV virus for example may either cause yellowing symptoms at lower or middle leaves or purpling or may not cause symptoms at all depending on the varieties. There were mostly no visual observable symptoms in the clean in *vitro* plantlets and this may be due to minute among of virus infection or single virus infection and this is inconformity with Opare-Obisaw *et al*. (2000) who stated that, when three sweetpotato varieties were evaluated, with single virus infection of SPFMV induced no observable symptoms in all the three sweetpotato varieties.

In the first phase of virus scoring (virus 1) it was observed that, the Farmer's materials showed more viral symptoms compared to Field generated materials, and *in vitro* generated materials showed very minimal or negligible virus symptom per plot. The second phase of (Virus 2) scores which was taken from eighth week after planting showed similar result but severity increased across all source of planting materials as well as varieties. This could be attributed to the fact that, the more sweetpotato plants (host) are exposed to viral transmitting vectors on the field, the more virus symptoms may manifest. This agreed with Brunt (1996) findings which stated that, increment in host plant stress by increasing the duration of exposure to disease causing vectors or source of contamination leads to more symptoms expression.

However, some varieties within *in vitro* tissue culture plantlets and Field derived materials showed equivalent low virus symptoms score and this could be due to the inherent ability of some genotype among the Field materials to grow faster to regenerate and recover from virus symptoms. This agreed with Schaefers and Terry (1976); Gibson *et al*. (1998) findings which stated that the mild or no observable virus symptoms in most varieties is due to the variety ability to grow faster to restrict cell to cell movement and/or replication of the virus within the plants. Infection with the different viruses caused a range of symptoms on the different sweetpotato varieties and on *Ipomoea setosa.* The use of different sources of planting materials revealed different visual virus symptoms scores on the field. The NCM – ELISA result also revealed variations of different viruses with importance on different source of planting materials. Wambugu (1991); and Ateka *et al*. (2007) reported that viruses with economic importance that have been reported on Farmers' fields are SPFMV, SPCSV, SPMMV, SPCFV and CMV and the most widely spread in the major sweetpotato production areas are SPCSV, SPFMV and SPMMV. Virus serology test confirmed the field virus symptom score that were observed on the field were actually due to virus. The Farmer materials that have been on the field for about 5 years without virus re-cleaning were observed to have the severe virus symptoms than Field materials that were subjectively selected based on the absence of virus symptoms. The field materials were also found to be highly infected with more virus symptoms than the *in vitro* generated planting materials. The virology test result also proved that all different source of sweetpotato planting materials have different viruses load. This is possible because, in general the number of generations a sweetpotato planting materials replanted on field without tissue culture re-cleaning or protection from virus vector transmitters the higher the possibility of higher virus titer/load of infected virus and the severity of the virus symptoms may manifestation. This in conformity with early assessing report of Moyer and Salazar, (1989) which stated that all sweetpotatoes grown from non-virus-tested source of planting

materials revealed the presence of one or more viruses in them. Sweetpotato is propagated vegetatively and in the process, propagating material gradually accumulates pathogens, especially viruses that cause decline in yield and quality. There are different viruses that have been isolated from different sweetpotato cultivars (Clark *et al*., 2012). The virology result also revealed sources of planting materials have varying virus load. This observed variation of field virus scores based on visual virus symptoms on different varieties could be attributed to inherent ability some varieties to express the symptom of a particular virus type infected but does not express other viruses' type. Infection with the different viruses caused a range of symptoms on the different sweetpotato varieties and on *Ipomoea setosa.* These symptoms are induced on *I. setosa* after grafting (Bryan *et al*., 2003). This agreed with Gibson *et al*. (1998) who revealed that different sweetpotato varieties have different levels of tolerance to viruses (Valverde *et al*., 2007). Accumulation of difference virus in different planting materials proved to have varying degree of virus expression.

5.2 Effects of source of sweetpotato planting materials on chlorophyll content and vine weight

The chlorophyll (SPAD values) meter reading is an index of chlorophyll content which relates to greenness. In this experiment when cleaned *in vitro* generated planting materials were compared with the other two planting materials chlorophyll content increased relatively higher than nonvirus cleaned tested planting materials (Farmer's materials and Field materials). This variation of source of planting materials on chlorophyll content could be attributed to variation of virus load which consequently influence photosynthetic ability base on the green pigment matter in the leaves. This could be that, the uncleaned generated materials that were having higher levels of virus accumulation in them which lead to leave chlorosis, has resulted in low chlorophyll SPAD meter

readings in such source of planting materials which induces stunted growth which leads to low biomass production. Sweetpotato virus disease (SPVD), characterized by small, distorted leaves which are often narrow (strap-like) and wrinkled, with a chlorotic mosaic or vein clearing, stunting of plants and heavy yield losses, has been reported since 1944 (Karyeija, *et al.,* 1998 and Mukasa, *et al*., 2003). Infections have considerable effects on cell metabolism such as photosynthesis, respiration, and transpiration. Symptom induction is primarily by the perturbation of the cell metabolism and damage to cell organelles such as chloroplasts (Roger, 2009). This is in conformity with Jones (2007) studies, who reported that, Chlorosis, the loss of chlorophyll from plants, can be caused by virus infection and number of genetic and environmental factors. Severa1 strains (biotypes) of cucumber mosaic virus (CMV) are known that can induce chlorosis, although most strains induce a light green/dark green mosaic (Kaper and Waterworth, 1981). The M strain of CMV (M-CMV) induces chlorosis on many host species; on Nico- tiana species it induces an extreme yellow white chlorosis (Rao and Francki, 1982).

Also, inherent varietal variation among sweetpotato with respect to chlorophyll content was observed (Figure 11). Though there were vast different in chlorophyll content among the varieties but there were no significant difference for all varieties. The varying SPAD meters readings among varieties was due to inherent ability of some varieties to manufacture chlorophyll more than others. This agrees with the earlier report that states that, photosynthetic parameters significantly vary between the different crop varieties. Plant growth depends on photosynthesis, which is affected by environmental factors such as temperature and nutrition (Chen *et al*., 2010).

Also, the variations among source of planting materials of sweetpotato for vine weight was also observed. These variations could be due to different virus accumulation in the different source of planting materials leading to varying of green pigment matter in the plants and consequently vine production. This conformed to earlier study that states that, in plants infected with SPFMV and

SPMMV, most of assimilate produced are allocated/retained in the leaves and vines but less allocated to the storage roots reducing the root production when compared to the virus-free plants. This can be attributed to impaired translocation of assimilates, among other metabolic activities as reported for cassava infected with cassava mosaic Gemini viruses' s (Clark *et al.,* 2012). Infection with SPCSV and SPFMV + SPCSV reduced the vine yield and photosynthetic active radiation (PAR) interception of the sweetpotato plants. The reduction may be attributed to reduced photosynthetic activity due to reduced photosynthetic organs, leading to stunting.

5.3 Effects of source of planting materials and variety on total root yield

In this study, variations in total root yield among source of planting materials was observed. The results indicated that, tissue culture *in vitro* cleaned planting materials produce higher total root yield and this could be partly due to the fact that they have least or no virus accumulation in them as *Ipomoeas setosa* was used as indicator to declare them virus free planting materials. This is harmony with Meristem culture which has also been proposed as a dependable method in obtaining and maintaining sweetpotato seed stocks. Thus virus free plantlets derived from these seed stocks have been reported to increase the yield in sweetpotato production, (Manganaris *et al*., 2003

and Fonseca *et al*.,2003) has also compared tissue culture regeneration and conventional growing in sweetpotato and revealed that *in vitro* tissue culture materials were better in yield. Furthermore, Yildirim *et al*. (2011) reported the positive effect of in vitro plantlets in the field growing. Also, Gao *et al*. (2000) also reported that, differences in yields are mainly due to variation in quality of the material use for propagation, often taken from the previous season of farmer's fields. These fields are usually infected with several viruses' types, thereby increasing the effect on yields. Systematics virus accumulation in Farmer materials as well as Field derived materials as a result

of infection and re-infection could resulted in drastic reduction in yield in Farmer materials. This is in harmony with, Pozzer *et al*. (1995) who reported that, when sweetpotato virus index materials derived from meristems of heat-treated plants were compared to yields from cuttings taken from farmers' fields yield increases up to118 %. Similarly, is confirmed in Peru by Fonseca *et al.* (2003) the ability of virus index materials to increase productivity which confirms long held belief that pathogen tested material had various advantages, such as higher yields than virus infected materials. Farmer materials recorded the highest percentage of virus infection of either one or two or even more Viruses' types. This could be the reason of drastic root yield reduction in farmer materials because it has not been cleaned for about five years and are not protected from virus vectors transmitted virus. This is in harmony with Tesfaye *et al*. (2013) who reported that in Ethiopia, the root yield reduction due to the interaction effects of SPFMV and SPCSV was said to be 37% compare to single infection. Yield losses in extra of 70 % have been attributed to the synergistic result of SPFMV and SPCSV in Uganda (Gibson *et al*., 1998; Gibson and Aritua, 2002). According to the virology analysis (Table 3) in chapter four Farmer materials had more virus titer as compare to Field derived materials and pathogen tested materials and this could be the reason for drastic reduction in storage root yield of farmer materials. This is in harmony with early studies that states that virus infection is known to affect plant physiology dramatically, including decreased photosynthesis, increased respiration and altered carbohydrate levels (Shalitin and Wolf, 2000). The alteration of these physiological processes caused by viral diseases is one of the primary causes of yield reduction in crop productivity across the world (Agrios, 2005). Various methods of eliminating virus disease of sweetpotato have been developed. These include meristem-tip culture and thermotherapy (El-Far and Ashoub, 2009; Valverde *et al*., 2008; Wang *et al*., 2010) and the use of more advanced technology. Thus, the use of virus-tested plants can improve cultivar productivity by as much as 81 – 224 % (Clark and Hoy, 2006; Feng *et al*., 2000). Furthermore, the

yield of virus-tested clones generally decreases gradually with successive plantings as a result of re-infection by viruses. According to Mesfin *et al*. (2009) it has been reported that continuous cultivation of potato varieties using seeds from previous season, 41-62 % yield reduction was recorded after four years depending on the relative tolerance of each variety which was mainly due to the accumulation of virus that could cause degeneration of seeds. Hence, an effective production and operation system is needed to frequently replenish planting stock.

