SWEET POTATO VIRUSES IN UGANDA:

IDENTIFICATION OF A NEW VIRUS, A MILD STRAIN OF AN OLD VIRUS AND REVERSION

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DECLARATION

I certify that this work has not been accepted in substance for any degree, and is not concurrently submitted for any degree other than that of Doctor of Philosophy (PhD) of the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised the work of others.

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ABSTRACT

In 2009, a sweet potato begomovirus (sweepovirus) was detected for the first time in Uganda. An isolate was sequenced, providing the first full sequence of a sweepovirus from mainland Africa which differed from other sweepoviruses by at least 13%, discriminating this isolate as a new species, 'Sweet potato leaf curl Uganda virus' (SPLCUV). SPLCUV was quite common in cultivars (cvs) Ejumula, New Kawogo and 318L having uneven distribution in infected plants and reversion to healthy occurred, especially in cv New Kawogo. SPLCUV was observed not to be synergised by *Sweet potato chlorotic stunt virus* (SPCSV), apparently making it the first report of a sweet potato virus not synergised by SPCSV. Besides SPLCUV, a 'mild' SPCSV strain that induced purpling symptoms and 50% yield reduction similar to wild type SPCSV when infecting alone was identified from Busia district, Uganda. 'Mild' SPCSV was never observed to be co-infected with *Sweet potato feathery mottle virus* (SPFMV) in farmers' fields. Experimentally, 'mild' SPCSV induced mild symptoms in *Ipomoea setosa* and sweet potato plants and SPFMV titre was greater in co-infections of SPFMV and wild type SPCSV than in co-infections of SPFMV and 'mild' SPCSV. Both RNase3 (accession No. HE575406) and p22 (accession No. HE575409) genes on RNA1 of 'mild' SPCSV compared closely to those reported previously. Instead, RNA1 region appears to be expressed less in the 'mild' SPCSV infection than in the wild type SPCSV infection though RNA2 continues to be more expressed in the 'mild' SPCSV infection than in the wild type SPCSV infection. Recovery from SPVD symptoms and reversion from SPFMV were observed in cv Kampala White co-infected with 'mild' SPCSV and SPFMV. Reversion from SPFMV single infections occured in several landraces with higher rates observed in shoots of resistant than susceptible cultivars. Overall, cv NASPOT 11 was the fastest to revert while cv Beauregard was the slowest.

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CHAPTER ONE

INTRODUCTION

1.1 Morphology, origin and distribution of sweet potato

Though grown as an annual crop, sweet potato (*Ipomoea batatas* L.) is a dicotyledonous perennial plant. It has enlarged starch-filled edible storage roots which are long and tapered, with a variety of flesh and skin colours ranging from white to orange and purple, and from white to pink, red, purple and brown respectively. The plant has alternate heart-shaped or palmately lobed leaves. It belongs to the Morning Glory family, the *Convolvulaceae* (Austin, 1988) that comprises plants with funnel-shaped flowers. *I. batatas* is the only food crop out of the approximately 500 species in this family (Watson and Dallwitz, 1991; 1994; Onwueme and Charles, 1994). It has a high genetic diversity with 4x and, more commonly, 6x ploidy forms (2n = 4x = 60 or 2n = 6x = 90). The wild species may be economically important in breeding for natural resistance to pests, diseases and climatic conditions. However, many of these species are bitter and some are actually poisonous (Onwueme and Charles, 1994; Huang and Sun, 2000).

Sweet potato was perhaps among the first crops that were domesticated and it is speculated to have originated between Central and northern South America (Huang and Sun, 2000) more than 5,000 years ago. The south west Pacific islands are probably a secondary centre of sweet potato diversity (Zhang *et al.,* 2004). The crop is now grown the World over in tropical, subtropical and warm temperate regions between 40°N and 32°S latitude and at elevations up to 2,500 m (He *et al.,* 1995). It is believed that Columbus introduced the crop to Western Europe around 1492. Shortly thereafter, Portuguese explorers took sweet potato to India, South East Asia, East Indies and

Africa (Austin, 1988; Zhang *et al.,* 2004; Srisuwan *et al.,* 2006). The crop was introduced to East Africa during the 16th century by slave traders (Woolfe, 1992; Allemann *et al.,* 2004). Sweet potato may have reached Uganda along trade routes from both east and west. Since then, its cultivation here has greatly increased and the crop is now widely grown in all regions of the country.

1.2 Importance of sweet potato

1.2.1 Importance of sweet potato globally

Worldwide, expressed on a dry matter basis, sweet potato is the sixth most important food crop after rice, wheat, potatoes, maize and cassava. It ranks fifth in developing countries and is the second most important root crop after potato (CIP, 1999a). Its flexibility in farming and food systems, ability to do well in harsh as well as good environments and relative resistance/tolerance to pests and diseases make sweet potato an important crop (Woolfe, 1992). More than 95% of the total 105 million metric tonnes of sweet potato produced in the World each year comes from developing countries (Scott *et al.,* 1999; Hijmans *et al.,* 2001; FAO, 2007). Asia is the leading producer with over 90 million tonnes produced annually, 85 million tonnes being produced by China. Africa is the second largest producer (Table 1). In Africa, the crop is an important food crop in much of the sub-Saharan region and is grown on about 2.1 x 10^6 ha with an estimated production of 9.9 x 10^6 t (FAO, 2008). Sweet potato is especially important in countries in the Great Lakes region of East Africa, Uganda having the largest production (FAO, 2007; 2008).

Table 1. World sweet potato production (percentages in parentheses) citing the major sweet potato producing countries

	Production in million metric tonnes				
	1982	1992	2002	2005	2009
China	110.63 (85.1%)	105.75 (84.6%)	120.84 (85.1%)	(83%)	80.5 (75.6%)
Nigeria	*	*	2.84 (2%)	(2%)	3.3 (3.1%)
Uganda	1.43 (1.1%)	1.875 (1.5%)	2.84 (2%)	(2%)	2.7 (2.5%)
Vietnam	2.34 (1.8%)	2.5 (2%)	1.42 (1%)	*	1.32 (1.2%)
Indonesia	1.69 (1.3%)	2.25 (1.8%)	1.42 (1%)	*	1.88 (1.8%)
Others	16.51 (12.7%)	13.88 (11.1%)	12.64 (8.9%)	(13%)	16.8 (15.8%)
World total production	130	125	142	*	106.5

* production data is not given

Source: FAO (2009)

Sweet potato yields two to four times more food per unit area per unit time than grain crops. It plays a central role in the food security for millions of people across South America, Africa and Asia and has had a long historical importance in disaster relief; the Japanese used it when hurricanes demolished their rice fields, it saved lives of Chinese when they were hit by famine in the early 1960s, and the crop saved rural Ugandans from hunger when *East African cassava mosaic virus* (EACMV) epidemics hit the country in the 1990s (CIP, 2010).

In East Africa, sweet potato is grown predominantly by the poorest people especially women, for both home consumption and to supplement household income by sale to

local markets and urban centres; any effort towards improving the crop will therefore have a huge potential, especially for the poor African woman. Improving sweet potato is quite possible because of its natural biodiversity and high nutritive value. The International Potato Center (CIP) has a fundamental role in its research in developing countries, especially by providing local scientists as well as resource poor farmers with technologies and breeding material that better fit local conditions.

The energy density, carbohydrates and micronutrients of sweet potato are comparable to other starchy staples (Table 2). Sweet potato has as well got some therapeutic values. The crop is an excellent source of vitamin A [in the form of beta-carotene in orange fleshed sweet potato (OFSP)], important in sight and fighting disease, and vitamin C having healing properties as an antioxidant food. A few varieties have high levels of antioxidant anthocyanin which can also substitute for synthetic colouring agents (Wallerstein, 2000; Bovell-Benjamin, 2007). Sweet potato is the richest low-fat source of vitamin E contributing to heart health, with one-fourth the calories of bread. The crop also has considerable amounts of iron, potassium, zinc and essential trace elements such as manganese, chromium, selenium and molybdenum (Bovell-Benjamin, 2007). Sweet potato roots are usually boiled and eaten for a meal with other foods especially ones rich in protein such as beans, vegetables, peas and meat (CIP, 1999b). Sweet potato can be processed into animal feed, starch, flour, candy and alcohol. The flour is used to make mandazis, chapatis, cakes, doughnuts, biscuits, etc.

Table 2. Nutritional values for sweet potato (per 100g raw edible portion) as compared to some other staples

	Sweet potato	Banana	Cassava	Potato
Vitamin C (mg)	22.7	9.1	20.6	19.7
Calcium (mg)	22.0	6.0	16.0	7.0
Energy (kcal)	105.0	92.0	160.0	79.0
Water (g)	72.8	74.3	59.7	79.0
Protein (g)	1.7	1.03	1.4	2.1
Carbohydrate (g)	24.3*	23.4	38.1	18.0
Iron (mg)	0.6	0.3	0.3	0.8
Potassium (mg)	204.0	396.0	271.0	543.0

* is for low dry matter American varieties

Source: INIBAP (1999)

1.2.2 Importance of sweet potato in Uganda

Of all crops grown in Uganda, sweet potato is the most widely cultivated (Mwanga and Wanyera, 1988). The country produces 2 million tonnes of the crop each year, making it the third largest producer in the World after China and Nigeria (Table 1). Sweet potato is mainly for local consumption; Uganda is fifth in *per capita* production and consumption in the World after the Solomon Islands, Tonga, Rwanda and Papua New Guinea. Sweet potato is normally cheaper than cereal-based foods, especially for the low income people and, chipped and dried, is especially important during the dry season (Hall *et al.,* 1998). It is an important staple crop throughout the country and it ranks second to banana in western and central regions and second to cassava in eastern Uganda (Bashaasha *et al.,* 1995; Mwanga and Wanyera, 1988; FAO, 2008).

Sweet potato has a short growing period and its storage roots can be left in the ground after maturity but, once harvested, they have a short storage life. These attributes make the crop suitable for piece meal harvesting for subsistence families (Woolfe, 1992), although weevils limit its in-ground storage. Sweet potato is gaining importance in Uganda because of the loss of cassava due to cassava mosaic disease (CMD) and cassava brown streak disease (CBSD).

1.3 Sweet potato cropping systems and management in Uganda

Sweet potato is grown in all regions of Uganda; the drier regions of the eastern and northern parts of the country have the greatest production (FAO, 2008). Everywhere, the crop is grown mainly as a monocrop by resource poor farmers, especially women, on small plots of less than 0.5 ha (Bashaasha *et al*., 1995; Carey *et al*., 1998; Ebregt *et al*., 2004b; Abidin, 2004; Abidin *et al*., 2005).

Uganda experiences two types of rainy seasons. Some parts of northern Uganda get one long rainy season a year (so-called unimodal) while the other parts of eastern, and the whole of southern and western parts of the country get two, more-or-less evenly spaced but relatively short, rainy seasons a year (bimodal) (Basalirwa, 1995; Rugumayo *et al*., 2003). Regardless of the nature of seasons, sweet potato is mainly planted at the beginning of rains. Crop harvesting starts during the latter part of the rainy season and continues through at least to the start of the dry season (Bashaasha *et al*., 1995). The main management practices in sweet potato include seed bed preparation just before the onset of rains, making mounds of approximately 1 metre in diameter or ridges, selecting healthy looking cuttings for planting and weeding at least once during the growing period. It is not a common practice in Uganda to apply fertiliser or pestcides.

Intercropping is not recommended because of the growth habit of the plant which completely covers the ground; farmers normally grow different cultivars separately, although often in the same plot. However, in some highly populated areas, such as Kabale in western Uganda, intercropping with plants such as beans (when sweet potato crops are still young), cassava and banana is inevitable because of the high population pressure (Bashaasha *et al*., 1995). Crop rotation is quite common and crops mainly involved include sorghum, millet, maize, beans, sesame and groundnut (Ebregt *et al.*, 2004a).

1.4 Constraints to sweet potato production

Despite the many advantages offered by the cultivation of sweet potato, production is mostly in developing countries and by resource poor farmers (FAO, 2007). Sweet potato production has declined over the past two decades; in many developing countries, any increase in production is attributable to an increase in area under cultivation *per se* rather than yield increase per unit area (FAO, 2008). This low productivity is a result of many production constraints. Abiotic constraints such as inadequate soil fertility (or lack of fertilizers added) and lack of irrigation are probably the most important. The most important factors that severely limit sweet potato yields worldwide about which something can be done cheaply and/or quickly, however, include use of inferior low yielding cultivars, poor cultural practices and poor methods of utilization of the crop including post-harvest processing, pests and diseases. Diseases caused by fungi, bacteria, nematodes, mycoplasma and viruses have been described to affect sweet potato production (Clark and Moyer, 1988).

The most important pest limiting sweet potato production in Uganda and worldwide is the sweet potato weevil, *Cylas* spp. (Chalfant *et al*., 1990; Bashaasha *et al.*, 1995;

Nottingham and Kays, 2002). There are three species of sweet potato weevil; *Cylas puncticollis* and *C. brunneus* are found in Africa and *C. formicarius* is found in the United States, Asia and the Carribean. The weevil causes most damage when it attacks the storage roots but it can survive on sweet potato foliage and crop debris thereby potentially damaging the crop throughout the year. Under optimal conditions for the weevil, total yield loss can occur mainly through the tunnelling effect caused in the storage roots and stems (Sutherland, 1986; Chalfant *et al.*, 1990). Sweet potato weevils also render the storage roots unfit for consumption as the affected roots become bitter, often coupled with a bad smell (Akazawa *et al.*, 1960; Uritani *et al.*, 1975; Sato *et al*., 1981).

Apart from the low potential yield of the varieties grown and weevils, viruses pose the next most important biotic constraint (Clark and Moyer, 1988). More than 35 sweet potato viruses are known worldwide (Table 3 and Appendix 1) of which 12 are recognised by the International Committee of Taxonomy of Viruses (ICTV) (Sheffield, 1957; Winter *et al*., 1992; Gibson *et al*., 1998; Lotrakul *et al*., 1998; Kreuze, 2002; Clark and Hoy, 2007; Clark *et al*., unpublished). In recent surveys of the Ugandan crop for viruses (Carey *et al*., 1998; Mukasa *et al*., 2003; Aritua *et al*., 2007), *Sweet potato feathery mottle virus* (SPFMV: *Potyvirus*; *Potyviridae*), *Sweet potato chlorotic stunt virus* (SPCSV: *Crinivirus*; *Closteroviridae*), *Sweet potato mild mottle virus* (SPMMV: *Ipomovirus; Potyviridae*), *Sweet potato chlorotic fleck virus* (SPCFV: *Carlavirus*; *Flexiviridae*) and *Sweet potato caulimo-like virus* (SPCaLV: *Caulimovirus*, *Caulimoviridae*), in decreasing order of prevalence, have been identified. Sweet potato leaf curl Uganda virus (SPLCUV: *Begomovirus*; *Geminiviridae*) has also been identified there and the limited survey data suggest it may come after SPFMV and SPCSV in importance (Wasswa *et al*., 2011).

1.5 Problem statement

Sweet potato viruses are next to weevils in economic importance (Clark and Moyer, 1988). The most important disease is the sweet potato virus disease (SPVD) due to the synergistic effect of SPFMV and SPCSV. SPVD can reduce root yield of affected plants by up to 98% and the leaf area is greatly reduced (Schaefers and Terry, 1976; Ngeve, 1990; Ngeve and Bouwkamp, 1991; Gibson *et al*., 1998; Karyeija *et al*., 2000; Gutierrez *et al*., 2003). SPCSV also synergises other viruses; the synergistic effect of SPCSV and SPFMV is less common but results in sweet potato severe mosaic disease (SPSMD) and can reduce yield by 80% (Mukasa *et al*., 2006). The yield effect of other disease complexes involving SPCSV such as SPCSV and *Sweet potato mild speckling virus* (*Potyviridae, Potyvirus*), and SPCSV and Cucumber mosaic virus (*Bromoviridae, Cucomovirus*) is not yet known.

Sweet potato single virus infections can also have significant effects on yield. SPFMV, the most prevalent virus in Uganda reduces yields of infected plants by 50% with smoother and less mature storage roots but induces no or only mild leaf symptoms in infected plants (Gibson *et al*., 1997; Njeru *et al*., 2004). Some strains of SPFMV can also lead to root cracking (Campbell *et al*., 1974; Usugi *et al*., 1994) and internal corkiness (Milgram *et al*., 1996; Karyeija *et al*., 1998a). SPCSV the second in prevalence also reduces yields by 50% (Gibson *et al*., 1998; Mukasa *et al*., 2006) and stunts the infected plants (Schaefers and Terry, 1976; Gibson *et al*., 1998). *Sweet potato leaf curl virus* (SPLCV) has been reported to reduce yields of infected cv Beauregard by 26% and results in dark skin and grooving of storage roots (Clark and Hoy, 2006) which become unusable and unsaleable.

A number of strategies including use of resistant varieties, phytosanitation and chemical control of the vectors have been used to control sweet potato viral diseases. In Uganda, several cultivars such as NASPOT series have been bred and selected for yield, quality and resistance to single virus infections but resistance breaks down upon co-infection of SPFMV and SPCSV. On the other hand, there is no commercially available chemical treatment to cure diseases of viral origin (Bock, 1994) yet, chemical control of vectors is an expensive approach to many resource poor farmers in developing countries like Uganda. Also, a number of insecticides have effectively controlled plant pests in the past but resistance has developed rapidly and notably, *Bemisia tabaci* (a vector for SPCSV, SPLCUV, SPMMV and many other plant viruses) has developed resistance to organophosphorus insecticides (Prabhaker *et al*., 1985; Toscano *et al*., 1997). *Aphis gossypi* (a vector for many other plant viruses including SPFMV) has developed resistance to pyrethroids (Ahmad *et al.*, 2003). The use of *in vitro* raised virus free material is also very expensive and not feasible for the case of Uganda. There was therefore, a need for alternative approaches for management of sweet potato viruses.

1.6 Justification of the study

Vegetative propagation should result in build up of viruses from generation to generation (Okpul *et al*., 2011). However, apparently because of the natural resistance mechanisms causing recovery and reversion, virus incidence appears generally not to reach 100% in sweet potato fields planted with local varieties despite the abundance of virus vectors and lack of symptoms in most of infected plants preventing positive selection. This scenario apparently relies on reversion to ensure a proportion of virus free plants at all times and, in the absence of immune cultivars, the benefits of selecting

symptomless (which are often virus free; Gibson *et al*., 1997) cuttings in the control of virus diseases cannot be overlooked (Clark *et al*., 2003; Basilio *et al*., 2005).

There are a few reports on recovery or reversion from SPFMV in sweet potato landraces in Uganda (Gibson *et al*., 1997; Aritua *et al*., 1998b). Aritua *et al*. (1998b) observed reversion from SPFMV infection in the landraces New Kawogo and Tanzania. Cultivar New Kawogo is very resistant and cv Tanzania is moderately resistant to SPFMV (Mwanga *et al*., 1995). In view of the apparent importance of this mechanism, my research study examined reversion in a range of resistant and susceptible Ugandan sweet potato landraces and improved varieties infected with SPFMV and tested if a low SPFMV titre is common to cultivars that revert.

Previous research has paid particular attention to SPFMV and SPCSV mainly because of the ubiquitous nature of these viruses (Cohen *et al*., 1992; Winter *et al*., 1992; Hoyer *et al*., 1996; Alicai *et al*., 1999b) and their roles in (SPVD) development (Hanh, 1979; Ngeve, 1990; Gibson *et al*., 1998; Karyeija *et al*., 2000; Gutierrez *et al*., 2003). However, many sweet potato viruses which apparently have limited distribution, and totally new viruses may be presesnt in Uganda. This is indirectly indicated by yield degeneration (Clark and Valverde, 2001; Nakazawa, 2001) rumoured to occur in Ugandan landraces such as New Kawogo resistant to known viruses. Also, Mukasa *et al*. (2003) found out that 11% of sweet potato plants in Uganda displaying virus like symptoms failed to react to the antisera used. Two symptomless plants of sweet potato cv New Kawogo from a garden in Kampala, central Uganda, were established in a quarantine greenhouse at NRI, UK. Scions were side-grafted to *I. setosa* seedlings which surprisingly developed leaf curling symptoms, symptoms unlike those of known

viruses in Uganda. This suggested that an unknown virus was present and its aetiology needed to be confirmed.

Similarly symptomless sweet potato cultivars were collected from various districts of Uganda in November 2008 with the intention of having virus free material. These were maintained in a screenhouse at the Makerere University Agricultural Research Institute at Kabanyolo (MUARIK), near Kampala. These plants were tested for viruses using NCM-ELISA, and one plant of cv Kampala White from a field in Busia had SPCSV by itself, causing only mild chlorosis symptoms in *I. setosa*. This is unusual because most plants infected with SPCSV quicky become infected also with SPFMV (Gibson *et al*., 1998). This strain of SPCSV seems therefore not to, or only weakly, synergise SPFMV and there was therefore a need to closely investigate this phenomenon. The overall objective of this study was therefore to identify new viruses and virus strains in sweet potato landraces in Uganda, their involvement in synergism and the implication of SPFMV titre on the phenomenon of reversion.

1.6.1 Objectives of the study

The specific objectives were;

i. To characterise the leaf curling virus and determine its prevalence in Ugandan sweet potato landraces
ii. To understand how the SPCSV isolate in Busia was not co-infected with SPFMV
iii. To confirm that sweet potato landraces do revert from SPFMV and whether titre of the virus or some other factor determines whether plants of different varieties revert

Chapter 2 reviews literature related to this study and expands on the knowledge gaps identified that required further study. Chapter 3 describes the identification and characterization of a new sweet potato virus in Uganda. Chapter 4 examines the synergistic effect of the 'mild' SPCSV as compared to the wild type SPCSV in plants co-infected with SPFMV and seeks a mechanism for the differences observed. Chapter 5 examines the phenomenon of reversion in Ugandan sweet potato landraces with varying levels of resistance and if virus titre is an indicator of cultivars that revert from SPFMV. Chapter 6 provides a general discussion, conclusion and recommendations based on the research described in chapters 3 to 5 and previous literature.

CHAPTER TWO

LITERATURE REVIEW

2.1 Viruses of sweet potato in the World

Sweet potato viruses were first reported in the U.S.A and East Africa in the 1930s and many occur wherever the crop is grown (Moyer and Salazar, 1989). Initially, bioassays including host range, symptoms and vector transmission were the major means of characterising them (Moyer and Salazar, 1989). With advances in microscopy, serology and molecular techniques, virus identification and characterisation have greatly improved (Torrance and Jones, 1981; Torrance, 2005) and many virus and virus-like particles have been identified (Moyer and Salazar, 1989; Onuki and Hanada, 1998; Loebenstein *et al*., 2003). There are at least 35 putative virus species currently known to infect sweet potato in the World but only 12 of these are recognised by ICTV (Table 3). Apart from SPCSV and SPFMV, which are ubiquitous (Hollings *et al*., 1976; Cohen *et al*., 1992; Winter *et al*., 1992; Hoyer *et al*., 1996; Colinet *et al*., 1996; Alicai *et al*., 1999b), most of the viruses are restricted to some regions or have not yet been identified elsewhere (Table 3 and Appendix 1). However, there is a likelihood of identifying more sweet potato viruses given the widespread presence of vectors for viruses (Gerling, 1990; Blackman and Eastop, 2000) and the movement of plant material that may be infected with vectors and/or viruses. The phytosanitary restrictions on the movement of sweet potato material between and within regions should be strictly followed to prevent the rapid spread of sweet potato viruses and virus strains.

Table 3. Some of the sweet potato viruses that have been reported worldwide and recognised by ICTV

Virus	Family	Genus	Vector	Distribution	Reference(s)
Sweet potato chlorotic stunt virus (SPCSV)	*Closteroviridae*	*Crinivirus*	Whiteflies	Worldwide	Cohen *et al*., 1992; Winter *et al*., 1992; Hoyer *et al*., 1996; Gibson *et al*., 1998; Alicai *et al*., 1999b.
Sweet potato mild mottle virus (SPMMV)	*Potyviridae*	*Ipomovirus*	Whiteflies	East Africa	Hollings *et al*., 1976; Colinet *et al*., 1996.
Sweet potato feathery mottle virus (SPFMV)	*Potyviridae*	*Potyvirus*	Aphids	Worldwide	Abad *et al*., 1992; Colinet and Kummert, 1993; Sakai *et al*., 1997; Kreuze *et al*., 2000; Abad *et al*., 2007.
Sweet potato latent virus (SPLV)	*Potyviridae*	*Potyvirus*	Aphids	Taiwan, Peru, China, Japan	Liao *et al*., 1979; Colinet *et al*., 1997; Yun *et al*., 2002.
Sweet potato mild speckling virus (SPMSV)	*Potyviridae*	*Potyvirus*	Aphids	Argentina, Peru, Brazil	Colinet *et al*., 1997.

Sweet potato virus G (SPVG)	*Potyviridae*	*Potyvirus*	Aphids	China, Egypt, U.S.A	Colinet *et al*., 1994; IsHak *et al*., 2003; Souto *et al*., 2003.
Sweet potato leaf curl virus (SPLCV)	*Geminiviridae*	*Begomovirus*	Whiteflies	U.S.A, Sicily, Kenya, China, Brazil, India, Italy, Japan	Lotrakul *et al*., 1998; Miano *et al*., 2006.
Sweet potato leaf curl Georgia virus (SPLCGV)	*Geminiviridae*	*Begomovirus*	Whiteflies	U.S.A	Lotrakul *et al*., 1998.
Ipomoea yellow vein virus (IYVV)	*Geminiviridae*	*Begomovirus*	Whiteflies	Spain	Banks *et al*., 1999.
Cucumber mosaic virus (CMV)	*Bromoviridae*	*Cucumovirus*	Aphids	Israel, Egypt, Kenya	Cohen and Loebenstein, 1991.
Sweet potato leaf speckling virus (SPLSV)	*Luteoviridae*	*Enamovirus*	Aphids	Peru, Cuba	Fuentes *et al*., 1996.
Tomato spotted wilt virus (TSWV)	*Bunyaviridae*	*Tospovirus*	Thrips?	U.S.A, Canada	Clark and Hoy, 2007

? = unassigned vector

2.2 Viruses of sweet potato and their epidemiology in Uganda

The first suspected viral disease of sweet potato in Eastern and Central Africa was reported in 1939 from the Ituri province of the Democratic Republic of Congo and then in Uganda in 1944 (Hansford, 1944). It is now thought to have been caused by SPFMV and SPCSV, which still commonly occur together in sweet potato fields in Uganda causing SPVD. In contrast, only a few cases of SPMMV, SPCaLV and SPCFV have been reported in the country (Carey *et al*., 1998; Gibson *et al*., 1998; Aritua *et al.,* 2002; Mukasa *et al*., 2003; Aritua *et al*., 2007). Wasswa *et al*. (2011) reported SPLCUV (*Begomovirus; Geminiviridae*) for the first time in Uganda and in the World. This was the second time for a *Begomovirus* to be reported in sweet potato in sub Saharan Africa, the first time being in Kenya by Miano *et al*. (2006). Mainly whiteflies or aphids are involved in the transmission of sweet potato viruses (Table 3). It is apparent, however, that the use of virus infected planting material is also an effective means of perpetuating and disseminating sweet potato viruses (Stevenson and Hagedorn, 1973; Carrol, 1981; Wang and Maule, 1994).

2.2.1 Family *Potyviridae*

Two sweet potato viruses in this family [SPFMV (*Potyvirus;* monopartite, transimitted by ahids with type species *Potato virus Y*) and SPMMV (*Ipomovirus;* monopartite, transimitted by whiteflies with type species SPMMV)] are known to occur in Uganda. SPFMV is the most widespread virus and SPMMV probably ranks fourth after SPCSV and SPLCUV in prevalence (Carey *et al*., 1998; Aritua *et al.,* 2002; Mukasa *et al*., 2003; Aritua *et al*., 2007; Wasswa *et al*., 2011). Other genera of *Potyviridae* include *Tritmovirus* (monopartite, transimitted by mites with type species *Wheat streak mosaic virus*), *Bymovirus* (bipartite, transimitted by fungi with type species *Barley yellow mosaic virus*),

Rymovirus (monopartite, transimitted by mites with type species *Ryegrass mosaic virus*) and *Macluravirus* (monopartite, transimitted by aphids with type species *Maclura mosaic virus*). Potyviruses are the economically most important and largest group of plant viruses (Shukla *et al*., 1994); they make up more than 30% of all known plant viruses (Shukla *et al*., 1994). Researchers have paid particular attention to SPFMV given its ubiquitous occurrence (Cohen *et al*., 1992; Winter *et al*., 1992; Hoyer *et al*., 1996; Alicai *et al*., 1999b) and involvement in synergism with SPCSV (Hanh, 1979; Ngeve, 1990; Gibson *et al*., 1998; Karyeija *et al*., 2000; Gutierrez *et al*., 2003), probably reducing the chances of identifying other sweet potato viruses.

SPFMV has a monopartite genome made of positive single stranded RNA (ssRNA) of *c*. 10.6 kb which is larger than the average (9.7 kb) of a potyvirus genome (Moyer and Kennedy, 1978; Shukla *et al*., 1994; Sakai *et al*., 1997; Souto *et al*., 2003). The virus has flexuous filamentous particles of about 850 nm in length. It forms 'pin wheel' inclusion bodies in the cytoplasm of infected cells (Souto *et al*., 2003). It has a coat protein (CP) of 38 kDa which is extremely large compared to other potyviruses. The SPFMV genome contains a single open reading frame (ORF) having untranslated regions (UTR) at both 5´- and 3´-ends (Carrington *et al*., 1989a). Virus-encoded proteases, P1, HC-Pro and NIa-Pro, process the large polyprotein (3493 aa) to mature proteins (Riechmann *et al*., 1992). While the NIa-Pro is responsible for the cleavage of the C-terminal two-thirds of the polyprotein (Dougherty and Carrington, 1988), the P1 and HC-Pro mediate their own cleavage from the polyprotein (Carrington *et al*., 1989a). The mature processed proteins and their functions are indicated in Table 4.

SPFMV is transmitted by sap and by many aphid species including *A. gossypii, A. Craccivora, Lipaphis erysimi* and *Myzus persicae* in a non-persistent manner (Stubbs and McLean, 1958). While a few strains can infect *Nicotiana benthamiana* and *Chenopodium species* (Clark *et al*., 1986) SPFMV mostly infects plants in the family *Convolvulaceae* especially the genus *Ipomoea* (Campbell *et al*., 1974). Two strains of SPFMV isolates, common strain (C) and russet crack (RC), were identified and characterized on the basis of symptoms, host range and serology (Moyer and Kennedy, 1978; Moyer *et al.*, 1980; Cali and Moyer, 1981; Usugi *et al*., 1994). Using serology, Cali and Moyer (1981) showed the RC strain to have a number of sub-strains. Following phylogenetic analysis of the CP sequences by Kreuze *et al*. (2000), two more strains were added; strain group O and strain group East Africa (EA). Many other strains such as the S strain (Sakai *et al*., 1997) are also known to exist.

