KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

STUDIES ON SWEETPOTATO VIRUSES FROM THE MAJOR SWEETPOTATO GROWING AGRO-ECOLOGIES OF GHANA

BY

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BSc (Hons) MOLECULAR BIOLOGY AND BIOTECHNOLOGY

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A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHILOSOPHY IN CROP PROTECTION (PLANT VIROLOGY)

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DECLARATION

I, LINDA APPIANIMAA ABROKWAH, do hereby declare that this thesis is my own work and that to the best of my knowledge and belief, contains no materials previously published or written by another person nor material which to a substantial extent has been accepted for an award of any degree of an institution or any other institution of higher learning except where due acknowledgement has been made in the text.

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ABSTRACT

Sweetpotato virus disease complex (SPVD) is the most destructive viral disease in Africa. It can cause yield loss up to 50%. In Ghana, not much work has been done on the identification and detection of sweetpotato viruses from the major sweetpotato growing agro-ecologies. A study was conducted to ascertain the incidence of sweetpotato viruses from the major sweetpotato producing areas and to ascertain the effects of sweetpotato virus diseases (SPVD) on the crop in Ghana. Sweetpotato viral disease samples were collected from all agro-ecologies in Ghana where the crop is grown and then preserved in the screenhouse for diagnostic purposes. Nitrocellulose membrane (NCM) enzyme-linked immunosorbent assay (ELISA), using specific virus antibodies and PCR techniques involving the use of specific and degenerate primers were used for the diagnostics. Virus diagnostics were done directly on virus-infected sweetpotato samples collected from the field and also on Ipomoea setosa indicator plants after they have been grafted with virus-infected sweetpotato collected from the various locations. In all, 127 samples were assayed. Effects of SPVD were assessed on three sweetpotato varieties, namely; 'Dadanuei', 'Ligri' and 'Bohye,' which are all varieties released by the CSIR-Crops Research Institute, Fumesua, Ghana. These were put under four levels of disease regimes; tissue culture cleaned and virus indexed planting materials, apparently healthy planting material collected from the field, virus infected planting material collected from field and artificially (using whiteflies) infected planting materials. There were four treatments and each treatment was repeated three times in a randomized complete block design (RCBD). Virus diagnostics, using NCM-ELISA, detected the following viruses; SPFMV (85.71%), SPCSV (16.67%), SPCaLV (6.35%), SPVG (4.76%), SPMSV (4.76%), SPCFV (1.57%) and CMV (3.97%). RT-PCR and PCR confirmed the detection of SPFMV and SPCSV as well as Begomoviruses in some of the samples. Several mixed infections were also detected in samples collected mostly from local varieties whilst the released varieties had mainly single virus

infections. The study has also optimized serological detection (NCMELISA) and RT-PCR protocols for the effective diagnosis of sweetpotato virus isolates in Ghana. Across board, tissue culture cleaned virus-indexed planting materials of the three varieties produced the largest yield with a mean of 12.00 tons/ha, whilst artificially infected (whitefly inoculated) planting materials produced the least yield of 0.78 tons/ha. The study revealed planting tissue culture cleaned virus indexed planting materials can affect yield of the crop positively whilst it showed the usefulness in planting improved varieties, compared to the local varieties in term of virus infections.

DEDICATION

I dedicate this research to my children, Marilyn Benewa Ofori-Oppong and Nathan Boah Ofori-Oppong, my husband Dr. Allen Oppong and the Abrokwah family for their inspiration and support in my education.

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CHAPTER ONE

1.0 INTRODUCTION

Sweetpotato (*Ipomoea batatas* L.) is a dicotyledonous, perennial plant that produces edible tuberous roots with lots of economic importance (Woolfe, 1992). According to FAOSTAT (2012), sweetpotato is the third most important vegetatively propagated crop in the world after Irish potato and cassava, with annual production of 126 million tons. Area harvested for sweetpotato in Ghana is estimated at 74,000 ha (FAOSTAT, 2012) which comes next to cassava and yam in order of importance among root crops.

Sweetpotato has a short growing period, is usually useful in crop rotations, helps in famine as a reserve crop, and grows well in marginal soils, producing large yields per unit area per unit time, and in some areas three harvests per year can be achieved (Karyeija *et al.*, 1998). Because of its robust nature and wide flexibility, it can be grown in several agro ecological zones hence, providing a sustainable food supply when other crops fail (Jana, 1982). Nutritionally, the tuberous root is rich in dietary fibre (pectin, cellulose, hemi-cellulose and lignin), proteins, vitamins (B1 and B2, C and E), β-Carotene (beneficial in fighting vitamin A deficiency in youngsters beneath the age of five years and breast-feeding mothers), mineral contents (mainly K, Fe and Ca) and carbohydrates (Low *et al.*, 1997).

The yellow and orange fleshed varieties represent the least expensive year-round source of dietary vitamin A available to deprived families in Africa (CIP, 1999). They are also used as animal feed and provide raw materials for alcohol production (Woolfe, 1992). Sweetpotato has high anthocyanin content which pigments are highly stable making the crop a healthier substitute to synthetic colouring elements in food. All these benefits make sweetpotato a high priority crop for food security (CIP, 2000).

For the reason that sweetpotato has vast genetic diversity (Zhang *et al.*, 1998) and the accompanying diversity in phenotypic and morphological traits, the crop has great potential for

further development to accommodate specific uses. However, its production is beset with abiotic and biotic limitations (Geddes, 1990).

Among the biotic stresses, viral diseases are the second most important constraint. This comes after the sweet potato weevil (Qaim, 1999), causing extensive losses worldwide (CIP, 2000). Virus complexes influence disease symptom severity thereby affecting yield losses considerably.

Sweetpotato virus disease (SPVD) is the most alarming complex condition of sweetpotato viruses caused by co-infection of *Sweetpotato chlorotic stunt virus* (SPCSV) and *Sweetpotato feathery mottle virus* (SPFMV). SPCSV is whitefly-borne and transmitted in a semi-persistent manner while SPFMV is aphid-borne and transmitted in a non-persistent manner. The combined infection of the two viruses causes the most severe disease of sweetpotato in Africa (Karyeija *et al.*, 2000; Gibson and Aritua, 2002; Mukasa *et al.*, 2003; Cuellar *et al.*, 2008). SPVD can cause yield reduction as high as 56-98% in Africa (Gibson *et al.*, 1998a) and in numerous countries throughout the world (Clark and Moyer, 1988; Carey *et al.*, 1999)

The costs of viral infections are not only restricted to decrease in crop yield, but also undermine the efforts in genetic improvement for yield and quality, since farmers normally abandon susceptible but otherwise high yielding varieties (Aldrich, 1963) which are also rich in starch and vitamin A. Also, the existence of lone virus infections may compromise the usage of farmer-saved vines, especially in regions where insect vectors are predominant. The reason being that single virus-infected vines can become sources of inocula for vector spreads, leading to mixed infections of different viruses.

Under field conditions, sweetpotato frequently develops complexes of mixture of viral diseases of up to three viruses and in rare situations, four viruses which reduce the quality of planting materials (Mukasa *et al.*, 2003).

In Ghana, not much work has been done in the identification and characterization of sweetpotato viruses in the major growing areas. Sweetpotato production is only popular and restricted to a few ecologies where leaves and roots are mostly consumed as staple. However, in these areas, farmers normally grow landraces and, in some cases, improved varieties which are susceptible to viruses.

Vine cuttings from mature crops are used to establish new fields, which are potential sources of infection in the newly planted fields. Even for the new improved varieties that have been produced over the years and adopted by farmers, not much has been done to preserve the true-to-type virus-tested foundation seed stocks.

Virus-tested varieties, produced from tissue culture plants that have been confirmed virusfree, have actually been found free of these viruses. Planting diseased vine cuttings or storage roots is the greatest collective source of sweetpotato viruses, but clean planting material can rapidly be re-infected by some viruses, particularly those spread by aphids and whiteflies. In Ghana, almost 70% of the crop is propagated from vines chosen and kept by farmers or bartered and traded locally.

Sweetpotato cultivars increasingly lose their resistance over time after release and are often replaced within 20 years. It is believed that this leads to virus accumulation in the propagating material.

Virus complexes rank second to weevils in causing yield reduction in sweetpotato. However, it is important to know the extent of yield losses caused by sweetpotato viruses in Ghana to guide breeders in the development of resistant cultivars. Similarly, information on sweetpotato viruses and their detection with effective methods can enhance the management of the SPVD. The convenience of accurate viral investigative procedures and practice of providing virus-indexed clean planting material for farmers can improve productivity in farmers' fields. It is, therefore,

important to know the different types of sweetpotato viruses and their distribution in the different ecologies so that management strategies can be implemented against them.

The main objective of this study was to optimize sweetpotato virus detection protocols, detect sweetpotato viruses in the important growing areas and estimate their effects on yield.

The specific objectives were to:

i. detect different sweetpotato viruses in the target ecologies, ii. optimize the effectiveness of sweetpotato virus detection protocols for the screening of sweetpotato virus isolates in Ghana, and iii. estimate the effect of SPVD infection on yield.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of Sweetpotato

Sweetpotato (*Ipomoea batatas* L.) is a herbaceous and perennial vine grown by vegetative propagation, using either stem cuttings or storage roots (Woolfe, 1992). Its growth habit is predominantly prostrate, with its vine system that expands rapidly horizontally to the ground. Sweetpotato growth habits include: erect, semi-erect, spreading and very spreading.

The storage roots are lateral roots which store photosynthetic products (Chua and Kays, 1981). The storage roots have no lignification, are fleshy and thick. The shape and size vary from round and long irregular, depending on the variety and environmental factors (Woolfe, 1992).

The "skin" of the storage root ranges from white to dark purple and the flesh colour varies from white to orange and purple in various distributions (Laurie and Niederwieser, 2004). Sweetpotato bears simple leaves which are normally alternate, heart-shaped or palmately lobed. The flowers are usually medium sized, depending on the variety (https://en.wikipedia.org/wiki/sweet_potato).

2.2 Economic Importance and Distribution of Sweetpotato

Sweetpotato is the seventh most important food crop in the world in terms of production (Loebenstein, 2009). It is the fifth most important tuber crop in the developing countries and an important staple food in Africa (FAOSTAT, 2009). The world's total harvested area is a little over 8.1m ha, producing 108m tons with an average yield of 13.3 ton/ha (FAOSTAT, 2012). Africa accounts for 15% of total world's sweetpotato production (Loebenstein, 2009). Sweetpotato tubers are large, starchy, sweet-tasting and generally consumed as vegetable with a high-energy value and rich in carbohydrates. It is also rich in vitamins such as vitamins A, C and some vitamin B complex (Miranda, 2002). Genotypes which have high to moderate amounts of vitamin A have been earmarked as food source for the alleviation of child blindness in Africa (Low *et al.*, 1997). In Africa, sweetpotato is often referred to as the 'poor man's crop' (Ndolo *et al.*, 2001).

It is particularly important in countries surrounding the Great Lakes in Eastern and Central Africa; Malawi, Angola and in Mozambique and Madagascar in Southern Africa and Nigeria and Ghana in West Africa (Woolfe, 1992; Low *et al.*,2009)

Sweetpotato gives more biomass and nutrients per hectare than any other food crop in the world (Karyeija *et al.,* 1998). It thrives well on fertile tropical soils and produces tubers without fertilizer application or irrigation, and it is one of the crops with a unique role in the relief of famine (Loebenstein, 2009). The young leaves and shoots are sometimes eaten as a vegetable.

In Ghana, the harvested area for sweetpotato is about 74,000 ha (FAOSTAT, 2012) which comes next after cassava and yam in importance among the root crops . Sweetpotato is not produced in all the regions or all the agro-ecologies in Ghana. It serves as food security crop for most farmers who depend on it for their subsistence with a few farmers growing it for

commercial purposes. The crop is grown and is an important staple in the Central, Volta, Eastern and Upper East Regions of Ghana (Carey *et al.,* 2011).

The agro-ecological zone of Central Region is coastal vegetation and grassland with mean annual rainfall of 800mm that of Volta Region is Guinea Savanna with mean annual rainfall of 1,100mm. Eastern Region is moist Semi-deciduous with mean annual rainfall of 1,500mm and Upper East is Guinea and Sudan Savanna agro-ecological zone with mean annual rainfall of 1,000mm (Oppong-Anane, 2001).

2.3 Sweetpotato Cultivation

Sweetpotato in Ghana is mainly cultivated for the carbohydrate-rich tuber but the foliage has the potential for use as vegetable and animal feed (Otoo *et al.,* 2001). It grows best between 20 and 30°C. Annual average temperatures range from 26.1^0C in places near the coast to 28.9^0C in the extreme north (Ministry of Food and Agriculture, 2010)

Sweetpotato can be cultivated between 2300-2500m above sea level (Del Carpio, 1969) and grows well in tropical and subtropical climates with the ability to tolerate harsh climatic conditions (Van den Berg and Laurie, 2004). It requires a minimum of three to five months (depending on the temperature and variety) to mature. Sweetpotato needs 500mm of rainfall in a growing season (Ahn, 1993) and can be grown all year round on wide varieties of soils (especially moderately acidic sandy to sandy loam) but does well on well-drained light and medium coarse soil with an ideal pH range of 5.8-6.0 (Mutandwa and Gadzirayi, 2007).

High rainfall enhances the growth of more vines (Obigbesan, 2009). Six to seven weeks after planting is the critical period for the development of storage roots. If soil oxygen is low, either because the soil is flooded or because it is so dry , the storage roots do not develop well and yields are reduced (http://www.ncsweetpotatoes.com).

Lower temperatures off-set the setting of roots and injure them. Heavy, poorly aerated soils inhibit adequate growth of storage roots, resulting in poor shapes. Sweetpotato can be cultivated asexually from vine cuttings or sexually from seed. (Woolfe, 1992).

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

2.4 Symptoms of Sweetpotato Virus infection

Viruses are amongst the smallest organisms known. They are very simple organisms and can only survive and multiply inside their host (Stathers *et al.*, 2005). Sweetpotato viruses are the most damaging group of disease-causing organisms affecting the crop in Africa. Usually, most viruses are carried from plant to plant, mostly by insect vectors such as aphids and whiteflies which feed on the plant sap.

Once a virus enters a cell of its host, it takes over part of the management of the cell's processes, and causes the cell to produce more viruses identical to itself. The virus then takes over the cell machinery of the host plant. These new virus particles then spread through the plant to infect more cells.