Also, the variation in storage root yield could also be due to difference in genotyping ability of the varieties used. Due to the fact that different genotypes respond to different virus infection differently.

Studies conducted to evaluate 19 sweetpotato varieties yield by Pozzer *et al*. (1995), Missah *et al*. (1991) revealed that, some varieties may have performed better than other although they are infected to the same virus. Yields of sweetpotato recorded in Ghana at the subsistence level are quite low compared with the IITA varietal studies.

5.4 Effects of source of planting materials and variety on marketable and non-marketable root

The result revealed that there were generally increased in yield of all *in vitro* tissue cultured pathogen tested planting materials across all varieties selected. The beneficial effects of pathogen tested materials on yield has been reported in several investigations. Non-pathogen materials that were generated from field and Farmers could be infected with certain amount viruses that could induce leaves chlorosis leading to low chlorophyll content and consequently caused reduction in yield of roots that are not marketable. The result obtained from the experiment is in conformity

with Hadidi *et al*. (1998) who stated that, Crop species that are vegetative propagated are particularly prone to losses caused by viruses that are transmitted from generation to generation in the planting materials. The *in vitro* generated platelets produced more marketable root yield of 11.23 % and 17.33 % better than Farmer's and Field materials respectively. Also Trials conducted at Bundaberg, Cudgen and Gatton in Queensland have indicated that Beauregard had a superior marketable yield as high as 24 tonnes per hectare, about 1 to 2 tonnes higher than Northern Star (Dennien, 2012). Pozzer *et al*. (1995), revealed that for 14 cultivars-trial at Bundaberg, an average of 38 % increase in total storage root yield for all cultivars Beauregard is significantly improved in marketable and total storage root yield of up to 470 % and 148 % respectively. Pozzer *et al*. (1995), also stated that, the findings showed the marketability of Higaturu (L3) was higher than Beauregard. Similarly, Sweetpotato is a vegetative propagated crop, and systemic pathogens like viruses can persist and spread over successive crop cycles (Bryan *et al*., 2003). Also, Pozzer *et al*. (1995), in Brazil, reported that, yield increases of 118 % were observed when virus-tested cuttings derived from meristems of heat-treated plants were compared to yields from cuttings taken from fields. Laurie *et al*. (2000) studies also indicated that elimination of viruses can lead to an increase in yield up to 80 %. Similarly, In South Africa, the virus-free sweetpotato scheme at ARC-VOPI is effective in maintaining the performance of sweetpotato cultivars in terms of yield (Laurie *et al*., 2000). By contrast, in Uganda no significant (P = 9 %) yield loss was recorded for Field generated planting material matched to virus-cleaned material of the same cultivar (Gibson *et al*., 2009).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study assessed three sweetpotato planting materials sources with different health status. The sources were *in vitro* generated, apparently 'clean' Field generated and Farmer's planting materials. Four varieties of the crop were selected under each planting material source. Data were recorded on viral symptoms and load, chlorophyll content, vine yield, root yield and marketable root yield.

The *in vitro* generated planting materials recorded least viral symptoms score. The Field materials recorded lower viral symptom score than the Farmer's material. The NCM-ELISA test also confirmed the symptomatic score which revealed very low viral load in the *in vitro* generated planting materials.

The chlorophyll content was determined by using SPAD meter. The Farmer's planting material source recorded lower chlorophyll content than the other two sources. The *in vitro* generated planting material had higher chlorophyll content than the Field planting material source. Materials from clean source produced more vine and root yield, thus *in vitro* planting material produced 22.8 % and 51.3 % higher vine tonnage than Field and Farmer's material respectively.

The *in vitro* generated planting material produced more root and more marketable proportion than the two other sources. The Apparently 'clean' field planting materials also did better than the Farmer's planting material source. Thus the cleaner the planting material the higher the root yield. The four varieties produced higher root under *in vitro* planting material source than the field source though the differences were not significant.

However, the difference was significant when the varieties were produced using Farmer's planting materials. Under cleaner planting material source, *in vitro* and Field, Dadanyuie and Apomuden recorded higher root yield. However, in the Farmer's planting material source, perceived to be unclean, Bohye which was the least performer under clean planting material sources outperformed the other three varieties.

6.2 Recommendations

The study therefore recommends that:

- Farmers should be encouraged to use tissue culture clean sweetpotato planting materials every five years since the viral load increases with time after generations of replanting.

- Farmers should practice vine multiplication in insect secluded areas to prevent virus insect vectors.
- Farmers should avoid using deteriorated vines that are more than three months old because vines older than three months and growing unprotected could be virus infected.
- Where farmers do not get clean planting material they should cultivate Bohye variety which can give higher yield under viral stress condition.

REFERENCES

Abd El-Baky, M. M. H., Ahmed, A. A., Abd El-Aal, F. S. and Salman, S. R. (2009). The causes and control of virus diseases in developing countries: Is sweetpotato virus disease the main problem International Potato Center Program Report, pp 241-248.

Abidin, P.E. (2004). Sweetpotato breeding for northeastern Uganda: Farmer varieties, farmer participatory selection, and stability of performance. PhD Thesis, Wageningen University, the Netherlands, 152 pp. ISBN 90- 8504-033-7.

ACIAR (2009). Soil fertility in sweetpotato-based cropping systems in the highlands of Papua New Guinea, 71, The Australian Centre for International Agricultural Research (ACIAR) Canberra, Australia.

Agrios, G.N. (2005). Plant Pathology 5th Edition. Virus diseases. Academic Press, San Diego, CA

Alam, M., Banu, M., Swaraz A., Parvez S., Hossain M., Khalekuzzaman, M. and Ahsan, N. (2004). Production of virus free seeds using meristem culture in tomato plant under tropical conditions. J Plant Biotechnol. 6: pp. 221–227.

Albuquerque, L. C., Inoue-Nagata, A. K., Pinheiro, B., Resende, R. O., Moriones, E. and NavasCastillo, J. (2012). Genetic diversity and recombination analysis of sweepoviruses from Brazil. Virology Journal 9, pp. 241–62.

Alemu, T. (2004). Characterization of Viruses of Pepper (Capsicum spp.) and Sweetpotato (*Ipomea batatas*) from Ethiopia. Doctoral Dissertation. University of Bonn. pp. 126.

Alicai, T., Adipala, E. and Gibson, R. W. (1999). Seasonal changes in whitefly numbers and their influence on incidence of sweetpotato chlorotic stunt virus and sweetpotato virus disease in sweetpotato in Uganda. International Journal of Pest Management 45: pp. 51-55.

Alemu, T. (1996). Characterization of Viruses of Pepper (Capsicum spp.) and Sweetpotato (*Ipomea batatas*) from Ethiopia. Doctoral Dissertation. University of Bonn. pp. 126.

Antia, B. S., Akpan, E.J., Okon, P.A. and Umoren, I.U. (2006). Nutritive and anti-nutritive evaluation of sweetpotatoes (*Ipomoea batatas*) leaves. Pakistan J Nutr 2: pp.166–8.

Anyaegbunam, H. N., Asumugha., G. N., Mbanasor, E. O., Ezulike, T. O. and Nwosu, K. I. (2008). Guide to Improved Sweetpotato Production in Nigeria. National Root Crops Research Institute, Umudike, Extension Guide 24, pp.1-9.

Aritua, V. (1999). Yield and reaction of non-indigenous sweetpotato clones to sweetpotato virus disease in Uganda. Proceedings of International Workshop on Sweetpotato Cultivar Decline Study, Miyakonojo, Japan, pp. 48-54.

Aritua, V., Olanya, O. M., El-Bedewy, R., Ewell, P.T., (1999). Yield sweetpotato clones to sweetpotato virus disease in NIgeria. Proceedings of International Workshop on Sweetpotato Cultivar Decline Study, Miyakonojo, Japan, pp3-23.

Aritual (1998). Nutritive and anti-nutritive evaluation of sweetpotatoes (*Ipomoea batatas)* leaves. Pakistan J Nutr 2: pp.166–8.

Ateka, E.M., Barg, E., Njeru, R.W., Thompson, G. and Vetten, H.J. (2007). Biological and molecular variability among geographically diverse isolates of sweetpotato virus 2. Arch. Virol. 152.

Austin, D. F. (1992). The *Ipomoea batatas* complex. I. Taxonomy. Bull. Torrey Botanical Club: 10(2): pp. 114-129.

Averre, C.W., Abad, J. A. and Jester, W.R. (1993). Sweetpotato virus-free micro propagated Jewel and Beauregard evaluation 1990 –1991. Biol. Cult. Tests Control Plant Dis. 8:31.

Beetham, P. and Mason, A. (1992), Production of pathogen-tested sweetpotato, ACIAR technical report 21, Department of Agriculture, Burnley, Australia.

Belehu, T. (2003). Agronomical and physiological factors affecting growth, development and yield of sweet potato in Ethiopia', Phd thesis, Doctor of Philosphy thesis, Pretoria.

Bhojwani, S.S. and Razdan. M.K. (1996). Production techniques of pathogen-tested sweetpotato, Page Count: 766 View all volumes in this series: Studies in Plant Science. Published Date: 8th November Select country/region pp. 67.

Bourke, R. M. (2009). The sweetpotato in Oceania, 22, Springer Science and Business Media B.V., Canberra, Australia.

Bourke, R. M. (1989). The sweetpotato in Oceania, 22, Springer Science + Business Media B.V., Canberra, Germine.

Bourke, R. M. (1991). The sweetpotato in Oceania, 22, Springer Science and Business Media B.V., Canberra, Gambia.

Bourke, R. M. (2006). Sweetpotato in Papua New Guinea ', paper presented to ACIAR Sweetpotato Workshop, Madang, Papua New Guinea, June 2006.

Bovell-Benjamin, A.C. (2007). Sweetpotato: a review of its past, present, and future role in human nutrition. Adv. Food Nutr Res 52: pp. 1–5.

