Norwegian University of Life Sciences
Faculty of Biosciences
Department of Plant Sciences

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Sweet Potato Production in South Sudan: Current status, virus infections and whitefly vector diversity

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Beatrice Langwa

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Abbreviations

AfDB	African Development Bank
cDNA	Complementary DNA
CIAT	International Center for Tropical Agriculture
CMV	*Cucumber mosaic virus*
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation of the United Nations
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
INGOs	international non-governmental organizations
IOM	International Organization for Migration
IYVV	*Ipomoea yellow vein virus*
MAF-GoSS	Ministry of Agriculture and Forestry-Government of South Sudan
NASH	nucleic acid spot hybridization
NCASP	Norwegian Church Aid Sudan Program
NGS	next generation sequencing
PCR	polymerase chain reaction
PDU	Project Development Unit (PDU)
qPCR	Quantitative polymerase chain reaction
RCA	rolling circle amplification

RT-PCR	Reverse-transcription polymerase chain reaction
SPCFV	*Sweet potato chlorotic fleck virus*
SPCSV	*Sweet potato chlorotic stunt virus*
SPCV	Sweet potato collusive virus
SPFMV	*Sweet potato feathery mottle virus*
SPLCESV	*Sweet potato leaf curl Spain virus*
SPLCGV	*Sweet potato leaf curl Georgia virus*
SPLCLaV	*Sweet potato leaf curl Lanzarote virus*
SPLCSPV	*Sweet potato leaf curl Sao Paulo virus*
SPLCUV	Sweet potato leaf curl Uganda virus
SPLCV	*Sweet potato leaf curl virus*
SPLCV-CN	*Sweet potato leaf curl China virus*
SPLV	*Sweet potato latent virus*
SPMMV	*Sweet potato mild mottle virus*
SPPV	Sweet potato pakakuy virus
SPSMV-1	Sweet potato symptomless virus 1
SPV2	*Sweet potato virus 2*
SPVC	*Sweet potato virus C*
SPVCV	Sweet potato vein clearing virus
SPVG	*Sweet potato virus G*
SRDS	small-RNA deep-sequencing

TAS-ELISA	Triple antibody sandwich enzyme-linked immunosorbent assay
UN	United Nations
WFP	World Food Programme

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Summary

Sweet potato (*Ipomoea batatas* (L.) Lam.) is an important staple food crop that assures food security in sub-Saharan Africa, especially East Africa. In South Sudan, sweet potato is a main staple food crop for many farming communities. The production of sweet potato worldwide is limited by many biotic constraints of which virus diseases are the second most important after sweet potato weevils (*Cylas spp.*). There is currently no information available on viruses infecting sweet potato and insect vectors of sweet potato viruses in South Sudan. This is due to decades of war that has led to limited access to technological advances in crop production, and little effort on research of crop plants in South Sudan.

This thesis presents the first report on the identity, incidence and distribution of viruses infecting sweet potato in South Sudan. In addition, the genetic diversity of the whitefly (*Bemisia tabaci*) (virus transmitting vector) populations collected from South Sudan is reported for the first time. As a first step in this study, a first baseline information on farmers' knowledge and perceptions on pests and diseases of sweet potato and production constraints, is also presented.

The baseline survey was conducted in five counties in three states of South Sudan: Wau County (Western Bahr el Ghazal state), Magwi County (Eastern Equatoria State), Lainya, Morobo and Yei counties (Central Equatoria state). Using a structured questionnaire, 180 farm households were interviewed. The results show that farmers were aware of the damage caused by pests and diseases on sweet potato but most of them (64.3%), had limited knowledge on how to identify diseases affecting sweet potato. Insect pests were perceived to be more damaging and sweet potato weevil was considered the most serious insect pest by 42.7% of the farmers. The majority of farmers (60.2%) did not practice pests and diseases control methods. Key production constraints facing farmers in order of importance were: lack of extension services (55%), lack of improved varieties (48.9%), low sale prices of sweet potato (43.9%), lack of credit services (43.3%), price fluctuations (42.8%), field pests (41.7%), drought (40.6%) and diseases (38.3%).

A survey of sweet potato viruses in sweet potato fields in the five counties revealed virus and virus-like symptoms with moderate disease incidence and mild symptom severity in the surveyed fields. Viruses infecting sweet potato were detected from sweet potato samples collected from fields in the five surveyed counties using double antibody and triple antibody sandwich enzyme-linked immunosorbent assay (DAS- and TAS-ELISA), reverse-transcription

polymerase chain reaction (RT-PCR) and small-RNA deep-sequencing (SRDS). Altogether 15 viruses were detected belonging to 6 genera, including two potyviruses, one crinivirus, seven begomoviruses, one cavemovirus, one mastrevirus and three badnaviruses. These results indicate the diversity of sweet potato viruses in South Sudan. The two most important viruses, *Sweet potato feathery mottle virus* (SPFMV; genus *Potyvirus*) and *Sweet potato chlorotic stunt virus* (SPCSV; genus *Crinivirus*) and their co-infections, which cause the severe sweet potato virus disease (SPVD), were identified in four of the five surveyed counties (Wau, Magwi, Lainya and Yei). This confirms the prevalence of SPVD in South Sudan and supports previous reports that SPFMV and SPCSV are the most commonly occurring and the most important viruses of sweet potato in East Africa. The begomovirus, *Sweet potato leaf curl virus* (SPLCV) was also widespread, identified in all the counties surveyed.

The genetic variability of *B. tabaci* populations were investigated. Due to the civil insecurity in the country at the time of this study, data were accessible in only one geographical location. One hundred and sixty-two *B. tabaci* individuals were collected from sweet potato, cassava, tomato and squash from 10 locations in Juba County, Central Equatoria State, South Sudan. Determination of phylogenetic relationships between sampled *B. tabaci* using sequences of mitochondrial DNA cytochrome oxidase I (mtCOI) revealed 6 distinct genetic groups of *B. tabaci* including three non-cassava haplotypes (Mediterranean MED, Indian Ocean (IO) and Uganda) and three cassava haplotypes (Sub-Saharan Africa 1 sub-group 1 (SSA1-SG1), SSA1-SG3 and SSA2). MED was the predominant haplotype on sweet potato and SSA2 on cassava in all the sampled locations. The Uganda haplotype was also widespread and was identified from five of the sampled locations. SSA2 was previously associated with the severe cassava mosaic disease epidemic in Uganda in the 1990s but has been largely replaced by SSA1 in all other parts of East and Central Africa. South Sudan is currently the only country in sub-Saharan Africa where SSA2 continues to predominate on cassava.

The information provided in this study will be useful for future research and can serve as a basis for the development of virus and whitefly vectors management and control strategies to improve sweet potato production in South Sudan. A comprehensive assessment of the diversity and geographical distribution of sweet potato viruses and *B. tabaci* in all sweet potato production areas in South Sudan is proposed.

Keywords: Sweet potato (*Ipomoea batatas*), pests, diseases, production constraints, virus detection, distribution, *Bemisia tabaci*, genetic diversity, haplotype.

Sammendrag

Søtpotet (*Ipomoea batatas* (L.) Lam.) er en viktig basismatvare som bidrar mye til matsikkerhet i Afrika sør Sahara, særlig i Øst Afrika. I Sør Sudan er søtpotet den viktigste komponenten I kosten på landsbygda. Søtpotetavlinger blir redusert av mange biologiske faktorer, hvorav virus er nummer to i viktighet etter søtpotetgresshoppe (*Cylas spp.*). Det finnes ingen informasjon om hvilke virus som finnes hos søtpotet i Sør Sudan eller hvilke vektorer som er der. Dette skyldes flere tiår med borgerkrig i landet, dette har medført begrenset tilgang til forbedrede metoder i landbruket og svært liten innsats innen forskning på kulturplanter i denne perioden.

Denne doktoravhandlingen er det første vitenskapelige arbeidet omkring identiteten, forekomst og utbredelse av virus hos søtpotet i Sør Sudan. I tillegg er dette den første studien av den genetiske diversiteten i populasjoner hos vektoren som overfører virus, kvitfly (*Bemisia tabaci*) som er samlet inn i Sør Sudan. Som første del av arbeidet, ble det utført en spørreundersøkelse blant bønder for å få en basis for kunnskapsnivået omkring faktorer som påvirker avlinger I søtpotet i landet, samt kunnskapen omkring virussykdommer og tiltak for å begrense skadeomfanget.

Spørreundersøkelsen ble utført i fem fylker i tre av statene i Sør Sudan: Wau County (Western Bahr el Ghazal state), Magwi County (Eastern Equatoria State), Lainya, Morobo og Yei counties (Central Equatoria state). Ved å bruke et strukturert spørreskjema ble 180 gårdshusholdninger intervjuet. Resultatene viser at bønder er klar over skadene fra skadedyr og sykdommer hos søtpotet, men flesteparten (64.3%) hadde bregenset kunnskap om hvordan man identifiserer sykdommer som fører til skader i søtpotet. Skadeinsekter ble antatt å være mest skadelige og søtpotetgresshopper ble angitt som den mest alvorlige hos 42.7% av bøndene. Flesteparten av bøndene (60.2%) gjorde ingen kontrolltiltak mot skadedyr og sykdommer. De viktigste avlingsreduserende faktorene, nevnt etter oppgitt viktighet var: mangel på rådgivingstjenester (55%), mangel på forbedrete sorter (48.9%), lave priser på avlingen av søtpotet ved salg (43.9%), mangel på kredittmuligheter (43.3%), prisfluktueringer (42.8%), skadedyr pests (41.7%), tørke (40.6%) og sykdommer (38.3%).

Ved prøvetaking av søtpotet i alle de samme fem fylkene, viste det seg at det var moderate virus og virusliknende symptomer i de undersøkte områdene. Virus som angriper søtpotet ble undersøkte ved hjelp av doble og triple antistoff ved ELISA-tester (sandwich enzyme-linked immunosorbent assay (DAS- and TAS-ELISA), reverse-transcription polymerase chain reaction (RT-PCR) og small-RNA deep-sequencing (SRDS). Tilsammen ble det funnet 15

virus som tilhører 6 virusslekter, som innebefatter to potyvirus, en crinivirus, syv begomovirus, one cavemovirus, en mastrevirus og tre badnavirus. Dette gir en indikasjon på diversiteten hos søtpotetvirus i Sør Sudan. De to viktigste virusene, *Sweet potato feathery mottle virus* (SPFMV; genus *Potyvirus*) og *Sweet potato chlorotic stunt virus* (SPCSV; genus *Crinivirus*) og deres saminfeksjon, som sammen forårsaker den alvorlige tilstanden som kalles søtpotetvirussykdom (sweet potato virus disease (SPVD)), ble funnet i fire av de undersøkte fem fylkene (Wau, Magwi, Lainya and Yei). Dette bekrefter den vide utbredelsen av SPVD i Sør Sudan og støtter samtidig tidligere undersøkelser i andre land som sier at SPFMV og SPCSV er de mest utbredte og viktigste virusene hos søtpotet i Øst Afrika. Begomoviruset, *Sweet potato leaf curl virus* (SPLCV) var også svært utbredt og ble funnet i prøver fra alle de fem fylkene som ble undersøkt.

Den genetiske diversiteten hos populasjoner av virusvektoren kvitfly (*B. tabaci)* ble undersøkt. På grunn av pågående borgerkrig, var det bare mulig å samle insektene i ett geografisk område. Ett hundre og sekstito *B. tabaci* individer ble samlet inn på søtpotet, cassava, tomat og squash fra 10 steder i Juba County, Central Equatoria State, Sør Sudan. Bestemmelse av fylogentisk slektsskap mellom innsamlede *B. tabaci* individer ble utført ved å bruke sekvensen til mitokondrie-DNA cytochrome oxidase I (mtCOI). Analysen viste seks distinkte genetiske grupper av *B. tabaci,* derav tre non-cassava haplotyper (Mediterranean MED, Indian Ocean (IO) og Uganda), samt tre cassava haplotyper (Sub-Saharan Africa 1 sub-group 1 (SSA1-SG1), SSA1-SG3 og SSA2). MED var den dominerende haplotypen på søtpotet og SSA2 var den dominerende på cassava på alle stedene innsamlingen var foretatt. Uganda haplotypen var også utbredt og ble identifisert fra fem av ti innsamlingssteder. SSA2 har tidligere av forbundet med alvorlige epidemiske angrep av cassava mosaic disease i Uganda på 1990-tallet, men har siden blitt for en stor grad blitt erstattet av SSA1 i alle andre deler av Øst- og Sentral Afrika. Sør Sudan er for tiden det eneste landet i Afrika sør for Sahara der SSA2 fortsatt er den dominerende på cassava.

Informasjonen fra denne doktoravhandlingen vil være svært nyttig i den videre forskningen I Sør Sudan og kan tjene som et utgangspunkt for utvikling av virus og vektorbekjempelse for å forbedre avlingene hos søtpotet i Sør Sudan. Det er foreslås at det blir gjennomført mer omfattende undersøkelser av diversiteten og geografisk utbredelse av virus og *B. tabaci* hos søtpotet i alle deler av Sør Sudan der søtpotet dyrkes, når det er trygt å ferdes der.

Nøkkelord: Søtpotet (*Ipomoea batatas*), skadedyr, sykdommer, avlingsreduserende faktorer, virusbestemmelse, virusutbredelse, *Bemisia tabaci*, genetisk diveristet, haplotype.

Appendix

Papers I-IV

This thesis contains the following papers, which will be referred to by their Roman numerals.

I. **Beatrice C. Misaka**, James P. Legg, Philip W. Marchelo-d'Ragga and Anne Kathrine Hvoslef-Eide (2019). Farmers' perceptions of pests, diseases and production constraints affecting sweet potato in South Sudan. Manuscript.

II. **Beatrice C. Misaka**, James P. Legg, Philip W. Marchelo-d'Ragga and Anne Kathrine Hvoslef-Eide (2019). Survey and detection of viruses infecting sweet potato (*Ipomoea batatas* (L.) Lam) in South Sudan. Manuscript.

III. **Beatrice C. Misaka**, Carl J. Spetz, Dereje Haile Buko, James P. Legg, Philip W. Marchelo-d'Ragga and Anne Kathrine Hvoslef-Eide (2019). Detection of viruses in sweet potato cultivars from South Sudan by small-RNA deep-sequencing techniques. Manuscript.

IV. **Beatrice C. Misaka**, Everlyne N. Wosula, Philip W. Marchelo-d'Ragga, Anne Kathrine Hvoslef-Eide and James P. Legg (2019). Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) colonizing sweet potato and cassava in South Sudan. Manuscript.

1. Introduction

1.1 Viruses

Viruses are sub-microscopic, obligate intracellular parasites of cellular organisms and can cause disease. They are living biological entities that control their replication autonomously. Viruses have a nucleic acid genome with functional genes, and can evolve and adapt to different hosts, vectors, and environments. In addition, they are capable of exchanging genetic information with other viruses, and even with their hosts (Wilson, 2014). Hull (2014) defined a virus as a set of one or more nucleic acid template molecules, either RNA or DNA, normally encased in a protective coat or coats of protein or lipoprotein, that is able to organize its own replication only within suitable host. It can usually be transmitted horizontally between hosts. Within the hosts cells, virus replication is (i) dependent of the host's protein-synthesizing machinery, (ii) organized from pools of the required materials rather than by binary fission, (iii) located at sites that are not separated from the host cells contents by a continuous lipoprotein bilayer membrane, and (iv) continually giving rise to variants through various kinds of change in the viral nucleic acid.

As cellular parasites, viruses cause so many diseases in all living organisms including vertebrates, invertebrates, plants, fungi and bacteria. Historically, viruses have been known to cause disastrous diseases in humans such as influenza, polio, rabies, smallpox, ebola hemorrhagic fever, acquired immunodeficiency syndrome (AIDS) and the severe acute respiratory syndrome (SARS) are caused by viruses (Agrios, 2005). In plants they have been reported to be responsible for yield losses and quality reductions in many important crops and sometimes can lead to crop failures resulting in famines. For example, in the 1990s, an epidemic of a severe cassava mosaic disease (CMD), caused by cassava mosaic geminiviruses (Family *Geminiviridae*: Genus *Begomovirus*) devastated the cassava crop in parts of East Africa, especially Uganda (Legg 1999). This CMD pandemic has had a significant impact on food supply and food security for subsistence farmers in the poorer regions of East Africa who normally rely on cassava as a staple food.

1.2 Classification of plant viruses

Viruses are distinguished as RNA viruses and DNA viruses. They are classified based on whether they are double-stranded or single-stranded, positive sense or negative sense,

filamentous or isometric, and whether they have common replication strategies (Agrios, 2005; Wilson, 2014). Plant viruses are classified into six major groups based on the nature of the genome (King et al., 2012):

(i) Double-stranded DNA (dsDNA); The *Caulimoviridae* is the only family of plant viruses with dsDNA genome. Viral replication is characterized by reverse transcription without a phase of RNA intermediate. Replication does not require integration step into the host genome for RNA transcription but is from an episomal minichromosome and the template for replication is circular dsDNA (Hohn & Rothnie, 2013; Hull, 2014).

(ii) Single-stranded DNA (ssDNA); This group of viruses replicate through double-stranded intermediates in the nuclei of infected plant cells. They also assemble into minichromosomes and are transcribed in infected cells. The genome organization of ssDNA viruses is either monopartite or bipartite. There are two families in this group, *Geminiviridae* and *Nanoviridae* (Hanley-Bowdoin et al., 2000; Hull, 2014).

(iii) Reverse-transcribing viruses (retroviruses); Replication in retroviruses involves integration into the host genome for transcription of viral RNA. Replication is aided by the reverse transcriptase enzyme that converts the viral RNA genome into double-stranded DNA. Plant retroviruses belong to the family *Pseudoviridae* (King et al., 2012).

(iv) Double-stranded RNA (dsRNA); Plant viruses in this group include members of the families *Reoviridae* and *Partiviridae*. Replication and transcription occur within viral capsids by RNA-dependent RNA polymerase (RdRP) in the cytoplasm (Patton & Spencer, 2000).

(v) Negative sense single-stranded RNA (ssRNA); the families *Rhabdoviridae* and *Bunyaviridae* belong to this group. In the *Rhabdoviridae* family, Rhabdovirus replication generally occurs in the cytoplasm following receptor-mediated endocytosis. Some plant rhabdoviruses replicate in the nucleus (Walker et al., 2018).

(vi) Positive sense single-stranded RNA (ssRNA+); majority of the plant virus families are placed in this group including *Closteriviridae*, *Potyviridae*, *Alfaflexiviridae, Betaflexiviridae, Tymoviridae, Secoviridae, Luteoviridae, Comoviridae, Virgaviridae* and *Bromoviridae*. In this group of viruses, replication involves a replication complex that constitutes the template, newly synthesizes RNA, and the virus-encoded replicase and host factors. Replication occurs in three phases, initiation, elongation, and termination (Hull, 2014).

1.3 Sweet potato

Sweet potato (*Ipomoea batatas* L.), is a dicotyledonous, perennial plant but normally grown as an annual crop for its edible storage roots. It is vegetatively propagated using either vine cuttings or storage roots. The crop varies vastly in taste, size, shape, texture, skin and flesh color. The skin color varies from yellow, red, orange and brown whereas the flesh color ranges from white, orange, yellow, purple, red, pink, and violet (Bovell-Benjamin, 2007; Loebenstein & Thottappilly, 2009; Padmaja, 2009). Sweet potato belongs to the family *Convolvulaceae*, the Morning Glory, and genus *Ipomoea* (Austin, 1987). It is the only species of economic importance in the genus *Ipomoea* (Woolfe, 1992). The genus *Ipomoea* is thought to contain over 500 species with ploidy levels ranging from 2x to 6x (Ozias-Akins & Jarret, 1994). It has both 4x and 6x forms (2n = 4x = 60 or 2n = 6x = 90). *I. batatas* probably originated from a cross between *I. trifida* and another wild *Ipomoea sp.* at least 500 years ago, with its primary center of origin and diversity in Central or Northern South America (Huang & Sun, 2000; Roullier et al., 2011; Srisuwan et al., 2006; Zhang et al., 2000).

All varieties of sweet potato are good sources of vitamins C, B2 (Riboflabin), B6 and E as well as dietary fiber, potassium, copper, manganese and iron. In addition, they are low in fat and cholesterol, rich in carbohydrates and the orange-fleshed root color varieties are particularly rich in β-carotene, the vitamin A precursor (Bovell-Benjamin, 2007; Loebenstein & Thottappilly, 2009; Tang et al., 2015; Woolfe, 1992). Consumption of orange-fleshed sweet potatoes in either fresh or processed form can contribute in alleviating dietary vitamin A deficiency (Burri, 2011; Hagenimana et al., 1998; Van Jaarsveld et al., 2006). Other uses of sweet potato include vegetables, animal feed, and industrial uses (fermentation, source of starch for food processing and production of chemical stocks) (Huang et al., 2003; Loebenstein & Thottappilly, 2009; Widodo et al., 2015).

Sweet potato is distributed widely in the tropical and subtropical regions of the world. It ranged seventh in global food crop production and is the third most important root crop after potato and cassava with annual production of 112.8 million metric tonnes in an estimated area of 9.2 million hectares (FAOSTAT, 2017; Loebenstein, 2015). China is the world's leading producer with 72 million metric tonnes amounting to 63.8% of world production. This is followed by Africa with about 27.7 million metric tonnes amounting to 24.6% of world production. In Africa, about 64.6% of the sweet potato production is in East Africa (FAOSTAT, 2017). Sweet potato in an important staple crop in third world countries, especially in parts of Africa, Asia,

and the Pacific (Bovell-Benjamin, 2007; Woolfe, 1992) but are only a secondary foodstuff in developed countries (Scott et al., 2000). It is highly productive, requires low production inputs, adapted to different soil types, and is resilient to climatic changes compared to other major staple food crops (Claessens et al., 2009; Low et al., 2009; Maquia et al., 2013). The crop is highly valued by subsistence farmers and is an important food security and income generating crop in East Africa because of its high harvest per unit area (Gibson et al., 2009). In areas having bimodal rainfall, sometimes sweet potato can be harvested three times per year. Sweet potato provides food security during famines caused by drought when main crops like maize fail (Karyeija et al., 1998). For example, people in Japan relied on sweet potato when typhoons demolished their rice fields, and during famines in China in the 1960s and in East Africa when cassava mosaic virus devasted cassava fields in the 1990s (Loebenstein & Thottappilly, 2009).

Despite the advantages sweet potato offers to subsistence farming, its production worldwide, is limited by many biotic constraints of which virus diseases are the second most important after sweet potato weevils (*Cylas spp.*) (Horton, 1989; Ngailo et al., 2016; Shonga et al., 2013). Management of viral diseases require correct identification of the causal viruses, understanding their interactions with host plants and the mechanisms involved in virus evolution and spread.

1.4 Sweet potato viruses

Pathogens, especially viruses, are considered the most important stimuli that are associated with yield variations and cultivar decline in sweet potato (Carroll et al., 2004; Clark et al., 2002; Clark & Hoy, 2006). Because sweet potato is produced by vegetative propagation, it may accumulate pathogens, particularly viruses, in the planting material over years resulting in yield and quality decline of the crop (Bryan et al., 2003; Clark et al., 2002; Lewthwaite et al., 2011). Viruses infecting sweet potato are widely distributed where ever sweet potato is grown and have earlier been reported in USA and East Africa in the 1930s (Brunt et al., 1996; Moyer & Salazar, 1989). In the past detection and identification of viruses relied on bioassays and serological techniques to explain the aetiology of various viral diseases of sweet potato (Carey et al., 1999; Moyer & Salazar, 1989). Study of several of these viruses has been limited due to lack of simple detection techniques. Advances in molecular methods of virus detection and identification has led to the understanding of the composition of sweet potato virus complexes, the effect of virus diseases on production systems, the biology of virus-host interaction, and management approaches to sweet potato virus diseases. Globally, more than 30 viruses belonging to nine families have been reported to infect sweet potato. These families include

Bromoviridae, Bunyaviridae, Caulimoviridae, Closteroviridae, Comoviridae, Flexiviridae, Geminiviridae, Luteoviridae, and Potyviridae. Half of these viruses are DNA viruses found in the families *Geminiviridae* and *Caulimoviridae* (Clark et al., 2012). More than 23 of these viruses have been assigned a formal taxonomic position by the International Committee on Taxonomy of Viruses (ICTV), some are presented in (Table 1).

The most important and widespread virus infecting sweet potato is *Sweet potato feathery mottle virus* (SPFMV; family *Potyviridae,* genus *Potyvirus*,) (Loebenstein, 2015; Moyer & Salazar, 1989). SPFMV by itself causes mild or no symptoms on sweet potato infected plants and no significant effect on yields (Clark & Hoy, 2006; Gutierrez et al., 2003; Karyeija et al., 2000). However, it is an important virus because it is a component of the severe sweet potato virus disease (SPVD), the most devastating disease affecting sweet potato worldwide (Gibson et al., 1998; Gutierrez et al., 2003; Milgram et al., 1996; Untiveros et al., 2007).

Another widespread virus of sweet potato is *Sweetpotato chlorotic stunt virus* (SPCSV; family *Closteroviridae,* genus *Crinivirus*,). Infection by SPCSV typically stunts sweet potato plants and causes either a purpling or chlorotic yellowing of middle and lower leaves (Gibson et al., 1998).

Symptoms are sometimes mild or absent depending on the isolate or environmental conditions. SPCSV by itself can cause up to 50% reduction in yield of storage roots (Adikini et al., 2016; Mukasa et al., 2006). SPCSV is a component of the SPVD. Its importance lies on its role in causing severe synergistic disease complexes with several unrelated viruses from different genera also infecting sweet potato (Cuellar et al., 2011a; Gibson et al., 1998; Karyeija et al., 2000; Kokkinos & Clark, 2006a; Mukasa et al., 2006; Untiveros et al., 2007).

Begomoviruses (Family *Geminiviridae*, genu *Begonovirus*) infecting sweet potato, referred to as sweepoviruses, are also widespread and have been reported in many countries including the United states (Lotrakul et al., 2003; Zhang & Ling, 2011), Peru (Fuentes & Salazar, 2003), Brazil ((Albuquerque et al., 2011; Paprotka et al., 2010), Spain (Lozano et al., 2009), Italy (Briddon et al., 2006), China (Bi & Zhang, 2012; Luan et al., 2007), Uganda (Wasswa et al., 2011), Kenya (Miano et al., 2006), South Africa (Esterhuizen et al., 2012) and India (Prasanth & Hegde, 2008). Symptoms caused by sweepoviruses consists of leaf curling and vein yellowing depending on the specific host, although most of the infection on sweet potato plants

Table 1. Some viruses that have been reported in sweet potato crops.

Virus	Family	Genus	Vector	References
Sweet potato feathery mottle virus (SPFMV)	*Potyviridae*	*Potyvirus*	Aphid	1, 8, 3, 6, 9
Sweet potato latent virus (SPLV)	*Potyviridae*	*Potyvirus*	Aphid	2, 3, 9
Sweet potato virus 2 (SPV2)	*Potyviridae*	*Potyvirus*	Aphid	7, 9
Sweet potato virus C (SPVC)	*Potyviridae*	*Potyvirus*	Aphid	9, 10, 11, 12
Sweet potato virus G (SPVG)	*Potyviridae*	*Potyvirus*	Aphid	8, 9
Sweet potato mild mottle virus (SPMMV)	*Potyviridae*	*Ipomovirus*	Whitefly	4, 3, 6, 5
Sweet potato chlorotic stunt virus (SPCSV)	*Closteroviridae*	*Crinivirus*	Whitefly	5, 10, 25, 13
Cucumber mosaic virus (CMV)	*Bromoviridae*	*Cucumovirus*	Aphid	29, 18
Sweet potato collusive virus (SPCV) (synonym Sweet potato caulimo-like virus)	Caulimoviridae	Cavemovirus		14, 15
Sweet potato pakakuy virus (SPPV)* (synonyms Sweet potato badnavirus A and B)	*Caulimoviridae*	*Badnavirus*		17, 10, 16, 18
Sweet potato vein clearing virus (SPVCV)*	*Caulimoviridae*	*Solendoviruss*		15
Sweet potato symptomless virus 1 (SPSMV-1)*	*Geminiviridae*	*Mastrevirus*		9, 16, 17
Ipomoea yellow vein virus (IYVV)	*Geminiviridae*	*Begomovirus*	Whitefly	22
Sweet potato leaf curl virus (SPLCV)	*Geminiviridae*	*Begomovirus*	Whitefly	8, 9, 18, 19
Sweet potato leaf curl Georgia virus (SPLCGV),	*Geminiviridae*	*Begomovirus*	Whitefly	10, 20, 22
Sweet potato leaf curl China virus (SPLCV-CN),	*Geminiviridae*	*Begomovirus*	Whitefly	21, 22
Sweet potato leaf curl Lanzarote virus (SPLCLaV)	*Geminiviridae*	*Begomovirus*	Whitefly	22
Sweet potato leaf curl Spain virus (SPLCESV)	*Geminiviridae*	*Begomovirus*	Whitefly	22, 24
Sweet potato leaf curl Uganda virus (SPLCUV)*	*Geminiviridae*	*Begomovirus*	Whitefly	23, 16
Sweet potato leaf curl Sao Paulo virus (SPLCSPV)	*Geminiviridae*	*Begomovirus*	Whitefly	16, 24
Sweet potato chlorotic fleck virus (SPCFV)	*Flexiviridae*	*Carlavirus*		3, 6, 9, 14
Sweet potato C-6 virus*	*Flexiviridae*	*Carlavirus*		25

* Tentative species. 1) Gibson et al., 1998; 2) Colinet et al., 1998; 3) Njeru et al., 2008; 4) Colinet et al., 1996; 5) Mukasa et al., 2003; 6) Ateka et al., 2004; 7) Ateka et al., 200); 8) Kokkinos & Clark, 2006b; 9) Kwak et al., 2014; 10) Kashif et al., 2012; 11) Tairo et al., 2005; 12) Ma et al., 2019; 13) Qin et al., 2014; 14) Aritua et al., 2007; 15) Cuellar et al., 2011b; 16) Mbanzibwa et al., 2014; 17) Kreuze et al., 2009; 18) Gu et al., 2014; 19) Ling et al., 2010; 20) Lotrakul et al., 2003; 21) Luan et al., 2007; 22) Lozano et al., 2009; 23) Wasswa et al., 2011; 24) Albuquerque et al., 2011; 25) Untiveros et al., 2007.

can be symptomless. Yield reductions of between 10% and 80% have been reported in infected sweet potato plants (Clark & Hoy, 2006; Ling et al., 2010).