Some common symptoms of virus infection in plants including sweetpotato are:

- stunted or diminished growth of the plants and leaves remain small,
- chlorosis (paleness, even whitening) of the leaf tissue. This chlorosis may be general or in a pattern, often either between the leaf veins in a mosaic or less well defined mottle, or along the veins to form a chlorotic network,

- misshapen/deformed leaves with an uneven or curled appearance (roll-down/ roll –up with mottle/mosaic, leaf distortion),
- pigmented leaves, often purple or yellow generally or in spots or rings, and
- reduced yield (quality and quantity) of sweetpotato storage roots.

2.5 Sweetpotato Virus Disease (SPVD)

SPVD has been reported to cause up to 50% yield loss (Carey *et al.*, 1999) and in many cases, associated with the disappearance of once elite cultivars (Gibson *et al.*, 1997b). SPVD seriously undermines efforts to genetically improve yield and quality of the crop. It is caused by co-infection of the whitefly-borne *Sweetpotato chlorotic stunt virus* (SPCSV) and the aphid-borne *Sweetpotato feathery mottle virus* (SPFMV) (Winter *et al.*, 1992).

The SPVD derived from dual infection with SPFMV and SPCSV was first reported in West Africa (Schaefers and Terry, 1976) and later in East Africa (Gibson *et al.*,1998b). SPVD was initially reported in Ghana in 1960 (Clark, 1960). Two viruses 'A' and 'B' were isolated by (Sheffield, 1958) from a Kenyan SPVD-infected plant.

Virus 'A' is aphid-borne and considered to be SPFMV (Karyeija *et al.*, 1998). Virus 'B' is whitefly-borne which is SPCSV (Cohen *et al.*, 1992). For plants that had been used for isolation of 'Virus B' by whitefly transmission (Shefield, 1958), the wide host range, sap transmission, apparent diversity of isolates and role in induction of SPVD of virus 'B' might then have resulted from the combined symptoms of both SPCSV and *Sweetpotato mild mottle virus* (SPMMV).

These symptoms expressed were not characteristic of SPCSV but of SPMMV (*Ipomovirus;* Potyviridae), another whitefly-borne virus commonly infecting sweetpotato in East Africa (Hollings *et al.*, 1976). SPCSV, SPFMV and SPMMV may be found together in sweetpotato with typical SPVD symptoms (Gibson *et al.*, 1998a). In plants infected with both viruses, SPCSV and SPFMV, SPCSV synergies the multiplication of SPFMV, the titre of both coat

protein (Gibson *et al.*, 1998b) and viral RNA (Karyeija *et al.*, 2000) of the SPFMV increases several hundred fold.

2.5 Sweetpotato Reaction to Viruses

Through cell to cell and vascular movement (Valkonen, 1994; Carrington *et al.*; 1996). Some plants are able to entirely subdue virus multiplication while others impede spread (Fraser; 1990). Sweetpotato resistance to viruses has been suggested to be associated with restricted virus movement (Nakashima *et al.*; 1993). From host resistance induced virus localization, the freshly budding organs may be symptomless, a phenomenon known as recovery (Fondong *et al.*, 2000).

After, the recovered portions, cuttings from these parts can be propagated and may not show symptoms (Fondong *et al.*, 2000). Most sweetpotato cultivars seem to be rather resistant to single infections of either SPFMV or SPCSV, showing mild symptoms from which they recuperate (Mwanga *et al.*, 2002a; Mukasa *et al.*, 2006; Gasura *et al.*, 2009).

2.6 Types of Sweetpotato Viruses

More than 14 viruses of sweetpotato have been reported by Moyer and Salazar (1990) and Brunt *et al.*, (1996). Study of these viruses of sweetpotato has been hampered by the lack of simple detection techniques. In Africa, infection by different viruses ranks second to weevils in causing yield reduction in this crop (Geddes, 1990).

Some of the viruses known to infect sweetpotato include : *Sweetpotato feathery mottle virus* (SPFMV), *Sweetpotato chlorotic stunt virus* (SPCSV), *Sweetpotato mild mottle virus* (SPMMV), *Sweetpotato chlorotic fleck virus* (SPCFV), *Sweetpotato latent virus* (SPLV), *Sweetpotato caulimolike virus* (SPCa-LV), *Cucumber mosaic virus* (CMV), *Sweetpotato virus*

C-6 (Untiveros *et al.,* 2007), *Sweetpotato virus G* (SPVG) and *Sweetpotato mild speckling virus* (SPMSV) (Ishak *et al.,* 2003; Mukasa *et al.,* 2003; Ateka *et al.,*2004; Tairo *et al.,* 2004; Miano *et al.,* 2006).

Viruses spread quickly through the vascular system of a plant to infect the whole plant. Therefore, any portions of an infected plant that are used as planting materials (vines or roots), almost always become diseased. These then carry the disease to the next generation of plants.

2.6.1 Sweetpotato Feathery Mottle Virus (SPFMV)

Sweetpotato Feathery Mottle Virus belongs to the family *Potyviridae,* genus *Potyvirus* and is the most common virus of sweetpotato found everywhere and with different strains identified wherever sweetpotato is grown. The range of symptoms associated with SPFMV infection are as much a function of the host genotype and the environment as they are of the virus strain or isolate (Alconero, 1972; Campbell *et al.,* 1974; Moyer and Kennedy,1978; Cali and Moyer, 1981; Moyer and Cali, 1985; Moyer, 1986)

Symptoms on sweetpotato leaves may consist of the classic irregular chlorotic patterns (feathering) associated with the leaf midrib as well as faint or distinct chlorotic spots which in some genotypes have purple pigmented borders. These symptoms are observed predominantly on the older leaves. Vein clearing, vein banding and chlorotic spots are predominant symptoms observed in the indicator host *Ipomoea setosa* (Ker Gawler). Symptoms may be mild and leaves produced after the initial flush may be symptomless.

Some strains of SPFMV cause necrotic lesions on the exterior of the roots (russet crack disease) while other strains induce symptoms in the interior of root (internal cork disease) (Cali and Moyer, 1981; Karyeija *et al.,* 1998; Valverde *et al.,* 2007). SPFMV alone generally causes no symptoms in sweetpotato (Gibson *et al.,* 1997) and either latent in many plants or spread rapidly by itinerant aphids. Leaf symptoms for SPFMV vary with cultivar susceptibility,

climatic conditions, plant age and strain virulence (Thompson and Mynhardt, 1986; Clark and Moyer, 1988; Ames *et al.,* 1997).

SPFMV is transmitted by several aphid species in a non-persistent manner (Stubbs and McLean, 1958) and by other aphid species that do not colonize (Wambugu, 1991; Aritua *et al.,* 1998). Co-infection by SPFMV with an unknown virus is frequently a problem in determining the etiology of disease complexes. Meanwhile many different strains exist and symptoms induced by these strains differ, many names have been used to describe the virus.

Among the names given include internal cork virus, Sweetpotato leaf spot virus, Sweetpotato ringspot virus, sweetpotato virus A and russet crack virus (Moyer and Salazar,1989). The most distinctive symptom of the virus, irrespective of strain, present the chlorotic feathering of the leaf midrib and in some genotypes, the expression of chlorotic spots with purple rings(Moyer and Salazar,1989).

2.6.2 Sweetpotato Chlorotic Stunt Virus (SPCSV)

Sweetpotato Chlorotic Stunt Virus was formerly known as Sweetpotato Vein Virus (Hoyer *et al.,* 1996a; Milgram *et al.,*1996; Ames *et al.,* 1997; Alicai *et al.,* 1999) and associated with SPVD (Winter et al., 1992). It is a member of the family Closteroviridae, *genus Crinivirus* (Aritua *et al.,* 1998; Fauquet and Mayo, 1999 and Karyeija *et al.,* 2001) with a positive stranded RNA genome (Karyeija *et al.,* 2000; Gibson and Aritua, 2002). SPCSV remains confined to the phloem and at similar or slightly lower titre in the SPVD-affected plants. SPCSV is transmitted in a semi-persistent manner by whitefly, *Bemisia tabaci* (Schaefers and Terry, 1976; Larsen *et al.,* 1991).

In Africa, infection by SPCSV alone is generally limited. Consequently, SPVD quickly follows any initial symptoms of SPCSV. Prevalence of SPVD is closely related to abundance of whiteflies (Aritua

et al., 1998) and control of SPVD essentially involves limiting the spread of SPCSV. SPCSV stunts growth and causes yellowing or purpling of lower leaves (Gibson *et al.*, 1998a).

SPCSV can infect plants by itself and it has been identified as a component of synergistic complexes with other viruses such as SPFMV and SPMSV (Schaefers and Terry, 1976; Gibson *et al.*, 1998a; Di Feo *et al.*, 2000; Gibson and Aritua, 2002). Experimentally, SPCSV can induce synergism with all tested potyviruses, CMV as well as Carlaviruses, and is always associated with an increase in the titre of the co-infecting virus and reduced yield of storage roots (Kreuze and Fuentes, 2008).

2.5.3 Sweetpotato Mild Mottle Virus (SPMMV)

Sweetpotato Mild Mottle (SPMMV) was primarily isolated in East Africa. It was formerly referred to as SPV-T in preliminary reports and may be the same as 'Virus B' isolated by Sheffield (1958). SPMMV belongs to the family *Potyviridae* and the *g*enus *Ipomovirus* and symptoms include leaf mottling, veinal chlorosis, dwarfing and poor growth. With four leaves or more, SPMMV infected on grafted *I. setosa* exhibited a bright yellow veinal chlorosis. Subsequent leaves are symptomless (Hollings *et al.*, 1976).

Although the morphology of SPMMV and its cytoplasmic inclusions are similar to those of other viruses, most notable among the divergent characteristics is the host range of SPMMV which includes 45 species in 14 plant families (Hollings *et al.*, 1976; Brunt *et al.*, 1996).

2.6.4 Sweetpotato Chlorotic Fleck Virus (SPCFV)

Sweetpotato Chlorotic Fleck Virus is an associate of the family Flexiviridae, *g*enus *Carlavirus* (CIP and Nolasco, 1992, Gibson et sl., 1997). This was initially isolated in 1992 from CIP germplasm collection on grafted *I. setosa*. SPCFV exhibit symptoms of chlorosis, leaf distortion, and

vein clearing. It is one of numerous viruses naturally infecting sweetpotato (Aritua *et al.*, 2007) and seems to be distinct serologically from other filamentous viruses infecting sweetpotato, including *Sweetpotato Feathery Mottle Virus, Sweetpotato virus G,* and *Sweetpotato Latent Virus, Sweetpotato Mild Mottle Virus, Sweetpotato Chlorotic Stunt Virus* and the C-6 virus.

2.6.5 Sweetpotato Latent Virus (SPLV)

Sweetpotato Latent Virus fits to the family *Potyviridae* genus *Potyvirus* and was at first called 'Sweetpotato Virus N' which was originally reported in Taiwan (Chung *et al.*, 1986). As the name implies, infection of several sweetpotato cultivars does not result in obvious foliar symptoms. While it induces mild symptoms in *I. setosa*, it can be simply detected in host plants using serological producers.

SPLV exhibits the numerous characteristics of Potyviruses with production of cytoplasmic inclusions. The host range of SPLV includes many *Convolvulus, Chenopodium* and some *Nicotiana s*pecies such as *N. benthamiana* (Domn). Yet, SPLV cannot be transmitted mechanically or by vector inoculation; neither aphid nor whitefly transmission as all efforts have been futile.

2.6.6 Sweetpotato Mild Speckling Virus (SPMSV)

Sweetpotato Mild Specking Virus (SPMSV) belongs to the Potyviridae and genus *Potyvirus.* SPMSV was originally identified in Argentina from plants with chlorotic dwarf compound disease that also involved SPFMV and SPCSV (Di Feo *et al.*, 2000). In sweetpotato, SPMSV presents sporadic chlorotic speckling, vein clearing, and blistering and leaf deformation. SPMSV shows mosaic in *I. nil* and *I. setosa*; vein clearing, and reduction, leaf deformation and roll down of leaves in *N. benthamiana* (Di Feo *et al.*, 2000; Loebenstein, 2009). It is spread by aphids in a non-persistent manner. It can also be transmitted through mechanical inoculation and grafting (Di Feo *et al.*, 2000; Loebenstein, 2009).

2.6.7 Sweetpotato Caulimo-Like Virus (SPCaLV)

Sweetpotato Caulimo-Like Virus (SPCaLV) is an individual member of the genus *Cavemovirus*. SPCaLV was originally detected in sweetpotato from Puerto Rico. It was identified in a complex with other viruses (SPFMV) from the South Pacific region including Tonga, Papua New Guinea, New Zealand, Solomon Islands, and Australia (Salazar and Fuentes, 2000; Pearson and Grisoni, 2002; Tairo *et al.,* 2006; Rännäli *et al.,* 2008, and Spain, Kenya, Uganda, and USA (Mukasa *et al.,* 2003). Former efforts to characterize SPCaLV failed because of its complexity, compared to other members of the Caulimoviridae.

Contrasting other viruses, it can be rightly detected in sweetpotato, thus saving time in routine virus indexing using a quick DNA extraction protocol and PCR primers or use of Nitro Cellulose Membrane-ELISA (De Souza and Cuellar, 2011).Sweetpotato plants infected with SPCaLV typically display no discrete viral symptoms. SPCaLV produces faint chlorotic spots or tiny areas of vein clearing which may progress into general chlorosis, wilting and premature death of leaves.

SPCaLV is not transmitted by aphids, mechanical means and seed or by contact between plants. Its vector is unknown. The impacts on yield are unknown (Riis-Jacobsen, 2011).

2.6.8 Sweetpotato Virus G

Sweetpotato Virus G was primarily reported in China, where it is common (Colinet *et al.,* 1998). It has afterwards been reported in the USA, Australia, Peru, Spain and Egypt (Clark and Moyer, 1988; Ishak *et al.,* 2003; Souto *et al.,* 2003; Ateka *et al.,* 2004; Tairo *et al.,* 2006; Clark and Hoy, 2006b; Trenado *et al.,* 2007 and Untiveros *et al.,* 2007). Formerly, the virus was found in areas around the Pacific Ocean (Rännäli *et al.,* 2008).