Boyette, M.D. (2009). The investigation of negative horizontal ventilation for long-term storage of sweetpotatoes', Applied Engineering in Agriculture, 5, pp. 701-8.

Brunt, A.A. Abad, J. A. and Jester, W.R. (1996). Sweetpotato ringspot a newly recognized Nepolike virus from Ipomoea batatas (Attract). Int. Virol congress, Berlin. pp: 6: pp.19 – 24.

Brunt, A.A. (1990). Sweetpotato ring like virus a newly recognized Nepo -like virus from *Ipomoea batatas* (Attract). Int. Virol congress, 6: pp. 19 – 24.

Bryan, A. D. (2002). Impact of Sweetpotato feathery mottle virus and micro propagation on yield, root quality, and virus incidence in commercial sweetpotato production systems. MS thesis. N.C. State Univ., Raleigh.by western blotting. J. Gen. Plant Pathol. 2: pp.182–184.

Bryan, A. D., Schultheis, J. R., Pesic-van Esbroeck, Z. and Yencho, G. C. (2003). Cultivar decline in sweetpotato: II. Impact of virus infection on yield and storage root quality in 'Beauregard' and 'Hernandez'. J. Am. Soc. Hortic. Sci. 128: pp. 856-863.

Burban, D.E, Van Etten, J. L., Xia Y. and Meints, R. H. (1992). Growth cycle of a virus, PBCV-1, that infects Chlorella-like algae. Viro. l pp.17- 25.

Byamukama, E., Gibson, R. Aritua, V. Mpembe, I. and Kayongo, J. (2004). Control strategies for sweetpotato virus disease in Africa. Virus Research 100, pp. 115-122.

Byrne, D. N., Bellows, T. S. Jr. and Parella, M. P. (1990). Whiteflies in agricultural systems. Whiteflies: their bionomics, pest status and management. Gerling, D. (ed) Great Britain, Intercept. pp. 227-261.

Cadena – Hinojosa B. and Cambell S. (1981). Sweetpotato in Papua New Guinea ', paper presented to ACIAR Sweetpotato Workshop, Madang, Papua New Guinea, June 1981.

Campbell, R. Gibson, R. Aritua, V. Mpembe, I. and Kayongo, J. (1997). Sweetpotato in Papua Nger. Paper presented to ACIAR Sweetpotato Workshop, Madang, Papua New Guinea, June 1997.

Carey, E. E., Gibson R.W., Fuentes, S., Machmud, M., Mwanga, R.O.M., Turya-mureeba, G., Zhang, L. and Salazar, L.F. (1999). The causes and control of virus diseases in developing countries: Is sweetpotato virus disease the main problem? International Potato Center Program Report for 1997-98, pp. 241-248.

Carey, E. E., Mwanga, R.O.M., Fuentes, S., Kasule, S., Macharia, C., Gichuki, S.T. and Gibson, R.W. (1997). Sweetpotato viruses in Uganda and Kenya: results of a survey.Proc. Sixth Triennial Symp. Inter. Soc. Tropical Crops – Africa Branch pp. 22–28.

Carey, E. E., Mwanga, R.O.M., Fuetes, S., Kasue, S., Macharia, C., Gichuki, S.T. and Gibson, R.W. (1996). Sweetpotato viruses in Uganda and Kenya: Results of a survey. Proceedings of Sixth Triennial Symposium of the International Society of Tropical Root Crops- Africa Branch (ISTRC-AB) 22-28 October1995, Lilongwe, Malawi pp. 457- 461.

Carey, E. E., Mwanga, R.O.M., Fuentes, S., Kasule, S., Macharia, C., Gichuki, S.T. and Gibson,

R.W. (1999). Sweetpotato viruses in Uganda and Kenya: results of a survey.Proc. Sixth Triennial Symp. Inter. Soc. Tropical Crops – Africa Branch pp. 22–28.

Carrington, J. C., Kasschau, K. D., Makajan, S. K. and Schaad, M. C. (1996). Cell-to-cell and long-distance transport of viruses in plants. Plant Cell: 8, pp.166-168.

Carroll, H.W., Villordon, A.Q., Clark, C.A., La Bonte, D.R. and Hoy, M.W. (2004). Studies on Beauregard sweetpotato clones naturally infected with viruses. Int. J. Pest Manag. 50: pp.101–106.

Chen, W. (2012). Exogenous hydrogen peroxide positively influences root growth and exogenous hydrogen peroxide positive influences root growth and metabolism in leaves of sweetpotato seedlings. Aus. J. Crop Sci. 11: pp. 1572-1578.

CIP (1987). Sweetpotato Facts: Production, Utilization, Consumption, CIP (International Potato Centre).

CIP (1998). Sweetpotato Facts: Production, Utilization, Consumption, CIP (International Potato Centre).

CIP (1999). Sweetpotato Facts: Production, Utilization, Consumption, CIP (International Potato Centre).

CIP. (1992). Exogenous hydrogen peroxide positively influences root growth and exogenous hydrogen peroxide positive Influences root growth and metabolism in leaves of sweetpotato seedlings. Aus. J. Crop Sci. 11: pp. 1572-1578.

CIP (2009). Procedures for the evaluations and analysis of sweetpotato trials.

Cipriani, G., Fuentes, S., Bello, V., Salazar, L. F., Ghislain, M. and Zhang, D. P. (2001). Transgene expression of rice cysteine proteinase inhibitors for the development of resistance against sweetpotato feathery mottle virus. Scientists and farmer: Partners in research for the 21st Century, pp. 267–271.

Clark, C. A. and Moyer, J. W (1988). Compendium of Sweetpotato Diseases, APS Press, The American Phytopathological Society, St. Paul, MN, USA, pp.74.

Clark, C. A. and Hoy, M. W. (2006). Effects of common viruses on yield and quality of Beauregard sweetpotato in Louisiana', Plant Disease, 1, pp. 83-87.

Clark, C. A., Davis, J. A., Abad, J. A., Cuellar, W. J. and Fuentes, S. (2012). Sweetpotato viruses: 15 years of progress of understanding and managing complex diseases. Plant Dis 96: pp.168–185.

Clark, C. A., Valverde, R. A., Fuentes S., Salazar, L.F. and Moyer J.W. (2002). Research for improved management of sweetpotato pests and diseases. T. Ames (ed.). Proceedings of

the first international conference on sweetpotato food and health for the future. Acta Hort. 583. p. 103 –112.

Cohen, J, Salomon, R. and Loebenstein, G. (1988). An improved method for purification of sweetpotato feathery mottle virus directly from sweetpotato', Phytopathology, 6, pp. 11-809.

Cohen, J., Franck, A., Vetten, H.J., Lesemann, D.E. and Loebenstein, G. (1992). Purification and properties of clostero virus-like particles associated with a whitefly-transmitted disease of sweetpotato. Ann. Appl. Biol. 121: pp.257–268.

Cohen, J., Franck, A., Vetten, H.J., Lesemann, D.E. and Loebenstein, G. (1990). Virus properties of clostero particles associated with a whitefly-transmitted disease of sweetpotato. Ann. Appl. Biol. 121: pp.17–28.

Coleman, E, Maltby, J, McCrystal, R, O'Donnell, W. and Playford, C. (2006). Developing smooth skin easy to peel sweetpotatoes, HAL project VG02114, Department of Primary Industries and Fisheries, Queensland Government, Sydney, Australia.

Coleman, E, McCrystal, R, Maltby, J. and O'Donnell, W. (2003). Development of smooth skinned easy to peel sweetpotato literature review., Queensland Department of Primary Industries and Fisheries, Brisbane, Australia.

Coleman, E. (2011). Developing smooth skin easy to peel sweetpotatoes, HAL project VG02114, Department of Primary Industries and Fisheries, Queensland Government, Sydney, Australia.

Coleman, E. (2006). Developing smooth skin easy to peel sweetpotatoes, HAL project VG02114, Department of Primary Industries and Fisheries, Queensland Government, Sydney, Australia.

Colinet, D., Nguyen, M., Kummert, J., Lepoivre, P. and Xia, F.Z. (1998). Differentiation among potyviruses infecting sweetpotato based on genus- and virus-specific reverse transcription polymerase. Appl. Biol. 21: pp. 27–26.

CRI. (2002). Crop Research Institute of Council for Scientific and industrial Resource, Ghana. Sweetpotato: The crop of the future. Factsheet, November, 2002. pp.6.

Dangler, J. M. (1994). Sweetpotato foundation programs provide sweetpotato growers with high quality pathogen-free stock. Compendium of sweetpotato foundation programs. Hort. Technology. 4: pp. 222 - 224.

De Souza, J and Cuellar, W.J. (2011). Sequence analysis of the replicas gene of 'sweet potato caulimo-like virus' suggests that this virus is a distinct member of the genus Cavemovirus', Archives of Virology, 156, 3, pp. 535-7.

Degras, L. (2003). Sweetpotato: The tropical Agriculturalist. Macmillan publishers Ltd. Lima, Peru. J. pathology. Virology 7: pp.16-19.

Dennien, S, Homare, D, Hughes, M, Lovatt, J, Coleman, E and Jackson, G.V.H. (2013). Growing healthy sweetpotato Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia.

Dennien, S. (2012). Sweetpotato varieties to meet market needs, HAL Project (VG09009), Queensland Department of Agriculture, Fisheries and Forestry, Brisbane, Australia. 7: pp. 13-39.

Diaz, J., Fermin dela, P. and Austin, D. F. (1992). Enlargement of fibrous roots in *Ipomoea batatas* (Convolvulaceae). Economic Botany Dis. 90: pp. 1347 –1352.

Du Plooy, D. P., Wagner, J. J. and Bosch S. E. (1988). Cultivation of Sweetpotatoes. Printed and Published in the Republic of South Africa by the Department of Agriculture and Water Supply, pp 1 -8.

Eastop, V. F. (1983). The biology of the principal aphid virus vectors. Plumb, R. T.; Thresh, J. M. ed. Oxford, England, Blackwell Scientific Publications. In: Plant virus epidemiology pp. 115-132.