Table 4. Known functions of some genome encoded proteins of *Potyviridae*

Protein	Functions	Reference(s)
P1 proteinase (P1-Pro)	Protease Modulator of gene silencing Virus replication	Carrington *et al*., 1990; Anandalakshmi *et al*., 1998; Verchot and Carrington, 1995
Third protein (P3)	Genome amplification Movement	Merits *et al*., 1999; Suehiro *et al*., 2004
Cylindrical inclusion protein (CI)	RNA helicase Cell- to-cell movement	Lain *et al*., 1990; Carrington *et al*., 1998; Roberts *et al*., 1998

Helper component proteinase (HC-Pro)	Protease Aphid transmission Suppressor of gene silencing Cell-to-cell and systemic movement Virus replication	Carrington *et al*., 1989b; Atreya *et al*., 1992; Sasaya *et al*., 2000; Wang and Maule, 1994; Kasschau and Carrington, 1998; Klein *et al*., 1994; Kasschau *et al*., 1997; Rojas *et al*., 1997; Kasschau and Carrington, 1995
6 kDa protein 1 (6K1)	Virus replication	Riechmann *et al*., 1992
6 kDa protein 2 (6K2)	Symptoms Long distance movement Virus replication	Spetz and Valkonen, 2004; Rajamäki and Valkonen, 1999; Restrepo-Hartwig and Carrington, 1994
Nuclear inclusion protein a (NIa)/ NIa proteinase (Pro)	Protease Virus replication	Dougherty *et al*., 1989; Daros and Carrington, 1997.
NIa/ viral protein genome linked (VPg)	Binds to initiation factor eIF(iso)4E Cell-to-cell and systemic movement Virus replication	Wittman *et al*., 1997; Schaad *et al*., 1997; Schaad *et al*., 1996

Nuclear inclusion protein b (NIb)	RNA-dependent RNA polymerase	Hong and Hunt, 1996
CP	Encapsidation of RNA Cell-to-cell and systemic movement Aphid transmission Virus replication	Jagadish *et al*., 1993; Dolja *et al*., 1994; Dolja *et al*., 1995; Atreya *et al*., 1995; Wang and Maule, 1994; Haldeman-Cahill *et al*, 1998

SPMMV, the type member of the genus *Ipomovirus* (Colinet *et al*., 1996), was isolated in East Africa from sweet potato plants in the 1970s (Hollings *et al*., 1976). SPMMV morphology, virion size, cytoplasmic 'pin wheel' inclusions and the viral genome organisation are similar to those of potyviruses (Hollings *et al*., 1976; Colinet *et al*., 1998). It has flexuous filamentous particles between 830-850 nm in length (Hollings *et al*., 1976). The positive single stranded RNA genome is about 10.8 kb (Sakai *et al*., 1997; Colinet *et al*., 1998). Despite the similarity to other potyviruses, sequences in the CP core region of SPMMV show many differences from other members of the *Potyviridae* (Colinet *et al*., 1996; 1998). Also, SPMMV has a wide host range that includes species in 14 plant families (Hollings *et al*., 1976) as compared to SPFMV whose host range is mainly limited to *Ipomoea species* (Clark *et al*., 1986). SPMMV occurs in Uganda at low incidences in single and multiple infections with other sweet potato viruses (Aritua *et al*., 2002; Mukasa *et al*., 2003). The virus is thought to be spread by *B. tabaci* in a persistent manner (Hollings *et al*., 1976).

2.2.2 Family *Closteroviridae*

The family *Closteroviridae* is characterized by flexuous filamentous virions ranging from 700-2000 nm in length (Cohen *et al*., 1992). *Closteroviridae* has three taxonomic groups based on virus genome organization and type of vector needed for virus transmission. These groups include genus *Closterovirus* (monopartite, transmitted by aphids), genus *Ampelovirus* (monopartite, transmitted by mealybugs) and genus *Crinivirus* (bipartite, transmitted by whiteflies) (Wisler *et al*., 1998).

SPCSV, a crinivirus, is the only virus of the family *Closteroviridae* known to infect sweet potato. It is a bipartite phloem-limited virus that is transmitted by two whitefly species, *B. tabaci* and *Trialeurodes abutilonea,* in a semi-persistent non-circulative manner (Sheffield, 1957; Schaefers and Terry, 1976; Cohen *et al*., 1992; Wisler *et al*., 1998; Sim *et al*., 2000). It is not sap transmitted. SPCSV has a wide host range including various *Ipomoea* species, *N. benthamiana, N. clevelandii, Amaranthus palmeri* and wild species of Lisianthus (*Eustoma grandiflorum*) (Cohen *et al*., 2001). Its genome comprises two ssRNAs (Agranovsky, 1995) encapsidated by the major coat protein (CP) - encapsidating about 95% of RNA and the minor CP (CPm) - encapsidating only about 5% of RNA. SPCSV possesses the second largest ssRNA genome among plant viruses after *Citrus tristeza virus* (Kreuze *et al*., 2002); the genome probably has 12 ORFs, RNA1 (9407 nt) having five putative ORFs and RNA2 (8223 nt) containing seven putative ORFs. The 3´-end does not have a poly (A) (Agranovsky *et al*., 1991) tail and the 5´-terminus has a methylated nucleotide cap (Cohen *et al*., 1992).

Sweet potato plants singly infected with SPCSV show only mild symptoms (Gibson *et al*., 1998; Mukasa *et al*., 2006). However, the virus synergises heterogeneous viruses in co-infections causing severe symptoms (Gibson *et al*., 1998; Karyeija *et al*., 2000; Mukasa *et al*., 2006). The disease caused when SPFMV is synergised is known as sweet potato virus disease (SPVD) and is the commonest of these complexes. SPCSV isolates can be differentiated into East African (EA) and West African (WA) isolates, named after the regions from which they were first described (Alicai *et al*., 1999b). Serological and molecular characteristics show that all East African isolates and some South American isolates belong to the EA group while isolates from elsewhere in the World including West Africa belong to the WA group (Alicai *et al*., 1999b; Fenby *et al*., 2002; IsHak *et al*., 2003). Fewer of the genome encoded protein functions are known compared to those encoded by *Potyviridae* genomes (Table 4 *versus* 5).

Table 5. Known functions of some genome encoded proteins of closteroviruses

Protein	Functions	Reference(s)
L-Pro/P-Pro	Proteinase Cell-to-cell movement Replication	Agranovsky *et al*., 1994; Peng *et al*., 2001 Peng *et al*., 2001; Peremyslov *et al*., 1998; Peng and Dolja, 2000
Met-Hel-RdRp	RNA dependent RNA Polymerase Localized to membranes	Peremyslov *et al*., 1998; Yeh *et al*., 2000; Erokhina *et al*., 2001

SHP	Cell-to-cell movement	Alzhanova *et al*., 2000
HSP70h	Cell-to-cell movement Virion assembly Attached to virion	Peremyslov *et al*., 1999; Alzhanova *et al*., 2001; Napuli *et al*., 2000
P60	Cell-to-cell movement Virion assembly	Satyanarayana *et al*., 2000; Alzhanova *et al*., 2000
CP	RNA encapsidation Cell-to-cell movement	Agranovsky *et al*., 1995; Alzhanova *et al*., 2000: 2001
mCP/CPd	RNA encapsidation Cell-to-cell movement vector transmission	Agranovsky *et al*., 1995; Tian *et al*., 1999; Alzhanova *et al*., 2000; 2001; Tian *et al*., 1999

2.2.3 Family *Geminiviridae*

Viruses in the family *Geminiviridae* have particles that resemble paired spheres (Bock *et al*., 1974; Lazarowitz, 1992) made of a protein coat and ssDNA that is either single component for monopartite viruses or two components referred to as DNA A and DNA B for bipartite viruses (Lotrakul, 2000). The circular ssDNA has a number of ORFs varying amongst the different genera. However, the ORF encoding CP maps to similar positions on

the single component genome and DNA A of the two component viruses. Also, the ORFs involved directly in DNA replication are found on the complementary sense of the DNA of both monopartite viruses and of DNA A of bipartite viruses. Geminiviruses have a highly conserved AT-rich non-coding intergenic region (IR) (TAATATTAC) that is in a similar position in both monopartite and bipartite viruses (Lazarowitz, 1992) and which is essential for replication (Stanley, 1995).

Geminiviruses infect both monocotyledonous (Bosque-Pérez, 2000; Hang *et al.*, 2001) and dicotyledonous plants (Moffit, 1999; Legg and Fauquet, 2004; Ling *et al*., 2008) and are transmitted by three groups of insects, leafhoppers, treehoppers or whiteflies (Chung *et al*., 1985; Lotrakul, 2000; Lotrakul *et al*., 2001). The family is divided into four genera (*Mastrevirus, Begomovirus, Curtovirus* and *Topocuvirus***)** on the basis of genome organization, virus host range and virus vector species (Fauquet and Stanley, 2003; Stanley *et al.*, 2005). Geminiviruses infecting sweet potato are restricted to two genera, *Mastrevirus* and *Begomovirus*.

2.2.4 Genus *Mastrevirus*

The name *Mastrevirus* was derived from the type species *Maize streak virus*. Mastreviruses are monopartite, infect both monocotyledonous (Lazarowitz, 1988) and dicotyledonous plants (van Regenmortel *et al*., 2000) and are transmitted by leafhoppers in a persistent manner. The *Mastrevirus* genome is *c.* 2600 to 2800 nucleotides and contains two conserved IR, the large IR (LIR) and small IR (SIR) located at opposite ends of the viral genome (Fig. 1i; Gutierrez, 1999; Hang *et al*., 2001). In addition, the genome has four ORF, two in the virion strand designated V1 and V2 which are essential for virus

movement (Lazarowitz *et al*., 1989; Boulton *et al*., 1993) and the other two in the complementary strand designated C1 and C2 (Fig. 1i). V1 encodes a protein involved in virus movement from cell-to-cell of host plants. V2, C1 (also known as Rep A gene) and C2 (also known as Rep B gene) encode virus replication associated proteins (Boulton, 2002; Palmer and Rybicki, 1998). The Rep A gene occurs exclusively in mastreviruses (Boulton, 2002). Some sweet potato contain a mastrevirus which is identifiable only by deep sequencing, which appears to cause no symptoms and which is present only at very low titres (Mbanzibwa *et al*., 2011).

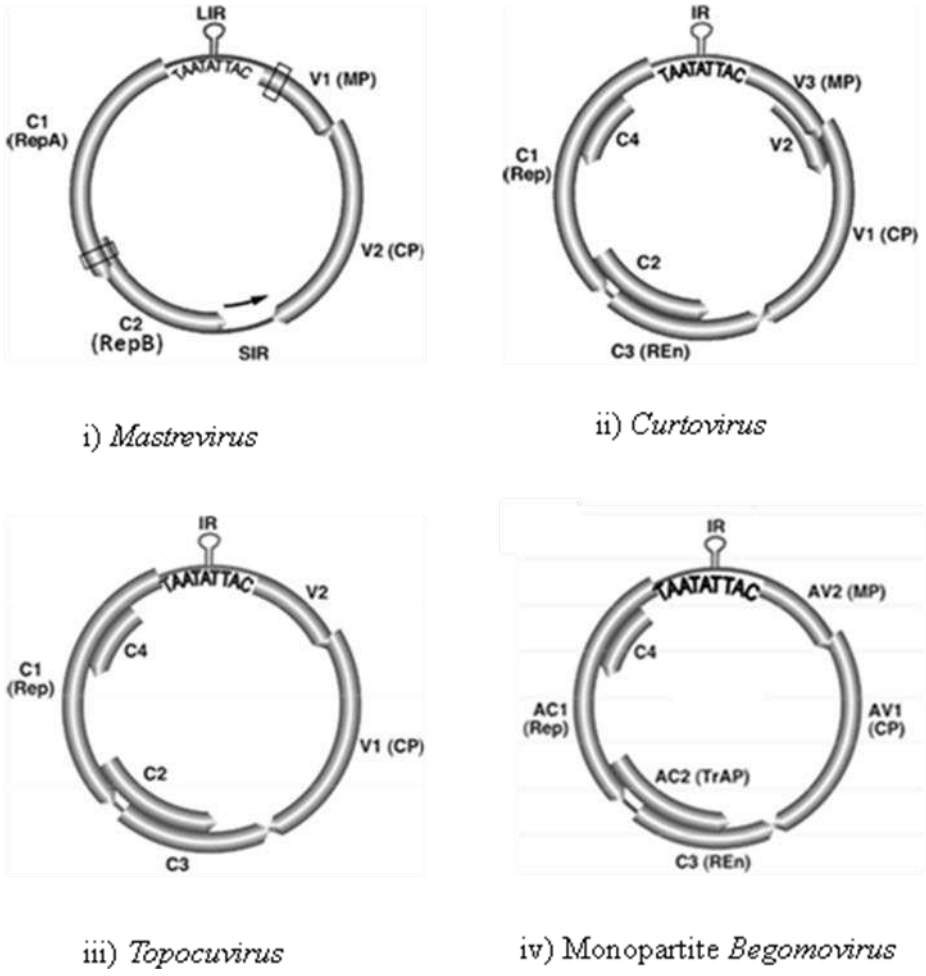

Figure 1. Genome organisation of the four *Geminiviridae* genera. The curved sections each represent an ORF and the circle going through all the curved sections is the ssDNA

Source: Fauquet *et al*. (2005).

2.2.5 Genus *Curtovirus*

Curtoviruses, type species *Beet curly top virus,* are monopartite viruses transmitted by leafhopper species (*Circulifer tenellus*) in a persistent manner and infect only dicotyledonous plants (Mumford, 1974; Stanley *et al*., 1986). The genome is *c.* 3000 nucleotides and has one IR and seven ORFs, four in complementary strand and three overlapping in the virion strand (Fig. 1ii). V1 encodes CP, V2 encodes proteins involved in regulation of ss and dsDNA, and V3 encodes movement protein (MP) that facilitate cell-to-cell movement of the virus within host plant. The complementary strand ORF C1 encodes replication (Rep) protein, C2 a protein of unknown function, C3 Rep enhancer (REn) protein and C4 a protein that can initiate cell division (Hormuzdi and Bisaro, 1995).

2.2.6 Genus *Topocuvirus*

There is only one virus, *Tomato pseudo-curly top virus* that is currently known to be a *Topocuvirus* and this occurs in the southern United States of America. It is monopartite and transmitted by treehoppers (*Micrutalis malleifera* Fowler) in a circulative (persistent) manner to only dicotyledonous plants (Briddon *et al*., 1996). The genome has a conserved IR and six ORFs, two in the virion strand that encode for CP and MP, and four in the complementary strand that encode for replication (Fig. 1iii; Gutierrez, 2002).

2.2.7 Genus *Begomovirus*

Most geminiviruses are begomoviruses: there are at least 102 species and 48 putative species (Fauquet *et al*., 2003). The type species is *Bean golden yellow mosaic virus.* Begomoviruses infect only dicotyledonous plants and are transmitted by *B. tabaci* and *B. argentifolii* whiteflies in a persistent manner (Markham *et al*., 1994). Most begomoviruses

are bipartite (Stanley and Gay, 1983) with their genome comprising circular DNA A and DNA B components (Revill *et al.,* 2003) with different nucleotide sequences, in addition to a conserved IR in each genomic component (Onuki *et al*., 2000; Lotrakul, 2000; Lotrakul *et al*., 2003). However, an increasing number of begomoviruses are being identified, including all sweet potato geminiviruses (sweepoviruses), which have a single genomic component equivalent to the DNA A component of bipartite begomoviruses (Kheyr-Pour *et al*., 1992; Briddon and Markham, 1995; Briddon, 2002) (Fig. 1iv). These also infect only dicotyledonous plants, are transmitted by whiteflies and are therefore retained within the genus *Begomovirus*. The ssDNA molecules of begomoviruses have *c.* 2500 to 2800 nucleotides. The monopartite genome encodes protein products responsible for various functions; AC1 is involved in replication, AC2 acts in transcription, AC3 encodes for REn protein, AC4 appears to influence symptom development and AV1 encodes coat protein which is essential for insect transmission (Briddon *et al*., 1989; Boulton *et al*., 1989; Lazarowitz *et al*., 1989; Regden *et al*., 1994).

Most begomoviruses cluster as either New or Old World but sweepoviruses appear to have no clear origin (Briddon *et al*., 2010). All the 'New World' and most of the 'Old World' begomoviruses have two component genomes with only a few single component ones in the 'Old World' (Fig. 2). This geographical distribution is still clear despite the worldwide movement of infected plant material and the frequent inter-species recombination events common with begomoviruses (Garrido-Ramirez *et al.,* 2000). Following phylogenetic analysis, begomoviruses reported from sweet potato and other *Ipomoea* species appear distinct from all other begomoviruses, branching from the main line of begomoviruses early in their phylogeny (Fig. 2), and have been named sweepoviruses (Fauquet and

Stanley, 2003; Briddon *et al*., 2005). Sweepoviruses include SPLCV, ICLCV, SPLCGV and IYVV (references are in Table 3 and Appendix 1). A number of putative species have been reported from Spain (Lozano *et al*., 2009) and Uganda (Wasswa *et al*., 2011).

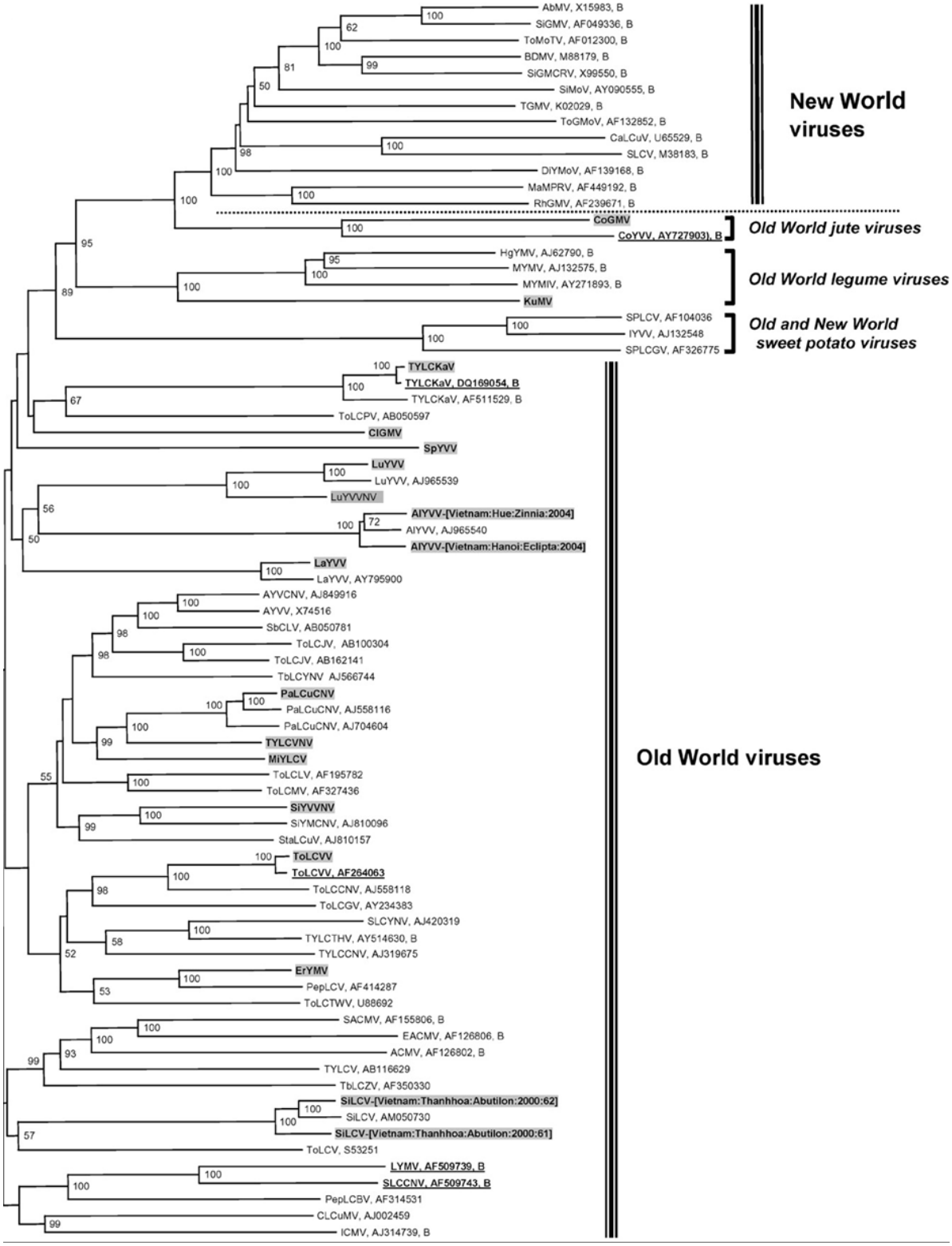

Figure 2. Phylogenetic tree of begomoviruses showing position of sweepoviruses. 'B' after some GenBank accession numbers indicates 'bipartite' viruses (Ha *et al*., 2008).

2.2.7.1 Taxonomic criteria for species demarcation in family *Geminiviridae*

The basic requirement for considering any particular virus as a distinct species is that more than one discriminating character should be considered for distinguishing species (Mayo and Pringle, 1998; van Regenmortel *et al*., 2000; Fauquet *et al*., 2005). The following characteristics have been established for classification of geminiviruses (Padidam *et al*., 1995; Brown *et al*., 2001; Fauquet and Stanely, 2003; Fauquet *et al*., 2003; Briddon *et al*., 2008; Fauquet *et al*., 2008).

i. Presence or absence of the DNA B component determining whether the virus is monopartite or bipartite.
ii. Presence or absence of ORF AV2. 'Old World' begomoviruses have both AV1 and AV2 whereas AV2 is missing in 'New World' begomoviruses.
iii. Natural host range and symptom expression. Some geminiviruses infect either dicotyledonous or monocotyledonous plants while others infect both.
iv. Different vector species. Insects involved in transmission of geminiviruses include leafhoppers, treehoppers and whiteflies.
v. Nucleic acid sequence identity. Identity <89% is generally indicative of a distinct species.
vi. Amino acid sequence identity. Identity <90% may be indicative of new species. In addition, virions of different virus species should react differently with key antibodies.
vii. Use of Rep protein to trans-replicate genomic component. If the Rep protein cannot trans-replicate the genomic component, it indicates a distinct species.

viii. Use of pseudorecombinants (only possible for bipartite viruses). For a distinct species, there should be no pseudorecombination between components of already known virus species.

2.2.8 Family *Flexiviridae*

Virus members were first collectively assigned to the family *Flexiviridae* in 2004 (Adams *et al*., 2004) and, by the year 2009, three new families - the *Alphaflexiviridae*, *Betaflexiviridae* and *Gammaflexiviridae* - were split from the original family by the ICTV (Astier, 2008). Carlaviruses of the family *Flexiviridae* with type species *Carnation latent virus*, have a positive-sense ssRNA genome with virion size of 610 - 690 nm in length. Virus particles are highly flexible and filamentous thus the name *Flexiviridae* (Foster and Taylor, 1998; Astier, 2008). The genus *Carlavirus* contains thirty-five described species and twenty-nine tentative species (Astier, 2008) and is transmissible by sap and in a semi-persistent manner by the aphids. The vector of SPCFV is currently not known (Appendix 1). SPCFV forms 'pin wheel' aggregations in the cytoplasm of infected cells (Adams *et al*., 2004). The virus infects sweet potato and some plant species in the families *Convolvulaceae*, *Chenopodiaceae* and *Solanaceae* but causes mild or no symptoms in infected sweet potato plants (Foster and Taylor, 1998; Aritua *et al*., 2009).

2.3 Virus identification

Determining viruses present in plants is pre-requisite to any control measure (Agrios, 2005). There are several ways of identifying plant viruses. Virus infected plants can be identified through symptom expression in host plants but symptoms vary with crop species or varieties, season, pathogen strain and whether the plant is infected with one or more

viruses (Moyer and Salazar, 1989; Mukasa *et al*., 2003). In latent infections, plants can be considered virus free when they are actually infected making it difficult for farmers, extension workers or even researchers to select for virus free plants (Hollings, 1965). More reliable techniques used for virus identification include electron microscopy, serological tests, plant infectivity assays (indicator plants/biological tests) and nucleic acid analysis (Torrance and Jones, 1981; Torrance, 2005; Lievens and Thomma, 2005; James *et al.*, 2006).

2.3.1 Plant infectivity assays

Different plants respond differently to viruses; some show clear and distinctive symptoms while others remain symptomless after infection with different viruses. Those which are very susceptible and show clear symptoms are used as indicator plants (Lister, 1959; De Bokx, 1970; Allen and Matteoni, 1991; Candresse, 2001). Plant species commonly used include *Gomphrena globosa, Phaseolus vulgaris, Chenopodium quinoa, C. amaranticolor, Petunia hybrida, N. debneyi, N. benthamiana, N. tabacum* and *Ipomoea* species (Lister, 1959; De Bokx, 1970; Allen and Matteoni, 1991). For sweet potato viruses, *I. nil* and *I. setosa* are commonly used. *I. setosa* is considered to be a near-universal indicator plant for sweet potato viruses (Clark and Moyer, 1988). Different indicator plants have different optimal growth stages at which they should be inoculated with the virus for the best response. The cotyledons of faba beans are very sensitive to some viruses (Allen and Matteoni, 1991) while plants like *Chenopodium* can be used up to the ten leaf stage (De Bokx, 1970). However, most indicator plants like *I. setosa* are best used at the two leaf stage.

There are quite a number of ways used to inoculate indicator plants with the virus, some being better than others for a given plant and virus type. Those most commonly used include use of vectors, sap and grafting (Lister, 1959; Carraro *et al*., 2008). With insect inoculation, virus free insect vectors reared for generations on confirmed healthy plants in cages are collected taking care not to damage them. Vectors are starved for a period of 3-4 hours and then fed on diseased plants for a period ranging from a few minutes to a couple of days depending on the relationship between the virus and vector (Girardeau and Ratcliffe, 1960; Schaefers and Terry 1976). The relationship is either non-persistent, semi-persistent or persistent (Smith, 1924; Watson, 1960; Watson and Plumb, 1972; Brown and Bird, 1992; Bedford *et al*., 1994; Wisler *et al.,* 1998; Sim *et al*., 2000; Johnson *et al*., 2002). The viruliferous vectors are then transferred to healthy indicator plants in cages for inoculation feeding for 3-4 hours (Cohen and Duffuss, 1989; Escriu *et al*., 2000).

Sap inoculation involves preparing a virus suspension from infected plants. The virus in sap is then introduced into healthy indicator plants. To avoid inhibitors in plant sap, purified virus suspensions can be used (Lister, 1959; Semancik and Weathers, 1965). There are several ways of introducing the inoculum to indicator plants. Use of a spray gun, often coupled with the use of an abrasive injects inoculum deep into the tissues of the host plant. Also inoculation can be done by stroking the plants with a finger or even a soft brush contaminated with virus; entry wounds for viruses are produced on leaves of indicator plants by using an abrasive such as carborundum or celite (Garnsey, 1974). Indicator plants react by forming localized lesions on the inoculated leaves or by showing systemic symptoms on new leaves in a week or so (Lister, 1959; Garnsey, 1974).

Often, inoculation of *I. setosa* is by grafting. During grafting, a vascular union is formed between stock and scion. Because plants are almost always systemically infected by viruses, viruses spread through the union (Navarro *et al.*, 1984; Galipienso *et al*., 2001). Grafting is time consuming but is less laborious for a few plants and more sensitive than some techniques like ELISA. Although the technique is in most cases limited to closely related plants, some distantly related species, for example, of solanaceous plants like potato, tomato and thorn apple are graft compatible (Nauriyal *et al.*, 1958; Ashkennazi, 1974; Schneider, 1978). Parasitic dodder plants (*Cuscuta* species) can also be used in cases of some species where virus transmission through grafting is rarely successful though it is quite cumbersome. Species such as *C. campestris* and *C. subinclusa* can be used to transmit viruses, connecting the vascular systems of the diseased and test plants (Carraro *et al.*, 2008).

Plant infectivity is non-specific and, using graft-inoculated *I. setosa*, detects nearly all viruses that infect sweet potato. The technique is slow requiring a long time period for symptoms to show. In addition, symptom expression is influenced by factors such as environment and virus strains (Lister, 1959; Garnsey, 1974) and it is important that the plants be kept long enough to allow the symptoms to appear. However, it can be very sensitive and indicator plants are often used as the first of a series of tests.

2.3.2 Electron microscopy

Virus particles can be visualised only using an electron microscope (MacRae and Mukesh, 1998). Tissue extracts are negatively stained with an electron dense stain such as 2% sodium phospho-tungstate (PTA) or uranyl acetate and viewed under an electron

microscope (Brenner and Horne, 1959). The advantage of electron microscopy is that it does not require virus specific reagents and can identify a broad range of viruses (Nebesářová1 and Vancová 2007). The technique also does not require special storage of samples; it has been used by Schoepp *et al*. (2004) for the detection of viruses in infected tissue preserved for decades in unknown solutions. Elongate virus particles such as SPFMV and SPCSV which are flexuous rods and rod shaped or filamentous viruses are readily distinguished from spherical ones such as SPLCV but may not be identified further. Electron microscopy also works well only if high concentrations of viruses are present. The equipment is very expensive and cumbersome (MacRae and Mukesh, 1998). Moreover, unlike ELISA and polymerase chain reaction (PCR) techniques (Hu, 1995), electron microscopy is not suitable for screening large numbers of samples. Because of these limitations of electron microscopy, immunological and molecular methods have been developed (Boltovets *et al.*, 2002; Mumford *et al*., 2006).

2.3.3 Serology

Serology involves use of antibodies which are produced in animals after injection with specific antigens (Cadena-Hinojosa and Campbell, 1981; Bazin, 1982; Cerovska *et al.*, 2002). Plant viruses are essentially made of proteins and nucleic acids, and a protein component, generally the coat protein, provides antigens when a suspension of purified virus particles is injected into an animal, for example, a rabbit or mouse; it is against these antigens that antibodies are formed (Bazin, 1982; Nikolaeva *et al*., 1995; Abouzid *et al*., 2002; Meng *et al*., 2003). Antiserum produced from a single cell type extracted from the spleen of an immunized mouse contains one type of immunoglobulin or antibody and is termed a monoclonal antibody (MAb; Bazin, 1982; Hammond *et al*., 1992). These

antibodies are mostly used for specific virus detection though some are quite broad. Polyclonal antibodies (PAb) are a mixture of immunoglobulins naturally produced in rabbits or other animals when they are injected with viral antigen (Cerovska *et al.,* 2002) and are used for slightly broader spectrum virus detection, for example, all strains of a virus (Hammond *et al*., 1992).

Serology provides quick methods and the procedure is simple. However, serological techniques are limited mainly to detecting virus coat protein, and coat proteins are prone to degradation by proteases in sample material when not kept under special conditions (-80^{o}C) for long periods (Torrance and Jones, 1981). Viroids consist of naked RNA unencapsidated by a protein coat (Tsagris *et al*., 2008; Diener, 1971) and cannot be detected serologically. Again if the virus titre is very low, a serological technique may give false negative results (Owens and Diener, 1981; Esbenshade and Moyer, 1982; Candresse *et al*., 2000). Furthermore, serology is less effective where changes such as recombination (common in geminiviruses; Garrido-Ramirez *et al.,* 2000; Pita *et al.,* 2001) occur in the coat protein of viruses. Serology includes techniques such as serologically-specific electron microscopy (SSEM) and enzyme linked immunosorbent assay (ELISA); ELISA is most widely used today.

2.3.3.1 Serologically-specific electron microscopy

The technique combines both serology and electron microscopy (Kapikian *et al*., 1972). SSEM involves coating of the nitrocellulose-coated copper EM grids with antiserum. The grids are floated on sap extracts and the virus is then retained by the antibodies. The grid is then stained and viewed. Trapped virus can also be ‘decorated’ by coating it again with

further antibodies before staining. The detection can be enhanced by tagging the antibodies with gold or similar labelling (Kapikian *et al*., 1972). SSEM is quite sensitive and fairly quick but involves the use of electron microscope which makes it expensive (MacRae and Mukesh, 1998) and it is probably most suited to analysis of a few samples.

2.3.3.2 Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay (ELISA) is rapid, simple (all steps are or can be done at room temperature), economical in use of antisera and relatively sensitive as it can detect as little as 10 ng of virus. The technique is suited to testing large numbers of samples (Hammond *et al.*, 1992) and can be used simultaneously to detect and quantify the virus by observing and measuring the intensity of colour development.

Different types of ELISA are known depending on the number of antibodies used during the reaction and whether direct or indirect and these include, in increasing order of sensitivity, nitrocellulose membrane (NCM-ELISA; that used by the CIP uses two antibodies: one to find the virus and another, tagged with an enzyme, to detect this), double antibody sandwich (DAS-ELISA; uses two antibodies: one to trap and another, tagged with an enzyme such as alkaline phosphatase, to detect) and triple antibody sandwich (TAS-ELISA; uses three antibodies: one to trap, one to find the virus and another, tagged with an enzyme, to detect this). The sensitivity of the technique increases with the increase in number of trapping antibodies (Hobbs *et al.*, 1987; Hammond *et al* 1992; Stipkovits *et al.*, 1993). ELISA procedures are either direct (Hobbs *et al.*, 1987) or indirect (Stipkovits *et al.*, 1993) in two ways; there is direct *versus* indirect ELISA based on whether the antigen is directly trapped by the substrate (NCM-ELISA) or indirectly by an antibody (DAS- and

TAS-ELISA), or whether the virus is directly detected by a labelled antibody (DAS-ELISA) or indirectly detected by first using a virus specific monoclonal murine antibody (MAb) followed by a labelled anti-mouse antibody (TAS-ELISA).