It causes mottling in *I. nil*, and chlorotic spotting in *I. setosa* and *I. tricolor* (Souto *et al.*, 2003). In sweetpotato, co-infections with SPFMV occur and it is difficult to differentiate the symptoms triggered by the two viruses (Clark *et al.*, 2012). The virus is transmitted in a nonpersistent manner by aphids; *A. gossypii* and *M. persicae* (Souto *et al.*, 2003; Wosula *et al.*, 2013). The virus can similarly be mechanically transmitted to several *Ipomoea* species (Brunt *et al.*, 1996; Souto *et al.*, 2003).

2.6.9 Sweetpotato Virus C-6

Sweetpotato Virus C-6 was initially isolated from a sweetpotato cultivar *Sosa 29* from the Dominican Republic at International Centre for Potato (CIP) in 1989 (Fuentes and Salazar, 1989). This cultivar showed symptoms of chlorotic spots. Sweetpotato Virus C-6 is transmitted at a low efficiency by mechanical inoculation and could also be transmitted by grafting. However, the virus could not be transmitted by aphids, notwithstanding a number of attempts (Fuentes and Salazar, 1989).The virus has a host range restricted to the family Convolvulaceae, hence it is suspected to be a member of the *Carlavirus* (Loebenstein, 2009). Its symptoms include chlorotic spots and vein clearing in *I. setosa* and *I. nil*.
Sweetpotato Virus C-6 causes a severe synergistic disease when plants are co-infected with SPCSV, causing about 50% reduction in yield loss and even more severe disease when SPFMV is included (Cohen *et al.*, 1997, Gibson *et al.*, 1998b).

2.6.10 Cucumber Mosaic Virus (CMV)

Cucumber mosaic virus, genus Cucumovirus, family Bromoviridae) is normally found infecting sweetpotato together with SPCSV and usually also SPFMV, producing symptoms similar to SPVD and causing up to 80% yield reduction (Loebenstein *et al.*,2009). It was initially observed in Israel where it severely affected sweetpotato yields (Clark and Moyer,

1988). It was shown that CMV could only infect sweetpotato if the plants were first infected with SPCSV, suggesting that SPCSV acted as a helper virus.

CMV is synergized by SPCSV (Untiveros *et al.,* 2007) which is of epidemiological importance because it affects virus accumulation in infected plants and may enhance transmission of CMV by aphids in countries where both CMV and SPCSV occur in sweetpotato (Ishak *et al.,* 2003). CMV is easily transmitted to sweetpotato plants mechanically by aphid inoculation of the acceptor plant which carries the whitefly- transmitted virus. Infection with each virus separately causes only mild or no symptoms in sweetpotato. Symptoms of CMV include stunting, chlorosis and yellowing (Cohen and Loebestein, 1991; Cohen *et al.,* 1992).

2.6.11 Begomoviruses

Begomoviruses belong to the family of Geminiviruses. They are plant viruses characterized by twin icosahedral particles (Briddon *et al.,*2010) Begomoviruses are transmitted by whiteflies and have either bipartite genome (known as DNA-A and DNA-B, both being ssDNA genomes of approximately 2.7 kb size, and contain ~ 220 bp at the common region) or they may have a monopartite genome.

Occurrence of Begomoviruses in sweetpotato is widespread and associated with most, if not all, geographic regions where sweetpotatoes are grown (Valverde *et al.,* 2007). Sweetpotato and science of these viruses have improved, therefore many new species have been discovered. There is evidence that there is considerable variability among the strains of Begomovirus (Lotrakul and Valverde, 1999; Lotrakul *et al.,* 2002).

For instance, the Sweetpotato Leaf Curl Virus (SPLCV) symptoms suggest that these viruses were present long before they were reported. Some of the strains either do not induce symptoms or induce very mild, transient symptoms in the standard indicator host, *I. setosa*. Besides, for

every ten to twenty percent of sweetpotato accessions tested at CIP, originating from different parts of the world tested positive for Begomoviruses (CIP, 1999).

The differences among sweetpotato Begomoviruses reported (Cohen *et al.,* 1997; Banks *et al.,* 1999; Onuki *et al.,* 2000 -and Lotrakul *et al.,* 2002), may also indicate that these viruses undergo a high rate of recombination, similar to reports for other Gemini viruses (Seal *et al.,* 2006). Sweetpotato-infecting Begomoviruses are phylogenetically distinct from the new and old world Begomoviruses, and are called 'sweep viruses' as a group (Fauquet and Stanley, 2003; Briddon et al., 2010; Wasswa *et al.,* 2011). The sweep viruses are generally symptomless in sweetpotato, even in double infection with SPCSV (Wasswa *et al.,* 2011), but they are able to attain high titres and they can spread undetected within the sweetpotato germplasm to new areas. The latent nature of Begomoviruses was reported by Clark and Hoy(2006) who found that yields of 'Beauregard' sweetpotato were reduced by twenty-five to thirty percent by SPLCV, notwithstanding the fact that no symptoms were observed on the plants.

2.7 Yield Loss Estimate due to Sweetpotato Virus Diseases

Viruses are indisputably the most important element of yield loss in sweetpotato production (CIP, 1989). Diseases due to viruses have remained the main reason restraining viable sweetpotato production (Gao *et al.,* 2000; Zhang et al., 2005; Wang *et al.,* 2010). Report from a study piloted in China (world's largest producer of sweetpotato) indicated that sweetpotato viruses effected an average yield loss of about 20 to 30% (Gao *et al.,* 2000), with severe losses of up to 78% (Shang *et al.,* 1996.)

Moreover, virus infested-sweetpotato plants were established to be far extra susceptible, than the healthy plants, to fungi *Monilochaetes infuscans* (Halst.exHarter) and *Ceratocystis*

fimbriata (Ellis et Halsted) and nematode *Pratylenchus coffeae* (Zimmerman) (Yang *et al.*, 1998; Wang *et al.*, 2000), consequently triggering greater yield losses.

2.8 Methods of Detection for Sweetpotato Viruses

The detection and identification of sweetpotato viruses remain cumbersome procedures (Karyeija *et al.*, 2000). Study of several viral diseases has been hampered by simple detection technique (Carey *et al.*, 1999). This is complicated by frequent occurrence of mixed infections.

After the virus has been identified, one can develop indexing procedures, search for sources of resistance or develop other control methods. Currently, progress has been made in developing sensitive techniques for several sweetpotato viruses (Kokkinos and Clark, 2006; Mukasa *et al.*, 2006; Tairo *et al.*, 2006). Sweetpotato viruses have been detected by observing symptom expression in the field and host range studies (Chavi *et al.*, 1997) and some by their vector relationship (Schaefers and Terry, 1976).

The difficulty in detecting sweetpotato viruses in sweetpotato is usually due to low virus titers rather than inhibitors or problems with the assays (Karyeija *et al.*, 2000; Kokkinos and Clark, 2006). This is complicated by frequent occurrence of mixed infections and synergistic complexes as in SPVD (Moyer and Kennedy, 1978), diverse viral strains, and uneven virus distribution within the plant. The primary test to detect sweetpotato viruses are bioassays on indicator plants by observing symptoms, vector transmission procedures, serology using diagnosis based on Enzyme Linked Immunosorbent Assay (ELISA) (Moyer and Salazar,1989; Chavi *et al.*, 1997) and PCR mostly because of their reliability and sensitivity (Mehta *et al.*, 1994).

2.8.1 Grafting

Indexing based on grafting unto susceptible indicator plants such as *I. setosa* is widely used to assay many sweetpotato viruses (Green and Lo, 1989; Loebenstein *et al.*, 2003). This is presumed to be a reliable method for the detection of most sweetpotato viruses that cannot be mechanically transmitted (Walkey, 1991). Grafting onto indicator plant is also used when virus titres are so low to enhance the virus symptoms.

Plants for the virus testing are grown in the screen house to produce stems, which are later assayed by grafting to *I. setosa* (Esbenshade and Moyer, 1982; Moyer and Salazar, 1989). The sweet potato cuttings grafted onto the side of the *I. setosa* induce symptoms such as vein clearing, puckering, leaf deformation and chlorotic spotting which start showing three to five weeks, depending on the temperature, age of the plant and the virus concentration or titre (Winter *et al.*, 1992; Gibson *et al.*, 1998a; Jericho and Thompson, 2000).

2.7.2 Insect Transmission

Myzus persicae (Sulzer), *Aphis gossypii* (Glover), *Macrosiphum euphorbiae* (Thomas) and *Bemisia tabaci* (Gennadius) are the most common insect vectors of sweetpotato viruses

(Brunt *et al.*, 1996). Plant viruses are spread from plant to plant by sap-sucking insects. Sweetpotato as well as other plants are essential in the life cycle of many viruses and their vectors, for the reason that viruses and vectors are able to live in adverse conditions and intervals between crop cycles in weed hosts, volunteer crop plants, abandoned crops, and vegetative plant parts (Dennien and Henderson, 2015). Insects transmit viruses via:

non-persistent transmission and persistent transmissions or ways.

2.7.2.1 Non-persistent transmission

When the insect feeds from the plant sap of virus-infected plant, viral particles get attached to the mouthparts of the insect. The virus particle is then carried onto the next plant that the insect

feeds on. The insect needs a short time (some few minutes) to transmit the virus between plants (Dennien and Henderson, 2015)

2.7.2.2 Persistent transmission

When the insect feeds on virus-infected plant, viral particles are carried in through the mouthparts into the gut of the insect (and stored in the salivary glands). The viral particles are then transferred to the next plant it feeds on. This is a slow process since the insect feeds for some hours to obtain the virus. Once the insect obtains the virus, it becomes infected with that virus throughout its life cycle (Dennien and Henderson, 2015).

2.7.3 Serological Detection

Enzyme Linked Immunosorbent Assay (ELISA) has been used for many years to detect plant viruses since its introduction in 1976 (Voller *et al.,* 1976; Clark and Adams, 1977; BarJoseph *et al.,* 1979). The procedure is based on the covalent linkage of an enzyme to an antibody, processing the incidence of an antigen-antibody complex via swift enzymatic clearly coloured product development (Converse *et al.,* 1990). ELISA is the chief check to detect plant viruses with polyclonal or monoclonal antibodies (Converse *et al.,* 1990; Walkey, 1991) which is done by collecting bioassay on indicator plants.

Nitrocellulose Membrane–Enzyme Linked Immunosorbent Assay (NCM-ELISA) is also used for detecting up to 10 plant viruses including SPFMV and SPCSV in sweetpotato as well as *I. setosa* (Abad and Moyer, 1992*;* Karyeija *et al.,* 1998, Karyeija *et al.,* 2000; Jericho and Thompson, 2000). It yields results stable to those gained using Triple Antibody Sandwich ELISA (TAS-ELISA) (Gibson *et al.,* 1997). One important aspect about ELISA technique is the assortment of the proper tissue (starting material) and the timing of the assay which are very critical (Esbenshade and Moyer, 1982).

Advantages of ELISA include its ability to detect viruses in small amounts/quantities or in low concentrations. Also, ELISA gives a speedy reaction (Walkey, 1991)

2.7.4 Polymerase Chain Reaction (PCR)

Classification of numerous viruses at the sequence level depends on their detection, using PCR methods. PCR is the practice of copied acid probes or the *in vitro* amplification of the precise nucleic acid sequence in a genome (in this case, the virus genome) that are then used to detect the presence of a particular disease (Dalla Rosa and Giroux, 2001). Intended for repetitive detection assays, PCR involves degenerate primers for recognition of the variants and strains of the virus.

The dearth of development in virus identification and classification is due to the recurrent occurrence of mixed infections and synergistic complexes besides inhibitory compound(s) in the sweetpotato that can disturb sensitivity and consistency of virus detection by PCR (Clark and Moyer, 1988; Moyer and Salazar, 1989; Fenby *et al.*, 1998). Thus, an interior optimization is desired to convert the superiority of the nucleic acid extracts used in PCR assays to classify and characterize viruses infecting sweetpotato. This technique is the utmost fit for viruses which are challenging to purify or which follow in mixed infections (Colinet *et al.*, 1994).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), using universal degenerate primers and strain-specific primers, has permitted discovery and classification of several Potyviruses that premeditated from nucleotide sequences of these viruses (Gibbs and Mackenzie, 1997; Colinet *et al.*, 1998; Souto *et al.*, 2003; Ateka *et al.*, 2004; Chen *et al.*, 2008). Generic and virus-specific primers have been castoff to distinguish and ascertain sweep viruses in *in-vitro* plantlets and greenhouse-developed sweetpotato plants, and in indicator *Ipomoea* plants (Li *et al.*, 2004; Lozano *et al.*, 2009; Paprotka *et al.*, 2010; Wasswa *et al.*, 2011). Increase of virus sequence information has improved the design of virus taxonspecific primers and

probes, explicit detection of viruses, certification of the results (Colinet *et al.*, 1998; Mukasa *et al.*, 2003; Souto *et al.*, 2003) and meaningfully upgraded resolution of incompletely characterized viruses.

While PCR-based approaches can amplify viruses existing in low titres, false negative reactions with well-known infected plants have been found with Potyviruses (Souto *et al.*, 2003) and with sweep viruses (Li *et al.*, 2004; Kokkinos and Clark,2006; Wasswa *et al.*, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of Experiments

Viral effect on yield assessment was carried out in the field at Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI), Fumesua, Kumasi, Ghana. Virus detection work, using NCM-ELISA and PCR was done at the Virology and the Molecular Biology Laboratories also at CSIR-CRI.

3.2 Sweetpotato sample collections

Fifty five (55) infected/diseased sweetpotato plant samples were collected from farmers' fields from the six regions of Ghana where the crop is grown. These genotypes are those planted in the areas sampled. These areas are Eastern, Central, Upper East, Greater Accra, Volta and Ashanti Regions. The agro-ecological zone of Eastern Region is moist SemiDeciduous with mean annual rainfall of 1,500mm.

Central and Greater Accra Regions are Coastal scrub and Grassland with mean annual rainfall of 800mm. Upper East is Guinea Savanna with an annual rainfall of 1,100mm.

Ashanti Region specifically Ejura, is Forest-transitional zone with an annual rainfall of

1,300mm (Oppong Anane, 2001).In the Volta Region sweetpotato is planted in the Guinea Savanna with mean annual rainfall of 1,100mm.

The samples collected were planted in pots in the screenhouse at CSIR-CRI and maintained as the source of inocula for laboratory diagnostics. Table 3.1 below shows the names of samples collected from each region.

