MAKERERE UNIVERSITY

REVERSION FROM *Sweet potato feathery mottle virus* IN BI-PARENTAL POPULATION OF NEW KAWOGO AND BEAUREGARD

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DECLARATION

I, IKALIZA IVAN, declare that this research report is my original work and is submitted for the award of Bachelor of Science degree in Agriculture of Makerere University. This report has never been submitted to any university for any award whatsoever.

Signature..... **Date** ..... 18/09/2018 .....

APPROVAL

This research report has been under my supervision and is now ready for submission to Makerere University for examination.

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ABSTRACT

Sweetpotato is a major food crop grown mainly by the rural poor who make up most of Uganda's population. In terms of dry matter production, it is the sixth most important food crop, after rice, wheat, potatoes, maize and cassava and the second most important root crop after potato in the entire world. However, its production is greatly constrained by virus infections. The most common viruses are *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV) found almost everywhere sweetpotato is grown. Methods have been proposed for virus disease control such as use of chemicals, biological control of insect vectors and use of resistance. Resistance through reversion from virus infections is a natural mechanism among resistant plants by which previously infected plants become virus free. Reversion from sweetpotato viruses has been observed mainly in east African sweetpotato landraces such as New Kawogo whereas the American varieties such as Beauregard seldom revert. Information is lacking whether reversion is heritable. The objective of this study was to evaluate heritability of reversion from SPFMV infections in a biparental population of the resistant cultivar, New Kawogo and the susceptible cultivar Beauregard. New Kawogo and Beauregard were planted in a crossing block and were cross bred upon maturity to obtain seeds for planting to the next generation. Fifty progeny seeds were germinated, and plants graft inoculated with SPFMV. Plants were evaluated for reversion for a period of 6 weeks after graft inoculation using the indicator plant *Ipomoea setosa*. Many (> 50%) of the progeny plants were found to show significant ($p \leq 0.05$) reversion potential from SPFMV infection. The results show that reversion is heritable and can provide a cost-effective way of managing SPFMV infection among susceptible varieties through crossing them with resistant ones.

LIST OF ABBREVIATIONS

cDNA- Complementary Deoxyribonucleic Acid

CIP- International Potato Center

DNA- Deoxyribonucleic Acid

ACMV- *African cassava mosaic virus*

ELISA-Enzyme Linked Immunosorbent Assay

FAO- Food and Agricultural Organization

FAOSTAT- Food and Agricultural Organization Statistics

INIBAP- International Network for the Improvement of Banana and Plantain

Mt- Metric tons

PCR- Polymerase Chain Reaction

RCA- Rolling Circle Amplification

RNA- Ribonucleic Acid

RT-PCR- Reverse Transcription Polymerase Chain Reaction

SPCaLV- *Sweet potato caulimo-like virus*

SPCFV- *Sweet potato chlorotic fleck virus*

SPCSV- *Sweet potato chlorotic stunt virus*

SPFMV- *Sweet potato feathery mottle virus*

SPLCUV- *Sweet potato leaf curl Uganda virus*

SPLCV- *Sweet potato leaf curl virus*

SPMMV- *Sweet potato mild mottle virus*

SPVD- Sweet Potato Virus Disease

SSEM- Serologically Specified Electron Microscope

DEFINITION OF TERMS

Anti-body- also known as an immunoglobulin, is a large, Y-shaped protein produced mainly by plasma cells that is used by the immune system to neutralize pathogens such as pathogenic bacteria and viruses.

Crossbreeding - mixing different species or varieties of animals or plants to produce hybrids

Cultivar - refers to an assemblage of plants selected for desirable characters that are maintained during propagation

Landrace- a domesticated, locally adapted, traditional variety of a species of animal or plant that has developed over time, through adaptation to its natural and cultural environment of agriculture and pastoralism, and due to isolation from other populations of the species.

Molecular marker - a molecule contained within a sample taken from an organism or other matter used to reveal certain characteristics about the respective source.

Morphological characteristics -gross structure of an organism or taxon and its component parts.

Persistent transmission - when the insect feeds on virus-infected plant, and viral particles are carried in through the mouthparts into the gut of the insect and stored in the salivary glands.

Semi persistent transmission- when the insect feeds from the plant sap of virus-infected plant, and viral particles get attached to the mouthparts and/or any other insect body parts commonly the stylet.

Recovery- when a plant showing clear symptoms of infection starts to produce organs which are symptomless

Reversion- is the ability of an infected plant to provide uninfected cuttings

Synergistic interaction- the interaction or cooperation of two or more organisms to produce a combined effect greater than the sum of their separate effects.

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CHAPTER ONE
INTRODUCTION

1.1 Description of sweetpotato

Sweetpotato (*Ipomoea batatas L.*) is an important food crop widely grown in the tropical, sub-tropical and warm temperate regions. Although it is grown as an annual, it is a perennial crop that belongs to the morning glory family (or convolvulaceae). It forms large fleshy-edible storage roots on the underground stem nodes. It has trailing or twinning stems up to 4m long with heart-shaped or halberd-shaped leaves and roots at the nodes. Sweetpotato is an indeterminant plant without a defined physiological maturity, and as such, storage roots may continue to enlarge for a long time. Its flowers have purplish throats and white margins, rarely bloom and may (or may not) produce seeds. The tubers are variable in size, shape and color. The skin and flesh vary in color, texture, moisture and quality (Valverde *et al*., 2007; Kumari *et al*., 2014).

Sweetpotato is highly diverse (genetically), more commonly with 6x and sometimes 4x ploidy forms (2n=6x=90 or 2n=4x=60). This can be attributed to gene flow between different ploidy levels of *Ipomoea* species closely related to *I. batatas* such as diploid *I. trifida* and *I. triloba* as a result of fertilization by non-reduced gametes (Srisuwan *et al.*, 2006). Out of about 500 species in its family, *I. batatas* is the only food crop and the rest of its wild relatives are mainly economically important in breeding for natural resistance to pests, diseases and abiotic stresses like drought (Austin, 1988; Huang and Sun, 2000).

1.2 Origin of Sweetpotato

The center of origin of *I. batatas,* basing on its morphological characteristics was thought to be between the Yucatan Peninsula of Mexico and the mouth of River Orinoco in Venezuela. Sweetpotato was originally domesticated at least 5000 years ago in tropical America (Austin, 1988). However, more recently, the use of molecular markers has been used to reveal that the primary center and probably the most likely center of origin of *I. batatas* is Central America (Huang and Sun, 2000; Zhang *et al*., 2000). This is owing to the great diversity and the richness of its wild relatives in this region (Huang and Sun, 2000). It is also suggested by recent studies that through non-human or natural transfer, the Oceania sweetpotato probably came from Central America. On the converse, some studies based on linguistic links between the Quenchua and Polynesian names of sweetpotato were used to assert the Peruvian origin of

sweetpotato. However, this assertion was greatly refuted by studies based on Molecular markers (Zhang *et al*., 2000).