In NCM-ELISA, sap or semi-purified virus is first spotted onto the nitro-cellulose membrane and allowed to dry for 30 minutes. The blotted membrane can then be kept at room temperature conditions for future analysis allowing samples to be taken to different laboratories to continue the detection. Remaining absorption sites are then blocked with a high protein solution. The membrane is then, following CIP protocol, probed with the first virus specific antibody (IgG) for reacting with the antigen (Priou, 2001) followed by anti-rabbit antibody which finds the first antibody. Its sensitivity is comparable to that of DAS-ELISA yet it is simpler to perform than DAS-ELISA and TAS-ELISA (Banymandhub-Munbodh, 1997). Unlike with DAS- and TAS-ELISA, after adding the substrate, NCM-ELISA positive reactions stored at room temperature remain stable for years. For positive reactions, colour development is proportional to the amount of virus in the sample thus allowing some virus quantification (Priou, 2001). However, NCM-ELISA is generally less reliable than DAS- and TAS-ELISA for both detection and quantification of the virus because colour intensity is measured by visual observation.

With DAS- and TAS-ELISA, microtitre plates are used as a support for reagents instead of a nitrocellulose membrane. DAS-ELISA first involves the use of polyclonal IgG to coat the plate, then the test samples are applied. This is followed by the anti-virus conjugate (IgG-AP) diluted in a high protein conjugate buffer and then by an appropriate substrate for colour development. Results are assessed by visual inspection and spectrophotometric

measurement of absorbance (Clark and Adams, 1977). With TAS-ELISA, polyclonal IgG coating antibody again is used to coat the plate followed by the test samples. However, this is followed by a monoclonal IgG antibody (detecting antibody diluted in a high protein conjugate buffer) prepared in a mouse against the antigen (virus). This is followed by an antibody against mouse conjugated with alkaline phosphatase raised in rat or goat (IgG-AP diluted in a high protein conjugate buffer) that 'finds' the anti-antigen mouse antibody (or Mab above) (Clark and Adams, 1977). Substrate is added and, like the DAS-ELISA, results are assessed by spectrophotometric measurement of absorbance (Clark and Adams, 1977). The results are semi-quantitative.

By using different antibodies in separate microtitre plates, it is possible to simultaneously test plants for different viruses (Hammond *et al*., 1992; Ndowora and Lockhart, 2000; James *et al.,* 2006). Also, using a mixture of specific antibodies, several viruses can be detected (but not distinguished) at the same time using the same membrane or microtitre plate for example, for phytosanitory purposes. However, ELISA has got some limitations. Antibodies that can detect SPFMV, SPMMV and SPCSV are readily available but antibodies for the detection of some viruses like SPLCV are not yet developed. The technique is less sensitive than some techniques such as PCR (Hu, 1995) especially if the virus titre in the plants is low. ELISA also requires polyclonal or monoclonal antibodies specific for each virus of interest that do not cross react with plant proteins (Hammond *et al*., 1992; Abouzid *et al*., 2002).

2.3.4 Nucleic acid analysis

Nucleic acid analysis procedures are based on the principle of nucleic acid hybridization between two more-or-less complementary single strands of nucleic acid (Strachan and Read, 1999). Nucleic acid detection is very sensitive and can detect virus in plant tissue with low virus titres that could not be detected by serology (Hu, 1995). The technique can also be used to detect viroids which lack the CP and could thus not be detected by immunology. Several nucleic acid analysis procedures are now available, many designed to increase sensitivity (Peters *et al*., 2004), alter specificity (Kutyavin *et al*., 2000) or allow automation of detection (Rutledge, 2004; Lievens and Thomma, 2005; Mumford *et al*., 2006).

2.3.4.1 Nucleic acid spot hybridization

Nucleic acid spot hybridization involves immobilizing DNA or complementary DNA (cDNA) on a nylon membrane. The nucleic acid is then probed with a labelled sequence by molecular hybridization (Cox and Singer, 2004), different methods being used afterwards depending on the label. DNA or RNA probes are used of which each has got advantages and disadvantages. Unlike DNA probes, single-stranded RNA probes can hybridize with the target sequence without re-annealing and RNA-RNA hybrids are more stable than DNA-RNA hybrids. However, the potential risk of degradation of RNA probes due to RNAase contamination during hybridization and high costs of generating such probes make the use of DNA probes more common in virus detection assays (Cox and Singer, 2004). Nucleic acid spot hybridization can detect both single and double stranded forms of nucleic acids of viruses. The technique is more sensitive than ELISA, capable of detecting

5-10 pg of virus. However, nucleic acid spot hybridisation is labour intensive and takes longer than ELISA (Hu, 1995).

2.3.4.2 Polymerase chain reaction

PCR starts with nucleic acid extraction from the plant tissue by methods such as CTAB (cetyl trimethyl ammonium bromide; Lodhi *et al* 1994) and TRizol (Stewart and Via, 1993). These methods are designed to extract DNA and/or RNA. PCR involves *in vitro* reactions synthesizing several copies of DNA. For RNA viruses including SPFMV and SPCSV, a cDNA strand complementary to the virus has to be synthesised first using reverse transcriptase (RT) enzyme thus the name reverse transcription polymerase chain reaction (RT-PCR). Like for ELISA, multiplex PCR can be used to simultaneously detect several viruses in a sample (Bertolini *et al.*, 2001; Wittwer *et al*., 2001). PCR/RT-PCR uses forward and reverse oligonucleotide primers which bind to opposite ends (3′-5′ and 5′-3′, respectively) of the viral nucleic acid region of interest (Gibbs and Mackenzie, 1997). Some primers can be designed using regions of the viral genome which are conserved among viruses of one group and these are used to detect more than one virus within a group (Rojas *et al*., 1993; Wyatt and Brown, 1996; Gibbs and Mackenzie, 1997; Saldarelli *et al*., 1998; Chen and Adams, 2001). On the other hand, specific primers are designed to anneal to specific regions of a virus isolate, strain or species.

Multiple copies of DNA are made using Taq DNA polymerase in repetitive steps of denaturation, annealing and extension carried out in a thermocycler rapidly changing temperatures in a cycle. Final results are obtained at the end of the reaction by using a gel to separate the different components, staining using ethidium bromide and finally

visualizing using ultraviolet (UV) light. PCR assays are highly sensitive (Hu, 1995) but the technique requires prior knowledge of the viral genome of at least two sections of about 25 nucleotides upon which forward and reverse primers are designed (Gibbs and Mackenzie, 1997).

2.3.4.3 Real-time quantitative polymerase chain reaction

Real-time quantitative polymerase chain reaction (qPCR) uses the principle of conventional PCR (Gibson *et al*., 1996b; Giulietti *et al*., 2001; Liu and Saint, 2002). Like for PCR, if the target nucleic acid is RNA, it is changed first to cDNA but fluorescent labels are added. During the reaction, signals from the labels are captured by the computer and displayed. qPCR streamlined the problem of quantifying gene expressions in organisms (Ginzinger, 2002) and the important feature with qPCR is that the amplified DNA is simultaneously detected and quantified as it accumulates in the reaction. There are two methods commonly used in qPCR; the SYBR green and the fluorescent probe method (Bustin, 2004).

2.3.4.3.1 SYBR green method

SYBR green is a dsDNA intercalating dye for non probe assays (Zipper *et al*., 2004). This green dye which binds to all dsDNA and emits light that is detected and transmitted to a computer during the reaction is added to the usual PCR mixture (Fig. 3). This dye only fluoresces when bound to dsDNA. Therefore, an increase in DNA product during PCR leads to an increase in fluorescent intensity detected by computer (Liu and Saint, 2002). Though sensitivity partly depends on cDNA synthesis conditions (Lekanne *et al*., 2002), the SYBR green method is generally less sensitive than fluorescent probe method as the

dye binds non-specifically to all dsDNA including primer-dimers (Peters *et al*., 2004). SYBR green qPCR sensitivity is greatly improved by reducing primer-dimer formation (Vandesompele *et al*., 2002; Peters *et al*., 2004).

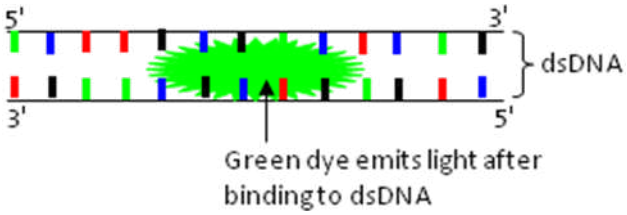

Figure 3. dsDNA-dye complex showing light being emitted

2.3.4.3.2 Fluorescent probe method

In the fluorescent probe method, a normal PCR mixture is prepared (Liu and Saint, 2002) and, in addition to the forward and reverse primers, a probe with a quencher at the 3′ end and fluorescent reporter dye at the 5′ end is added. Some of the dyes used for the quencher are TAMRA, non florescent quencher (NFQ), black hole quencher (BHQ), or dark quencher while reporter dyes include FAM, VIC or JOE among others. Because the reporter dyes have a short wave length (normally green), they can fluoresce while the quencher dyes (normally red), as they have a long wave length, remain unaffected during the reaction. In addition, the probe is phosphorylated at the quencher (3'-) end to stop it from extending during the reaction. The probe is designed to hybridize between the two primers (Fig. 4). As with the primers, the probe is designed to be complementary to the target DNA/cDNA, and both RNA and DNA probes can be used (Kutyavin *et al*., 2000; Costa *et al*., 2004). When intact, the probe will not fluoresce (it is quenched). During a PCR reaction, the primers and probe anneal to the respective complementary DNA strands. The forward primer is extended and the probe is displaced by the new strand. In addition,

the Taq polymerase has an exonuclease activity that cleaves the reporter dye from the 5′ of the probe. Separation of the reporter dye from the quencher molecule results in fluorescence (Fig. 4). Fluorescence increases exponentially as the number of amplified copies increases until reagents reduce resulting in a linear phase and finally into a plateau when all reagents are depleted (Costa *et al*., 2004). Applied Biosystems developed the florescent probe method described above and this type of qPCR is often known as TaqMan after their brand name.

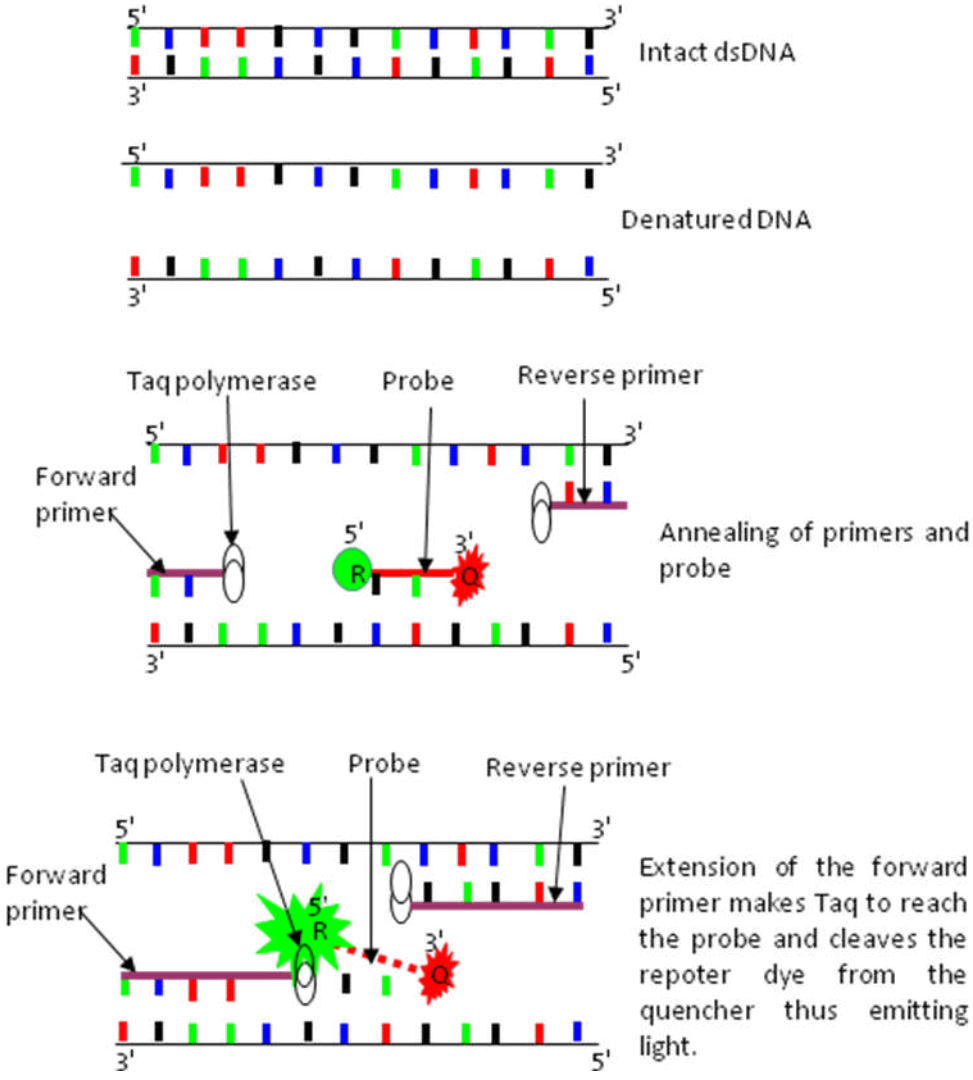

Figure 4. The mechanism of TaqMan qPCR/RT-qPCR reaction

Source: http://www.nature.com/emboj/journal/v20/n9/fig_tab/7593722a_F2.html

As the reaction proceeds for both SYBR green and fluorescent probe methods, the amplicon accumulation is detected and translated into a qPCR sigmoid graph (Fig. 5). The graph has three phases: an exponential phase (reagents are abundant and the curve grows exponentially), a linear phase (reaction starts slowing down as a result of decrease in reagents) and a plateau phase (reagents depleted thus reaction stops) (Rutledge, 2004). The

y-axis shows the number of nucleic acid amplified copies (or normalized reporter-Rn) while the x-axis shows the cycle number in the reaction.

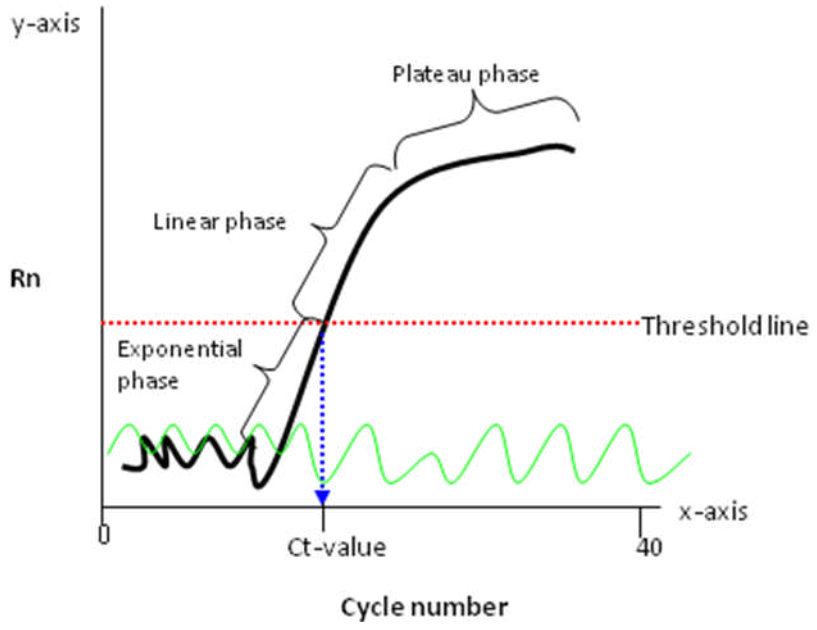

Figure 5. The qPCR amplification graph

The exponential phase is the most important and reliable phase for detection and quantification of nucleic acid. Therefore, two important values of the curve are computed within this phase. One is the threshold line value (red line, Fig. 5) that indicates the value for amplified nucleic acid copies at which background fluorescence can no longer interfere with the target reaction fluorescence. The other value is the cycle threshold (Ct) value (blue line, Fig. 5) that shows the cycle number at which significant increase in amplicon was first detected at the threshold line. These two values are used to calculate the relative quantity of target nucleic acid using for instance, the $2^{(\_\text{Delta Delta C(T)})}$ method (Livak and Schmittgen, 2001) taking into account a housekeeping gene to normalise differences in target nucleic acid concentrations between samples (Weller *et al.*, 2000; Van Hiel *et al.*, 2009). Because qPCR amplicons are detected as they accumulate after every cycle, the

technique can be used for simultaneous detection and quantification of DNA/RNA. The greater the starting copy of the targeted nucleic acid, the sooner a significant increase in fluorescence is observed and the smaller the Ct value. A negative reaction (green line, Fig. 5) shows no Ct value and is instead indicated as 'undetermined' or negative (Livak and Schmittgen, 2001). Although any Ct value above 38 may be treated as suspect, the reaction continues for 40 cycles. The fluorescent probe method is a more sensitive and reliable technique than the SYBR green method but is more expensive (Bustin and Nolan, 2004).

With conventional PCR, results are obtained at the end of the reaction by running the gel, staining using ethidium bromide and finally visualizing using UV light. qPCR is a faster, more sensitive and safer method. It detects the PCR products as they accumulate in the reaction after every cycle and avoids the use of gel, dangerous ethidium bromide and UV light (Souaze *et al*., 1996; Ginzinger, 2002).

2.4 Symptoms and yield effects of sweet potato viruses

There are many sweet potato viruses reported worldwide but only a few have been shown to affect yield and especially when they occur in co-infections. Damage is to the quality and size of leaves and of storage roots. Some viruses of major economic importance include SPFMV, SPCSV, SPMMV and SPLCV. SPFMV is found worldwide wherever sweet potato is grown (Table 3) and some strains can induce root cracking (Campbell *et al*., 1974; Usugi *et al*., 1994) and/or internal corkiness in susceptible cultivars (Milgram *et al*., 1996; Karyeija *et al*., 1998a). SPFMV-infected plants can yield 50% less than uninfected controls with smoother and less mature storage roots but foliage weight seems to remain unaffected (Gibson *et al*., 1997; Njeru *et al*., 2004). SPFMV rarely induces leaf

symptoms in infected plants except when first infecting (Brunt *et al*., 1996; Kokkinos, 2006) when symptoms can include leaf mottling, vein chlorosis, dwarfing and poor growth (Fig. 6). Effects are much worse if SPFMV co-infects with SPCSV, resulting in synergism of the SPFMV; plants develop leaf symptoms (Fig. 6) typical of a potyvirus (mosaic, stunting, vein clearing etc). The disease is called SPVD and it reduces root yields by up to 98% (Schaefers and Terry, 1976; Ngeve, 1990; Ngeve and Bouwkamp, 1991; Gibson *et al*., 1998; Karyeija *et al*., 2000; Gutierrez *et al*., 2003).

SPMMV also rarely causes symptoms of leaf chlorosis and rugosity (Fig. 6) in susceptible sweet potato plants when it occurs as single infection and it seems to have no effect on yield. The virus is, like SPFMV, also synergised by SPCSV; leaf symptoms then include chlorosis, rugosity, leaf strapping and dark green islands (Fig. 6), and the disease is called sweet potato severe mosaic disease (SPSMD) and it reduces the storage root yield by up to 80% (Mukasa *et al*., 2006).

By itself, SPCSV stunts sweet potato plants and can cause reddening, purpling or chlorotic yellowing of leaves (Fig. 6) (Schaefers and Terry, 1976; Gibson *et al*., 1998). These symptoms can be mild or absent but yield loss of up to 50% has been reported (Gibson *et al*., 1998; Mukasa *et al*., 2006).

SPLCV is reported to have reduced the yields of cultivar Beauregard in U.S.A by 26% and caused grooving and skin darkening (Clark and Hoy, 2006) but the disease rarely causes interveinal chlorosis and leaf curling symptoms in infected plants (Bock *et al*., 1974). SPLCUV was found not to be synergised when co-infected with SPCSV, perhaps the only

sweet potato-infecting virus not to be (Wasswa *et al*., 2011). There is a need to combat the effects of these viruses by exploiting the possible resistance mechanisms to viruses of economic importance. This can sustain sweet potato production in developing countries.

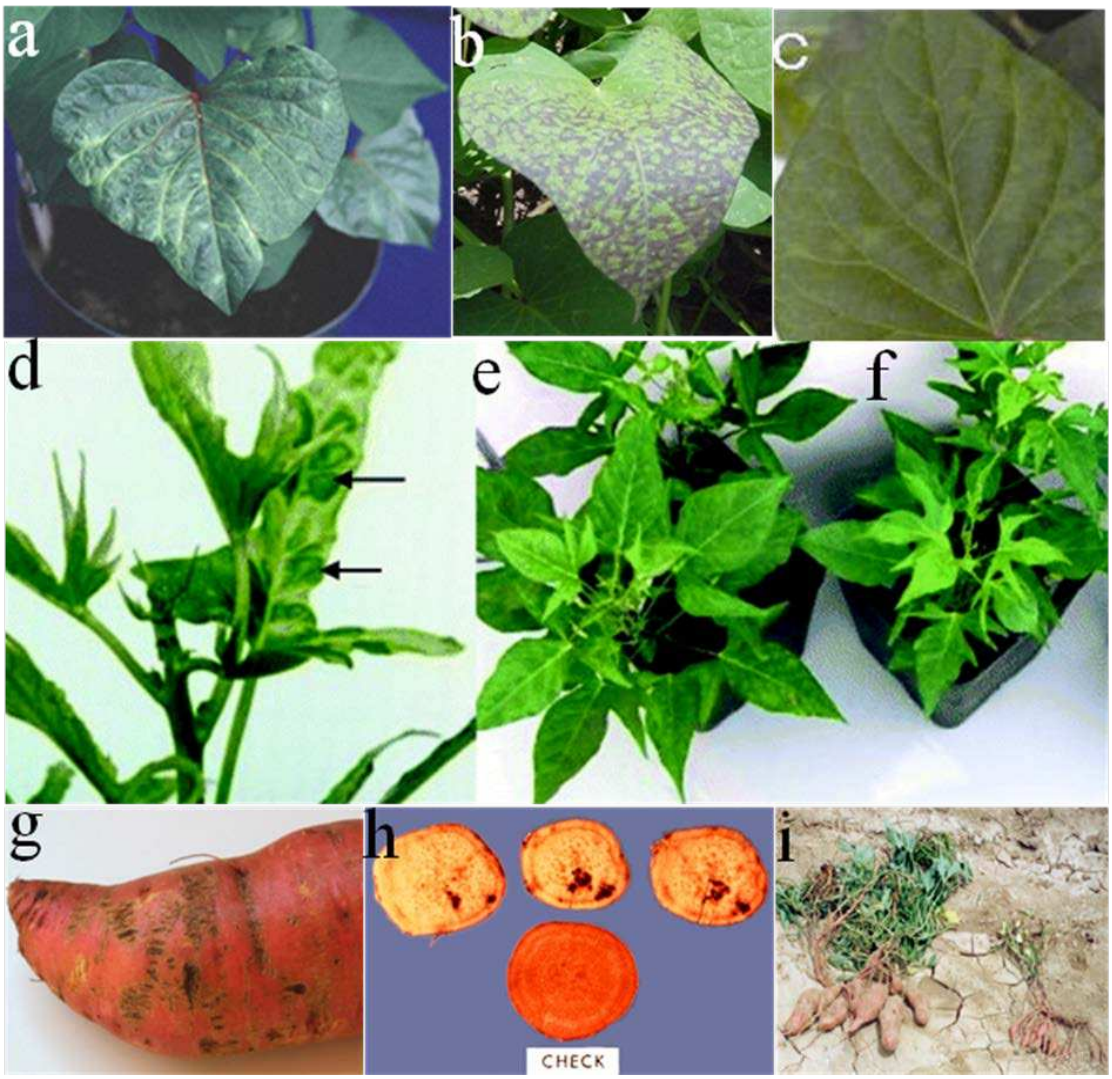

Figure 6. Virus associated symptoms in sweet potato plants. a) plant with SPFMV showing vein clearing, b) purpling and chlorotic spots typical of SPCSV, c) mottling characteristic of SPMMV, d) dark green islands (arrows) associated with co-infection of SPMMV + SPCSV, e) and f) severe symptoms due to co-infection of SPMMV + SPCSV and SPFMV + SPCSV, respectively, g) external root cracking induced by the russet crack strain of SPFMV, h) internal corkiness attributed to SPFMV and i) storage roots of healthy plants (left) and SPVD affected plants (right) (Mukasa *et al*., 2003; 2006; also contribution by S. Fuentes and L. Salazar)

2.5 Control of viruses

Unlike some fungal and bacterial diseases, plant viral diseases cannot be chemically controlled (Hadidi *et al*., 1998; Clark *et al*., 2002). Therefore, strategies for viral disease control have to focus on preventive measures, especially ones that can supplement each other. These strategies must be designed to be simple, inexpensive and within the limited capacity of the farmers. Preventive measures can be achieved in several ways which include quarantine, resistant varieties, virus free planting material and chemicals to control the vector (Hollings, 1965; Hadidi *et al*., 1998; Allam, 2000; Clark *et al*., 2003). Biological control of vectors can be effective for a few viruses but the cost of producing and releasing natural enemies may be very high (Bauer *et al.,* 2008) and it is often the insect vectors that move to a crop that are important to virus transmission rather than the resident population. Seed propagation could also be used to control viral diseases as seeds of some virus infected plants may be virus free. However, this may not be an option in sweet potato because it is mainly propagated vegetatively and there being high variability in the seed derived progeny.

2.5.1 Chemical control

Viruses cannot be directly controlled by use of chemicals but this can be indirectly achieved through controlling their vectors (Palumbo *et al*., 2001). Even then, chemical control is effective only for vectors that have to feed on a crop for several hours (semi-persistently and persistently transmitted viruses like SPCSV and SPLCV, respectively) which gives enough time for the vector to succumb to the pesticide before it transmits the virus. If the vector needs to probe a diseased plant for only a short time to transmit the virus (i.e. non-persistently transmitted viruses like SPFMV), then chemical control is

unlikely to be effective (Perring *et al.,* 1999). Also, several insecticides controlled pests in the past but many pests have now developed resistance. *B. tabaci*, a vector for SPCSV, SPLCV and many other viral diseases of sweet potato and other crops, has developed resistance to organophosphorus insecticides (Prabhaker *et al*., 1985; Toscano *et al*., 1997). Ahmad *et al.* (2003) reported cases of *A. gossypi*, a vector for SPFMV, developing resistance to pyrethroids. Besides, chemical control is an expensive strategy to many resource poor farmers in Africa and is mostly feasible only in screenhouses.

2.5.2 Quarantine

There is considerable evidence that currently ubiquitous viruses (SPCSV and SPFMV; references are in Table 3) were taken to areas where they had never existed before. In fact in some areas, these viruses have been identified only recently (Valverde *et al*., 2004; Abad *et al*., 2007). Many other sweet potato viruses still have a restricted distribution (Table 3 and Appendix 1). Many areas where these viruses do not currently occur appear to have favourable environments for the viruses and vectors and in some areas vectors are rife. Thus, the focal point in the control of potential disease epidemics by such viruses is firstly to restrict the movement of any plant material likely to carry the virus between regions. The government of Uganda should implement rigorous use of inspection, certification and movement permits for sweet potato material as the newly introduced strains may have more severe effects on the local varieties and the government may have limited capacity to contain them.

2.5.3 Use of resistant cultivars

The use of resistant or tolerant varieties has obvious advantages in decreasing the losses due to virus diseases (Aritua *et al*., 1998a; 1998b). Resistant cultivars can be developed through a conventional breeding programme (Fraser, 1990; Valkonen, 1994) or through transformation using viral genes (Abel *et al*., 1986). Resistance to SPCSV and SPFMV is controlled by two separate recessive genes inherited in a hexasomic or tetradisomic manner (Mwanga *et al*., 2002; Diaz-Pendon *et al*., 2004). It is therefore complicated (but not impossible) to breed for.

It may also be possible in the future to transfer resistance genes from wild sweet potato-related species (Valkonen, 1994; Huang and Sun, 2000). Karyeija *et al*. (1998b) identified a wild *Ipomoea species* that exhibited extreme resistance to SPFMV, SPCSV and a combination of both viruses. However, accessions of this wild species need to be studied to verify whether or not this was an aspect of non-host resistance described by Heath (2000). Moreover, most wild species are diploid in nature whereas sweet potato is a hexaploid complicating the process of incorporating resistance from the wild species through cross breeding (Diaz *et al*., 1996). Wild plants also contain numerous undesirable traits. Apart from all that, conventional breeding is laborious and requires much time (Jennings, 1957). Each generation takes a minimum of sweet potato growing season and a series of backcrosses are needed to remove the undesirable characteristics (Wilson *et al*., 1989; Morales, 2001).

Because sweet potato is vegetatively propagated, it may eventually succumb to multiple virus infections and currently, there are no cultivars with total natural resistance to various

virus infections. Sweet potato landraces such as New Kawogo and many others from East Africa have considerable resistance to SPFMV (Mwanga *et al*., 1995; Mukasa *et al*., 2006) but resistance breaks down upon co-infection with SPCSV (Gibson *et al*., 1998) and they are relatively poor yielding (Gibson *et al*., 2000). In single infections, these cultivars show mild and transient symptoms or no symptoms at all (Gibson *et al.*, 1998; Mukasa *et al*., 2003). Considerable effort has been made by breeders and many cultivars have been bred and selected for yield, quality and resistance. However, such varieties may be resistant to one or a few viruses but be susceptible to others. So, breeding for virus resistance might require genes from several source plants further delaying and complicating the process. Breeding for resistance may also eventually give rise to more severe virus isolates (van den Bosch *et al.*, 2006; van den Bosch *et al.*, 2007).

Of late, some breeding programs have involved farmers, researchers, extension workers and other stakeholders working together (Witcombe *et al*., 1996; Toomey, 1999; Soleri *et al*., 1999; Gibson *et al*., 2008; Mwanga *et al*., 2011). These participatory plant breeding programs involve all plant breeding beneficiaries at most steps of breeding, farmers and researchers in particular being involved at an early stage. The technique avoids losing farmers' preferred attributes in the new varieties and results in their better adoption (Sthapit *et al*., 1996; Kornegay *et al*., 1996; Pandey and Rajataserrekul, 1999; Sperling *et al.*, 2001; Witcombe *et al.*, 2003; Gibson *et al*., 2008; Mwanga *et al*., 2011). Farmers will easily adopt a new variety if, in addition to the new characteristics incorporated, such a variety retains a considerable percentage of its original attributes. Otherwise, farmers have a tendency of retaining much of their own stock (planting material) for replanting even though they know that such stock is not doing so well. Some of the attributes that make

farmers retain their own planting material are good eating quality, early maturity and good storage root size.

Strategies have been sought to overcome the many limitations of conventional breeding. This has mainly been through transformation exploiting the viral CP gene thereby mimicking CP-mediated cross protection (Baulcombe, 1996; Beachy, 1997; Fraser, 1998; Savenkov and Valkonen, 2001; Cipriani *et al*., 2001; Okada *et al*., 2001). Transgenic resistance to a virus using viral CP was first achieved by Abel *et al*. (1986); thereafter several virus-derived genes (Beachy, 1997) and untranslatable sequences (Lindbo and Dougherty, 1992) have been successfully used to obtain transgenic plants resistant to viruses. Since SPCSV plays a pivotal role in the development of synergism and SPVD (Gibson *et al*., 1998; Karyeija *et al*., 2000), transgenic resistance to at least SPCSV might be appropriate to control SPVD but SPCSV breaks CP-mediated resistance (Kreuze *et al*., 2008). Also, achieving transformations without causing accidental deleterious effects on the plant variety in the meantime is difficult and viruses may develop mechanisms to suppress gene silencing in resistant transgenic plants (Mitter *et al*., 2001).