1.4.1 SPFMV

SPFMV is a member of the family *Potyviridae,* genus *Potyvirus*. Potyviruses constitute the largest and the most economically important group of plant viruses with more than 200 members (Valli et al., 2015; van Regenmortel et al., 2000), comprising more than 30% of all known plant viruses. The virions of potyviruses are flexuous, filamentous particles without an envelope 680-900 nm long and 11-15 nm wide (van Regenmortel et al., 2000).

SPFMV has flexuous filamentous particles between 830-850 nm in length. The genome is positive-sense single stranded RNA (ssRNA) of about 10.6 kb, which is larger than the average (9.7 kb) of a potyvirus genome (Sakai et al., 1997; Shukla et al., 1994; van Regenmortel et al., 2000). SPFMV genome has a single open reading frame (ORF), flanked by an un-transcribed region (UTR) at both the 5'-end and 3'-end encoding a large polyprotein. The coat protein (CP) of SPFMV is exceptionally large (38 kDa) in contrast to other potyviruses (Abad & Moyer, 1992).

SPFMV is non-persistently transmitted by aphids *M. persicae* (Sulzer), and *A. gossypii* Glover (Souto et al., 2003; Wosula et al., 2012). The host range of SPFMV is restricted primarily to the *Convolvulaceae* family (Wosula et al., 2012). It can be mechanically transmitted to various *Ipomoea spp*. such as *I. batatas*, *I. setosa*, *I. nil*, *I. incarnata* and *I. purpurea*, and some strains of *Nicotiana benthamiana*, *N. clevelandii*, *Chenopodium amaranticolor* and *C. quinoa* (Brunt et al., 1996). SPFMV is the most worldwide distributed virus of sweet potato and the best characterized consisting of four distinct strains: East African (EA); ordinary (O), russet crack (RC) and common (C), classified based on the CP sequences (Moyer & Salazar, 1989; Tairo et al., 2005). Isolates of strains RC, O and EA are closely related to each other, but are phylogenetically distant from strain C (Tairo et al., 2005). The common strain C was separated to be a distinct species of potyvirus due to differences in the P1 region and named *Sweet potato virus C* (SPVC) (Untiveros et al., 2010). Strains RC, O and C are distributed worldwide (Tairo et al., 2005) whereas isolates of the EA strain have been largely restricted to countries in East Africa (Kreuze et al., 2000; Tairo et al., 2005; Tugume et al., 2010). However, EA strain was

later reported in Peru (Untiveros et al., 2008), Easter Island and Zimbabwe (Rännäli et al., 2009).

1.4.2 SPCSV

SPCSV belongs to the family *Closteroviridae,* genus *Crinivirus* (van Regenmortel et al., 2000). The family *Closteroviridae* has positive-sense, single-stranded RNA (ssRNA) viruses that have the largest genomes among plant viruses (Dolja et al., 2006) of which SPCSV is the second largest virus after *Citrus tristeza virus* (CTV; genus *Closterovirus*) (Kreuze et al., 2002). The unique characteristic of the genus *Crinivirus* is in its significant divergence in ORFs downstream of the replication proteins and variability of genome organization amongst its members.

SPCSV has flexuous particles of 850 to 950 nm in length and total genome length of 17630 nucleotides (nt) (Dolja et al., 2006; Kreuze et al., 2002). The genome is bipartite consisting of RNA1 (9407 nt) and RNA2 (8223 nt) molecules. RNA1 contains five putative ORFs while RNA2 contains seven putative ORFs. SPCSV has several novel features of criniviruses, such as nearly identical 3′UTRs on both genomic RNAs (Kreuze et al., 2002). The distinct feature of SPCSV is that it contains two novel ORFs encoding a Class 1 dsRNA-specific RNase III enzyme (RNase3) and a p22 gene located on the RNA1 genome, the two RNAs have nearly identical 208-nt-long 3′ terminal and the ORF for a putative small hydrophobic protein (p7) found at a novel position in RNA1 (Kreuze et al., 2002). Both RNase3 and a p22 suppress RNA silencing-based antiviral defense in sweet potato plants (Cuellar et al., 2008; Kreuze et al., 2005). However, RNase3 enhances the RNA-silencing suppression activity of p22 (Kreuze et al., 2005) and RNase3 is capable of mediating RNA-silencing in the absence of p22 (Cuellar et al., 2008). Most isolates of SPCSV characterized lack the p22 gene which has been found only in isolates from Uganda (Cuellar et al., 2011a; Tairo et al., 2005; Tugume et al., 2013). SPCSV is transmitted by whiteflies (e.g. *Bemisia tabaci*) in a semi-persistent manner (Schaefers & Terry, 1976; Sim et al., 2000; Valverde & Moreira, 2004). It is a phloem-limited virus (Karyeija et al., 2000; Nome et al., 2007) and has limited host range confined mainly to the genus *Ipomoea* and some species of *Nicotiana* and *Amaranthus palmeri* (Cohen et al., 1992). SPCSV can be distinguished into two distantly related strains East African (EA) and West African (WA) based on serology and nucleotide sequence data (Hoyer et al., 1996; Tairo et al., 2005). The EA strain is found only in East Africa but has also been reported in Peru

where is co-occurs with the WA strain (Gutierrez et al., 2003). The WA strain is distributed worldwide except occasionally in East Africa.

1.5 Sweet potato virus disease complexes

Virus disease complexes in sweet potato are common and very often results from synergistic interactions in mixed virus infections which influence symptoms and yield losses (Carey et al., 1999; Clark & Hoy, 2006; Di Feo et al., 2000; Karyeija et al., 2000; Mukasa et al., 2006; Untiveros et al., 2007). The most important and common disease of sweet potato worldwide is sweet potato virus disease (SPVD). This disease is caused by the synergistic interaction of SPCSV and SPFMV (Gibson et al., 1998; Gutierrez et al., 2003; Mukasa et al., 2006; Untiveros et al., 2007; Valverde & Moreira, 2004). SPVD was first reported in eastern Belgian Congo (now Democratic Republic of Congo) in 1939 (Carey et al., 1999). This disease is characterized by severe disease symptoms including chlorosis, small, deformed leaves, and severe stunting. Significant yield reductions between 50% to 100% have been reported (Gibson et al., 1998; Karyeija et al., 1998; Mukasa et al., 2006; Njeru et al., 2004). SPCSV break down resistance to SPFMV in plants coinfected with the two viruses leading to high accumulation of SPFMV titers and severe diseases symptoms (Karyeija et al., 2000; Mukasa et al., 2006).

Other viral disease complexes described in sweet potato in most cases involve SPCSV as a mediator of synergistic interaction. It has been reported to synergize the potyviruses, russet crack strain of SPFMV (SPFMV-RC), *Ipomoea vein mosaic virus* (IVMV), and *Sweet potato virus G* (SPVG), with significant increase in virus titres of these viruses (Kokkinos & Clark, 2006a). Di Feo et al. (2000) reported the chlorotic dwarf (CD), an important disease of sweet potato in Argentina caused by the synergistic interaction of three viruses, SPCSV, SPFMV and *Sweet potato mild speckling virus* (SPMSV). Mukasa et al. (2006) showed increased titres of SPFMV and *Sweet potato mild mottle virus* (SPMMV; *Ipomovirus*) by approximately 1000-fold following coinfection with SPCSV whereas SPCSV titres were reduced twofold, indicating an antagonistic interaction. Synergistic effects of SPCSV has been observed with viruses from the genus *Cucumovirus* (*Cucumber mosaic virus;* CMV), genus *Carlavirus* (*Sweet potato chlorotic fleck virus;* SPCFV and C-6 virus) (Untiveros et al., 2007), genus *Cavemovirus* (sweet potato collusive virus; SPCV and sweet potato vein clearing virus; SPVCV) (Cuellar et al., 2011b) and genus *Begomovirus* [(*Sweet potato leaf curl virus* (SPLCV), Sweet *potato leaf curl Georgia virus* (SPLCGV) and *Sweet potato leaf curl South Carolina virus* (SPLCSCV)] (Cuellar et al., 2015). Mixed infections of sweet potato with two or more potyviruses has been

reported to cause yield decrease in infected plants (Clark & Hoy, 2006). In all reports on the synergism of SPCSV with viruses from different genera, reduction in SPCSV titres and enhancement of tires in the other viruses has been observed with varing degree in addition to variability in symptom expression and yield decrease.

1.6 Detection of viruses

Methods for detection and identification of plant viruses, both in plants and vectors are of high importance in viral diseases management and successful crop production. Detection and identification of sweet potato viruses has previously relied on biological indexing and electron microscopy. Recently, progress has been made in developing sensitive and reliable techniques for detection of several sweet potato viruses including enzyme-linked immunosorbent assay (ELISA), and nucleic acid-based techniques such as nucleic acid spot hybridization (NASH), polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), real-time or quantitative (qPCR), multiplex PCR methods, rolling circle amplification (RCA), and next generation sequencing (NGS) (Colinet et al., 1998; Crosslin & Hamlin, 2011; Kokkinos & Clark, 2006b; Kreuze et al., 2009; Kwak et al., 2014; Tairo et al., 2006).

1.6.1 Biological indexing

This method is based on grafting of sweet potato scions onto *Ipomoea setosa,* a sensitive indicator plant, has been the accepted method for detection and transmision of sweet potato viruses (Cohen et al., 1997; Feng et al., 2000; Li et al., 2004). Other indicator plants such as *I. nil* 'Scarlet O Hara', *Nicotiana benthamiana*, *N. clevelandii*, *Chenopodium quinoa* and *I. aquatica* has been used to detect viruses that do not express symptoms on *I setosa* as a susceptible host (Li et al., 2004; Lotrakul et al., 1998; Moyer & Salazar, 1989). These indicator plants readily show symptoms which may not be expressed in the original sweet potato plants due to low virus titres (Karyeija et al., 2000). The increase in virus titres is an advantage for dowmstream serological as well as nucliec acid-based testing. In addtion, unlike sweet potato, *I. setosa* plants do not contain inhibitors that may interfere with virus detection (Kokkinos & Clark, 2006b). However, biological indexing is relatively insensitive, not species-specific, time consuming, require labour and greenhouse space (Clark et al., 2012; Valverde et al., 2007).

1.6.2 Electron microscopy

Electron microscopy visualizes virus particles providing information on its identity and/or taxonomic group (Wilson, 2014). It has been used in the detection of sweet potato caulimo-like virus (Atkey & Brunt, 1987), sweet potato virus exhibiting closterovirus-like particles (Winter et al., 1992) and sweet potato potyvirus (Souto et al., 2003). The technique can detect unknown pathogens without species-specific reagents, but requires very expensive equipment and operators with specialised skills (Wilson, 2014).

1.6.3 ELISA techniques

ELISA method carried out on a nitrocellulose membrane or a microtiter plate has been widely used for virus detection in sweet potato (Aritua et al., 2007; Ateka et al., 2004; Gutierrez et al., 2003; Karyeija et al., 2000; Opiyo et al., 2010a; Souto et al., 2003; Untiveros et al., 2007), particularly in developing countries where the use of other methods is limited by the available resources. It is generally efficient, sensitive, robust, easy to use, cost effective and can be utilize in testing large number of samples (Boonham et al., 2014; Torrance & Jones, 1981). The sensitivity and specificity of ELISA has been attained using specific antisera or monoclonal antibodies (Alicai et al., 1999). In cases where viruses cannot be purified, sensitive and specific virus detection by ELISA has been achieved using antisera raised against recombinant virus proteins (Steel et al., 2010). Although ELISA has been used widely for virus detection in plants, it has several drawbacks (Boonham et al., 2014). Viruses in sweet potato plants have low titre concentration and irregular distribution (Cadena-Hinojosa & Campbell, 1981; Karyeija et al., 2000). Thus, ELISA detects sweet potato viruses mainly from symptomatic sweet potato plants. Sweet potato tissues have high amounts of phenolics, latex and inhibitors that adversely affect reagents used in the tests (Abad & Moyer, 1992; Kokkinos & Clark, 2006b). Grafting scions of the tested sweet potato plants onto *I. seto*sa and testing of the systemically infected indicator plants instead of the original, sampled sweet potato plant has been a solution to these problems.

1.6.4 Nucleic acid spot hybridization (NASH)

This technique detects viral RNA and DNA and is more sensitive than ELISA. In NASH, the target virus nucleic acid is bound onto a physical matrix such as nitrocellulose paper. The sap extracted from the plant is denatured by heat or alkaline treatment before spotting onto a membrane (Wilson, 2014). Radio-labelled probes are commonly added and allowed to bind to the target or sometimes non-radioactive reporters such as conjugate enzymes. A radioactive-

sensitive film or a substrate or enzyme is used for the detection of the added label (Wilson, 2014). Detection of sweet potato viruses by NASH has been reported (Abad & Moyer, 1992; Müller et al., 2002; Valverde et al., 2004b).

1.6.5 Polymerase Chain Reaction (PCR)

This method is more sensitive and specific for the detection of plant viruses than ELISA (Boonham et al., 2014; Vunsh et al., 1990). It can detect very low virus titres in plant samples, and a wider range of viruses or virus strains within a particular taxonomic group (Li et al., 2004; Wilson, 2014). The PCR techniques require specific primers and probes that can be easily designed based on available sequence information for detection of specific viruses and virus strains (Colinet et al., 1998; Wilson, 2014). PCR only amplifies DNA, thus can be used directly for detection of DNA viruses. For the detection of RNA viruses, reverse-transcription polymerase chain reaction (RT- PCR) method is used where the RNA is first converted to complementary DNA (cDNA) by reverse-transcription (Wilson, 2014). The PCR and RT-PCR methods have been successfully used in the detection and identification of sweet potato viruses, and in the differentiation of mixed infections with more than one strain of virus (Alicai et al., 1999; Ateka et al., 2007; Colinet et al., 1998; Kwak et al., 2015; Li et al., 2004; Li et al., 2008; Tairo et al., 2006). Although conventional PCR and RT-PCR methods are highly sensitive and specific for virus detection, they have problems of post-PCR contamination. Opening of tubes after thermal cycling release small amounts of DNA into the laboratory environment which could eventually be detected by the PCR method. This has resulted in recurring problems with false positive results (Boonham et al., 2014). This problem has been solved by the development of real-time PCR or quantitative PCR (qPCR) as a modification to conventional PCR (Higuchi et al., 1992).

1.6.6 Real-time or quantitative (qPCR)

This technique is highly sensitive in virus detection compared to conventional PCR (Boonham et al., 2004; Boonham et al., 2009). It is suitable for detecting viruses where their RNA could not code for any proteins or antibodies could not be used (Boonham et al., 2004). It is possible to handle large sample numbers using robotic liquid handling systems. In qPCR, DNA/cDNA amplification and detection steps are performed in the same closed PCR tube. Detection of virus DNA/cDNA is done by capturing and measuring fluorescence released by fluorescently-tagged probes. The fluorescent signal is generated within the closed PCR tube and could be

detected either during amplification ('real-time') or at the end of it ('end point') which effectively reduces the post-PCR contamination risk. The technique is simple to perform, requires less time, can be multiplexed and is high throughput (Boonham et al., 2014; Espy et al., 2006). qPCR has been employed in the detection of both DNA and RNA viruses of sweet potato (Barkley et al., 2011; Kokkinos & Clark, 2006a and b).

1.6.7 Multiplex PCR and RT-PCR techniques

This protocol enables simultaneous detection of multiple viruses in a single assay. The methods are simple, rapid, sensitive, reliable, and cost-effective (Chiquito-Almanza et al., 2017; Gambino & Gribaudo, 2006; Li et al., 2012; Wintermantel & Hladky, 2010). Multiplex methods can identify viruses in mixed infections and discriminate among known viruses infecting different crops (Kwak et al., 2014; Wintermantel & Hladky, 2010). They have been successfully used in the detection and differentiation of sweet potato viruses (Kwak et al., 2014; Lan et al., 2018; Li et al., 2012; Opiyo et al., 2010b).

1.6.8 Rolling circle amplification (RCA)

Further improvements in virus diagnostics is the RCA universal method for detection of circular DNA viruses. RCA method use DNA polymerase of the Bacillus subtilis bacteriophage φ 29 (Dean et al., 2001). The enzyme possesses both, polymerase and strand displacement activity, thus allowing circular DNA to be replicated to nearly unlimited extent using a RCA. This method has been used in the detection of sweet potato begomoviruses (Haible et al., 2006; Paprotka et al., 2010). However, this technique is limited only to DNA viruses.

1.6.9 Next generation sequencing (NGS) techniques

NGS technologies has offered the possibilities of detecting and identifying multiple known and novel viruses in crop plants without prior knowledge of the virus (Boonham et al., 2014; Jones et al., 2017). Upon infection, virus infected plants accumulate virus-derived small interfering RNAs (vsiRNAs) that are produced in response to viral infection, a plant defense mechanism called RNA silencing (Llave, 2010; Mlotshwa et al., 2008). In eukaryotes, small interfering RNAs (siRNAs) direct antiviral immunity through RNA interference which generates small 21–24 nucleotide small RNA (sRNA) molecules corresponding to invading viruses (Mlotshwa et al., 2008). During this process vsiRNAs are enriched in the host and can be selectively purified for deep sequencing and assembly (Wu et al., 2010). Virus detection by RNA-

sequencing require the use of bioinformatic tools designed for analysis of sequenced data. The main elements of many of these tools include quality control of the raw reads, assembly of raw reads into contigs, the removal of host sequences by alignment to a host genome, and identification of viral reads by mapping to a virus database (Flygare et al., 2016; Jones et al., 2017; Zheng et al., 2017). In sweet potato, known and novel viruses have been detected and identified using NGS (Kashif et al., 2012; Kreuze et al., 2009; Mbanzibwa et al., 2014; Qin et al., 2016), and complete genome sequencing of virus genomes (Cuellar et al., 2011a; Kreuze et al., 2009). NGS has also been applied in the field of vector-enabled metagenomics (VEM) in the identification of viruses present in the environment from their insect vectors (Ng et al., 2011; Rosario et al., 2013).

1.7 Transmission of plant viruses

Most of the viruses infecting plants are transmitted to their host plants by homopterans vectors (Fereres & Moreno, 2009; Hogenhout et al., 2008). Plants infecting viruses utilize specific vectors to facilitate their movement from one host plant to another in order to survive (Bragard et al., 2013). Virus transmission requires series of steps that include host searching or pre-alighting behaviour, probing on superficial tissues, settlement and stylet penetration to the target feeding tissues and salivation and continuous sap ingestion from the preferred feeding site (Fereres & Moreno, 2009; Nault, 1997). Before viruses are transmitted to their hosts, they bind to specific sites in or on vectors and are retained there (Hogenhout et al., 2008; Ng & Falk, 2006; Whitfield & Rotenberg, 2015). Two strategies are recognized for viruses that are transmitted by insect vectors: capsid strategy and the helper strategy (Blanc et al., 1998; Chen et al., 2011; Nault, 1997; Ng & Falk, 2006). In the capsid strategy, viruses encode for structural proteins on the surface of the virion that are essential for transmission. For example, in whitefly transmission of *Lettuce infectious yellows virus* (LIYV), transmission is determined by a minor coat protein (CPm)-mediated virion retention mechanism in the anterior foregut or cibarium of whitefly vectors (Chen et al., 2011). In the helper strategy, viruses encode additional non-structural proteins that act to bridge the virion to the vector binding site as in the case of aphid transmitted *Cauliflower mosaic virus* (CaMV) where the viral transmission helper protein P2 is required (Bak et al., 2013). Based on acquisition and inoculation thresholds, as well as retention of the virus by its vector(s), four basic types of insect vector-plant virus transmission relationship have been described. These include non-persistent (non-circulative); semi-

persistent; persistent-circulative and persistent-propagative (Hogenhout et al., 2008; Nault, 1997; Ng & Falk, 2006).

1.7.1 Whitefly vectors of plant viruses

Whiteflies, (Hemiptera: *Aleyrodidae*) are of economic importance in world agriculture due to the direct and indirect damage they cause on plants. They feed directly on plants via stylet mouthparts which pierce plant tissues and suck phloem sap (Liburd et al., 2015; Oliveira et al., 2001). The indirect damage of whiteflies on plants is by excretion of honeydew onto surfaces of leaves and fruits that may support the growth of sooty mold fungi (*Capnodium* spp.) which interfere with photosynthesis (Liburd et al., 2015; Martin et al., 2000; Oliveira et al., 2001). Honeydew causes stickiness in cotton lint, resulting in difficulties in the ginning and spinning processes (Butler Jr et al., 1988; Miyazaki et al., 2013). The major indirect damage of whiteflies on plants is that some species are vectors of plant viruses that can cause severe diseases and yield losses (Bedford et al., 1994; Gilbertson et al., 2015). They transmit viruses in semipersistent or persistent manner. Whiteflies in the genera *Bemisia* and *Trialeurodes* are the virus vectors. In the genus *Bemisia, B. tabaci* (Gennadius) (the sweet potato whitefly) and *B. afer* are virus vectors (Gamarra et al., 2010; Jones, 2003; Malumphy, 2003) whereas in the genus *Trialeurodes*, *T. vaporariorum* (the greenhouse whitefly), *T. abutilonea* and *T. ricini* are vectors of viruses (Duffus et al., 1996; Jones, 2003; Wintermantel, 2004).

1.7.2 *Bemisia tabaci* (Gennadius)

B. tabaci is a polyphagous and highly destructive pest to many crops of economic importance including food, fiber and ornamental crops (Byrne & Bellows, 1991; Gilbertson et al., 2015). It has a host range of over 1000 plants species (Oliveira et al., 2001; Simmons et al., 2008). Over 350 plant virus species belonging to five genera are transmitted by *B. tabaci*. These include the genus *Begomovirus*, genus *Crinivirus,* genus *Ipomovirus,* genus *Torradovirus,* and genus *Carlavirus* (Gilbertson et al., 2015; Jones, 2003; Navas-Castillo et al., 2011; Polston et al., 2014; Verbeek et al., 2014). Begomoviruses are the bigest group of viruses vectored by *B. tabaci*.

B. tabaci is genetically diverse and complex. The level of diversity in the genetic complex of *B. tabaci* has been examined based on the mitochondrial cytochrome oxidase I (mtCOI) gene (Boykin et al., 2012; Brown, 2000; De Barro & Ahmed, 2011; De Barro, 2012; Dinsdale et al., 2010; Tay et al., 2012). These studies have revealed at least 34 cryptic species that are

morphologically indistinguishable. Two members of the species complex, Mediterranean (MED) and Middle East-Asia Minor 1 (MEAM1), are highly invasive and have been spread globally causing extensive damage to many agricultural crops (Dalton, 2006; De Barro & Ahmed, 2011). In sub-Saharan Africa, two major groups of *B. tabaci* are known to prevail as important vectors of plant viruses. One group comprise several putative species, including: Indian Ocean (IO), MED, MEAM1 and Uganda (Sseruwagi et al., 2005; Tocko-Marabena et al., 2017). This group colonizes sweet potato, tomato, cucurbits, eggplant, cotton and legumes and other crops. The second group colonizes only cassava but does not colonize other crops which includes Sub-Saharan Africa 1 to 5 (SSA1–5). SSA1 has been divided into 5 sub-groups: SSA-subgroup1 (SSA1-SG1), SSA1-SG2, SSA1-SG3, SSA1-SG4 and SSA1-SG5 (Esterhuizen et al., 2013; Ghosh et al., 2015; Gnankine et al., 2013; Legg et al., 2002; Legg et al., 2014; Mugerwa et al., 2012). Recently the cassava colonizing group has been reclassified into six major groups comprising Sub-Saharan Africa East and Central Africa (SSA-ECA), Sub-Saharan Africa East and Southern Africa (SSA-ESA), Sub-Saharan Africa Central Africa (SSA-CA), Sub-Saharan Africa West Africa (SSA-WA), Sub-Saharan Africa 2 (SSA2) and Sub-Saharan Africa 4 (SSA4) (Chen et al., 2019; Wosula et al., 2017).

2. Study justification

Plant viral diseases cause enormous economic yield losses in crop plants and are important limiting factors in several crop production systems (Hanssen et al., 2010; Jeger et al., 2004; Jones et al., 2017). Viruses are difficult to control or eradicate. No antiviral products are available for plant disease management; hence, management of viral diseases is through avoidance of infection and introduction of genetic resistance in the host plants (Hanssen et al., 2010; Wilson, 2014). Plants viruses are vectored by a number of organisms including insects, mites, nematodes, fungi and plasmodiophorids (Whitfield & Rotenberg, 2015). Most of these viruses are transmitted to their host plants by homopterans vectors and Whiteflies (Hemiptera: Aleyrodidae) are the most important vectors of plant viruses worldwide (Fereres & Moreno, 2009; Gilbertson et al., 2015; Jones, 2003). The rate and extend of disease incidence depend critically upon vector population dynamics and behaviour, plant resistance to the viruses and the vectors, and the transmission processes, for a given set of environmental and host conditions (Spence, 2001). Therefore, detection and identification of viruses, understanding the biology of virus-host interaction, and insect vector-plant-virus transmission relationship is crucial for effective disease control and management decision (Jones et al., 2017; Simmons et al., 2009).

Viruses are the second most important biotic constrain to sweet potato production after sweet potato weevils (*Cylas* spp.) (Horton, 1989; Ngailo et al., 2016; Shonga et al., 2013). In Africa SPVD is the most serious disease of sweet potato and is responsible for about 50% to 100% yield losses in East Africa (Adikini et al., 2016; Mukasa et al., 2006; Ndunguru et al., 2009). The most important constraint to sweet potato production in developing countries facing poor and small-scale growers is control of sweet potato viruses through varietal resistance, quality planting material, and crop management (Fuglie, 2007). Production of sweet potato in East Africa is largely by resource-poor farmers who grow local landraces for home consumption, hence strategies to control SPVD should be suitable to these conditions (Gibson et al., 2004).

Like many farming communities in East Africa, sweet potato equally has a significant role for food security and household incomes for many communities in South Sudan, especially in the Greater Equatoria and Western Bahr el Ghazal states. Sweet potato was reported the crop that sustained six thousand South Sudanese internally displaced people who had taken shelter in a church near South Sudan's border with Congo due to insecurity imposed by the ongoing war

in South Sudan (Jeffrey, 2017). Although sweet potato is largely grown by resource-poor farmers for home consumption, it is also being grown for income purposes and is progressively becoming more commercialized due to urbanization. Despite the importance of sweet potato to farm households as well as urban populations, very little documentation on its production is available and no research has been done on pest and diseases affecting sweet potato in South Sudan due underdevelopment of agriculture.

Underdevelopment of agriculture in South Sudan is a result of several challenges which include lack of investment, low productivity, an insecure land tenure system, inadequate support services, poor infrastructure, lack of extension services, and lack of agricultural data and information flow (AfDB, 2013; Chokerah & Horvath, 2012; CIAT et al., 2011). However, these challenges have been exacerbated by war-related destruction and insecurity, and population displacements that has led directly to severe food insecurity (IOM, 2013). Following decades of war and limited access to technological advances in crop production, there has been little effort on research of crop plants in South Sudan (AfDB, 2013; Kaka & Oyik, 2008). As such, not much has been done to identify and manage pest and diseases affecting crop plants in South Sudan of which sweet potato is one of the most important.

A few historical records of research on crop improvement in South Sudan before the war and during the war has been reviewed by (CIAT et al., 2011). Crop research in South Sudan between 1970 and 2010 was done by the Project Development Unit (PDU) and Norwegian Church Aid Sudan Program (NCASP) who focused mainly on plant breeding. Most of the work was done on screening both introduced and local crop varieties for yield performance and adaptation, and susceptibility to common pests and diseases of South Sudan with little or no attention to pest and diseases management. Introduced plant materials were from East, Central and West Africa which proved most relevant. During the peak of war between 1987 and 2005, international non-governmental organizations (INGOs) and United Nations (UN) agencies took the lead in agricultural interventions in Southern Sudan. Moreover, there was no system in place to coordinate and monitor agricultural research in Southern Sudan during the war. Many agricultural research reports were confined to the archives of NGOs, which are not easily accessible to the public. These agencies concentrated on introduction of improved crop varieties from neighbouring countries to mitigate food insecurity. After independence of South Sudan from Sudan in 2011, research policies and strategies have been developed. Several agencies, with Food and Agriculture Organisation of the United Nations (FAO) as lead of UN

agency, have been involved in promoting programs to improve crop production and productivity, however, the conflict that broke out again in South Sudan in 2013 has led to the improper implementation of these research programs. Currently, there is no proper research running on crop plants. Due to the ongoing war, INGOs and, to a lesser extent, the Ministry of Agriculture and Forestry-Government of South Sudan (MAF-GoSS) focus in relief seed supply to the war affected farmers mostly importing from neighboring Kenya and Uganda and to some extend buying from local farmers (CIAT et al., 2011). In view of the above challenges, no research has been undertaken to improve sweet potato production, and to identify production constraints, viruses affecting sweet potato and insect vectors that transmit sweet potato viruses. South Sudan is neighbouring Uganda, an East African country which is reported to have SPVD as the most damaging to sweet potatoes production (Adikini et al., 2016; Mukasa et al., 2003), and currently there is large-scale trade between the two countries. Mwanga and Ssemakula (2011) reported the selling of sweet potato vines planting material, as well as products to farmers and NGO's in South Sudan (Sudan), by a Ugandan farmer from Soroti District, north-eastern Uganda. Quarantine controls at border crossings are currently very limited. Therefore, there is high probability of occurrence of sweet potato viruses in South Sudan likely through sweet potato vines planting material.

2.1 Aim of the study

This study sought to provide basic data on the identity, diversity and distribution of viruses infecting sweet potato and insect vectors of sweet potato viruses in South Sudan.

2.2 Specific Objectives

i. To assess farmers' knowledge and perception of sweet potato production practices, pest and diseases and their management, and production constraints;

ii. To determine the identity, distribution and importance of the main viruses infecting sweet potato;

iii. To describe the effects of the main sweet potato viruses on sweet potato varieties;

iv. To identify and characterize whitefly vectors of sweet potato viruses.