The crop was then introduced to China (the largest producer) in the late 16th century, most likely from Luzon in the Philippines (O'Brien, 1972) and other probable regions such as Vietnam, India and Burma. The crop was transferred from West Indies to Western Europe by Columbus, in 1492 and then to India, South East Asia, East Indies and Africa in the 16th century by the Portuguese (Zhang *et al*., 2000). The crop might have reached Uganda, from the East and the West along trade routes.

1.3 Production and distribution

Sweet potato is cultivated in about 111 countries with a total of 110.75 million tons produced in 2013. It is grown on about 8.1 million hectares with an average yield of about 13.3 tons/ha. The main producers are the Asian countries, particularly China catering for 71% of the world production. In the year 2013, China took the lead with a gross production of 70,526,000 Mt followed by Tanzania in East Africa with a gross production of 3,470,304 Mt. Uganda was in the fifth position with a gross production of 1,810,000 Mt following after Nigeria and Indonesia with 3,450,000 Mt and 2,386,729 Mt respectively (Table 1; FAO, 2017). China has 6.6 million hectares of cultivated area accounting for approximately 70% of world's total cultivated area thus dominating the distribution. Second to it is East Africa where most of the production is concentrated around the shores of Lake Victoria (Loebenstein, 2009).

Table 1: World Sweet potato production in metric tonnes (percentages in parentheses) showing the major sweet potato producing countries

Year Country	2002	2005	2009	2013	2016
China	120.84 (85.1%)	83%	80.5 (75.6)	70.526 (68.6%)	70.526 (68.6%)
Nigeria	2.84 (2%)	-2%	3.3 (3.1%)	3.45 (3.35%)	3.9 (3.7%)
Uganda	2.84 (2%)	-2%	2.7 (2.5%)	1.81 (1.7%)	2.1 (2%)
Vietnam	1.42 (1%)	*	1.32 (1.2%)	*	*
Indonesia	1.42 (1%)	*	1.88 (1.8)	2.387 (2.3%)	2.3 (2.2%)
Others	12.64 (8.9%)	-13%	16.8 (15.8%)	24.5 (24%)	26.1 (24.9%)
World Production	142	*	106.5	102.8	105

*Production data is not given

Source; FAOSTAT (2017)

1.4 Importance of sweetpotato

In terms of dry matter production, sweetpotato is the sixth most important food crop after rice, wheat, potatoes, maize and cassava and the second most important root crop after potato in the entire world. Under certain circumstances, sweetpotato can produce more edible energy per hectare per day than any other crop. It has the highest production, in terms of biomass and nutrients in comparison to other food crops in the world (CIP 2000 and 2010).

The storage roots are used as animal feeds, raw materials for alcohol production and most importantly as staple food in most parts of sub Saharan Africa. Due to its ability to thrive under conditions with limiting growth requirements such as poor soil fertility and low soil moisture levels, sweetpotato is used as a famine relief crop in most developing countries (CIP, 2010).

Compared to other crops, its tubers are important sources of carbohydrates and other nutrients (Table 2). The leaves are used as greens. In processed form, sweetpotato is used for making pasta in most of China, as well as in confectionary to make candies, sweets and sugar coated

or salted crisps for snack foods. In Uganda, it is used in pie fillings and sources such as Tomato sauce. Its flour is used as supplement for wheat flour in baking bread, biscuits or cakes.

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	20.0	19.7
Calcium (mg)	22.0	6.0	16.0	7.0
Energy (kca)	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	271.0	543.0

*is for low dry matter American varieties

Source: INIBAP (1999)

1.5 Constraints to production of sweetpotato

Sweetpotato production is constrained by a number of factors including poor management of crop pests, diseases, soil and water resources and generally poor production systems. The major crop pests are the weevils (*Cylus spp*) in most of Africa. The commonest species of weevils are; *Cylus puncticolis* and *C. brunneus* in Africa and *C. formicarious* in the United States, Asia and Carribean. In mild infestations, their damage is due to defoliation of plant leaves but can in severe cases attack the storage roots. Their tunneling effect in both stems and roots is of most important significance. They significantly lower the quality of the storage roots causing them to become bitter and attain a bad smell (Sato *et al*., 1981).

Virus diseases are second to weevils in importance. They occur wherever sweetpotato is grown (Brunt *et al.,* 1996). The most important of these is the Sweet potato virus disease (SPVD),

caused by a co-infection between *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV) (Mukasa *et al*., 2003; Cuellar *et al*., 2008). The disease is the most devastating of all sweetpotato diseases as it leads to massive reduction in yields and quality of storage roots (Clark and Moyer., 1988). Massive yield losses have been reported; 30-50% in the United States (Clark and Hoy, 2006), 56-98% in Africa (Gibson *et al*., 1998) and as great as 90% in East Africa including Uganda (Clark *et al*., 2012).

1.6 Problem statement

Sweetpotato production is greatly constrained by virus infections. Methods have been proposed for virus disease control where use of resistance is considered as the most sustainable control measure. Resistance through reversion, where previously SPFMV-infected plants became virus free has been reported (Gibson *et al*., 2014). Here, the East African white fresh landraces such as New Kawogo were observed to be more reverting than the American orange flesh cultivars such as Beauregard. It was not known if the reversion potential is heritable and if it could be used to improve sweetpotato varieties. This study evaluated reversion in a bi-parental population of cultivars Beauregard and New Kawogo.

I.7 Justification of the Study

SPFMV is the most prevalent virus worldwide and commonly involved in synergism with SPCSV with a great impact on yield. Heritability of reversion from SPFMV infections will provide a fundamental basis for promoting resistance in susceptible varieties through crossing such varieties with known resistant ones. This will provide a cheaper and more convenient SPFMV management option. This will substantially reduce the SPFMV infections and yield losses among the resource poor farmers who cannot afford other control options for virus management.

1.8 Study objectives

1.8.1 Main objective

The main objective of the study was to contribute to virus management in sweetpotato through evaluating heritability of reversion from SPFMV infections in a bi parental population of New Kawogo and Beauregard.

1.8.2 Specific objectives

I. To make crosses and obtain seeds between New Kawogo and Beauregard
II. To determine reversion from SPFMV infection in progenies of New Kawogo and Beauregard

1.9 Hypotheses

I. When cultivars New Kawogo and Beauregard are crossed, they produce viable seeds which germinate to produce hybrid offsprings

II. Progenies of a cross of New Kawogo and Beauregard revert from SPFMV infections in a segregated manner

CHAPTER TWO
LITERATURE REVIEW

2.1 Common viruses of sweetpotato and their distribution

Over 35 viruses belonging to different genus have been reported worldwide. However, only six of these have been reported in Uganda which include; *Sweet potato mild mottle virus, Sweet potato chlorotic stunt virus, Sweet potato leaf curl Uganda virus, Sweet potato feathery mottle virus, Sweet potato chlorotic fleck virus, and Sweet potato caulimo-like virus* (Wasswa *et al*., 2011*;* Clark *et al*., 2012).