The natural resistance of sweet potato plants to viruses is through mechanisms such as gene silencing and reversion. In past studies, Gibson *et al*. (1997) and Mukasa *et al*. (2006) found many cultivars in Uganda to be naturally quite resistant to SPFMV and SPMMV strains, showing only mild initial symptoms, from which they usually recover and may revert to being uninfected (Aritua *et al*., 1998b).

2.5.3.1 Gene silencing

Upon infection with viruses, plants employ a mechanism known as gene silencing against the foreign genes entering the plant (Waterhouse *et al*., 2001a; Vaucheret, 2001; Voinnet, 2001). Gene silencing is either at the post-transcriptional (Palaqui *et al*., 1997; Hamilton and Baulcombe, 1999; Hammond *et al*., 2000; Dalmay *et al*., 2001) or transcriptional (Vaucheret, 2001) level. At the post-transcriptional level, the silencing mechanism targets messenger RNA (mRNA) before it is translated into respective proteins. The silencing system is very specific and precise, degrading only foreign and unusual mRNA, first at sites of infection. Shortly thereafter, a systemic signal is sent to distal parts of the plant to degrade any particles homologous to mRNA perceived by the plant to be aberrant (Voinnet and Baulcombe, 1997; Palaqui *et al*., 1997; Ruiz *et al*, 1998; Llave *et al*., 2002).

Double stranded RNA (dsRNA) from a replicating virus or secondary structured virus has a crucial role in RNA silencing as it produces small interfering RNAs (siRNAs) (Waterhouse *et al*., 2001b) through the action of the RNase III enzyme Dicer (Bernstein *et al*., 2001; Tijsterman and Plasterk, 2004; Pham *et al*., 2004). siRNAs are 21-25 nucleotide double-stranded RNAs and it is these siRNAs that mediate degradation of any mRNAs from foreign particles (Hannon, 2002). A slightly similar pathway involves the use of microRNAs (miRNA) instead of siRNAs (Carrington and Ambros, 2003). miRNAs are produced from factors encoded as stem-loop precursors in the genome which are then processed into miRNA through the action of RNase III enzyme Drosha together with Dicer (Lee *et al*., 2003). miRNAs and siRNAs closely resemble each other in size, structure and function (Carrington and Ambros, 2003). Although miRNAs can target and destroy mRNA, they mostly act by binding and inhibiting the translation of mRNA. At the

transcriptional level, RNA silencing is before transcription; here the gene is made inaccessible to transcriptional machinery by RNA silencing mechanism (Baulcombe, 1996).

Despite RNA silencing, many viruses (both DNA and RNA viruses) manage to infect their host plants quite successfully (Voinnet *et al*., 1999; Yelina *et al*., 2002). This is because viruses, in turn, deploy virus-encoded proteins which suppress both transcriptional and post transcriptional RNA silencing (Anandalakshmi *et al*., 1998; Kasschau and Carrington, 1998; Voinnet *et al*., 1999; Voinnet *et al*., 2000; Ahlquist, 2002; Moissiard and Voinnet 2004). Several viral RNA silencing suppressors have been reported and these include the HC-Pro (potyviruses; Kasschau and Carrington, 1998), 2b (cucumoviruses; Brigneti *et al*., 1998), P1 (sobemoviruses; Voinnet *et al*., 1999), p19 (tombusviruses; Lakatos *et al*., 2004), the 25 kDa movement protein (Potexviruses; Voinnet *et al*., 2000), the AC2 (geminiviruses; Voinnet *et al*., 1999), RNase III endonuclease (RNase3) and 22 kDa RNA silencing suppressor protein (p22) (criniviruses; Kreuze *et al*., 2002; Carmell and Hannon, 2004) genes.

Cuellar *et al*. (2009) showed that sweet potato plants transformed with RNase3 gene from SPCSV synergised SPFMV and other viruses. RNase3 also cleaved synthetic double-stranded siRNAs of 21, 22 and 24 base pairs (bp) *in vitro* to products of approximately 14 bp that are inactive in RNA interference (RNAi) and affected total siRNA isolated from SPFMV-infected sweet potato plants, suggesting a viral mechanism for suppression of RNAi by cleavage of siRNA. These results implicated RNase3 in suppression of antiviral defence in sweet potato plants. By synergising a range of unrelated viruses including

SPFMV to cause SPVD, RNase3 is revealed as a protein that can mediate viral synergism, a function previously described only for P1/HC-Pro. A role for p22 as a pathogenicity enhancer of SPCSV is provided by complementary expression of p22 in transgenic sweet potato plants (Cuellar *et al*., 2011). However, Cuellar *et al*. (2008) showed that many isolates of SPCSV apart from those from Uganda do not have a p22 gene yet synergise SPFMV. Therefore, p22 is not essential for synergy but appears to assist it, as the symptoms were more severe with the p22-encoding isolates. Cañizares *et al*. (2008) showed in another closterovirus (*Tomato chlorosis virus*) that a p22 gene has a suppressing effect; no RNA virus other than SPCSV encodes an RNase3 or uses two independent proteins cooperatively for RNA silencing suppression (Kreuze *et al*., 2005).

2.5.3.2 Recovery and reversion in sweet potato plants

In turn, plants evolved an even greater level of host resistance that restrain virus-encoded RNA silencing suppression (Li *et al*., 1999). This is manifested through possibilities of diseased plants to recover from disease symptoms and subsequently have low virus titres (Gibson and Otim-Nape, 1997; Mukasa *et al*., 2006), or totally reverting from virus infection (Gibson *et al*., 1997; Aritua *et al*., 1998b; Fondong *et al.*, 2010). Recovery was first reported in the 1930s when symptomatic cassava plants infected with *African cassava mosaic virus* (ACMV) started to produce new leaves without symptoms (Storey and Nichols, 1938). Since then, several other vegetatively propagated plants have been reported to suppress virus symptoms completely, a phenomenon known as recovery. Cuttings from recovered portions may grow without the virus and this is then called reversion (Storey and Nichols, 1938; Gibson *et al*., 1997). Researchers have observed both

recovery (Gibson *et al*., 1997; Aritua *et al*., 1998b; Mukasa *et al*., 2006; Gasura *et al.*, 2009) and reversion (Aritua *et al*., 1998b) in sweet potato.

Although viruses are known to systemically infect their host plants (Petty *et al*., 1990), resistance to viruses in sweet potato has been attributed to the ability of plants to restrict virus movement (Toussaint *et al*., 1984; Carrington *et al*., 1996). Inconsistent with this, however, Kreuze, (2002) found resistance to SPFMV to be attributed to virus degredation rather than virus movement. In addition, genotype has also been reported to influence recovery (Gasura *et al.*, 2008) and probably reversion in sweet potato plants. Because recovery and reversion seem to be important phenomena behind survival of sweet potato landraces by the selection and use of virus free planting material by farmers (Gibson *et al*., 1997: Aritua *et al*., 1998b), this study looked at ways of exploiting the phenomenon, especially finding if a low virus titre is a common factor to cultivars that revert.

2.5.4 Selection and use of symptomless sweet potato planting material

Farmers in Uganda have considerable experience in growing sweet potato and grow a wide range of traditional cultivars and varieties developed by researchers including the NASPOT series bred in Uganda (Mwanga *et al*., 1995). NASPOT 11 (Tomulabula) was selected by both farmers and researchers during participatory breeding. The local elite cultivars commonly grown include Tanzania, Dimbuka, New Kawogo, Semanda and Ejumula. Farmers normally collect healthy looking cuttings from old standing crop especially in the neighbouring fields, thus vegetatively propagating the crop.

As stated previously, sweet potato plants rarely express symptoms when infected with single viruses (Kokkinos, 2006; Mukasa *et al*., 2006) but that most of the symptomless sweet potato is actually virus free (Gibson *et al*., 1997) confirms the farmers' decision to select symptomless material as being largely correct. However, some of the material may be infected and so farmers may suffer some yield loss because of this. In co-infection with SPCSV, many sweet potato viruses produce severe foliar symptoms which can easily be identified (Hanh, 1979; Ngeve, 1990; Gibson *et al*., 1998; Karyeija *et al*., 2000; Gutierrez *et al*., 2003; Mukasa *et al*., 2006) and again farmers can easily select not to choose such SPVD affected planting material.

2.5.5 Virus elimination

Several deliberate methods such as electrotherapy, chemotherapy, cryopreservation, tissue culture and thermotherapy can be employed to eliminate viruses from propagation material. Electrotherapy involves the application of electrical pulses to eliminate viruses from either *in vivo* or *in vitro* plants and the technique has been successfully used to eliminate *Almond mosaic virus* from almond trees (Quacquarelli *et al*., 1980) and *Potato virus X* in potatoes (Lozoya-Saldaña *et al*., 1996). Chemotherapy involves use of antimetabolite chemicals such as ribavirin, 5-Azacytidine, and 3-Deazauridine that block the virus nucleic acid synthesis/replication. Chemical treatment is either done directly by spraying *in vivo* plants or indirectly by adding these chemicals to media of *in vitro* plants (Bittner *et al*., 1987; De Fazio, 1987; Toussaint *et al*., 1993). Thermotherapy (see next section) involves keeping plants or tissue cultured material close to the upper temperature tolerance of the material usually for several weeks. Cryopreservation (cold treatment) may be used to eliminate viroids [e.g., Potato spindle tuber viroid (PSTVd)] whose replication

and accumulation are favoured by high temperatures (30^{o}C - 40^{o}C) so thermotherapy (heat treatment) is ineffective (Lizarraga *et al.*, 1980; Brison, 1997; Lizarraga *et al.*, 1991; Helliot, 2002).

Tissue culture may include use of callus tissue or apical meristems in tissue culture or meristem tip grafting and meristem tip culture (Jayasree *et al.*, 2001; Lizarraga *et al.*, 1991). With callus, somatic embryos and adventitious buds have to be derived from the callus (organogenesis). Somatic embryogenesis from ovules or seedlings derived apomictically from nucellus tissue can also result into virus free plants (George, 1993). Meristem tip grafting is mainly used for virus elimination in tree species rather than herbs. The rootstock is obtained from virus free seedlings and the scion can consist either of a meristem tip removed directly from the diseased *in vivo* mother plant, or be a small shoot resulting from *in vitro* culture of a meristem tip (Bhojwani and Razdan, 1983). Meristem tip culture involves the excision of meristematic domes (*c.* 0.1 mm long) from infected plants and growing them on tissue culture medium (Kaiser and Teemba, 1989; Allam, 2000). To enhance virus elimination efficiency, meristem tip culture usually follows thermotherapy and may eliminate several plant viruses (Berg and Bustamante, 1973; Wu and Su, 1991; Wasswa *et al*., 2010).

2.5.5.1 Thermotherapy for virus elimination

Thermotherapy, the most common method of freeing planting material of virus, is the exposure of diseased plants to hot air or water for a period of time usually measured in days or weeks. The high temperature inactivates and eventually eliminates the plant viruses (Kassanis, 1950; Nyland and Goheen, 1969; Walkey, 1976; Wang and Hu, 1980; Kaiser

and Teemba, 1989; Cheema *et al*., 1999; Wasswa *et al*., 2010). Thermotherapy can be done either on potted *in vivo* plants or *in vitro* plants before meristem tip excision (Zapata *et al.*, 1995; Cheema *et al*., 1999). The temperature and duration of exposure depend on the virus and heat tolerance of the plant (cultivar) (Kerr and Mahmood, 2001) but is generally above the optimum for growth.

In vivo thermotherapy has been done on a number of plants to eliminate several viruses as well as virus complexes. For instance, Keg ler (1967, 1968) observed elimination of *Plum pox virus* (PPV) from plum trees using a temperature of 37°C for 2-3 weeks, followed with grafting the recovered green shoots on virus-free rootstocks. Janečková (1993) used a combined method of *in vivo* thermotherapy and *in vitro* chemotherapy to eliminate viral complexes of PPV, *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) from four plum cultivars. *In vitro* thermotherapy alone is also sufficient for elimination of some viruses; ACMV was eradicated from cassava when *in vitro* plants were kept at 37^{o}C for 6 weeks under a 16 hours light and 8 hours dark period (Kaiser and Teemba, 1989). Similarly, *Cassava brown streak virus* (CBSV) was eradicated from meristem tip derived plantlets of *in vitro* cassava plants heat treated at 36^{o}C for 8 hours darkness and 40^{o}C for 16 hours light for a period of 4 weeks (Wasswa *et al*., 2010). In sweet potato, thermotherapy routinely used by CIP involves a temperature of 38°C for one month before meristem culture to eliminate various viruses (Lizarraga *et al*., 1992). According to Panta *et al*. (2007) and El Far and Ashoub (2009), SPFMV was freed from plants by thermotherapy followed by meristem culture using temperature range of 35-37°C with light intensity of 5000 lux for 30 days.

Virus free material is multiplied into thousands of plants firstly *in vitro* and later acclimatized *in vivo* in vector free screenhouses. Virus free plants are then used as mother plants either for direct *in vivo* multiplication or *in vitro* micropropagation. Multiplication is then done at the county/subcounty level in open fields of selected farmers for mass generation of cuttings for distribution to subsistence and commercial farm use. The use of sweet potato virus free plants can restore cultivar's original excellent yield and quality for a period of over three years (Basilio *et al.,* 2005).

CHAPTER THREE

FIRST IDENTIFICATION OF A SWEET POTATO BEGOMOVIRUS (SWEEPOVIRUS) IN UGANDA: CHARACTERIZATION, DETECTION AND DISTRIBUTION

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3.1 Introduction

Sweet potato, *Ipomoea batatas*, an important food crop in much of sub-Saharan Africa, is grown on about 2.1 x 10^6ha with an estimated production of 9.9 x 10^6t (FAO, 2008). Africa has the second largest production after Asia; here, the crop is especially important in countries in the Great Lakes region of East Africa, Uganda having the largest production (FAO, 2008). Viruses pose the second most important biotic constraint after weevils (Clark and Moyer, 1988). In recent surveys, several viruses including SPFMV, SPCSV, SPMMV, SPCFV and SPCaLV have been identified infecting sweet potato crops in Uganda (Carey *et al*., 1998; Mukasa *et al*., 2003; Aritua *et al*., 2007). The most important disease is the synergistic SPVD caused by dual infection of SPCSV and SPFMV (Gibson *et al.*, 1998).

Several Ugandan landraces, such as New Kawogo, show high resistance to SPFMV and SPCSV (Mwanga *et al*., 1995; Gibson *et al.,* 1997) but this and some other cultivars are rumoured to be declining in yield, suggesting at least one more virus is widespread in sweet potato in Uganda. SPLCV (*Begomovirus*; *Geminiviridae*) has recently been reported in sub-Saharan Africa but only in Kenya (Miano *et al*., 2006), though otherwise having a wide distribution including the U.S.A, China, Japan and Spain (Lotrakul *et al*., 1998; Luan *et al*., 2007; Lozano *et al*., 2009).

Members of the *Geminiviridae* have particles that resemble paired spheres containing single stranded (ss) DNA either as a single component for monopartite viruses or as two components referred to as DNA-A and DNA-B for bipartite viruses. The family is subdivided into four genera mainly on the basis of genome organization, vector species and host range. *Begomovirus* is the largest genus, containing viruses transmitted by whitefly, particularly *B. tabaci* (Gennadius), and infecting dicotyledonous plants (Fauquet *et al*., 2003). Phylogenetically, all begomoviruses reported from sweet potato and other *Ipomoea* spp are monopartite, diverge basally from other begomoviruses and have been named sweepoviruses (Fauquet and Stanley, 2003; Briddon *et al*., 2005), a name we have adopted through out this thesis. Other sweepoviruses include ICLCV (Cohen *et al*., 1997), SPLCGV, IYVV (Banks *et al*., 1999) and other several putative species reported from Spain (Lozano *et al*., 2009). Most begomoviruses can be grouped as either ‘New’ or ‘Old’ World but sweepoviruses appear to have no clear origin (Briddon *et al*., 2010). In this chapter, we report a new sweepovirus from Uganda including its full length sequence (GenBank accession no FR751068), the first for a sweepovirus from mainland Africa, its detection by indicator plants and molecular methods, a mechanism of host plant resistance

in Ugandan sweet potato varieties, and information on sweepovirus distribution and prevalence in Uganda.

3.2 Materials and Methods

3.2.1 Initial source of material and evidence for a sweepovirus

Two symptomless plants of sweet potato cv New Kawogo were collected in November 2008 from a garden in Kampala, central Uganda, and established in a quarantine greenhouse at NRI, UK. Scions were side-grafted to one week old *I. setosa* seedlings, the almost universal indicator plant for sweet potato viruses (Clark and Moyer, 1988). Within 4 weeks, symptoms of upward leaf-curling, vein thickening, yellow mottle and leaf chlorosis resembling those of begomovirus infection were observed on the *I. setosa* (Fig. 7). Other sweet potato plants with SPVD were collected and similarly established at NRI and were diagnosed for viruses as described below.

3.2.2 DNA extraction and PCR

Total nucleic acid (TNA) was extracted from the above sweet potato plants inducing leaf curl symptoms in grafted *I. setosa* and from the affected *I. setosa* using a cetyl trimethyl ammonium bromide (CTAB) method, described originally by Lodhi *et al*. (1994) and later modified (Maruthi *et al*., 2002).

The CTAB extraction buffer [2% (w/v) CTAB, 1.4 M NaCl, 0.2% (v/v) 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0] was preheated to 60ºC for 10 minutes. Mercaptoethanol is always added fresh to the buffer. Approximately 100 mg of

diseased plant leaf tissue was placed into a thick gauged plastic bag. The tissue was ground using a roller and mixed with 10 volumes (1 ml) of CTAB extraction buffer. About 750 µl of the sample was poured into a 1.5 ml eppendorf tube and the samples were heated at 60ºC for 30 minutes. The samples were mixed with an equal volume (750 µl) of phenol: chloroform: isoamylalcohol (25:24:1), briefly vortexed and centrifuged at 13000 rpm for 10 minutes. The top aqueous phase (500 µl) was transferred into a new 1.5 ml eppendorf tube. The DNA was precipitated by adding 0.6 volumes (300 µl) of cold (-20ºC) isopropanol and incubated at -20ºC for at least 1 h. The samples were centrifuged at 13000 rpm at 4ºC for 10 minutes and the supernatant was discarded. The pellet was washed in 0.5 ml 70% ethanol by vortexing and then centrifuged for 5 minutes at 13000 rpm. The ethanol was removed and the pellet was vacuum dried for 5 minutes. The dried pellet was suspended in 100 µl 1X TE buffer and stored at -20ºC. Extractions were diluted 1:100 fold in sterile distilled water (SDW) before being used in PCR amplifications.

Generic sweepovirus primers, virus sense primer SPG3 (5'-ACT TCG AGA CAG CTA TCG TGC C-3') and anti-sense primer SPG4 (5'-AGC ATG GAT TCA CGC ACA GG-3') designed to anneal to nucleotide sequences in the coat protein gene (V1) and ORF C2 of SPLCV were used to amplify the intervening part of the sweepovirus genome (Li *et al.*, 2004). The expected band size for this product is 1148 base pairs (bp). The PCR was done in 25 µl reaction mixture of 2.5 µl 10X PCR buffer, 1.5 µl of 25 mM $MgCl_2$, 2 µl of 2.5 mM dNTP mix, 1.0 µl of each primer (20 µM), 0.1 µl (5 U/µl) of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), 1 µl of 1:100 fold diluted DNA extract and 15.9 µl of SDW. PCR conditions included an initial step of 94°C for 2 minutes; 11 cycles of 94°C for 40 seconds, 50°C for 40 seconds, 72°C for 90 seconds; 24 cycles of 94°C for 40 seconds,

52°C for 40 seconds, 72°C for 90 seconds; and 72°C for 10 minutes. PCR products were assessed by electrophoresis in 1.2% agarose gel in Tris-acetate (TAE) buffer, stained with ethidium bromide and viewed under UV light (Fig. 8).

Other PCR primer pairs known to react with sweepoviruses (PW 285-1 and PW 285-2 and the degenerate SPG1 and SPG2; Li *et al*., 2004) and degenerate primer pairs reacting broadly to non-sweepovirus begomoviruses (Deng A and Deng B; Deng *et al*., 1994) and PAL1c1960 and PAL1v1978, PAR1v722 and PAR1c715 (Rojas *et al*., 1993) were also tested on the sweepovirus-infected extracts using the same protocol.

3.2.3 Real-time quantitative PCR

SPLCV is considered to be detected inefficiently by PCR (Lotrakul *et al.*, 1998; Kokkinos and Clark, 2006b) whereas qPCR is more efficient (90% detection by qPCR *versus* 45% detection by PCR) (Kokkinos and Clark, 2006b). Despite this, qPCR equipment is not commonly available in Africa and requires expensive consumables, so the efficiency of the indicator plant, *I. setosa,* was compared to qPCR as a means of detecting the sweepovirus. Total nucleic acid was extracted from two middle leaves of each test plant using the above CTAB method. The quality and quantity of DNA were determined using a NanoDrop® ND-1000 Spectrophotometer. All samples were then diluted to a concentration of 10ng/µl before the qPCR assay.

A primer/probe set was designed to amplify a part of the DNA of SPLCV. The primers were SPLCV-543F (5'-GGG CTT ACC CAT CGT TTG G-3') and SPLCV-612R (5'-CCA TCC AAA CTT TAC CAT CAA-3') with SPLCV-562P as a probe (5'-TAA GAG TGT

GTG TGT TAA GTC TAT GGG CA-3'). qPCR was performed on a PE 7900 Sequence Detection System using PCR-96 M2-HS-C microplates sealed with optical adhesive covers (Applied Biosystems). The total reaction volume mixture of 25 µl contained 2.5 µl of 10X buffer A, (this buffer and 25mM $MgCl_2$ are specifically supplied with TaqMan® 1000 RXN Gold Pack, Part No. 430441), 5.5 µl of 25 mM $MgCl_2$, 2 µl of 2.5 mM dNTP mix, 1.0 µl of each primer (7.5 p mol/µl), 0.5 µl of TaqMan probe (5 p mol/µl), 0.125 µl of AmpliTaq Gold polymerase (5 U/µl), 1 µl of template DNA and 11.375 µl of molecular grade water. A negative control (molecular grade water), a positive control and a housekeeping gene, cytochrome oxidase [cox (Weller *et al.*, 2000)], were included on each plate. Cox was included to normalise for differences in DNA concentrations between samples. qPCR thermal cycler conditions used included 50ºC for 2 minutes, 95ºC for 10 minutes (AmpliTaq Gold activation) followed by 40 cycles of denaturation at 95ºC for 15 seconds and annealing/extension at 60ºC for 1 minutes. Each sample was duplicated to reduce pipetting errors.

3.2.4 Stability for detection by PCR and qPCR of the sweepovirus in:

3.2.4.1 Dry leaves

Sweet potato leaves from the naturally sweepovirus-infected plants of cv New Kawogo and graft-inoculated plants of cv Beauregard were air dried and kept at room temperatures for time intervals of 28, 56 and 84 days. Numbers of sweepovirus detections by PCR and qPCR were recorded (Table 6a).

3.2.4.2 Dry extracts

Nucleic acid extracts (extracted using CTAB method above) from the fresh leaves of cvs New Kawogo and Beauregard were divided into 5 aliquots of 10 µl each. Aliquots were vacuum-dried for 5 minutes on a low heat until they appeared completely dry. They were then kept dry at room temperature for 0, 3, 7, 14 and 21 days after which they were re-diluted in 10 µl of molecular grade water and stored at -20^{o}C. The samples at time interval 0 were re-suspended in 10 µl of molecular grade water immediately after drying and stored at -20^{o}C. Fresh sample extracts were included as a control of the drying process and nucleic acid dry storage. Sample extracts were tested using qPCR (Table 6b).

3.2.5 Sequencing the complete genome of the sweepovirus and phylogenetic analysis

(This work was done largely by Dr. Bettina Otto)

I. setosa plants were infected with the sweepovirus by grafting with the original infected New Kawogo plants from Kampala and grown for 4 weeks. DNA was extracted using the modified CTAB method described before. The complete genome was amplified using SPLCUV-*Bam*HI primers (SPLCUV-*Bam*HI: F 5'-GGA TCC TTT GAC GTT TGT ACA GGC-3' and SPLCUV-*Bam*HI: R 5'-GGA TCC TTA TTA GGC CTC CTA TCT-3'), resulting in one large (2.9 kb) molecule. This molecule was cloned into a pGEM-T Easy vector (Promega UK Ltd., Southampton). Clones were checked for the correct length of insert before sequencing (GeneService London, UK). Three independent clones were sequenced to ensure sequence identity and reliability. The sequence obtained was compared to other sweepoviruses found during a BLAST search (Table 7). Multiple sequence alignment was carried out using the software package CLUSTAL W (Higgins *et al*., 1994) before phylogenetic analyses were carried out to generate parsimonious trees

using PAUP 4.0 version 10 beta for Mac (Swofford, 2003) with bootstrapping for 1000 replicates (Fig. 9). A search for recombination events was done using RDP (Recombination Detection Programme) 3 Alpha 44 (Martin *et al*., 2010). Open reading frames (ORFs) and amino acid sequences of these other sweepoviruses were obtained by using the ORF Finder NCBI and the ExPASy Molecular Biology Server, Swiss Institute of Bioinformatics, Geneva, Switzerland and sequence identities were compared.

3.2.6 Distribution of the sweepovirus in sweet potato plants as determined by PCR

Different parts (petiole, midrib and lamina) of upper, middle and lower leaves of 12 sweet potato plants cv New Kawogo clonally-derived from the original infected plants were tested by PCR. Virus distribution in a plant was assessed from the numbers of samples that tested positive for each leaf position/site (Table 9).

3.2.7 Prevalence of sweepovirus(es) in different Ugandan sweet potato cultivars and locations

The middle leaves of 207 asymptomatic plants of 8 sweet potato cultivars were randomly selected from farmers' fields in Soroti district in eastern Uganda and from farmers' and researchers' fields in Luwero and neighbouring Wakiso districts in central Uganda. The cultivars were the landraces New Kawogo, Dimbuka, Ejumula, Araka White and Tanzania, the improved popular variety NASPOT 1, and two clones selected by farmers during participatory breeding coded 1081L and 318L. Midrib leaf samples were tested by PCR as described above. Because earlier results had shown that PCR at best detected only 2 of every 3 sweepovirus infected plants, two samples from each leaf of all New Kawogo and

Ejumula (the main varieties tested) samples were tested; the combined results are shown in Table 10.

3.2.8 Reversion from sweepovirus infection in Ugandan landraces as determined by grafting to *I. setosa* and qPCR

A cutting was taken from naturally-infected plants of cvs New Kawogo, Dimbuka and NASPOT 1 obtained from central Uganda. Each resulting plant was grown in the glasshouse at NRI and the terminal tip [about 2cm long] was removed after 4 months and grafted to one week old *I. setosa* seedlings which were examined for leaf curling symptoms 3 weeks later. Removing the tip caused buds along the main stem to sprout and also promoted growth of already-established shoots at the base of the plant. Two weeks later, tips of new shoots from along the main stem and from basal shoots were grafted to individual *I. setosa* and also examined for leaf curling symptoms 3 weeks later. All shoots of cvs Dimbuka and NASPOT 1 tested by grafting were also tested by qPCR using the first lower leaf of the scion before being grafted to *I. setosa* (Table 11).

3.3 Results

3.3.1 Initial graft inoculations and PCR tests

The *I. setosa* plants grafted with scions from two symptomless plants cv New Kawogo collected in Kampala developed upward leaf curling, vein thickening and leaf chlorosis, symptoms typical of sweepovirus infection (Fig. 7). PCR tests on these symptomatic *I. setosa* and asymptomatic source sweet potato using the sweepovirus-generic primer set SPG3 and SPG4 were positive (Fig. 8). Three other plants of sweet potato from Uganda

maintained at NRI because they had SPVD and were dually infected with SPCSV and SPFMV were also found to be infected with a sweepovirus when tested with PCR using primer set SPG3 and SPG4. These plants had typical symptoms of SPVD and showed no additional symptoms, for example, of leaf curling. The primer sets SPG1 and SPG2, and PW 285-1 and PW 285-2 previously reported to detect SPLCV-Taiwan (Li *et al*., 2004) also amplified a part of the Ugandan sweepovirus genome. The other degenerate primer sets tested, which had been developed to detect non-sweepovirus begomoviruses (Rojas *et al*., 1993; Deng *et al*., 1994), failed to amplify a part of the Ugandan sweepovirus genome.

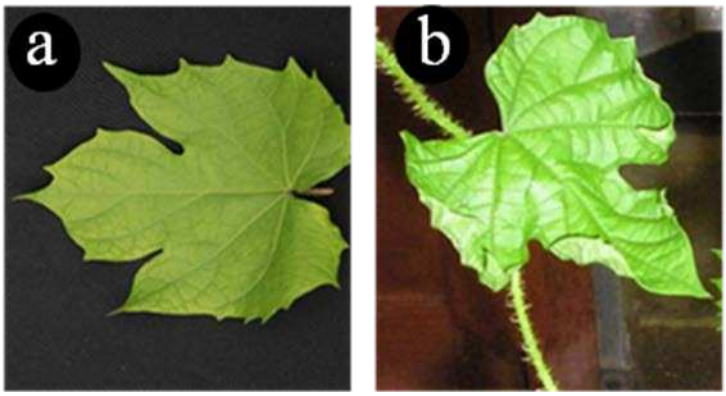


Figure 7. *I. setosa* leaves showing leaf curling symptoms induced by SPLCUV. Plate a shows healthy control leaf. Plate b shows *I. setosa* graft inoculated with SPLCUV

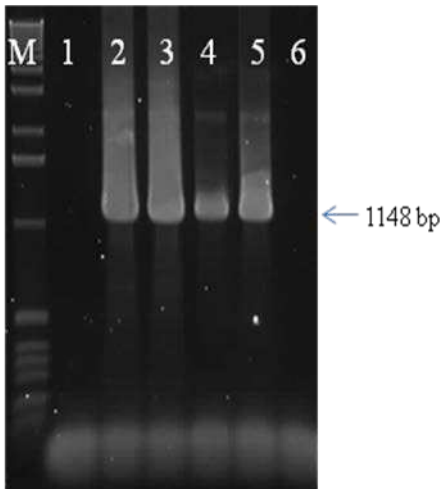


Figure 8. PCR identifying a sweepovirus in graft-inoculated *I. setosa* plants and in source sweet potato plants cv New Kawogo. Lanes: M = 1kb Marker, 1 = *I. setosa* grafted with healthy New Kawogo sweet potato, 2 = *I. setosa* grafted with infected New Kawogo plant 1, 3 = *I. setosa* grafted with infected New Kawogo plant 2, 4 = Sweet potato cv New Kawogo plant 1, 5 = Sweet potato cv New Kawogo plant 2, 6 = SDW. Primers SPG3 and SPG4 were used. The arrow indicates the band of the expected size.

3.3.2 Stability of the sweepovirus in dry leaves and extracts

The Ugandan sweepovirus was detected in dried leaves stored for up to 84 days and in dried DNA extracts for up to 21 days. qPCR detected the Ugandan sweepovirus in all infected leaf samples whereas PCR detected it in most of the infected samples (Table 6).