Edmond, J. B. Eastop, V. and Ammerman, G.R. (1971). Sweetpotatoes: Production, processing, marketing, Westport, CT: The AVI Publishing. pp. 334.

El-Far, M. M. M. and Ashoub, A. (2009). 'Utility of thermotherapy and meristem tip for freeing sweetpotato from viral infection', Australian Journal of Basic and Applied Sciences, 3, (1), pp. 9-153.

Ellis, W. O., Oduro, I., Fianko, K. and Otoo, J. A. (2001). Quality of Gari from Eighteen Sweetpotato Varieties. In Proceedings of the 8th ISTRC AB Symposium, Ibadan Nigeria, 2001. pp. 146-50.

FAO (2009). FAO statistics http://apps.fao.org:Food and Agricultural Organization.

FAO (2004). FAO statistics http://apAguayo VM, Baker SK, Crespin X, Hamani H, mamadoulTa¨ıbou A. 2005.Development Partners website: www.tzdpg.or.tz).FAO website (www.fao.org).

FAO (2012). FAO production statistics. Available on http://www.fao.org/ assessed on 12/08/2013.

FAOSTAT (2008). Statistical database (online) of Food and Agriculture Organization of the United Nations.

FAOSTAT (2001). Food and Agriculture Organization of the United Nations, Production Statistics (http://www.apps.fao.org/).

FAOSTAT (2006). Statistic Database (online) available online at http://apps.fao.org assessed on 12/08/2013.

FAOSTAT (2007). FAO Statistical Databases. URL http://apps.fao.org/ Fletcher, J.D., Lewthwaite.

Feng, G., Yifu, G. and Pinbo, Z. (2000). Production and deployment of virus-free sweetpotato in China', Crop Protection, vol. 19, no. 2, pp. 105-11Field evaluation of yield effects on the U.S.A. heirloom sweetpotato cultivars infected by Sweetpotato leaf curl virus Crop Protection, 7: pp. 757-65.

Fonseca, G., Mohapatra, S., Panda, S. H., Sahoo, S. K., Sivakumar, P. S. and Ray, R. C. (2003). Beta-carotene-rich sweetpotato curd: production, nutritional and proximate composition. Internat J. Food Sci. Tech. 42: pp. 5 –14.

Fuentes, L. F. (2007). First report of sweetpotato leaf curl virus in Peru', Plant Disease, 8: pp. 98.

Feng, G., Yifu, G. and Pinbo, Z. (2000). Production and deployment of virus-free sweetpotato in China', Crop Protection, (2): pp. 105-11.

Gamarra, H. A., Fuentes, S., Morales, F. J., and Barker, I. (2008). *Bemisia afer*, a vector of Sweetpotato chlorotic stunt virus. Plant Dis. (submitted).

Gao, F., Gong, Y. F., and Zhang, P. B. (2000). Production and development of virus-free sweetpotato in China. Crop Protect. 19: 105 – 111.

Gibb, K.S. and Padovan, A.C. (1994). Detection of sweetpotato feathery mottle Potyvirus in sweet potato growing in Northern Australia using an efficient and simple assay ', International Journal of Pest Management. 9: pp. 223-8.

Gibbons, S. (2000). Transgenic sweetpotato research collaboration: A case study of ABSP involvement in Kenya. Linkages Newsletter Fourth Quarter Feature Article. pp.34.

Gibson, R. and Aritua, V. (2002). clostero virus-lik particles associated with a whitefly-transmitted disease of sweetpotato. Virology J. Crop Protect. 9: 15 – 19.

Gibson, R. Aritua, V., Byamukama E, Mpembe I, Kayongo, J. (2004) Control strategies for sweetpotato virus disease in Africa. Virus Research 100: pp. 115-122.

Gibson, R. W., Jeremiah, S. C., Aritua, V., Msabaha, R., P., Mpembe, I. and Ndunguru, J. (2000). Sweetpotato virus disease in sub-Saharan Africa: Evidence that neglect of seedlings in the traditional farming system hinders the development of superior resistant landraces. Journal of Phytopathology 148: pp. 441-444.

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

Gosukonda, R. M., Prakash, C.S.and Dessai, A.P., (1995). Shoot regeneration in vitro from diverse genotypes of sweetpotato and multiple shoot production per explant. Hort. Sci. 30(5): pp. 1074–1077.

Gruden, K., Pompe-Novak, M., Baebler, S., Krecic-Stres, H., Toplak, N., Hren, M., Kogovsek, P., Gow, L., Foster, G.D., Boonham, N. and Ravnikar, M. (2008). Expression microarrays in plant- virus interaction', in GDJIEHYNPD Foster (ed.), Methods in Molecular Biology, 451: pp. 583-613.

Guidoni, S., Mannini, F., Ferrandino, A., Argamante, N. and Di Stefano, R. (1997). The effect of grapevine leafroll and rugose wood sanitation on agronomic performance and berry and leaf phenolic content of a Nebbiolo clone (Vitis vinifera). Am J. Enol Viticult pp.55- 57.

Gurmu, F., Hussein, S. and Laing, M., (2015) Diagnostic assessment of sweetpotato production in Ethiopia: constraints, post-harvest handling and farmers' preferences. Research on Crops; 16(1): pp.104-115.

Gutierrez, D. L., Fuentes, S. and Salazar, L. F. (2003). Sweetpotato virus disease (SPVD) distribution, incidence, and effects on sweetpotato yield in Peru. Horticulture J. 16:535–537. Plant Dis 87: pp: 297–302.

Hadidi, R.K., Khetarpal, Q. and Koganezawa, H. (1998). Economical losses due to plant viruses St. Paul, MN, USA: APS Press.

Hagenimana, V., (2001). Final Technical Report, Project R7036 (ZB0099). An enterprise approach to commodity system improvement: Sweetpotato in Uganda and Kenya. Plant Dis. 87:297302.

Holwede, R.K. and Ekaganezawa, H. (1998). Economical losses due to plant viruses St. Paul, MN, USA: APS Press.

Hall, M.R (1994), 'Combined heating applications increased plant production from bedded sweetpotato roots', HortScience, vol. 29, no. 9, pp. 10-422.

Hirorko, S., Noriko, I., Satoshi, T., Tadahiro and Akio, M. (2000). Nutritive Evaluation of chemical composition of leaves, stalks and Stem of sweetpotato (*Iponea Balakas Poir*), Food Chem., 68: pp. 35- 67.

Huang, A. S., Tanudjaja, L.and Lum, D. (2000). Contents of Alpha-, Beta-carotene, and dietary Fiber. HortScience, 29(19): pp. 10-42.

Hughes, M. and Dennien, S. (2013). Sweetpotato viruses in Australia, Brisbane, Australia, 26 June Personal communication.

IsHak, J.A., Kreuze, J.F., Johansson, A., Mukasa, S.B., Tairo, F., El-Abbas, F.M.A. and Valkonen, J.P.T. (2003). 'Some molecular characteristics of three viruses from SPVD-affected sweetpotato plants in Egypt', Archives of Virology. 12, pp. 49-60.

Islam, S. (2006). Sweetpotato (*Ipomoea batatas* L.) leaf: its potential effect on human health and nutrition. J. Food Sci. 71, (2), pp.13.

Jones, R. A. C. and Dwyer, G. I. (2007). Detection of sweetpotato chlorotic fleck virus and sweetpotato feathery mottle virus - strain O in Australia', Australasian Plant Pathology. 6: pp. 51-54.

Jones, R. A. C. characteristics of three viruses from SPVD-affected sweetpotato plants in Egypt Archives of Virology, vol. 6, no. 12, pp. 19-21.

J R T, (2000). Japan Soc for Root and Tuber Crops, Mini White paper, sweetpotato in Japan 2: pp. 27-32.

Kaper, J. M., and Waterworth, H. E. (1981). Cucumoviruses. In Plant Wood Cliffs, NJ: PrenticeHall). Virus lnfections and Comparative Diagnosis, E. Kurstak, ed (New York: ElsevierIHolland Biomedical Press), pp. 257-332.

Kapinga, R. and Ndunguru, J. (2007). Viruses and virus-like diseases affecting sweetpotato subsistence farming in southern Tanzania. African J. Agric. Res. 2: pp.232–239.

Kapinga, R. P. T., Ewell, Jeremiah, S.C. and Kileo, R.D. (2007). Sweetpotato in Tanzanian farming and food system: Implications for Research. A farming systems survey report by the Tanzania National Root and Tuber Crops and Farming Systems Research Programme and the International Potato Centre. Nairobi, Kenya. pp. 27-32.

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

Karyeija, R. F., Gibson, R. W. and Valkonen, J.P.T. (1998). Resistance in sweetpotato virus disease (SPVD) in wild East African Ipomoea. Ann. Appl. Biol. 133: pp. 39 – 44.

Kays, S. J. (2005) Sweetpotato production worldwide: assessment trends and the future. Acta Horticulture 670, pp.2-19.

Khan, V.A, Stevens, C, Fyffe, AE, Rhoden, E.G, Bartlett, J.R, Kittrell, DM and Peek, W, (2008). The use of plasticulture technology in sweetpotato production, Recent advances in agriculture.

Tjintokohadi, K, Atu, L, Laranang, L, Velasco, V, Joe, J, Timothy, M, Francis Siuromea, Tuhuna, M, Jackson, G and Ezeta, F. (2007). A report of sweetpotato survey held in Guadalcanal, Solomon Islands', ACIAR sweet potato project.

Kim, Y.C. (1961), 'Effects of thermoperiodism on tuber formation in *Ipomoea batatas* under controlled conditions', Plant Physiology, 5: pp. 680.

Kokkinos, C. D. and Clark, C. A. (2006). Interactions among Sweetpotato chlorotic stunt virus and different potyviruses and potyvirus strains infecting sweetpotato in the United States. J Plant Biotechnology 6: pp.21–27.

Kokkinos, C.D and Clark, C.A. (2006). Real-time PCR assays for detection and quantification of sweetpotato viruses. Plant Diseases 90: pp. 783 - 8.