Table 3: The six sweet potato viruses found in Uganda

Virus	Family	Genus	Vector	Distribution	References
Sweet potato chlorotic stunt virus	Closteroviridae	Potyvirus	White fly	World wide	Gibson *et al*., 1998; Alicai *et al*.,1999
Sweet potato mild mottle virus	Potyviridae	Ipomovirus	White fly	East Africa	Hollings *et al*., 1976
Sweet potato feathery mottle virus	Potyviridae	Potyvirus	Aphids	Worldwide	Abad *et al*., 2007
Sweet potato leaf curl uganda virus	Geminiviridae	Begomovirus	White fly	USA, Sicily, Kenya, China, Uganda	Miano *et al* 2006
Sweet potato caulimo-like virus	Caulimoviridae	Caulimovirus	*	*	*
Sweet potato chlorotic fleck virus	Flexiviridae	Carlavirus	*	*	*

2.1.1 *Sweet potato mild mottle virus*

Sweet potato mild mottle virus (SPMMV) is a white fly-borne virus found commonly infecting sweet potato in East Africa. It may be found together with SPCSV in sweetpotato causing typical SPVD symptoms (Mukasa *et al*., 2006). Symptoms of SPMMV include leaf mottling, venal chlorosis, dwarfing and poor growth. According to Hollings (1976), with four leaves or more, SPMMV infected *Ipomoea setosa* exhibits a bright yellow venal chlorosis but subsequent leaves become symptomless.

2.1.2 *Sweet potato chlorotic stunt virus*

Out of over 35 viruses known to infect sweet potato, *Sweet potato chlorotic stunt virus* (SPCSV) is the most devastating, being distributed worldwide and has been detected in all sweet potato areas except those in pacific region It was previously known as *Sweet potato sunken vein virus*. The virus is phloem limited transmitted by white fly species, *Bemicia tabaci* and *Trialeurodes abutilonea* in a semi persistent fashion (Clark *et al*., 2012; Quin *et al*., 2014). Its symptoms are aggravated when it coinfects with SPFMV to cause Sweet potato virus disease.

2.1.3 *Sweet potato leaf curl virus*

This group of viruses is distributed worldwide, associated with most, if not all geographic regions where sweetpotatoes are grown. They belong to a phylogenetically distinct group of Begomoviruses known as sweepoviruses. They are generally symptomless, even in double infection with SPCSV. This limits selection of virus free materials for propagation when efficient virus detection methods are unavailable (Valverde *et al*., 2007; Albuquerque *et al*., 2012).

They are transmitted by several biotypes of the white fly vector *Bemisia tabaci* (Simmons *et al*., 2009; Trenado *et al*., 2011). According to Kim *et al*, (2015), sweepoviruses are also transmitted through true seed of sweetpotato. Sweepovirus infection results in leaf curling and vein yellowing on some hosts. In one study involving naturally infected wild host species, SPLCV was identified in mixed infection with *Merremia leaf curl virus* in *Merremia* species (Qiu *et al*., 2007).

2.1.4 *Sweet potato feathery mottle virus*

Single infections by SPFMV are always symptomless, but the symptoms become more severe if a coinfection occurs with SPCSV. This encourages further perpetuation of the virus since

farmers select such symptomless but infected plants for propagation in absence of efficient detection methods (Untiveros *et al*., 2007). Some studies have shown that infections by SPFMV alone can lead to yield reductions of up to 46%, depending on variety and environment (Domola *et al*., 2008; Adikini *et al*., 2016).

The virus is ubiquitous, infecting sweetpotato all over the world. Its infections are characterized by inclusion bodies in the cells cytoplasm. It is transmitted by many aphid species including *A. gossypii* and *Myzus persicae* in a non-persistent manner. It majorly infects plants in the family convolvulaceae, most especially the genus *Ipomoea*. According to phylogenetic analysis, four strains of SPFMV have been described which include the Common, Russet Crack, Yellow Vein or East African and the Ordinary strains (Campbell *et al*., 1974; Untiveros *et al*., 2007). However, many other strains such as the S strain also do exist (Sakai *et al*., 1997).

2.2 Detection of viruses

The detection and identification of sweetpotato viruses is complicated by frequent occurrence of mixed infections and synergistic complexes, low virus titers, diverse viral strains, and presence of asymptomatic infections (Karyeija *et al*., 2000; Valverde *et al*., 2007). Currently, progress has been made in developing sensitive techniques for several sweet potato viruses through various ways described below.

2.2.1 Serological detection

When an animal is injected with a pathogen, it produces certain proteins (called antibodies) in response, which are specific to the pathogen injected. These antibodies either deactivate the pathogens or neutralize them thus preventing further infection. Serological detection involves the use of such antibodies so as to effect reactions used to identify presence or absence of a virus. There are two main techniques used in serological detection, that is, Serologically Specified Electron Microscope (SSEM) and Enzyme Linked Immunosorbent Assay (ELISA). ELISA is the most widely used.

ELISA was introduced in 1976. It is based on the covalent linkage of an enzyme to an antibody. Advantages of ELISA include its ability to handle very large samples, its speedy reaction and it is also relatively economical (Voller *et al*., 1976; Bar Joseph *et al*., 1979).

ELISA techniques include Nitrocellulose Membrane–Enzyme Linked Immunosorbent Assay (NCM-ELISA), Double Anti-body Sandwich ELISA (DAS-ELISA) and Triple Antibody Sandwich ELISA (TAS-ELISA). NCM-ELISA is carried out by first spotting sap on to a

Nitrocellulose membrane and allowing it to dry for about 30 minutes while ensuring that remaining absorption sites are blocked with a high protein solution. The membrane is then probed with two successive antibodies following CIP protocol. In this method, color development which is proportional to amount of virus present is measured by visual observation (Valverde *et al*., 2007).

DAS- and TAS-ELISA clearly differ from NCM-ELISA in their use of micro titer plate as a support for reagents instead of a nitrocellulose membrane. The former involves first coating the plate with a polyclonal antibody, followed by an antibody-virus conjugate and then a color development substance. With the later, a polyclonal antibody is used to coat the plate followed by a monoclonal antibody then followed by another antibody and to the mixture, a substrate is added. The results in both cases are determined by spectrophotometric measurement of absorbance thus making it more reliable than NCM-ELISA (Voller *et al*., 1976; Bar Joseph *et al*., 1979).

2.2.2 Molecular methods

2.2.2.1 Molecular hybridization

It employs the use of labeled viral DNA or RNA with labels for detection of viruses. This method has also been used for detection of both SPFMV and SPLCV. It provides greater detection sensitivity than the immunoassay approach because of its ability to escape interference by host factors (Valverde *et al*., 2007; Abad and Moyer, 1992).