Table 6. Number of dried leaves and DNA extracts that tested positive for a sweepovirus after storage

a) Dried leaves

Dried leaf storage time (days)	No. of dried leaves that tested positive by:			
	PCR		qPCR	
	Cv New Kawogo	Cv Beauregard	Cv New Kawogo	Cv Beauregard
28	4/5	5/5	5/5	5/5
56	4/5	3/5	5/5	5/5
84	5/5	4/5	5/5	5/5

b) Dried DNA extract

Dried DNA extract storage time (days)	No. of dried DNA extracts that tested positive using qPCR	
	Cv New Kawogo	Cv Beauregard
0	2/2	2/2
3	2/2	2/2
7	2/2	2/2
14	2/2	2/2
21	2/2	2/2

3.3.3 Sequencing the complete genome of the sweepovirus and phylogenetic analysis

(This work was done largely by Dr. Bettina Otto)

The complete genome sequence of the Ugandan sweepovirus was 2799 nt long and contained six ORFs, as expected for a typical monopartite begomovirus. Two ORFs were in the sense direction and four ORFs were in the antisense direction. The two ORFs found on the viral sense strand encode the coat protein (V1) and the partially overlapping pre-coat protein (V2). The four ORFs found on the complementary strand encode the replication-associated protein (C1), transactivator protein (C2), replication enhancer protein (C3) and C4 protein. Based on comparison with other sweepoviruses, the Ugandan sweepovirus showed highest identity to *Sweet potato golden vein associated virus* (SPGVaV)-PB1[BR:Sou1] and *Sweet potato leaf curl Lanzarote virus* (SPLCLaV)-[ES:CI:BG27:02] at 87% nt identity [Table 7; (see Table 8 for full version of virus names)]. The phylogenetic relationship between it and other sweepoviruses including several SPLCV isolates revealed that it grouped together with other sweepoviruses [Fig. 9; (see Table 8 for full version of virus names)]. Overall comparison of nucleotide sequences revealed that none of the other sweepoviruses was more than 87% identical, making it a separate virus species considering the cut-off point of 89% identity established for separating species of the genus *Begomovirus* (Fauquet *et al*., 2003). There was no evidence of recombination events.

Table 7. Percent nucleotide identities for the complete genome and ORFs of SPLCUV with other sweepoviruses

	Open reading frames						
Virus name	DNA	V1 (coat protein)	V2	C1	C2	C3	C4
SPLCCV	84.0	98.0	97.3	79.4	70.1	79.9	69.4
SPGVaV-PB1[BR:Sou1]	87.0	94.9	89.2	90.0	77.7	77.1	63.5
SPLCLaV-[ES:CI:BG27:02]	87.0	92.9	88.5	94.3	80.4	79.2	88.2
SPLCV-RS1-[BR:Tav1]	86.9	94.1	91.4	94.8	79.1	79.2	83.5
SPLCLaV-[ES:Mal:BG30:06]	86.6	92.5	89.4	95.1	77.0	77.1	89.4
SPLCV-[MerN4]	86.0	95.7	87.6	87.1	79.1	75.7	70.6
SPLCV-[PR80]	85.9	95.7	92.0	86.8	79.1	75.7	70.6
SPLCV-Japan	85.4	95.7	90.6	94.6	66.7	68.8	87.1
SPLCV-ES[ES:CI:BG12:02]	85.4	95.3	91.2	86.8	80.4	84.4	68.2
SPLCCaV-[ES:CI:BG21:02]	85.3	93.3	90.3	90.0	62.2	67.4	67.1
SPLCV-ES[ES:CI: BG1:02]	85.3	94.5	90.3		80.4	84.7	68.2

Table 8. Virus names, accession numbers and abbreviations

Virus name	Origin	Accession No.	Abbreviation
Ipomoea yellow vein virus	Spain	AJ132548	IYVV-[ES98]
Ipomoea yellow vein virus	Spain	EU839576	IYVV-[ES:Mal:IG1:06]
Ipomoea yellow vein virus	Spain	EU839577	IYVV-[ES:Mal:IG3:06]
Ipomoea yellow vein virus	Spain	EU839578	IYVV-[ES:Mal:IG5:06]
Merremia leaf curl virus	Puerto Rico	DQ644561	MLCV
Sweet potato golden vein associated virus	Brazil	FJ969829	SPGVaV-PA-[BR:Bel1]
Sweet potato golden vein associated virus	Brazil	FJ969830	SPGVaV-PB1[BR:Sou1]
Sweet potato leaf curl Canary virus	Spain	EF456741	SPLCESV-[ES:CI:BG1:02]
Sweet potato leaf curl Canary virus	Spain	EF456742	SPLCCaV-[ES:CI:BG4:02]
Sweet potato leaf curl Canary virus	Spain	EF456745	SPLCCaV-[ES:CI:BG7:02]
Sweet potato leaf curl Canary virus	Spain	EU856365	SPLCCaV-[ES:CI:BG21:02]
Sweet potato leaf curl Canary virus	Spain	FJ151200	SPLCESV-[ES:Mal:IG2:06]
Sweet potato leaf curl Canary virus	Spain	FJ529203	SPLCCaV-[ES:CI:BG25:02]

Sweet potato leaf curl Georgia virus	U.S.A	AF326775	SPLCGV-[US:Geo:16]
Sweet potato leaf curl Korean virus	South Korea	FJ560719	SPLCKrV
Sweet potato leaf curl Lanzarote virus	Spain	EU839579	SPLCLaV-[ES:Mal:BG30:06]
Sweet potato leaf curl Lanzarote virus	Spain	EF456746	SPLCLaV-[ES:CI:BG27:02]
Sweet potato leaf curl virus	Brazil	FJ969832	SPLCV-CE-[BR:For1]
Sweet potato leaf curl virus	Brazil	FJ969833	SPLCV-RS1-[BR:Tav1]
Sweet potato leaf curl virus	Brazil	FJ969834	SPLCV-RS2-[BR:Est1]
Sweet potato leaf curl virus	Brazil	FJ969835	SPLCV-RS2-[BR:Mac1]
Sweet potato leaf curl virus	Brazil	FJ969836	SPLCV-RS2-[BR:Poa1]
Sweet potato leaf curl virus	Brazil	FJ969837	SPLCV-RS2-[BR:Ros1]
Sweet Potato leaf curl China virus	China	DQ512731	SPLCCV
Sweet potato leaf curl virus	China	EU253456	SPLCV-[RL31]
Sweet potato leaf curl virus	China	EU267799	SPLCV-[RL7]
Sweet potato leaf curl virus	Eastern China	FJ176701	SPLCV-China
Sweet potato leaf curl virus	India	FN432356	SPLCV-Bengal
Sweet Potato leaf curl virus	Italy	AJ586885	SPLCV-[IT:Sic:02]
Sweet potato leaf curl virus	Japan	AB433786	SPLCV-Japan
Sweet potato leaf curl virus	Puerto Rico	DQ644562	SPLCV-[PR80]

Sweet potato leaf curl virus	Puerto Rico	DQ644563	SPLCV-[MerN4]
Sweet potato leaf curl virus	U.S.A	AF104036	SPLCV-[US:Lou:94]
Sweet potato leaf curl virus Spain	Spain	EF456744	SPLCV-ES[ES:CI:BG6:02]
Sweet potato leaf curl virus Spain	Spain	EU856364	SPLCV-ES[ES:CI:BG12:02]
Sweet potato leaf curl virus Spain	Spain	EU856366	SPLCV-ES[ES:CI:BG13:02]
Sweet potato mosaic-associated virus	Brazil	FJ969831	SPMaV-[BR:BSB1]
Non-sweepo begomoviruses: Old World			
Papaya leaf curl virus	India	DQ629103	PaLCV-New-Dehli
Stachytarpheta leaf curl virus	China	AJ495814	StaLCV-Hn5

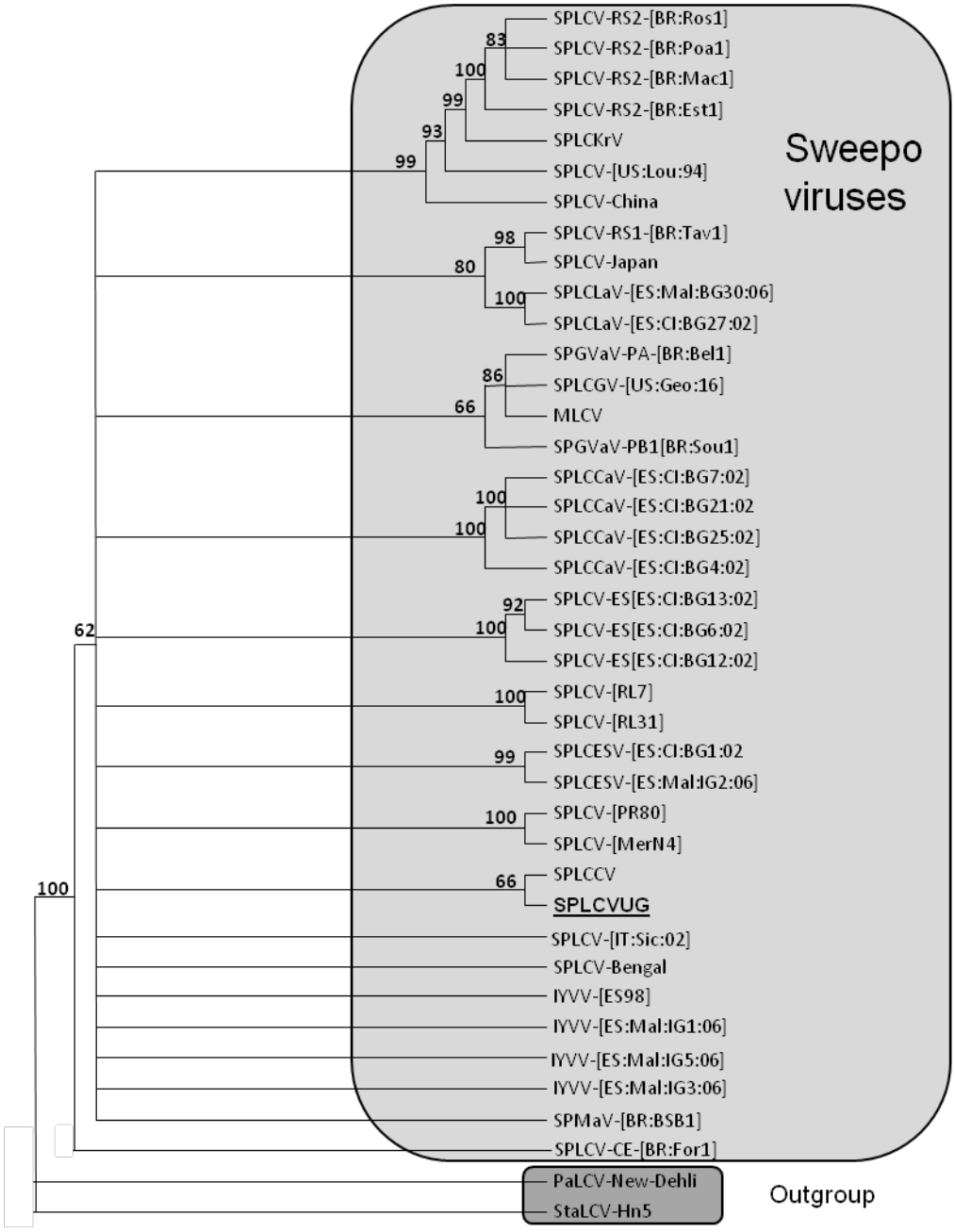


Figure 9. Consensus parsimonius tree made from the nucleotide alignments of complete genomes showing the relationship between SPLCUV and other begomoviruses. Numbers at nodes indicate percent bootstrap scores using 1000 replicates (stringency of 60%)

3.3.4 Distribution of the sweepovirus in sweet potato plants as determined by PCR

Overall, PCR detected the sweepovirus at most in only about 2 of every 3 samples from 12 infected clonally propagated New Kawogo plants, detections being mostly in middle or lower leaves and in the midrib region of these leaves (Table 9).

Table 9. Detection and distribution of sweepovirus (SPLCUV) in 12 infected plants of cv New Kawogo

	Upper leaves	Middle leaves	Lower leaves	Total
Petiole	0/12 (0%)	2/12 (17%)	0/6* (0%)	2/30
Midrib	3/12 (25%)	7/12 (58%)	3/6* (50%)	13/30
Lamina	2/12 (17%)	4/12 (33%)	4/6* (67%)	10/30
Total	5/36	13/36	7/18	

* Only six plants had physiologically old lower leaves

3.3.5 Prevalence of sweepovirus(es) in different Ugandan sweet potato cultivars and locations

Sweepovirus(es) were detected in plants of cvs New Kawogo, Ejumula and 318L and in plants originating in Wakiso, Luwero and Soroti Districts. Initially, 12 New Kawogo and 3 Ejumula tested positive; re-testing the negative samples detected a further 3 positive New Kawogo samples and 2 positive Ejumula samples (Table 10). Infected plants appeared to be more common in farmers' fields than at a research station though samples were too few and unbalanced in terms of variety for this to be statistically analysed.

Table 10. Prevalence of sweepovirus(es) in different locations and cultivars in Uganda

Sweet potato cultivar	Source of samples		No of infected samples	
		District	positives/total	%
New Kawogo	University field collection	Unknown origin	1/2	50
New Kawogo	Farmer's field	Luwero	8/25	32
New Kawogo	NaCRRI	Wakiso	3/26	11.5
New Kawogo	Farmer's field	Luwero	2/25	8
New Kawogo	Farmer's field	Luwero	1/25	4
Subtotal			15/103	15
318 L	Farmer's field	Luwero	6/15	40
1081 L	NaCRRI	Wakiso	0/15	0
NASPOT 1	NaCRRI	Wakiso	0/14	0
Dimbuka	NaCRRI	Wakiso	0/15	0
Tanzania	NaCRRI	Wakiso	0/15	0
Tanzania	Farmer's field	Soroti	0/10	0
Ejumula	NaCRRI	Wakiso	0/13	0
Ejumula	Farmer's field	Soroti	5/10	50

NaCRRI = National Crops Resources Research Institute

3.3.6 Reversion from sweepovirus infection in Ugandan landraces as determined by grafting to *I. setosa* and qPCR

Some terminal shoots, side shoots and basal shoots of known infected single plants of cvs New Kawogo, NASPOT 1 and Dimbuka, were identified as sweepovirus-free by use of qPCR as well as graft-inoculation to *I. setosa* (Table 11). All scions that tested positive with *I. setosa* also tested positive with qPCR and those that tested negative with *I. setosa* tested negative with qPCR. This reversion was more often observed in cv New Kawogo, the terminal shoot, all the side shoots and 3 of the 7 basal shoots of this plant testing negative (Table 11). Only one of the 3 side shoots from both Dimbuka and NASPOT 1 tested negative and none of the terminal shoots reverted (Table 11). In addition to these tests, infection was frequently lost during routine maintenance of the New Kawogo plants using cuttings.

Table 11. Reversion from SPLCUV in sweet potato cultivars as determined by grafting to *I. setosa* and qPCR

Cultivar	No. of shoots testing negative/No. of shoots tested					
	Terminal		Side		Basal	
	I. setosa	qPCR	*I. setosa*	qPCR	*I. setosa*	qPCR
New Kawogo	1/1	*	7/7	*	3/7	*
NASPOT 1	0/1	0/1	1/3	1/3	**	**
Dimbuka	0/1	0/1	1/3	1/3	0/1	0/1

*****qPCR not performed

****** No basal shoots to test

3.4 Discussion

The presence of a begomovirus infecting sweet potato (sweepovirus) in Uganda has been demonstrated for the first time by the development of typical leaf curl symptoms on graft-inoculated *I. setosa*, by its detection by PCR and qPCR only with sweepovirus-specific primers (Li *et al*., 2004) and by the similarity of its genome both in terms of its length, organisation into six ORFs typical of other sweepoviruses (Paprotka *et al*., 2010) and nucleic acid sequence (Lozano *et al*., 2009) (Table 7). This is the first reported complete genome sequence of a sweepovirus from mainland Africa and is also only the second time a sweepovirus has been reported there. The sequence of the DNA of the Ugandan sweepovirus we sequenced differs from those of other reported sweepoviruses by at least 13%, which is beyond the begomovirus species demarcation limit (Fauquet *et al*., 2003) and we have therefore tentatively named it Sweet potato leaf curl Uganda virus (SPLCUV). Phylogenetic analysis separates sweepoviruses basally from other begomoviruses (Fauquet and Stanley, 2003) and, consistent with this, degenerate primer pairs designed for detection of non-sweepovirus begomoviruses (Rojas *et al*., 1993; Deng *et al*., 1994) failed to detect SPLCUV. Unlike other begomovirus clusters, the sweepovirus cluster has no clearly identifiable continental origin (Briddon *et al*., 2010) and, again consistent with this, sweepoviruses most closely related to SPLCUV occur in countries in different continents – Brazil (Paprotka *et al*., 2010) and Spain (Lozano *et al*., 2009) (Table 7 and 8). Sweet potato storage roots can survive several months and sprout readily, so providing a means by which infected planting material is likely to have been transferred between continents historically as well as recently; this may explain the basal divergence of sweepoviruses from other begomoviruses, their lack of a clear geographical origin and

the poor correspondence between geographical distribution and apparent phylogenetic relationships.

The low efficiency of PCR at detecting SPLCUV in samples from known infected plants is in agreement with earlier observations by Lotrakul *et al.* (1998) who were unable to amplify SPLCV-US DNA products by PCR from sweet potato in quantities that could be visualised by ethidium bromide. We, like Kokkinos and Clark (2006b), could detect SPLCUV by PCR in sweet potato and not all PCR tests of known infected plants/samples were positive. qPCR is perhaps a 1000 fold more sensitive than PCR (Kokkinos and Clark, 2006b) and we similarly found a higher rate of positive samples using this technology. SPLCUV, however, appears not to be evenly distributed in plants of Ugandan sweet potato landraces, highest rates of detection by PCR being in the region of the midrib of mature leaves (Table 9). Even testing midrib samples did not give 100% positive results with PCR, qPCR and graft-inoculating *I. setosa* seedlings and it seems likely that some of the failures to detect result from the restricted distribution of SPLCUV in plants rather than the insensitivity of the test. We have shown that PCR detected SPLCUV with similar efficiency to fresh samples in dried leaf samples and in dried DNA extracts kept for nearly 3 months and 3 weeks respectively at room conditions (Table 6), providing the valuable opportunity of allowing samples to be sent to specialist centres for analysis. qPCR, as well as being more sensitive, is also safer than PCR as it avoids using ethidium bromide and UV light but it requires special instruments and very expensive reagents which many laboratories in developing countries like Uganda do not have. Graft-inoculating *I. setosa* plants seems a cheaper test for sweepoviruses, especially with the back-up of PCR to provide specificity.

SPLCUV has been overlooked in the past years, perhaps partly because it is asymptomatic. Interestingly, the lack of leaf curl symptoms in sweet potato also extended to sweet potato plants co-infected with SPCSV and SPFMV, the symptoms of such plants remaining as typical SPVD and preliminary qPCR assays, not described herein, not revealing unusually high titres of SPLCV. This seems unusual as most sweet potato viruses (Gibson *et al.*, 1998) and another sweepovirus (WJ Cuellar, personal communication) are synergised by SPCSV; indeed, SPLCUV may be the first sweet potato-infecting virus that is not synergised.

Sweepovirus infection appears to be widespread and fairly common in Uganda now, being detected by PCR in crops in both central and eastern Uganda and in 3 sweet potato cultivars. No leaf curl symptoms were, however, observed following graft inoculations to *I. setosa* of 116 symptomless field sweet potato plants in Uganda in the mid-1990s, including plants of cv New Kawogo (Gibson *et al*., 1997). In more recent and extensive virus surveys of sweet potato in both Uganda (Mukasa *et al*., 2003; Aritua *et al*., 2007) and Kenya (Ateka *et al*., 2004b), both diseased and symptomless field sweet potato plants were grafted to *I. setosa*. Some leaf curling symptoms on sweet potato and on graft-inoculated *I. setosa* were noted by Mukasa *et al*. (2003) but Aritua *et al*. (2007) could not confirm similarly diseased plants to be infected by a begomovirus by PCR. Ateka *et al*. (2004b) grafted 607 symptomless field plants onto *I. setosa* but no unusual symptoms were noted; although some symptoms of unknown aetiology were observed on sweet potato plants, none were observed when these were graft-inoculated to *I. setosa*. Tairo *et al*. (2004) also reported no evidence of a begomovirus in a survey in neighbouring Tanzania. Since sweepovirus infections induce clear leaf curl symptoms on *I. setosa*, these results suggest

that sweepovirus(es) may have been rare in, if not absent from, East Africa until recently. However, a probable sweepovirus was reported in 1984 in Nigeria, the disease causing upward rolling of sweet potato leaves and being transmitted by whiteflies (Rossel and Thottappilly, 1988).

This previous rarity may have resulted from it being a recent invader or from the resistance in landraces to infection evidenced by the limited distribution and reversion to healthy observed in scion tips and from entire cuttings taken from infected plants during routine propagation. The partial sequence reported by Miano *et al.* (2006) is not identical with the comparable region in our isolate consistent with a diversity of sweepoviruses evolving and/or arriving in Africa over a long time period – hence we are not sure if infection in the field is with one or several sweepoviruses. In cassava, the most recent epidemic of begomovirus infections was associated with a massive increase in population of its whitefly vector, *B. tabaci*, on the crop (Gibson *et al*., 1996a), perhaps associated with a change in its biotype (Legg *et al*., 1994), and with a recombination event occurring between cassava begomoviruses to create a more virulent strain (Zhou *et al*., 1997). This epidemic devastated the Ugandan crop and spread throughout East Africa and beyond (Otim-Nape *et al*., 2000). The surveys of whitefly numbers on sweet potato in Uganda from 1996 to 1998 (Aritua *et al*., 1998a; 1999; Alicai *et al*., 1999a) pre-date our common detection of sweepovirus infection so a similar upsurge of whiteflies on sweet potato could have occurred undetected. Sweepoviruses also naturally recombine their DNA (Lozano *et al*., 2009), providing a fast means of evolving additional to individual gene mutation. No evidence for a recombinant origin of the SPLCUV genome was found but all other available complete sweepovirus sequences included in the analysis are non-African and are

therefore unlikely candidates for recombination. Infected plants yielded 26% less than virus-free controls in trials in the U.S.A (Clark and Hoy, 2006); recent spread of sweepovirus(es) may already have had a role in the decline in productivity rumoured in some varieties by farmers in Uganda.

The vital importance of this food crop to poor rural and peri-urban families throughout much of sub-Saharan Africa suggests there is an immediate need to repeat and extend the whitefly surveys in Uganda using the same method, to do a comprehensive survey of sweepovirus prevalence and diversity and to measure sweepovirus yield effects on common African varieties.

3.5 Observations made after publication: effect of environment on SPLCV symptom expression in *I. setosa*

Introduction

A sweepovirus was first identified in Uganda by its induction of clear leaf curling symptoms in *I. setosa* (Fig. 7) in a glasshouse in the UK. This was later confirmed by PCR (Fig. 8) and sequencing (Fig. 9) (Wasswa *et al*., 2011). However, previous researchers (Gibson *et al*., 1997; Mukasa *et al*., 2003; Aritua *et al*., 2007) had grafted thousands of plants to *I. setosa* in screenhouses in Uganda in search for sweet potato viruses but failed to detect SPLCUV. Despite the current relatively high prevalence of the virus in the country (Wasswa *et al*., 2011), hundreds of sweet potato plants were also grafted to *I. setosa* in this study in Uganda to screen for virus free plants but none of the symptoms

displayed resembled the leaf chlorosis and leaf curling symptoms normally induced by SPLCUV.

Material and Methods

To see if there is difference in SPLCV symptom expression (probably because of differing greenhouse conditions) between Uganda and UK, 41 cuttings of symptomless cv New Kawogo were randomly selected from 5 farmers' fields in Wakiso district, Uganda. Each cutting was divided into two and one of each of these cuttings was established in a screenhouse at NaCRRI, Wakiso district - Uganda for 4 weeks. Shoot tips were then obtained from these plants and were grafted to *I. setosa* grown at NACRRI and observations made for leaf curl symptoms 4 weeks after grafting (work done by Ms Scovia Adikini). The remaining halves of the cuttings were taken to the quarantine glasshouse at the University of Greenwich (NRI), UK, and grown in a glasshouse for 4 weeks. Thereafter, shoot tips were cut from 31 successfully established plants, grafted to *I. setosa* and observations made for leaf curl symptoms for 4 weeks. The ambient temperature in a greenhouse at NaCRRI ranged between 26^{o}C and 40^{o}C while that at NRI ranged from 24^{o}C to 36^{o}C.

Results

None of the 41 grafted *I. setosa* plants in Uganda showed leaf curl symptoms. However, 7 plants from 3 farms out of the 31 plants from 5 farms grafted in a glasshouse in the UK developed very clear leaf curling that was confirmed using PCR to be induced by a sweepovirus.

Discussion

These results suggest that sweepovirus symptoms in *I. setosa* are affected by environmental factors, such that they occur on *I. setosa* in UK greenhouse conditions but do not occur in Ugandan greenhouse conditions. It is thus possible that the sweepovirus(es) has, maybe for decades, been present but unnoticed in Uganda and neighbouring countries such as Kenya (Ateka *et al*., 2004b) and Tanzania (Tairo *et al*., 2004), where viruses have been diagnosed using this indicator plant. Its presence could thus be among the factors responsible for the occasional cultivar degeneration apparently observed in the country. This strengthens the need for an extensive survey of sweepoviruses in the country using nucleic acid analysis techniques, also to determine its effect on the yield of Ugandan landraces and to understand the environmental factors that influence symptom expression.

CHAPTER FOUR

IDENTIFICATION OF A 'MILD' STRAIN OF *SWEET POTATO CHLOROTIC STUNT VIRUS* IN UGANDA: ABSENCE OF CO-INFECTION WITH SPFMV IN THE FIELD AND IMPACT ON TITRES OF EXPERIMENTALLY CO-INFECTED SPFMV

4.1 Introduction

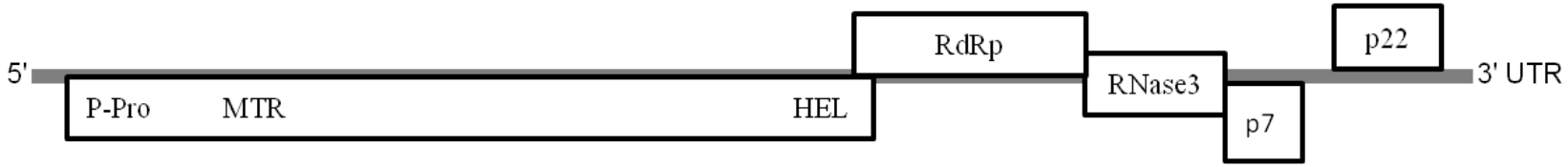
Among all viruses of the family *Closteroviridae*, only SPCSV infects sweet potato. It has a worldwide distribution and can be differentiated into two strains, denoted as EA (East Africa) and WA (West Africa) after the geographical location of their first description using serological and molecular characteristics. The EA group includes all isolates from East Africa, including Uganda, and some from South America whereas isolates from elsewhere in the World belong to the WA group (Alicai *et al*., 1999b; Fenby *et al*., 2002; IsHak *et al*., 2003).

SPCSV by itself causes mild symptoms in sweet potato (purpling or yellowing of lower leaves and lack of vigour) (chapter 2; Fig. 6) and yield losses of up to 50% (Gibson *et al*., 1998; Mukasa *et al*., 2006). Likewise, plants infected with SPFMV alone may have up to a 50% yield loss (Gibson *et al*., 1997; Njeru *et al*., 2004) but seldom show symptoms (Brunt *et al*., 1996; Kokkinos, 2006). However, SPCSV synergises SPFMV and other viruses to cause very severe symptoms of plant stunting, leaf distortion, chlorosis, mosaic or vein clearing (chapter 2; Fig. 6); co-infection with SPFMV results in the common severe disease known as SPVD (Gibson *et al*., 1998). SPVD can reduce the yield of affected

plants by up to 98% (Hanh, 1979; Ngeve and Bouwkamp, 1991; Gibson *et al*., 1998; Gutierrez *et al*., 2003) and is the main way in which these viruses directly constrain the yield of sweet potato in Uganda and in much of the Tropics including Africa.

The SPCSV genome is one of the largest of plant viruses (Fig. 10) (Kreuze *et al*., 2002), probably having up to 12 ORFs. It is bipartite, RNA1 (9407 nt) possessing 5 putative ORFs and RNA2 (8223 nt) containing 7 putative ORFs (Agranovsky *et al*., 1991; Cohen *et al*., 1992). In Uganda, a variant of SPCSV is found which possesses RNase3 as well as p22 genes on RNA1 (Fig. 10; Kreuze *et al*., 2002; Cuellar *et al.,* 2008). The RNase3 gene, together with the p22 gene, breaks down the host plant's resistance based on RNA silencing, probably by cleaving the small interfering (si)RNAs on which RNA silencing is based (Kreuze *et al*., 2002; Kreuze *et al*., 2005). When SPFMV co-infects, it benefits from this destruction of the host's resistance, resulting in an increase of up to 600 fold in its titres (Mukasa *et al*., 2006) and causing the severe symptoms of SPVD (Ngeve and Bouwkamp, 1991; Gibson *et al*., 1998; Karyeija *et al*., 2000; Gutierrez *et al*., 2003; Mukasa *et al*., 2006), while leaving SPCSV titres little affected (Gibson *et al*., 1998) or slightly reduced (Kokkinos and Clark, 2006a).

RNA1



RNA2

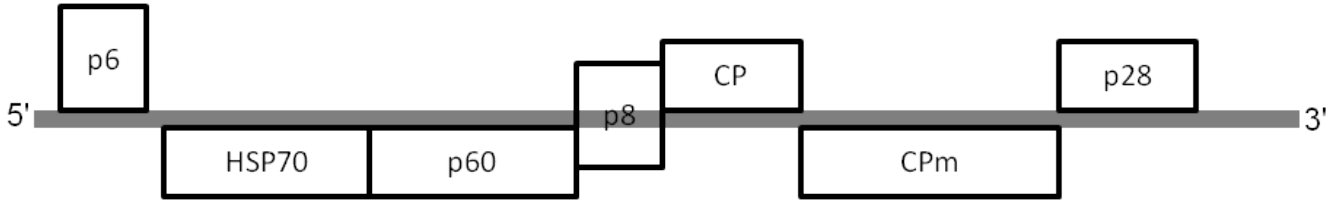


Figure 10. Genomic structure of SPCSV showing RNA1 and RNA2 regions with ORFs indicated by boxes. P-Pro, putative papain-like leader proteinase; MTR, methyltransferase domain; HEL, helicase domain; RdRp, RNA-dependant RNA polymerase domain; RNase3, RNase III-like domain; HSP70h, heat shock protein 70 family homologue; CP, coat protein; CPm, minor coat protein (Kreuze *et al*., 2002)

Because of the key role RNase3 plays in the development of SPVD and the difficulty in incorporating natural resistance to SPCSV in elite cultivars, efforts to control the disease have been targeted towards knocking out RNase3 gene. Cuellar *et al*. (2008) used engineered resistance to SPCSV to target RNA silencing against the SPCSV polymerase region (viral RNA-dependent RNA polymerase, RdRp) with an intron-spliced hair pin construct. However, only 10 out of 20 transgenic events challenged with SPCSV alone showed significant reduction in virus titres and even this was not sufficient to prevent SPVD upon co-infection with SPFMV.

Alternative means of immunity to SPCSV are still being sought to control SPVD. In 2008, 255 apparently healthy sweet potato cultivars from farmers' fields were collected in Uganda as a potential source of farmer-preferred virus free elite cultivars. Plants were established in pots in a screenhouse for 4 months before being tested for viruses. During this time, the netting of the screenhouse was torn, exposing the plants to vectors for more than 3 months after which the plants were tested using NCM-ELISA kit originating from CIP, Peru. One plant of cv Kampala White from a field in Busia had SPCSV alone; 18 of the other plants were infected with SPFMV + SPCSV and 44 were infected with SPFMV alone (192 appeared to be virus free). This single SPCSV infection caused only mild chlorosis symptoms in the indicator plant, *I. setosa*. It was investigated further, partly in case it could cross protect and so provide means of controlling the wild type. This led eventually to a study of the mechanism behind the absence of co-infecting SPFMV with this 'mild' SPCSV.

4.2 Materials and Methods

4.2.1 Survey of farmer-grown sweet potato cultivars and prevalence of SPFMV, SPCSV and SPVD in Busia district

A survey of farmer-grown sweet potato cultivars for SPFMV, SPCSV and SPVD prevalence was done in 2010 on five farms in Busia district where the original 'mild' SPCSV was obtained. Plants were assessed by visually observing symptoms because 'mild' SPCSV was observed to induce quite distinct purpling in infected sweet potato plants, and SPVD affected plants, in normal cases, showed more severe symptoms including mosaic or vein clearing on affected leaves. The distance between sampled fields was about 6 km and, in each field, all mounds were closely inspected for disease symptoms, each mound being an observational unit. Incidences of SPCSV and SPVD were calculated as the percentage of the vines showing SPCSV or SPVD symptoms out of the total number of vines assessed in a field. The total number of vines in a field was obtained by multiplying the number of mounds by 6, the number of vines normally planted in each mound in Busia district. Local names for cultivars were used. Sample cuttings were collected and established in a screenhouse at MUARIK, Wakiso district, central Uganda. These were later grafted to *I. setosa* to check visual observations and to assess the prevalence of SPFMV infecting by itself.