Koruza, B. and Jelaska, S., (1993). Influence of meristem culture and virus elimination on phonotypical. J Plant Biotechnology 4: pp. 22–24.

Kreuze, J. F., Karyeija, R. F, Gibson, R. W. and Valkonen, J. P. T. (2000). Comparisons of coat protein gene sequences show that East African isolates of Sweetpotato feathery mottle virus form a genetically distinct group. Archives of Virology 145, pp.567-574.

Laurie, S. M., Thompson, G. J. and Van den Berg, A. A. (2000). Present state of quality and yield of Southern African sweetpotato cultivars. In: Proceedings of international workshop on sweetpotato cultivar decline study, 55–61 (Eds Y Nakazawa and K Ishiguro). Department of Upland Farming, Kyushu National Agricultural.

Lebot, V. (2010). Sweetpotato', in J.E Bradshaw (ed.), Root and Tuber Crops, Springer New York, vol. 7, pp. 97-125.

Lee, Q and Pitrat, M. (2007). Integrated control of aphid-borne viruses on the in muskmelon. Pp. 19-26 in: Plant virus epidemiology. Oxford, England, Blackwell Scientific Publication.

Lenné, J.M. (1991). Diseases and pests of sweetpotato: South-East Asia, the Pacific and East Africa. Natural Resources Institute Bull. 46: pp. 116.

Lepoivre, P. (1998) Production and field evaluation of healthy micro propagated sweetpotato [Internet]. CTA. Available from: <http://internships.cta.int Accessed 28 December 2011.

Ling, K. S., Jackson D.M., Harrison, H., Simmons, A. M., Pesic, V. and Esbroeck, Z. (2010). Field evaluation on the yield effects of U.S. heirloom sweetpotato cultivars infected by Sweetpotato leaf curl virus.

Liu, Q. C., Zhang, L. M, Wang, Q. M.and Wang, Q. C (2011). Sweetpotato in China. In: Loebenstein G, Thottappilly G, editors. Biology and biotechnology of sweetpotato. Springer; pp. 25 –58.

Loebenstein, G., Fuentes, S., Cohen, J. and Salazar, L. (2003). Sweetpotato. G, Virus and viruslike diseases of major crops in developing countries. Kluwer Academic Publishers, London, pp. 223–248.

Low J.W and Van Jaarsveld P. J. (2008). The potential contribution of bread buns fortified with beta-carotene-rich sweetpotato in Central Mozambique. Food Nutr Bull 29: pp.8–107.

Magagula, N. E. M., Ossom, E. M., Rhykerd, R. L. and Rhykerd, C. L. (2010). Effects of chicken manure on soil properties under Sweetpotato [*Ipomoea batatas* (L). Lam.] Culture in Swaziland. American-Eurasian Journal of Agronomy, 3 (2): pp. 36-43.

Manganaris, G.A., Economou, A.S., Bouborakas, I.N., Katis, N.I., (2003). Elimination of PPV and PNRSV through thermotherapy and meristem-tip culture in nectarine. Plant Cell Rep. 22 pp. 195-200.

Mannini, F., Argamante, N. and Credi, R., (1996). Improvements in the quality of grapevine 'Nebbiolo' clones obtained by sanitation. Acta. Hort. 427: pp.106-111.

Maule, A. J., Caranta, C. and Boulton, M. T. (2007). Sources of natural resistance to plant virus: status and prospects. Molecular plant pathology; 9: pp. 223-231.

Meludu, N. T., Ajala, C. G. and Akoroda, M. O. (2003). Poverty Alleviation through the Processing of Sweetpotato Tubers into Toasted Granules and Consumer Preferences in Nigeria. African Journal of Root and Tuber Crops, 5(2), pp. 56-59.

Mesfin, T., Wondirad, M. and Bekele, K. (2009). Review of Research on Diseases of Root and Tuber Crops in Ethiopia In. Abraham. T, eds: Increasing Crop Production through Improved Plant Protection Volume II. Plant Protection Society of Ethiopia (PPSE). Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa, Ethiopia. pp.169-202.

Miano, D, Labonte, D. and Clark, C. (2008). Identification of molecular markers associated with sweetpotato resistance to sweetpotato virus disease in Kenya. Euphytica; 160: pp. 15-24.

Milgram, M., Cohen, J. and Loebenstein, G. (1996). Effects of sweetpotato feathery mottle virus and sweetpotato sunken vein virus on sweetpotato yields and rate of reinfection on virus free planting material in Israel. Phytoparasitica. Modifications of grapevine (Vitis vinifera L), cv. Refosk. Vitis 3 2: pp. 59 - 60.

Missah, G., Wondirad, M. and Bekele, K. (1991). varieties performance infected to the same virus trial. Virology Horm 75: pp. 97–115.

Moyer J, Jackson G. and Frison, E. (1989) FAO/IBPGR technical guidelines for the safe movement of sweetpotato germplasm. FAO/IBPGR, Rome.

Moyer, J. W. and Salazar, L. F. (1989). Viruses and virus-like diseases of sweetpotato. Plant Disease 73, pp.451-455.

Moyer, J. W. and Kennedy, G.G. (1978). Purification and properties of sweetpotato feathery mottle virus', Phytopathology, 68(7) : pp. 998-1004.

Moyo, J.W., Jackson, G.V.H., and Frison, E.A. (eds.), (2004). FAO/IBPGR Technical Guidelines for the Safe Movement of Sweetpotato Germplasm.

Mukasa, S. B, Rubaihayo, P. R.and Valkonen, J.P.T. (2003). Sequence variability within the 3proximal part of the Sweetpotato mild mottle virus genome. Archives of Virology 148, pp.487-496.

Mukherjee A. (2002). Effect on NaCl on in vitro propagation of sweetpotato (*Ipomoea batatas* L.). App. Biochem. Biotech. pp. 431– 441.

Mwanga R., Moyer J., Zhang D., Carey E. and Yencho G. (2002) Nature of resistance of sweetpotato to sweetpotato disease. Acta. Hort .583: pp. 113 –119.

Mwanga, R.O.M., p'Obwoya, C.N.O., Otim-Nape, G.W. and Odongo, B. (1991). Sweetpotato improvement in Uganda. In: Alvarez MN, Asiedu R (Eds). The Role of Root Crops in Regional Food Security and Sustainable Agriculture, Proceed-ings of the Fourth Eastern and Southern African Root Crops Workshop, IITA, Ibadan, Nigeria, pp. 59-67.

Nameth, S. T.; Dodds, J. A.; Paulus, A. O.; Laemmlen, F.F. (1986). Cucurbit viruses of California: an ever-changing problem. Plant disease 70: pp. 8-12.

Ndolo, P. J., Carey, E.E., Gichuki, S.T., Kamau, J. W., Maisiba, G., Lusweti, T., Ngungi and Maina, D.K. (1997). Evaluation of elite sweetpotato clones in Kenya. Proceedings of the 4th Trienniel Congress of the African Potato Association. Pretoria, South Africa. pp. 23-28.

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

Njeru, R. W., Mburu, M. W. K., Cheramgoi, mE., Gibson, R. W., Kiburi, Z. M., Obudho, E. and Yobera, D. (2004). Studies on the physiological effects of viruses on sweetpotato yield in Kenya. Ann. Appl. Biol. 145: pp.71-76.

North Carolina Sweetpotato Commission, (2011). Sweet potatoes: industry, viewed 1 November 2011, <http://www.ncsweetpotatoes.com/sweet-potato-industry/growing-sweet-potatoesin-north-carolina/growing-your-seedstock.

O' Brien, P.J. (1972). The sweetpotato: Its origin and dispersal ', in American Anthropology.

Okada, Y., Nishiguchi, M., Sonoda, S., Mori, M., Kimura, T., Hanada, K., Sakai, J., Murata, T., Matsuda, Y., Fukuoka, H., Miyazaki, T., Nakano, M., Usugi, T. and Saito A. (2001). Sweetpotato.

Okonkwo, J. C. (2002). Effect of time of introducing soybean into potato on the performance of potato/soybean intercrop in Jose Plateau, Nigeria. Journal of Sustainable Agriculture and the Environment, 4(2): 185191. Plant Biology, 1(1): pp.10-15.

Onwueme, I. C. (1991). Field crop production in tropical Africa- Technical centre for Agricultural and Rural Co-operation CTA publicatio, Ede.The Netherlands..CTA publication, Ede. The Netherlands. chain reaction. Plant Dis. 82: pp. 223 – 229.

Onwueme, I. C. (1978). The tropical tuber crops. Yams, cassava, sweet potato, and cocoyams, vol. xii + 234pp. ISBN 0-471-99608 - 4, the tropical tuber crops. Yams, cassava, sweet potato, and cocoyams.

Opare-Obisaw, C., Danquah, A. O., Doku, E. V., Boakye, B. B. and Ansah-Kissiedu, D. (2000). Consumer Evaluation of Five New Sweetpotato (Ipomeas batatas) Varieties. Journal of Consumer Studies and Home Economics, 24, 61-65. http://dx.doi.org/10.1046/j.13652737.2000.00125.

Osagle, A. U. (1998). Nutritional quality of plant foods Department of Biochemistry University of Benin. Biological agriculture and horticulture 13: 29-34.

Owour, O., Lima, M.I. and Kitajima, E. (2000). Local trial for virus-free sweetpotato. In: KUZA [newsletter], issue no.1, p.4 (Eds S Kandi and M Kinua). Monsanto Central Africa, Nairobi, Kenya.

Pardales, J.R, Jr., Banoc, D.M., Yamauchi, A., Iijima, M. and Kono, Y. (1999). 'Root system development of cassava and sweetpotato during early growth stage as affected by high root zone temperature', Plant Production Science, 4: pp. 247-51.

Porter-Dosti, M., Mills, J. P., Simon, P. W. and Tanumihardjo, S. A. (2006). Bioavailability of beta-carotene (BC) from purple carrots is the same as typical orange carrots while high BC carrots increase BC stores in Mongolian gerbils (Meriones unguiculatus). Br. J. Nutr. 96: pp. 258– 67.