2.2.2.2 Rolling Circle Amplification

Rolling circle amplification has been successfully used to amplify the circular, single stranded DNA viruses, such as sweepoviruses. Among the advantages, is that no previous knowledge of the sequence is required and full-length genomes can be amplified which provides more information about genetic diversity. Whereas among the disadvantages, is that it is limited to the detection of circular DNA viruses. Among other limitations, methodological problems have been reported due to the presence of defective DNA and host plant mitochondrial plasmids, which are amplified non-specifically (Wyant *et al*., 2012).

2.2.2.3 Polymerase Chain Reaction

Polymerase Chain Reaction is the practice of copied acid probes or the *in vitro* amplification of the precise nucleic acid sequence in a genome (in this case, the virus genome) that are then used to detect the presence of a particular disease. For RNA viruses such as SPFMV and SPCSV, Reverse Transcription-Polymerase Chain Reaction is employed whereby a cDNA

strand complementary to the virus has to first be synthesized using the enzyme reverse transcriptase. It involves using both forward and reverse oligonucleotide primers which bind to the opposite ends of the viral nucleic acid region of interest. Specific primers can be designed to anneal to specific regions of a virus. The limitation with this technique is that though it can amplify viruses existing in low titers, false negative reactions with well-known infected plants have been found with Potyviruses and with sweepoviruses (Souto *et al*., 2003; Li *et al*., 2004; Wasswa *et al*., 2011; Qin *et al*., 2013).

2.2.3 Grafting

Grafting onto susceptible indicator plants is widely used to ascertain many sweetpotato viruses (Loebenstein *et al*., 2003). A number of universal indicators may be used though the criteria for choosing among the indicators varies, depending on the interests of the researcher. Most commonly used indicators include; *Chenopodium quinoa, Ipomoea nil* and *Ipomoea setosa.* For sweetpotato viruses, *I. nil* and *I. setosa* are the most preferred. *I. setosa* is considered to be a near universal indicator for sweetpotato viruses (Clark and Moyer, 1988; Valverde *et al*., 2007).

The advantage of this method is that it enhances the titer of the viruses in a susceptible indicator host, leading to improved detection using serological and nucleic acid-based methods. However, the method is limited in that it does not confirm the identity of the viruses, given that some symptoms are common among viruses. It also requires maintenance of plants in greenhouse or growth chambers making it uneconomical to the resource poor (Clark *et al*., 2012).

2.3 Control of plant viruses

2.3.1 Chemical control

Since there is no discovery of any chemical treatment for virus diseases unlike fungal and bacterial infections which can be chemically controlled, chemical control is achieved indirectly by applying chemicals to kill vectors (insects) which transmit the viruses. This is however costly and can be uneconomical for the rural poor since sweet potato is grown mainly in the developing countries. It is also effective in controlling only a small spectrum of pests. This is because the insect vectors may require some time of exposure to the chemical, for it to have significant effect on them. This also limits chemical control to viruses that are transmitted by a few insect vectors thus making it an ineffective approach. In addition to the above limitations,

cases of resistance to several chemicals by insect vectors have been reported for example the resistance of *A gossypi*, a vector for SPFMV, to pyrethroids (Palumbo *et al*., 2001)

2.3.2 Biological control

This involves the use of natural enemies which predate on the vectors. It can be effective though costly in terms of producing and releasing the natural enemies.

2.3.3 Quarantine

Though introduction of more germplasm may be necessary to provide the diversity to improve the African crop, for the sake of minimizing virus infections, there is a need to exclude the movement of nonindigenous strains of viruses.

2.3.4 Cultural methods

This refers to systems which require temporal discontinuity in cultivation of host plants. Such systems provide more epidemiological and evolutionary bottlenecks than encountered in continuous cropping systems (Kirthi *et al*., 2002). This approach is challenged by the many alternative wild and crop hosts for both virus and vector, and the difficulty in coordinating plant hosts more so among small holder farmers.

2.3.5 Genetic engineering

This approach has been used in response to difficulty in breeding for virus resistance in sweetpotato. In this way, resistance against SPFMV was achieved by Okada *et al*, (2002), and by the Monsanto scientists by introducing the SPFMV coat protein encoding region into the sweetpotato genome. The main challenge to this approach is failure to attain durable resistance in the fields and greenhouses which is attributed to a number of reasons such as when the transgene is not from a locally prevalent SPFMV strain and/or if the plants became infected with SPCSV (Clark *et al.*, 2012).

2.3.6 Resistance

2.3.6.1 Selection of SPFMV-resistant cultivars by African farmers

Farmers select cuttings from those plants which seem to be virus free. Growing of enormous varieties of cultivars in an area and the free exchange of planting material among neighboring farmers greatly contributes to selection of resistant varieties (Kapinga *et al*., 1995). Although selection is hampered by the presence of asymptomatic plants, its effectiveness is confirmed by the occurrence of virus-resistant cultivars among local landraces such as New Kawogo.

2.3.6.2 Recovery and reversion

Recovery is a phenomenon when a plant showing clear symptoms of infection starts to produce organs which are symptomless. This could be due to the ability of the plant to suppress virus multiplication and/or to inhibit virus spread. If cuttings obtained from the recovered portion are able to grow without symptoms and the virus, the plant is said to have undergone reversion. Therefore, reversion is the ability of an infected plant to provide uninfected cuttings (Fondong *et al*., 2000).

This mechanism was first reported in the 1930s in Cassava but has since then been reported among several vegetatively propagated plants where by cuttings from such plants, which have reverted grow without the virus (Gibson *et al*., 1997). In Uganda, certain sweetpotato varieties have been found to be resistant to sweetpotato viruses from which they revert (Wasswa *et al*., 2011; Gibson *et al*., 2014).

2.4 Breeding for Resistance against SPFMV

Breeding in sweetpotato has been greatly hindered because the crop is highly heterozygous, has many self and cross incompatibilities and many genotypes fail to bloom and set seed. This hinders the regular use of backcross or test cross populations for genetic analysis of sweetpotato. Backcrosses also encourages strong inbreeding depression. As a result, F1 progeny resulting from crosses between clones are used to study inheritance of traits in sweetpotato. Studies have revealed that resistance to SPFMV is controlled by a recessive gene which is expressed by reduced symptom severity and reduced virus multiplication. However, such resistance has been found to break down when SPFMV coinfects with SPCSV resulting into severe SPVD symptoms (Karyeija *et al*., 2000; Ngailo *et al*., 2013).