4.2.2 Re-testing cv Kampala White to confirm single infection by 'mild' SPCSV

A cutting of cv Kampala White infected with 'mild' SPCSV was established in a greenhouse at FERA and re-tested for SPFMV and SPCSV by grafting to one week old *I. setosa* seedlings and using RT-qPCR. Observations for symptom development were made

up to 4 weeks after graft inoculation. Total nucleic acid (TNA) was extracted from leaves of 'mild' SPCSV infected cv Kampala White and *I. setosa* using CTAB method, described originally by Lodhi *et al*. (1994) and later modified (Maruthi *et al*., 2002) (chapter 3, section 3.2.2). The quality and quantity of RNA were determined using a NanoDrop® 2000 Spectrophotometer. All samples were then diluted to a concentration of 10ng/µl before being used in the RT-qPCR amplifications.

Degenerate SPCSV primers designed for the heat shock protein 70 (HSP70) region [EA-SPCSV-38F (5'-GGA GTT TAT TCC CAC CTG TYT ATC TG-3'), EA-SPCSV-126R (5'-GGT AAT TGC GAA GAA TCY AAA ACC-3') and the probe EA-SPCSV-67P* (5'-CGG CTA CAG GCG ACG TG-3')] and specific SPFMV primers designed using the CP region [SPFMV-Uni-818F (5'-CGC ATA ATC GGT TGT TTG GTT T-3'), SPFMV-Uni-925R (5'-TTC CTA AGA GGT TAT GTA TAT TTC TAG TAA CAT CAG-3') and the probe SPFMV-Uni-847P * (5'-AAC GTC TCC ACG CAA GAA GAG GAT GC-3')] were used. The TaqMan probe method of RT-qPCR reaction was performed on Mastercycler® ep realplex Sequence Detection System using PCR microplates (twin.tec PCR plate 96, skirted) that were sealed with optical adhesive covers (Applied Biosystems).

The total reaction volume mixture of 25 µl contained 2.5 µl of 10X buffer A, 5.5 µl of 25 mM $MgCl_2$, 2 µl of 2.5 mM dNTP mix, 1.0 µl of each primer (7.5 p mol/µl), 0.5 µl of TaqMan probe (5 p mol/µl), 0.05 µl of RT (M-MuLV 200U/µl), 0.125 µl of AmpliTaq Gold polymerase (5 U/µl), 1 µl of template RNA and 11.325 µl of molecular grade water. A negative control (molecular grade water), a positive control (RNA from SPVD affected sweet potato plant) and a housekeeping gene [cytochrome oxidase (cox)] (Weller *et al.,*

2000), were included on the plate and each sample was duplicated to reduce pipetting errors. Cox was added to correct for differences in RNA concentrations between samples. RT-qPCR thermal cycler conditions used included 48°C for 30 minutes (cDNA synthesis), 95°C for 10 minutes (AmpliTaq Gold activation) followed by 40 cycles of denaturation at 95ºC for 15 seconds and annealing/extension at 60ºC for 60 seconds.

4.2.3 Effect of temperature on reversion from SPFMV in single infection and co-infection involving 'mild' SPCSV + SPFMV, and wild type SPCSV + SPFMV

Cultivar Kampala White plants infected with SPFMV alone, SPFMV + 'mild' SPCSV, and SPFMV + wild type SPCSV and cv Resisto having single infection of SPFMV were used. Each plant by virus combination was replicated twice and plants were grown in incubators (Leec, UK), one set at 28^{o}C with 12 h of light and 12 h of darkness and the other set at 35^{o}C with 12 h of light and 12 h of darkness for 2 weeks. Observations were made on leaf symptom development. Leaf samples were collected just before exposure of plants to heat treatment and also weekly during heat treatment, and SPFMV titre determined using RT-qPCR. At the end of 2 weeks of heat treatment, shoot tips were cut from each sweet potato plant and separately grafted to one week old *I. setosa* seedlings and observations were made on symptom development for 5 weeks. Leaf samples were collected at week 3, 4 and 5 and SPFMV titre determined using RT-qPCR.

4.2.4 Comparison of RNase3 and p22 of 'mild' SPCSV and wild type SPCSV strains

(Work done largely by Dr. Bettina Otto)

TNA was extracted using the CTAB method from an SPVD affected plant of an unknown cultivar from Kampala and the 'mild' SPCSV-infected cv Kampala White. cDNA was

synthesized from 6 µl of genomic RNA in a 22.5 µl reaction mixture using Superscript ™ III reverse transcriptase (200 U/µl) primed with random primers. Primer pairs; RNase3 F (5'-TCG TCG TTT CGY AAG ATT TTC G-3') and RNase3 R (5'-ARA CCA AAG TAG KGC CAC ATC AA-3') and p22 F (5'-CTT TGA ACG ATG AGT TCT GG-3') and p22 R (5'-CTA CCC TAA TAT CTT TAT CG-3') designed to anneal to specific regions of RNase3 and p22 genes, respectively, were used for PCR amplification of the respective cDNA templates.

The 25 µl PCR reaction mixture for each gene consisted of 2.5 µl of 10X reaction buffer, 0.5 µl of 25mM $MgCl_2$, 2.0 µl of 10 mM dNTPs, 0.5 µl of 20 mM of forward primer, 0.5 µl of 20 mM of reverse primer), 0.1 µl of 2.5 U of Taq DNA polymerase (Invitrogen), 2.0 µl of cDNA and sterile water to bring to 25 µl volume. cDNA was denatured at 94^{o}C for two minutes, 35 cycles of 94^{o}C for 30 seconds (denaturing), 52^{o}C (annealing) for 30 seconds, and 72^{o}C (extension) for 1 minute. The reaction was ended with a final extension step at 72^{o}C for 10 minutes. Amplicons were separated on a 1% agarose gel containing 0.1 µl of ethidium bromide per gram of gel in 1X Tris ethylene diamine tetra acetic acid (Tris-EDTA) buffer at 90 volts for 50 minutes and then viewed under UV light. Comparison was made on RNase3 band size between 'mild' SPCSV and wild type SPCSV strains. PCR products of both RNase3 and p22 of the 'mild' SPCSV strain were then separately cloned into pGEM-T Easy vectors (PromegaUKLtd). Clones were checked for the correct length of insert before sequencing (GeneService London). Three independent clones were sequenced for each gene to ensure sequence identity and reliability. The sequences obtained were compared to other sequences of RNase3 and p22 genes of SPCSV found in the GenBank (http://blast.ncbi.nlm.nih.gov) and in the literature (Tugume, 2010).

4.2.5 Determining the spread of 'mild' SPCSV and effect on yield

Further samples of 'mild' SPCSV-infected sweet potato cv Kampala White were collected from Busia in 2010. These were established in a screenhouse at NaCRRI and indexed for virus diseases using *I. setosa*. 120 plants were found to have 'mild' SPCSV and none had SPFMV. This material was multiplied and used to set up a field trial in a farmer's field in Busia district (eastern Uganda) and at MUARIK (central Uganda). Each experiment comprised four plots of healthy plants and four plots of 'mild' SPCSV-infected plants arranged in a replicated randomised Latin square. Each plot had four mounds each approximately 1 m in diameter. In Busia, 6 cuttings were planted per mound with 3 points each having 2 cuttings (this is the normal practice in Busia). At MUARIK, 3 cuttings were planted per mound with 3 points each having a single cutting (this is the normal practice in Wakiso). Whiteflies and aphids were counted in the fields at 19 weeks after planting. Observations were made at 25 weeks on the spread of SPVD and 'mild' SPCSV. Root quality and yield were evaluated; and mean and P values were generated by subjecting the yield data to ANOVA using GenStat 14.0 for Windows.

4.2.6 Effect of 'mild' SPCSV strain on wild type SPCSV in field and in greenhouse sweet potato plants and *I. setosa*

Field trials were planted in Busia and at MUARIK in Uganda using sweet potato cultivars Dimbuka (healthy material – tested by *I. setosa*) and Kampala White (healthy and 'mild' SPCSV-infected material – tested by *I. setosa*). Each experimental plot for each cultivar (and healthy status) had 36 mounds (6 x 6) with the 4 mounds in the centre planted with SPVD (SPFMV + wild type SPCSV) infectors of cv Kyebandula. Each mound was about 1 m in diameter and each experimental plot was replicated twice at each site. Plots were 5 m

apart with a barrier of maize planted between them. Using symptoms, comparison was made of the spread of SPCSV and SPVD from the SPVD infector to the healthy Dimbuka and Kampala White and the 'mild' SPCSV-infected Kampala White in both MUARIK and Busia to monitor if 'mild' SPCSV stops wild type SPCSV from bringing SPVD and whether Kampala White is resistant to SPFMV. Fifty cuttings were randomly selected from each of the experimental blocks and grafted to *I. setosa* to confirm visual observations. Mean virus/disease spread values and the significance of virus/disease spread were established by subjecting the data to ANOVA using GenStat 14.0 for Windows.

The greenhouse experiments were done at NRI. Shoot tip scions were cut from sweet potato plants of 'mild' SPCSV-infected cv Kampala White, wild type SPCSV-infected cv Beauregard and SPFMV-infected cv Resisto. SPFMV single infections and co-infections of SPFMV + 'mild' SPCSV, SPFMV + wild type SPCSV, and SPFMV + 'mild' SPCSV + wild type SPCSV were established by side grafting these to both *I. setosa* seedlings and healthy plants of cv Kampala White. The virus and virus combinations were done once in *I. setosa* but replicated twice in cv Kampala White. Plants were established from cuttings taken from the inoculated Kampala White plants to avoid any effects from the virus infector cuttings. Observations of foliar symptoms were done for 4 weeks in both *I. setosa* and cv Kampala White and several leaf samples from different stem positions from each of the plants were separately collected at the end of 4 weeks. In another experimental set up, cv Kampala White plants were separately graft inoculated with SPFMV alone, 'mild' SPCSV alone, SPFMV + 'mild' SPCSV, and SPFMV + wild type SPCSV. Observations for symptom development were done for 9 weeks and the oldest leaf samples were collected weekly from each of the plants and stored at -80^{o}C. At the end of 9 weeks, shoot

tips from 5 consecutive shoots along the main stem of each of the infected cv Kampala White were grafted to *I. setosa* and observations for symptom development done for 4 weeks. Healthy Kampala White plant was also grafted to *I. setosa* to act as a control.

All *I. setosa* and cv Kampala White leaf samples collected were used for determining SPFMV titre (using TaqMan probe method; see section 4.2.2) and SPCSV titre (using SYBR green method) using primer pairs based on the coat protein and HSP70 (RNA2) genes respectively. RNA1 titre (using P-Pro, MTR, HEL, RdRp, RNase3 and p22 genes) in co-infections of SPFMV + 'mild' SPCSV, and SPFMV + wild type SPCSV was also determined using SYBR green method of RT-qPCR. SPCSV primers designed using the HSP70 region [EA-SPCSV-38F and EA-SPCSV-126R (see section 4.2.2), P-Pro primers [P-Pro F (5'-GCG ACG AAA ACC GGA TTC TTT GAT CC-3') and P-Pro R (5'-GTA GGG CCC ATT CTA CCG AAC C-3')], MTR primers [MTR F (5'-GCA GGC GCT GTA TTC TCA AGG TC-3') and MTR R (5'-GAG GAC CTT AGT ACT AAA CTG CCT ATA A-3')], HEL primers [HEL-F (5'-CCA CCG TAR ACG CTG AAC YRA GT-3') and HEL-R (5'-CCT CTT CAA CGA CCA ACT TAG ATG TRG-3')], RdRp primers [RdRp-F 5'-TTT CAG TCG ACC TCC TGC GTC G-3' and RdRp-R (5'-ATG GTT AGG TCT CCT ACA GGT GGT AAT-3')], RNase3 primers [RNase3-F2 (5'-CCC GAC CAA ATG CAG TTG TG-3') and RNase3-R2 (5'-GCA CAA CCA ACY AAC CAA CG-3')] and p22 primers [p22-F1 (5'-CCC TAA AAT CAC TAA TCG ATG AG-3') and p22-R1 (5'-AAA GAT GAG GAT GCA ATC GTT G-3')] were used.

The quality and quantity of RNA were determined using a NanoDrop® 2000 Spectrophotometer. All samples were then diluted to a concentration of 10ng/µl using

molecular grade water. The diluted samples were DNase treated prior to cDNA synthesis and the total reaction volume mixture of 10 µl contained 4 µl of sample, 1 µl of RQ1 RNase free DNase 10X reaction buffer, 1 µl of RQ1 RNase free DNase and 4 µl of molecular grade water. The mixture was incubated at 37^{o}C for 30 minutes. 1 µl of RQ1 DNase stop solution was then added to terminate the reaction. The mixture was incubated at 65^{o}C for 10 minutes to inactivate the DNase. cDNA was synthesized from 6 µl of genomic RNA in a 22.5 µl reaction mixture using Superscript ^{TM} III reverse transcriptase (200 U/µl) primed with random primers. The 25 µl SYBR green qPCR reaction mixture consisted of 12.5 µl of SYBR, 8.5 µl of molecular grade water, 0.75 µl of 5 mM of each primer and 2.5 µl of cDNA. A negative control (molecular grade water) and a housekeeping gene [cytochrome oxidase (cox)] (Weller *et al.*, 2000), were included on the plate (twin.tec PCR plate 96, skirted) and each sample was duplicated to reduce pipetting errors. Plates were sealed with optical adhesive covers (Applied Biosystems). The reaction was performed on Mastercycler® ep realplex Sequence Detection System and qPCR thermal cycler conditions used include 95°C for 15 minutes (SYBR activation) followed by 40 cycles of denaturation at 94°C for 15 seconds, annealing at 52ºC for 30 seconds and extension at 72^{o}C for 30 seconds. Relative virus titre and gene expression data were analysed from the raw fluorescence data [Ct values at which a change in normalised reporter (ΔRn) crosses the threshold] using the $2^{(\_\text{Delta Delta C(T)})}$ method (Livak and Schmittgen, 2001). The fold change in virus titre (target gene) relative to the reference gene (cox) was determined by the equation: $Cq = 2^{-\Delta\Delta Ct}$: $Cq = 2^{-}[(Cq_{\text{target gene}}) - (Cq_{\text{reference gene}})] - [(\text{mean } Cq_{\text{target gene}}) - (\text{mean } Cq_{\text{reference gene}})]$, where Cq = cycle quantity, ΔΔCq = differences in Cq values between the target gene and reference gene.

4.3 Results

4.3.1 Survey of farmer-grown sweet potato cultivars and prevalence of SPFMV, SPCSV and SPVD in sweet potato fields in Busia district

Cultivar Kampala White was the predominant cultivar grown in Busia district found in fields on all the 5 farms visited (Table 12). Other cultivars grown included Bunduguza, Silk, and Mubirigwambidi and were either in separate fields or mixed with Kampala White. Although SPCSV symptoms were common, SPVD was rarely observed at any of the farms visited (Table 12). In confirmation, plant samples observed as having SPCSV or SPVD symptoms in the field reacted when grafted to *I. setosa* either by showing mild chlorosis for SPCSV or severe mosaic symptoms typical of plants with SPFMV + SPCSV. None of the plants sampled reacted as infected with SPFMV alone.

Table 12. Prevalence (by symptom observation and grafting samples to *I. setosa*) of SPFMV, SPCSV and SPVD (percentage in parenthesis) in sweet potato cultivars grown in Busia district

Farm No.	Cultivars grown	Total vines	Number of vines infected with:			No. of vines not infected
			SPCSV	SPFMV	SPCSV + SPFMV	
1	Kampala White	3300	165 (5%)	0 (0%)	0 (0%)	3135 (95%)
	Bunduguza	750	0 (0%)	0 (0%)	8 (1%)	742 (99%)
	Silk	450	0 (0%)	0 (0%)	0 (0%)	450 (100%)
2	Kampala White	2550	1260 (49.4%)	0 (0%)	3 (0.12%)	1287 (50.47%)
	Mubirigwambidi	600	0 (0%)	0 (0%)	30 (5%)	570 (95%)
	Silk	750	0 (0%)	0 (0%)	0 (0%)	750 (100%)
3	Kampala White	4350	216 (5%)	0 (0%)	0 (0%)	4134 (95%)
	Mubirigwambidi	2100	48 (2.3%)	0 (0%)	0 (0%)	2052 (97.7%)
	Bunduguza	1650	0 (0%)	0 (0%)	0 (0%)	1650 (100%)
	Silk	360	0 (0%)	0 (0%)	0 (0%)	360 (100%)
4	Kampala White	11700	1521 (13%)	0 (0%)	1 (0.01%)	10178 (86.99%)
5	Kampala White	10050	302 (3%)	0 (0%)	1 (0.01%)	9747 (96.98%)
Total		38610	3512 (9.1%)	0 (0%)	43 (0.1%)	35055 (90.8%)

4.3.2 Re-testing cv Kampala White to confirm single infection by 'mild' SPCSV

The 'mild' isolate of SPCSV when infecting alone produced purpling and chlorosis of leaves and general stunting typical of SPCSV (Fig. 11b). This isolate induced milder chlorotic symptoms compared to the more severe chlorotic symptoms induced by the wild type SPCSV in *I. setosa* (Fig. 11d *versus* 11e). Both the infected cv Kampala White plant and graft inoculated *I. setosa* tested positive only for SPCSV using RT-qPCR with Ct values of 22.03 and 20.48, respectively (Table 13).

Table 13. Ct values for the 'mild' SPCSV and SPFMV in sweet potato cv Kampala White and *I. setosa*

Ct value			
Kampala White		*I. setosa*	
SPCSV	SPFMV	SPCSV	SPFMV
22.03	(-)	20.48	(-)

(-) indicates negative plant sample

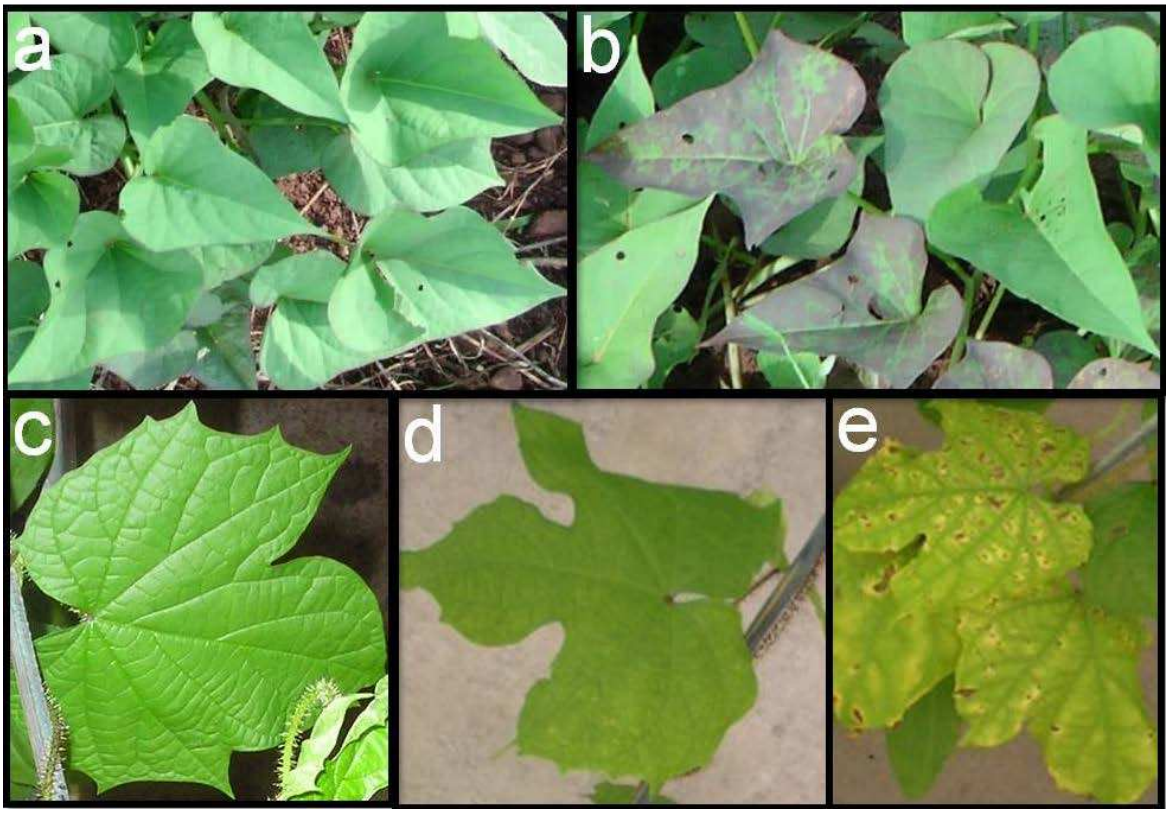


Figure 11. Mild SPCSV symptoms induced in *I. setosa* and purpling in sweet potato plants: a) Healthy field sweet potato plants of cv Kampala White, b) cv Kampala White infected with 'mild' SPCSV showing leaf purpling, c) healthy *I. setosa* leaf d) *I. setosa* leaf infected with 'mild' SPCSV showing mild chlorotic symptoms, and e) *I. setosa* leaf infected with wild type SPCSV with severe chlorotic symptoms

4.3.3 Effect of temperature on reversion from SPFMV in single infection and co-infection involving 'mild' SPCSV + SPFMV, and wild type SPCSV + SPFMV

Although cv Resisto did not revert from SPFMV, there was a clear observable effect of temperature on virus titre in this cultivar and in singly infected plants of cv Kampala White and plants co-infected with SPFMV + 'mild' SPCSV (Table 14). Cv Kampala White singly infected with SPFMV reverted at 2 weeks after heat treatment at 35^{o}C. SPFMV titre

was comparable between the two temperatures (28^{o}C and 35 ^{o}C) for co-infections of SPFMV + wild type SPCSV and there was no clear overall trend in virus titre over time (Table 14). Cv Kampala White which reacted negative for SPFMV using RT-qPCR also did not induce any symptoms in *I. setosa* whereas plants that reacted positive induced clear leaf symptoms typical of SPFMV or SPVD.

Table 14. SPFMV titre (average $2^{-\Delta\Delta Ct}$) over time (weeks) in heat treated sweet potato plants infected with SPFMV, SPFMV + 'mild' SPCSV, and SPFMV + wild type SPCSV and in *I. setosa* graft inoculated using heat treated plants

		Average $2^{-\Delta\Delta Ct}$							
		28°C				35°C			
		Cv Resisto	Cv Kampala White			Cv Resisto	Cv Kampala White		
	Weeks	SPFMV	SPFMV	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV	SPFMV	SPFMV	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV
Sweet potato	0	0.664*	0.094*	3.672*	34.114*	0.619*	0.058*	3.474*	42.399*
	1	0.137	0.048	6.407	52.936	0.035	0.006	1.517	40.218
	2	0.033	0.01	0.87	21.918	0.021	(-)	0.699	30.511
I. setosa	3	0.075	0.061	1.117	57.381	0.01	(-)	0.395	10.176
	4	0.164	0.097	9.884	49.912	0.015	(-)	0.766	25.027
	5	0.012	0.124	0.926	23.377	0.005	(-)	0.337	36.66

* just before heat treatment and (-) indicates negative reaction

4.3.4 Comparison of RNase3 and p22 genes of ‘mild’ SPCSV and wild type SPCSV

(Work done largely by Dr Bettina Otto)

RT-PCR results revealed the same size band of RNase3 from SPVD affected and ‘mild’ SPCSV-infected sweet potato plants (Fig. 12). Full sequences of RNase3 of ‘mild’ SPCSV (accession No. HE575406) compared closely to those RNase3 found in GenBank (http://blast.ncbi.nlm.nih.gov); only two amino acid (aa) changes at position 34 (G to D) and 159 (H to Y) were found between ‘mild’ RNase3 and those RNase3 found in the GenBank but showed no change to those RNase3 reported by Tugume (2010). p22 (accession No. HE575409) was also found in the ‘mild’ SPCSV and showed no change to other p22 sequences found in GenBank (http://blast.ncbi.nlm.nih.gov) and literature (Tugume, 2010).

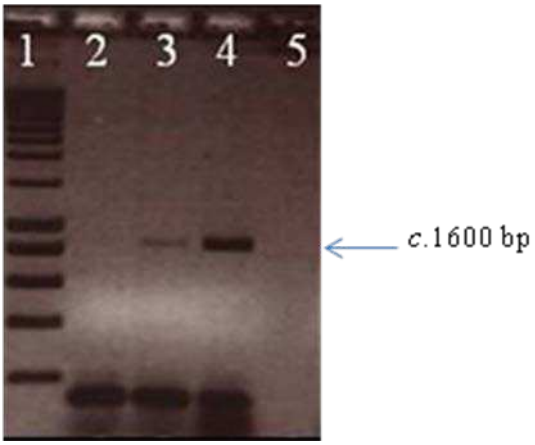


Figure 12. RT-PCR gel showing amplified products of RNase3 of ‘mild’ and wild type SPCSV. Lanes: 1 = 1 kb ladder; 2 = Kampala White healthy, 3 = RNase3 from wild type SPCSV, 4 = RNase3 from ‘mild’ SPCSV, 5 = Negative control (SDW)

4.3.5 Determining the spread of 'mild' SPCSV and effect on yield

The trial planted in Busia had very limited spread of SPCSV and there was no spread of SPVD. Only 6 plants on 4 mounds out of 96 plants on 16 mounds became infected despite having completely-infected neighbouring plots (Table 15). Whiteflies were few; only 4 whiteflies were counted per 5 minutes in the whole field when the field was 19 weeks old. Only 2 aphids (apterae) were counted per 5 minutes.

In the trial planted at MUARIK, SPCSV spread rapidly, 33 plants out of 48 plants on 16 mounds becoming infected with SPCSV (Table 15). Some of this spread was of wild type SPCSV because SPVD developed on some plants. Interestingly, SPVD was slightly more common and more severe (Fig. 13) on plants that were originally healthy (7 plants on 6 mounds were affected by SPVD of 48 plants on 16 mounds) than those that were originally infected with 'mild' SPCSV (4 plants on 4 mounds with SPVD of 48 plants on 16 mounds) (Table 15). The whitefly population was very high with 225 whiteflies counted per 5 minutes when the field was 19 weeks old. Aphid population density was still low but higher than in Busia; 4 aphids (apterae) counted per 5 minutes.

'Mild' SPCSV had a significant ($P \leq 0.01$) effect on the root yield in Busia district (Table 17); here, where 'mild' SPCSV hardly spread to healthy plots, it reduced yield by over 50% (Table 16). The difference was less evident (Table 16) and non significant ($P \leq 0.05$; Table 17) at MUARIK where many of the originally healthy plants also became infected with SPCSV or SPCSV + SPFMV and a few of the originally 'mild' SPCSV-infected plants developed SPVD. 'Mild' SPCSV did not have any effect on root colour, texture or shape.

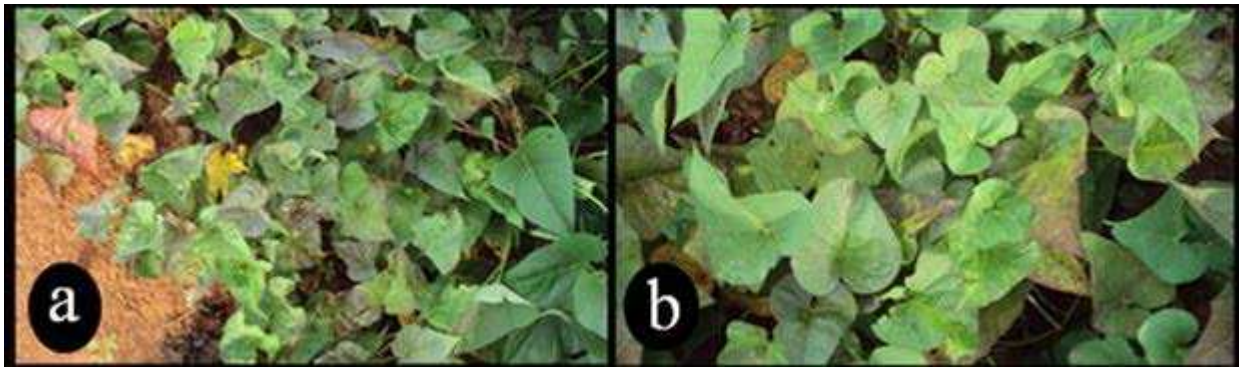


Figure 13. Varying SPVD symptoms severity observed on (a) originally healthy and (b) originally 'mild' SPCSV-infected Kampala White in MUARIK trial. Picture was taken 19 weeks after setting the trial

Table 15. Spread of SPCSV in field trials of cv Kampala White

	Busia trial	MUARIK trial
Number of plants in originally healthy plots that became infected with SPCSV	6/96	33/48
Number of plants in originally healthy plants that became affected by SPVD	0/96	7/48
Number of plants in originally 'mild' SPCSV-infected plants that became affected by SPVD	0/96	4/48

Table 16. Storage root weight of 'mild' SPCSV-infected and healthy cv Kampala White

	Storage root weight (Kg) per 16 mounds	
	Healthy	'mild' SPCSV
Busia	39	16.8
MUARIK	19	13

Table 17. ANOVA to test for differences in sweet potato yield between symptomless and 'mild' SPCSV-infected cv Kampala White in MUARIK and Busia field trials

Source of variation	d.f	Mean squares	Mean squares
		Storage root yield in MUARIK	Storage root yield in Busia
Replication	3	0.833	12.138
Healthy status	1	4.500^{ns}	61.605^{**}
Error	3	3.667	1.338
Total	7		

** indicates significance at $P \leq 0.01$

^{ns} indicates non significance at $P \leq 0.05$

4.3.6 Effect of 'mild' SPCSV strain on wild type SPCSV in field and in greenhouse sweet potato plants and *I. setosa*

There was less SPCSV spread and fewer SPVD-affected plants observed in Busia than MUARIK trials (Table 18) and SPCSV, but not SPVD, spread varied significantly ($P \leq 0.05$) between the two locations (Table 19a). At MUARIK, there was more SPCSV spread than SPVD development to originally healthy cv Kampala white; in healthy Dimbuka, there were less SPCSV-infected plants than SPVD affected plants. Grafting to *I. setosa* indicated that SPCSV single infections were generally of 'mild' SPCSV. Overall, plots that originally had 'mild' SPCSV had fewer cases of SPVD than plots planted with either symptomless cvs Dimbuka or Kampala White (Table 18), but SPVD and SPCSV spread did not vary significantly ($P \leq 0.05$) between 'mild' SPCSV-infected cv Kampala White and symptomless cvs Dimbuka and Kampala White (Table 19b).

Table 18. Experiment on 'cross protection' of 'mild' SPCSV strain against wild type SPCSV

	Total no. of vines	No. of vines that got infected with SPCSV ('mild' or wild type)	No. of vines that got affected by SPVD
MUARIK trial			
'Mild' SPCSV-infected cv Kampala White	192	N/A	11
Symptomless cv Kampala White	192	97	15
Symptomless cv Dimbuka	192	12	27
Busia Trial			
'Mild' SPCSV-infected cv Kampala White	384	N/A	1
Symptomless cv Kampala White	384	2	6
Symptomless cv Dimbuka	384	3	7

N/A = not applicable

Table 19a. ANOVA to test for differences in SPVD and SPCSV spread between the two locations (Busia and MUARIK) planted with 'mild' SPCSV-infected cv Kampala White and symptomless cvs Dimbuka and Kampala White

Source of variation	d.f	Mean squares SPVD spread	d.f	Mean squares SPCSV spread
Replication	1	2.08	1	18
Location	1	126.75 ^{ns}	1	1352 *
Error	9	14.45	5	376.3
Total	11		7	

* indicates significance at $P \leq 0.05$

^{ns} indicates non significance at $P \leq 0.05$

Table 19b. ANOVA to test for differences in SPVD and SPCSV spread between 'mild' SPCSV-infected cv Kampala White and symptomless cvs Dimbuka and Kampala White

Source of variation	d.f	Mean squares SPVD spread	d.f	Mean squares SPCSV spread
Replication	1	2.08	1	18
Healthy status X cultivar	2	30.58^{ns}	1	882^{ns}
Error	8	24.46	5	470.3
Total	11		7	

^{ns} indicates non significance at $P \leq 0.05$

Cv Kampala White plants graft inoculated with co-infection of 'mild' SPCSV and SPFMV had very mild or no symptoms whereas Kampala White plants graft inoculated with wild type SPCSV and SPFMV developed typical SPVD (Fig. 14). *I. setosa* plants graft-inoculated with SPFMV + 'mild' SPCSV also showed less severe symptoms, plants continuing to grow (Fig. 15a). *I. setosa* plants co-infected with SPFMV + wild type SPCSV were severely stunted and started dying from the top (Fig. 15b). It was also observed that sweet potato and *I. setosa* graft inoculated with wild type SPCSV + 'mild' SPCSV + SPFMV developed intermediary symptoms (Fig. 14 and 16, respectively).