3. Thesis: Main results and discussions

Prior to this study, no research on sweet potato production has ever been conducted in South Sudan. This study presents the first report on the identification and distribution of viruses affecting sweet potato in South Sudan. Also, another first report on the genetic diversity of *B. tabaci* whitefly populations collected from South Sudan is presented. In addition, results from baseline survey on sweet potato farmers' perceptions on sweet potato pest and diseases and production constraints are highlighted. The information provided in this study can thus serve as a basis for the development of virus and whitefly vectors management and control strategies to improve sweet potato production in South Sudan.

3.1 Farm household surveys

3.1.1 Importance of sweet potato to poor and small-scale farmers in South Sudan

We conducted a farm households' survey in August and September 2015 in three states of South Sudan comprising five counties namely, Wau County (Western Bahr el Ghazal state), Lainya, Morobo and Yei counties (Central Equatoria) and Magwi County (Eastern Equatoria state). In total 180 households were interviewed (60 households per state) using a structured questionnaire. Results showed that sweet potato is a major food crop and a source of both food and income to the farm households (Table 2). Most of the farm households (81.7%) valued sweet potato as food security crop (Table 2). Average land area cultivated to sweet potato across the three states was 0.42 hectares with mean income from sweet potato sales amounting to 1618.7 South Sudanese pounds, equivalent to 107.9 USD (Table 3). Western Bahr el Ghazal state registered the largest area devoted to sweet potato (0.53 ha) and the highest earnings (162.5 USD).

Sweet potato become largely commercialized in South Sudan as a result of high demand in urban markets. Urbanization in South Sudan has expanded after the country's independence in 2011. This motivated farmers to increase acreages dedicated to sweet potato production as they earned substantial income when their produce was transported to urban markets especially farmers from Magwi and Lainya counties where both feeder and main road networks allow the farmers to access urban markets. Similar results were reported in Kenya where increased commercialization of sweet potato was a direct result of consumer driven demand (Kivuva et al., 2014).

Table 2. Response of interviewed farm households to the importance of sweet potato in their farming systems.

Sweet potato importance	State			Overall mean	χ^2
	Western Bahr el Ghazal	Central Equatoria	Eastern Equatoria		
Reason for growing sweet potato (%)					9.6*
Source of food	8.3	13.3	26.7	16.1	
Source of income	8.3	3.3	3.3	5	
Source of both food and income	83.3	83.3	70	78.9	
Value of sweet potato in household farming (%)					14.9*
Staple crop	25	56.7	33.3	38.3	
Major crop	50	33.3	50	44.4	
Minor crop	25	10	16.7	17.2	
Sweet potato as a food security crop (%)					10.4*
Yes	93.3	96.7	80	90	
No	6.7	3.3	20	10	
Reasons for considering sweet potato as food security crop (%)					6.2
Production can be on limited land	7.2	17.2	20.8	15.9	
Early maturity	92.9	79.3	72.9	81.7	
Drought tolerant	0	1.7	4.2	1.9	
Others	0	1.7	2.1	1.2	
Number of respondents	60	60	60	180	

* Values are statistically different at $P \leq 0.05$

We found that farm households valued sweet potato as a food security crop because it is early maturing. Farm households regarded sweet potato as a rescue crop when their main crops are not yet harvested or when other crops fail due to drought, other stresses or pests and diseases. Early maturing varieties of sweet potato can be harvested within 4 to 5 months and it can be harvested on piecemeal basis. Storage roots can be sliced and airdried for preservation. Okonya and Kroschel (2014) reported piecemeal harvesting periods extended between 6 to 8 months in sweet potato producing districts in Uganda. Motsa et al. (2015) mentioned that the benefit of early and piecemeal harvesting of sweet potato over several months ensures a lasting source of food. It gives an improved food access, availability and stability, hence, food security.

Table 3. Means of reported area cultivated to sweet potato and sweet potato income (in the previous year 2014) according to farmers in the three surveyed states of South Sudan.

Category	State			Overall mean	P-value
	Western Bahr el Ghazal	Central Equatoria	Eastern Equatoria		
Area cultivated to Sweet potato in acres (hectare)*	1.31±0.102[a] (0.53)*	0.80±0.087[b] (0.32)*	0.99±0.084[b] (0.40)*	1.033±0.055 (0.42)*	0.001
Sweet potato income in South Sudanese pounds (USD)	2437.9±361.1[a] (162.5)*	1501.1±208.3[b] (100.1)*	875.9±123.043[b] (58.4)*	1618.7±154.4 (107.9)*	0.0001

Means followed by same letters in the same row are not significantly different at $P \leq 0.05$ (LSD)

* Area cultivated to sweet potato in hectare and sweet potato income in US dollars.

3.1.2 Sweet potato production constraints

In this survey, key constraints to sweet potato production reported by interviewed households in order of importance included lack of extension services (55%), lack of improved varieties (48.9%), low sale prices (43.9%), lack of credit services (43,3%), price fluctuations (42.8%), field pests (41.7%), drought (40.6%) and diseases (38.3%) (Table 4, Paper I).

Inadequate extension services are a significant problem to farmers in all agricultural systems throughout South Sudan (AfDB, 2013). This shows that farmers remotely rely of their own knowledge and experience in coping with production problems. The identification of lack of extension services as a number one constraint indicates the readiness of sweet potato framers to adopt new production technologies if available and provided to them. Therefore, delivery of adequate extension services is crucial for sweet potato farmers to acquire knowledge on accurate crop pest and diseases identification, pest and diseases management skills, and new production technologies to improve sweet potato production. Our results concur with Ngailo et al. (2016) who reported that one reason to low sweet potato production was lack of provision of extension services to farmers. Asfaw et al. (2012) reported the role of adequate extension services on effective change of livelihoods of chickpea and pigeon pea farmers in Ethiopia and Tanzania. They mentioned that farmers were able to adopt new production technologies which resulted in increased productivity due to frequent contacts of farmers with extension agents.

The lack of improved sweet potato varieties was reported as the second most important constrain to sweet potato production in this study. Improved productivity is determined by quality planting material, that is high yielding, resistant to pests and diseases, drought tolerant and early maturing. Farmers' access to such material would result in increased yields, improved food security, better incomes, reduced poverty and enhanced livelihoods. Gibson et al. (2009) noted that improvements in quality of planting material free from weevils and sweet potato virus disease and physiological vigour are of major importance to improve livelihoods of poor people throughout the sub-Saharan countries. This can be achieved through evaluation, development and dissemination of improved pest and disease-resistant sweet potato varieties.

The use of credit can enhance productivity and income greatly. Small-scale sweet potato farmers who have access to credit can purchase planting materials and hire labour that can increase production area and productivity. In contrast, unavailability of credit limits the production potential of small-scale sweet potato farmers. In this study the lack of credit services was highlighted as a constraint to sweet potato production by sweet potato farmers. This indicates the importance of the government and other financial supporting agencies to provide farmers with capital to boost sweet potato production. In Nigeria, Olagunju (2007) reported that sweet potato farmers that were provided with credit had more efficient use of resources than those without credit.

Low sale prices and price fluctuation were a discomfort to sweet potato farmers in South Sudan. Most of the sweet potato farmers had no access to urban markets but sell their sweet potato produce in local markets. This results in low prices due to oversupply. Inaccessibility to domestic, regional and international markets due to lack of rural and feeder roads is a common hindrance to increased agricultural production in South Sudan (AfDB, 2013). Farmers would be encouraged to increase production of sweet potato if alternative markets and easy affordable transportation systems are available. Improvements in market system components such as price information and the network of permanent roads are essential elements for the development of agricultural value chains including that for sweet potatoes in South Sudan.

Storage pests and access to vines (planting material) were reported as additional important sweet potato production constraints in Central Equatoria and Western Bahr el Ghazal States (Table 4, Paper I). Sweet potato is propagated vegetatively from vine cuttings. Therefore, availability of vines to farmers at the onset of rains is a limiting factor especially in areas with unimodal rainfall. Training farmers how to conserve planting material before the start of rains

is vital to avail planting material during the onset of rains. Gibson et al. (2009) pointed out that a partial solution to conserve planting material is to advise farmers to use domestic wastewater to raise planting material. Alternatively, the maintenance of planting materials in moist valley bottoms is feasible. Another extensive solution is to bury small- to medium-sized roots at the start of the dry season in a small nursery bed and water them for 4 – 6 weeks before the onset of rains. Such roots will sprout to produce large amounts of planting material.

Ngailo et al. (2016) observed that the lack of extension services and credit along with pests and diseases, drought and low prices were major constraints for sweet potato production in Tanzania. Also, the absence of improved varieties was reported as a limiting factor for increased sweet potato production in sub-Saharan Africa (Fuglie, 2007) and price fluctuation in Uganda (Okonya & Kroschel, 2016). Shortage of clean and enough planting material was a constraint to increased sweet potato production in Kenya and Tanzania (Kivuva et al., 2014; Mmasa et al., 2012).

3.1.3 Farm households' knowledge on pests and diseases of sweet potato and their control

Insect pests reported by sweet potato farmers were sweet potato weevil (*Cylas spp*.), whiteflies (*Bemisia tabaci* (Gennadius)), butterflies (*Acraea acerata* Hewitson), and aphids (*Aphis spp.*) (Figure 3b, Paper I). Other pests reported were millipedes and ants. Sweet potato weevil was perceived by most of the farmers (42.7%) as the most damaging insect pest. Thirty-two-point seven percent of the farmers were aware of the presence of whiteflies in their fields but had no knowledge that whiteflies are vectors of plant viruses. The majority of the farmers (64.3%) were not able to identify diseases that were affecting their sweet potato crop, even after being shown photos of disease symptoms on sweet potato (Figure 3c, Paper I). Conversely the minority could identify the symptoms but did not know the name of the disease that caused the symptoms. Overall 60.2% of the farmers did not use any method of pest and disease control. A few of the farmers practiced early planting, crop rotation and use of traditional pesticides (ash spray), and the use of resistant varieties and pesticides were mentioned by farmers who returned from exile in Uganda (Figure 3d, Paper I).

Sweet potato storage root damage by weevils was a concern to farmers. Some farmers in Yei County, Central Equatoria State, who could not access urban markets due to transportation difficulties, reduced total acreage planted to sweet potato because of weevil infestation. Meanwhile, farmers in Bilinya village (Magwi County, Eastern Equatoria State), where sweet

potato is their main food crop, choose not to leave their sweet potato storage roots for long in the soil and instead they are harvested and preserved as sliced dried roots, to avoid damage by weevils. We found that farmers were aware of the presence of whiteflies in their fields but did not associate whiteflies to any disease symptoms on sweet potato. This might probably be because farmers do not know that insects can transmit plant viruses. Training of farmers in South Sudan to gain knowledge and skills in insect pest identification and the effect they cause on their crop is vital for a better control of insect transmitted viral diseases.

Weevil damage as a constraint to sweet potato production have been reported in Tanzania (Kagimbo et al., 2018; Ngailo et al., 2016), in Uganda (Okonya et al., 2014), in Ethiopia (Shonga et al., 2013) and in Papua New Guinea (Gurr et al., 2016). Kivuva et al. (2014) in Kenya, in their study reported farmers awareness of the presence of whiteflies on their sweet potato crop. Okonya and Kroschel (2016) reported similar results for potato farmers in Uganda. Okonya et al. (2014) in Uganda reported the inability of farmers to consider whitefly as an important pest of sweet potato because they lack the knowledge that whiteflies are vectors of sweet potato viruses.

In this survey, farmers were unable to identify diseases affecting sweet potato indicating that farmers had never received any knowledge about diseases affecting sweet potato. This is clearly reflected on farmers' reports of a lack of extension services as the major constraint in sweet potato production. In order to set strategies to address management of diseases, it is important to train farmers in disease identification, epidemiology and management. It is also crucial for farmers to have knowledge of being able to distinguish symptoms of individual disease infection from other diseases as well as insect vectors of viral diseases. Such knowledge is beneficial to farmers to identify disease problems early in their crop and to help experts on plant health to deliver to the farmers the appropriate control strategies at the right time.

Adam et al. (2015), reported that sweet potato farmers in Tanzania were able to identify infected sweet potato plants but were unable to identify the specific type of disease affecting the plants from both direct and photographic observations. Schreinemachers et al. (2015) in a survey on vegetables and legumes in Asia, found that farmers in Tamil Nadu who have received training on virus diseases and their mode of transmission were able to distinguish between symptoms caused by viruses and those caused by other diseases. In contrast, most of the farmers in Thailand and Vietnam, who have not received any training on virus diseases, were

unable to identify virus disease symptoms and had no knowledge about the role of insect vectors in virus transmission.

We found that the methods for pest and diseases control were not practiced by most of the farmers. This might be due to the farmers' lack of knowledge of pest and disease management. Most of the farmers commented that they did not receive any assistance on sweet potato plant health, from either the agricultural extension workers nor from Non-Governmental Organizations (NGOs). This indicates the importance of delivering plant health services to farmers and providing training on pest and disease management methods. Gurr et al. (2016) reported that low level of pest and disease management practices among sweet potato farmers in Papua New Guinea was due to scarcity in biological and technical knowledge. Okonya and Kroschel (2016) pointed out the necessity of training farmers and extension workers on insect pest identification, biology and control on potato.

3.2 Detection of viruses infecting sweet potato (Paper II and III)

3.2.1 Virus and virus-like disease incidence and symptom severity

Sweet potato farmers' fields were surveyed between August and September 2015 in five counties of three states of South Sudan including Wau County (Western Bahr el Ghazal state), Magwi County (Eastern Equatoria state), Lanya, Yei and Morobo counties (Central Equatoria state (Figure 1, Paper II). The results showed distinct and severe virus-like and virus disease symptoms in all the three surveyed states. Symptoms observed were purpling of lower leaves, purple rings and feathering, leaf curling, mosaics, vein clearing, chlorosis and stunting (Figure 2A to G, Paper II). Mean disease incidence was relatively high (21.7% to 30.3%), but mild disease severity scores (1.7 to 2.1) in the surveyed fields (Figure 3, Paper II) suggesting that the occurrence of coinfections in sweet potato plants in the surveyed fields were not common. Most infections of sweet potato with single viruses are latent showing mild or no symptoms, depending on the cultivar, and in some cases, no symptoms even on indicator plants (Clark et al., 2012; Cuellar et al., 2015; Karyeija et al., 2000). Severe disease symptoms in sweet potato is associated with co-infection of sweet potato plants with two or more viruses (Di Feo et al., 2000; Karyeija et al., 2000; Mukasa et al., 2003; Valverde et al., 2007).

3.2.2 Viruses detected in sweet potato by ELISA and RT-PCR

In order to confirm the occurrence of viruses in the surveyed sweet potato fields, we collected a total of 201 sweet potato leaf samples, 108 symptomatic and 93 asymptomatic, from the surveyed fields. One symptomatic and 31asymptomatic vines were also collected from different sweet potato cultivars grown by farmers in the three surveyed states. The sweet potato leaf samples were serologically tested by DAS- and TAS-ELISA for five sweet potato infecting viruses (SPFMV, SPCSV, SPMMV, SPV2 and CMV). We detected two viruses, SPFMV and SPCSV, in 24 (22.2%) of the symptomatic leaf samples either in single infection or in co-infection (SPCSV + SPFMV) in four of the surveyed counties except Morobo County (Table 1, Paper II). Single infections by SPFMV were common in samples from Magwi County whereas single infections by SPCSV were common in samples from Yei County (Table 1, Paper II). The asymptomatic leaf samples tested negative for all the assayed viruses.

After the establishment of the 31 asymptomatic and 1 symptomatic sweet potato vines collected from different cultivars in farmers' fields, leaf samples were tested by ELISA for the same above assayed viruses. SPFMV and SPCSV were detected from the symptomatic cultivar, namely, Mali-Mali-2 (Table 2, Paper II). All the leaf samples from the asymptomatic cultivars tested negative for all the assayed viruses. However, after grafting the asymptomatic sweet potato cultivars onto *I. setosa*, symptoms typical of SPFMV were observed on *I. setosa* leaves in 14 (45%) of the cultivars (Figure 2H and I, Paper II). ELISA test of the symptomatic *I. setosa* leaf samples detected only SPFMV in these 14 cultivars (Table 2, Paper II). The sero-positive samples were from cultivars collected from Magwi, Lainya and Yei counties. Cultivars collected from Wau and Morobo counties were sero-negative (Table 2, Paper II). The presence of SPFMV and SPCSV in the sero-positive cultivars was validated by RT-PCR (Figure 1).

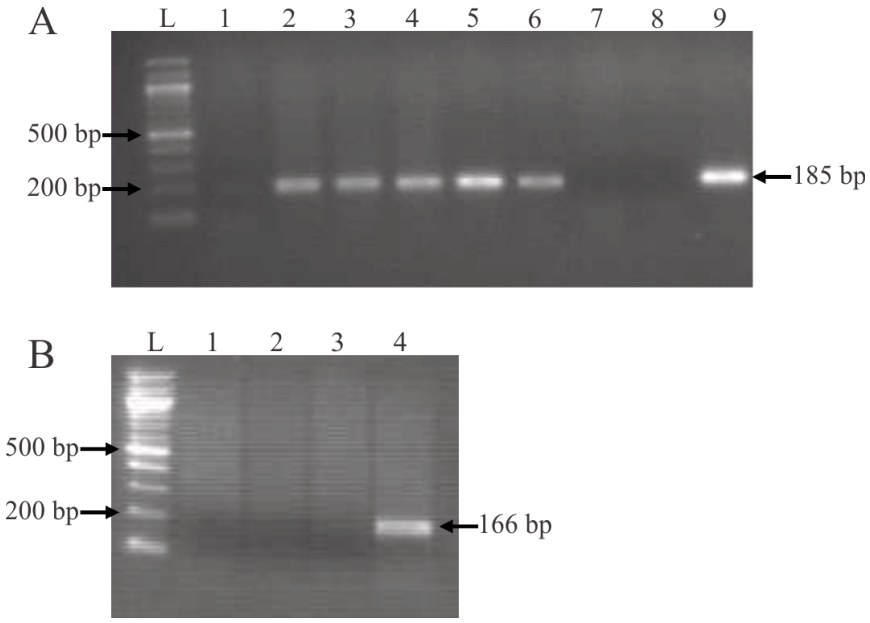

Figure 1. Virus-specific detection of SPFMV (185 bp primer) (A) and SPCSV (166 bp primer) (B) by RT-PCR. Amplification products were analyzed by electrophoresis through a 1 % agarose gel. (A) SPFMV, Lane 1 to 8 show positive and negative samples of some of the tested sweet potato cultivars. Lane 9 shows sample from the co-infected cultivar Mali-Mali-2. (B) SPCSV, Lane 4 shows positive sample from the co-infected cultivar Mali-Mali-2. Lane L shows 100bp DNA marker. Nucleotide sequences of the various primers used are presented in Paper II (adopted from Kathurima et al 2011).

The detection of SPFMV and SPCSV in all the three surveyed states clearly demonstrates the occurrence of these two important sweet potato viruses in South Sudan. SPFMV and SPCSV have been reported as the most commonly occurring viruses in East Africa, either in single or dual infection (Aritua et al., 2007; Ateka et al., 2004; Mukasa et al., 2003; Ndunguru et al., 2009). SPFMV was the most detected in the assayed samples. This may explain the prevalence of SPFMV in sweet potato fields. SPFMV shows no or mild symptoms and no reduction in yield when infecting sweet potato plants alone (Clark & Hoy, 2006; Karyeija et al., 2000). Farmers usually select symptomless plants as the source of planting material for their next crop. Since sweet potato is vegetatively propagated, pathogens, particularly viruses, may accumulate in the planting material over generations and can cause deline in yield and quality of the crop (Lewthwaite et al., 2011). It is likely that SPFMV or other symptomless viruses can be carried to the next generations through the symptomless cuttings. This can cause effect on yield of sweet potato when mixed infections with complexes of potyviruses, and/or SPCSV or other viruses are involved. The effect of SPFMV on sweet potato yield is significant when in

coinfection with other potyviruses (Clark & Hoy, 2006) and more drastic yield decreases are observed when in coinfection with the crinivirus, SPCSV (Untiveros et al., 2007).

Like SPFMV, SPCSV was also detected in sweet potato samples from all the three surveyed states, but more prevalent in Yei County (Central Equatoria state). SPCSV infecting sweet potato by itself can cause clear synmptoms and yield reduction of up to 50% (Adikini et al., 2016; Mukasa et al., 2006). However, synergistic interaction of SPCSV with SPFMV results in SPVD, the most important disease of sweet potato that causes severe symptoms and drastic yield loss (Clark & Hoy, 2006; Mukasa et al., 2006; Untiveros et al., 2007). SPCSV has also been reported to mediate synergistic interactions with viruses from other genera including *Cucumovirus, Carlavirus, Cavemovirus* and *Begomovirus* (Cuellar et al., 2011b; Cuellar et al., 2015; Untiveros et al., 2007). In this study SPVD was detected in four of the five surveyed counties indicating that this important disease of sweet potato is widespread in the sweet potato production areas of South Sudan. Therefore, it is worth noting that SPCSV should be the target of any sweet potato virus control strategy in South Sudan.

In this study, about 80% of the symptomatic sweet potato samples did not react to any of the antisera of the assayed viruses. It might be that these samples were infected with other viruses, which were not assayed in this study, suggesting that more viruses are infecting sweet potato in South Sudan. Similar findings have been reported in East Africa (Ateka et al., 2004; Ndunguru & Kapinga, 2007; Opiyo et al., 2010a). Another explanation of the failure to detect viruses from the symptomatic leaf samples could be that virus symptoms were confused with symptoms of non-viral agents. Symptoms of non-viral agents, such as mineral deficiency, may resemble virus symptoms (O'Sullivan et al., 1997). Alternatively, one could argue that the failure to detect viruses from the symptomatic samples by ELISA could be because the samples were silica-dried preserved. Situations where ELISA failed to detect viruses from dried leaf samples has been reported in citruses (Hung et al., 2000; Saponari et al., 2008).

All the asymptomatic leaf samples gave negative ELISA results. Virus titres in asymptomatic sweet potato plants are unevenly distributed with very low concentrations to be detected by ELISA. Thus ELISA detection of sweet potato viruses is reliable only on symptomatic plant samples (Cadena-Hinojosa & Campbell, 1981; Karyeija et al., 2000).

3.2.3 Viruses detected by small-RNA deep-sequencing

Small-RNA deep-sequencing (SRDS) has been reported as an efficient method for detecting and identifying multiple known and novel viruses in crop plants without prior knowlegde of the viruses (Jones et al., 2017; Boonham et al 2014). It can also detect viruses at low titre levels even in asymptomatic plants (Kreuze et al., 2009). In Paper III, we selected 7 cultivars from the 14 detected positve to SPFMV by ELISA and RT-PCR, the co-infected cultivar Mali-Mali-2, and 8 cultivars from those which were sero-negative in ELISA for all of the assayed viruses. These 16 cultivars were representative from all the five counties surveyed (3 from Wau County, 5 from Magwi County, 2 from Lainya County, 3 from Morobo County and 3 from Yei County). Sweet potato samples from these 16 cultivars were sequenced using SRDS to confirm the presence of SPFMV and SPCSV previously detected by ELISA and RT-PCR in these cultivars, to confirm the absence of SPMMV, SPV2 and CMV previously undetected by ELISA in this cultivars and to detect possible new viruses in these cultivars.

A total of 8,669,846 to 21,248,090 siRNAs reads (size range between 18 to 50 nt) were generated for each of the 16 samples. Most of the reads (7,724,893 to 18,535,925) were between sizes of 18 to 26 nt showing two main peaks at the sizes of 21 and 24 nt. These results concur with previous reports on the production of vsiRNAs of 21nt, 22nt and 24nt sizes by Dicer-like (DCL) enzymes in response to the presence of positive-strand RNA viruses in virus-infected plants (Donaire et al., 2009; Llave, 2010; Xie et al., 2004).

Alignment of small-RNA (sRNA) virus contigs to retrieved reference virus genomes confirmed the presence of SPFMV in the infected cultivars (Ladwe achel, Koda, Goli, Lobel, Kajamingi, Senja Moko-2, Bakaya and Mali Mali-2) and the presence of SPCSV in cultivar Mali-Mali-2 (Table 1, Paper III). SRDS confirmed the absence of SPFMV and SPCSV in the other 8 cultivars (Yankar-2, Mayenduro, Mviro, Jivi, Lupandura, Singa Na kilo, Apana Lipa and Karamojo-1) previously tested negative by ELISA and RT-PCR (Table 1, Paper III) and the absence of SPMMV, SPV2 and CMV from all the cultivars tested. Small-RNA contigs covered 86.3% of the complete genome sequence of SPFMV-EA strain (FJ155666; isolate Piu3) whereas coverage of the complete genome sequences of RNA1 and RNA2 of SPCSV-EA strain (AJ428554, AJ428555; isolate Uganda) was 83.4% and 91.5%, respectively (Table 2, Paper III). These indicate that as in Uganda, Kenya and Tanzania (Ateka et al., 2004; Cuellar et al., 2011a; Tairo et al., 2005), the East African strains of SPFMV and SPCSV occur in South Sudan. It might be probable that SPFMV and SPCSV were introduced into South Sudan

through trade of sweet potato planting material between South Sudan and the neighbouring, Uganda and Kenya. It could also have been introduced by planting material brought by South Sudan returnees who came home after the peace agreement was signed. Currently, there is large-scale trade between South Sudan, Uganda and Kenya and quarantine controls at border crossings are very limited.

SRDS was able to detect 13 more viruses from the 16 sequenced samples belonging to five genera including one potyvirus [*Sweet potato virus C* (SPVC)], 7 begomoviruses [*Sweet potato leaf curl virus* (SPLCV), *Ipomoea yellow vein virus* (IYVV), *Sweet potato mosaic associated virus* (SPMaV), *Sweet potato leaf curl Sao Paulo virus* (SPLCSPV), *Sweet potato golden vein associated virus* (SPGVV), *Sweet potato leaf curl Georgia virus* (SPLCGV), and *Sweet potato leaf curl Uganda virus* (SPLCUV)], one cavemovirus [*Sweet potato caulimo-like virus* (SPCV)], one mastrevirus [*Sweetpotato symptomless mastrevirus 1* (SPSMV-1)], and 3 badnaviruses [*Sweet potato badnavirus A* (SPBV-A), *Sweet potato badnavirus B* (SPBV-B) and *Sweet potato badnavirus C* (SPBV-C), collectively known as *Sweet potato pakkakuy virus* (SPPV)] (Table 2, Paper III). Coverage of virus reference genomes sequences by sRNA virus contigs was significant and ranged from 50.2% (SPCV) to 98.7% (SPVC) (Table 2, Paper III). These results are consistent with previous findings that detected similar viruses using SRDS (Gu et al., 2014; Kashif et al., 2012; Kreuze et al., 2009; Mbanzibwa et al., 2014; Qin et al., 2016). Interestingly, SRDS detected viruses from those samples that tested negative for the viruses assayed in ELISA and RT-PCR (Table 1, Paper III).

Begomoviruses (sweepoviruses) were detected most in the sequenced samples. The most commonly occurring virus was SPLCV detected in 14 (87%) of the samples (Figure 2, Paper III) indicating the prevalence of this virus in the three surveyed states. Sweepoviruses can cause no clear symptoms on sweet potato infected plants, depending on the cultivar (Cuellar et al., 2015). However, yield reduction has been reported in sweet potato plants infected with SPLCV alone (Clark & Hoy, 2006; Ling et al., 2010), and synergistic interaction of SPCSV and sweepoviruses has been observed to show no clear symptoms but strong increase in virus titres (Cuellar et al., 2015). Mixed infections in sweet potato by sweepoviruses are common (Lozano et al., 2009; Zhang & Ling, 2011). Recombination has been shown to be one of the main driving forces in the evolution of sweepoviruses (Albuquerque et al., 2012; Wasswa et al., 2011). Recombination analysis has revealed many recombination hotspots within members of the sweepovirus group (Albuquerque et al., 2012). Thus, it is conceivable that members

within the sweepovirus group have evolved due to recombination events that occurred during mixed infections. Therefore, it is worth mentioning that the high number of sweepoviruses detected in this study may pose a threat to sweet potato production in South Sudan. This suggests the need to investigate the diversity and distribution of sweepoviruses in South Sudan given the increasing geographical expansion of the whitefly (*Bemisia tabaci*), a vector of begomoviruses.

Other prevailing viruses were SPBV-A, detected in 93.8% of the sequenced samples, and SPBV-B and SPSMV-1 detected in 100% of the sequenced samples (Figure 2, Paper III). Badnaviruses are significant in other tropical crops including banana, black pepper, cocoa, citrus, sugarcane, taro, and yam (Bhat et al., 2016). However, in sweet potato, they have no economic impact but are vertically transmitted persistent viruses living in commensal or mutualistic relationship with sweet potato (Kreuze et al., 2017). Badnaviruses are efficiently transmitted by seed and cuttings, cause no visible symptoms on sweet potato or indicator host plants, and cannot be affected by virus elimination therapy. Likewise, mastreviruses have been reported to cause yield losses in maize and chickpeas (Martin & Shepherd, 2009; Schwinghamer et al., 2010; Shepherd et al., 2010) but their effect on sweet potato is not yet elucidated. Cao et al. (2017) pointed out the necessity of understanding the significance of SPSMV-1 on sweet potato production.

3.2.4 Distribution and coinfection of sweet potato viruses in the five counties surveyed

Our results show that, altogether, 15 sweet potato virus species were identified from the sweet potato samples tested in this study and were distributed in all the three states surveyed. SPFMV and SPCSV were detected in 4 counties except Morobo County (Table 1, Paper II). SPVC was detected in 4 counties except Wau (Table 1, Paper III). Except SPLCV, it is notable that the sweepoviruses were most prevalent in samples from Wau County (Table 1 Paper III). Moreover, SPGVV was also detected in Magwi County whereas IYVV in Lainya and Morobo counties. SPCV was identified in Magwi County. SPBV-A, SPBV-B and SPMV-1 were detected in samples from all the surveyed counties. These results indicate a high diversity of viruses infecting sweet potato in South Sudan.