CHAPTER THREE
METHODOLOGY

3.1 Location of study

The crosses and reversion from SPFMV in bi-parental population of New Kawogo and Beauregard were conducted at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). The institute is located 19 km North of Kampala, 0˚281'N and 32˚271'E; at altitude, 1204m above sea level. The soils are deep, well drained highly weathered latisols with a characteristic red color and a pH of 5.6. The area is sub humid, with an average annual rain fall of 1234mm well distributed throughout the year. The distribution of rainfall in this area follows a bimodal pattern with the wettest months being April to May and October to November with mean daily minimum and maximum temperatures of 17˚C and 27˚C respectively.

3.2 Breeding to obtain the bi parental population

3.2.1 Source of planting material

The sweetpotato cultivars used to obtain the bi-parental population (that is, New Kawogo and Beauregard) were provided by the PEARL project (ID OPP1112152).

3.2.2 Procedure followed in breeding

Vines for the east African resistant landrace New Kawogo and the American susceptible variety, Beauregard were planted in rows in a crossing block. At flowering, the mature unopened flower buds of both cultivars were clipped in the late evenings by placing pieces of drinking straws on their tips to prevent normal opening and unwanted pollination. On the next day, early in the morning, flowers to be used as males (Beuaregard) were obtained, broken off their peduncles and their petals removed. Flowers used as females (New Kawogo) were simply opened by removing the straws. The anthers of the males were rubbed on to the stigmas of the female, whilst ensuring that enough pollen is retained on the stigmas. The females were again closed using a ribbon to prevent further pollination by insects. These flowers were tagged to differentiate them from the unused flowers.

3.2.2.1 Data collection and analysis

The above crosses were regularly monitored to identify any pollination failures or abortions. Successfully pollinated seeds were harvested at full maturity between five to six weeks. Data was collected on the number of crosses made, number of abortions got and number of seeds

obtained from the crosses. The seeds were then counted and placed in well labelled test tubes. Data was analyzed using descriptive statistics.

3.2.3 Seed scarification and planting

The seeds were properly dried in air dry conditions. The dried seeds were then soaked in concentrated sulphuric acid for five minutes. The sulphuric acid was decanted and the seeds rinsed under running tap water. The seeds were then planted in two-liter plastic pots containing well mixed soil, sand and animal manure in a 3:1:1 ratio and the contents placed in an insect proof screen house. The seeds started germinating after 3-4 days and they were subsequently multiplied.

3.2.3.1 Data collection and analysis

Data was collected on number of seeds that germinated and the seedling vigor of the germinated seeds. Data was analyzed using descriptive statistics (percentages).

3.3 Testing for reversion in the bi-parental population of New Kawogo and Beauregard

Eleven healthy plants form each of the fifty progenies of the bi-parental population were planted in two-liter plastic pots for two weeks. Ten of these were then side-graft inoculated with SPFMV-infected scion from *I. setosa* inoculum and one non-inoculated healthy control plant was included for each progeny. Plants were tested for reversion from SPFMV for 6 weeks starting at one week after graft inoculation. Detection was by use of *I. setosa* and was done by assessing for symptoms of infection. The number of plants that were successfully inoculated and those which reverted were recorded for each progeny.

3.3.1 Data collection and analysis

Depending on the number of plants which reverted, the percentage rate of reversion for each progeny was calculated and reversion was categorized as very low/no reversion (00-20%), low (21-40%), moderate (41-60%), high (61-80%) and very high (81-100%).

CHAPTER FOUR
RESULTS

4.1 Cross breeding to obtain the biparental population

The number of crosses made was 120 out of which some aborted and others produced viable seeds (Table 4). 84% of the seeds germinated and 88% of those which germinated produced vigorous seedlings (Table 5).

Table 4: Number of crosses, abortions and seeds harvested

Number of crosses	120
Number of abortions	52
Number of seeds	68

Table 5: Number of seeds planted, germinated and vigorous seedlings produced

Number of seeds planted	68
Number of seeds that germinated	57 (84%)
Number of plants with vigor	50 (88%)

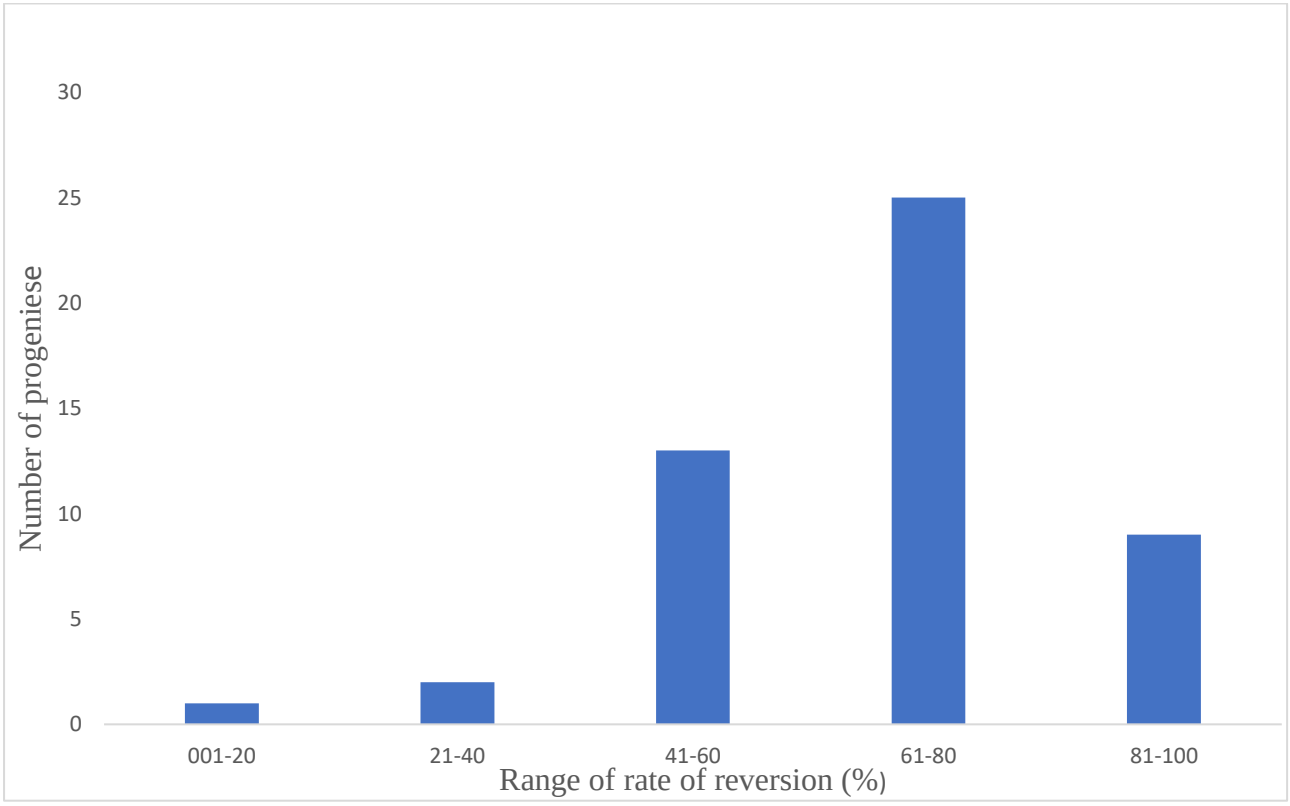
4.2 Reversion from SPFMV by the biparental population

Only 2% and 4% of the progenies were found with very low and low reversion potential, respectively. 18% of the progenies were found with very high reversion potential. Most of the progenies (26% and 50%) had moderate to high reversion potential, respectively (Table 6). Reversion potential in the progenies of a biparental population of New Kawogo by Beauregard was skewed to the right (Figure 1).