Region/Locality/Geographical Position	Name of Cultivar	Cultivar/Variety Type
<u>Eastern</u> 06^{0}03'N 00^{0}17W Begoro	Anago-1	Local
	Anago-2	Local
<u>Volta</u> 46'N 0^{0}41'E		
Kpeve	Ogyefo	Improved
	Okumkom	Improved
	Santom Pona	Improved
	Hi-Starch	Improved
	Apomuden	Improved
	Sauti	Improved
Ohawu	Okumkom	Improved
	Santom Pona	Improved
	Ogyefo	Improved
	Hi-Starch	Improved
	Apomuden	Improved
	Sauti	Improved
	Trotroyeye	Local
Xetorlogo	Gloglobo	Local
Kudzordzikope	Trotroyeye	Local
Davego	Shashango	Local
Vume	Shashango	Local
Atipke	Shashango	Local
Agorve	Gloglobo	Local

Central

Jukwa-Abudu	Santom Yellow	Improved

Table 3.1. Sweetpotato Genotypes samples collected and their locations during the

sample collection

Komenda	Okumkom	Improved
Region/Location/Geographical Position	Name of Cultivar	Cultivar/Variety Type
	Apomuden	Improved
	Hi-Starch	Improved
	Sauti	Improved
	Santom Pona	Improved
	Ogyefo	Improved
Greater Accra 05035'N 00006'N		
Pokuase	Sauti	Improved
	Santom Pona	Improved
	Okumkom	Improved
	Ogyefo	Improved
	Hi-Starch	Improved
	Apomuden	Improved
Afiaman-Amasaman	Nagoli	Local
	Nagoli	Local
Upper East 11^{0}03'N 00^{0}19'W		
Navrongo	Seed (Ogyefo)	Local
	Ogyefo	Improved
Bawku	Apomuden	Improved Local
	Nabdam 1	
Bolgatanga		Local
	Nabdam 2	Local
	Seedling (Apomuden)	Local
	Navorongo	Local
	Bawku	Improved
	Bolga S1	Improved
	Bolga S2	
Ashanti 1.3^{0}1196'N 7.46761^{0}N Ejura	Farmer's Field 1	Local
	Farmer's Field 2	Local
	Research Field 1	Improved
	Research Field 2	Improved
	Research Field 3	Improved
	Research Field 4	Improved
	Research Field 5	Improved
		Improved

	Research Field 6a Research Field 6b	Improved

Table 3.1. Sweetpotato Genotypes samples collected and their locations during the sample collection

3.3 Graft Inoculation

Ipomoea setosa seeds obtained from International Centre for Potato (CIP) Office, CSIR-CRI, Ghana, were planted in pots in the insect-proof screen house at the CSIR-CRI, Fumesua. The seeds were first scarified by soaking in 95% concentrated sulphuric acid for 1h and then rinsed three times with distilled water to completely wash off the acid. The scarified seeds were placed on a wet paper towel in sterilized 9-cm Petri dishes for 72h and allowed to germinate (Schaefers and Terry, 1976).

These seedlings were transferred into square plastic pots of 20cm in length and 20cm in breath at a density of two seedlings per pot. The plastic pots were filled with 6.4kg (2:1:1) soil media consisting of 2 parts of sand to 1 part of loam and 1 part of decomposed poultry manure.

A disposable sterile blade was used to cut a wedge on the stem of each of the sweetpotato plants/cultivars collected from the surveys. Sweetpotato cultivars of four-week- old, 2cm vine length with three nodes were used for the grafting. Each sweetpotato cultivar was grafted onto a four-week old *I. setosa* arranged in a Complete Randomised Design (CRD) and the union was pegged to prevent the sweetpotato scion from falling off. Thirty six (36) sweetpotato diseased samples from the 55 that were collected were grafted on the *I. setosa*

(Plate 3.1) with each replicated once making a total of seventy-two.

Plate 3.1. Sweetpotato scions grafted onto *I. setosa* stalks in the screenhouse (union arrowed)

3.4 Identification of viral symptoms and determination of viral severity of the grafted *I. setosa* in the Screen house

The severity of the sweetpotato virus symptoms on all the grafted *I. setosa* were scored three times at 10 days intervals until the 30th day. A five-point rating scale (Hahn, 1979) where; 1 = no visible symptoms; 2 = mild symptoms; 3 = moderate symptoms; 4 = severe symptoms; and 5 = very severe symptoms was used.

3.5 Detection of Sweetpotato Viruses with NCM-ELISA

Leaf discs of one centimeter diameter were taken each from three sweetpotato leaves. These sweetpotato genotypes were collected from the areas sampled and were planted in pots in the screen house. The leaves were randomly taken; one from the top, middle and bottom along the

sweetpotato vines and were harvested from symptomatic and symptomless plants. A total of 127 plants were used (36 of the sweetpotato plants that were collected which were grafted and replicated to give 72 plants, 55 from all infected sweetpotato cultivars that were collected) for the detection. Polyclonal antisera specific to 10 viruses (that can be detected by NCM-ELISA), namely SPFMV, SPCSV, SPMMV, SPCFV, SPMSV, SPCaLV, SPLV, C-6, SPVG and CMV as well as negative and positive controls were obtained from the International Potato Center (CIP), Lima, Peru.

The samples were subjected to serological assays for viruses, using NCM-ELISA kits as described by Gibb and Padavan (1993). This was done by grinding the leaf discs in plastic bags filled with 3ml extraction buffer [Tris-buffered saline (TBS) pH 7.5 containing 0.2% sodium sulphite]. The sap was allowed to stand for 30 min at room temperature till the plant sap phased out.

After the 10 membranes had been labelled each with the name of the virus, they were placed over three sheets of filter paper and then immersed in TBS buffer in a 9-cm Petri dish for 2 min. The liquid on the surface of the pre-wet membrane was allowed to be absorbed for 3 min and then a micropipette was used to pick 20µl of the clear supernatant from the bag onto the centre of the square on the membrane, a process called Blotting. The membranes were transferred onto dry pieces of filter paper and air-dried for 20 min. About 30ml of blocking buffer was poured in 10 dishes of 9 cm Petri dishes and the air-dried membranes were immersed in it for 60 min.

This was done along with the positive control membrane strips that came along with the kit for every virus and sweetpotato plant from tissue culture (virus-free), indexed as negative control. After incubation, the membranes were washed once with TBS rapidly for eight seconds. Virus-specific antibody solutions were prepared for all the various viruses and added to the washed

membranes. With the Petri-dishes covered, they were left on a shaker at a gentle agitation at 50rpm and incubated overnight.

The next morning, the virus-specific antibody solutions, as well as the unbound antibody, were removed by washing the membranes with 30ml T-TBS with constant agitation four times for 3 min each. After washing, the membranes were placed between towel papers on a flat surface and pressed gently to remove air bubbles, then the membranes were placed in Petri-dishes containing 30ml of antibody conjugated with enzyme (conjugate antibody) and incubated for 1 h.

The conjugate solution was discarded and the unbound antibodies were removed by washing with T-TBS as done before.

Afterwards, the membranes were placed in between paper towels to remove excess solution.

A 25ml of substrate colour development solution were poured onto each membrane in the Petri dishes and the reaction allowed to take place for 30 min for all the viruses, except for SPCSV which was allowed a reaction period of 1 h because this virus takes quite a longer time for the colour to develop. The colour development reaction was stopped by discarding the substrate solution and immersing the membrane in tap water, then washed with distilled water for 10 min.

The membranes were then air-dried on filter paper and the results recorded. The development of a purple colour on nitrocellulose membrane confirmed virus positive samples and the intensity of the purple colour denotes the concentration of the virus in the sample. The colour intensity was scored on a scale of 1 + to 6 + where, + = very very light purple, ++ = very light purple, +++ = modest purple, ++++ = deep purple, +++++ = very deep purple and ++++++ = very very deep purple (Gutiérrez *et al.*, 2003). Samples that tested negative were denoted with a '-'sign.

3.6 Nucleic Acid Extraction

Total nucleic acid (genomic DNA and RNA) was extracted from 127 (72 from grafted *I. setosa* and 55 from sweetpotato accessions collected from the surveys), four-week-old tender leaves located towards/near the apex of the *I. setosa* and infected sweetpotato samples collected from the surveys. About 200 mg of the fresh young leaf tissue per plant was harvested for extraction of total nucleic acid, using the modified protocol described by Egnin *et al.*, (1998). The leaves were weighed with an electronic balance and placed into 2ml eppendorf tubes and ground with a pestle in liquid nitrogen to a fine powder.

About 800µl of lysis buffer was added to the fine powder and incubated for 10 min in water bath at 90^0C with occasional inverting up and down to mix. This was allowed to cool at room temperature for 2 min and then 400µl of 5M Potassium acetate added to precipitate the proteins and polysaccharides. It was then incubated on ice for 30 min and centrifuged at 14,000 rpm for 15 min. About 900µl of the clear supernatant was pipetted into another 2ml tube and 540µl of cold isopropanol as well as 90µl of 3M Sodium acetate added.

This was incubated at -20^0C for 1h to precipitate most of the genomic nucleic acid. Subsequently, centrifugation at 14,000 rpm was done. The supernatant was decanted and the pelleted nucleic acid washed with 80 % ethanol. The pellets were air-dried and dissolved in 500µl of 1XTE solution.

About 250µl of 7.5M Ammonium acetate was added to further precipitate the proteins out. This mixture was incubated on ice for 3 min and centrifuged at 13,000rpm. About 700µl of the supernatant was pipetted into a 1.5ml eppendorf tube and equal volume of cold isopropanol was added to precipitate the total genomic acid out. This was centrifuged at 14,000 rpm and the pelleted genomic acid air-dried. After drying, the pellet was again dissolved in 100ul 1XTE solution and the quality of the genomic nucleic acid was determined.

3.7 Nucleic Acid Quantification and Gel electrophoresis

The integrity of the extracted DNA and RNA was evaluated by electrophoresis in 0.8% agarose gel stained with ethidium bromide and 1x TAE (Tris-acetic EDTA) buffer. Electrophoresis of the nucleic acid was carried at 100V for 40minutes and then visualized with a UV transilluminator. The concentration of the RNA and DNA was projected by the intensity and comparison to 1kb lambda DNA mass ladder (1kb, Invitrogen). The quantification of the DNA and RNA was further evaluated by reading absorbance at 260nm and 280nm with the Nanodrop 2000C Spectrophotometer (Thermo Scientific, USA).

3.8 Polymerase Chain Reaction (PCR) Amplification

PCR amplification of DNA (using degenerate universal primers)/ RNA was carried out with sequence specific primers to the viruses on an AB (Applied Biosystem) Thermocycler. The PCR conditions were optimized for cycling number, concentrations of the primer, $MgCl_2$ and DNA/RNA template. The reaction mixture (10µl) contained a final concentration of 1X PCR Buffer, 3.5mM of $MgCl_2$ (25mM), 0.25mM dNTPs (20mM), primer 0.25µM (10µM) of each forward and reverse primer, 0.8U of Supertherm Taq Polymerase (5U/l) and 1µl of 10ng DNA/RNA template.

In each PCR run, a negative control was included to detect contaminations. The PCR products were electrophoresed on 1.5 % agarose gel and bands were stained with ethidium bromide visualised under ultraviolet transilluminator (AlphaImager^{TM} 2200, UVP, USA).
Images were captured with MiniBus GelCapture (DNA Bio Imaging System, Israel).

The bands were scored as present (+) for amplification of the band or absent (-) for absence of the band. Table 3.2 below shows the sequence of the degenerate and specific primers and the PCR/RT-PCR cycling conditions used for each of the primers. These sequences and protocols were obtained from the CIP Virology Laboratory, Lima, Peru.

3.9 Evaluation of sweetpotato for yield of sweetpotato in the field

For the field establishment, the land was cleared and ploughed. Ridges of 5m length and a width of 1m between rows were made for the planting of the materials. For each block, the experiment was carried out on a total plot size of 246.4 m^2. Planting was done on 29th September, 2014.

The three sweetpotato varieties used were; 'Ligri,' 'Dadanuei' and 'Bohye' (Table 3.3) obtained from the CSIR-CRI, Fumesua. Four different treatments (nature of planting material) for each of the varieties were as follows:

(i) Diseased field collections

(ii) Healthy field collections

(iii)tissue culture clean materials, and

(iv) artificially inoculated materials, using insect vectors (whiteflies and aphids).

The artificial inoculation was done by rearing whiteflies (*B. tabaci)* and Aphids sp. for two weeks and they were transferred onto healthy (tissue culture cleaned virus indexed) sweetpotato in the screenhouse for 48h. Afterwards, they were transplanted in the field.

Table 3.2. Sweetpotato genotypes used for the field establishment and their characteristics.

	Characteristics		
Sweetpotato Genotypes	Yield/t/ha	Months to maturity (Months)	Tolerance to SPVD
Bohye	22	3-5	Tolerant
Ligri	22	3-5	Tolerant
Dadanuei	18	3-5	Tolerant

These three materials were selected because they are newly released varieties that have been certified to be tolerant to SPVD. Again, the months to maturity are the same which eliminates any form of bias in the experiment. Furthermore, the expected yields are almost the same per hectare.

For each of the three genotypes; 34 vines per plot were planted in two rows on ridges, spaced at 0.3m within rows. Each vine cutting with a length of about 30cm was inserted at a slant with two-thirds buried below the soil surface. The genotype 'Ogyefo' was grown at the edges of the field to serve as border. The field was weeded as needed, using a hoe. The experiments were conducted under rain-fed conditions.

Eight samples from each genotype (that is two each from the nature of planting material) with the different treatments (source of planting material) were taken at random for serology (NCM-ELISA) and PCR /RT-PCR techniques as described above to determine the type of viruses present.

3.10 Harvesting

Harvesting was done 90 days after planting. Plants were harvested by uprooting the central 10 plants out of the 17 of each row, leaving plants at both edges, which served as borders. Vines were cut with cutlass and the storage roots were uprooted with hoe. Data were taken on the following parameters:

- yield of tubers (t/ha) calculated by weighing on a balance the total number of tubers harvested per plot
- no. of tubers per plot
- foliar weight per plot/kg

All of these were converted to per hectare.

3.11 Virus Incidence and Severity on sweetpotato in the field

Number of diseased plants (incidence) was counted. Disease severity of sweetpotato virus symptoms on the field was scored, using a five-point severity rating scale by Hahn (1979) described in section 3.4. Data were taken for mean disease incidence and severity from one month to three months after planting.

3.12 Experimental Design and Data Analysis

Factorial arrangement in RCBD with three replications was used for the field trial. GenStat Discovery Edition (Version 4, VSN International Limited, UK) was used for data analysis. Least significant difference, (LSD) was used to separate treatment means at 5%. The count data for incidence was transformed, using the square root transformation.