Looking at the virus titre, SPFMV titre was highest in both *I. setosa* and Kampala White plants co-infected with wild type SPCSV + SPFMV followed by plants co-infected with 'mild' SPCSV + SPFMV and least in plants singly infected with SPFMV (Table 20 and 21). In *I. setosa* plants, 4 weeks after graft inoculation, SPFMV titre was 3.2 times and 12.9 times more in co-infections of 'mild' SPCSV + SPFMV and wild type SPCSV + SPFMV, respectively whereas in cv Kampala White, SPFMV titre increased 7.2 fold and 42 fold in co-infections of 'mild' SPCSV + SPFMV and wild type SPCSV + SPFMV, respectively (Table 20). Cv Kampala White singly infected with SPFMV started showing uneven virus distribution 5 weeks after graft inoculation and by this time SPFMV titre started reducing in co-infection of 'mild' SPCSV + SPFMV, though never reaching zero for the period of 9 weeks, but did not reduce in co-infection of wild type SPCSV + SPFMV (Table 21). SPCSV titre (using the HSP70 gene) of both 'mild' SPCSV single infection and in co-infection of 'mild' SPCSV + SPFMV was comparable (Table 22) but it was 4.7 times less in co-infections of wild type SPCSV + SPFMV compared to co-infections of 'mild' SPCSV + SPFMV (Table 24). All genes on RNA1 region of the 'mild'

SPCSV were at a much lower titre than of the wild type SPCSV in co-infections with SPFMV (Table 23). Specifically, both RNase3 and p22 genes were over 64 fold and 177 fold, respectively, greater in co-infection of wild type SPCSV + SPFMV compared to co-infection of 'mild' SPCSV + SPFMV (Table 24). Grafting results of cv Kampala White to *I. setosa* at the end of 9 weeks showed reversion from SPFMV single infections in 3 out of 5 shoots. Reversion from SPFMV in co-infection of SPFMV + 'mild' SPCSV occurred in 1 out of 5 shoots; the shoot tip that showed reversion did not induce any SPVD symptoms in *I setosa* but instead induced only development of mild leaf chlorosis. No reversion was observed in co-infection of SPFMV + wild type SPCSV (Table 25).

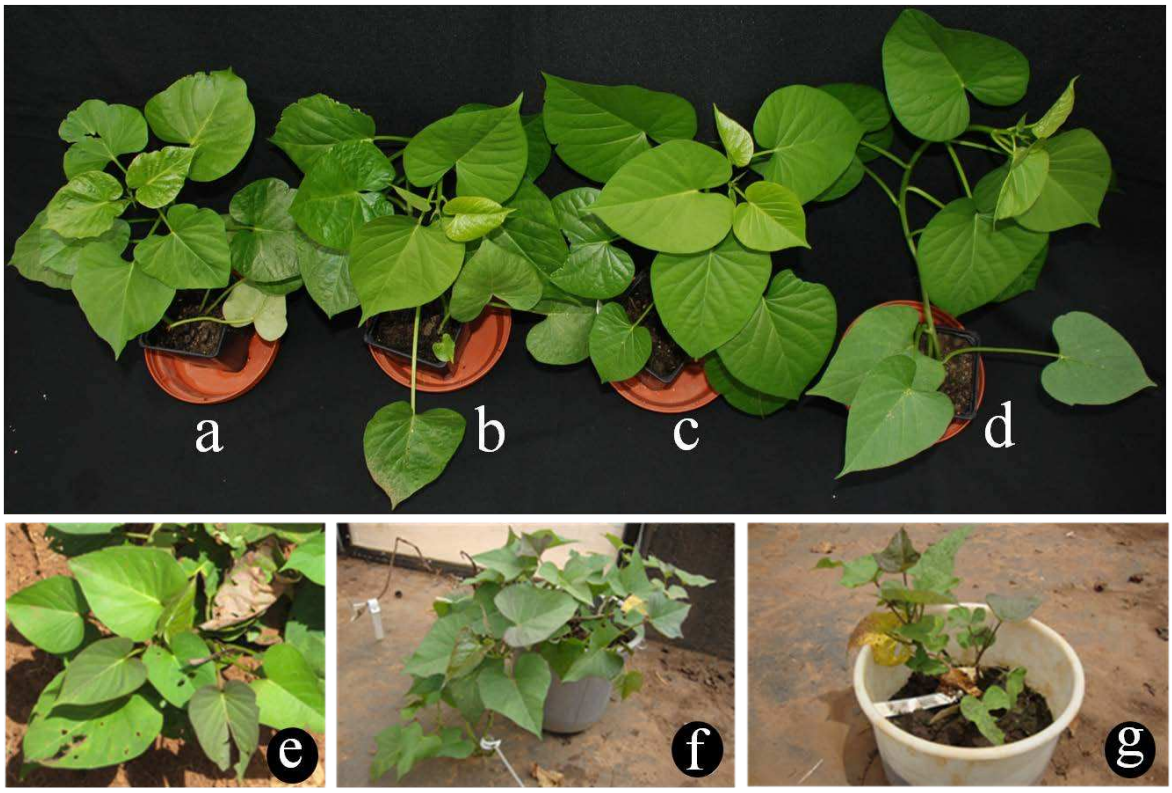


Figure 14. Cv Kampala White infected with different virus(es) showing differences in synergism between 'mild' SPCSV and wild type SPCSV when co-infect with SPFMV. a) co-infection of wild type SPCSV + SPFMV; b) co-infection of 'mild' SPCSV + SPFMV; c) SPFMV single infection; d) healthy plant ; e) healthy field plant; f) plant graft inoculated with SPFMV + 'mild' SPCSV + wild type SPCSV; g) plant graft inoculated with SPFMV and wild type SPCSV. Pictures a to d were taken 8 weeks after graft inoculation but 4 weeks after cutting off plants and replanting from continuous inoculums while pictures f and g were taken 16 weeks after graft inoculation

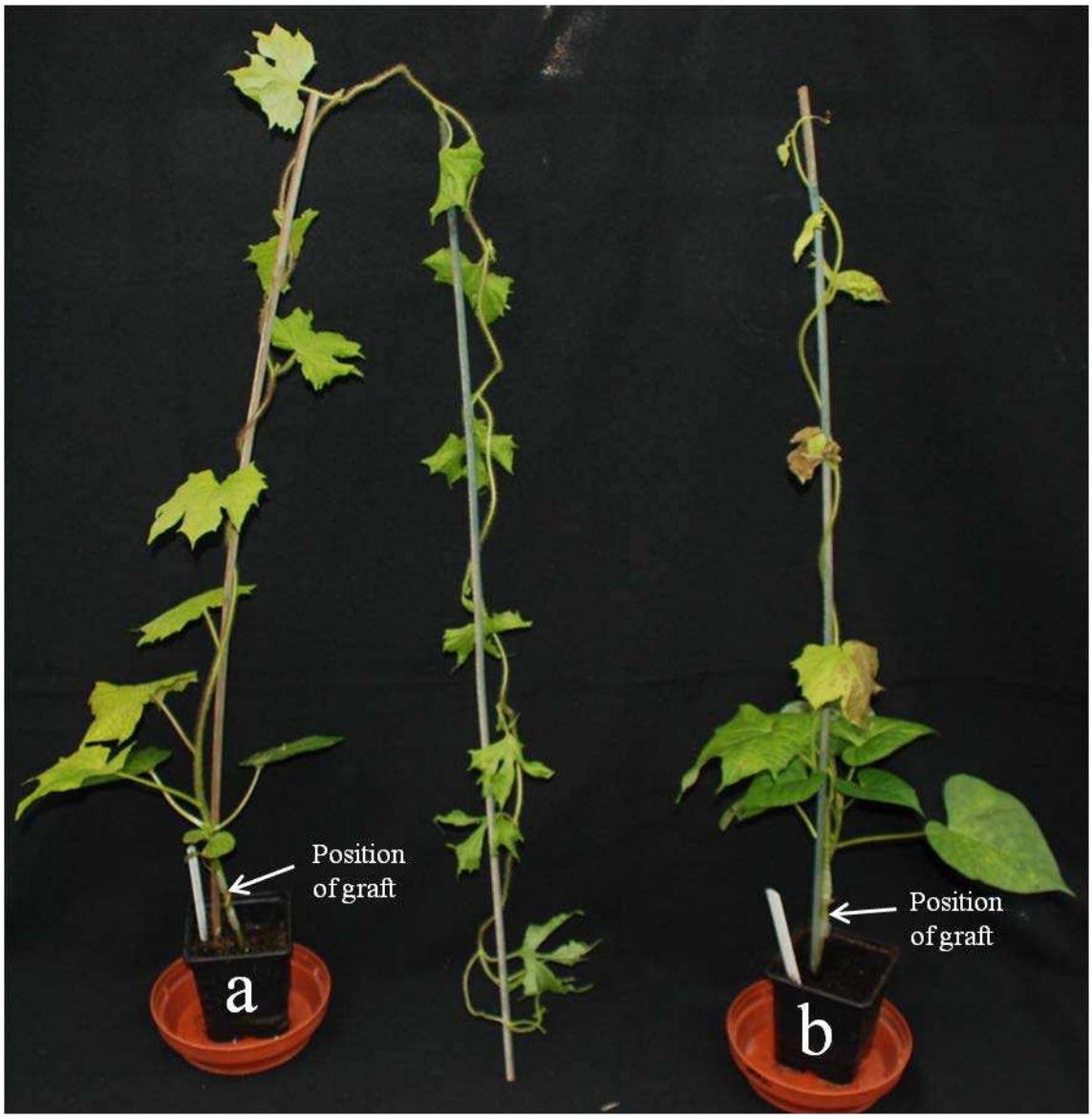


Figure 15. a) *I. setosa* graft inoculated with SPFMV and 'mild' SPCSV and b) *I. setosa* graft inoculated with SPFMV and wild type SPCSV showing differences in synergism between 'mild' SPCSV and wild type SPCSV 4 weeks after graft inoculation

Figure 16. *I. setosa* plants graft inoculated with different virus combinations to compare synergism of SPFMV by 'mild' SPCSV and wild type SPCSV. a) healthy plant; b) plant infected with wild type SPCSV; c) plant infected with 'mild' SPCSV; d) plant infected with SPFMV; e) plant infected with SPFMV + wild type SPCSV + 'mild' SPCSV; f) plant infected with SPFMV + 'mild' SPCSV; and g) plant infected with SPFMV + wild type SPCSV. Picture was taken 3 weeks after graft inoculation

Table 20. Mean fold change in SPFMV titre (average $2^{-\Delta\Delta Ct}$) at 4 weeks after graft inoculation of cv Kampala White and *I. setosa* plants with SPFMV, SPFMV + 'mild' SPCSV, and SPFMV + wild type SPCSV (leaf positions on a plant or plant number are in parentheses)

	Average $2^{-\Delta\Delta Ct}$					
	I. setosa			Cv Kampala White		
	SPFMV	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV	SPFMV	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV
	1.006 (2†)	2.057 (2†)	58.473 (2†)	1.000 (1*)	3.563 (1*)	38.663 (1*)
	1.239 (3†)	4.227 (4†)	9.522 (5†)	0.395 (2*)	6.483 (2*)	19.907 (2*)
	5.492 (5†)	11.188 (5†)	6.5162 (6†)			
	2.258 (6†)	7.152 (8†)				
	1.207 (7†)					
	0.329 (8†)					
Mean of means	1.921	6.156	24.837	0.698	5.023	29.285

† indicates leaf position

*indicates plant number

Table 21. Mean fold change in SPFMV titre (average $2^{-\Delta\Delta Ct}$) over time (weeks) in cv Kampala White plants infected with SPFMV, SPFMV + 'mild' SPCSV, and SPFMV + wild type SPCSV

	Average $2^{-\Delta\Delta Ct}$		
	SPFMV	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV
Weeks			
1	1.095	252.55	4970.342
2	1.708	103.121	2319.939
3	1.012	115.099	7814.851
4	1.001	532.382	3201.673
5	(-)	1.2151	230.089
6	1.001	1.469	3898.812
7	(-)	1.045	822.033
8	1.134	4.175	798.401
9	(-)	1.420	360.783

(-) indicates negative sample

Table 22. Mean fold change in HSP70 titre (average $2^{-\Delta\Delta Ct}$) between single infection of 'mild' SPCSV and co-infection of 'mild' SPCSV + SPFMV in cv Kampala White plants at 9 weeks after graft inoculation

	Average $2^{-\Delta\Delta Ct}$	
Leaf position	'mild' SPCSV infected plant	'mild' SPCSV + SPFMV infected plant
Bottom	1.122	1.031
Middle	0.071	0.137
Top	0.667	0.68
Mean of means	0.620	0.616

Table 23. The average Ct values ± Standard error of mean (SEM) for different genes along RNA1 and RNA2 of 'mild' SPCSV and wild type SPCSV for 4 weeks fter graft inoculation

	Ct values							
	RNA1						RNA2	COX
SPCSV strain	P-Pro	MTR	HEL	RdRp	RNase3	p22	HSP70	
'Mild' SPCSV	33.6±1.87	37.3±1.61	35.6±1.54	37.8±1.06	32.2±1.36	36.2±1.86	24.0±0.96	25.0±0.24
Wild type SPCSV	24.1±1.81	31.7±0.39	26.9±2.65	29.2±3.14	24.3±1.59	27.3±2.86	32.4±7.37	24.4±0.07

Table 24. Mean fold change in HSP70, RNase3 and p22 titres (average $2^{-\Delta\Delta Ct}$) over time (weeks) in cv Kampala White plants infected with SPFMV + 'mild' SPCSV, and SPFMV + wild type SPCSV

	Average $2^{-\Delta\Delta Ct}$					
	HSP70		RNase3		p22	
	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV
Weeks						
1	0.001	0.01	2.435	147.02	2.131	109.433
2	0.0005	0.075	0.445	31.306	2.588	738.856
3	0.00004	0.123	0.028	78.025	0.111	862.262
4	0.01	0.014	0.179	3.156	1.295	126.359
5	1.009	0.0005	(-)	0.130	(-)	1.331
6	0.052	0.002	0.011	0.267	(-)	3.83
7	0.0001	0.004	0.042	2.156	1.913	261.235
8	0.002	0.0005	0.023	1.238	0.123	64.504
9	0.001	0.0003	(-)	0.71	(-)	2.545
Mean of means	0.1195	0.025	0.452	29.334	1.360	241.151

(-) indicates a negative sample

Table 25. Reversion from SPFMV (as determined by grafting to *I. setosa*) in sweet potato cv Kampala White infected with SPFMV alone, SPFMV + 'mild' SPCSV, and SPFMV + wild type SPCSV for 9 weeks

	Cv Kampala White			
	Healthy	SPFMV	SPFMV + 'mild' SPCSV	SPFMV + wild type SPCSV
Shoot position				
5 (most top)	–	–	–	+
4	–	–	+	+
3	–	–	+	+
2	–	+	+	+
1 (most bottom)	–	+	+	+

+ and – indicate presence and absence of SPFMV symptoms, respectively

4.4 Discussion

From previous surveys conducted before the identification of 'mild' SPCSV, it was clear that SPCSV rarely occured by itself (Gibson *et al*., 1998). In fact, from the country-wide survey during this study, the only case of SPCSV single infection in sweet potato was from Busia, the district where 'mild' SPCSV is prevalent. Furthermore, the plants had been exposed to the insect vectors for more than three months in a high disease pressure zone of MUARIK when the screenhouse got damaged. Though this exposure of plants to vectors could have affected the virus incidence data, thus ending up having fewer virus free plants compared to what Gibson *et al*. (1997) observed in their work, it did not lead to the 'mild' SPCSV-infected plant succumbing to SPVD. In the subsequent survey in Busia district during this study, only a very few SPVD-affected plants (normally associated with SPFMV + wild type SPCSV) and no cases of SPFMV single infections were observed; meanwhile, plants with symptoms of 'mild' SPCSV were common in the district (Table 12). Rossel and Thottappilly (1988) noted that cultivars can develop such mild SPVD symptoms that farmers cannot readily distinguish between affected and unaffected plants. Similarly, Alicai *et al*. (1999b) by use of serology, identified isolates of $SPCSV_{EA}$ differing in the severity of the associated SPVD. However, these previous reports involve relatively mild SPVD in farmers fields; our report describes an absence of SPFMV and no (or a very few severe) SPVD in farmers' fields in Busia, and only getting mild SPVD in experimental trials.

The availability of a 'mild' SPCSV strain suggested a possible opportunity for the control of SPCSV and so SPVD through cross protection. However, it appeared to cross protect only poorly if at all and, although the 'mild' SPCSV strain caused no SPVD symptoms, it

is not really mild in sweet potato (even though it may be in *I. setosa*). It caused clear purpling and stunting of infected sweet potato plants (Fig. 11) typical of wild type SPCSV infection (Duffus, 1995), including the $SPCSV_{EA}$ serotype (Gibson *et al.,* 1998). The virus also causes yield loss of up to 50% (Table 16) which is similar to yield losses observed by Gibson *et al*. (1998) and Mukasa *et al.* (2006) working with the wild type SPCSV. Infection by 'mild' SPCSV did, however, cause seedlings of *I. setosa* to develop only mild chlorotic symptoms (Fig. 11). This is in contrast to observations made by Hoyer *et al.* (1996) probably working on $SPCSV_{EA}$, Gibson *et al.* (1998) working on $SPCSV_{EA}$ and Cohen *et al.* (1992) working on $SPCSV_{WA}$ who all noted stunting of *I. setosa* with small, brittle, and yellow leaves. An isolate of $SPCSV_{WA}$ from Argentina caused mild mosaic symptoms in *I. setosa* but, unlike 'mild' SPCSV, this isolate caused mild mosaic in old and new leaves of sweet potato. Co-infection of SPFMV and 'mild' SPCSV in sweet potato plants in a glasshouse took abnormally long to produce SPVD symptoms and even then symptoms were inconsistent and less severe than those that were observed when SPFMV co-infects with wild type SPCSV (Fig. 14). Furthermore, when looking at the virus titre in both *I. setosa* and sweet potato plants, SPFMV titre was greatest in co-infections involving SPFMV and wild type SPCSV followed by co-infections of SPFMV and 'mild' SPCSV and least in SPFMV single infections (Table 20 and 21).

Upon infection in normal situations, SPCSV breaks down the resistance of sweet potato to virus through RNA silencing suppression (Kreuze *et al*., 2002; 2005). As a result, SPFMV easily infects such plants and multiplies to reach high titres (Mukasa *et al*., 2006), leading to the development of SPVD (Gibson *et al*., 1998). In bipartite criniviruses, the 3' proximal end of RNA1 contains the RNA silencing suppressor genes, RNase3 and p22

(p22 is only reported in Ugandan $SPCSV_{EA}$ isolates) (Kreuze *et al*., 2002; 2005). The 'mild' SPCSV has both RNase3 and p22 genes and these genes were within the range of variation of other apparently wild type isolates described by Tugume (2010). SPCSV isolates that do not encode for p22 gene still successfully synergise heterologous viruses (Cuellar *et al*., 2008). Kreuze *et al*. (2005) showed that the p22 gene somehow enhances silencing. Cuellar *et al*. (2008) found that most SPCSV do not possess a p22 gene (Ugandan SPCSV isolates are apparently exceptional) but that the synergy is greater when SPCSV encoding p22 co-infects with SPFMV than when a non p22 encoding SPCSV co-infects with SPFMV, confirming that the p22 gene, if present, confers some additive effect in synergism. On the contrary, the 'mild' SPCSV strain encodes a p22 gene and one that is identical to the wild type SPCSV p22 genes in GenBank (http://blast.ncbi.nlm.nih.gov) and literature (Tugume, 2010) yet its synergistic effects are far less dramatic than wild type SPCSV.

Thus, the discrepancy in synergism between 'mild' and wild type SPCSV seems unlikely to be due to deficiencies in either RNase3 or p22 gene. However, all genes (including RNase3 and p22) on RNA1 are much less expressed (Table 23 and Table 24) in the 'mild' SPCSV than in a wild type isolate (results that incidentally would not obviously occur if the RNase3 and/or p22 of 'mild' SPCSV were simply aberrant) and this lesser expression seems to be the likely cause of this isolates failure to synergise well SPFMV. This suggests a reduction in the efficacy in any sub-genomic (sg) mRNA promoter(s) in the RNA1 region (RNA2 continues to occur 'normally' in 'mild' SPCSV as shown by the 'normal' expression of its HSP70 gene). Indeed, it seems unlikely (though not impossible) that separate sg mRNA promoters should be responsible; for all genes to be affected

similarly, it seems more likely that they share a common sg mRNA promoter, especially since they are located very close to each other on RNA1 (Kreuze *et al*., 2002).

Field plants that originally had 'mild' SPCSV infections appeared to show less severe SPVD symptoms than their counterparts that started as healthy material [but more severe than 'mild' SPCSV experimentally co-infected with SPFMV] (Fig. 13 *versus* 14). Experimentally, wild type and 'mild' SPCSV were also able to be made to co-exist together and the outcome when infected also with SPFMV appears to be one of intermediate severity, a circumstance that was particularly obvious in *I. setosa* (Fig. 15). The mechanism responsible for RNA silencing suppression is the same as that for cross protection (Ratcliff *et al*., 1999; Kreuze *et al*., 2002) and it may be that the RNase3 and p22 genes of SPCSV (of maybe the wild type only), by hindering RNA silencing, allow more than one strain of the same virus to co-exist in the same plant, without dominance of one or the other.

SPVD was rarely observed in Busia district though 'mild' SPCSV is prevalent especially in the predominant cv Kampala White. There are several probable reasons that could be contributing to this SPVD rarity. Experimentally, SPFMV was observed not to be fully synergised by 'mild' SPCSV (Tables 20 and 21) and Kampala White plants were observed quite often totally recovering from the mild SPVD symptoms with a dramatic decline in SPFMV titre (Table 21) from which they eventually reverted (Table 25). Previous studies have shown that aphids seldom acquire SPFMV if the titres are as low as in singly infected plants (Aritua *et al*., 1998b). In addition, aphids were few on sweet potato in Busia district - though aphids are normally few in sweet potato fields (Aritua *et al*., 1998b; Kantack *et*

al., 1960) and SPFMV is speculated to be transmitted by itinerant alate aphids of species that do not colonise sweet potato. Because of the low virus titre and rarity of aphids, SPFMV may not be efficiently spread in fields in Busia district leading to less SPVD. It may also be due to the fact that cv Kampala white, the predominant cultivar in Busia, is relatively resistant to SPFMV as it was observed to revert from SPFMV single infections (also see chapter 5; Table 30). In addition, heat treatment was observed to enhance reversion from SPFMV in singly infected plants and kept SPFMV titre even lower in plants co-infected with SPFMV and 'mild' SPCSV (Table 14). This suggests the possibility of sweet potato field plants maintaining a low SPFMV titre, from which they probably eventually revert, in areas such as Busia which are normally associated with long dry seasons (Basalirwa, 1995; Rugumayo *et al*., 2003) and with relatively high temperatures.

SPVD-affected plants remain very small and have pronounced symptoms for farmers to select against (Gibson *et al*., 2004). 'Mild' SPCSV singly infected sweet potato plants manage to grow almost normally, so infected plants do not have a reduced visibility to whiteflies and farmers may find it difficult to select against such plants. In addition, in normal SPVD, wild type SPCSV titre is reduced (Karyeija *et al*., 2000; Mukasa *et al*., 2006; Kokkinos and Clark, 2006a). This was indirectly confirmed in this study by two ways; firstly when the titre of the HSP70 gene (RNA2) of SPCSV was observed to be greater (4.7 fold) in co-infection of 'mild' SPCSV + SPFMV than in co-infection of wild type SPCSV + SPFMV (Table 24) [consistent with at least the titre of the wild type SPCSV being reduced in co-infections with SPFMV (Kokkinos and Clark, 2006a)] and secondly when the titre of SPCSV (HSP70) remained more-or-less the same both in single

infection of ‘mild’ SPCSV and co-infection of ‘mild’ SPCSV + SPFMV (Table 22). Just like the case for aphids acquiring SPFMV, the unaffected titre of this ‘mild’ SPCSV in co-infections with SPFMV is likely to be more easily acquired by whiteflies than the lower wild type SPCSV titre in an SPVD affected plant. All these give advantage to ‘mild’ SPCSV and it may be expected that this poorly synergising strain of SPCSV will become much more widespread in Uganda. Indeed, it is already the prevalent isolate in Busia district.

Additional surveys for the ‘mild’ SPCSV in Uganda are needed. Although ‘mild’ SPCSV causes less damage on an individual plant basis than the wild type which readily synergises SPFMV to cause SPVD, it still causes large losses in yield and total yield losses to viruses may thereby increase if the ‘mild’ SPCSV becomes much more common than wild type SPCSV as a result of its apparent numerous survival advantages. The ‘mild’ strain also is an intriguing example of a virus evolving to change its molecular behaviour so that it no longer is able to be ‘parasitized’ by other viruses ‘piggy-backing’ on its ability to combat RNA silencing by the host sweet potato. How it does this without losing the advantages of destroying the plant’s RNA silencing mechanism is intriguing. SPCSV is a phloem-limited virus whilst SPFMV occurs more widely distributed in plants. It may be that ‘mild’ SPCSV is more fit than wild type SPCSV and has succeeded in suppressing RNA silencing only within the phloem, giving advantage mainly only to itself, whereas wild type SPCSV suppresses silencing in the entire plant, giving advantage to itself but also to a wide range of other viruses. Further studies of this ‘mild’ SPCSV will no doubt provide more detail of RNA silencing by plants and how viruses suppress it.

CHAPTER FIVE

REVERSION FROM VIRAL INFECTION IN UGANDAN LANDRACES

5.1 Introduction

Viruses pose the second most important biotic constraint after weevils to sweet potato (Clark and Moyer, 1988; CIP, 2000) and several sweet potato viruses have been reported in Uganda (Carey *et al*., 1998; Mukasa *et al*., 2003; Aritua *et al*., 2007; Wasswa *et al*., 2011). Much emphasis has been put on co-infection of SPFMV and SPCSV which results in the severe disease SPVD (Gibson *et al*., 1998). However, some single virus infections can also result in yield reduction and may cause cultivar decline/degeneration. For instance, SPFMV, the most prevalent virus in Uganda, appears to cause yield loss of up to 50% (Gibson *et al*., 1997; Njeru *et al*., 2004). SPCSV is the second most prevalent virus and can cause up to 50% yield loss by itself (Gibson *et al*., 1998; Mukasa *et al*., 2006). Preliminary survey results show that SPLCUV follows SPCSV in prevalence (Wasswa *et al*., 2011) and yield studies in the U.S.A of the related SPLCV show it can cause up to 26% yield loss (Clark and Hoy, 2006). Other viruses that follow closely, in decreasing order of prevalence, include SPMMV, SPCFV and SPCaLV (Carey *et al*., 1998; Mukasa *et al*., 2003; Aritua *et al*., 2007) but their effect on yield has not been studied.

Previous research has shown reversion from SPFMV in resistant (New Kawogo) and moderately resistant (Tanzania) cultivars of sweet potato in Uganda (Aritua *et al*., 1998b) but did not consider reversion in susceptible cultivars. The natural potential of sweet potato landraces to recover/revert from single virus infections should be exploited but its

importance in sustaining the productivity of sweet potato has not been evaluated. Reversion in a wider range of cultivars is described in this chapter. Information on SPFMV titres (using RT-qPCR, DAS- and TAS-ELISA) in resistant and susceptible sweet potato cultivars is also reported.

5.2 Materials and Methods

5.2.1 SPFMV titre and recovery from SPFMV in *I. setosa*

Two plants of one week old *I. setosa* were side grafted with SPFMV infected scions of cultivar Resisto. Plants were observed for SPFMV symptoms for a period of 4 weeks. Leaf portions from the first leaf to show symptoms were collected weekly for 4 weeks and stored at -80^{o}C. At the end of the 4 weeks, up to 11 consecutive leaf samples, starting with the first true leaf just after the graft (considered as leaf 1) were collected. The 8th leaf was the first to show recovery at week 4. Leaf samples were used for determining SPFMV titres using RT-qPCR method as described in chapter 4, section 4.2.2.

5.2.2 Ugandan sweet potato landraces with potential to revert from SPFMV as determined by grafting to *I. setosa* and RT-qPCR

Sweet potato cultivars New Kawogo, Araka White, Beauregard and NASPOT 1 growing in a screenhouse at NRI, UK and previously confirmed as being singly infected with SPFMV by grafting to *I. setosa* were used. Terminal shoot tips were cut off and separately graft-inoculated to *I. setosa* to confirm infection. This also promoted growth of more lateral and basal shoots in the plants. Three weeks after decapitating the main stem, shoot tips which had appeared along the main stems and tips of all basal shoots of each cultivar

were cut and each separately grafted to *I. setosa*. The *I. setosa* were examined for symptoms of SPFMV for 4 weeks after grafting. Plants that reacted negative with *I. setosa* were confirmed using RT-qPCR.

5.2.3 SPFMV titre and reversion in different sweet potato cultivars

Cuttings from healthy sweet potato plants of cvs New Kawogo, Beauregard, Ejumula, Dimbuka and NASPOT 1 were separately established in small pots in a screenhouse at NaCRRI, Uganda. Two weeks later, SPFMV infected scions from cv Resisto were independently grafted to three plants of each cultivar. Uninoculated controls of each cultivar were also included. Plants were tested for successful inoculation by grafting them to *I. setosa*. Samples were taken from top, middle and bottom leaves (as a single sample for each plant) of successfully inoculated plants 5 weeks after grafting and tested for SPFMV using DAS-ELISA (Clark and Adams, 1977). At 9 weeks, shoot tips (*c.* 5 cm long) were cut from all plants and re-planted so they were independent from the scions. At 3 and 6 weeks after replanting, samples were collected from top, middle and bottom leaves (as a single sample for each plant) and tested for SPFMV using TAS-ELISA (Clark and Adams, 1977). Three shoot tips from each of these plants were finally grafted to *I. setosa* to test for reversion.

SPFMV titre was also determined in sweet potato field plants from NaCRRI and MUARIK. Planting material used in planting these fields was selected from healthy looking plants and the material picked for testing was from at least 4 months old symptomless plants. Six different cultivars were tested using TAS-ELISA and these

included Beauregard, Ejumula, Dimbuka, Semanda, NASPOT 1 and New Kawogo. Healthy controls for each cultivar were included on the plate.

The same grafting experiment as above was repeated at NRI, grafting SPFMV infected cv Resisto onto two plants each of cvs Huachara, New Kawogo, Kampala White, Munyera, Peruanita, Araka White, Beauregard, NASPOT 1 and NASPOT 11. Two weeks later (considered as week zero in the Results section), leaf samples were collected from the plants, stored at -80^{o}C and the shoots were cut off and re-planted, again to stop any interfering virus inoculum from the scions. At the same time, tips of the replanted shoots were cut from each of these cultivars and grafted to *I. setosa* to test for successful inoculations, observing for symptoms for 4 weeks. Leaf samples were taken weekly for storage at -80^{o}C from the successfully graft inoculated replanted sweet potato cuttings for 12 weeks; SPFMV titre determined at the end of the experiment by RT-qPCR. At the end of 12 weeks, shoot tips from 5 consecutive shoots along the main stem of each sweet potato plant were grafted to *I. setosa* and observations for symptom development made for 4 weeks. The main stems ranged between 3-4 feet in length and the lateral shoots were distributed along the whole length of the stem from bottom to top.