Table 6: Reversion from SPFMV infection by the biparental population of New Kawogo and Beauregard determined by grafting on Ipomoea setosa.

Category	Rate of reversion (in percent)	Number of progenies	Percentage progenies per category
Very low	0-20	1	2
Low	20-40	2	4
Moderate	40-60	13	26
High	60-80	25	50
Very high	80-100	9	18

Figure 1: Percentage reversion by the different progenies from the New Kawogo Beauregard biparental population.



CHAPTER FIVE

DISCUSSION OF RESULTS

Despite the fact that sweetpotato crossing has a lot of incompatibilities (Gurmu *et al*., 2013), a cross between New Kawogo and Beauregard seems to be compatible. New Kawogo and Beauregard when crossed produced viable seeds (84%) which gave rise to a vigorous F1 biparental population. This agrees with observations from previous researchers who managed to cross cultivars New Kawogo and Beauregard (Yada *et al*., 2017). Most of the progenies of the biparental population in this study showed significant reversion (at $p \leq 0.05$) from SPFMV infection (Appendix 1). This reversion was better than the reversion displayed by the parent Beauregard (Gibson *et al*., 2014) though mostly less than reversion displayed by cultivar New Kawogo (Gibson *et al*., 2014). New Kawogo was reported to undergo complete reversion while cultivar Beauregard was seldomly found to revert (Gibson *et al*., 2014; Adikini *et al*., 2016). The significant reversion observed in this study in F1 biparental population suggests the possibility of inheritance of genes for reversion from the resistant cultivar New Kawogo.

Reversion in the progenies was skewed to the right indicating that most of the progenies had acquired the reversion phenomenon from the reverting parent New Kawogo. The limited or no reversion that was observed in a few progenies could be attributed to the fact that the same genetic resistance can be expressed differently among the progenies as in the inheritance of genes for resistance against cassava viruses (Fargette *et al*., 1996). In addition, resistance to viruses including SPFMV, is a quantitative character and controlled by several sets of genes which makes additive gene action an important phenomenon (Mwanga *et al*., 2002) This makes the resistance character to segregate and express differentially. Also, since resistance to viruses in sweetpotato is due to ability to restrain virus encoded RNA silencing suppression and restriction of virus movement (Cuellar e*t al*., 2009), the virus might either shift or drift to evade the resistance mechanisms.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

The crosses of New Kawogo and Beauregard produce viable seeds which germinate into vigorous F1 progenies. The progenies show good reversion when graft inoculated with SPFMV. A few of the progenies show no or limited reversion. This implies that resistance genes are inherited and in a segregating manner. Therefore, cross breeding of resistant (reverting) varieties with susceptible (non-reverting) ones can be employed as an effective way of managing SPFMV infections. This will significantly improve sweetpotato production. .

However, the cross should also be evaluated for reversion from the other prevalent viruses such as SPCSV, SPMMV and SPLCV.

REFERENCES

Abad, J. A., & Moyer, J. W. (1992). Detection and distribution of sweetpotato feathery mottle virus in sweetpotato by in vitro-transcribed RNA probes (riboprobes), membrane immunobinding assay, and direct blotting. *Phytopathology*, *82*(3), 300-305.

Abad, J. A., Parks, E. J., New, S. L., Fuentes, S., Jester, W., & Moyer, J. W. (2007). First report of sweet potato chlorotic stunt virus, a component of sweetpotato virus disease, in North Carolina. *Plant Disease*, *91*(3), 327-327.

Adikini, S., Mukasa, S. B., Mwanga, R. O., & Gibson, R. W. (2016). Effects of Sweet Potato Feathery Mottle Virus and Sweet Potato Chlorotic Stunt Virus on the Yield of Sweetpotato in Uganda. *Journal of Phytopathology*, *164*(4), 242-254.

Albuquerque, L. C., Inoue-Nagata, A. K., Pinheiro, B., Resende, R. O., Moriones, E., & Navas-Castillo, J. (2012). Genetic diversity and recombination analysis of sweepoviruses from Brazil. *Virology journal*, *9*(1), 241.

Alicai, T., Fenby, N. S., Gibson, R. W., Adipala, E., Vetten, H. J., Foster, G. D., & Seal, S. E. (1999). Occurrence of two serotypes of sweet potato chlorotic stunt virus in East Africa and their associated differences in coat protein and HSP70 homologue gene sequences. *Plant Pathology*, *48*(6), 718-726.

Austin, D. F. (1988). The taxonomy, evolution and genetic diversity of sweet potatoes and related wild species. *Exploration, maintenance, and utilization of sweetpotato genetic resources*, 27-60.

Bar-Joseph, M., Garnsey, S. M., Gonsalves, D., Moscovitz, M., Purcifull, D. E., Clark, M. F., & Loebenstein, G. (1979). The use of enzyme-linked immunosorbent assay for detection of citrus tristeza virus. *Phytopathology*, *69*(2), 190-194.

Brunt, A. A., Crabtree, K., & Dallwitz, A. J. (1996). Analítico: Viruses of plants; descriptions and lists from the VIDE database.

Campbell, R. N., Hall, D. H., & Mielinis, N. M. (1974). Etiology of sweet potato russet crack disease. *Phytopathology*, *64*(2), 210-218.

CIP, 2000. ANNUAL report. International Potato Center.

CIP, 2010. CIP facts and figures about Sweet Potato. International potato Center, Lima, Peru.

Clark, C. A., & Hoy, M. W. (2006). Effects of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. *Plant Disease*, *90*(1), 83-88.

Clark, C. A., & Moyer, J. W. (1988). *Compendium of sweet potato diseases*. American Phytopathological Society.

Clark, C. A., Davis, J. A., Abad, J. A., Cuellar, W. J., Fuentes, S., Kreuze, J. F., ... & Valkonen, J. P. (2012). Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Disease*, *96*(2), 168-185.

Cuellar, W. J., Kreuze, J. F., Rajamäki, M. L., Cruzado, K. R., Untiveros, M., & Valkonen, J. P. (2009). Elimination of antiviral defense by viral RNase III. *Proceedings of the National Academy of Sciences*, pnas-0806042106.