Co-infection rate was very high in the sequenced samples. Five different combinations were observed which ranged from 5 to 10 species of viruses detected in a single plant (Figure 3 and Table 1, Paper III). However, in sweet potato leaf samples collected from farmers' fields co-

infection with SPFMV and SPCSV that cause the severe SPVD were detected in all the counties except Morobo County. The identification of SPVD and its distribution in the four counties of South Sudan supports previous reports that SPVD is the most serious disease in East Africa (Mukasa et al., 2003; Opiyo et al., 2010a; Tairo et al., 2004). The absence of SPVD in all samples collected from Morobo County is an advantage. This may explain that farmers were likely to be able to select virus-free vine cuttings. Moreover, this may not indicate that Morobo County is free from SPVD since only few samples were tested.

The high rate of mixed infections observed in this study highlight the need to investigate the prevalence of sweet potato infecting viruses in all sweet potato growing areas in South Sudan and set strategies to prevent their spread. Currently, most farmers have no supply of virus-free planting material but select asymptomatic planting material from their previous crop and share, or sell it, without applying any phytosanitary practices. The provision of resistant varieties and phytosanitation services to farmers should be a priority. Control of whitefly and aphid vectors that transmit SPCSV and SPFMV respectively, is vital in the fight against the spread of SPVD. Gibson et al. (2004) reported that the deployment of resistant varieties accompanied with phytosanitation practices is an effective control strategy for SPVD for small-scale farmers.

3.3 Genetic variability of *Bemisia tabaci* (Gennadius) (Paper IV)

Bemisia tabaci (Gennadius) is a polyphagous, highly destructive pest capable of vectoring viruses in most agricultural crops (Gilbertson et al., 2015; Jones, 2003; Navas-Castillo et al., 2011; Polston et al., 2014; Verbeek et al., 2014). In sweet potato it transmits begomoviruses, the crinivirus, SPCSV and the impomovirus, SPMMV (Dombrovsky et al., 2014; Gamarra et al., 2010; Hassan et al., 2016; Ling et al., 2011; Simmons et al., 2009; Valverde et al., 2004a).

In this study we investigated the genetic variability in 162 *Bemisia tabaci* individuals collected from four host plants: sweet potato (*Ipomeoa batata* L.), cassava (*Manihot esculenta* Crantz), tomato (*Solanum lycopersicum* L.) and squash (*Cucurbita pepo* L. 'Zucchini') from 10 locations in Juba County, Central Equatoria State, South Sudan (Table 1and figure 1, Paper IV). Phylogenetic analysis using mitochondrial DNA cytochrome oxidase I (mtCOI) revealed a high level of variability among the *B. tabaci* populations which defined six distinct groups: three non-cassava haplotype groups obtained from sequences from sweet potato, tomato and squash, and three cassava haplotype groups obtained from sequences from cassava (Figure 2,

Paper IV). A total of 45 selected sequences representing haplogroups found in this study have been submitted to GenBank under the following accession names (MN318379 - MN318423).

The three phylogenetically distinct groups of the non-cassava haplotypes included Mediterranean (MED), Indian Ocean (IO) and Uganda (Figure 2, Paper IV). MED was the predominant haplotype found in all locations and constituted 90 whiteflies (55.5%) of all the whiteflies collected. Of these 72 whiteflies (44.4%) were found on sweet potato. The haplotypes Uganda and IO had 11 whiteflies (6.8%) and 4 whiteflies (2.5%) respectively (Table 3, Paper IV). Haplotype analysis of *B. tabaci* MED revealed six haplotypes among the samples collected (Table 4, Paper IV). Two of these are previously described African MED haplotypes, while the other four are new unique haplotypes falling within the MED group. Haplotype diversity (0.51), nucleotide diversity (0.012) and a positive significant Tajima's D (2.07283: $P < 0.05$) suggest that the population is undergoing balancing selection and has not undergone rapid recent expansion. The analysis of IO and Uganda haplotypes identified two haplotypes for IO and one for Uganda (Table 4, Paper IV). The clustering of the MED, IO and Uganda into distinct groups separate from the cassava colonizing clade is consistent with previous findings in Sseruwagi et al. (2005) and, Tocko-Marabena et al. (2017).

The haplotype MED was found as the predominant haplotype on sweet potato, tomato and squash distributed in all locations suggesting that this haplotype is an important pest of sweet potato and other crops in Juba County and other parts of South Sudan. *B. tabaci* MED is an important haplotype group worldwide which is thought to have originated from Africa. It has been reported to be extremely polyphagous and invasive infesting both field and greenhouse crops (De Barro et al., 2011; Horowitz et al., 2003) including numerous crops and weed hosts (Gnankine et al., 2013; Romba et al., 2018; Sseruwagi et al., 2005). Under intensive production systems, MED has developed resistance to various insecticides (Horowitz & Ishaaya, 2014; Roditakis et al., 2009; Wang et al., 2010). Moreover, SPCSV, one of the most important viruses infecting sweet potato in East Africa (Mukasa et al., 2006) is transmitted by *B. tabaci* (Gamarra et al., 2010). It is likely that this is the main vector of this virus in South Sudan. However, future investigations should ascertain the relative abilities to transmit SPCSV of each of the three *B. tabaci* haplotypes occurring on sweet potato since no similar studies have been conducted anywhere else in sub-Saharan Africa. This represents an important gap in the existing understanding of the relationship between *B. tabaci* haplotype groups and the viruses that they vector.

In this study, haplotype Uganda, was clearly defined as a distinct monophyletic group in our mtCOI sequence analysis. Haplotype Uganda has previously been identified as a genetically distinct haplotype occurring in East Africa (Hadjistylli et al., 2016; Legg et al., 2002). Our sequences of the Uganda haplotype were identical to the original Uganda haplotype sequence also obtained from a whitefly adult collected from sweet potato in Uganda (33NamSP-AY057174) (Legg et al., 2002). However, its occurrence on other crops other than sweet potato in Uganda and on common beans in Kenya has been reported (Sseruwagi et al., 2005; Wainaina, 2019) suggesting that this haplotype is confined to East Africa with a relatively narrow host range. Our results represent the northernmost record of haplotype 'Uganda' and the third country report.

The sequences from cassava were grouped into three cassava haplotypes SSA1-SG1, SSA1-SG3 and SSA2. SSA2 was the second most abundant haplotype with 43 whiteflies (26.5%), all of them were found on cassava distributed in all locations. SSA1-SG1 also found on cassava had 13 whiteflies (8%) and was identified only at two locations. SSA1-SG3 was the least abundant whitefly haplotype with only one whitefly (0.6%) and was found on sweet potato in only one location (Figure 2 and Table 3 Paper IV). Haplotype analysis identified two SSA2 haplotypes (Table 4). Haplotype diversity (0.509), nucleotide diversity (0.00205) and a positive significant Tajima's D (257824: $P < 0.05$) suggest that the population has not undergone recent expansion but is instead experiencing balancing selection. These results concur with previous studies from countries growing cassava in Africa where haplotypes from cassava clustered into a distinct clade separate from non-cassava colonizing *B. tabaci* (Sseruwagi et al., 2005; Tocko-Marabena et al., 2017; Wosula et al., 2017).

We found that SSA2 is the predominant haplotype in the cassava group while SSA1-SG1 is less frequent. These results contrast with recent findings from East Africa where SSA1-SG1 was shown to be the predominant *B. tabaci* haplotype on cassava (Legg et al., 2014; Tocko-Marabena et al., 2017; Wosula et al., 2017). SSA2 was previously associated with the cassava-CMD pandemic spread in Uganda (Legg et al., 2002). However, recent reports show that SSA2 has been absent in whitefly collections from cassava in Uganda and western Kenya (Mugerwa et al., 2012; Sseruwagi et al., 2006), and replaced by SSA1-SG1 (Legg et al., 2014), even though this haplotype prevailed in low frequencies in Uganda and Kenya between 2004 and 2010. Moreover, the presence of SSA2 has been recently noted on cassava in western Kenya and weedy hosts in Uganda (Chen et al., 2019; Manani et al., 2017; Mugerwa et al., 2018).

Furthermore, a recent continent-wide assessment of cassava-colonizing *B. tabaci* in sub-Saharan Africa found that SSA2 was the most widely distributed of the haplotypes recorded (Chen et al., 2019). It simultaneously occurs with others throughout its geographic range but in all cases, it appears to be less frequent than SSA1 haplotypes. Our data suggest that South Sudan could be an exception to this pattern, since there were more than three times as many SSA2 individuals recorded compared to those of SSA1-SG1.

Dafalla and Ahmed (2005) reported that in the 1990s, CMD caused high damage to cassava in Western Equatoria Province of pre-independence southern Sudan. Furthermore, the viruses African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), and East African cassava mosaic virus-Uganda (EACMV-UG) were reported to infect cassava in Eastern and Western Equatoria states (Ntawuruhunga et al., 2007). Although SSA2 has been displaced by SSA1-SG1 in Uganda, the predominance of SSA2 in our sequences show that SSA2 is the main cassava-colonizing *B. tabaci* haplotype in present day South Sudan. This indicates that the change in Uganda may not have happened further north in South Sudan. The explanation to this extreme difference in change of population of SSA2 in Uganda and South Sudan are currently not clear but would be a useful topic for future study.

Although *B. tabaci* whiteflies were sampled from a relatively small portion of South Sudan, 6 haplotype groups were discovered. This suggests that, like Uganda, this part of East Africa has a high level of whitefly diversity. This provides a strong indication that this part of Africa may have been a source for MED whiteflies that have had devastating global impacts as an invasive pest (Ramos et al., 2018).

4. Conclusions

- Based on the farm households baseline survey, farmers were aware of the damage caused by pests and diseases on sweet potato but had limited knowledge on how to identify diseases affecting sweet potato. Insect pests were perceived to be more damaging and sweet potato weevil was considered the most serious insect pest. The majority of farmers did not use any control measures for pests and diseases on sweet potato. The most important production constraints facing sweet potato farmers, in order of importance were: lack of extension services, lack of improved varieties, low sale prices of sweet potato, lack of credit services, price fluctuations, field pests, drought and diseases.

- The current study is the first to assess the identity, incidence and distribution of viruses and virus diseases that infect sweet potato in South Sudan. Altogether 15 viruses were detected including SPCSV, SPFMV, SPVC, SPLCV, IYVV, SPMaV, SPLCSPV, SPGVV, SPLCGV, SPLCUV, SPCV, SPSMV-1, and SPBV-A, SPBV-B, and SPBV-C (collectively known as SPPV) in the assayed sweet potato samples from all the three states surveyed. Thirteen of these viruses were detected by SRDS, which supports the effectiveness of this method as useful for simultaneous detection of viruses in sweet potato infected with multiple viruses without the need to use virus-specific primers or antibodies.

- The most important viruses of sweet potato, SPFMV and SPCSV and their co-infections were identified in four of the five counties surveyed confirming their prevalence in South Sudan. This supports previous reports that SPFMV and SPCSV are the most commonly occurring and the most important viruses of sweet potato in East Africa.

- Seven begomoviruses, SPLCV, IYVV, SPMaV, SPLCSPV, SPGVV, SPLCGV and SPLCUV were identified. SPLCV was the most distributed detected in samples from all locations.

- Six *B. tabaci* haplotype groups, including three non-cassava groups (MED, Indian Ocean and Uganda) and three cassava groups (SSA1-SG1, SSA1-SG3 and SSA2) were identified. MED and SSA2 were the predominant haplotypes and most widely

distributed amongst the sampled locations. The Uganda haplotype is also widespread and was identified from five of the sampled locations.

- The MED species group of *B. tabaci* includes some of the most insecticide-resistant populations of whiteflies. Therefore, although *Bemisia* whiteflies may not be present on sweet potato and other host plants at high abundance levels, any future management efforts will need to apply extreme caution in the application of chemical insecticides in order to preclude the development of whitefly resistance.

5. Future perspectives

- Delivery of extension services and training to farmers is paramount for the acquisition of better knowledge on the crop, its pests and diseases and their management. Support from government and other finance and development agencies to strengthen the economic status of farmers by providing credit loans is crucial. Vibrant markets for sweet potato products can greatly encourage farmers to increase production.

- The 13 viruses detected by SRDS were not confirmed by RT-PCR of PCR. However, the coverage of reference genomes by sRNA virus contigs were significant (above 80%). This suggests that additional tests to verify the occurrence of these viruses in the tested cultivars may not be necessary. However, to gain an understanding of the prevalence of these viruses in sweet potato in South Sudan, it is essential to conduct comprehensive surveys in all sweet potato growing areas.

- In this study ELISA, RT-PCR and SRDS were used to identify viruses infecting sweet potato. Thirteen of the viruses were simultaneously identified by SRDS indicating that the detection of viruses using only the conventional methods is not enough to explain the actual causal agents of viral diseases, especially in the case of South Sudan where no studies on viral diseases has ever been conducted before. Hence, a comprehensive study on the occurrence and distribution of sweet potato viruses in South Sudan might need a combination of methods to identify and characterize the different causal agents of viral diseases to allow accurate formulation of control strategies. Moreover, the current war situation in South Sudan could not allow effective use of molecular methods for virus detection and identification in the country. These techniques are expensive to implement, require sophisticated tools, extreme controlled conditions and qualified expertise. Therefore, one could recommend ELISA and PCR methods currently for South Sudan since they are easy to establish and robust in their use, even with new and inexperienced users.

- The prevalence of SPFMV and SPCSV in all of the surveyed states is significant. Future research strategies should prioritize the control of these two viruses since their synergy results in SPVD, the single most important disease of sweet potato worldwide. Currently, sweet potato farmers in South Sudan have no source of virus-free planting

material but select asymptomatic cuttings from their previous crop. The provision of resistant varieties and phytosanitation services to farmers should be a priority.

- Whitefly populations observed in South Sudan were associated with transmission of viruses causing damaging diseases in cassava and sweet potato. Improving the understanding of the dynamic interactions between vector and virus will be important for each of these crop-virus-vector pathosystems. An essential first step in this task will be to conduct a comprehensive assessment of the genetic diversity, geographical distribution, population dynamics and host range of *B. tabaci* species in South Sudan. This new knowledge will then provide the basis for the development of effective whitefly management strategies.

- Due to the inevitable limitations imposed by civil war in the country, this research could not cover all sweet potato producing areas of South Sudan. As such the number of sweet potato samples used in this study were only representative. Furthermore, the collection of whiteflies (*Bemisia tabaci*) vectors of viruses was confined to only one geographical location, Juba County in Central Equatoria state. Future research studies should conduct a comprehensive survey in all sweet potato producing areas of South Sudan to elucidate the identity, incidence, distribution and effect of viruses on sweet potato farming in South Sudan. Surveys of whitefly virus vectors in all agricultural regions of South Sudan is essential for better management of whiteflies in sweet potato as well as alternative crops.

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Errata list

PhD candidate: Beatrice Clarence Misaka Langwa

Thesis: **Sweet Potato Production in South Sudan: Current status, virus infections and whitefly vector diversity**

Date: 28/10/2019

Side	Line	Original text	Corrected text
1. Introduction			
1.4.1 SPFMV	194	Sweet potato virus C	*Sweet potato virus c*
1.5 Sweet potato virus disease complxes	251	sweet potato mild speckling virus	*Sweet potato mild speckling virus*
1.6.1 Biological indexing	284	titeres	titres
1.6.2 Electron microscopy	291	detecetion	detection
3. Thesis: Main results and discussions			
3. Thesis: Main results and discussions	549	**3. Thesis: Main results and discussion**	**3. Thesis: Main results and discussions**
3.1.1 Importance of sweet potato to poor and small-scale farmers in South Sudan	580	Table 2. Response of interviewed farm households to the importance of sweet potato in their farming systems	Table 2. Response of interviewed farm households to the importance of sweet potato in their farming systems.
		Table 3.	The position of table 3 in the text is changed (moved to next page).
3.1.2 Sweet potato production constraints	649	rains	of rains

	655	Tanzania of (Kivuva et al., 2014;	Tanzania (Kivuva et al., 2014;
Paper I, II, III and IV		No page numbers.	Page numbers inserted.
Paper I	Figure 1	Figure 1. Map of South Sudan study locations in five counties in three states: Western Bahr el Ghazal, Eastern Equatoria and Central Equatoria.	Figure 1. Map of South Sudan showing study locations in five counties in three states: Western Bahr el Ghazal, Eastern Equatoria and Central Equatoria.
Paper III			
2.1 Plant material		Marshello-Dragga	Marchelo-d'Ragga
3.2 Detection of sweet potato viruses by SRDS		Marshello-Dragga	Marchelo-d'Ragga
3.4 Distribution and coinfection of sweet potato viruses in the five locations studied	Figure 3		The position of figure 3 in the text is changed (moved to previous page).
Paper IV	Reference	Cuellar, W. J., Galvez, M., Fuentes, S., Tugume, J. & Kreuze, J. (2015). Synergistic interactions of be-gomoviruses with Sweet potato chlorotic stunt virus (genus C rini-virus) in sweet potato (I pomoea batatas L.). Molecular Plant Pa-thology, 16 (5): 459-471.	Cuellar, W. J., Galvez, M., Fuentes, S., Tugume, J. & Kreuze, J. (2015). Synergistic interactions of begomoviruses with *Sweet potato chlorotic stunt virus* (genus *Crinivirus*) in sweet potato (Ipomoea batatas L.). Molecular Plant Pathology, 16 (5): 459-471.

Appendix

PAPER I-IV

PAPER I

Farmers' perceptions of pests, diseases and production constraints affecting sweet potato in South Sudan

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Abstract

Sweet potato is an important staple food crop for subsistence farmers in South Sudan. Its production has remained unexploited due to decades of war and limited access to production technologies. The aim of this study was to identify farmers' knowledge and perceptions of sweet potato varieties, varietal attributes, pests, diseases, plant health management and production constraints. A baseline survey was conducted in various regions: Western Bahr el Ghazal, Central Equatoria and Eastern Equatoria States in South Sudan August - September 2015. Using a structured questionnaire, 180 farm households were interviewed in five counties. The key production constraints reported by farmers were lack of extension services (55%), lack of improved varieties (48.9%), low prices (43.9%), lack of credit services (43.3%), price fluctuations (42.8%), field pests (41.7%), drought (40.6%) and diseases (38.3%). Farmers were conscious of pests and diseases affecting sweet potato but the majority (64.3%) could not identify specific diseases. On average, 42.7% of the farmers perceived sweet potato weevils (*Cylas spp*.) as the most serious pests, and 60.2% took no action to control pests or diseases. Preferred varietal attributes were high yield (98.9%), early maturity (97.8%), good taste (97.2%), white flesh (86.1%), long storage (63.3%), drought tolerance (62.8%), disease resistance (62.8%), and pest resistance (56.1%). Tackling these production constraints is essential to improve sweet potato production in South Sudan. Nutritional security would be enhanced through the introduction of orange-fleshed sweet potato varieties into sweet potato production areas, whilst enlightening farmers on its value as a natural source of vitamin A.

Key words: *Ipomoea batatas,* pests, diseases, production constraints

1 Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.), family Convolvulaceae, is a tuberous root crop, which has its primary centre of origin and diversity in Central America (Zhang et al. 2000). North-western South America and parts of Central America are the centres of the highest diversity of sweet potato (Huang and Sun 2000). It is widely distributed in all tropical and sub-tropical areas of the world and is the third most important root crop globally after potato and cassava. The total production is about 112.8 million t/year on about 9.2 million ha (FAOSTAT 2017).

Sweet potato is an important staple food crop in many developing countries and has a significant role in assuring food security and generating household incomes in eastern and southern African countries (Abidin 2004; Gibson et al. 2009; Low et al. 2009; Miyazki et al. 2013; Mwanga and Ssemakula 2011). In South Sudan, it is a main staple food crop for many communities, especially in the Greater Equatoria and Western Bahr el Ghazal States, and provides an important source of income and food security. It was reported as the crop that sustained six thousand South Sudanese internally displaced people who had taken shelter in a church near South Sudan's border with Congo (Jeffrey 2017). The crop is consumed in high quantities both in the rural and urban communities and large quantities are imported from neighbouring Uganda.

Several abiotic and biotic factors constrain sweet potato production in sub-Saharan Africa. The abiotic factors include limited access to improved varieties and planting material, poor post-harvest handling technology, and weak socio-economic and policy structures (Fuglie 2007; Kapinga and Carey 2003; Ngailo et al. 2016). The most important biotic constraints are sweet potato weevils and sweet potato virus disease (SPVD) (Mukasa et al. 2003; Ndunguru et al. 2009; Ngailo et al. 2016; Shonga et al. 2013). Very little documentation on the production and production constraints of sweet potato is available for South Sudan due to underdevelopment of agriculture. Agricultural production has remained largely unexploited in South Sudan which can partly be explained by decades of war, little effort on crop research, and limited access to technological advances in crop production (AfDB 2013; Kaka and Oyik 2008). Underdevelopment of agriculture in South Sudan is also a result of several challenges which include lack of investment, low productivity, an insecure land tenure system, inadequate support services, weak infrastructure and lack of extension services (AfDB 2013; Chokerah and Horvath 2012). However, these challenges have been exacerbated by insecurity that has

led directly to severe food insecurity (IOM 2013). In view of the above challenges, very little research has been undertaken into how to improve sweet potato production, storage, processing, marketing, and to identify production constraints. The study reported here aimed to provide a first step in this research process through a baseline survey. This was designed to improve understanding of the status of sweet potato production by subsistence farmers and identify research gaps where interventions are needed. The objectives of the farm household survey were to identify farmers' knowledge and perceptions of: (1) sweet potato varieties; (2) perceived varietal attributes; (3) pests and diseases of sweet potato and their management; and (4) production constraints.

2 Materials and methods

2.1 Study area

The study was carried out in three States: Western Bahr el Ghazal, Central Equatoria and Eastern Equatoria in August and September 2015. Locations were chosen based on the importance of sweet potato production and consumption in these areas. In total five counties were surveyed (Figure 1). Information on geographical coordinates, elevation, rainfall, cropping seasons and soil characteristics of the surveyed counties are presented in Table 1.

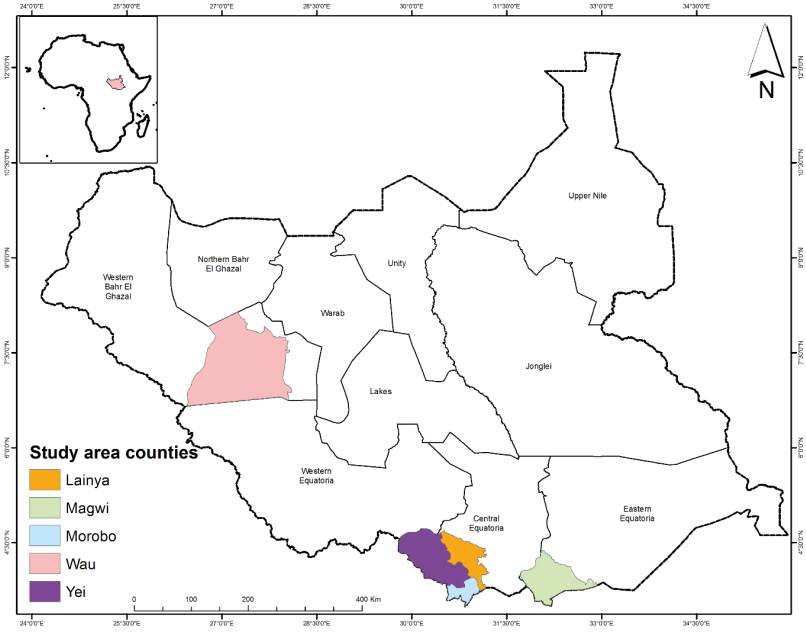

Figure 1. Map of South Sudan showing study locations in five counties in three states: Western Bahr el Ghazal, Eastern Equatoria and Central Equatoria.

Table 1. Geographical coordinates, elevation, rainfall, cropping seasons and soil characteristics of the five surveyed counties.

Descriptors	State				
	Western Bahr el Ghazal	Eastern Equatoria	Central Equatoria		
	County				
	Wau	Magwi	Lainya	Yei	Morobo
Longitude(°E)	27.75 – 7.84	32.08 - 32.99	30.7 – 30.87	30.61–30.67	30.78 – 30.91
Latitude(°N)	7.59 – 7.66	3.6 – 4.21	4.15 – 4.27	3.59 – 4.09	3.7 – 3.76
Elevation (masl)	507 - 532	647 - 918	872 - 889	838 – 895	1025 - 1191
Rainfall (mm per annum)	1100 – 1300 (unimodal) [2]	1100 - 1300mm (bimodal) [1, 2]	1100-1500 (bimodal) [2]	1100-1500 (bimodal) [2]	Average 1552 (bimodal) [1, 2]
Cropping season(s)	One: April to November [2]	Two: April/May to July and September/October to December [1, 2]	Two: March to June and July to November [2]	Two: March to June and July to November [2]	Two: March/April to July and August to October/November [1]
Soil characteristics	Plinthic ferrasols with sandy clay texture [2, 3]	Plinthic ferrasols with heavy and loamy clay Soil texture [1, 3]	loamy clay and sandy soils [2]	loamy clay and sandy clay soils [2]	Eurtric nitosols with clay loam soil texture [1, 3]

[1](Hoffmann et al. 2012)
[2] (FEWS-NET 2013)
[3] (Odero 2008)

2.2 Data collection

Using a structured questionnaire, 180 households were interviewed, with 60 households per State. The number of bomas (villages) selected in each County differed. In Wau County, two bomas (Ngisa and Ngoalema); in Magwi County, six bomas (Abara, Agora, Magwi, Omeo, Matara and Bilinya); in Lainya County, one boma (Limbe); in Yei County, two bomas (Yei and Mongo); and in Morobo County, three bomas (Kendila, Girili and Kindi) (Table 2). Prior to interviewing households in each County, a one-day training workshop was conducted to train enumerators who were to be involved in administering questionnaires and interviewing farm households. This was to help the enumerators to understand the content of the questionnaires and clarify any questions. Due to the diverse ethnicity, enumerators were selected who knew the language of the households in surveyed communities. The following data were collected:

Table 2. Geographical coordinates of bomas (villages) selected in each county surveyed relative to South Sudan and its states.

State	County	Payam	Boma (Village)	Longitude (^{0}E)	Latitude (^{0}N)	Elevatiom (masl)
Western Bahr el Ghazal	Wau	Ngisa	Ngisa	27^0 45.082´	07^0 34.578´	516
	Wau	Ngisa	Ngoalema	27^0 50.360´	07^0 39.471´	532
Eastern Equatoria	Magwi	Iwire	Abara	$32^0$10.160´	$04^0$05.169´	816
	Magwi	Magwi	Agora	32^0 12.493	04^0 05.208´	854
	Magwi	Magwi	Magwi	32^0 20.203´	04^0 08.842´	911
	Magwi	Magwi	Omea	32^0 14.469´	04^0 12.560´	875
	Magwi	Nimule	Matara	32^0 05.109´	03^0 35.774´	665
	Magwi	Mogali	Bilinya	32^0 08.683´	03^0 37.707´	658
Central Equatoria	Lanya	Kenyi	Limbe	30^0 51.745´	04^0 09.630´	889
	Yei	Otogo	Mongo	30^0 37.047´	03^0 55.690´	888
	Yei	Yei	Yei	30^0 39.969´	04^0 04.982´	838
	Morobo	Gulumbi	Kendila	30^0 51.796´	03^0 44.785´	1046
	Morobo	Gulumbi	Girili	30^0 48.429´	03^0 43.529´	1180
	Morobo	Gulumbi	Kindi	30^0 46.635´	03^0 41.751´	1191

(i) Socio-demographics of the farm households. This included gender, age, type of household, level of education, type of farming, and land ownership.

(ii) Sweet potato varieties grown and preferred varietal attributes. Farm households were asked to list all the sweet potato varieties they grow, the most preferred varieties and varietal attributes.

(iii) Farmers' knowledge and perceptions of pests and diseases of sweet potato and their control methods. To assess farmers' knowledge on insect pests and diseases, farmers were asked if they experience pest and disease attack on their sweet potato crop, and which part of the crop was most damaged (Table 3). This was supplemented by showing farm households pictures of sweet potato common pest and disease symptoms on leaves and roots. To assess farm households' knowledge on pest and disease control methods, farmers were given options and their selections were ticked appropriately. The options included (1) spray with chemicals; (2) use traditional pesticides; (3) do crop rotation; (4) intercrop; (5) plant resistant varieties (6) plant early; and (7) don't use any method of control.

(iv) Sweet potato production constraints. Farm households were asked to mention the constraints in sweet potato production and rate them in order of importance. A three-point scale was used (Table 3).

Table 3. Criteria followed for scoring farmers' knowledge of pests and diseases and production constraints.

Pests and Diseases			Production constraints		
Score	Knowledge level	Criteria	Score	Extent of problem	Criteria (tick as appropriate)
1	Yes	Farmer identified one pest and type of damage caused	1	severe	Farmer sees the problem as very important to him/her and serious.
2	No	Farmer could not identify any pest or diseases or the damaged caused	2	moderate	Farmer sees the problem but not so important.
			3	low	Farmer sees the problem as minor

2.3 Data analysis

The Statistical Package for Social Sciences (IBM SPSS Statistics 23) was used to analyse the collected data. The analysis included descriptive statistics and cross tabulations. Results are presented as percentages of respondents of interviewed farm households.

3 Results

3.1 Sociodemographic information

Overall 77.8% of the households interviewed were males and 85% of the households were male-headed (Figure 2a and b). Generally, the majority of the respondents were in the active age groups of, 19-30 and 31-40 years of age, 30.6 % and 43.9% respectively (Figure 2c). Most of the respondents (58.8%) received primary education (Figure 2d). In all, 63.9 % of the respondents practiced small-scale farming (Figure 2e) and more than half (57.2%) had customary lands while 36% had their own title (Figure 2f).