5.3 Results

5.3.1 SPFMV titre and recovery from SPFMV in *I. setosa*

Recovery from SPFMV symptoms was observed in *I. setosa* with the upper leaves starting from leaf 8 growing with no symptoms 4 weeks after graft inoculation. SPFMV titre was observed to decline up the plant in samples collected at fourth week after graft inoculation

but never reached zero. SPFMV titre was more-or-less constant in the first leaf to show symptoms for the period of 4 weeks (Table 26).

Table 26. Mean fold change in SPFMV titre (average $2^{-\Delta\Delta Ct}$) over time (weeks) in *I. setosa* first leaf to show symptoms (leaf portions from this same leaf were collected weekly) and 11 consecutive leaves at week 4 including first leaf to show symptoms and recovered leaves

	Average $2^{-\Delta\Delta Ct}$				
	Week 1	Week 2	Week 3	Week 4	
				Plant 1	Plant 2
Leaf no.					
1				1.023	1.0
2 (1st leaf to show symptoms)	3.34971*	1.087735*	2.151198*	1.162	3.350
3				1.621	0.22
4				1.652	0.048
5				1.807	0.012
6				1.561	0.001
7				1.465	0.0004
8 (1st leaf to show recovery)				0.391	0.000005
9 (recovered leaf)				0.245	
10 (recovered leaf)				0.275	
11 (recovered leaf)				0.091	

* indicates average value for plant 1 and 2

5.3.2 Ugandan sweet potato landraces with potential to revert from SPFMV as determined by grafting to *I. setosa* and RT-qPCR

Reversion from SPFMV occurred more in shoots of some cultivars than in others. Cultivars New Kawogo and Araka White completely reverted from SPFMV while none of the branches of cultivar Beauregard reverted. All shoots of NASPOT 1 also showed reversion while the main stem did not revert (Table 27). Plants that tested negative with *I. setosa* also tested negative with RT-qPCR.

Table 27. Reversion in sweet potato cultivars from SPFMV as determined by grafting to *I. setosa* and confirmed by RT-qPCR

Cultivar	Main stems reverted	Lateral shoots reverted	Basal shoots reverted
New Kawogo,	1/1	4/4	2/2
NASPOT 1	0/1	2/2	1/1
Araka White,	1/1	3/3	3/3
Beauregard,	0/1	0/3	0/1

5.3.3 SPFMV titre and reversion in different sweet potato cultivars

SPFMV titre results from DAS- and TAS-ELISA showed that cultivars with more potential to revert have lower virus titres than those with low reversion potential (Table 28). A general decline in virus titre was also observed across all cultivars (both susceptible and tolerant) over time (Table 28). SPFMV titre in cultivars New Kawogo and NASPOT 1 with high potential to revert was comparable to their respective healthy controls 12 weeks

after inoculation (WAI). However, at 12 WAI cvs Beauregard, Ejumula and Dimbuka with low reversion potential still had a high SPFMV titre compared to their respective healthy controls and it took at least 18 WAI for these cultivars to develop negligible virus titres. Grafting all these cultivars at the end of 18 weeks confirmed reversion in 2 of 3 cuttings of each of the cultivars Beauregard, Dimbuka and Ejumula while all cuttings from cultivars New Kawogo and NASPOT 1 reacted negatively on *I. setosa*. In sweet potato field plants collected from MUARIK, SPFMV occurred more often in susceptible cultivars than tolerant ones and susceptible cultivars had a slightly higher virus titre than resistant cultivars. Overall SPFMV incidence was 26% in these field plants (Table 29).

RT-qPCR results generally showed the American cv Beauregard to maintain a higher virus titre and stay infected for a longer period (11 weeks) than any other cultivar (Table 30). Peruvian cvs Huachara and Peruanita and the Ugandan cv Munyera showed occasional high titres and all the rest (Ugandan cultivars) showed very low titres. Overall, RT-qPCR results showed cv Beauregard to be the most susceptible and cv NASPOT 11 (Tomulabula) as the most resistant (Table 30). RT-qPCR results were confirmed by results from graft inoculation to *I. setosa* (Table 31) which showed cvs Beauregard and Peruanita as the most susceptible and cv NASPOT 11 (Tomulabula) as the most resistant with the rest of the cultivars showing moderate resistance. Generally, shoots from the top of the main stems were more often virus free than shoots from the bottom (Table 31).

Table 28. Trend in SPFMV titre at 405 nm in different sweet potato cultivars for the period of 18 weeks

	DAS-ELISA		TAS-ELISA			
	5 WAI (still attached to inoculum)		12 WAI (detached from inoculum)		18 WAI (detached from inoculum)	
Cultivar	+ SPFMV	Healthy	+ SPFMV	Healthy	+ SPFMV	Healthy
Beauregard	1.574	0.127	0.430	0.195	0.247	0.239
Ejumula	1.722	0.020	0.309	0.153	0.264	0.249
Dimbuka	1.541	0.270	0.191	0.109	0.252	0.178
NASPOT 1	0.333	0.030	0.222	0.109	0.200	0.250
New Kawogo	0.609	0.100	0.173	0.091	0.233	0.209

Table 29. Prevalence of SPFMV (percentage in parenthesis) and virus titre (at 405 nm) in field plants of different sweet potato cultivars

Cultivar	Source of sample	TAS-ELISA	
		No. of positives	Average OD value of infected plants
Beauregard	NaCRRI	7/20 (35%)	0.723
Ejumula	NaCRRI	8/20 (40%)	0.721
Dimbuka	Kabanyolo	5/20 (25%)	0.837
Semanda	Kabanyolo	6/20 (30%)	0.651
NASPOT 1	Kabanyolo	2/20 (10%)	0.586
New Kawogo	Kabanyolo	3/20 (15%)	0.620
Total positives		31/120 (26%)	

Table 30. SPFMV titre (average $2^{-\Delta\Delta Ct}$) as determined by RT-qPCR in different sweet potato cultivars for the period of 12 weeks

	Cultivars								
Weeks	Beauregard	Araka White	Munyera	New Kawogo	Huachara	NASPOT 1	Kampala White	NASPOT 11	Peruanita
0	1.025	0.000001	0.00007	0.000006	0.0004	*	*	*	*
1	0.19	0.012	0.001	0.0003	0.001	0.006	0.008	0.0000004	0.001
2	0.812	0.211	0.002	0.00002	0.001	0.0001	0.0004	0.000002	0.012
3	0.811	0.102	0.147	0.006	0.069	0.001	0.037	0.002	0.066
4	0.161	0.213	0.017	0.0008	0.005	0.002	0.0003	0.004	0.094
5	0.573	0.108	0.127	0.009	(-)	0.055	0.115	(-)	0.367
6	0.782	(-)	0.028	(-)	(-)	(-)	(-)	(-)	0.03
7	0.662	(-)	1.146	(-)	0.262	(-)	(-)	(-)	(-)
8	0.235	(-)	0.564	(-)	1.086	0.399	(-)	(-)	0.115
9	0.538	0.00001	0.000002	(-)	(-)	(-)	0.000006	(-)	0.000006
10	0.732	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
11	0.445	(-)	0.922	(-)	0.067	(-)	(-)	(-)	0.938
12	(-)	7.718	8.674	1.597	(-)	(-)	(-)	(-)	(-)

*not determined; (-) indicates negative sample

Table 31. Reversion from SPFMV as determined by grafting to *I. setosa* of sweet potato shoots graft inoculated for 12 weeks

	Cultivar																	
	Huachara		Peruanita		Beauregard		Kampala White		NASPOT 1		New Kawogo		Munyera		Araka White		NASPOT 11	
Plant number	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2*	1	2
Shoot position																		
5 (most top)	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	**	–	–
4	–	+	+	–	+	+	–	–	–	–	–	–	+	–	–	**	–	–
3	–	+	+	+	–	+	+	–	+	+	–	–	+	+	+	**	–	–
2	+	+	+	+	+	+	+	+	–	+	+	–	+	–	+	**	–	–
1 (most bottom)	–	+	+	+	+	+	+	+	+	+	+	–	–	+	+	**	–	–

+ and – indicate presence and absence of SPFMV symptoms, respectively

***** and ** indicate that plant died and (thus) no shoots to test, respectively

5.4 Discussion

The observation of reversion from SPFMV confirms previous observations and reports: Gibson *et al.* (1997) reported that field plants of some varieties did not appear to be infected with SPFMV and attributed this to some factor such as reversion whilst Aritua *et al*. (1998b) showed there was reversion from SPFMV in Ugandan cvs New Kawogo and Tanzania, a very resistant and moderately resistant cultivars respectively (Mwanga *et al*., 1995). In addition to confirming these results, the current studies revealed reversion from SPFMV in more Ugandan sweet potato landraces and improved varieties ranging from very resistant to very susceptible cultivars. Recovery, but not reversion, was also observed in the indicator plant *I. setosa*.

That at least Ugandan cultivars, including apparently even relatively susceptible ones, can revert from SPFMV infections, suggests reversion is important in combating the effects of viruses on yields. Indeed, reversion seems to indirectly influence the selection of cultivars grown by farmers. Cultivars, such as New Kawogo that have a greater reversion potential are more commonly grown by Ugandan farmers (Bashaasha *et al*., 1995) than foreign cultivars such as Beauregard with a lesser reversion potential. Likewise, Gasura *et al.* (2009) noted that cultivars with ability to recover were common in the high SPVD pressure zones in central and western regions of Uganda. The possibility to select against sweet potato cultivars with low reversion potential is also evidenced by the observed selection by farmers of symptomless planting material. Although SPFMV rarely induces symptoms in infected sweet potato plants (Hollings, 1965), most of the symptomless material is actually virus free (Gibson *et* al., 1997). This was also confirmed in the current study but with relatively greater SPFMV incidence (Table 29) compared to Gibson *et al*. (1997)

observation. This is probably because of the greater SPFMV-susceptibility of some of the cultivars tested as well as the high virus incidence at MUARIK. Even for susceptible cultivars that could not completely revert, at least some of their shoots (mainly top shoots) were able to revert (Table 31). When replanting, farmers use the top most part (8-10 inches) of the shoot ideally indicating the likelihood of picking reverted plant parts even from the susceptible cultivars. Farmers may also abandon cultivars because of poor yields, probably due to their failure to revert, SPFMV decreasing storage root yield of infected plants by 50% (Gibson *et al*., 1997; Njeru *et al*., 2004). These scenarios indirectly show how farmers may select for reversion. The practice of selecting for symptomless planting material, of which most is virus free, and the natural potential of plants to revert thus explains why sweet potato viruses do not build up to 100% incidence in the field despite the high abundance of vectors and the vegetative propagation of the crop (Gibson *et al*., 1997). However, this does not necessarily mean that virus indexing should be overlooked; relying on only symptomless material may mean accepting some yield loss because some of the planting material is infected and it is not possible to tell which from the symptoms. Instead, selecting for symptomless material and indexing to confirm such material should supplement each other.

Although viruses are generally known to systemically infect their host plants (Petty *et al*., 1990), SPFMV has long been shown to have an uneven distribution within infected plants (Frison and Ng, 1981; Green *et al*., 1988; Abad and Moyer, 1992; Gibb and Padovan, 1993), allowing healthy parts to be selected. Resistance to viruses in sweet potato has been attributed to the ability of plants to restrict virus movement (Toussaint *et al*., 1984; Carrington *et al*., 1996) and the varying pathogen concentration throughout infected plants

(Mori and Hosokawa, 1977). Other researchers have attributed resistance to SPFMV to destruction of virus mRNA through RNA silencing (Kreuze, 2002). The uneven virus distribution may be due to restriction of virus movement or uneven destruction of virus within the plant; its presence was confirmed in this study for cultivars such as Huachara and Peruanita (Table 30). Almost similar to our observations on *I. setosa*, Salomon (1989) found recovery from SPFMV in an indicator plant *I. nil* which he correlated with an increase in a proteolytic activity that cleaves SPFMV capsid protein. It has not been reported if such increased proteolytic activity also occurs in SPFMV-infected sweet potato and *I. setosa.*

The ability of sweet potato landraces to revert varied between cultivars and that susceptible cultivars took longer to lose the virus (Table 27 and 31) implies a genetic basis: Gasura *et al.* (2008) found some F_1 progenies from a cross between paternal sweet potato cultivar Munyera with recovery potential and four maternal parents to be displaying some levels of recovery. Recovery and reversion from ACMV in cassava are also genotype dependent (Jennings, 1957; 1960; Fargette *et al*., 1996; Gibson and Otim-Nape, 1997) and may also vary with the virus, infection with a severe strain of ACMV (actually now shown to be EACMV) delaying reversion as compared to typical ACMV (Gibson and Otim-Nape, 1997). However, environmental factors may also have a significant role in the phenomenon of reversion. Indeed, heat treatment at 35^{o}C resulted in apparent reversion from SPFMV infection 2 weeks after graft inoculation (chapter 4; Table 14). Rossel and Thottappilly (1985) noted that resistant plants in the tropics often grow without virus disease symptoms especially during the hot season. Also, Bertschinger *et al*. (1995) observed a positive correlation between temperature and systemicity of viruses in potato

and Gibson and Otim-Nape, (1997) commented that cassava plants in Uganda recover from ACMV symptoms more often in the dry hot season than cool wet season. Correspondingly, Aritua *et al*. (1998b) suggested a possible influence of environmental conditions on reversion from SPFMV by sweet potato cvs New Kawogo and Tanzania as reversion progressed more rapidly in field than in screenhouse conditions. Therefore genotype by environment interaction may also have a crucial role in reversion observed in sweet potato landraces in Uganda and environmental factors that promote reversion therefore need further studies.

In summary, our results show that several sweet potato cultivars, including susceptible ones, revert from SPFMV, perhaps especially in hot conditions. The results can be used to identify sweet potato cultivars with high reversion potential, virus titre even being a selection criterion in resistance breeding. Reversion linked with virus testing can also be used to produce virus free sweet potato material. This avoids the use of expensive and labour intensive *in vitro* virus elimination techniques that are not feasible in many developing countries like Uganda.

CHAPTER SIX

GENERAL DISCUSSION

The overall objective of this study was to identify new viruses and virus strains in sweet potato landraces in Uganda, their involvement in synergism and the implication of SPFMV titre on the phenomenon of reversion. Specifically, the study identified SPLCUV and described its prevalence in Uganda and how some cultivars reverted, showed how the SPCSV isolate in Busia was not co-infected with SPFMV, based on poor expression of its RNase3 and p22 genes which would otherwise suppress resistance due to plant-based RNA silencing, and confirmed that sweet potato landraces do revert from SPFMV infection, probably based on natural resistance based on RNA silencing and that titre of the virus is at least one indicator of reversion in different cultivars. The main conclusions from the studies of this thesis are:

i. A new virus (SPLCUV: *Begomovirus*) is described from Uganda. This is the first evidence of a *Begomovirus* infecting sweet potato in Uganda and is the second report of a sweepovirus in the sub Saharan region, the first report being in Kenya. Sweepovirus seems to be common in Uganda.

ii. SPLCUV is not synergised by SPCSV and sweet potato landraces revert from SPLCUV infection.

iii. A strain of SPCSV is described which poorly synergises SPFMV. Sweet potato plants co-infected with SPFMV and this ‘mild’ SPCSV strain also revert from SPFMV infection. This ‘mild’ SPCSV provides only limited cross-protection against wild type SPCSV, evidence that RNA silencing based resistance has been partially

switched off by the RNase3 and p22 genes of the SPCSV. However, yields of plants only infected with 'mild' SPCSV are still halved and the symptoms of 'mild' SPCSV seem identical to those of wild type SPCSV when infecting alone so, cross protection would probably not be a useful attribute anyway.

iv. The difference in SPVD severity (wild type SPCSV + SPFMV *versus* 'mild' SPCSV + SPFMV) is due to differences in the expression levels of RNase3 and p22 genes of SPCSV; both genes are far more expressed in the wild type SPCSV than in the 'mild' SPCSV. The difference in RNase3 and p22 gene expression is likely due to the mutation(s) in the sg mRNA promoter(s) for these genes rather than any change in the genes themselves.

v. Virus titre is indicative of cultivars that revert and the time needed for cultivars to revert varies between resistant and susceptible cultivars. However, at some point in time, especially a few days after infection/inoculation, resistant cultivars can also have high virus titres like susceptible ones. Amongst all cultivars tested, cv NASPOT 11 is the fastest to revert from SPFMV single infection while cv Beauregard is the slowest.

A begomovirus infecting sweet potato, SPLCUV, was identified for the first time in Uganda; this is only the second time a sweepovirus has been identified in mainland Africa, the first time being in neighbouring Kenya (Miano *et al*., 2006), and it is the first time a full-length sequence has been described in Africa. The sweepovirus identified in Uganda differed from the Kenyan one and all others in the World by at least 13% (Table 7); this is beyond the species demarcation limit for begomoviruses (Fauquet *et al*., 2003) and was tentatively named SPLCUV. That is not to say that other sweepoviruses do not occur in

Uganda, including the Kenya one; one only was sequenced. Areas that still remain in great need of further study are the diversity of sweepoviruses present in Uganda and elsewhere in Africa, and the effect sweepovirus(es) have on yield. Sweepoviruses diverge basally from other begomoviruses (Fauquet and Stanley, 2003) and have no clear geographical origin (Briddon *et al*., 2010). Both of these observations were confirmed by this study when (a) the degenerate primer pairs designed for non-sweepovirus begomoviruses (Rojas *et al*., 1993; Deng *et al*., 1994) failed to detect SPLCUV and (b) when SPLCUV was found to be most closely related to sweepoviruses from countries in continents (Paprotka *et al*., 2010; Lozano *et al*., 2009) other than Africa (Fig. 9 and Table 8). This basal divergence of sweepoviruses and their unclear geographical origin are probably because of the movement of infected sweet potato material including the storage root that can readily sprout.

Many viruses are asymptomatic in sweet potato and they may have been overlooked, with most emphasis put on SPVD-associated viruses (SPCSV + SPFMV). However, sweepovirus(es) is probably common and widespread in Uganda yet it has only now been detected. However, previous researchers did not find it when they grafted what probably amounted to several hundreds of scions from symptomless plants in Uganda to *I. setosa* in their search for sweet potato viruses in the country (Gibson *et al*., 1997; Mukasa *et al*., 2003; Aritua *et al*., 2007). These results suggest that sweepovirus(es) may be a recent introduction in the country but spread quickly, perhaps aided by its lack of symptoms when infecting sweet potato plants and the high abundance of whiteflies. On the other hand, however, it is possible that SPLCUV symptom expression in *I. setosa* is dependent on environmental factors. Results [section 3.5; chapter 3 (observations made after publication)] showed that, while *I. setosa* in Ugandan screenhouses did not show any

symptoms, *I. setosa* grafted in a glasshouse in UK using the same sweet potato plants developed clear leaf curling symptoms; this was confirmed to be sweepovirus by PCR. I also had previously failed to detect sweepoviruses in many graft-inoculations to *I. setosa* in Uganda and the lack of symptoms developing on *I. setosa* in Uganda is perhaps the more likely explanation of why it was not previously detected. Interestingly, the lack of leaf curl symptoms in sweet potato also extended to sweet potato plants also infected with SPCSV and SPFMV which only developed typical SPVD symptoms. This lack of synergy with SPCSV, apparently the first report of a sweet potato virus not synergised by SPCSV, may also have had a role in it previously being undetected in the country.

Several previous researchers have reported a number of SPCSV strains, some having more severe synergistic effects than others (Rossel and Thottappilly, 1988; Gibson *et al*., 1998; Alicai *et al*., 1999b; Cuellar *et al*., 2008). According to Rossel and Thottappilly (1988), cultivars can develop very mild SPVD symptoms that farmers cannot readily distinguish between affected and unaffected plants. However, all these still seem to involve no lack of synergy by SPCSV. In this study, a mild strain of SPCSV experimentally observed to synergise SPFMV only partially was identified in Ugandan sweet potato fields. The RNase3 and p22 genes of SPCSV are responsible for breaking down RNA silencing-based resistance to SPFMV (Kreuze *et al*., 2002; Kreuze *et al*., 2005; Cuellar *et al*., 2008) and thus, in co-infections with SPCSV, severe symptoms of SPFMV normally develop (Gibson *et al*., 1998). All SPCSV strains have the RNase3 gene but the p22 gene has been found exclusively in Ugandan isolates so far. Here, it apparently boosts the synergising effect of the RNase3 gene and makes symptoms more severe (Kreuze *et al*., 2005; Cuellar *et al*., 2008). The ‘mild’ SPCSV has both RNase3 and p22 genes; its p22 is very similar to the

p22 genes in the GenBank and literature whereas RNase3 has some variations, specifically with those RNase3 reported in the GenBank (http://blast.ncbi.nlm.nih.gov) but negligible with those reported by Tugume (2010). Instead, both the RNase3 and p22 genes were far less expressed for the 'mild' SPCSV than for wild type SPCSV (Tables 23 and 24) and it seems likely that a change in the RNase3 and/or p22 sg mRNA promoter(s) is responsible for the lesser gene expression in 'mild' SPCSV rather than any change in the genes themselves. This lesser expression of the RNase3 and p22 genes may be the explanation of their lack of synergising effect on co-infecting SPFMV, rather than any mutation in the genes themselves. Their lesser expression may instead be caused by mutation in a sg mRNA promoter presumed to drive their expression. Lam *et al*. (1989) observed that site specific mutations at the TGACG motif of activation sequence factor 1 (ASF-1) of the 35S promoter of *Cauliflower mosaic virus* (CaMV) alters gene expression pattern for virus resistance in transgenic plants. The mechanisms behind the different interactions of the 'mild' type SPCSV in host plants co-infected with SPFMV, especially looking at the possibility of mutations in sg mRNA promoters of RNase3 and p22 genes, require further study.

In fields in Busia district, SPVD was rare and, if present, associated with the presence of wild type SPCSV despite the relatively much greater abundance of 'mild' SPCSV. This was consistent with field experiments; trials that started as healthy succumbed more to SPVD from SPVD infector than trials that were originally infected with 'mild' SPCSV (Table 15 and 18). The possibility of 'mild' SPCSV partially cross-protecting against the wild type SPCSV may account for this rarity of SPVD. Other reasons perhaps more likely to be responsible for the rarity of SPVD and high prevalence of 'mild' SPCSV in Busia

district are: plants infected with the ‘mild’ SPCSV continue to have almost normal growth and so do not suffer reduced visibility to whiteflies and are not easily selected against by farmers when they are selecting planting material. Also, unlike in normal SPVD where SPCSV titre is less than when it infects by itself (Karyeija *et al*., 2000; Mukasa *et al*., 2006; Kokkinos and Clark, 2006a), experimental co-infections involving ‘mild’ SPCSV and SPFMV left SPCSV titre unaffected. Such unaffected virus titre is likely to lead to ‘mild’ SPCSV being more readily acquired by whiteflies than the wild type with a reduced titre in normal SPVD. In addition, experimental co-infections of SPFMV and ‘mild’ SPCSV had a low titre of SPFMV (Table 20 and 21) allowing plants to recover and eventually revert to being infected only with ‘mild’ SPCSV. Such low SPFMV titres may also result in it not easily being acquired by aphids (Aritua *et al*., 1998b) and so it again may not spread to infect other ‘mild’ SPCSV-infected plants; aphids also seemed rare in Busia district. However, the occurrence of ‘mild’ SPCSV, despite its name, provides little hope in the control of SPCSV because yield loss of affected plants was still around 50% and is likely to become more (or is already) widespread because of its better survival characters. Extended surveys for prevalence of ‘mild’ SPCSV in Uganda are needed and a careful watch kept on its spread. Wild type SPCSV faces a big penalty as it synergises SPFMV to very high titres and is thus easily selected against in severely SPVD affected plants.

Upon infection with viruses, plants employ an RNA silencing mechanism against all foreign genes entering the plant (Waterhouse *et al*., 2001a; Vaucheret, 2001; Voinnet, 2001). However, many viruses, in turn, deploy virus-encoded proteins which suppress RNA silencing allowing them to infect their host plants quite successfully (Anandalakshmi

et al., 1998; Kasschau and Carrington, 1998; Voinnet *et al*., 1999; Voinnet *et al*., 2000; Ahlquist, 2002; Moissiard and Voinnet 2004). RNA silencing suppression is more evident for the case of virus synergism where infected plants become severely affected. In turn, however, plants also evolved an even greater level of host resistance that restrain virus-encoded RNA silencing suppression (Li *et al*., 1999) which is manifested through possibilities of diseased plants to revert from virus infection. Reversion also seems to work on the RNA silencing mechanism (Ratcliff *et al*., 1999; Kreuze *et al*., 2002) but, severely affected SPVD plants do not show any signs of reversion; the mechanism seems to be commonly deployed for single virus infections and reversion was observed for two unrelated viruses, SPLCUV and SPFMV. Reversion from SPFMV in SPVD affected plants was only once observed when 'mild' SPCSV co-infected with SPFMV. This is not the first time reversion has been observed in sweet potato plants in Uganda. Gibson *et al*. (1997) observed that many field plants were healthy when graft-indexed on *I. setosa* (and reversion was the most obvious explanation) and Aritua *et al*. (1998b) observed actual reversion from SPFMV in the very resistant cv New Kawogo and resistant cv Tanzania. In addition to confirming these results, the current study observed reversion in some very susceptible sweet potato cultivars such as Beauregard (Table 28, 30 and 31). That reversion occurred from unrelated viruses and even in the susceptible cultivars suggests it is an important phenomenon in the control of sweet potato viruses. Of all cultivars tested, the Ugandan cv NASPOT 11 (Tomulabula) was the fastest to revert while the American cv Beauregard was the slowest to revert from SPFMV. The landrace New Kawogo was the fastest to revert from SPLCUV. NASPOT 11 is a product of participatory plant breeding.

Several hypotheses have been put to explain reversion. For example, SPFMV is known not to be uniformly distributed in the infected plants (Frison and Ng, 1981; Green *et al*., 1988; Abad and Moyer, 1992; Gibb and Padovan, 1993). In this study, SPLCUV was also observed to have uneven distribution in infected plants (Table 9) which is consistent with an earlier observation by Kokkinos and Clark (2006b) that they could not detect SPLCV in all parts of the plant. Several other viruses in other crops such as CBSV and ACMV in cassava are also now known not to be uniformly distributed within the plant (Mori and Hosokawa, 1977; Rossel *et al*., 1987; 1994; Njock *et al*., 1994) and disagrees with earlier observations that one of the characteristics of plant viruses is that they systemically infect their hosts (Petty *et al*., 1990). Other researchers have attributed reversion from SPFMV in East African sweet potato cultivars to destruction of virus RNA through RNA silencing (Kreuze *et al*., 2002) rather than limited virus movement and uneven distribution – but destruction of virus could still be the mechanism accounting for the limited virus movement and uneven distribution of virus. Irrespective of the mechanism, it is likely to be very important in ensuring that sweet potato planting material is mainly virus free. It is not yet reported whether pathogen genotype influences reversion in sweet potato but it seems a likely outcome as part of the continual battle between plant and pathogen. Environmental factors also seem to play a significant role in reversion, heat treatment enhancing reversion from SPFMV (Table 14). Further work is needed on the influence of genotype and environment on the phenomenon of reversion. Reversion can be exploited in maintaining and ensuring farmers with virus free planting material without necessitating researchers going for the more expensive *in vitro* techniques which are anyway not feasible for the case of sweet potato in Uganda. Virus titre results can be used by breeders to select for

cultivars with high reversion potential and such cultivars can be used to breed for virus resistance.

Research gaps cited above open up opportunities for the academic virologists and/or researchers. Besides that, exploitation of the results particularly on the mechanism of reversion will help plant health experts in designing virus control packages that are apporopriate for the case of Uganda. Overall, the results of this study will contribute to enhanced integrated sweet potato viruses management in sweet potato fields in Uganda and elsewhere in the World and thus sustain sweet potato productivity.

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APPENDIX

Appendix 1. Sweet potato viruses not yet recognised by ICTV

Tentative species		Putative Genus	Vector	Distribution	References
Sweet potato virus Y (SPVY)	*Potyviridae*	*Potyvirus*	?	Taiwan, U.S.A	Ateka *et al*., 2004a; Souto *et al*., 2003.
Sweet potato leaf curl Uganda virus (SPLCUV)	*Geminiviridae*	*Begomovirus*	Whiteflies	Uganda	Wasswa *et al*., 2011.
Sweet potato chlorotic fleck virus (SPCFV)	*Flexiviridae*	*Carlavirus*	?	S. America, East Africa	Aritua *et al*., 2003; Ateka *et al*., 2004a; Tairo *et al*., 2004.
Sweet potato yellow dwarf virus (SPYDV)	*Potyviridae*	*Ipomovirus*	?	Taiwan, Far East	Liao *et al*., 1979.

Sweet potato vein mosaic virus (SPVMV)	*Potyviridae*	*Potyvirus?*	Aphids	Argentina	Nome *et al*., 1974.
Sweet potato ringspot virus (SPRSV)	*Comoviridae*	*Nepovirus*	?	Papua New Guinea	Brown *et al*., 1988.
Sweet potato caulimo-like virus (SPCaLV)	*Caulimoviridae*	*Caulimovirus?*	?	Puerto Rico, Uganda	Atkey and Brunt, 1987; Mukasa *et al*., 2003.
Ipomoea crinkle leaf curl virus (ICLCV)	*Geminiviridae*	*Begomovirus*	?	U.S.A	Cohen *et al*., 1997
Sweet potato virus 2 (SPV2)	*Potyviridae*	*Potyvirus*	Aphids	Nigeria	Rossel and Thottaplilly, 1988
Sweet potato badnavirus A (SPBV-A)	*Caulimoviridae*	*Badnavirus*	?	Tanzania	Mbanzibwa *et al*., 2011
Sweet potato badnavirus B (SPBV-B)	*Caulimoviridae*	*Badnavirus*	?	Tanzania	Mbanzibwa *et al*., 2011

Sweet potato badnavirus C (SPBV-C)	*Caulimoviridae*	*Badnavirus*	?	Tanzania	CIP, unpublished
Sweet potato badnavirus D (SPBV-D)	*Caulimoviridae*	*Badnavirus*	?	Tanzania	CIP, Unpublished
Sweet potato vein clearing virus (SPVCV)	*Caulimoviridae*	*Cavemovirus*	?	East Africa, Central America and the Caribbean islands	CIP, Unpublished
Sweet potato C-3 virus	*Bunyaviridae*	*Phlebovirus?*	?	Taiwan	CIP, Unpublished
Sweet potato C-6 virus	*Flexiviridae*	*Carlavirus?*	?	Taiwan	CIP, Unpublished
Sweet potato golden vein associated virus (SPGVaV)	*Geminiviridae*	*Begomovirus*	?	Brazil	Paprotka *et al* 2010
Sweet potato leaf curl Canary virus (SPLCCaV)	*Geminiviridae*	*Begomovirus*	Whiteflies	Spain	Lozano *et al*., 2009
Sweet potato leaf curl China virus (SPLCV-CN)	*Geminiviridae*	*Begomovirus*	?	China	Luan *et al*., 2007

Sweet potato leaf curl Lanzarote virus (SPLCLaV)	*Geminiviridae*	*Begomovirus*	Whiteflies	Spain	Lozano *et al*., 2009
Sweet potato leaf curl Spain virus (SPLCESV)	*Geminiviridae*	*Begomovirus*	Whiteflies	Spain	Lozano *et al*., 2009
Sweet potato mosaic associated virus (SPMaV)	*Geminiviridae*	*Begomovirus*	?	Brazil	Paprotka *et al* 2010
Sweet potato symptomless mastrevirus 1 (SPMV)	*Geminiviridae*	*Mastrevirus*	?	Tanzania	Mbanzibwa *et al*., 2011
Sweet potato virus C (SPVC)	*Potyviridae*	*Potyvirus*	Aphids	U.S.A	Kokkinos and Clark, 2006a

? = Unassigned genus or vector