Cuellar, W. J., Tairo, F., Kreuze, J. F., & Valkonen, J. P. (2008). Analysis of gene content in sweet potato chlorotic stunt virus RNA1 reveals the presence of the p22 RNA silencing suppressor in only a few isolates: implications for viral evolution and synergism. *Journal of General Virology*, *89*(2), 573-582.

Domola, M. J., Thompson, G. J., Aveling, T. A. S., Laurie, S. M., Strydom, H., & Van den Berg, A. A. (2008). Sweet potato viruses in South Africa and the effect of viral infection on storage root yield. *African Plant Protection*, *14*(1), 15-23.

Fargette, D., Colon, L. T., Bouveau, R., & Fauquet, C. (1996). Components of resistance of cassava to African cassava mosaic virus. *European Journal of Plant Pathology*, *102*(7), 645-654.

Fondong, V. N., Thresh, J. M., & Fauquet, C. (2000). Field experiments in Cameroon on cassava mosaic virus disease and the reversion phenomenon in susceptible and resistant cassava cultivars. *International Journal of Pest Management*, *46*(3), 211-217.

Food and Agriculture Organization of the United Nations, Statistics Division (FAOSTAT). 2017. Retrieved 25 April 2018.

Gibson, R. W., Mpembe, I., Alicai, T., Carey, E. E., Mwanga, R. O. M., Seal, S. E., & Vetten, H. J. (1998). Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathology*, *47*(1), 95-102.

Gibson, R. W., Mwanga, R. O. M., Kasule, S., Mpembe, I., & Carey, E. E. (1997). Apparent absence of viruses in most symptomless field-grown sweet potato in Uganda. *Annals of Applied Biology*, *130*(3), 481-490.

Gibson, R. W., Wasswa, P., & Tufan, H. A. (2014). The ability of cultivars of sweetpotato in East Africa to 'revert'from Sweet potato feathery mottle virus infection. *Virus research*, *186*, 130-134.

Gurmu, F., Hussein, S., & Laing, M. (2013). Self-and cross-incompatibilities in sweetpotato and their implications on breeding. *Australian Journal of Crop Science*, 7(13), 2074.

Hollings, M., Stone, O. M., & Bock, K. R. (1976). Purification and properties of sweet potato mild mottle, a white-fly borne virus from sweet potato (Ipomoea batatas) in East Africa. *Annals of applied biology*, *82*(3), 511-528.

Huang, J. C., & Sun, M. (2000). Genetic diversity and relationships of sweetpotato and its wild relatives in Ipomoea series Batatas (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. *Theoretical and Applied Genetics*, *100*(7), 1050-1060.

INIBAP, 1999. Banana, plantains and INIBAP. Annual Report, 1999. INIBAP, Montpellier, France, 56p.

Kapinga, R. E., Ewell, P. T., Jeremiah, S. C., & Kileo, R. (1995). Sweet potato in Tanzanian farming and food systems. *Implications for Research. International Potato Center (CIP) Sub-Saharan Africa Regional Office, Nairobi, Kenya, and Ministry of Agriculture, Dar-es-Salaam, Tanzania*, *47*.

Karyeija, R. F., Kreuze, J. F., Gibson, R. W., & Valkonen, J. P. T. (2000). Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. *Virology*, *269*(1), 26-36.

Kim, J., Kil, E. J., Kim, S., Seo, H., Byun, H. S., Park, J., ... & Yang, J. W. (2015). Seed transmission of Sweet potato leaf curl virus in sweet potato (Ipomoea batatas). *Plant Pathology*, *64*(6), 1284-1291.

Kirthi, N., Maiya, S. P., Murthy, M. R. N., & Savithri, H. S. (2002). Evidence for recombination among the tomato leaf curl virus strains/species from Bangalore, India. *Archives of Virology*, *147*(2), 255-272.

Kumari, R., Kumar, R., Open Source Drug Discovery Consortium, & Lynn, A. (2014). A GROMACS tool for high-throughput MM-PBSA calculations. *Journal of chemical information and modeling*, *54*(7), 1951-1962.

Li, R., Salih, S., & Hurtt, S. (2004). Detection of geminiviruses in sweetpotato by polymerase chain reaction. *Plant disease*, *88*(12), 1347-1351.

Loebenstein, G., Fuentes, S., Cohen, J., & Salazar, L. F. (2003). Sweet potato. In *Virus and virus-like diseases of major crops in developing countries* (pp. 223-248). Springer, Dordrecht.

Loebenstein, G., Thottappilly, G., Fuentes, S., & Cohen, J. (2009). Virus and phytoplasma diseases. In *The sweetpotato* (pp. 105-134). Springer, Dordrecht.

Miano, D. W., LaBonte, D. R., Clark, C. A., Valverde, R. A., Hoy, M. W., Hurtt, S., & Li, R. (2006). First report of a begomovirus infecting sweetpotato in Kenya. *Plant Disease*, *90*(6), 832-832.

Mukasa, S. B., Rubaihayo, P. R., & Valkonen, J. P. (2003). Incidence of viruses and virus like diseases of sweetpotato in Uganda. *Plant Disease*, *87*(4), 329-335.

Mukasa, S. B., Rubaihayo, P. R., & Valkonen, J. P. T. (2006). Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfected sweetpotato plants. *Plant Pathology*, *55*(3), 458-467.

Mwanga, R. O., Yencho, G. C., & Moyer, J. W. (2002). Diallel analysis of sweetpotatoes for resistance to sweetpotato virus disease. Euphytica, 128(2), 237-248.

Ngailo, S., Shimelis, H., Sibiya, J., & Mtunda, K. (2013). Sweet potato breeding for resistance to sweet potato virus disease and improved yield: progress and challenges. *African Journal of Agricultural Research*, *8*(25), 3202-3215.

O'BRIEN, P. J. (1972). The Sweet Potato: Its Origin and Dispersal 1. *American anthropologist*, *74*(3), 342-365.

Okada, Y., Nishiguchi, M., Saito, A., Kimura, T., Mori, M., Hanada, K., ... & Murata, T. (2002). Inheritance and stability of the virus-resistant gene in the progeny of transgenic sweet potato. *Plant breeding*, *121*(3), 249-253.

Palumbo, J. C., Horowitz, A. R., & Prabhaker, N. (2001). Insecticidal control and resistance management for Bemisia tabaci. *Crop protection*, *20*(9), 739-765.

Qin, Y., Zhang, Z., Qiao, Q., Zhang, D., Tian, Y., & Wang, Y. (2013). Molecular variability of sweet potato chlorotic stunt virus (SPCSV) and five potyviruses infecting sweet potato in China. *Archives of virology*, *158*(2), 491-495.

Qiu, B., Coats, S. A., Ren, S., Idris, A. M., Xu, C., & Brown, J. K. (2007). Phylogenetic relationships of native and introduced Bemisia tabaci (Homoptera: Aleyrodidae) from China and India based on mt COI DNA sequencing and host plant comparisons. *Progress in Natural Science*, *17*(6), 645-654.