Pozzer, l., Dusi, A.N., Lima, M.I. and Kitajima, E.W. (1995). Characterization of a Brazilian isolate of sweetpotato feathery mottle virus infecting sweetpotato. Fitopat. Brasil. 20: pp. 65–71.

Qaim, M. (1999). The economic Effects of Genetically Modified Orphan Commodities Projection for Sweetpotato in Kenya. ISAAA Briefs No. 13. International Services for Acquisition for Agribiotech Application, Ithaca, New York and ZEF: 32 pp. Rees, D.J., Njue, E.K., Makini F.W. and Mbugua D.M.

Rao, A. L. N., and Francki, R.I.B. (1982). Distribution of determinants for symptom production and host range on the three RNA components of cucumber mosaic virus. J. Gen. Virol. 61, 197-205.

Ravi, V and Indira, P. (1999), 'Crop physiology of sweetpotato', Horticultural Reviews, 23: pp. 277-338.

Riis-Jacobsen, J. (2011). List of sweetpotato viruses with links to fact sheets, viewed 31 October 2011, < http://sweetpotatoknowledge.org/crop-management/deseases/viruses/list-of-sweetpotato-viruses-with-links-to-fact-sheets.

Roger, H. (2009). Comparative Plant Virology 2nd Edition, Academic Press, San Diego, CA

Rossel. E. A. and Thottappilly, H. (1988) Partial characterization and molecular cloning of a clostero virus from sweetpotato infected with the sweetpotato virus disease complex from Nigeria. Phytopathology, 8(8): pp. 69-75.

Salazar, L.F. and Fuentes, S. (2000). Current knowledge on major virus diseases of sweetpotatoes', in Y Nakazama and K Ishiguro (eds), International workshop on sweetpotato cultivar decline study, 42: pp 80-82.

SARI (2004). Savannah Agricultural Research Institute annual 2014 report.

Sato, K. (1981). 'Growth and development of potato plants influence by combination of air-temperature and soil temperature', Japanese Journal of Crop Science, 50, (3): pp. 267- 75.

Schaefers, G.A and Terry, E.R (1976). Insect transmission of sweet potato disease agents' in Nigeria', Phytopathology.,5: pp. 642-5.

Shalitin, D, Wolf, S. (2000). Cucumber mosaic virus infection affects sugar transport in melon plants. Plant Physiology 123: pp.597 – 604.

Smith, P.R, Stubbs, L.L. and Taylor, R.H. (1967). Plant virology, Research Institute Reports, Victoria, Australia.

Souto, E. R., Sim, J. Chen, J., Valverde, R. A., and Clark, C. A. (2003). Properties of strains of Sweetpotato feathery mottle virus and two newly recognized potyviruses infecting sweetpotato in the United States. Plant Dis. 87: pp.1226-1232.

Spiegel, S., Frison, E. A, Converse, R. H. (1993). Recent development in therapy and virusdetection procedures for international movements of clonal plant germ plasm. Plant Dis.77: pp.176-1180.

Steinbauer, C. E. and Kushman, L.J. (1971). 'Sweetpotato culture and diseases', Agriculture Handbook, Agricultural Research Service, United States Department of Agriculture, pp. 388.

Tairo, F., Jones, R.A.C. and Valkonen, J.P.T. (2006). Potyvirus complexes in sweetpotato: occurrence in Australia, serological and molecular resolution, and analysis of the Sweet potato virus 2 (SPV2) component. 9: pp. 1120-8.

Tairo, F., Mukasa., S. B., Jones, R.A.C., Kullaya, A, Rubaihayo R., Valkonen, J.P.T. (2005). Unraveling the genetic diversity of the three main viruses involved in Sweetpotato Virus Disease (SPVD), and its practical implications. Molecular Plant Pathology 6: pp.199-211.

Tanumihardjo, S. A. (2008). Food-based approaches for ensuring adequate vitamin A nutrition. Comp. Rev. Food Sci Food Safety 7: pp. 81-373.

Tanumihardjo, S. A. (2009). Sweetpotato beta-carotene bioefficacy is enhanced by dietary fat and not reduced by soluble fiber intake in Mongolian gerbils. J. Nutr. 139: pp. 44–50.

Tanumihardjo, S. A. (2006). Bioavailability of beta-carotene (BC) from purple carrots is the same as typical orange carrots while high BC carrots increase BC stores in Mongolian gerbils (Meriones unguiculatus). Br. J. Nutr. 96: pp.58–67.

Tesfaye, T., Fikre, H. and Mesele, G. (2013). Prevalence, incidence and distribution of sweetpotato virus: Its effect on the yield of sweetpotato in Southern Region of Ethiopia. International Journal of Science and Research; 2(1): pp. 591-595.

Trendo, H.P., Lozano, G., Valverde, R.A., and Navas-Castillo, J. (2007). First report of Sweetpotato virus G and Sweetpotato virus 2 infecting sweetpotato in Spain. Plant Dis. 91: 1687 (Abstr.).

Tumwegamire, S. (2011) Hand book of Sweetpotato Integrated Crop Management: Research Outputs and Programs for East Africa. pp. 23.

Turyamureeba, G., Odongo, B., Odongo, G., Alajo, G. C., Yencho, R.W., Gibson, N.E., Smit, J.M and Carey, E.E. (2008). Release of six sweetpotato cultivars (‘NASPOT 1’ to ‘NASPOT J. Nutr. 17: pp: 29 – 33.

Udoession, H. J. and Ifo, G. M. (1990). Prevalence, incidence and distribution of sweetpotato virus: Its effect on the yield of sweetpotato in Southern Region of Ethiopia. International Journal of Science and Research; 2: pp. 591-595.

Untiveros, M., Fuentes, S. and Kreuze, J. (2007). Molecular variability of sweetpotato feathery mottle virus and other potyviruses infecting sweetpotato in Peru. Arch. Virol.Online (DOI). USDA (2007). USDA National Nutrient Database for Standard Reference, Release 20. http://www.nal.usda.gov./fnic/foodcomp/search.

USDA. ARS. (2010). Three filamentous viruses isolated from sweetpotato in Japan. Ann. Phytopathol. Soc. J. 57: pp. 512–521.

Valverde, R. A., Clark, C. A. and Valkonen, J.P.T. (2007), Viruses and virus diseases complexes of sweetpotato, 15 March 2007 edn, vol. 1, Global Science Books, Baton Rouge LA, USA, viewed 14 July 2013.

Valverde, R. A., Clark, C. A., Kokkinos, C. D. and Gutierrez, D. L. (2008), Sweetpotato viral diseases, Characterization, diagnosis and management of plant viruses. Volume 1: industrial crops.

Valverde, R.A., Lozano, G., Navas-Castillo, J., Ramos, A. and Vald´es, F. (2004). First report of Sweetpotato chlorotic stunt virus and Sweetpotato feathery mottle virus infecting sweetpotato. Plant diseases. pp.23.

Van Jaarsveld P. J and Ossom, E. M., Rhykerd, R. L. and Rhykerd, C. L. (2005). Vir. J. 7: (2) pp.6-9

Villordon, A., Sheffield, R., Rojas, J. and Chiu, Y. L. (2011). 'Development of simple Bayesian Belief and Decision Networks of visualization tools for determining optimal In- row

Villordon, A., Solis, J., LaBonte, D. and Clark, C. (2010). 'Development of a prototype Bayesian Network model representing the relationship between fresh market yield and some agroclimatic variables known to influence storage root initiation in sweetpotato', Hort. Sci. 8 pp. 1167-77.

Villordon, A. Q. and LaBonte, D. R. (1996) Genetic variation among sweetpotatoes propagated through nodal and adventitious sprouts. Am Soc. Hortic. Sci. 12: pp.70 –78.

Villordon, A.Q., LaBonte, D. and LaBonte, D.R. (2003) Genetic variation among sweetpotatoes propagated through nodal and adventitious sprouts. Am Soc. Hort. Sci. pp: 21: pp. 70 –74.

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

Vuylsteke, D. and Talengera, D. (1998) Postflask management of micro propagated bananas and plantains. International Institute of Tropical Agriculture (IITA), Ibadan. 10: pp73–75.

Wambugu, F. M. (2003). Development and transfer of genetically modified virus-resistant sweetpotato for subsistence farmers in Kenya. Nutr. Rev. 61: S110–S113.

Wambugu, F. (1991). In Vitro and Epidemiological Studies of Sweetpotato (*Ipomea batatas*) (L.) Lam. Virus Diseases in Kenya. Ph.D. Thesis, University of Bath, UK.

Wambugu, F. (2004). Notes in New Scientist, 181 No. 2433, 7 February 2004.

Wang, Q., Zhang, L., Wang, B., Yin, Z., Feng, C. and Wang, Q. (2010). 'Sweetpotato viruses' in China', Crop Protection. 29: pp. 4 - 110.

Wang, Q. and Valkonen, R. (2008). Characterization of Sweetpotato from detection and distribution. Plant Pathology 60: pp. 100 –190.

Warammboi, J. G., Dennien, S., Gidley, M. J. and Sopade, P. (2011). Characterization of Sweetpotato from Papua New Guinea and Australia: Physicochemical, Pasting and lllGelatinisation Properties. Food Chemistry, 126: pp.1759-1770. http://dx.doi.org/10.1016/j.foodchem.2010.12.077.

Wilson, L. G. and Averre. C. A. (1989). Growing and marketing quality sweetpotatoes. N.C. Agr. Ext. Serv., N.C. State Univ., Raleigh.

Wilson, LA and Lowe, SB (1973). Anatomy of root system in West Indian sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars', Annals of Botany, 37: pp. 633.

Wilson, LA and Lowe, SB (1992). Anatomy of root system in West China sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars', Annals of Botany 37: pp. 633.

Woolfe, J. (1992). Sweetpotato: an untapped food resource. Cambridge University Press and the International Potato Center (CIP), Cambridge.

Yang, C., Shang, Y., Zhao, J., Li, C., Yang, C. L., Shang, Y. F., Zhao, J. H. and Li, C. S. (1998). Produce techniques and practice of virus-free sweet potato', Acta Phytophylacica Sinica, 25: 1, pp. 51-5.