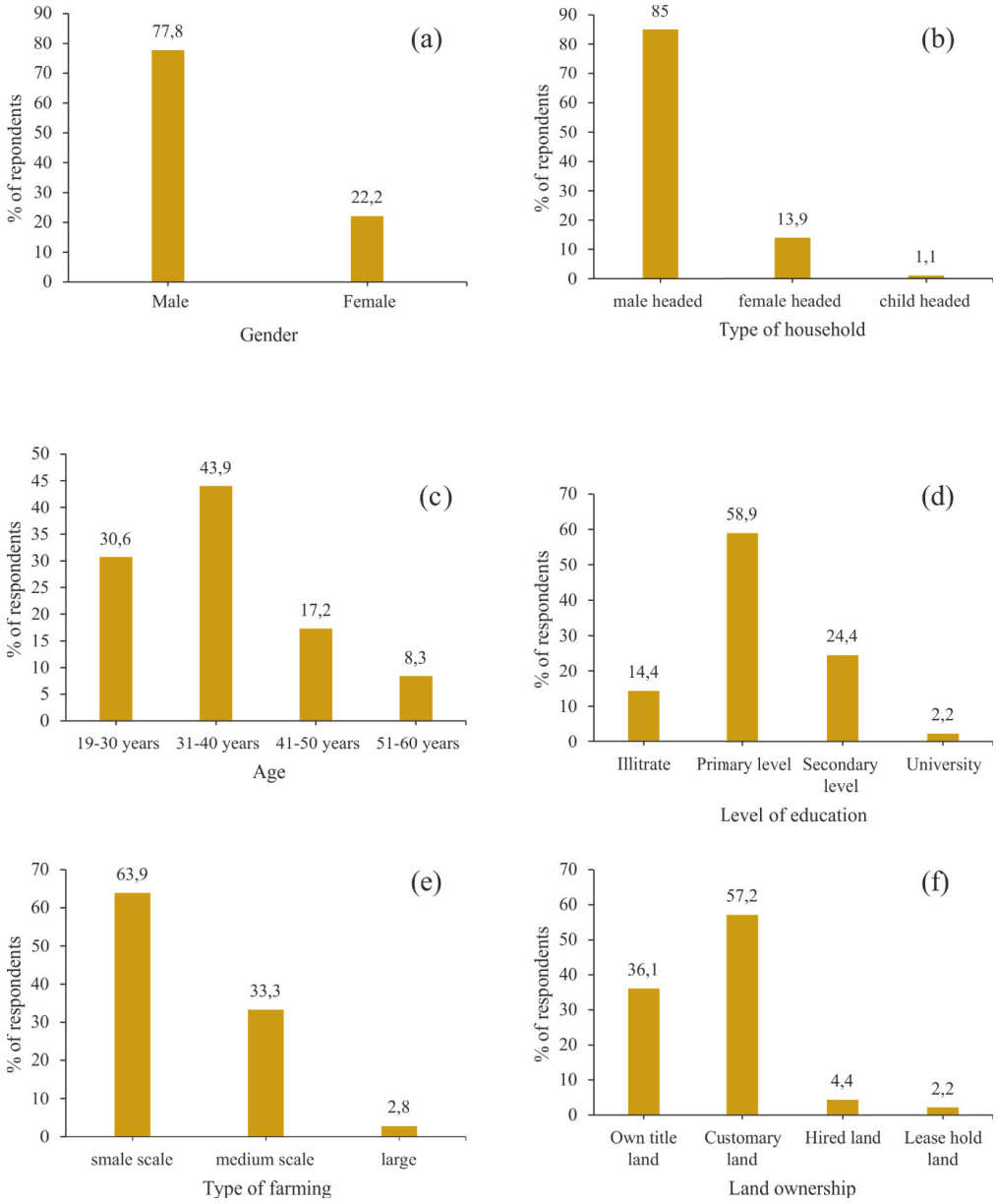

Figure 2. Sociodemographic information of respondents. (a) Gender, (b) Type of household, (c) Age, (d) Level of education, (e) Type of farming, (f) Land ownership.

3.2 Sweet potato production constraints

Generally, a number of constraints were reported by the interviewed households the most important of which were the lack of extension services (55%), lack of improved varieties

(48.9%), low prices (43.9%), lack of credit services (43,3%), price fluctuation (42.8%), field pests (41.7%) and drought (40.6%) (Table 4). Storage pests and access to vines (planting material) were other important constraints in Central Equatoria and Western Bahr el Ghazal States.

Table 4. Constraints to sweet potato production identified by farmers in the surveyed states (percentage of respondents).

No.	Constraints	Western Bahr el Ghazal	Central Equatoria	Eastern Equatoria	Overall	χ^2
1	Lack of extension services	40	75	50	55	17.40*
2	Lack of improved varieties	46.7	60	40	48.9	8.07
3	Low price	40	50	40	43.9	7.17
4	Credit services	38.3	61.7	30	43.3	15.55*
5	Price fluctuation	43.3	41.7	43.3	42.8	2.54
6	Field pests	48.3	38.3	38.3	41.7	2.79
7	Drought	33.3	41.7	46.7	40.6	5.32
8	Diseases	36.7	38.3	40	38.3	0.16
9	Store pests	43.3	53.3	16.7	37.8	18.49*
10	Lack of market	26.7	41.7	35	34.4	7.23
11	Access to vines (planting material)	31.7	51.7	15	32.8	20.99*
12	Weeds	30	30	25	28.3	22.23*
13	Poor soil	20	6.7	18.3	15	5.74
14	Land shortage	16.7	20	3.3	13.3	9.43
15	Poor yield	3.3	15	15	11.1	7.05

* Values are statistically different at $P \leq 0.05$

3.3 Farm households' knowledge on pests and diseases of sweet potato and their control

Overall, 95% of respondents were aware of damage caused by pest and diseases on sweet potato (Figure 3a). Sweet potato weevil (*Cylas spp*.) damage was recognized by 42.7% of the respondents. 32.7% of the farmers were aware of the presence of whiteflies (*Bemisia tabaci* (Gennadius)) in their fields but did not consider whiteflies as a pest of sweet potato or relate them to any damage on their crop (Figure 3b). In addition to butterflies (*Acraea acerata* Hewitson), and aphids (*Aphis gossypii* Glover), other pests reported were millipedes and ants. Overall, 64.3% of respondents were not able to identify the diseases affecting sweet potato (Figure 3c). Across States, most of the farm households (60.2%) did not use any method of

pest and disease control on their sweet potato crop. However, 12.9% practiced early planting, 11.7% used traditional pesticides (ash spray) and 11.1% practiced crop rotation (Figure 3d). The smallest percentage of the respondents (1.2% and 2.9%), many of whom had returned from exile in Uganda, indicated resistant varieties and use of pesticides as a means of pest and disease control respectively.

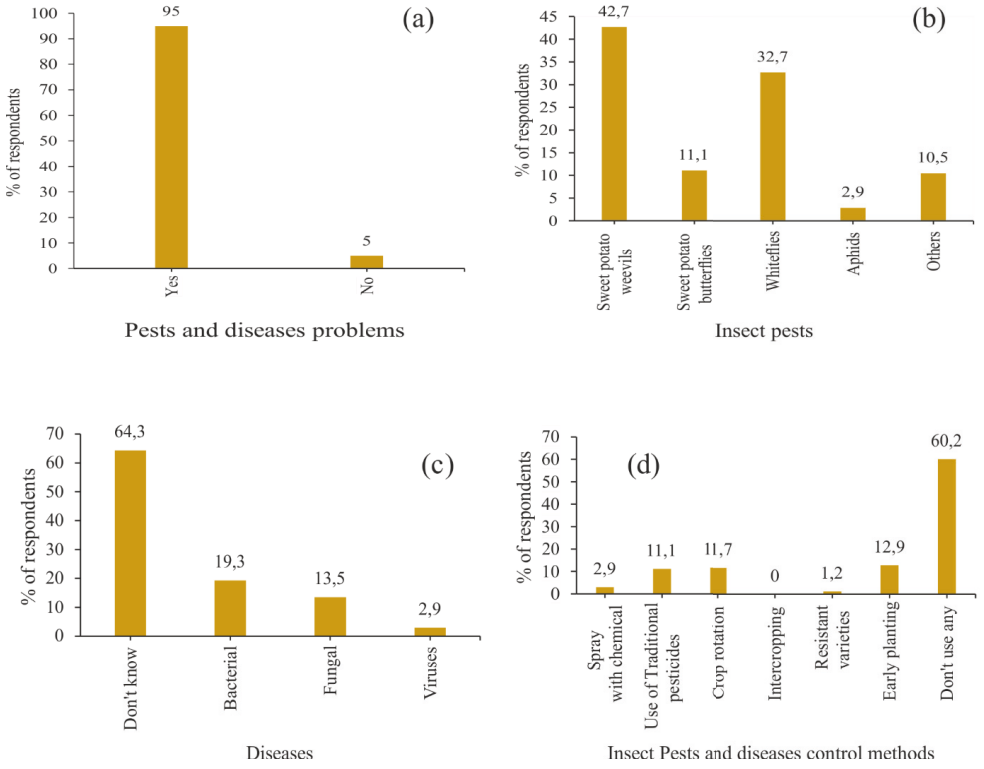

Figure 3. Farmers' knowledge of pests and diseases of sweet potato. (a) Pests and diseases problems, (b) Insect pests, (c) Diseases, (d) Insect pests and diseases control methods

3.4 Sweet potato varieties grown by farmers and their preferred varietal attributes

Several sweet potato varieties were being grown by farmers in each County surveyed although each County had its preferred variety(ies) which was grown by almost all farmers (Table 5). Most of the varieties were white-fleshed, however, yellow-fleshed varieties were also found in each of the surveyed counties and only one orange fleshed variety. The varieties Mavede (Wau County), Lupandura (Lainya County), Apana Lipa (Yei and Morobo counties), and

Table 5. Sweet potato varieties grown by farmers in each state and county surveyed.

State	County	Sweet potato variety	Flesh color	Most preferred variety(ies)	Total
Western Bahr el Ghazal	Wau	Mavede	white	Mevede	7
		Yankar-2	Purple		
		Yankar-1	white		
		Vayunduka	white		
		Mayenduro	white		
		Mviro	yellow		
		Kolingwa	white		
Central Equatoria	Lainya	Lupandura	white	Lupandura	4
		Kajamingi	cream		
		Senja Moko-1	yellow		
		Gweregwere	cream		
	Yei	Meridi Meridi	white	ApanaLipa	9
		Senja moko-2	white		
		Iribu	white		
		Manya Manya	white		
		Kedi Kedi	yellow		
		Bakaya			
		Mali Mali	white		
		Karamojo-2	orange		
		Apana Lipa	white		
	Morobo	Singa na kilo	white	Apana Lipa	3
		Apana Lipa	white		
		Karamojo-1	yellow		
Eastern Equatoria	Magwi	Lachan Mati Pii	white	Lachan Mati Pii	9
		Layelo	yellow	Goli	
		Ladwe Achel	cream		
		Lobel	white		
		Jogo	white		
		Koda	white		
		Goli	white		
		Nilo	white		
		Jivi	purple		

Lachan Mati Pii and Goli (Magwi County) were grown by most of the farmers. Wau, Lainya, Yei, Morobo and Magwi counties each had one yellow-fleshed variety (Mviro, Senja Moko-1, Kedi Kedi, Karamojo-1 and Layelo), respectively. The varieties, Yankar-2 and Givi from Wau and Magwi counties respectively, had a purple flesh color. The orange fleshed variety (Karamojo-2) was found in Yei County. Table 6 presents varietal attributes preferred by farmers. Overall, preferred varietal attributes included: high yield (98.9%), early maturity (97.8%), good taste (97.2%), white flesh (86.1%), long storage period in the soil (63.3%), drought tolerance (62.8%), disease resistance (62.8%) and pest resistance (56.1%). Yellow-fleshed sweet potatoes were not preferred by most of the farm households across States. However, 36.7% of farmers in Central Equatoria and 40% in Eastern Equatoria consumed yellow-fleshed sweet potatoes.

Table 6. Farmers' perception on preferred varietal attributes of sweet potato (percentage of respondents).

Attribute	Western Bahr el Ghazal	Central Equatoria	Eastern Equatoria	Overall mean	χ^2
Good yield	96.7	100	100	98.9	4.05
Early maturity	95	98.3	100	97.8	3.58
Good eating quality (taste)	98.3	93.3	100	97.2	5.35
White fleshed	96.7	78.3	83.3	86.1	9.01*
Longer storage period	78.3	58.3	53.3	63.3	9.04*
Drought tolerant	81.7	55	51.7	62.8	13.89*
Disease resistance	80	60	48.3	62.8	13.17*
Pest resistance	78.3	45	45	56.1	18.05*
Yellow fleshed	16.7	36.7	40	31.1	8.92*

* Values are statistically different at $P \leq 0.05$.

4 Discussion

4.1 Socio-demographic information

South Sudan has passed through several years of insecurity following the country's independence in 2011. This has made it difficult to conduct agricultural research work, although the needs for increased food production are arguably greater than anywhere else on the African continent. In 2015, there was a window of opportunity to conduct a survey of farmers' perceptions on sweet potato production. This made it possible to produce the first

extensive set of baseline data on the socio-economics and production constraints affecting this important food staple. The survey conducted in three important sweet potato growing States covered 180 households.

Our results show that 77.8% of the households interviewed were males and the majority were in the active age groups of, 19-30 (30.6%) and 31-40 (43.9%). The engagement of more males in sweet potato production than females could be because sweet potato has become more commercialized due to the expanding urbanization after South Sudan's independence in 2011 that led to a high demand of food commodities in urban markets. Okonya et al. (2014) reported more males than females being engaged in sweet potato farming because of the commercialization of sweet potato in Soroti District of Uganda. The high proportion of young farmers involved in sweet potato production is encouraging. This indicates the potential of increasing sweet potato production and the possibility for adoption of improved technologies. Farmers' awareness of sweet potato as an income-generating crop might have motivated young men to invest in growing the crop. Moreover, the presence of more young men in the rural sector may be attributed to the lack of access to higher education, which is a result of the protracted war in South Sudan. The war has led to most rural children growing up without access to further education opportunities. Over 1300 children dropped out of school in Magwi County, Eastern Equatoria, due to war violence that led to massive displacement while 70% of schools in the conflict-affected States of Jonglei, Unity and Upper Nile have not been functioning (Hodgkin and Thomas 2016). Farmers with better education have the opportunity to be involved in off-farm activities that can improve their level of income. In addition, a better education contributes to farmers acquiring new knowledge and technical skills, and consequently the tendency to adopt improved production technologies (Alene and Manyong 2007). Education broadens the knowledge of farmers on how to manage the crop and the importance of high-quality planting material (Adam et al. 2015).

In this study, most of the respondents practiced small-scale farming and had customary lands. The possibility of increasing production in customary land for commercial purposes is normally limited, since decision making on the land depends on how much share each member of the family can have. This may hinder the development of farming plans for those with a commercial interest (Fawole 2007). However, 36% of farmers had their own land title suggesting the possibility of expanding land usage for sweet potato production and trying out new production technologies.

4.2 Sweet potato production constraints

On average, key constraints to sweet potato production highlighted by interviewed households were lack of extension services, lack of improved varieties, low sale prices of their crop, lack of credit services, price fluctuation, field pests, as well as drought and diseases. Our results in South Sudan are consistent with those of Ngailo et al. (2016) who reported the lack of extension services and credit along with pests and diseases, drought and low prices as the major constraints for sweet potato production in Tanzania. The absence of improved varieties was reported as a limiting factor for increased sweet potato production (Fuglie 2007; Kagimbo et al. 2018) and price fluctuation was an important constraint of sweet potato production in Uganda (Okonya and Kroschel 2016). The present study observed storage pests and access to vines (planting material) as additional important sweet potato production constraints in Central Equatoria and Western Bahr el Ghazal States. These results are comparable with those of Kivuva et al. (2014) and Mmasa et al. (2012) who reported the unavailability of clean and sufficient planting material as constraints to farmers in Kenya and Tanzania. In order to improve productivity in the surveyed areas of South Sudan, farmers need access to quality planting material that is high yielding, resistant to pests and diseases, drought tolerant and early maturing. If farmers were able to access such material, it would result in increased yields, improved food security, better incomes, reduced poverty and enhanced livelihoods. Access to extension services in the surveyed areas is paramount for farmers to acquire better knowledge of crop pests and diseases and management skills to improve sweet potato production. Previous reports revealed that the inadequate extension service support to agricultural and livestock farmers is a substantial concern for farmers throughout South Sudan (AfDB 2013). Unavailability of credit for sweet potato farmers limits their ability to purchase planting material and hire labour to increase production, as the use of credit can enhance productivity and income greatly. In Nigeria, Olagunju (2007) reported that sweet potato farmers provided with credit had more efficient use of resources than those without credit. This highlights the importance of the government and other financial supporting agencies investing in farmers with capital to boost sweet potato production. The majority of farmers sell their crop produce in local markets, resulting in low prices due to oversupply. The availability of alternative markets for sweet potato produce would encourage farmers to increase production to provide for more urban populations. Improvements in market system components such as price information and the network of permanent roads are also essential for the development of agricultural value chains in South Sudan.

4.3 Farm households' knowledge on pests and diseases of sweet potato and their control

This study revealed that farmers considered sweet potato weevils as the most serious insect pest. Most farmers reported that they could not leave their sweet potato roots for too long in the soil due to high infestation by weevils. A farmer in Yei County, Central Equatoria State, abandoned plans to plant a large area of sweet potato due to weevil infestation, adding that he lost his crop because of difficulties to access urban markets. Farmers in Western Bahr el Ghazal State, meanwhile, asked for immediate help to control sweet potato weevils. In Bilinya village, Magwi County in Eastern Equatoria State, where sweet potato is their main food crop, farmers harvest sweet potato roots earlier and preserve them as sliced dried roots to minimize damage by weevils. Sweet potato weevils have been reported to cause drastic reductions in yield and production of sweet potato in Eastern Tanzania (Ngailo et al. 2016), in Uganda (Okonya et al. 2014), in Ethiopia (Shonga et al. 2013) and also in Papua New Guinea (Gurr et al. 2016).

Our findings show that farmers were aware of the presence of whiteflies in their fields but did not relate whiteflies to any damage on sweet potato. This concurs with the findings in Kenya by Kivuva et al. (2014), who reported that 6% of interviewed farmers were able to identify whiteflies in their sweet potato crop. Okonya and Kroschel (2016) reported similar results where potato farmers in Kapchwora District of Uganda identified whiteflies in their crop with a local name, Kapata. The fact that farmers were unable to relate whiteflies to any damage on sweet potato, is probably because they did not have the knowledge that whiteflies are vectors of *Sweet potato chlorotic stunt virus* (SPCSV) (Closteroviridae; *Crinivirus*), which when in synergy with *Sweet potato feathery mottle virus* (SPFMV) (Potyviridae; *potyvirus*), causes the devastating sweet potato virus disease (SPVD). SPVD has been reported to cause reduction in yield of sweet potato ranging from 50% to 100% (Ndunguru et al. 2009; Ngailo et al. 2013). Our results concur with those of Okonya et al. (2014) who reported that farmers did not consider whitefly as an important pest of sweet potato because they lack the knowledge to relate the presence of the insect vector to virus symptoms or crop damage and yield loss.

Although farmers were aware of diseases affecting their crop, the majority were unable to identify the diseases even when aided with photographs of symptoms and damage that the diseases cause. This shows that farmers were not exposed to any education of diseases of the crop and consequently could not comprehend the different diseases of sweet potato. This is evidenced by farmers' reports of a lack of extension services as the major constraint in sweet potato production. This is in accordance with Adam et al. (2015) who in their study suggested

that the reason for farmers being unable to classify diseases and know the cause of plant root damage could be due to lack of access to agricultural extension services. (Ngailo et al. 2016), reported that improved extension services are one of the prerequisites to boosting sweet potato production and productivity.

Two-thirds of farm households in the studied areas managed pests infesting their sweet potato crop: 12.9% practiced early planting, 11.7% used traditional pesticides (ash spray) and 11.1% practiced crop rotation. Use of crop rotation by farmers has been reported as a means of controlling sweet potato pests in Tanzania and Cuba (Kagimbo et al. 2018; Lagnaoui et al. 2000) and wood ash application has been described from Uganda (Okonya et al. 2014). Sex pheromones, *Beauveria bassiana* (naturally occurring insect-killing fungus), and predatory ants were reported as the most important methods for controlling sweet potato weevil in Cuba (Lagnaoui et al. 2000). In Uganda resistant varieties and phytosanitation have both been reported to be effective control strategies for sweet potato virus disease (Gibson et al. 2004; Ngailo et al. 2013).

The majority of farm households (60.2%) did not use any method of pest and disease control on their sweet potato crop. Similar results have been reported in Papua New Guinea (Gurr et al. 2016) where majority of sweet potato farmers did not practice plant health management. Farmers' lack of awareness on control methods of sweet potato pests and diseases might be the reason why most farmers did not use any of the pest and disease management methods. In all the surveyed States, farmers reported that they had never received any assistance concerning how to control pests and diseases of sweet potato from either agricultural extension workers or Non-Governmental Organizations (NGOs), and they had no access to pesticides. This indicates that it is crucial to show farmers how to identify and control the major pests and diseases of sweet potato. Okonya and Kroschel (2016) pointed out the necessity of training farmers and extension workers in insect pest identification, biology and control on potato.

4.4 Sweet potato varieties grown by farmers and their preferred varietal attributes

The present study showed that farmers had knowledge about sweet potato varieties and their attributes. Most of the varieties grown were white-fleshed. Farmers occasionally grow yellow-fleshed varieties, but orange-fleshed ones were rare. According to farmers in Eastern and Central Equatoria States, most of the sweet potato landraces had disappeared during the course of the prolonged war. Most of the present sweet potato varieties, especially the preferred ones

– as the names indicated – had been introduced from Uganda or the Democratic Republic of Congo (DRC) by those farm households who had returned from exile. However, not all varieties were grown by farmers, but those that were high-yielding, early-maturing and that tasted good were preferred. This shows that improved planting material was scarce which is why almost all farmers had to rely on one variety because of limited options. This calls for attention from researchers and extension workers to evaluate, multiply and disseminate a wider diversity of sweet potato varieties so that farmers are able to access a greater choice of varieties with a diversity of quality traits. Associated with this activity, farmers need to be provided with training on how to maintain and multiply their own planting material in a healthy condition.

The respondents preferred white-fleshed sweet potato, partly because of a lack of knowledge of the health benefits of yellow and orange-fleshed varieties coupled with the lack of availability of improved yellow or orange-fleshed varieties. However, the low dry matter content of many of these varieties is frequently cited as a reason for the preference for the traditional white-fleshed varieties, which have been selected over generations for their 'mealiness' (equivalent to high dry matter content). Orange-fleshed sweet potato has been reported to have a high content of β-carotene, a vitamin A precursor (Ishiguro et al. 2010). Our results differ from the findings of Kivuva et al. (2014) in kenya, who reported that varieties with high β-carotene were mostly preferred by farmers. The reason for the popularity in Kenya could be due to the large-scale promotion of orange-fleshed sweet potato and its associated health benefits there, which has been driven by the International Potato Centre (CIP) together with national partners. Acceptance of orange-fleshed sweet potato has been reported also in Tanzania (Laurie and Magoro 2008; Ngailo et al. 2016), another country where similar large awareness-raising programmes have been run. In South Sudan, however, 36.7% of farmers in Central Equatoria and 40% in Eastern Equatoria consumed yellow-fleshed sweet potatoes. This indicates that farmers may be ready to accept orange-fleshed sweet potato if it is available. It also suggests that there may be some useful local varieties being cultivated that could be incorporated into any future sweet potato breeding work that aims to combine high β-carotene content with other farmer-preferred quality traits. However, in the current situation, there is clearly a need to encourage farm households to promote the production and consumption of yellow/orange-fleshed sweet potato for health benefits, and to introduce, evaluate and multiply improved orange-fleshed sweet potato germplasm. It also shows the need to focus on improving the yellow and orange-fleshed sweet potato varieties to better suit the needs of farmers and consumers. An important topic for research focus during this process will be to

ensure that newly developed varieties have adequate levels of resistance to the major pests and diseases of sweet potato, as many exotic varieties with the highest β-carotene content are deficient in this respect.

In this study, the preferred varietal attributes by farmers across States included: high yield (98.9%), early maturity (97.8%), good taste (97.2%), white flesh (86.1%), long storage period in the soil (63.3%), drought tolerance (62.8%), disease resistance (62.8%) and pest resistance (56.1%). These results are comparable with those of previous studies conducted in Uganda, where farmers considered resistance to pests and diseases, drought tolerance, higher yield, good taste, and duration to maturity as the main traits for selection of good sweet potato varieties and adoption of new cultivars (Gibson et al. 2008; Zawedde et al. 2014). Kagimbo et al. (2018) also reported high yield, good taste, early maturity, drought tolerance and disease resistance as the most important varietal selection attributes for farmers in Tanzania. This indicates that the introduction of new improved sweet potato varieties to farmers through breeding programmes should be geared according to farmers' consumer preferences in order to ease adoption.

5 Recommendations from households for improved sweet potato production

Throughout the studied areas, farm households emphasized their most important needs as being the provision of extension services and training, the supply of credit by the government, and increased access to an adequate supply of improved varieties. Other needs mentioned included access to markets through the improvement of road and transport facilities, and the provision of pesticides for pest control. There was also a strong demand for support in increasing the mechanization of agriculture through schemes to introduce tractors. With the improved civil security situation realized at the end of 2018, there is renewed hope that it will be possible to make progress in addressing several of these needs in the near future.

6 Conclusions

This study has provided an understanding of the baseline status of sweet potato production in the studied areas. Farmers grew several sweet potato varieties, but a few were preferred over others as a result of their low yield. The preferred varieties were all white-fleshed. A few yellow-fleshed varieties were available, but orange-fleshed varieties were rare. Farmers' preferred varietal attributes were high yield, early maturity, good taste, white flesh, longer storage period in soil, tolerance to drought and resistance to pests and diseases. Farmers were aware of damage caused by pests and diseases on sweet potato but had limited knowledge on

how to identify diseases affecting sweet potato. Insect pests were perceived to be more damaging and sweet potato weevil was considered the most serious insect pest. The majority of farmers did not use any control measures for pests and diseases on sweet potato. The most important production constraints facing sweet potato farmers, in order of importance were: lack of extension services, lack of improved varieties, low sale prices of sweet potato, lack of credit services, price fluctuations, field pests, drought and diseases. Farmers recommended delivery of extension services and training, provision of credit and improved varieties, improvement of roads and transport facilities to access markets as well as the provision of pesticides and agricultural machinery such as tractors. Productivity could be improved in the surveyed areas by providing farmers with quality planting material that is high yielding, good quality, resistant to pests and diseases, drought tolerant and early maturing. This would result in increased yields resulting in improved food security, better incomes, less poverty and enhanced livelihoods for sweet potato farmers. There is a need to introduce better yielding orange-fleshed sweet potato varieties into the sweet potato producing areas. It is important to make farmers aware of the importance of orange-fleshed sweet potato as one of the natural sources of vitamin A. Delivery of extension services and training to farmers is paramount for the acquisition of better knowledge on the crop, its pests and diseases and their management. Support from government and other finance and development agencies to strengthen the economic status of farmers by providing credit loans is crucial. Vibrant markets for sweet potato products can greatly encourage farmers to increase production.

Baseline information gained from this study will assist research institutions in South Sudan in structuring research strategies oriented towards sweet potato production that are based on the needs of small-scale farmers. This study only focused on the status of sweet potato production in areas that were accessible during this survey due to the on-going war. Further investigations need to be conducted elsewhere in South Sudan to have a more comprehensive picture of sweet potato production in the country. It is to be hoped that security conditions will improve in the near future such that this important goal can be achieved.

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PAPER II

Survey and detection of viruses infecting sweet potato (*Ipomoea batatas* (L.) Lam) in South Sudan

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Abstract

A survey of sweet potato viruses was conducted in 66 sweet potato fields in three states of South Sudan between August and September 2015. Virus and virus-like symptoms were observed in 81.8% of the fields in all the surveyed states. Common symptoms observed included purpling of lower leaves, purple rings and feathering, leaf curling, mosaic, vein clearing, chlorosis, and stunting. Moderate disease incidence (21.7%-30.3%) and mild symptom severity (1.7-2.1) was encountered in the surveyed fields. One hundred and eight symptomatic and 93 asymptomatic sweet potato leaf samples and 31 asymptomatic plants were collected from the surveyed fields. These were tested using double antibody and triple antibody sandwich enzyme-linked immunosorbent assay and reverse-transcription polymerase chain reaction for *sweet potato chlorotic stunt virus* (SPCSV), *sweet potato feathery mottle virus* (SPFMV), *sweet potato mild mottle virus*, *sweet potato virus 2* and *cucumber mosaic virus*. Two viruses, SPCSV and SPFMV, and their co-infections were identified in 22.2% of the symptomatic samples. The asymptomatic leaf samples were seronegative for all tested viruses. SPFMV was detected the most prevalent in 10.2% of the symptomatic leaf samples and 35.5% of the asymptomatic plants after graft inoculation to *Ipomoea setosa.* SPCSV and co-infections of SPCSV+SPFMV were detected in 6.5% and 5.6% of the symptomatic samples, respectively. This is the first report of the occurrence of viruses on sweet potato in South Sudan. A comprehensive survey of all sweet potato production areas is proposed to clarify the identity, incidence and distribution of sweet potato viruses in South Sudan.

Keywords: *Ipomoea batatas*, viruses, detection, incidence, distribution, South Sudan

1. Introduction

Sweet potato, (*Ipomoea batatas* (L.) Lam) is the third most important root crop, after potato (*Solanum tuberosum* L.) and cassava (*Manihot esculentum* Crantz) worldwide and covers an estimated area of 9.2 million hectares, with a total production of 112.8 million metric tonnes (FAOSTAT, 2017). In Africa, the crop is grown primarily by small-scale farmers as a food security crop and household income; with the highest production concentrated in East Africa (Gibson et al., 2009; Stathers et al., 2005). Total sweet potato production in East Africa amounts to 17.9 million metric tonnes harvested from an area of 2.3 million hectares (FAOSTAT, 2017). In South Sudan, sweet potato is among the main food crops, which also include maize, sorghum millet, groundnuts, sesame, soybeans, cowpeas and cassava. Its cultivation is mainly concentrated in Bahr el Ghazal, Lakes, Eastern Equatoria, Central Equatoria, and Western Equatoria states (FEWS-NET, 2013). Although sweet potato is largely grown by resource-poor farmers for home consumption, it is also being grown for income purposes and is progressively becoming an income-generating crop due to urbanization. However, the production of sweet potato worldwide is limited by many biotic constraints of which virus diseases are the second most important after sweet potato weevils (*Cylas* spp.) (Horton, 1989; Mukasa et al., 2003; Ngailo et al., 2016; Shonga et al., 2013).