Sakai, J., Mori, M., Morishita, T., Tanaka, M., Hanada, K., Usugi, T., & Nishiguchi, M. (1997). Complete nucleotide sequence and genome organization of sweet potato feathery mottle virus (S strain) genomic RNA: the large coding region of the P1 gene. *Archives of virology*, *142*(8), 1553-1562.

Sato, K., Uritani, I., & Saito, T. (1981). Characterization of the Terpene-Inducing Factor Isolated from the Larvae of the Sweet Potato Weevil, *Cylas formicarius fabricicus* (Coleoptera: Brenthidae). *Applied Entomology and Zoology*, *16*(2), 103-112.

Simmons, A. M., Ling, K. S., Harrison, H. F., & Jackson, D. M. (2009). Sweet potato leaf curl virus: efficiency of acquisition, retention and transmission by Bemisia tabaci (Hemiptera: Aleyrodidae). *Crop protection*, *28*(11), 1007-1011.

Souto, E. R., Sim, J., Chen, J., Valverde, R. A., & Clark, C. A. (2003). Properties of strains of Sweet potato feathery mottle virus and two newly recognized potyviruses infecting sweet potato in the United States. *Plant Disease*, *87*(10), 1226-1232.

Srisuwan, S., Sihachakr, D., & Siljak-Yakovlev, S. (2006). The origin and evolution of sweet potato (Ipomoea batatas Lam.) and its wild relatives through the cytogenetic approaches. *Plant Science*, *171*(3), 424-433.

Trenado, H. P., Orílio, A. F., Márquez-Martín, B., Moriones, E., & Navas-Castillo, J. (2011). Sweepoviruses cause disease in sweet potato and related Ipomoea spp.: fulfilling koch's postulates for a divergent group in the genus Begomovirus. *PLoS One*, *6*(11), e27329.

Untiveros, M., Fuentes, S., & Salazar, L. F. (2007). Synergistic interaction of Sweet potato chlorotic stunt virus (Crinivirus) with carla-, cucumo-, ipomo-, and potyviruses infecting sweet potato. *Plant Disease*, *91*(6), 669-676.

Valverde, R. A., Clark, C. A., & Valkonen, J. P. (2007). Viruses and virus disease complexes of sweetpotato. *Plant Viruses*, *1*(1), 116-126.

Voller, A., Bartlett, A., Bidwell, D. E., Clark, M. F., & Adams, A. N. (1976). The detection of viruses by enzyme-linked immunosorbent assay (ELISA). *Journal of General Virology*, *33*(1), 165-167.

Wasswa, P., Otto, B., Maruthi, M. N., Mukasa, S. B., Monger, W., & Gibson, R. W. (2011). First identification of a sweet potato begomovirus (sweepovirus) in Uganda: characterization, detection and distribution. *Plant Pathology*, *60*(6), 1030-1039.

Wyant, P. S., Strohmeier, S., Schäfer, B., Krenz, B., Assunção, I. P., de Andrade Lima, G. S., & Jeske, H. (2012). Circular DNA genomics (circomics) exemplified for geminiviruses in bean crops and weeds of northeastern Brazil. *Virology*, *427*(2), 151-157.

Yada, B., Alajo, A., Ssemakula, G. N., Mwanga, R. O., Brown-Guedira, G., & Yencho, G. C. (2017). Selection of simple sequence repeat markers associated with inheritance of sweetpotato virus disease resistance in sweetpotato. *Crop Science*, 57(3), 1421-1430.

Zhang, D., Cervantes, J., Huamán, Z., Carey, E., & Ghislain, M. (2000). Assessing genetic diversity of sweet potato (Ipomoea batatas (L.) Lam.) cultivars from tropical America using AFLP. *Genetic Resources and Crop Evolution*, *47*(6), 659-665.

APPENDICES

Appendix 1: Reversion from SPFMV infection by the biparental population of New Kawogo and Beauregard determined by grafting on Ipomoea setosa.

Progeny number	Number of plants showing reversions	Number of plants which did not revert after infection	NUMBER OF SUCCESSFULL INNOCULATIONS	Percentage reversion	O-E	(O-E)^2	((O-E)2)/E
p1	3	0	5	60	-2	4	0.8
p2	6	0	10	60	-4	16	1.6
p3	7	0	10	70	-3	9	0.9
p4	6	0	9	66.66666667	-3	9	1
p5	4	0	10	40	-6	36	3.6
p6	4	0	4	100	0	0	0
p7	5	3	8	62.5	-3	9	1.125
p8	6	3	10	60	-4	16	1.6
p9	9	0	10	90	-1	1	0.1
p10	2	0	4	50	-2	4	1
p11	7	1	10	70	-3	9	0.9
p12	7	0	10	70	-3	9	0.9
p13	6	2	10	60	-4	16	1.6
p14	1	0	4	25	-3	9	2.25
p15	7	0	10	70	-3	9	0.9

p16	4	0	10	40	-6	36	3.6
p17	6	0	6	100	0	0	0
p18	4	0	9	44.44444444	-5	25	2.777777778
p19	2	0	4	50	-2	4	1
p20	5	0	5	100	0	0	0
p21	3	0	5	60	-2	4	0.8
p22	7	2	8	87.5	-1	1	0.125
p23	10	0	10	100	0	0	0
p24	5	4	10	50	-5	25	2.5
p25	5	0	7	71.42857143	-2	4	0.571428571
p26	4	0	10	40	-6	36	3.6
p27	6	0	10	60	-4	16	1.6
p28	4	0	10	40	-6	36	3.6
p29	6	0	6	100	0	0	0
p30	6	1	7	85.71428571	-1	1	0.142857143
p31	3	0	5	60	-2	4	0.8
p32	1	0	8	12.5	-7	49	6.125
p33	3	0	10	30	-7	49	4.9
p34	7	0	10	70	-3	9	0.9
p35	8	0	8	100	0	0	0
p36	2	0	5	40	-3	9	1.8
p37	2	0	6	33.33333333	-4	16	2.666666667
p38	6	2	8	75	-2	4	0.5

p39	7	1	10	70	-3	9	0.9
p40	3	0	10	30	-7	49	4.9
p41	6	0	7	85.71428571	-1	1	0.142857143
p42	6	0	8	75	-2	4	0.5
p43	5	1	9	55.55555556	-4	16	1.777777778
p44	6	0	7	85.71428571	-1	1	0.142857143
p45	4	0	10	40	-6	36	3.6
p46	2	0	4	50	-2	4	1
p47	7	0	8	87.5	-1	1	0.125
p48	3	0	6	50	-3	9	1.5
p49	0	0	3	0	-3	9	3
p50	7	0	10	70	-3	9	0.9
							74.77222222
							0.010304

P=0.010304

Significant at p≤0.05