Yildirim, E. R., Rodriguez, E. and Amaya, D. B. (2011). Food carotenoids; analysis, composition and alterations during storage and processing foods. Forum Nutr 56:35–7. Ruel MT. 2001. Can food-based strategies help reduce vitamin A and iron deficiencies? A review of recent evidence. Washington, D.C.: Intl. Food Policy Research Inst. pp.77.

Zhang, D. and Salazar, L. (2000). CIP's strategy for controlling sweetpotato virus diseases in developing countries. In: International workshop on sweetpotato cultivar decline study

Zhang, L. M., Wang, Q. M., Liu, Q. C. and Wang, Q. C. (2009). Sweetpotato in China. In: Loebenstein G, Thottappilly G, editors. Biology and biotechnology of sweetpotato. Springer; pp. 35–58.

Zuraida, N. (2003). Sweetpotato as an alternative food supplement during rice storage. J. Litbang Pertanian 22(4), pp.150 –155.

Analysis of variance
Variate: Virus_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	2.0556	1.0278	0.36	
rep.source stratum					
source	2	40.2222	20.1111	7.13	0.048
Residual	4	11.2778	2.8194	4.76	
rep.source.Varities stratum					
Varities	3	5.6667	1.8889	3.19	0.049
source.Varities	6	10.6667	1.7778	3.00	0.033
Residual	18	10.6667	0.5926		
Total	35	80.5556			

NCM ELISA TEST (VIROLOGY ANALYSIS)

Analysis of variance

Variate: SPCSV

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.0556	0.0278	0.10	
rep.source stratum					
source	2	33.5556	16.7778	60.40	0.001
Residual	4	1.1111	0.2778	0.61	
rep.source.Varities stratum					
Varities	3	6.7500	2.2500	4.96	0.011
source.Varities	6	1.3333	0.2222	0.49	0.808
Residual	18	8.1667	0.4537		
Total	35	50.9722			

Variate: SPFMV

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.7222	0.3611	0.49	
rep.source stratum					
source	2	38.2222	19.1111	25.96	0.005
Residual	4	2.9444	0.7361	1.59	
rep.source.Varities stratum					
Varities	3	15.2222	5.0741	10.96	<.001
source.Varities	6	4.4444	0.7407	1.60	0.204
Residual	18	8.3333	0.4630		
Total	35	69.8889			

Analysis of variance

Variate: SPFV

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	2.1667	1.0833	2.60	
rep.source stratum					
source	2	13.1667	6.5833	15.80	0.013
Residual	4	1.6667	0.4167	1.10	
rep.source.Varities stratum					
Varities	3	7.5556	2.5185	6.63	0.003
source.Varities	6	2.6111	0.4352	1.15	0.376
Residual	18	6.8333	0.3796		
Total	35	34.0000			

Analysis of variance
Variate: SPMV

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.5000	0.2500	0.43	
rep.source stratum					
source	2	40.1667	20.0833	34.43	0.003
Residual	4	2.3333	0.5833	1.34	
rep.source.Varities stratum					
Varities	3	7.2222	2.4074	5.53	0.007
source.Varities	6	2.9444	0.4907	1.13	0.386
Residual	18	7.8333	0.4352		
Total	35	61.0000			

Analysis of variance

Variate: SPSMV

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.5000	0.2500	1.20	
rep.source stratum					
source	2	28.6667	14.3333	68.80	<.001
Residual	4	0.8333	0.2083	0.94	
rep.source.Varities stratum					
Varities	3	8.3056	2.7685	12.46	<.001
source.Varities	6	0.4444	0.0741	0.33	0.910
Residual	18	4.0000	0.2222		
Total	35	42.7500			

Analysis of variance

Variate: CMV

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.7222	0.3611	2.36	
rep.source stratum					
source	2	22.3889	11.1944	73.27	<.001
Residual	4	0.6111	0.1528	0.59	
rep.source.Varities stratum					
Varities	3	8.3333	2.7778	10.71	<.001
source.Varities	6	2.5000	0.4167	1.61	0.202
Residual	18	4.6667	0.2593		
Total	35	39.2222			

Analysis of variance

Variate: Average Chlorophyll cont.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum					
	2	195.20	97.60	11.97	
rep.source stratum					
source	2	7310.28	3655.14	448.42	<.001
Residual	4	32.60	8.15	0.55	
rep.source.Varities stratum					
Varities	3	265.07	88.36	5.92	0.005
source.Varities	6	118.26	19.71	1.32	0.298
Residual	18	268.51	14.92		
Total	35	8189.94			

Analysis of variance

Variate: Vine yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	3.8510	1.9255	1.24	
rep.sources stratum					
sources	2	508.7347	254.3674	164.39	<.001
Residual	4	6.1895	1.5474	1.56	
rep.sources.Variety stratum					
Variety	3	86.6438	28.8813	29.12	<.001
sources.Variety	6	14.1333	2.3556	2.37	0.072
Residual	18	17.8542	0.9919		
Total 35	637.4066				

Analysis of variance

Analysis of variance

Variate: Marketable root yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	55.859	27.930	1.72	
rep.source stratum					
source	2	493.850	246.925	15.18	0.014
Residual	4	65.081	16.270	4.24	
rep.source.Varities stratum					
Varities	3	10.429	3.476	0.91	0.458
source.Varities	6	47.608	7.935	2.07	0.109
Residual	18	69.119	3.840		
Total	35	741.946			

Analysis of variance

Variate: Non-marketable root yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	5.0265	2.5133	2.20	
rep.source stratum					
source	2	5.0557	2.5279	2.21	0.226
Residual	4	4.5725	1.1431	2.96	
rep.source.Varities stratum					
Varities	3	8.1497	2.7166	7.04	0.002
source.Varities	6	10.8181	1.8030	4.67	0.005
Residual	18	6.9442	0.3858		
Total	35	40.5668			

Analysis of variance

Variate: Non-marketable root number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	1772.2	886.1	1.85	
rep.source stratum					
source	2	1748.7	874.3	1.82	0.274
Residual	4	1917.7	479.4	2.63	
rep.source.Varities stratum					
Varities	3	1844.1	614.7	3.38	0.041
source.Varities	6	1498.2	249.7	1.37	0.279
Residual	18	3278.2	182.1		
Total	35	12059.0			

Analysis of variance

Variate: Marketable root number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	108.50	54.25	0.87	
rep.source stratum					
source	2	2302.17	1151.08	18.47	0.010
Residual	4	249.33	62.33	5.66	
rep.source.Varities stratum					
Varities	3	57.89	19.30	1.75	0.192
source.Varities	6	202.94	33.82	3.07	0.030
Residual	18	198.17	11.01		
Total	35	3119.00			

Analysis of variance

Variate: Root yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	47.885	23.942	0.75	
rep.source stratum					
source	2	665.184	332.592	10.45	0.026
Residual	4	127.330	31.832	5.93	
rep.source.Variety stratum					
Variety	3	32.870	10.957	2.04	0.014
source.Variety	6	72.930	12.155	2.26	0.048
expeResidual	18	96.618	5.368		
Total	35	1042.816			

Analysis of variance

Virology scores

Variety	Source	plot No	rep	SPFMV	SPMMV	SPLV	SPCFV	SPC6V	SPMSV	SPCaLV	SPCSV	SPVG	CMV	Cotrol
Apomuden	Field Mat	1	1	+3	+2	-0	+3	-0	+2	-0	+3	-0	+2	+5
Bohye	Field Mat	2	1	+4	+3	-0	+1	-0	+1	-0	+1	-0	+1	+5
Ligri	Field Mat	3	1	+2	+1	-0	+2	-0	+2	-0	+1	-0	+1	+5
Dadanyuie	Field Mat	4	1	+1	+2	-0	+1	-0	+1	-0	+2	-0	+1	+5
Bohye	Lab. Mat	5	1	+1	+1	-0	+1	-0	-0	-0	-0	-0	-0	+5
Ligri	Lab. Mat	6	1	-0	-0	-0	-0	-0	-0	-0	-0	-0	-0	+5
Dadanyuie	Lab. Mat	7	1	-0	-0	-0	-0	-0	-0	-0	-0	-0	-0	+5
Apomuden	Lab. Mat	8	1	+1	+1	-0	+1	-0	+1	-0	-0	-0	+1	+5
Ligri	Farmer MaWT76t	9	1	+3	+2	-0	+2	-0	+2	-0	+2	-0	+2	+4
Dadanyuie	Farmer Mat	10	1	+2	+2	-0	+1	-0	+1	-0	+2	-0	+1	+5
Apomuden	Farmer Mat	11	1	+5	+4	-0	+3	-0	+3	-0	+4	-0	+3	+5
Bohye	Farmer Mat	12	1	+3	+3	-0	+2	-0	+2	-0	+3	-0	+1	+5
Bohye	Lab. Mat	13	1	+1	-0	-0	+1	-0	-0	-0	-0	-0	-0	+5
Ligri	Lab. Mat	14	1	-0	-0	-0	-0	-0	-0	-0	+1	-0	-0	+5
Dadanyuie	Lab. Mat	15	2	+1	+1	-0	-0	-0	-0	-0	-0	-0	-0	+5
Apomuden	Lab. Mat	16	2	+1	+1	-0	+1	-0	+1	-0	+1	-0	+1	+5
Ligri	Farmer Mat	17	2	+2	+4	-0	+1	-0	+2	-0	+3	-0	+2	+5