Over 30 viruses belonging to seven genera have been reported to infect sweet potato worldwide. The genera include: *Potyvirus, Crinivirus, Carlavirus, Cucumovirus, Ipomovirus*, *Badnavirus* and *Begomovirus* (Clark et al., 2012; Mukasa et al., 2006; Untiveros et al., 2007; Valverde et al., 2007). Viruses can cause yield losses of between 40% 98% in sweet potato (Adikini et al., 2016; Gutierrez et al., 2003; Ling et al., 2010; Milgram et al., 1996; Mukasa et al., 2006; Ngeve & Bouwkamp, 1991). Sweet potato infected by a single virus predominantly shows no symptoms, and in some cases, this is also true for indicator plants such as *Ipomoea setosa* (Clark & Hoy, 2006; Clark et al., 2012). Infection of sweet potato with complex viruses and multiple co-infections can cause severe disease giving rise to severe symptoms and significant yield losses (Adikini et al., 2016; Aritua & Adipala, 2006; Clark & Hoy, 2006; Di Feo et al., 2000; Gutierrez et al., 2003; Njeru et al., 2004; Opiyo et al., 2010; Untiveros et al., 2007). Sweet potato virus disease (SPVD) is the most economically important disease of sweet potato and is caused by the synergistic interaction of *sweet potato chlorotic stunt virus* (SPCSV), a whitefly-transmitted *Crinivirus,* and *sweet potato feathery mottle virus* (SPFMV), an aphid-transmitted *Potyvirus* (Gutierrez et al., 2003; Ndunguru et al., 2009; Ngeve &

Bouwkamp, 1991; Njeru et al., 2004). SPCSV also synergizes many other sweet potato viruses belonging to a wide range of taxonomic groups. These include members of the genera: *Potyvirus* (e.g. *Sweet potato latent virus* (SPLV) and *Sweet potato mild speckling virus* (SPMSV)); *Ipomovirus* (e.g. *Sweet potato mild mottle virus* (SPMMV)); *Cucumovirus* (e.g. *cucumber mosaic virus* (CMV)); *Carlavirus* (e.g. *Sweet potato chlorotic fleck virus* (SPCFV)); and *Begomoviruses* (*Sweepoviruses*) (Clark & Hoy, 2006; Cuellar et al., 2015; Mukasa et al., 2006; Untiveros et al., 2007; Valverde et al., 2007). In Africa, some of the viruses that are known to infect sweet potato include members of the genus *Potyvirus* (e.g. SPFMV, *Sweet potato virus G* (SPVG), SPLV, SPMSV and *sweet potato virus 2* (SPV2)); the genus *Cavemovirus* (e.g. *Sweet potato caulimo-like virus* (SPCa-LV)); the genus *Carlavirus* (e.g. SPCFV); the genus *Ipomovirus* (e.g. SPMMV); the genus *Crinivirus* (e.g. SPCSV); the genus *Begomovirus* (e.g. *Sweet potato leaf curl virus* (SPLCV)); and the genus *Cucumovirus* (e.g. CMV) (Ateka et al., 2004; Ateka et al., 2007; Miano et al., 2006; Mukasa et al., 2003; Mukasa et al., 2006; Ndunguru & Kapinga, 2007; Njeru et al., 2004; Opiyo et al., 2010; Sivparsad & Gubba, 2013; Wasswa et al., 2011). In East Africa, virus disease complexes can cause yield losses ranging from 50% to 100% (Gibson et al., 1998; Karyeija et al., 1998; Mukasa et al., 2006; Ndunguru et al., 2009; Njeru et al., 2004) and the 4 most frequently occurring viruses are often detected in single or multiple infections. These are: SPFMV, SPMMV, SPCSV and SPCFV (Aritua et al., 2007; Ateka et al., 2004; Mukasa et al., 2003; Ndunguru et al., 2009; Njeru et al., 2008; Opiyo et al., 2010; Tairo et al., 2004; Tairo et al., 2005). In South Sudan, there has been no research on viruses affecting sweet potato. This is due to decades of war that has led to limited access to technological advances in crop production, and little effort on research of crop plants in South Sudan (Kaka & Oyik, 2008). Therefore, there is little information about the identity and distribution of sweet potato viruses in South Sudan. However, in Uganda, a neighboring country to South Sudan, SPVD is reported to be the most damaging disease affecting sweet potato production (Aritua et al., 1999; Mukasa et al., 2003). Therefore, there is a high probability that sweet potato viruses occur in South Sudan, as they could be readily introduced through infected sweet potato vine planting material. (Mwanga & Ssemakula, 2011) reported the selling of sweet potato vine planting material, as well as products to farmers and non-governmental organizations (NGO's) in Sudan (South Sudan), by a Ugandan farmer from Soroti District, north-eastern Uganda. Currently, there is large-scale trade between the two countries and quarantine controls at border crossings are very limited. In order to counter this threat, the identification of virus species in South Sudan is of great importance for the design of management strategies for crops as well as for the development

of sweet potato virus-free production programs. The aim of this study is, therefore, to determine and classify the identity, incidence and distribution of the main viruses of sweet potato in South Sudan.

2. Materials and methods

2.1 Field survey and sweet potato sampling

The survey was conducted in three states of South Sudan, comprising five counties: Wau county in Western Bahr el Ghazal state, Magwi county in Eastern Equatoria state, and Lanya, Yei and Morobo counties in Central Equatoria state (Figure 1) between August and September 2015. In total 66 fields were sampled, 22 fields in each of the three states. Fields were selected along accessible rural roads at intervals of 3 km to 5 km depending on the availability of sweet potato fields. The study locations were chosen based on high production and consumption of sweet potato. These fields were examined for sweet potato virus-like disease symptoms and for sampling sweet potato leaves and vines for sweet potato virus detection.

Thirty plants were sampled along an X transect –15 plants along each of the two vertices (lines of the X) spaced at even intervals when walking the transects from one side of the field to the other (Aritua et al., 1999). Sweet potato crops 2-4 months old were selected. For each of the selected plants, sweet potato virus-like symptoms were described and then scored for disease severity, using the standard severity scoring system with a scale of 1-5 (where: 1= no symptoms, 2= mild, 3= moderate, 4= severe and 5= very severe symptoms). Sweet potato virus disease incidence was calculated as the percentage of symptomatic plants out of the total of 30 sampled plants assessed in the transect.

From the selected sweet potato plants, 201 leaf samples were collected. In each field, three plants were selected, two symptomatic and one asymptomatic. In fields with no virus symptoms, all sampled plants were asymptomatic. Three leaves (upper, middle and lower) were taken from each plant. Leaf samples were immediately placed in a quarantine net bag, labeled, stapled and placed in zip-lock plastic bags containing silica gel for dry preservation (Dennien et al., 2013). Samples dried in silica gel are easy to handle, can be stored at room temperature under dry conditions and can be placed in a freezer for long-term storage. Silica gel can be

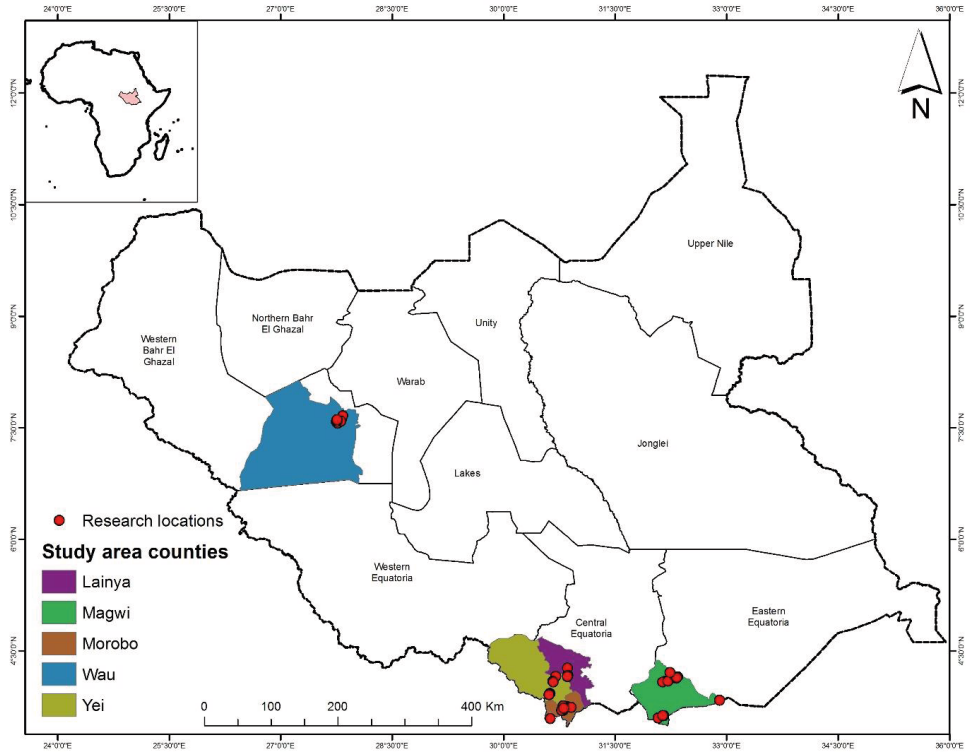

Figure. 1 Map of South Sudan showing surveyed sweet potato fields in five counties in three states: Western Bahr el Ghazal, Eastern Equatoria and Central Equatoria.

reused by drying it on a tray in an oven at 110˚C for 10-20 min (Dennien et al., 2013). Silica-dried leaf tissues have frequently been used for DNA extraction for use in PCR-based techniques (Chase & Hills, 1991; Narzary et al., 2015; Semagn, 2014). The preserved dry leaf samples were transferred to the Norwegian Institute of Bioeconomy (NIBIO), Norway for sweet potato virus detection. Photographs were taken of both symptomatic and asymptomaticsampled plants. Information on variety and location was recorded for each sampled field, in order to facilitate subsequent analysis and the correlation of symptoms with the specific viruses.

Thirty-one asymptomatic and one symptomatic vine cuttings were collected from 32 sweet potato cultivars grown by farmers in the five surveyed counties. Names of varieties, field background information, and location and variety characteristics were recorded. Vines were brought to the University of Juba, South Sudan where they were established in plastic pots in

an insect-proof screen house. After plant establishment, vine cuttings were transferred to Norwegian University of Life Sciences, Norway, where they were established in an insect-proof screen house for virus detection.

2.2 Detection of sweet potato virus

2.2.1 Serological assay

Of 201 silica-dried sweet potato leaf samples, the 108 symptomatic and 93 asymptomatic samples were subjected to serology-based virus testing using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) according to Clark and Adams (1977). The following viruses were assayed: SPCSV, SPFMV, SPMMV, SPV2 and CMV. Fresh leaf samples from 31 asymptomatic and one symptomatic (showing sweet potato virus disease symptoms) sweet potato plants were also tested. Dried leaf samples from three leaves (bottom, middle and top) was weighed to 0.2 g and double the volume of the extraction buffer (9ml) was added and homogenized. For the fresh samples, 0.5 g of leaf sample was weighed from three leaves (bottom, middle and top) and 4.5 ml of extraction buffer (not doubled) was added and homogenized. ELISA kits and positive controls for specific viruses were obtained from Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkultutrn GmgH, Plant Virus Department, Germany. Virus-specific polyclonal antibodies, immune-globulin (IgG) and enzyme conjugates were provided for use in DAS-ELISA for the viruses SPFMV, SPMMV, SPV2 and CMV whilst IgG and monoclonal antibody Mab were obtained for SPCSV in TAS-ELISA. Dilutions of antibodies were 1:1000 at coating and 1:1000 at detecting for all viruses assayed by DAS-ELISA. For SPCSV detection, using TAS-ELISA antibodies were diluted 1:1000 at coating and 1:100 for MAb when detecting. *Ipomoea setosa* extract was used as a negative control. In both DAS- and TAS-ELISA, Para-nitrophenyl-phosphate substrate at 1mg/ml (Sigma-Aldrich, San Luis, Misuri, USA) was dissolved in diethanolamine (pH 9.8) (Sigma-Aldrich, San Luis, Misuri, USA) buffer and added to microplates. After the microplates had been incubated at room temperature for 30-60 min and then again after two hours, the results were first assessed visually for varied degrees of yellow color development. Secondly, optical density (OD) was measured in a spectrophotometric ELISA plate reader at absorbance at 405nm (A405) after one- and two- hour incubations at room temperature. ELISA readings were blanked over the negative controls and samples were considered positive where the OD was greater than twice the mean value for the negative controls (Aritua et al., 2007).

2.2.2 Virus indexing

Virus titer accumulation in many sweet potato cultivars is low and direct virus detection from sweet potato tissue is often unreliable using a serological test. Therefore, grafting sweet potato scions on to *Ipomea setosa*, a universal indicator host for sweet potato viruses, was used to boost virus titer (Karyeija et al., 2000; Mukasa et al., 2003; Tairo et al., 2004). Sweet potato plants that tested negative to the assayed viruses by ELISA were indexed by grafting onto *I. setosa*. Scions from the sweet potato plants were top-grafted or side-grafted on to three-week old *I. setosa* plants. All the grafted plants were maintained at 25 ˚C in an insect proof green house and virus symptoms on *I. setosa* were monitored and recorded from three to six weeks after grafting. Symptomatic *I. setosa* leaves were then assayed for viruses by DAS- and TAS-ELISA as described above.

2.3 Reverse-transcription polymerase chain reaction (RT-PCR) amplification

2.3.1 Isolation of total RNA and cDNA synthesis

Sweet potato viruses that were identified serologically positive were confirmed by RT-PCR. Total RNA was extracted from 80-100 mg tissue powder of fresh sweet potato leave tissues using Trizol (Invitrogen, Carlsbad, California, USA) following the procedure provided by the manufacturer (Invitrogen, Carlsbad, California, USA). Fresh leaf tissues were ground to a fine powder with a mortar and pestle containing liquid nitrogen. Tissue powder was immediately stored at -80 ˚C before RNA extraction. Total RNA of 2.5 µg was used as a template for first-strand cDNA synthesis using SupperScript II reverse transcriptase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and random primers in a 20 µl final reaction volume according to the manufacturer's instructions (Invitrogen, Carlsbad, California, USA). One µl of random primers, 2.5 µg of total RNA, 1µl of dNTP Mix (10 mm each) were mixed and sterile distilled water was added to a volume of 12 µl in a 1.5 ml microtube. The mixture was heated to 65 ˚C for 5 min, chilled on ice, then briefly centrifuged. Four µl of 5x First-Strand buffer and 2 µl of 0.1 M DTT were added before incubation at 25 ˚C for 2 min. One µl of SupperScript II reverse transcriptase was added followed by 1 µl of sterile distilled water to make up to a 20 µl final volume. The reaction was incubated at 25 ˚C for 10 min, and 42 ˚C for 50 min after which it was inactivated by heating to 70 ˚C for 15 min.

2.3.2 RT-PCR detection of sweet potato viruses

One µl of cDNA from the first-strand reaction was used as a template for amplification in RT-PCR (total reaction volume 25 µl). Specific primers for SPCSV and SPFMV were used as described by (Kathurima et al., 2011). The primers SPCSV-A2 (5′-GCAGGTTTCTACGCATCTCTATC-3′) and SPCSV-B2 (5′-GAGCCCTGGCCCATTTCCTA-3′) for SPCSV, and SPFMV-A (5′-ACTACACCTGCACGTGCTAAAGAA-3′) and SPFMV-C (5′-TATTGCACACCCCTCATTCCTAAG-3′) for SPFMV. The expected product sizes for the primers were 166 bp and 185 bp for SPCSV and SPFMV respectively. PCR conditions were set at 94 ˚C for 2 min, 34 cycles of 94 ˚C for 20 s for denaturation, an annealing temperature of 53˚C and 52˚C for SPFMV and SPCSV respectively for 20 s, 72 ˚C for 1 min and final extension for 10 min at 72˚C. The PCR amplicons were subsequently run on a 1% agarose gel electrophoresis stained with ethidium bromide. A 100 bp ladder was used as a marker. Gel electrophoresis was undertaken at 110 v for 30 min and DNA bands were visualized using the Gel Doc™ XR+ Gel Documentation System (BIO-RAD).

3. Results

3.1 Virus-like symptoms observed in farmers' fields

Virus-like symptoms were observed in sweet potato fields surveyed in five counties of three states in South Sudan. Of the 66 fields surveyed, 54 (81.8%) had plants with virus-like symptoms. Symptoms observed included purpling of lower leaves, purple rings and feathering, leaf curling, mosaics, vein clearing, chlorosis and stunting (Figure 2). In Western Bahr el Ghazal state, common symptoms observed were purpling of lower leaves. Two fields were unusual as they had stunted plants and mosaics, in addition to leaf purpling. In Eastern Equatoria state, symptoms included mosaics, leaf purpling and curling, while in Central Equatoria State they comprised leaf purpling, feathering, vein clearing, chlorosis and stunting.

3.2 Virus and virus-like disease incidence and symptom severity

Thirty-two sweet potato cultivars were collected from farmers' fields. Though some farmers grew more than one cultivar in the same field, most of the fields surveyed were planted with

one cultivar. Five cultivars were extensively grown, namely: Mavede (Wau county), Lachan-Mati-Pii and Goli (Magwi county), Lupandura (Lainya county) and Apana-Lipa (Morobo and Yei counties).

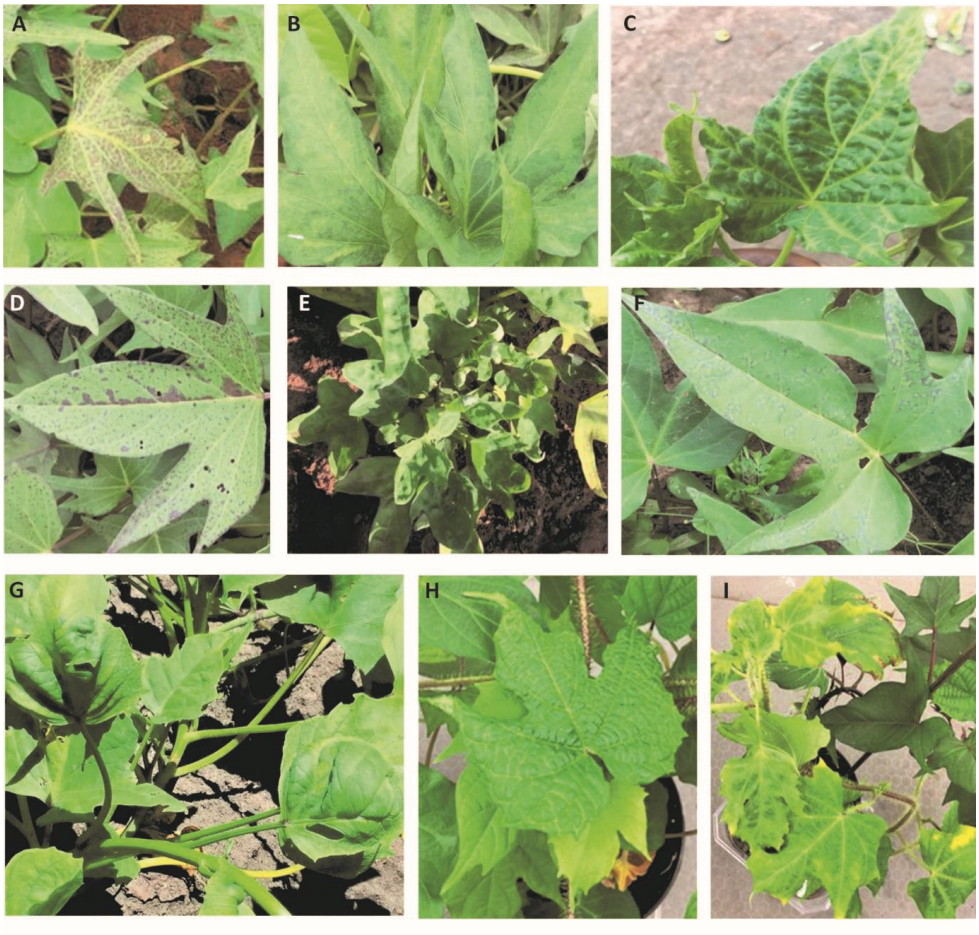

Figure 2. Virus and virus like symptoms observed in farmers' fields in the five surveyed counties of South Sudan. A, Severe leaf purpling on cv. Mavede; B, Mosaic leaf on cv. Mavede; C, Vein clearing and chlorosis on cv. Mali-Mali-2; D, leaf purpling on cv. Lupandura; E, Stunting and leaf distortion on cv. Mavede; F, Purple rings and feathering on cv. Senja-Moko-2; G, Downward leaf curling on cv. Ladwe-Achel; H and I, vein clearing on *I. setosa* leaf grafted with asymptomatic sweet potato scion.

The mean incidence of SPVD and virus-like symptoms observed in farmers' fields in the surveyed states ranged from 21.7% to 30.3% (Figure 3). There was no significant difference between states for SPVD incidence ($P > 0.05$). The highest incidence, 46.7%, was recorded in

Wau (Western Bahr el Ghazal state) and Lainya (Central Equatoria state) counties where most of the fields visited had diseased plants. Disease symptom severity was mild in all the surveyed states with mean severity scores ranging from 1.7 to 2.1 (Figure 3). The highest symptom severity score of 2.8% was recorded in Wau (Western Bahr el Ghazal state) and Yei (Central Equatoria State) counties.

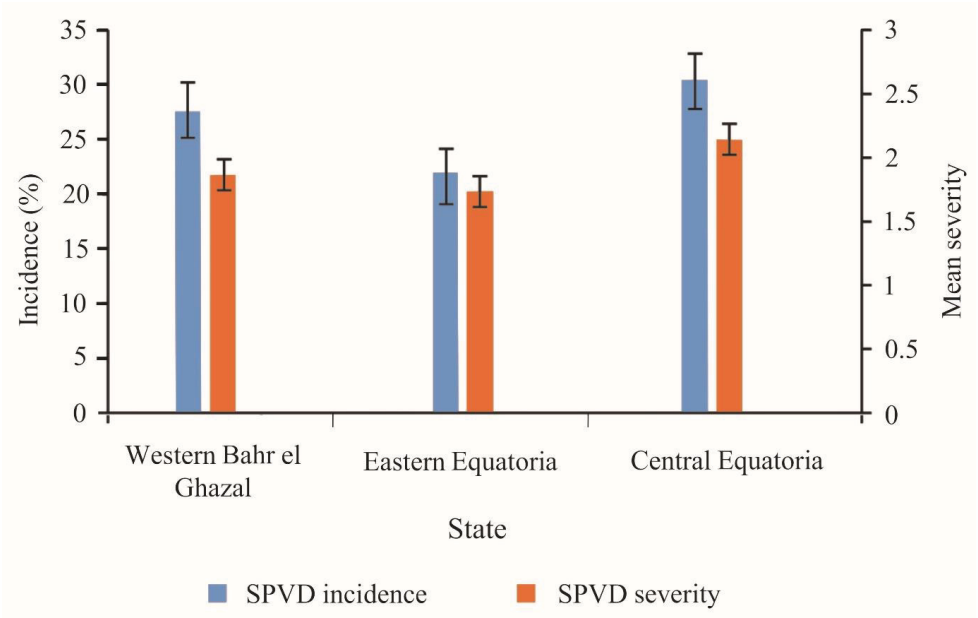

Figure 3. Virus and virus-like disease incidence and symptom severity observed in farmers' fields in the surveyed states of South Sudan.

3.3 Identification of viruses from silica-dried sweet potato leaf samples

Two-hundred and one sweet potato plant leaf samples, 108 symptomatic and 93 asymptomatic, were collected from 66 farmers' fields in the surveyed areas. Twenty-four (22.2%) of the symptomatic leaf samples were seropositive to two of the viruses tested, whereas, none of the asymptomatic samples reacted with any of the antisera (Table 1).

Of the five sweet potato viruses assayed, only SPCSV and SPFMV were detected in the plant samples assayed, either in single infections or in co-infections of SPCSV + SPFMV in all the surveyed counties except Morobo County. SPMMV, SPV2 and CMV were not detected in any

Table 1. Sweet potato symptomatic (S) and asymptomatic (A) leaf samples per county that reacted positively for SPCSV, SPFMV and SPCSV+SPFMV in ELISA.

State	County	Samples assayed		SPCSV		SPFMV		SPCSV +SPFMV	
		S	A	S	A	S	A	S	A
Western Bahr el Ghazal	Wau	34	32	2 (5.9)*	0	2 (5.9)	0	2 (5.9)	0
Eastern Equatoria	Magwi	34	32	0	0	7 (20.6)	0	1 (2.9)	0
Central Equatoria	Lainya	16	8	0	0	2 (12.5)	0	1 (6.3)	0
	Morobo	16	8	0	0	0	0	0	0
	Yei	8	13	5 (62.5)	0	0	0	2 (25)	0
Total		108	93	7 (6.5)	0	11 (10.2)	0	6 (5.6)	0

* Figures in parentheses are percentages of infected samples computed from the total of symptomatic samples. The viruses, SPMMV, SPV2 and CMV were tested for but not detected.

of the samples collected in all the surveyed areas. SPFMV was detected the most prevalent in 11 (10.2%) of the symptomatic samples occurring in single infections in three of the five counties surveyed (Table 1). Single infection with SPCSV amounted to 7 (6.5%) symptomatic samples from two of the five counties surveyed. Dual infections of plants with SPFMV + SPCSV were detected in 6 (5.6%) of the symptomatic samples in all the areas surveyed, except Morobo county. Single infection with SPCSV was most prevalent in Yei County, 62.5% whereas single infection with SPFMV was detected more in Magwi County (20.6%).

3.4 Identification of virus by indexing and RT-PCR

Thirty-one asymptomatic and one symptomatic sweet potato cultivars collected from farmers' fields in the three surveyed states were assayed for SPCSV, SPFMV, SPMMV, SPV2 and CMV by DAS- and TAS-ELISA. All the asymptomatic cultivars were seronegative for all the assayed viruses. The symptomatic cultivar, Mali-Mali-2 was seropositive for SPFMV and SPCSV (Table 2). The presence of SPFMV and SPCSV in the cultivar Mali-Mali-2 was

confirmed by RT-PCR. However, after grafting the asymptomatic sweet potato cultivars onto *I. setosa*, symptoms typical of SPFMV (Figure 2H and I) were observed on *I. setosa* leaves in 14 (45%) of the cultivars. The cultivars that showed symptoms on *I. setosa* were those collected from Magwi, Lainya and Yei counties. ELISA tests of the symptomatic *I. setosa* leaf samples detected only SPFMV in these 14 cultivars (Table 2). This was confirmed by RT-PCR. SPMMV, SPV2 and CMV that were not detected by ELISA neither in the sweet potato plant leaf samples nor in the *I. setosa* leaf samples.

4. Discussion

This survey of viruses in sweet potato in South Sudan was the first of its kind ever to be conducted. It covered five major sweet potato producing counties in three states of South Sudan. Other sweet potato producing areas in the country could not be visited due to the ongoing conflict and insecurity in those areas. The results of this study revealed distinct and severe virus-like and virus disease symptoms with relatively high incidence, but mild severity in all the surveyed states. This indicates the presence of viral diseases of sweet potato in the surveyed areas. The low overall severity scores are the result of only a small proportion of the plants exhibiting severe symptoms. It has been reported that severe symptoms in sweet potato plants is a result of co-infection with two or more viruses (Aritua et al., 2007; Mukasa et al., 2003; Valverde et al., 2007). The results obtained in this study suggests that the occurrence of dual infections in sweet potato fields in South Sudan is not very common.

Of the viruses assayed, only two viruses, SPFMV and SPCSV, were detected from the sweet potato plant samples collected in all the surveyed states in single or co-infections. This concurs with (Aritua et al., 2007, Ndunguru et al 2009) in Uganda and Tanzania who reported that SPFMV and SPCSV were the most commonly detected viruses in single and co-infection. SPFMV was the most widely distributed virus. It was detected in single and dual infections in symptomatic sweet potato plant samples and in the asymptomatic sweet potato plants after grafting to the *I. setosa* indicator plant. This follows a pattern reported from several other African countries, including South Africa (Sivparsad & Gubba, 2013), Kenya (Ateka et al., 2004; Opiyo et al., 2010), Ethiopia (Tesfaye et al., 2011), and Rwanda (Njeru et al., 2008). SPFMV exhibits mild or no symptoms in sweet potato cultivars infected with SPFMV alone (Karyeija et al., 2000). The common occurrence of SPFMV in single infection in the present study may explain the prevalence of SPFMV in sweet potato fields, since farmers may tend to

Table 2. Presence of sweet potato viruses in 31 asymptomatic sweet potato cultivars collected from farmers' fields in five surveyed counties detected by ELISA after grafting onto *I. setosa.*

State	County	Cultivar	Viruses detected by ELISA	
			SPFMV	SPCSV
Western Bahr el Ghazal	Wau	Mavede	-	-
		Yankar-1	-	-
		Yankar-2	-	-
		Vayunduka	-	-
		Mayenduro	-	-
		Mviro	-	-
		Kolingwa	-	-
Eastern Equatoria	Magwi	Ladwe-Achel	+	-
		Lachan-Mati-Pii	+	-
		Layelo	+	-
		Lobel	+	-
		Jogo	-	-
		Koda	+	-
		Goli	+	-
		Nailo	+	-
		Jivi	-	-
Eastern Equatoria	Lainya	Senja-Moko-1	+	-
		Kajamingi	+	-
		Gweregwere	-	-
		Lupandura	-	-
	Morobo	Singa-Nakilo	-	-
		Apana-Lipa	-	-
		Karamojo-1	-	-
	Yei	Meridi-Meridi	+	-
		Senja-Moko-2	+	-
		Mali-Mali-1	-	-
		Kedi Kedi	-	-
		Manya-Manya	+	-
		Bakaya	+	-
		Irubu	-	-
		Karamojo-2	+	-
		Mali-Mali-2*	+	+
		Total	15/32	1/32

* Viruses detected from symptomatic sweet potato leaf sample before grafting onto *I. setosa*. The viruses, SPMMV, SPV2 and CMV were tested for but not detected.

select asymptomatic sweet potato vines for their next crop resulting in the propagation of this virus (Ateka et al., 2004; Gibson et al., 1997; Njeru et al., 2008).