Table 3.3. Degenerate and specific primer sequences and their cycling conditions used for PCR/RT-PCR

Virus	Oligo Name	Sequence 5'-3'	Cycling Protocol
1. Begomoviruses (including Sweetpotato Leaf Curl Virus)	SPG1	CCCCKGTGCGWRAATCCAT	94^0C, 40 s $(72\text{-}n)^0$C 30 s 11 cycles (n=-1^0C per cycle) 72^0C 90 s 94^0C 40 s 60^0C 40 s 24 cycles 72^0C 90 s 72^0C 10 min, 4^0C Forever
	SPG2	ATCCVAAYWTYCAGGGAGCTAA	
2. Cucumber Mosaic Virus (CMV)	CMV-F	GCCGTAAGCTGGATGGACAA	95ºC, 5 min 96ºC, 5 s 6ºC, 5 s 35 cycles 72ºC, 30 s 72ºC for 1 min.
	CMV-R	TATGATAAGAAGCTTGTTTCCG	
3. Sweetpotato Chlorotic Stunt	SPCSV-F	ATCGGCGTATGTTGGTGGTA	Denaturation: 65^0C 5 min ,4^0C (ice) RT Condition:40^0C 60 min,95^0C 5min, 4^0C

Virus (SPCSV)	SPCSV-R	GCAGCAGAAGGCTCGTTTAT	(ice) PCR condition: 94^{0}C 2 min 94^{0}C 30 s 53^{0}C 30 s 72^{0}C 40 s 72^{0}C 10 min 10^{0}C Forever 35 cycles

Table 3.3. Cont'd Degenerate and specific primer sequences and their cycling conditions used for PCR/RT-PCR.

Virus	**Oligo Name**	**Sequence 5'-3'**	**Cycling Protocol**
4. Sweetpotato Feathery Mottle Virus (SPFMV)	SPFMV-F	GGATTAYGGTGTTGACGACACA	Denaturation: 65^{0}C 5 min ,4^{0}C (ice) RT Condition:40^{0}C 60 min, 95^{0}C 5min, 4^{0}C(ice) PCR condition: 94^{0}C 2 min 94^{0}C 30 s 60^{0}C 30 s 72^{0}C 1 min 20 s 72^{0}C 10 min 10^{0}C Forever 30 cycles
	SPFMV-R	TCGGGACTGAARGAYACGAATTTAA	

5. Sweetpotato Mild Mottle Virus (SPMMV)	SPMMV-F SPMMV-R	AGTATGGAGGTGTCTGGAAACATGG CCACTGTTGTTGCCTCTGAACTTCC	Denaturation: 65^0C 5 min ,4^0C (ice) RT Condition: Room temp 10 min,37^0C 60min, 70^0C 15 min, 4^0C(ice) PCR condition: 94^0C 2 min 94^0C 30 s 55^0C 30s 72^0C 1.5 min } 35 cycles 72^0C 10 min 10^0C Forever

Table 3.3. Cont'd Degenerate and specific primer sequences and their cycling conditions used for PCR/RT-PCR.

Virus	**Oligo Name**	**Sequence 5'-3'**	**Cycling Protocol**
6. Sweetpotato Virus G (SPVG)	SPVG-F	GTATGAAGACTCTCTGACAAATTTTG	Denaturation: 65^0C 5 min ,4^0C (ice) RT Condition:40^0C 60 min,95^0C 5min, 4^0C(ice) PCR condition: 94^0C 2 min 94^0C 30 s 60^0C 30 s } 30 cycles 72^0C 1 min 20 s 72^0C 10 min 10^0C Forever
	SPVG-R	TCGGGACTGAARGAYACGAATTTAA	
	SPVC6-F	GAATCTGATTATGARGCITTYGAYGC	Denaturation: 65^0C 5 min ,4^0C (ice)

7. Sweetpotato Virus C6 (SPVC6)	SPVC6-R	IAAATTCCAACCRCARAADGTIGG	RT Condition:40^{0}C 60 min,95^{0}C 5min, 4^{0}C (ice) PCR condition: 94^{0}C 2 min 94^{0}C 30 s 50^{0}C 30 s 35 72^{0}C 1 min 72^{0}C 10 min

Table 3.3. Cont'd Degenerate and specific primer sequences and their cycling conditions used for PCR/RT-PCR

Virus	**Oligo Name**	**Sequence 5'-3'**	**Cycling Protocol**
8. Sweetpotato Latent Virus (SPLV)	SPLV-F	GGGTGATGATGGACGGAGACA	RT Condition:42^{0}C 30 min,95^{0}C 5min, 4^{0}C(ice) PCR condition: 94^{0}C 30 s 50^{0}C 30 s 72^{0}C 90 s 72^{0}C 10 min 10 ^{0}C Forever
	SPLV-R	CCGATGATGTGTATTTGTGAGC	35 cycles
9. Sweetpotato Mild Speckling	SPMSV-F	GCCAAAACCAACAAGCATCA	RT Condition:95^{0}C 5 min,42^{0}C 30 min, 4^{0}C (ice) PCR condition:

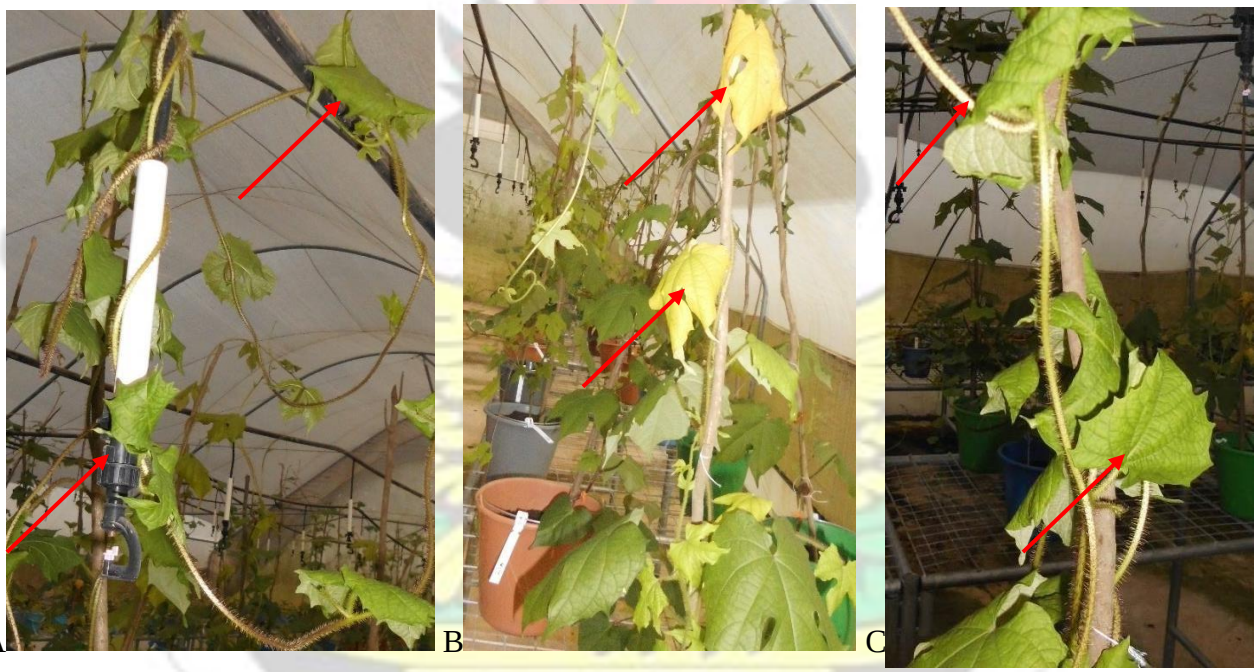
Virus (SPMSV)	SPMSV-R	ATTCGCATTTCCTCATCATCT	94^0C 30 s 50^0 C 30 s 72^0C 90 s 72^0C 10 min 10 ^{0}C Forever } 35 cycles
10. Sweetpotato Collusive Virus (SPCV)	SPCV-F	GTAGATATAATTCAGGAAC	PCR condition: 94^0C 2 min 94^0 C 30 s 53^0C 30 s 72^0C 60 s } 35 cycles 72 ^{0}C 10 min 10 ^{0}C Forever
	SPCV-R	GCATCATCTGTTCCATTTCT	

CHAPTER FOUR

4.0 RESULTS

4.1 Disease Symptoms observed on Grafted *I. setosa* in the Screenhouse

Disease symptoms observed on grafted *I. setosa* and sweetpotato cultivars (Table 4.1) in the screen house were mainly M = Mosaic, Cs = Chlorosis Spots, Y = Yellowing, Nv = Net venation, St = Stunting, Ld = Leaf deformation, Vb = Vein banding, Rd = Roll-down, Vc = Vein clearing, Lp = Leaf puckering, Nc = Necrosis and Ru = Roll-up. These symptoms were expressed from two weeks after inoculation on the grafted *I. setosa*.



A: Roll-down, vein clearing	B: Yellowing and general chlorosis	C: vein clearing

Plate 4.1 Some of the viral symptoms observed in the screen house on *I. setosa grafted* plants

4.1 Symptoms and viruses detected on grafted *I. setosa*

Thirty six sweetpotato diseased samples that were grafted on the *I. setosa* (replicated making 72) showed disease symptoms mainly mosaic, chlorosis/chlorotic spots, yellowing, net venation, stunting, leaf deformation, vein banding, roll-down, vein clearing, leaf purlin, necrosis and roll-up.

Table 4.1 shows the symptoms and the number of *I. setosa* that were infected

Symptoms Expressed on grafted *I. setosa*	**Number of Infected *I. setosa* in the screen house**
Mosaic	10
Chlorosis/ chlorotic spots	4
Yellowing	20
Net veination	16
Stunting	8
Leaf deformation	18
Vein banding	2
Roll-down	14
Vein clearing	9
Leaf puckering	1
Necrosis	7
Roll-up	4

The commonest symptoms displayed on the grafted *I. setosa* in the screen house was yellowing, followed by leaf deformation. The least occurring symptoms were leaf puckering and vein banding. Table 4.1 shows the symptoms and the number of *I. setosa* that were infected.

The viruses mainly detected were Sweetpotato Feathery Mottle Virus (SPFMV), Sweetpotato Chlorotic Fleck virus (SPCFV), Sweetpotato Caulimo–like virus (SPCaLV), Sweetpotato Chlorotic Stunt Virus (SPCSV) and Sweetpotato Virus G (SPVG). SPFMV was the most common virus detected, followed by SPCSV. The least occurring viruses were SPCaLV and SPVG. Table 4.2 shows the symptoms and viruses identified from the grafted *I. setosa.*

Table 4.2 Symptoms and viruses detected from grafted *I. setosa* with NCM-ELISA

		Viruses identified from the grafted *I. setosa* with NCM-ELISA				
Region/Locality/ Variety	**Symptoms**	**SPFMV**	**SPCFV**	**SPCaLV**	**SPCSV**	**SPVG**
Eastern, Begoro						
Anago-1	M,Cs	+++	-	-	-	-
Anago-2	Y,Nv,St	++	-	-	-	-
Central						
Jukwa-Abudu	M	++++	-	-	-	-
Santom Yellow						
Komenda	Y,RD,St	+++++	-	-	+	-
Hi- Starch	Nv,Y,RD,St	+++	-	-	-	-
Apomuden	Y,M,Ld	++++	-	-	+	-
Sauti	M,RD,Ld,Nc	++++	-	-	-	-
Santom Pona	Nc,St,RD,Y	++++	-	-	+++	-
Okumkom	RD,Nv,Y,Ld	+++	-	-	-	+
Ogyefo						
Volta,						
Kpeve						
Ogyefo	Y,Nv,Ld	++	-	-	-	-
Okumkom	Vb,Nv,Vc,RD	+++	-	-	-	-
Santom Pona	RD,Cs,Ld,Nv,Y	++++	-	-	-	-
Hi-Starch	Y,St,Nv,Vc	++++	-	-	+++	-
Apomuden	RD,Ld,Cs,Nv,Vc	++++	-	-	-	-
Sauti	Vc,Ld,RD	+++	-	-	-	-
Ohawu						
Okumkom	RD,Nv,Vc,Y	+++	-	-	+	-
Santom Pona	Ld,Nv,Vc	+++	-	-	-	-
Ogyefo	Nv,M	+++	-	-	++	+
Sauti	Y,Nc,RD,M	++++	-	-	-	-
Hi-Starch	Y,St	++++	-	-	++++	-
Apomuden	RD,Vc,Ld	+++	-	-	-	-
Xetorlogo						
Trotroyeye	Nc,Y,Ld,St	++++	-	-	-	-

Table 4.2 Symptoms and viruses detected from grafted *I. setosa*. Cont'd

Region/Locality/ Variety	Symptoms	Viruses identified from the grafted *I. setosa* with NCM-ELISA				
		SPFMV	SPCFV	SPCaLV	SPCSV	SPVG
Agorve			-	-	-	-
Gloglobo	Y,Nv,Vc,Vb	+++++				
Kudzordzikope						
Gloglobo	Y,Nc,Ld,RU	++++	-	-	-	-
Trotroyeye	Nv,M	++++	-	-	-	-
Davego						
Trotroyeye	RU,Ld,M	++++	-	-	-	-
Shashango	M,Y,Ld	++++	-	-	-	-
Vume						-
Shashango	Cs, RU, Nc	+++++	+	+	+	
Atipke			-	-	-	-
Shashango	Y, Ld	++++				
Greater Accra						
Pokuase						
Sauti	Nc,Y,St	+++	-	-	-	-
Santom Pona	Y,Ld,Lp,M	++++	-	-	-	-
Ogyefo	Ld,RD,Y,Nv	++++	-	-	-	-
Okumkom	RD,Ld	++	-	-	-	-
Apomuden	Nc,Y,Nv,Vc	+++	-	-	+	+
Hi-Starch	Nc,Y,Nv	++++	-	-	++	-
Afiaman-Amasaman						
Nagoli						
	Nc,Ld,RU	+++	-	-	-	-

Key: + = very very light purple, ++ = very light purple, +++= modest purple, ++++=deep purple, +++++= very deep purple. Samples that tested negative were denoted with a '-'sign

4.3 Mean disease incidence and severity of sweetpotato varieties planted on the field from the different sources of planting material.