Dadanyuie	Farmer Mat	18	2	+1	+3	-0	+2	-0	+3	-0	+2	-0	+1	+5
Apomuden	Farmer Mat	19	2	+4	+5	-0	+4	-0	+4	-0	+3	-0	+3	+5
Bohye	Farmer Mat	20	2	+3	+3	-0	+3	-0	+2	-0	+1	-0	+4	+5
Dadanyuie	Field Mat	21	2	+2	+1	-0	+1	-0	+1	-0	+2	-0	+1	+5
Apomuden	Field Mat	22	2	+3	+3	-0	+3	-0	+2	-0	+1	-0	+2	+5
Bohye	Field Mat	23	2	+2	+1	-0	+1	-0	+1	-0	+2	-0	+1	+5
Ligri	Field Mat	24	2	+1	+2	-0	+2	-0	+2	-0	+2	-0	+2	+5
Ligri	Farmer Mat	25	3	+3	+3	-0	+1	-0	+3	-0	+3	-0	+3	+5
Dadanyuie	Farmer Mat	26	3	+1	+2	-0	+2	-0	+2	-0	+2	-0	+1	+5
Apomuden	Farmer Mat	27	3	+4	+4	-0	+1	-0	+3	-0	+4	-0	+3	+5
Bohye	Farmer Mat	28	3	+3	+2	-0	+1	-0	+2	-0	+2	-0	+2	+5
Dadanyuie	Field Mat	29	3	+2	+1	-0	+1	-0	+1	-0	+1	-0	+1	+5
Apomuden	Field Mat	30	3	+4	+2	-0	+2	-0	+3	-0	+3	-0	+2	+5
Bohye	Field Mat	31	3	+2	+3	-0	+1	-0	+2	-0	+1	-0	+1	+5
Ligri	Field Mat	32	3	+4	+2	-0	+1	-0	+1	-0	+2	-0	+2	+5
Apomuden	Lab. Mat	33	3	+1	+1	-0	+1	-0	+1	-0	+1	-0	+1	+5
Bohye	Lab. Mat	34	3	-0	-0	-0	-0	-0	-0	-0	-0	-0	-0	+5
Ligri	Lab. Mat	35	3	-0	+1	-0	-0	-0	-0	-0	-0	-0	-0	+5

Dadanyuie	Lab. Mat	36	3	-0	-0	-0	+1	-0	-0	-0	-0	-0	-0	+5

Buffers stocks used and respective storage condition

NAME	CHEMICAL COMPOSITION	STORAGE CONDITIONS
TBS pH 7.5 (2,000 ml)	Tris Base 4.84 g (0.02 M) NaCl 58.44 g (0.5 M)	Dissolve in 1,990 ml distilled water and adjust to pH 7.5 with concentrated HCl (37%). Complete to 2,000 ml with distilled water.
TTBS (2,000 ml)	TBS 2,000 ml Tween-20 1.0 ml (0.05 %)	Store at 5 ± 3°C.
Extraction buffer	TBS buffer containing 0.2 % sodium sulfite	Store at 5 ± 3°C.
Blocking solution	TBS + 2 % milk + 2 % TRITON X-100	Prepare fresh for each test

Substrate buffer pH 9.5 (500 ml)	Tris Base 6.05 g (0.1 M) NaCl 2.92 g (0.1 M) MgCl2.6H2	Dissolve Tris Base, NaCl and MgCl2.6H20 in 450 ml distilled water. Adjust pH to 9.5 with concentrated HCl
	0 0.51 g (0.005 M)	(37%). Complete to 500 ml With distilled water.
Antibody and conjugate solution	TBS buffer containing 2% milk	Prepare fresh for each step.
NBT Stock solution	NBT 40 mg N,N-dimethylformamide (70%)1.2 ml	Mix well and store at 5 ± 3°C, protected from Light (dark bottle or cover it with foil).
BCIP Stock solution	BCIP 20 mg N,N-dimethylformamide 1.2 ml	Mix well and store at 5 ± 3°C, protected from light (dark bottle or cover it with foil).
Substrate solution	Substrate buffer 30ml NBT stock solution 90 *ul* BCIP stock, solution 90 *ul* solution	Add the NBT stock solution in 30 ml of Substrate buffer. Afterwards add the BCIP

Source CIP, 2010

Materials and equipment used

Name	Materials	Storage Conditions / Use
Membranes	Nitrocellulose	Store in a dry place at room Temperature.
Filter papers (Whatman # 4)	Filters	Store at room temperature
Sample bags 4"x6"x6 (wide, long and thickness, Respectively. Thickness in thousandth of a inch)	Polyethylene	Use one for each sample
Small tubes of 1 cm diam.	Glass	Use to cut disks from leaf Samples.
Thick test tube or piece of round wood		Use in sample maceration.
Micropipettes (20, 200 and 1000 µl)		Check according to Equipment Control procedure.
200 and 1000ul tips		
Surgical forceps		Use them when manipulating the membranes.

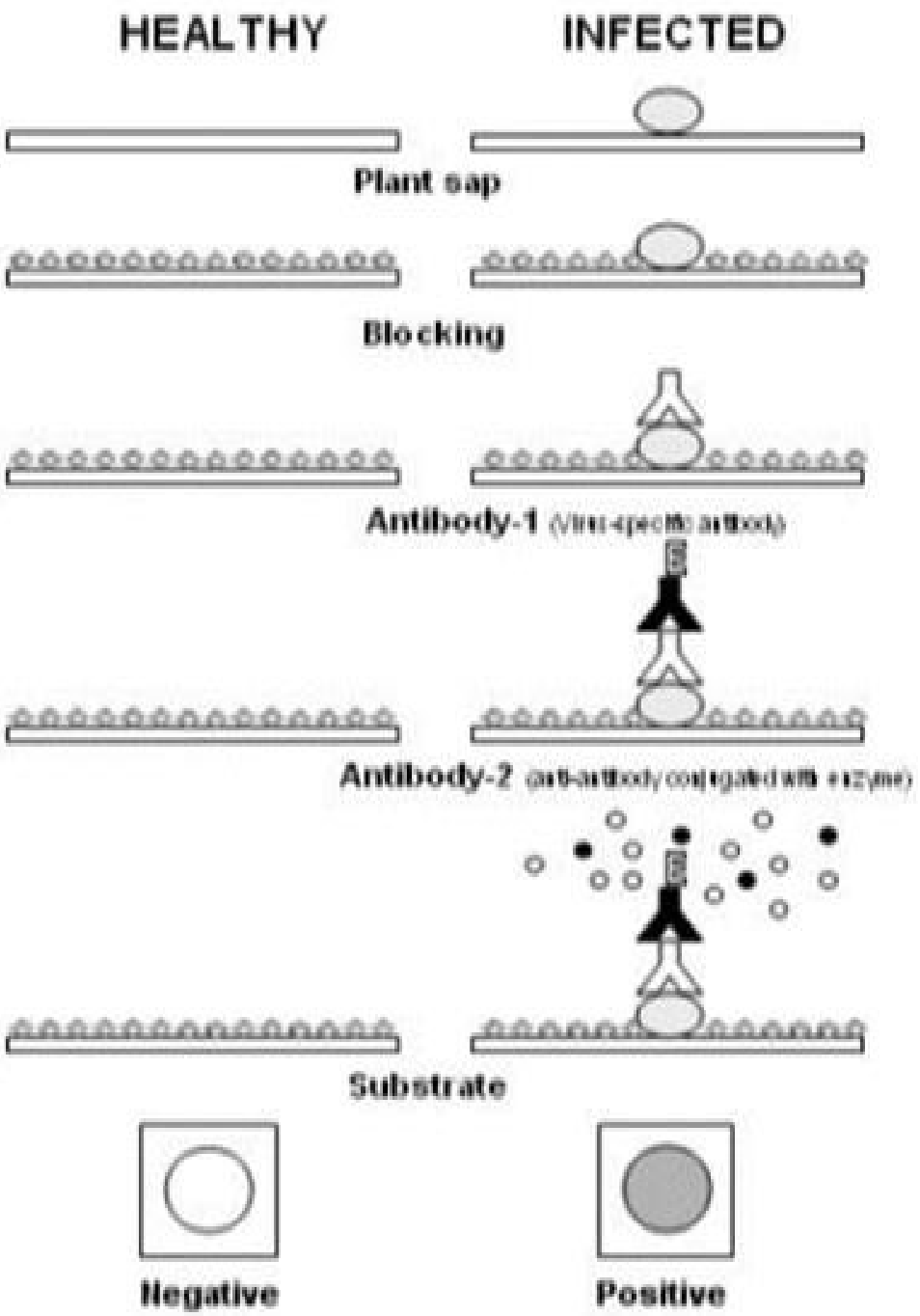
2000, 1000, 500, 250ml containers or glass bottles		
1000, 500, 250, 100, 50ml graduated cylinders		
Distilled water		Store at room temperature in enough volume to be used/refresh weekly
Refrigerator		5 ± 3°C
pH meter		
digital and analytical balances		
rotary shaker		
vacuum pump		
Milli-Q water purification apparatus		
Magentic stirrer		
Dot blotting apparatus		

Source CIP, 2010

Other reagents used

NAME	STORAGE
Antibodies : for each of the tested viruses	Store at 5 ± 3°C.
GAR: Goat anti-rabbit Ig G alkaline phosphate conjugate (BIO-RAD).	Store in a dark and cold place at 5 ± 3°C. Do not freeze
NBT: Nitro blue tetrazolium (BIO-RAD).	Store in a dark and cold place at -20°C.
BCIP: 5 Bromo-4-chloro-3-indolyl phosphate (BIO-RAD).	Store in a dark and cold place at -20°C.
DMF: N, N-dimethylformamide (SIGMA).	Store at room temperature.
HCl: Hydrochloride acid 37% (MERCK).	Store at room temperature.
MILK : Powdered cow milk	Store at room temperature.

Source CIP, 2010



The steps involve with NCM- ELISA virus detection test.

Reagent used in the serological process and its components

Tween-Tris buffered saline (T-TBS) consist of 2.42g Tris, 29.22g NaCl, 2.5ml HCI (18.5%) and 0.5ml Tween 20 in 1 liter distilled water.

Extraction buffer consist of 2g sodium sulfide (Na_2So_3) per liter of TBS (T-TBS minus Tween 20

Blocking buffer consist of 20g powdered cow milk and 20g triton in 1 liter TBS Antibody buffer consist of 20g powdered cow milk in 1-liter TBS.

Substrate buffer consist of 12. lg Tris, 5.8g NaCl, Ig Magnesium chloride (MgCI) and 2ml HCl (18%) in 1 liter of distilled water 21.