We observed that SPCSV was the second most common virus in sweet potato crops in South Sudan. Like SPFMV, it occurred in both single and dual infections. This contrasts with results obtained from several other countries where SPCSV was shown to be the most prevalent virus (Uganda: (Mukasa et al., 2003; Mukasa et al., 2006; Ndunguru et al., 2009), (Tanzania: (Ndunguru & Kapinga, 2007; Ndunguru et al., 2009) and Peru (Gutierrez et al., 2003). SPCSV by itself can cause considerable reduction in yield of storage roots and a drastic loss in yield when in synergy with SPFMV, resulting in sweet potato virus disease (SPVD) (Aritua & Adipala, 2006; Gibson et al., 1998; Gutierrez et al., 2003; Karyeija et al., 2000; Mukasa et al., 2006; Ngeve & Bouwkamp, 1991). Some East African sweet potato cultivars have been reported to revert from SPFMV infection or become resistant to SPFMV (Gibson et al., 1997; Gibson et al., 2014). However, SPCSV when in co-infection with SPFMV, breaks down resistance to SPFMV in sweet potato cultivars, thereby enhancing susceptibility to infection by SPFMV (Karyeija et al., 2000; Kreuze et al., 2005; Valverde et al., 2007). The high incidence of SPCSV in single as well as dual infections in Yei County, shows that this is clearly an important virus in South Sudan. Therefore, SPCSV should be a major target for control. Immediate support of resource-poor farmers with virus-free propagation programs by government, none governmental organizations (NGO's) and research agencies is of great importance to minimize the spread of SPCSV in farmers' fields. Development of resistant varieties and the control of whitefly and aphid vectors that transmit SPCSV and SPFMV respectively should be a priority in the fight against the spread of sweet potato viruses. Gibson et al. (2004) reported that the deployment of resistant varieties accompanied with phytosanitation practices is an effective control strategy for SPVD.

Almost 80% of the symptomatic sweet potato samples assayed were seronegative. We only tested for five viruses in this study. It is likely that the symptoms observed in the diseased samples in which the tested viruses were not detected, were the result of infection with different viruses. This suggests the presence of more viruses infecting sweet potato in South Sudan. Similar cases have been reported in East Africa (Ateka et al., 2004; Mukasa et al., 2003; Ndunguru & Kapinga, 2007; Opiyo et al., 2010; Tairo et al., 2004). However, confusion of virus symptoms with symptoms caused by non-viral agents, such as mineral deficiency (O'Sullivan et al., 1997), could be another reason. Alternatively, failure to detect viruses in

most of the symptomatic samples by ELISA could be because the samples were silica-dried. (Saponari et al., 2008) reported lower or occasionally negative ELISA A405 values from silica-dried samples in citrus samples infected with CTV, whereas (Hung et al., 2000) reported the failure of ELISA to detect CTV in oven-desiccated citrus samples.

The asymptomatic samples did not give positive reactions to any of the antisera of assayed viruses. However, after grafting the asymptomatic sweet potato cultivars to an indicator plant, *I. setosa*, a considerable proportion (45%) did react positively to SPFMV antiserum. Similar results were reported by (Aritua et al., 2007; Ateka et al., 2004; Gibson et al., 1997; Njeru et al., 2008; Tairo et al., 2004). The distribution of SPFMV titres in asymptomatic sweet potato plants is erratic and titres are often too low to be detected by ELISA (Cadena-Hinojosa & Campbell, 1981; Karyeija et al., 2000). The absence of sweet potato viruses in most of the asymptomatic sweet potato plant samples was an advantage. In Morobo county, no viruses were detected in either symptomatic or asymptomatic samples, even after grafting the collected cultivars to *I. setosa*. This shows that farmers are likely to be able to select virus-free vine cuttings. Currently, most farmers produce their own planting material from the previous crop and share or sell it without applying any phytosanitary practices.

5. Conclusions

The current study is the first to assess the identity, incidence and distribution of viruses and virus diseases that infect sweet potato in South Sudan. SPFMV, SPCSV, and their co-infection were the only viruses identified in all three surveyed states. Future research strategies should prioritize the control of these two viruses since their synergy results in SPVD, the single most important disease of sweet potato worldwide. Currently, sweet potato farmers in South Sudan have no source of virus-free planting material but select asymptomatic cuttings from their previous crop. The provision of resistant varieties and phytosanitation services to farmers should be a priority. More sweet potato viruses, other than those detected in this study, are likely to be present in South Sudan. We suggest further research to identify and determine their effects. Due to inevitable limitations imposed by civil war in the country, not all sweet potato producing areas could be covered during this study. Therefore, a comprehensive survey is required to elucidate the identity, incidence, distribution and effect of viruses on sweet potato farming in South Sudan.

Acknowledgments

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PAPER III

Detection of viruses in sweet potato cultivars from South Sudan by Small-RNA deep-sequencing techniques

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Abstract

Virus complexes are major challenges to sweet potato production worldwide. However, the prevalence of viruses infecting sweet potato in South Sudan has been largely unstudied. This study used small-RNA deep-sequencing (SRDS) analysis to detect viruses in sweet potato in 15 asymptomatic and one symptomatic sweet potato cultivars collected from five locations in three states of South Sudan. Assembly of small-RNA reads using VirusDetect program, identified 15 viruses belonging to 6 genera (*Potyvirus, Crinivirus, Begomovirus, Cavemovirus, Mastrevirus* and *Badnavirus*) as following: Two potyviruses [*Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato virus C* (SPVC)] each detected in 50% of the samples, one crinivirus [*Sweet potato chlorotic stunt virus* (SPCSV, 6.3%)], seven begomoviruses [*Sweet potato leaf curl virus* (SPLCV, 87.5%), *Ipomoea yellow vein virus* (IYVV, 25%), *Sweet potato mosaic associated virus* (SPMaV, 6.3%), *Sweet potato leaf curl Sao Paulo virus* (SPLCSPV, 12.5%), *Sweet potato golden vein associated virus* (SPGVV, 25%), *Sweet potato leaf curl Georgia virus* (SPLCGV, 6.3%), and *Sweet potato leaf curl Uganda virus* (SPLCUV, 12.5%)], one cavemovirus [*Sweet potato collusive virus* (SPCV, 6.3%)], one mastrevirus [*Sweet potato symptomless mastrevirus 1* (SPSMV-1, 100%)], and 3 badnaviruses [*Sweet potato badnavirus A* (SPBV-A, 93.8%), *Sweet potato badnavirus B* (SPBV-B, 100%) and *Sweet potato badnavirus C* (SPBV-C, 56.3%), collectively known as *Sweet potato pakkakuy virus* (SPPV)]. Coinfections were common, and samples simultaneously infected with five to 10 viruses. SRDS confirmed the presence of SPFMV and SPCSV previously detected by ELISA and RT-

PCR. These results indicate the prevalence of sweet potato viruses in South Sudan and highlight the importance of further investigation and comprehensive survey in sweet potato production areas.

Key words: sweet potato (*Ipomoea batatas*), virus detection, sRNAs deep sequencing

1. Introduction

Sweet potato (*Ipomea batatas* L.; family *Convolvulaceae*) is an import root crop grown widely in tropical and subtropical regions of the world and is the third most important root crop after potato (*Solanum tuberosum*) and cassava (*Manihot esculenta*) (Clark et al., 2012; Loebenstein, 2015). It is one of the most important subsistence and food security crops in developing countries (Gibson et al., 2009; Low et al., 2009; Low, 2011). In South Sudan, is an important food security and income generating crop with its production mainly concentrated in Greater Equatoria and Western Bahr el Ghazal States (Ntawuruhunga et al., 2007). Although sweet potato is highly valued as a food security crop its production is constrained by viral diseases (Clark & Hoy, 2006; Clark et al., 2012; Mukasa et al., 2003; Tairo et al., 2004).

Because sweet potato is vegetatively propagated accumulation and perpetuation of viruses can become a major problem to production that contributes to severe losses and cultivar decline (Adikini, S. et al., 2015; Bryan et al., 2003; Lewthwaite et al., 2011). Most of the viruses infecting sweet potato are symptomless in single infections but can develop more severe disease symptoms and yield losses when in mixed infections (Clark & Hoy, 2006; Clark et al., 2012; Karyeija et al., 2000; Valverde et al., 2007). More than 30 viruses have been reported to infect sweet potato found in 9 families (Clark et al., 2012). Half of these viruses were recently described as DNA viruses belonging to the families *Geminiviridae* and *Caulimoviridae* (Clark et al., 2012). The main viruses of sweet potato worldwide are *Sweet potato chlorotic stunt virus* (SPCSV; genus *Crinivirus*; family *Closteroviridae*), a whitefly transmitted virus, and S*weet potato feathery mottle virus* (SPFMV; genus, *Potyvirus*; family, *Potyviridae*), an aphid transmitted virus. These two viruses when in co-infection develop a severe sweet potato virus disease (SPVD) caused by the synergistic interaction of SPCSV and SPFMV (Karyeija et al., 2000; Mukasa et al., 2006; Untiveros et al., 2007). SPCSV is also reported to mediate synergistic interaction with other potyviruses (Kokkinos & Clark, 2006) and many viruses from different genera including *Ipomovirus*, *Carlavirus* and *Cucumovirus*, *Cavemovirus*,

Solendovirus and *Begomovirus* (Cuellar et al., 2011b; Cuellar et al., 2015; Mukasa et al., 2006; Untiveros et al., 2007).

Several reliable methods have been employed for detecting and identifying plant viruses including physical, biological, serological and molecular techniques such as reverse transcription-polymerase chain reactions (RT-PCR), Real time PCR, PCR and multiplex RT-PCR (Albuquerque et al., 2012; Cuellar et al., 2011b; Li et al., 2012; Ling et al., 2010; Pardina et al., 2012; Qin et al., 2016; Tairo et al., 2004; Untiveros et al., 2008). However, these techniques require prior knowledge of the viruses present and therefore, tests are specific to limited number of related viruses (Jones et al., 2017; Mumford et al., 2006). Recent advancement in virus diagnosis has been the use of next generation sequencing (NGS) technology which enables the detection of viruses on host plants without prior knowledge of the presence of the virus (Adams et al., 2013; Bi et al., 2012; Fonseca et al., 2018; Giampetruzzi et al., 2012; Hagen et al., 2012; Kashif et al., 2012; Kreuze et al., 2009; Mbanzibwa et al., 2014). This method enables deep sequencing and assembly of virus-derived small interfering RNAs (vsiRNAs) that accumulate in virus infected plants in response to viral infection, a plant defense mechanism called RNA silencing (Llave, 2010; Mlotshwa et al., 2008; Pantaleo et al., 2007). Studies on viruses infecting sweet potato has scarcely been done in South Sudan. In this study we used small RNA deep sequencing (SRDS) for the first time to confirm the presence of SPFMV and SPCSV previously detected by ELISA and RT-PCR in sweet potato cultivars from South Sudan and to detect possible new viruses in these cultivars. Our results confirm the presence of these two viruses in these cultivars. In addition, SRDS enabled us to identify 13 more sweet potato viruses previously not detection in South Sudan.

2. Materials and methods

2.1 Plant material

The plant material used in this study was selected from 31 asymptomatic and one symptomatic sweet potato cultivars originally collected from five locations in three states of South Sudan in August and September 2015. The locations included Wau County (Western Bahr el Ghazal state), Magwi County (Eastern Equatoria state), Lainya, Yei and Morobo counties (Central Equatoria state) (Figure 1). The collected sweet potato vines were first established in an insected proof screen house at the university of Juba, South Sudan. After plant establishment, vine cuttings from these cultivars were transferred to Norwegian University of Life Sciences

(NMBU), Ås, Norway. The vines were then established and maintained in an insect-proof green house for virus detection in the Norwegian Institute of Bioeconomy (NIBIO) virus laboratory. Fourteen of these cultivars were proven to be infected with SPFMV and one coinfected with SPFMV and SPCSV, by index grafting, doubled and triple antibody sandwich enzyme-linked immunosorbent assay (DAS- and TAS-ELISA) and RT-PCR. The viruses,

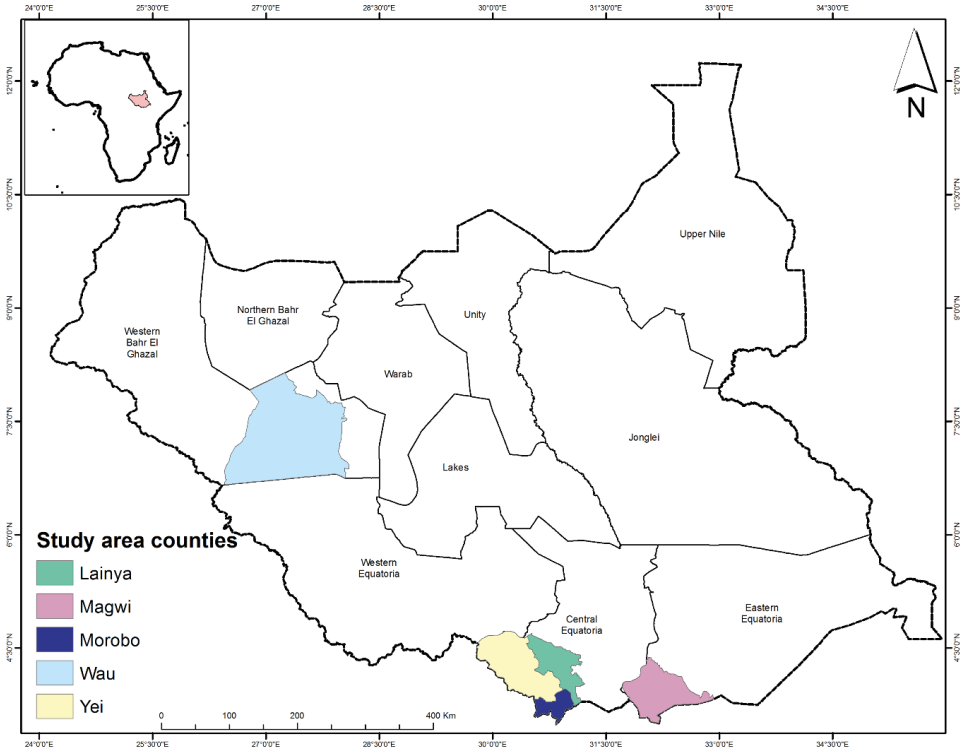

Figure 1. Map of South Sudan showing sampled locations in Western Bahr el Ghazal, Central Equatoria and Eastern Equatoria states.

sweet potato mild mottle virus (SPMMV), *sweet potato virus 2* (SPV2) and *cucumber mosaic virus* (CMV) were also tested for but not detected (B. C. Misaka, J. P. Legg, P.W. Marchelo-d'Ragga and A. K. Hvoslef-Eide, unpublished data).

For this study, 7 of the cultivars infected with SPFMV and one coinfected with SPFMV and SPCSV were selected. In addition, 8 of those cultivars which were not infected with any of the viruses assayed were also selected. In total, 16 cultivars (3 from Wau County, 5 from Magwi

County, 2 from Lainya County, 3 from Morobo County and 3 from Yei County), were used for total RNA isolation.

2.2 Total RNA isolation

Fresh sweet potato leaves were harvested from 16 sweet potato cultivars and ground in liquid nitrogen to a fine power using motor and pestle. Total RNA was then isolated from 90 to 110 mg of the powered plant leaf tissue using Spectrum Plant Total RNA kit (Sigma-Aldrich) according to the manufacturer's instructions. Total RNA was further purified by On-Column DNase 1 Digest Set (Sigma-Aldrich) and then eluted in 30 µl of elution solution provided in the Plant Total RNA kit. Quantity and quality of RNA was measured using spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and gel electrophoresis respectively. Purity, concentration and RNA integrity number (RIN) of total RNA were measured with Agilent 2100 Bioanalyzer.

2.3 Small-RNA deep sequencing

From each of the 16 samples, 10 µg of total RNA was taken and sent for deep sequencing services center (Fasteris) in Geneva, Switzerland. The procedure included small RNA library preparation using illumina TruSeq small RNA kit. Separation of sRNA from total RNA was performed by polyacrylamide gel electrophoresis and sRNA of size between 18 to 30 nucleotides were selected. These were then ligated with a single-stranded 3′ adapter and bar-coded 5́′ adapter followed by reverse-transcription and PCR amplification to generate a DNA library. The library was purified and diluted to 10nM concentration and deep sequenced using Illimina HiSeq 3000/4000 instrument following the manufacturer's protocol.

2.4 Sequence analysis

Small RNA reads in the size range of 18 to 50 nucleotides were analyzed using VirusDetect software, an automated bioinformatics pipeline (Zheng et al., 2017). In brief, sRNA sequences were mapped to known virus reference sequences using BWA (Li & Durbin, 2009) and de novo assembled using Velvet (Zerbino & Birney, 2008) at different K-mer lengths where the best K-mer length was then determined. De novo assembled siRNA contigs were pooled together with those generated from reference-guided assemblies, and then processed to remove redundant sequences (Zheng et al., 2011). Contigs were then compared to virus nucleotide and protein reference sequences using BLASTN and BLASTX programs. Contigs having hits to

retrieved virus sequences were identified as virus contigs. These were then merged and used to derive the coverage of the reference by virus contigs.

3. Results and discussion

3.1 small-RNA deep-sequencing (SRDS)

Virus diseases have been a major challenge to sweet potato production worldwide. SRDS of plant viruses has been proven to be an efficient method of virus detection in plants without prior knowledge of the viruses and can detect viruses at low titre levels (Jones et al., 2017; Kreuze et al., 2009). To perceive the distribution of sweet potato viruses in South Sudan we examined 16 sweet potato cultivars (15 asymptomatic and one symptomatic), collected from five locations in three states of South Sudan using SRDS. These cultivars were previously evaluated for infection of known sweet potato viruses including SPFMV, SPCSV, SPMMV, SPV2 and CMV using graft indexing, ELISA and RT-PCR (B. C. Misaka, J. P. Legg, P.W. Marchelo-d'Ragga and A. K. Hvoslef-Eide, unpublished data). Total RNA of 10µg from each of the cultivar was deep-sequenced separately. A total of 8,669,846 to 21,248,090 siRNAs reads (size range between 18 to 50 nt) were generated for each of the 16 samples. Most of the reads (7,724,893 to 18,535,925) were between sizes of 18 to 26 nt showing two main peaks at the sizes of 21 and 24 nt. These results concur with previous reports on the production of vsiRNAs of 21nt, 22nt and 24nt sizes by Dicer-like (DCL) enzymes in response to the presence of positive-strand RNA viruses in virus-infected plants (Donaire et al., 2009; Llave, 2010; Xie et al., 2004). Using VirusDetect software (an automated bioinformatics pipeline), reads were assembled into contigs and compared to reference virus nucleotide sequences using the BLASTN and BLATX programs.

3.2 Detection of sweet potato viruses by SRDS

As expected, alignment of sRNA virus contigs to reference virus genomes resulted in the detection of SPFMV in 8 of the cultivars (Ladwe achel, Koda, Goli, Lobel, Kajamingi, Senja Moko-2, Bakaya and Mali Mali-2) that were previously positive for this virus in ELISA and RT-PCR (Table 1) (B. C. Misaka, J. P. Legg, P.W. Marshello-d'Ragga and A. K. Hvoslef-Eide, unpublished data). The presence of SPCSV in one sample (Mali Mali-2) co-infected with SPFMV and SPCSV was also confirmed by SRDS (Table 1). The genome coverage was high for both SPFMV and SPCSV. Small-RNA contigs covered 86.3% of the complete genome sequence of SPFMV-EA strain (FJ155666; isolate Piu3) used as reference while coverage of

the complete genome sequences of RNA1 and RNA2 of SPCSV-EA strain (AJ428554, AJ428555; isolate Uganda) was 83.4% and 91.5%, respectively (Table 2). It is interesting that the genome coverages for these two important viruses, SPFMV and SPCSV, involved in SPVD are very high. This suggests that, as in Uganda, Kenya and Tanzania (Ateka et al., 2004; Cuellar et al., 2011a; Tairo et al., 2005) the East African strains of SPFMV and SPCSV occur in South Sudan. South Sudan borders Kenya and Uganda, thus, it is likely that there is trade of sweet potato planting material between the neighbouring countries or these materials were brought into South Sudan by refugees returning home after the peace agreement was signed.

SRDS confirmed the absence of SPFMV and SPCSV in the other 8 cultivars (Yankar-2, Mayenduro, Mviro, Jivi, Lupandura, Singa Na kilo, Apana Lipa and Karamojo-1) previously tested negative by ELISA and RT-PCR (Table 1) and the absence of SPMMV, SPV2 and CMV from all the cultivars. SPMMV, SPV2 and CMV were previously not detected by ELISA and RT-PCR in these cultivars (B. C. Misaka, J. P. Legg, P.W. Marchelo-d'Ragga and A. K. Hvoslef-Eide, unpublished data). SPMMV, SPV2 and CMV have been detected in East Africa. SPMMV is one of the common sweet potato viruses occurring in East Africa (Ateka et al., 2004; Mukasa et al., 2003; Tairo et al., 2004). SPV2 has been reported in Zambia, South Africa and Tanzania (Ateka et al., 2007; Mbanzibwa et al., 2014) while CMV has been reported in Kenya and Uganda (Adikini et al., 2015; Opiyo et al., 2010). This indicates the need to investigate the occurrence of these viruses in sweet potato growing areas of South Sudan.

3.3 SRDS reveals thirteen more sweet potato viruses from five genera

In addition to SPFMV and SPCSV, SRDS was able to detect 13 more viruses, which were not tested before by ELISA or RT-PCR in the sequenced sweet potato samples (Table 1). These viruses belong to five genera including one potyvirus [*Sweet potato virus C* (SPVC)], 7 begomoviruses [*Sweet potato leaf curl virus* (SPLCV), *Ipomoea yellow vein virus* (IYVV), *Sweet potato mosaic associated virus* (SPMaV), *Sweet potato leaf curl Sao Paulo virus* (SPLCSPV), *Sweet potato golden vein associated virus* (SPGVV), *Sweet potato leaf curl Georgia virus* (SPLCGV), and *Sweet potato leaf curl Uganda virus* (SPLCUV)], one cavemovirus [*Sweet potato collusive virus* (SPCV)], one mastrevirus [*Sweetpotato symptomless mastrevirus 1* (SPSMV-1)], and 3 badnaviruses [*Sweet potato badnavirus A* (SPBV-A), *Sweet potato badnavirus B* (SPBV-B) and *Sweet potato badnavirus C* (SPBV-C), collectively known as *Sweet potato pakkakuy virus* (SPPV)] (Table 2). Coverage of virus

Table 1. Sweet potato viruses detected by small RNA deep sequencing in sweet potato cultivars collected from five locations in South Sudan.

Location (State/county)	Cultivar	Virus species*														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Western Bahr el Ghazal																
Wau	Yankar-2[b]	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-
	Mayenduro[b]	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-
	Mviro[b]	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-
Eastern Equatoria																
Magwi	Ladwe Achel[a]	+	+	-	-	-	-	-	-	-	-	+	+	-	+	-
	Koda[a]	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+
	Goli[a]	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+
	Jivi[b]	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-
	Lobel[a]	+	+	+	+	-	-	-	+	-	-	+	-	-	+	+
Central Equatoria																
Lainya	Kajamingi[a]	+	+	-	+	+	-	-	-	-	-	+	-	-	+	+
	Lupandura[b]	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-
Morobo	Singa Na Kilo[b]	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-
	Apana Lipa[b]	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-
	Karamojo-1[b]	+	+	+	+	-	-	-	-	-	-	+	-	-	-	+
Yei	Senja Moko-2[a]	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+
	Bakaya[a]	-	+	-	+	-	-	-	-	-	-	+	-	-	+	+
	Mali Mali-2[a]	+	+	+	-	-	-	-	-	-	-	+	-	+	+	+

* (1) SPBV-A, (2) SPBV-B, (3) SPBV-C, (4) SPLCV, (5) IYVV, (6) SPMaV, (7) SPLCSPV, (8) SPGVV, (9) SPLCGV, (10) SPLCUV, (11) SPSMV-1, (12) SPCV, (13) SPCSV, (14) SPFMV, (15) SPVC. The viruses SPMMV, SPV2 and CMV were not detected.

[a] Samples previously tested positive for SPFMV and SPCSV in ELISA and RT-PCR (B. C. Misaka, J. P. Legg, P.W. Marshello-Dragga and A. K. Hvoslef-Eide, unpublished data).

[b] Samples previously tested negative for SPFMV, SPCSV, SPMMV, SPV2 and CMV in ELISA and RT-PCR (B. C. Misaka, J. P. Legg, P.W. Marshello-Dragga and A. K. Hvoslef-Eide, unpublished data).

Table 2. Viruses and percentage genome coverage by sweet potato viruses-derived siRNA contigs.

Virus	Family	Genus	Accession number	Reference genome size	Genome Coverage (%)
Sweet potato badnavirus A (SPBV-A; pakakay, SPPV)	*Caulimoviridae*	*Badnavirus*	KM000054	808	92
Sweet potato badnavirus B (SPBV-B; pakakuy, SPPV)	*Caulimoviridae*	*Badnavirus*	FJ560944	7961	73.2
Sweet potato badnavirus C (SPBV-C; pakakuy, SPPV	*Caulimoviridae*	*Badnavirus*	JQ902104	438	66.9
Sweet potato leaf curl virus (SPLCV)	*Geminiviridae*	*Begomovirus*	KF040464	2836	95.9
Ipomoea yellow vein virus (IYVV)	*Geminiviridae*	*Begomovirus*	EU839577	2783	87.5
Sweet potato mosaic associated virus (SPMaV)	*Geminiviridae*	*Begomovirus*	FJ969831	2803	64.9
Sweet potato leaf curl Sao Paulo virus (SPLCSPV)	*Geminiviridae*	*Begomovirus*	KF836891	2768	97.5
Sweet potato golden vein associated virus (SPGVV)	*Geminiviridae*	*Begomovirus*	HQ333143	2824	93.3
Sweet potato leaf curl Georgia virus (SPLCGV)	*Geminiviridae*	*Begomovirus*	AF326775	2773	88.2
Sweet potato leaf curl Uganda virus (SPLCUV)	*Geminiviridae*	*Begomovirus*	FR751068	2799	69.6
Sweetpotato symptomless mastrevirus 1 (SPSMV-1)	*Geminiviridae*	*Mastrevirus*	FJ560945	1012	97.7
Sweet potato collusive virus (SPCV)	*Caulimoviridae*	*Cavemovirus*	HQ694978	7723	50.2
Sweet potato chlorotic stunt virus-strain East Africa (SPCSV-EA) RNA1	*Closteroviridae*	*Crinivirus*	AJ428554	9407	83.4
Sweet potato chlorotic stunt virus-strain East Africa (SPCSV-EA) RNA2	*Closteroviridae*	*Crinivirus*	AJ428555	8223	91.5
Sweet potato feathery mottle virus (SPFMV-EA)	*Potyviridae*	*Potyvirus*	FJ155666	11004	86.3
Sweet potato virus C (SPVC)	*Potyviridae*	*Potyvirus*	GU207957	10820	98.7

reference genomes sequences by the assembled virus contigs from sRNA was significant and ranged from 50.2% (SPCV) to 98.7% (SPVC). These results demonstrate the effectiveness of SRDS as a powerful tool for detecting plant viruses without the use of specific primers or antibodies. Our results concur with the findings of (Gu et al., 2014; Kashif et al., 2012; Kreuze et al., 2009; Mbanzibwa et al., 2014; Qin et al., 2016) who detected similar viruses using SRDS. The detection of these viruses from asymptomatic plants, except SPCSV, agrees with previous findings that most sweet potato viruses cause no obvious symptoms on infected sweet potato plants (Cuellar et al., 2011b; Cuellar et al., 2015; Untiveros et al., 2007).

SPVC has the highest reference genome coverage (98.7%) by virus contigs and was identified in 8 (50%) of the samples (Table 2 and Figure 2). This potyvirus was detected in almost all cultivars previously positive to SPFMV (Table 1). It was also detected in one cultivar (Karamojo-1), previously tested negative for SPFMV by ELISA and RT-PCR (Table 1). SPVC has been reported to be distributed worldwide in Australia, Africa, Asia, and North and South America (Kashif et al., 2012; Tairo et al., 2005) and highly prevalent in China and Korea (Kwak et al., 2014; Ma et al., 2019).

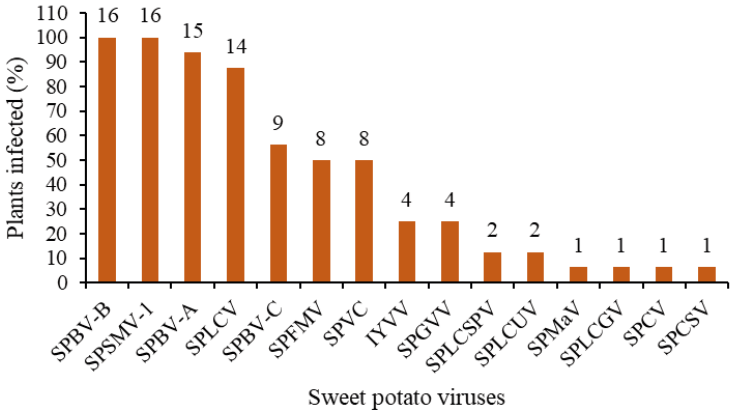

Figure 2. Percentage of sweet potato cultivars infected by a single virus species. The numbers above each bar indicate the number of cultivars infected by a single virus species.