Results of mean disease incidence and severity on the sweetpotato varieties planted on the field are shown in Table 4.3. The differences in disease incidence and severity between "Ligri" cultivated from infected field vines and whitefly inoculated materials were significant ($P<0.05$). The same was true for "Bohye".

'Dadanuei' produced from field infected, whitefly inoculated and field healthy materials revealed significant differences ($P<0.05$) for mean disease incidence matched to tissue culture. There were no significant differences ($P>0.05$) for varieties all showed from the tissue culture produced planting material. Field healthy source of planting material revealed significant difference ($P<0.05$) for mean disease incidence across the three varieties; Ligri, Bohye and Dadanuei.

'Ligri' variety indicated a significant difference ($P<0.05$) for mean disease incidence from the field infected source of planting material but Bohye and Dadanuei varieties were not significantly different ($P>0.05$) for the same source of planting material. 'Ligri' from whitefly inoculated source of planting material showed a significant difference ($P<0.05$) for mean disease incidence whereas 'Bohye' and 'Ligri' varieties were not significantly different ($P>0.05$).

Ligri' produced from field-infected and whitefly-inoculated materials showed significant differences ($P<0.05$) for mean disease severity, compared to tissue culture and field healthy. 'Bohye' produced from field infected, whitefly inoculated and field healthy source of planting materials revealed significant difference ($P<0.05$) for mean disease severity same to tissue culture. 'Dadanuei' produced from tissue culture, field healthy, field infected and whitefly inoculated materials showed significant differences ($P<0.05$) for mean disease severity.

'Ligri' variety was not significantly different ($P>0.05$) for mean disease severity from the tissue culture source of planting material. Consequently, Bohye and Dadanuei varieties showed no significant differences ($P>0.05$) for the mean disease severity from the same source of planting material. Field healthy source of planting materials for all the varieties revealed significant difference ($P<0.05$) for mean disease severity.

Subsequently, the field infected sources of planting materials for the same varieties indicate the no significant difference (P<0.05) for mean disease severity. Alternatively, 'Ligri was not significantly different (P<0.05) whereas 'Bohye' and Dadanuei' had a significant difference (P<0.05) for the mean disease severity from the whitefly inoculated source of planting material.

Table 4.3 Mean disease incidence and severity of the sweetpotato varieties planted in the field from the different sources of planting material

Sweetpotato Variety	Mean Disease incidence from one to three months after planting				Mean disease severity from one to three months after planting			
	Tissue Culture	Field Healthy	Field Infected	Insect Inoculated	Tissue Culture	Field Healthy	Field Infected	Insect Inoculated
Ligri	1.24	1.24	6.93	2.90	1.25	1.25	2.67	2.54
Bohye	1.95	10.00	9.64	4.61	1.42	2.75	2.83	2.08
Dadanuei	1.00	5.63	9.14	4.11	1.17	2.08	2.67	1.83
LSD(P>0.05)			1.47				0.46	
CV(%)			29.0				28.80	

4.4 Detection of sweetpotato viruses using NCM-ELISA from grafted *I. setosa* and

sweetpotato cultivars collected during the sample collection

Serological tests with NCM-ELISA detected seven viruses both from the indicator plant *I. setosa* and the sweetpotato plants directly. In all, from the 127 plants directly tested, 108 (85.71%) gave positive reaction to *Sweetpotato feathery mottle virus* (SPFMV), 21(16.67%) for *Sweetpotato chlorotic stunt virus* (SPCSV), 8 (6.35%) for *Sweetpotato caulimo-like virus* (SPCaLV*)*, 6 (4.76%) for *Sweetpotato virus G* (SPVG), 6 (4.76%) for *Sweetpotato mild speckling virus*(SPMSV), 2 (1.57%) for *Sweetpotato chlorotic fleck virus* (SPCFV) *and* 5 (3.97%) for *Cucumber mosaic virus* (CMV) . No sample reacted positively to the antiserum specific to *Sweetpotato latent virus* (SPLV), *Sweet potato C6 virus* (SPC6V*)* and *Sweetpotato mild mottle virus* (SPMMV). It was noted that the *I. setosa* reached higher concentrations (Table 4.3) for sweetpotato virus than in *I. batatas* plants (Table 4.4).

The ELISA reaction gave a weak to very strong purple colour intensity, depending on the concentration/titre of virus present, as shown in Plate 4.2. Multiple mixed infections were detected with the commonly occurring SPVD; SPFMV+SPCSV, SPFMV+SPVG, SPFMV+ SPCaLV , SPFMV+ SPMSV , SPFMV+ SPCFV and SPFMV+ CMV. Three samples 'Ogyefo' from Ohawu, a local variety 'Shashango' from Davego and another from Ejura; 'Ejura 6b' had mixed infections of three viruses (SPFMV+SPCSV+SPVG), (SPFMV +SPCaLV+ CMV) and (SPFMV+SPCFV+SPMSV), respectively.

Three samples had mixed infections of four viruses; a local variety 'Trotroyeye' from Davego had (SPFMV +SPCSV+SPVG+ SPCaLV), 'Shashango' from Kudzordzikope and 'Apomuden' from Komenda had mixed infections of (SPFMV +SPCSV +SPCaLV +CMV).

A landrace 'Gboglobo' from Agorve had mixed infections of five viruses (SPFMV +SPCSV +SPCaLV+ SPVG+CMV). Most of the local varieties had mixed infections of two or more viruses, while almost all the improved varieties had a single virus infections (Table 4.4).

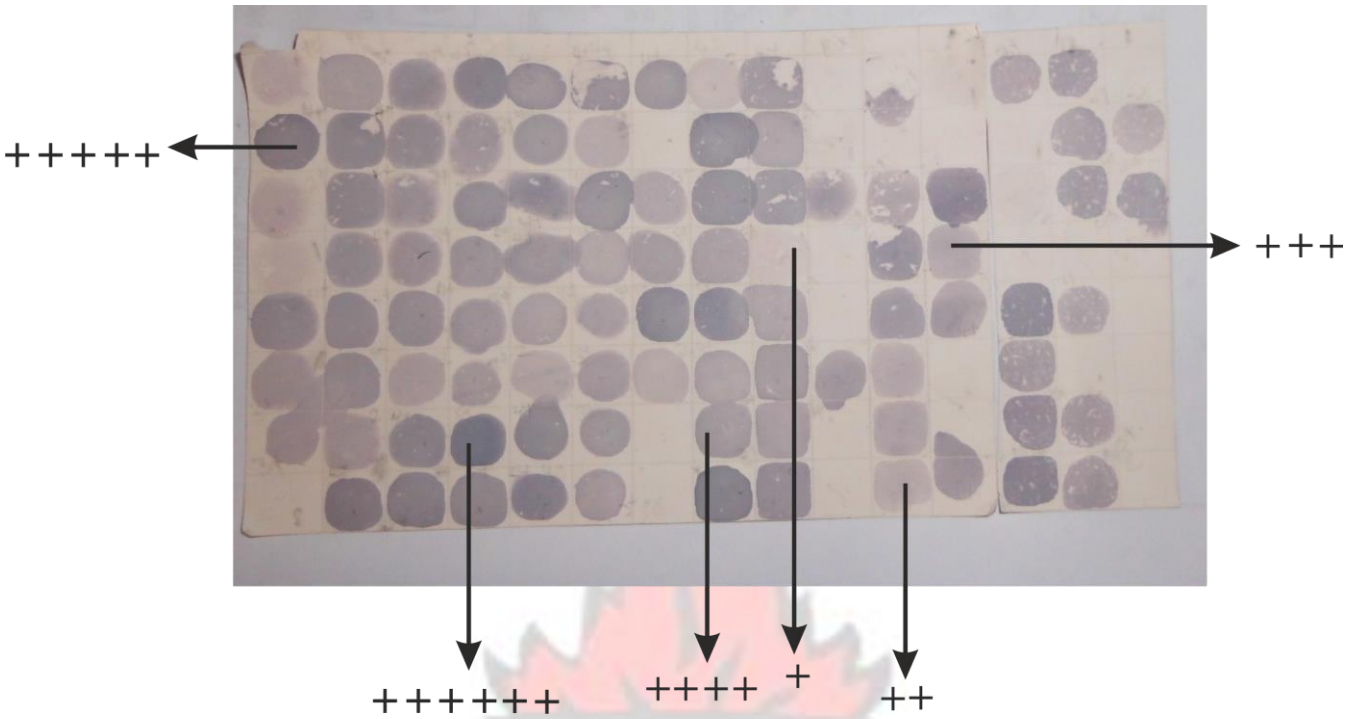


Plate 4.2 An NCM-ELISA results showing different purple intensities (+ to ++++++) for the detection of SPFMV

4.5 Symptoms and viruses detected from the sweetpotato plants collected from the major growing areas

Table 4.4 shows the symptoms and viruses that were detected from sweetpotato plants collected from the major growing areas. Symptoms showed by the 55 sweetpotato diseased samples were mainly mosaic, chlorosis/chlorotic spots, yellowing, net venation, stunting, vein banding, roll-down, vein clearing, necrosis and roll-up. Twenty five samples exhibited mosaic, 38 showed chlorosis or chlorotic spots, 15 showed yellowing, 25 samples had net veination and 17 had stunted growth. Other symptoms that were displayed included vein banding 27; roll-down 1; vein clearing 31; necrosis 13; and 28 for roll-up. The most common symptom on the sweetpotato plants in the screen house was chlorosis/chlorotic spots, followed by vein clearing, the least symptoms was roll-down and necrosis.

The viruses mainly detected were Sweetpotato Feathery Mottle Virus (SPFMV), Sweetpotato Caulimo–like virus (SPCaLV), Sweetpotato Chlorotic Stunt Virus (SPCSV), Sweetpotato

Virus G (SPVG), Sweetpotato chlorotic Fleck virus (SPCFV), Sweetpotato Mild Specking Virus (SPMSV) and Cucumber Mosaic Virus (CMV). SPFMV was the most occurring virus detected, followed by SPCSV. The least occurring viruses were SPVG and SPCFV.

Table 4.4 Symptoms and viruses detected directly from the sweetpotato plants collected from the major growing areas

Region/Locality/Variety	Symptoms	Viruses identified from the sweetpotato plants collected from the major growing areas						
		SPFMV	SPCaLV	SPCSV	SPVG	SPCFV	SPMSV	CMV
Eastern, Begoro Anago-2 Central,Jukwa-Abudu Santom Yellow	Nc,RU,Vb,Nv	-	-	-	-	-	-	-
	RU,Nv,Y	-	-	-	-	-	-	-
Central, Komenda Hi- Starch	Y,St,RU,Vc,Vb	++++	-	-	-	-	-	-
Apomuden	Y,Vc,Vb,Nc	++++	-	-	-	-	-	-
Sauti	M,Cs,Nv	++	-	-	-	-	-	-
Santom Pona	Y,M,Cs,Vc	++	-	-	-	-	-	-
	RD,Vc,M	++++	-	-	-	-	-	-
Okumkom	Cs,Nv,Vc,Vb, RU	-	-	-	-	-	-	-
Ogyefo								

Volta,								
Kpeve	Cs,Vb	++++	-	-	-	-	-	-
Ogyefo								
	Cs,Nv,M							
Okumkom		-	-	-	-	-	-	-
	RU,Nv ,Cs							
Santom Pona		-	-	-	-	-	-	-
	Vb,Vc,RU							
Hi-Starch		+++	-	-	-	-	-	-
	Y,Vb,Nv,Vc							
		+	-	-	-	-	-	-
Apomuden	St,Ld,RU,Vb, Vc	-	-	-	-	-	-	-
Sauti								

Table 4.4 Symptoms and viruses detected directly from the sweetpotato plants collected from the major growing areas Cont'd

Region/Locality/Variety	Symptoms
Volta	
Ohawu	Cs,Vc,Vb,Nv
Okumkom	RU,Cs,V,St
Santom Pona	Vc,Vb,M,Cs
Ogyefo	Cs,Vc,Vb,M, RU
Sauti	M,Cs,Nc
Hi-Starch	Cs,Nv,RU,Vc, M
Apomuden	St,Y,Vc,Vb,Cs ,RU
Xetorlogo	Cs,Nv,Y
Trotroyeye	Cs,Vc,Vb,M, RU
Agorve	
Gloglobo	
Kudzordzikope	
Gloglobo	
Davego	
Trotroyeye	Nc,RU,M,Nv, Vc,Vb
Shashango	RU,St,C,Vc,Vb, Nc
Vume	
Shashango	Cs, RU, Nc
Atipke	Cs, RU, Nc, M
Shashango	
Greater Accra	
Pokuase	
Sauti	Nc, Y, St
Santom Pona	Y, Ld,Lp,M
Ogyefo	Ld,RD,Y,Nv
Okumkom	RD,Ld
Apomuden	Nc,Y,Nv,Vc
Hi-Starch	

Nc,Y,Nv Viruses identified from the sweetpotato plants collected from the major growing areas

+++++	+		+		+		-		-		+		
++	-		-		-		-		-		-		
SPFMV	SPCaL V	SPVG	SPMSV	SPCSV	SPCFV	CMV	V						
++++	+		-		-		-		-		+		
+++	-	-		-									
	-	-		-									
	-	-											
+++	+		-		-		-		-		-		
-	-	-		-									
		-		-									
		-											
+++	-		-										
	-		-										
	-		-										
++	-		-										
	-		-										
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++	-		-		-		-		-		-		
-	-	-		-									
+++++	-		-		-		-		-		-		
		-		-									
-	-		-		-		-		-		-		
		-											
++++	-		-										
+	-		-		-		-		-		-		
	-		-										
+++	+		+		-		-		-		+		
+	+		+		-		-		-		-		
-	+	-		-									
		-		-									
		-											

Region/Locality/Variety Symptoms

Viruses identified from the sweetpotato plants collected from the major growing areas

SPCaL V SPCSV SPVG SPCFV SPMSV CMV CMV

Greater Accra	M,Cs,Nc	+++	-	-	-	-	-	-
AfiamanAmasaman Nagoli								
Upper East								
Navrongo Seed from Ogyefo	Y,Nc,RU,M,Cs	-	-	+	-	-	-	-
Navrongo local	Cs,Nc,Vc,Vb,Nv	-	-	+	-	-	-	-
Ogyefo	M,Y,Nc,Vb	++++	-	+	-	-	-	-
Apomuden	M,Y,Nc,Cs	+++	-	-	-	-	+	-
Seedling from Apomuden	St,Cs,Nc,M,RU,Nv		-	-	-	-	-	++
Bakwu								
Bakwu Local	RU,Cs,Nc,Ld	++++		-	-	-	+	-
Nabdam	RU,Cs,Nc,M,Vc,Vb	-						
Nabdam-1	M,RU,Nc,Cs,St,Nv,Vc		-	-	-	-	+	-
Nabdam-2		-	-	-	-	-	-	-
Bolga S1	RU,Nc,Cs,St,Nv,Vc St,Vc,Cs	+	-	+	-	-	-	-
Bolga S2		-	-	-	-	-	-	-
Ashanti								
Ejura	RU,Cs,Nv,Vc,							
Ejura A	Vb,Nc,M							
Ejura B	St,RU,Y,Vc,Nc Vb,Nv,M	++	-	-	-	-	-	-
Ejura 1	Y,St,Vc,Nc,VB,	-	-	-	-	-	-	-
Ejura 2	Nv,St,Nc,Vc,Cs	- ++++	-	-	-	-	-	-
Ejura 3	Ru,Nc,St,M,Vc,Vb,Nv,Y	-	-	-	-	-	-	-
Ejura 4	Nc,Cs,Nv,Vc Vb,	+++	-	-	-	-	+	-
Ejura 5	RU,Nc,Vc,M,Cs	-					-	-
Ejura 6a	St,Y,Nc,Cs,M	+++++	-	-	-	-	-	-
Ejura 6b		++	-	-	-	-	-	-
			-	-	-	+	-	-
			-	-	-	++	+	-

Table 4.4 Symptoms and viruses detected directly from the sweetpotato plants collected from the major growing areas Cont'd

4.6 Nucleic acid-based detection of viruses from grafted *I. setosa* and sweetpotato varieties collected from the major growing areas

Markers specific for SPFMV, SPCSV and degenerate primers for Begomovirus gave positive results to the presence of these viruses in the samples. However, primers specific for Sweetpotato Virus G (*SPVG*), Sweetpotato Caulimo-like Virus (*SPCaLV*) and Cucumber Mosaic Virus (*CMV)* gave negative (no amplification) results to the presence of these viruses in the samples. On the whole, 43 of the samples amplified at exactly 589bp for SPFMV, 56 of the samples amplified at 912bp for Begomovirus whereas 15 samples amplified at 486bp to establish the presence of the SPCSV. Plates 4.3 and 4.4 show results of Begomovirus SPG1/SPG2 at 912bp and SPFMV with SPF-F/SPFCG2-R at 589pb.

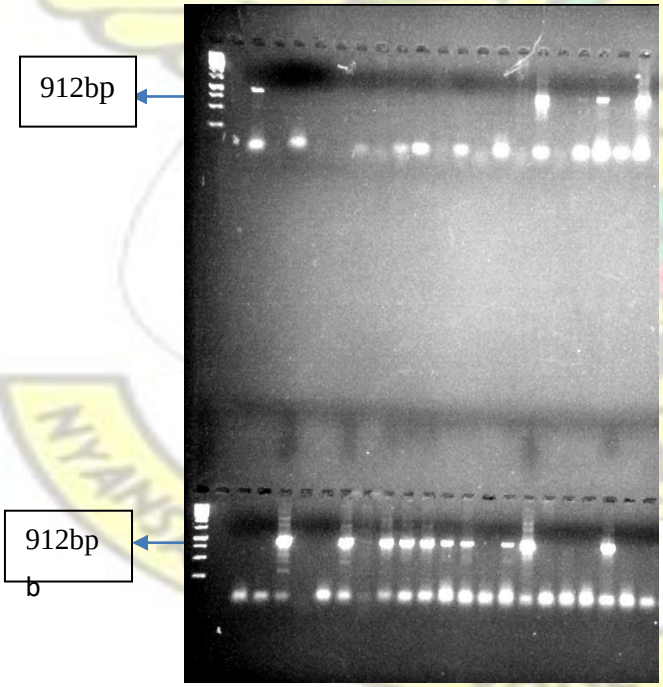

Plate 4.3. PCR, using degenerate primers SPG1/SPG2

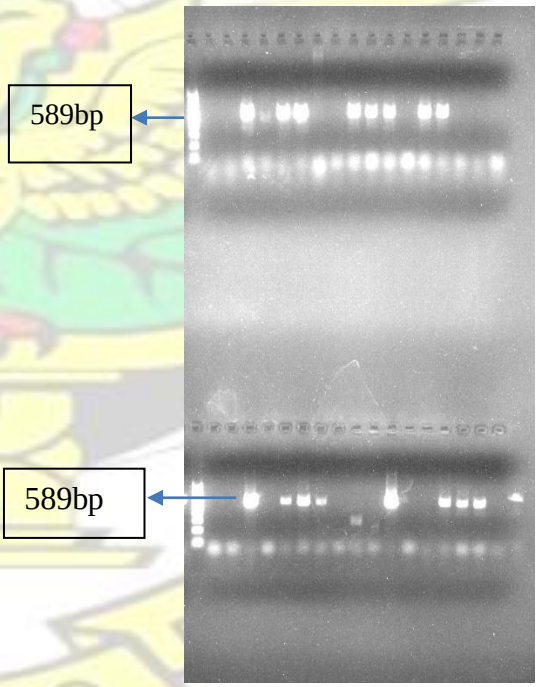

Plate 4.4. .RT-PCR, using specific primers SPF/SPFCG2-R

Markers specific for SPFMV, SPCSV and degenerate primers for Begomovirus gave positive results (+) to the presence of these viruses in the samples. Of the 127 samples that were tested, 62 gave positive results (+) for Begomoviruses, 43 gave positive results for SPFMV and 15 for SPCSV.

Table 4.5. Samples which amplified for SPFMV, SPCSV in the Reverse Trancriptase-PCR and Begomovirus in the PCR

Cultivar Number/ Locality	RT- PCR FMV	CSV	Begomovirus	Cultivar Number/Locality	RT-PCR FMV	CSV	Begomovirus
Eastern, Begoro		-		**Volta**			
Anago- 1	+		+				
				Kpeve			
				Ogyefo	-	-	-
Anago-1	-	-	+	Ogyefo,	-	-	-
Anago-2	-	-	-	Okumkom	-	-	-
Anago-2	-	-	-	Okumkom	-	-	+
				Santom Pona	-	-	+
Central				Ogyefo	-	-	-
Jukwa Abudu				Okumkom		-	-
Santom Yellow	-	-	-				
Komenda							
Santom Yellow	-	-	-	Santom Pona	-	-	+
Sauti	+	+	+	Hi-Starch	-	-	+
Santom Pona	-	-	+	Hi-Starch	-	-	-
Santom Pona	-	-	+	Apomuden	-	-	+
Ogyefo	+	-	+	Apomuden	-	-	+
Ogyefo	+	-	-	Sauti	+	-	+
Okumkom	+	-	+	Sauti	-	-	+
Okumkom	-	-	+	Xetrologo			
Apomuden	+	-	-	Trotroyeye	-	-	-
Apomuden	+	-	-	Trotroyeye	+	+	-
Hi- Starch	-	-	-	Agorve	-	-	-
				Gloglobo			
Hi- Starch	-	-	+	Gloglobo	+	-	-
				Shashango	+	+	-

Cultivar Number/ Locality	RT- PCR FMV	CSV	Begomovirus	Cultivar Number/Locality	RT-PCR FMV	CSV	Begomovirus
Volta				**Volta,**			
Ohawu				Davego			
				Shashango	+	-	-
				Trotroyeye	-	+	-
Okumkum	-	-	+				
Okumkum	-	-	+				
Santom Pona	-	-	+	Trotroyeye	-	-	-
Santom Pona	+	-	+	Kudzordzikope	-	-	-
				Gloglobo			
Ogyefo	+	-	+	Gloglobo	+	+	-
Ogyefo	-	-	+				
				Vume			
Hi- Starch	-	+	+				
Hi- Starch	+	-	+	Shashango	+	-	-
Apomuden	-	-	-	Shashango	+	+	-
Apomuden	+	-	-	Atipke			
				Shashango	+	+	+
Sauti	+	-	+	Shashango	+	+	-
Sauti	+	-	+				
Greater Accra							
Pokuase							
Sauti	+	-	+				
Ogyefo	+	-	+				
Hi-Starch	-	-	+				
Hi-Starch	-	-	-				
Apomuden	+	-	+				
Apomuden	+	-	-				
Sauti	+	+	+				
Santom Pona	+	-	+				
Okumkom	+	-	+				
Ogyefo	-	-	-				

Hi- Starch	-	-	-
Apomuden	-	-	-
Afiaman			
Amasaman			
Nagoli	-	-	-

Table 4.5. Samples which amplified for SPFMV, SPCSV in the Reverse Trancriptase-PCR and Begomovirus in the PCR. Cont'd

Table 4.5. Samples which amplified for SPFMV, SPCSV in the Reverse Trancriptase-PCR and Begomo virus in the PCR. Cont'd

Cultivar Number/ Locality	RT- PCR FMV	CSV	Begomovirus
Upper East			
Navorongo			
Navorongo local	-	-	+
Navorongo seed	+	-	-
Seed, Navorongo	-	-	-
Ogyefo (Navorongo)	-	-	-
Seedling (Navorongo)	-	-	-
Apomuden (Navorongo)			
Nabdam			
Nabdam 1	-	-	-
Nabdam 2, Nabdam	+	-	+
Bolga			
Bolga S1	+	-	+
Bolga S2	-	-	+
Ashanti			
Ejura A,Ejura	-	+	-
Ejura B,''	-	-	-
Ejura 1,''	-	-	+
Ejura 2,''	-	-	+
Ejura 3,''	-	-	-
Ejura 4,''	-	-	+
Ejura 5,''	-	-	+
Ejura 6a,''	+	-	+
Ejura 6b,''	-	-	+

Key + = very very light purple, ++= very light purple, +++= modest purple, ++++=deep purple, +++++= very deep purple. Samples that tested negative were denoted with a '-'sign

4.7 Viruses detected with NCM-ELISA, PCR and RT-PCR from samples planted in the field

Sweetpotato Feathery Mottle Virus (SPFMV) was detected as the virus most prevalent in the field where the sweetpotato samples were planted to assess the effect of viruses on yield. The development of a purple colour on nitrocellulose membrane confirmed virus positive samples and the intensity of the purple colour denote the concentration of the virus in the sample. Four samples gave positive reaction for the SPFMV with the NCM-ELSA. The PT-PCR for SPFMV amplified for three samples at 589bp. PCR results for Begomovirus with the same materials amplified for three samples at 912bp. RT-PCR for SPCSV gave no amplification for any of the samples. Table 4.6 shows viruses detected with NCM-ELISA, PCR and PTPCR from samples planted in the field.

4.6 Viruses detected with NCM-ELISA, PCR and RT-PCR from samples planted in the field

Sweetpotato Variety	Treatment	Viruses I			
		NCM-ELISA	RT-PCR/PCR		
		SPFMV	SPFMV (589bp)	Begomovirus (912bp)	SPCSV (486bp)
Bohye	Field Healthy	-	-	+	-
Ligri	Whitefly Inoculated	+++	+	-	-
Dadanuei	Field Infected	-	-	-	-
Ligri	Whitefly Inoculated	++++	-	-	-
Dadanuei	Tissue Culture	++++	-	-	-
Bohye	Field Infected	-	-	-	-
Dadanuei	Field Healthy	+++	+	+	-
Ligri	Field Infected	++++	+	+	-

Key: - = no purple colouration or absence of a band, + =presence of a band, +++ =modest purple, ++++ = deep purple

Table 4.7 Yield of tubers and foliage weight of sweetpotato varieties planted from tissue culture, field healthy, field infected and whitefly inoculated planting materials.

Sweetpotato Variety	Yield of tubers (t/ha)				Foliage weight (t/ha)			
	Tissue Culture	Field Healthy	Field Infected	Whitefly Inoculated	Tissue Culture	Field Healthy	Field Infected	Whitefly Inoculated
Ligri	12.00	4.89	3.89	1.00	8.56	8.78	8.00	3.11
Bohye	7.67	3.33	2.56	1.22	7.44	5.44	3.89	2.89
Dadanuei	6.89	6.22	1.11	0.78	13.33	11.11	2.11	3.67
LSD(P<0.05)			2.57				2.89	

4.8 Yield of tubers and foliage weight of sweetpotato varieties planted from tissue culture, field healthy, field infected and whitefly inoculated planting materials.

Results of yield of tubers and foliage weight of sweetpotato varieties planted on the field for the different sources of planting material is shown in Table 4.7. The differences between 'Ligri' produced from tissue culture, field healthy and field infected materials were significant (P<0.05) for tuber yield, with the whitefly inoculated vines producing the smallest yields. The differences between 'Bohye' produced from field-infected, field healthy and tissue culture materials were significant (P<0.05) for tuber yield, with that of whiteflyinoculated vines producing the smallest yields.

The differences between 'Dadanuei' produced from tissue culture and field healthy materials were significant (P<0.05) for tuber yield, with field-infected and whitefly-inoculated vines producing the smallest yields (Table 4.7)

The difference between 'Ligri' produced from tissue culture and field healthy source of planting materials were significant (P<0.05) for tuber yields. Further, field-infected and whitefly-inoculated vines for the same variety were significant (P<0.05) for tuber yields. The

difference between 'Bohye' produced from tissue culture and field heathy as well as fieldinfected and whitefly-inoculated materials were significant (P<0.05) for tuber yield. The difference between 'Dadanuei' from tissue culture, field healthy, field-infected and whiteflyinoculated vines were not significant (P>0.05) for tuber yield.

The difference between 'Ligri' produced from tissue culture planting material were significant (P<0.05) for tuber yield than 'Bohye' and 'Dadanuei' vines from the same treatment (tissue culture). The difference between tuber yields of field healthy planting vines were significant (P<0.05) across the three varieties. Tuber yields of field-infected source of planting vines were also significant (P<0.05) across the three varieties; with 'Dadanuei' producing the least yield of 0.78t/ha. Tuber yield of whitefly-inoculated vines were not significant (P>0.05) between the three varieties for tuber yield.

The difference between tuber yields produced form tissue culture and field healthy vines from the three varieties were significant (P<0.05) for 'Ligri' and Bohye' but not for 'Dadanuei'. 'Bohye and 'Dadanuei' from field-infected and whitefly-inoculated vines were not significant (P>0.05) compared to 'Ligri' which is significant (P<0.05).

Foliar weight of 'Ligri' produced from tissue culture, field-infected and field healthy vines were significant (P<0.05) compared with whitefly inoculated vines from the same variety (Table 4.7). Foliar weight of 'Bohye' produced from tissue culture vines were significant (P<0.05) for field-infected and whitefly-inoculated vines.

Foliar weight of 'Dadanuei' produced from tissue culture, field healthy, field infected and whitefly inoculated vines were significant (P<0.05). 'Ligri' and Bohye varieties were not significant (P>0.05) for foliar weight produced from tissue culture vines but that of Dadanuei' was significant (P<0.05). Foliar weight of field healthy vines for the three varieties were significant (P<0.05). Foliar weight of field-infected vines for the three varieties were significant (P<0.05) with Dadanuei producing the least foliar weight of 2.11t/ha.

For the whitefly-inoculated materials, 'Ligri, 'Bohye' and Dadanuei' were not significant different (P>0.05) (Table 4.7).

Plates 4.5. to 4.10 gave pictorial evidence of number and size of tubers of each of the varieties compared to their tissue culture as well as field -infected sources of planting material at harvest.

Plates 4.5. to 4.10 Tubers of each cultivar produced from tissue culture vines and field infected vines.

Plate 4.5Tubers of Bohye produced from tissue culture vines

Plate 4.6Tubers of Bohye produced from field infected material

Plate 4.7 Tubers of Ligri produced from field infected material

Plate 4.8 Tubers of Ligri produced from tissue culture vines

Plate 4.9 Tubers of Dadanuei produced from tissue culture vines

Plate 4.10 Tubers of Dadanuei produced from field infected material

CHAPTER FIVE

5.0 DISCUSSION

5.1 Mean Incidence and Severity of Viruses on Sweetpotato

Sweetpotato mean disease incidence and severity of virus infection for all the sources of planting materials across the varieties indicated that the tissue-cultured planting material was the most withstanding to viruses than all the other treatments. This advocates the effectiveness of tissue culture as a technique for virus cleaning in sweetpotato (Green and Lo (1989); CIP (1999); Wang and Valkonen (2008) ; Mashilo (2009); Feng *et al.*, (2011)). Mean incidence and severity of viruses in field-infected sources of planting material were highest across the varieties because vine cuttings from matured crops are used to plant new crops. Therefore, build-up and preservation of the virus increase (Valverde *et al.*, 2007).

Vegetative breeding of infected roots or vines offers a sound means of spreading viruses within the production cycle (Sivparsad, 2014). Among the varieties, 'Bohye' suffered the most viral infection, followed by 'Dadanuei' and 'Ligri'. This may be because dissimilar cultivars response or perform differently when subjected to different conditions or treatments (Rukarwa *et al.*, 2010).

5.2 Detection of sweetpotato viruses with NCM-ELISA

Sweetpotato Feathery Mottle Virus (SPFMV) was detected as the most frequent occurring virus in the study areas. This is in conformity with reports that SPFMV is the most common virus of sweetpotato found everywhere sweetpotato is grown (Campbell *et al.*, 1974; Ndunguru and Kapinga, 2007). Nevertheless, sweetpotato varieties infected with SPFMV showed no or only mild symptoms of mosaic, chlorotic spots, vein clearing, yellowing and net veination. This agrees with the findings of Gibson *et al.(*1997), Gibson *et al.(*1998a) and Clark *et al.*(2012) that lone infection of SPFMV causes no or mild symptoms in most sweetpotato cultivars.

Sweetpotato Chlorotic Stunt Virus (SPCSV) showed a low occurrence in the study areas, and corroborates the report by Sossah *et al.* (2015) who observed that SPCSV has a low occurrence in Ghana. However, Carey *et al.* (1999) and Mukasa *et al.* (2003) reported that SPCSV is prevalent mostly in East African countries.

The low incidence of SPCSV maybe because the West African serotype of SPCSV may not be common in Ghana (Carey *et al.,* 1999) or that its vector (whitefly) is not effective in the dissemination of the virus (Sossah *et al.,* 2015).

The other viruses, SPCaLV, SPVG, SPMSV, CMV and SPCFV that were detected with the NCM-ELISA, were similar to those reported by Sossah *et al.* (2015) who, reported of the presence of these viruses: SPCaLV, SPCFV and CMV in Ghana on sweetpotato. SPCaLV was detected in 6.4% of the materials together with SPFMV which is consistent with reports by Gao *et al.* (2000) and Uganda WISARD Project Information (1999). SPCFV and CMV were detected at lower occurrence as mixed infections. Whitefly transmitting SPCSV easily infect sweetpotato plants in mix infections with CMV, according to Cohen and Loebestein, (1991) and Loebenstein (2009).

SPVG and Sweetpotato Mild Speckling Virus (SPMSV) had the same incidences of 4.8%. These two potyviruses normally occur in mixed infections; SPVG acts as helper virus in coinfections with SPFMV, making it difficult to differentiate the symptoms triggered by the two viruses (Clark *et al.,* 2012). Mixed infections of SPFMV + SPCSV, SPFMV + SPVG, SPFMV + SPCaLV, SPFMV + SPMSV, SPFMV + SPCFV, SPFMV + CMV have also been reported (Domola *et al.,* 2008) which specify the multiple/mixture infections of viruses in sweetpotato. The high incidence of dual infection by SPFMV and SPCSV agrees with earlier findings by Mukasa *et al.* (2003); Ateka *et al,* (2004); Tairo *et al.* (2004); Miano *et al.* (2006)

and Nyaboga *et al.* (2008) that co-infection of SPFMV and SPCSV causes the most severe virus disease of sweetpotato.

SPVD reduces yield in Africa by 50% (Winter *et al.,* 1992; Gibson *et al.,* 1998b). Again, there were mixed infections of SPFMV+ SPCSV+ SPVG, SPFMV+ SPCaLV + CMV and SPFMV+ SPCFV+ SPMSV, indicating the complex nature of sweetpotato viral infections. The more complex the viral infection, the more severe the symptom on the host (Di Feo *et al.,* 2000).

Mixed infection of SPFMV + SPCSV + SPVG + SPCaLV resulted in more severe symptoms of chlorosis, mosaic, net veination, yellowing and stunting whereas multiple infection of SPFMV + SPCSV + SPVG + SPCaLV + CMV gave most severe symptoms of yellowing, stunting, roll-up, chlorosis, mosaic, vein clearing and vein banding. This indicates the difficulty in cleaning sweetpotato from viruses if all these viruses can infect one sample. Yet, a combination of meristem tip culture with cryopreservation and thermotherapy really increase the proficiency of virus eradication in sweetpotato (Wang and Valkonen (2008); Mashilo (2009) and Feng *et al., (*2011).

The other viruses were not detected for the antiserum specific for *Sweetpotato latent virus* (SPLV), *Sweet potato C6 virus* (SPC6V*)* and *Sweetpotato mild mottle virus* (SPMMV). It is reported that viruses are unevenly spread in infected sweetpotato and exist in very low amount that they might not be consistently detected and that there is a requisite to collect tissues from different parts of a plant for ELISA (Green and Lo, 1989). As representative samples were taken from all parts of the plants, failure to detect these viruses suggests that either their concentration was too low for ELISA detection or they were not present at all in all the samples tested. However, the lack of detection of sweetpotato virus infection by ELISA and even molecular methods such as RT-PCR is common (Souto *et al.,* 2003). Studies by Mukasa *et al.*

(2003) and Ateka *et al.* (2004) also indicated that these viruses were rare in farmers' fields in East Africa.

Most of the local varieties had mixed infection of two or more viruses, while almost all the improved varieties had a single virus. Sweetpotato is vegetatively propagated and accumulation of viruses in the planting stock can be a problem (Valverde *et al.* (2007); Clark *et al,* (2012). In Ghana, most of the improved varieties are developed to be tolerant to viruses. The single virus detected in these samples, perhaps, suggests the effectiveness of screening these varieties for virus infection before their release to farmers.

5.3 Nucleic Acid Based Detection of Viruses

The number of samples that amplified for SPFMV and SPCSV in the RT-PCR was smaller than the number of samples that gave positive result for the same viruses in NCM-ELISA. This was probably because sweetpotato viruses spread higher in concentrations in *I. setosa* than in sweetpotato plants (Kreuze and Fuentes, 2008) hence the less amplification. Also, sweetpotato tissues have polysaccharides and inhibitory components which interfere in PCR reactions (Clark *et al.*, 2012).

Sixty two (62) of the samples amplified at 912bp for Begomovirus (usually symptomless), suggesting that plants with no observable symptom may be infected with one or more viruses, signifying latent infection (Adane, 2010) hence, is large number of samples that amplified. Nevertheless, primers specific for Sweetpotato Virus G (*SPVG*), Sweetpotato Caulimo-like Virus (*SPCaLV*) and Cucumber Mosaic Virus (*CMV)* gave negative (no amplification) results to the presence of these viruses in the samples, even though they gave positive results with the NCM-ELISA. This can be attributed to antibodies used in NCM-ELISA which were polyclonal and they could have had cross reaction with closely related viruses (Atu, 2014).

This could be the case of the antiserum of SPFMV with SPVG and vice versa. Comparing the NCM-ELISA and RT-PCR result tables, the mild positive reaction of SPVG could be the cross

reaction with SPFMV. Ordinarily, a positive reaction in NCM-ELISA, must give positive reaction in RT-PCR, unless the reaction in the NCM-ELISA is to *Sweetpotato virus C* (*S*PVC*)* and not SPFMV (Souto *et al.,* 2003).

Initially, SPVC was considered as a strain of SPFMV (as SPFMV-C) some time ago and that SPFMV and SPVC are normally found together in some places (Untiveros *et al.,*2007); Souto *et al.,* (2003). In addition, symptoms recorded on the grafted *I. setosa* for SPFMV single-infected plants were mainly chlorotic spots and net venation, whereas the symptoms on mixture of SPFMV and SPVG were net venation, vein clearing, necrosis and yellowing, similar to symptoms expressed by infection of SPFMV alone. This indicates the probability that the mild SPVG detected by the NCM-ELISA is a cross reaction of the SPVG antiserum with SPFMV. Thus, subsequent testing is needed to resolve discrepancies between assays and confirm positive results (Tairo *et al.,* 2006). Similarly, CMV and SPCaLV not amplifying could be the presence of polysaccharides and inhibitor components in the sweetpotato plants that interfered with PCR reactions (Clark *et al.,*2012).

5.4 Evaluation of Viruses Detected from samples planted on the field

Sweetpotato feathery mottle virus (SPFMV) was the most prevalent virus detected in the field where the sweetpotato varieties were grown. This further confirms that SPFMV is the most common virus occurring wherever sweetpotato is grown (Loebenstein, 2009). In addition, previous survey and NCM-ELISA test results conducted on CSIR-CRI sweetpotato research field specified the presence of SPFMV, SPCSV and CMV as the viruses existing in the field (Dr. Allen Oppong, personal communication).

However, this study did not detect SPCSV or CMV with NCM-ELISA or RT-PCR from any of the samples planted on the field.. Perhaps, this could be as a result of low concentration/titre of the SPCSV virus in these samples (Gibson and Aritua, 2002).

5.5 Assessment of yield reduction due to SPVD

Among the different sources of planting material, tissue culture performed better in yield than all the other sources of planting material. The smallest yield was from the whitefly inoculated source of planting material. Tissue culture source of planting material producing the highest yield is expected. This is because tissue culture sources of planting materials were certified virus-free and they very vigorous right from initiation in the field. The vigour was maintained throughout the growing period of the experiment which culminated in the yield figures obtained in this study. Similar findings have been reported by Amankwah (2012).

The least yield produced by the whitefly-infected source of planting material was not expected. It was anticipated that possibly, the field infected source of material was going to produce the least yields. The possible reason that can be assigned to this observation is that probably, the tissue culture materials that were artificially inoculated suffered a setback, resulting from shock arising from the insect attack and the subsequent transplanting from the screenhouse to the field which might have affected crop development. All the three varieties were somewhat affected for both the tuber and foliar weights.

Again, it is also reported that initial stages of crop establishment is very critical for storage roots (http://www.ncsweetpotatoes.com) hence, disease pressure starting from this seedling stage of the crop could have adversely affected the yield obtained for the whitefly-inoculated source of planting material. The field-infected planting materials could not give the least yield could be due to the ability of some of the field infected materials might have recovered from the virus attack under natural field infection. This recovery is a natural phenomenon that can be attributed to resistance. Results from the NCM-ELISA and PT-PCR indicated that the samples (field infected materials) planted on the field were attacked by SPFMV lone infections and recovery has once been reported for single infections of SPFMV and SPCSV (Karyeija *et al.*,2000; Mwanga *et al.*,2002 and Mukasa *et al.*,2006). Also, as severity of virus symptoms

increased, yield decreased considerably (Amankwah, 2012). This is evident for yield as virus severity score for tissue culture source of planting material was 1 (healthy) whereas that of whitefly inoculated source of planting material was higher (2.54).

However, foliar weight was greatest for field healthy planting material with whitefly-inoculated source of planting material having the smallest. The source of planting material used have been shown to affect yield in a study of sweetpotato vines (Tewe *et al.*, 2003). One way to conserve good yields is that farmers must seek and use virus-free material and this material must be checked for virus build-up every two or three years (Laurie *et al.* (1999).

Nevertheless, reduced manifestation of the symptoms of virus on sweetpotato plants in the field highlights the need to breed for virus-free/tolerant cultivars as the solution for improved productivity of sweetpotato in the country.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

This study has detected seven different viruses in the important sweetpotato growing ecologies in Ghana. Using NCM-ELISA, SPFMV, SPCSV, SPVG, SPMSV, SPCaLV, SPCFV and CMV were detected from these areas. SPFMV was the most prevalent. Mixed infections were also detected, especially in the local varieties. PCR and RT-PCR also detected Begomoviruses, SPFMV and SPCSV in some of the samples. Yield reductions due to virus infection from four different sources of planting material have been assessed. Tissue culture virus indexed clean materials was the best source of planting material for sweetpotato propagation and gave greatest root yield.

6.2 RECOMMENDATIONS

It is recommended that farmers should be encouraged to use tissue cultured and virus-cleaned planting materials for propagation to obtain utmost yield. Breeders should develop and release only improved virus-free varieties because the study showed that levels of viral infection in the released varieties were low, compared to the local landraces. NCM-ELISA was able to detect most of the sweetpotato viruses, compared with that RT-PCR indicating that RT-PCR technique should be tried again. Also, the field work should be repeated to establish the authenticity of the work.

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