Genome coverage for the begomoviruses (sweepoviruses) was considerably high for all the seven identified species, SPMaV (64.9%), SPLCUV (69.6%), IYVV (87.5%), SPLCGV (88.2%), SPGVV (93.3%), SPLCV (95.9%) and SPLCSPV (97.5%) (Table 2). SPLCV was the most widely distributed sweepovirus, detected in 14 (87%) of the samples (Figure 2). IYVV

and SPGVV were each detected in 4 (25%) of the samples. SPLCSPV and SPLCUV were each detected in 2 (12.5%) of the samples while SPMaV and SPLCGV were each identified in one (6.3%) of the samples. Sweepoviruses are generally wide spread globally and have been found in many countries including Spain (Lozano et al., 2009), Italy (Briddon et al., 2006), Brazil (Paprotka et al., 2010), Peru (Fuentes & Salazar, 2003), USA (Kashif et al., 2012; Lotrakul et al., 2003), India (Prasanth & Hegde, 2008) and China (Bi & Zhang, 2012). In Africa SPLCV has been detected in Kenya (Miano et al., 2006), SPLCSPV in South Africa and Tanzania (Esterhuizen et al., 2012; Mbanzibwa et al., 2014), SPLCUV in Uganda and Tanzania (Mbanzibwa et al., 2014; Wasswa et al., 2011), SPGVV and SPLCGV in Tanzania (Mbanzibwa et al., 2014), and SPMaV in South Africa (Esterhuizen et al., 2012). This suggest that sweepoviruses are widely distributed in Africa. Although sweepoviruses have been reported to cause no clear symptoms on most sweet potato infected plants, yield reduction has been observed in plants infected with SPLCV alone (Clark & Hoy, 2006; Ling et al., 2010). Cuellar et al. (2015) reported that co-infection of sweet potato plants with SPCSV+SPLCV, SPCSV+SPLCGV and SPCSV+ SPLCSCV (*Sweet potato leaf curl South Carolina virus*) showed no clear symptoms but strong increase in sweepovirus titres was observed. Based on the afore reports, the high number of sweepoviruses identified in this study may cause significant impact on sweet potato production in South Sudan in the future. Mix infection in sweet potato by sweepoviruses are common (Lozano et al., 2009; Zhang & Ling, 2011). Within virus evolution, recombination events are one of the main driving forces (Wasswa et al 2011; Albuquerque et al 2012). Thus, it is conceivable that members within the sweepovirus group have evolved due to recombination events that occurred during mixed infections. Indeed, recombination analysis show many recombination hotspots within members of this group. Therefore, there is need to investigate the diversity and distribution of sweepoviruses in South Sudan and set strategies for the control of these viruses given the increasing geographical expansion of the whitefly (*Bemisia tabaci*), a vector of begomoviruses.

SPCV was detected in one sample (6.3%) and recovery of the genome was 50.2% (Figure 2 and Table 2). This virus has been reported in the neighbouring countries of Uganda and Kenya (Aritua et al., 2007; Cuellar et al., 2011b) and in Tanzania (Cuellar et al., 2011b; Mbanzibwa et al., 2014) indicating that it is widely distributed in East Africa. Cuellar et al. (2011b), reported that co-infection of *I. setosa* with SPCV and SPCSV resulted in increased virus symptoms and titres, suggesting the possibility of the occurrence of co-infections in the field

that may contribute to yield loss. Though SPCV is detected only in one sample in this study, it is worthwhile to investigate its distribution in South Sudan.

In this study SPSMV-1 was detected in all samples tested 16 (100%) (Figure 2) indicating that it is widely distributed. Reference genome sequence coverage by virus contigs was very high (97.7%) (Table 2). Occurrence of SPSMV-1 has also been reported elsewhere in, Peru (Kreuze et al., 2009), Central America (Clark et al., 2012), Korea (Kwak et al., 2014), Tanzania (Mbanzibwa et al., 2011; Mbanzibwa et al., 2014), and China (Cao et al., 2017; Wang et al., 2015). Matreviruses have been reported to cause yield losses in crops such as maize and chickpeas (Martin & Shepherd, 2009; Schwinghamer et al., 2010; Shepherd et al., 2010). Though SPSMV-1 is widely distributed in sweet potato, its effect on yield of infected sweet potato plants has not yet been determined. Therefore, understanding the significance of SPSMV-1 on sweet potato production remains a necessity (Cao et al., 2017).

Coverage of genome sequences for SPBV-A, SPBV-B and SPBV-C by virus contigs was relatively high, 92%, 73.2% and 66.9% respectively (Table 2). However, SPBV-B was detected in all the samples sequenced 16 (100%) and SPBV-A in 15 (93.8%) whereas SPBV-C was identified in 9 (56.3%) of the samples sequenced (Figure 2). This indicates that SPBV-B and SPBV-A are more prevalent than SPBV-C in the sampled locations. In agreement with our results, SPBV-A and SPBV-B have been reported in sweet potato in Peru (Kreuze et al., 2009), Central America (Kashif et al., 2012), China (Gu et al., 2014; Qin et al., 2016), Tanzania (Mbanzibwa et al., 2011; Mbanzibwa et al., 2014), and South Africa (Nhlapo et al., 2018) while SPBV-C has been reported in Tanzania (Mbanzibwa et al., 2014) indicating that they are widespread globally. Badnaviruses have been reported to infect a broad range of horticultural crops in the tropics including banana, black pepper, cocoa, citrus, sugarcane, taro, and yam (Bhat et al., 2016). For example, *Banana streak virus* (BSV), a badnavirus, has been reported as a constraint to banana production in Uganda (Iskra-Caruana et al., 2014; Kubiriba et al., 2001). In sweet potato the economic impact of badnaviruses (*Sweet potato pakkakuy virus* (SPPV) on sweet potato production is unknown. Moreover, Kreuze et al. (2017) reported that SPPV represent a new type of vertically transmitted persistent virus living in commensal or mutualistic relationship with sweet potato which may pose little effect to sweet potato production. In their study, they found that SPPV is not integrated into the sweet potato genome, occurs at extremely low titres, and shows no significant synergistic interaction with the important sweet potato viruses. They further pointed out that SPPV is efficiently transmitted

by seed and cuttings, cause no visible symptoms on sweet potato or indicator host plants, and cannot be affected by virus elimination therapy.

3.4 Distribution and coinfection of sweet potato viruses in the five locations studied

Our results show that the 15 sweet potato virus species identified were distributed in all the locations in the three states (Table 1). SPBV-A, SPBV-B, SPMV-1 and SPLCV were the most frequently distributed viruses detected in samples from all locations. It is notable that the sweepoviruses were most prevalent in samples from Western Bhar el Ghazal state. SPGVV was also detected in a sample from Eastern Equatoria state whereas IYVV in cultivars from Central Equatoria state. The potyviruses, SPFMV and SPVC were detected in at least one of the samples collected from Eastern and Central Equatoria. The cavemovirus, SPCV, was identified in one sample from Eastern Equatoria state. This result indicates a high diversity of sweet potato virus species in the sampled locations. Interestingly, SRDS enabled us to identify sweet potato viruses in cultivars from Wau and Morobo counties (Table 1) which were ones detected negative for viruses tested by ELISA and RT-PCR.

The coinfection rate was very high in our samples. Mixed infections ranged from 5 to 10 species of viruses infecting a single plant (Figure 3). Seven (43.8) of the samples were co-infected by 5 virus species, one (6.3%) had 6 virus species, 5 (31.3%) had 7 virus species and

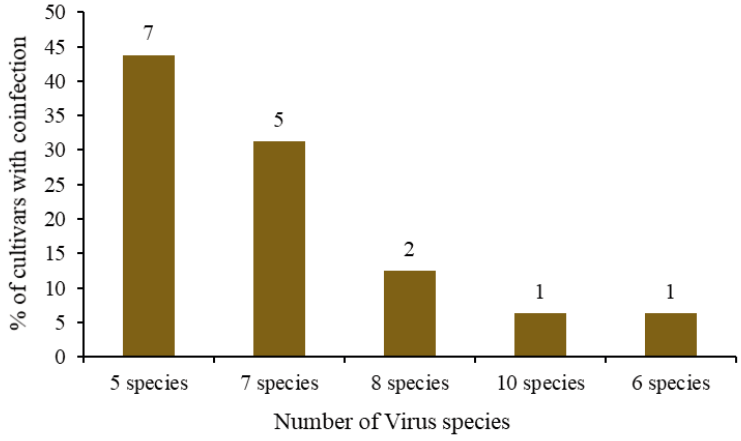

Figure 3. Proportion (%) of coinfections of sweet potato cultivars by different sweet potato viruses. The numbers above each bar indicate the number of cultivars coinfected by different sweet potato virus species.

2 (12.5%) had 8 virus species. One (6.3%) sample was co-infected by 10 species. The high rate of mixed infections observed in this study highlight the need to investigate the prevalence of sweet potato viruses in all sweet potato growing areas in South Sudan and set strategies to prevent their spread. Vegetative propagation allows accumulation of viruses in infected plants, thus can readily be disseminated through sharing of planting materials within and across regions or locations.

4. Conclusions

In this study we used SRDS for detection of viruses from 16 sweet potato cultivars from South Sudan. We were able to confirm the presence of two previously reported viruses, SPFMV and SPCSV, and the absences of three viruses: SPMMV, SPV2 and CMV previously tested for by ELISA and RT-PCR. In addition, we reported 13 more viruses including SPVC, SPLCV, IYVV, SPMaV, SPLCSPV, SPGVV, SPLCGV, SPLCUV, SPCV, SPSMV-1, and SPBV-A, SPBV-B, and SPBV-C (collectively known as SPPV). Nevertheless, two of this additionally detected viruses, SPSMV-1 and SPPV are not important sweet potato viruses. Taken together, the results support the effectiveness of deep sequencing of vsiRNAs as a useful method for simultaneous detection of viruses in sweet potato infected with multiple viruses without the need to use virus-specific primers or antibodies. Although the newly reported viruses in this study were not confirmed by RT-PCR of PCR, the genomes of most of the viruses identified were significantly covered (above 80%). Therefore, one could suggest that additional tests to verify the occurrence of these viruses in the tested cultivars may not be necessary. However, to gain understanding of the prevalence of these viruses in sweet potato in South Sudan, it is essential to conduct comprehensive survey in all sweet potato growing areas. The information provided in this study can serve as a bases for the development of virus management and control strategies in South Sudan.

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quarantine facilities for maintenance of the plant material brought from South Sudan for virus detection.

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PAPER IV

Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera:Aleyrodidae) colonizing sweet potato and cassava in South Sudan

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Abstract

Bemisia tabaci (Gennadius) is a polyphagous, highly destructive pest capable of vectoring viruses in most agricultural crops. There is currently no information available on the distribution of *B. tabaci* in South Sudan. Civil insecurity has impeded the progress of agricultural research in the country for many years, and at the time of the study reported here, data were accessible in only one geographical location. We investigated the genetic variability in 162 *B. tabaci* individuals collected from sweet potato, cassava, tomato and squash from 10 locations in Juba County, Central Equatoria State, South Sudan. Sequences of mitochondrial DNA cytochrome oxidase I (mtCOI) were used to determine the phylogenetic relationships between sampled *B. tabaci*. Six distinct genetic groups of *B. tabaci* were identified including three non-cassava haplotypes (Mediterranean (MED), Indian Ocean (IO) and Uganda) and three cassava haplotypes (Sub-Saharan Africa 1 sub-group 1 (SSA1-SG1), SSA1-SG3 and SSA2). MED predominated on sweet potato and SSA2 on cassava in all the sampled locations. The Uganda haplotype was also widespread, occurring in five of the sampled locations. SSA2 was associated with the epidemic of severe cassava mosaic disease that spread through Uganda in the 1990s but has been largely replaced by SSA1 in all other parts of East and Central Africa. South Sudan is currently the only country in sub-Saharan Africa where SSA2 continues to predominate on cassava. This study provides important information on the diversity of *B. tabaci* species in South Sudan. A comprehensive assessment of the genetic diversity, geographical distribution, population dynamics and host range of *B. tabaci* species in South Sudan is vital for effective management of its establishment and spread.

Keywords: *Bemisia tabaci*, genetic diversity, distribution, haplotype

1. Introduction

Cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas* (L.) Lam.) are key staple root crops that assure food security in sub-Saharan Africa. This is due to their high calorie content, low production inputs, adaptation to different soil types and resilience to climatic change compared to other major staple food crops (Claessens et al., 2008; Hillocks et al., 2002; Jarvis et al., 2012; Low et al., 2009). Total production of cassava in Africa amounts to 177.8 million tonnes, while that of sweet potato is 27.7 million tonnes (FAOSTAT, 2017). In South Sudan, cassava is the major food security crop after maize or sorghum in the Greenbelt and the Ironstone Plateau zones which include Western, Central and Eastern Equatoria (Greenbelt zone), Western Bahr el Ghazal State (Ironstone Plateau zone), and Lakes State (FAO/WFP, 2016). In 2015, the estimated production area for cassava was 75,910 ha and total production was 1.1 million tonnes. These estimates, however, may not represent the actual production due to the ongoing civil unrest in the country (FAO/WFP, 2016). Like cassava, sweet potato is widely grown by farmers in the cassava-producing areas of South Sudan. It is the third most widely grown crop in Eastern and Western Equatoria states after cassava and groundnut (Ntawuruhunga et al., 2007). Data on cultivation area and production of sweet potato are not available. Due to the ongoing war, agricultural surveys are mostly done by non-governmental organizations, which focus most on major staple crops like maize, sorghum, cassava and groundnuts.

One of the major biotic factors that constrains the production of cassava and sweet potato in sub-Saharan Africa is virus diseases (Alabi et al., 2008; Legg et al., 2006; Legg et al., 2014; Mukasa et al., 2003; Mukasa et al., 2006; Ngailo et al., 2016). The most important virus diseases of cassava in sub-Saharan Africa are cassava mosaic disease (CMD) caused by cassava mosaic begomoviruses and cassava brown streak disease (CBSD) caused by cassava brown streak ipomoviruses (Hong et al., 1993; Legg & Fauquet, 2004; Winter et al., 2010). The damage caused on cassava by CMD and CBSD can result in up to 82% yield losses (Legg et al., 2006; Owor et al., 2004). CMD is prevalent wherever cassava is grown. In the 1990s, CMD was reported to be the most destructive virus disease in Western Equatoria Province in Southern Sudan before the independence of South Sudan from Sudan (Dafalla & Ahmed, 2005). The cassava mosaic begomoviruses, *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), and *East African cassava mosaic virus*-Uganda (EACMV-UG) have been reported to be prevalent in South Sudan (Ntawuruhunga et al., 2007;

Tadu et al., 2006). *Sweet potato chlorotic stunt virus* (SPCSV; genus *Crinivirus*) is the most important virus affecting sweet potato due to its ability to mediate severe synergistic disease with several other sweet potato-infecting viruses which results in major yield losses (Cuellar et al., 2015; Kokkinos & Clark, 2006; Mukasa et al., 2006; Untiveros et al., 2007). SPCSV is a component of sweet potato virus disease (SPVD), the most devastating virus disease of sweet potato worldwide, which is caused by co-infection of SPCSV and *Sweet potato feathery mottle virus* (SPFMV), an aphid-transmitted potyvirus (Aritua & Adipala, 2006; Gutierrez et al., 2003; Karyeija et al., 2000; Ndunguru et al., 2009). Yield losses due to SPVD can amount to between 50% and 100% in East Africa (Mukasa et al., 2006; Ndunguru et al., 2009; Njeru et al., 2004). The occurrence of SPCSV and SPFMV has been detected in South Sudan in a survey of sweet potato viruses conducted in 2015 in the sweet potato growing areas of Western Bahr el Ghazal, Eastern and Central Equatoria states (Misaka et al., unpublished data).

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous highly destructive pest of food, fiber and ornamental crops globally (Byrne & Bellows, 1991; Gilbertson et al., 2015). It is known to damage host plants directly by feeding on phloem sap, and indirectly by excreting honeydew onto the surfaces of leaves, fruits and fiber, and transmission of plant viruses (Butler Jr et al., 1988; Byrne & Bellows, 1991; Liburd et al., 2015; Miyazaki et al., 2013; Polston et al., 2014). Honeydew secretion promotes the growth of sooty mold fungi (*Capnodium* spp.) on the surfaces of leaves and fruits and the contamination of cotton lint resulting in low quality grading (Liburd et al., 2015; Miyazaki et al., 2013). *B. tabaci* causes its most severe crop damage through vectoring plant viruses. The insect is a vector of more than 350 plant virus species belonging to five genera including begomoviruses (family *Geminiviridae*), Criniviruses (family *Closteroviridae*), ipomoviruses (family *Potyviridae*), torradoviruses (family *Secoviridae*), and some carlaviruses (family *Betaflexiviridae*). Most of the viruses transmitted are begomoviruses (Gilbertson et al., 2015; Jones, 2003; Navas-Castillo et al., 2011; Polston et al., 2014; Verbeek et al., 2014). *B. tabaci* is a vector of cassava mosaic begomoviruses (CMBs) and cassava brown streak ipomoviruses (CBSIs) that devastate cassava crops in East and Central Africa (Legg, 2010; Legg et al., 2015; Maruthi et al., 2017). SPCSV (crinivirus) is transmitted by *B. tabaci*, *B. afer* sensu lato and *Trialeurodes abutilonea* (the banded-winged whitefly) (Gamarra et al., 2010; Sim et al., 2000; Valverde et al., 2004). Other viruses of sweet potato vectored by *B. tabaci* include *Sweet potato leaf curl virus* (SPLCV), *Ipomoea leaf curl virus* (ILCV) (begomovirus) and *Sweet potato mild mottle virus* (SPMMV) (ipomovirus) (Dombrovsky et al., 2014; Hassan et al., 2016; Ling et al., 2011; Simmons et al., 2009;

Valverde et al., 2004). Outbreaks and spread of CMD and CBSD in cassava have been linked to the super-abundance of *B. tabaci* populations (Legg et al., 2006; Legg et al., 2014; Maruthi et al., 2005). Rapid spread of SPCSV has also been associated with high *B. tabaci* populations, leading to high incidences of SPVD (Aritua et al., 1998; Byamukama et al., 2004; Cohen et al., 1992).

Bemisia tabaci is genetically complex with at least 34 cryptic species that are morphologically indistinguishable. These have been identified mostly through sequencing of a portion of the mitochondrial cytochrome oxidase I (mtCOI) gene (Boykin et al., 2012; Brown, 2000; De Barro et al., 2011; De Barro, 2012; Dinsdale et al., 2010; Tay et al., 2012). In sub-Saharan Africa, *B. tabaci* is an important vector for plant viruses that infect cassava, sweet potato and other crops including tomato, cucurbits, eggplant, cotton and leguminous crops (Legg et al., 2002; Maruthi et al., 2005; Romba et al., 2018; Sseruwagi et al., 2005; Tocko-Marabena et al., 2017). Two major groups of *B. tabaci* are known to occur in sub-Saharan Africa. One group colonizes sweet potato, vegetables and other crops but does not colonize cassava (non-cassava types). This group includes several putative species, including: Indian Ocean (IO), Mediterranean (MED), Middle East Asia Minor 1 (MEAM1) and Uganda (Sseruwagi et al., 2005; Tocko-Marabena et al., 2017). The other cassava-colonizing group includes: Sub-Saharan Africa 1 to 5 (SSA1–5). SSA1 has been separated into 5 sub-groups: SSA-subgroup1 (SSA1-SG1), SSA1-SG2, SSA1-SG3, SSA1-SG4 and SSA1-SG5 (Esterhuizen et al., 2013; Ghosh et al., 2015; Gnankine et al., 2013; Legg et al., 2002; Legg et al., 2014; Mugerwa et al., 2012).

The mitochodrial DNA cytochrome oxidase I (mtCOI) marker has been the most widely used marker for phylogenetic studies of *B. tabaci*. It has a high degree of variability and has played an important role in characterizing genetic relationships between the *B. tabaci* cryptic species and haplotypes (Boykin et al., 2007; Brown, 2000; Dinsdale et al., 2010). MtCOI has been used extensively in assessing the genetic variability, phylogeographic distribution, and identification of new invasive species of *B. tabaci* in Africa (Berry et al., 2004; Esterhuizen et al., 2013; Legg et al., 2014; Sseruwagi et al., 2005), and elsewhere (Hu et al., 2011; Islam et al., 2018; Khatun et al., 2018). Recent SNP-genotyping using NextRAD sequencing, however, revealed that mtCOI sequencing is not completely effective at distinguishing cassava-colonizing *B. tabaci* genotypes (Wosula et al., 2017). These were reclassified into six major groups designated: Sub-Saharan Africa East and Central Africa (SSA-ECA), Sub-Saharan Africa East and Southern

Africa (SSA-ESA), Sub-Saharan Africa Central Africa (SSA-CA), Sub-Saharan Africa West Africa (SSA-WA), Sub-Saharan Africa 2 (SSA2) and Sub-Saharan Africa 4 (SSA4) (Wosula et al., 2017; Chen et al., 2019). Information on the occurrence and distribution of *B. tabaci* in sub-Saharan Africa is available, but there are no data for South Sudan. Assessing the nature of the problem posed by *B. tabaci* and the viruses it transmits in South Sudan and developing appropriate control strategies are currently impeded by the instability caused by the ongoing civil war. Data are urgently required on the genetic groups, haplotype diversity, geographical distribution and the phylogenetic relationships of *B. tabaci* in South Sudan. As a first step, this study sought to address this need by sampling and characterizing *B. tabaci* collected from cassava and sweet potato in Juba county, Central Equatoria State, South Sudan. We therefore aimed to provide the first description of the diversity of *B. tabaci* on sweet potato and cassava in South Sudan.

2. Materials and methods

2.1 Whitefly sampling

Adult whiteflies (*Bemisia tabaci*) were collected from sweet potato and cassava fields across 10 locations in Juba County (Central Equatoria State, South Sudan) between July and August 2018 (Table 1 and Figure 1). Whiteflies were also sampled from tomato and squash plants adjacent to sweet potato and cassava fields. *B. tabaci* adults collected from sweet potato plants in greenhouses at the University of Juba in August 2017 were also added to the field collections. In total 24 fields were sampled from the 10 locations. Whiteflies were aspirated alive and immediately preserved in 95% ethanol in vials, before being stored in the freezer at -20°C. Sweet potato and cassava leaves that contained *B. tabaci* nymphs were cut into small pieces, put into vials and also preserved in 95% ethanol before being stored in the freezer. *B. tabaci* were collected from several plants in each sampled field and at least 20 whiteflies were collected from each field.

2.2 DNA Extraction

DNA was extracted from single whiteflies, which were either adults or fourth instar nymphs. Insects were added to 3µl of lysis buffer in a 1.5ml Eppendorf tube then macerated. The lysis buffer contained 10mM Tris-HCL (pH 8.0, 50 mM KCL, 2.5 mM $MgCL_2$, 0.45% Tween-20, 0.01% Gelatine, and 60 ug/ml Proteinase). The mixture was then vortex shaken and spun down and immediately incubated on ice for 15 min. This was followed by incubation at 55˚C in a

water bath for 30 min and the lysate was stored at -20˚C for downstream use. For PCR use, the lysate was diluted using sterile DPEC treated water in a ratio of 1:9.

Table 1. Geographical information of sampling sites in Juba County, Central Equatoria State, South Sudan.

Host plant	Area/Payam	Sampling site	Latitude (˚N)	Longitude (˚E)	Elevation (masl)	Date
Cassava	Rajaf West	Lologo 2	04˚ 48.456′	031˚ 35.463′	468	31.07.2018
	Northern Bari	Lemon Gaba	04˚ 52.051′	031˚ 29.873′	487	03.08.2018
	Kondokoro	Juba Na Bari-Jezira	04˚ 51.167′	031˚ 37.430′	457	04.08.2018
	Munuki	Gudele 1 Block 7	04˚ 52.383′	031 ˚ 33.025′	478	04.08.2018
	Northern Bari	Gudele 2 Jopa	04˚ 52.852′	031˚ 31.742′	472	04.08.2018
Tomato	Kondokoro	Juba Na Bari-Jezira	04˚ 50.945′	031˚ 37.392′	460	04.08.2018
	Munuki	Gudele 1 Block 5	04˚ 52.849′	031˚ 33.770′	466	27.07.2018
	Munuki	Gudele 1 Block 7	04˚ 52.383′	031˚ 33.025′	478	04.08.2018
Squash	Rajaf East	Tokiman	04˚ 46.396′	031˚ 36.334′	460	04.08.2018
Sweet potato	Rajaf West	Beitery	04˚ 51.323′	031˚ 32.009′	508	19.07.2018
	Munuki	Mouna-Suk Hajer	04˚ 31.130′	031˚ 34.483′	490	24.07.2018
	Northern Bari	Lemon Gaba	04˚ 51.688′	031˚ 30.104′	506	25.07.0218
	Northern Bari	Lemon Gaba	04˚ 31.965′	031˚ 30.140′	490	27.07.2018
	Munuki	Gudele 1 Block 5	04˚ 52.849′	031˚ 33.770′	466	27.07.2018
	Northern Bari	Lemon Gaba	04˚ 51.850′	031˚ 30.083′	498	28.07.2018
	Rajaf West	Beitery	04˚ 51.669′	031˚ 32.117′	488	28.07.2018
	Rajaf West	Lologo 2	04˚ 48.443′	031˚ 35.564′	473	30.08.3018
	Rajaf West	Lologo 2	04˚ 46.447′	031˚ 35.496′	476	31.07.2018
	Rajaf West	Lologo 2	04˚ 48.344′	031˚ 35.302′	474	01.08.2018
	Munuki	Gudele 1 Block 5	04˚ 52.662′	031˚ 33.690′	469	02.08.2018
	Northern Bari	Lemon Gaba	04˚ 52.051′	031˚ 29.873′	487	03.08.2018
	Kondokoro	Juba Na Bari-Jezira	04˚ 50.945′	031˚ 37.392′	460	04.08.2018
	Northern Bari	Gudele 2 Jopa	04˚ 52.766′	031˚ 31.757′	470	04.08.2018
	Juba	University of Juba (Green house)	04˚ 50.327′	031˚ 35.225′	494	10.08.2017

2.3 Mitochondrial COI (MtCOI) PCR amplification and sequencing

DNA extracted from 228 individual whiteflies from all the sampled locations was used for PCR amplification. Two sets of primers were used for amplification of a partial fragment of mtCOI, primer MM1: (5′-CTGAYATRGCKTTTCCTCG-3′-F, 5′-TTACTGCAYWTTCTGCCAC-3′-R) (IITA lab) and primer set: 2195-Bt-F (5′-TGRTTTTTTGGTCATCCRGAAGT-3′) and C012-Bt-sh2-R (5′- TTTACTGCACTTTCTGCC-3′) (Mugerwa et al., 2018). These primers

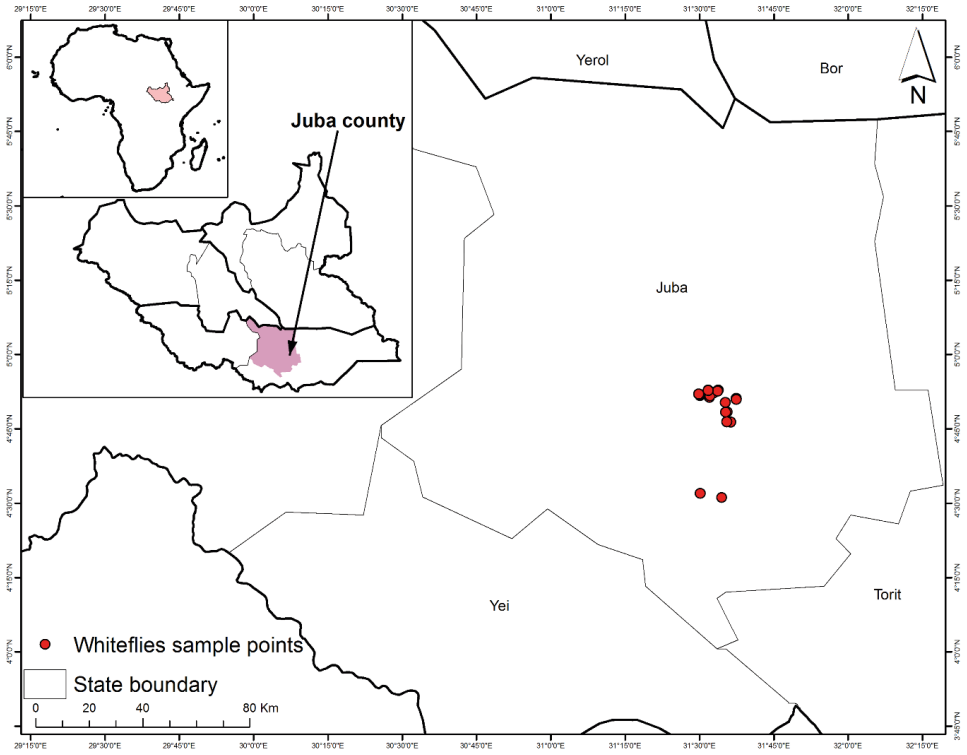

Figure 1. Map of South Sudan showing sampling sites of *Bemisia tabaci* populations on sweet potato, cassava, tomato and squash in Juba County, Central Equatoria State, South Sudan.

amplified ~1300bp and ~867bp respectively portions of the mtCOI gene. The PCR reaction contained 1X QuickLoad Master Mix (New England Biolabs, UK), 1mM $MgCl_2$, 0.24µM of each primer, 2 µl DNA, and sterile distilled water to achieve the desired reaction volume of 25 µl. PCR was carried out at 95°C for 5min initial denaturation of template DNA, followed by 35 cycles at 94°C for 40s, 56°C for 30sec for annealing, and 72°C for 90sec for extension, with a final extension at 72°C for 10min. PCR products were run on a 1% agorose gel in 1 x TAE buffer stained with GelRed™ (Biotium, Fremont, CA, USA). DNA bands were visualized using a Gel Doc™ XR+ Gel Documentation System and only samples with intact bands were selected for sequencing. PCR products were sent to Macrogen Inc. (Maryland, USA) for purification and direct sequencing. DNA sequences were manually edited using Ridom Trace Edit v1.1.0 software. Sequences were assembled into contigs using CLC Main Workbench 7.0.2 (QIAGEN, Aarhus, Denmark). Multiple alignment of edited sequences was performed

using Clustal W in MEGA version 7.0.26 (Kumar et al., 2016) and sequences were trimmed to 744 nt. Construction of a maximum-likelihood phylogenetic tree was performed using MEGA with 1000 bootstrap replicates. Sequences were blasted using GenBank's (NCBI) Blastn and selected reference sequences with 99% to 100% identity to our mtCOI sequences were included in the phylogenetic tree for comparison with previously published haplotypes. The extent of nt sequence variation within the identified *B. tabaci* groups was examined. Estimates were obtained for number of haplotypes, polymorphic sites (S), average number of nucleotide differences (k), nucleotide diversity (Pi), haplotype diversity (Hd), Theta per sequence and Theta per site and significance values using the mismatch distribution procedure of Dna-SP 6.12.03 (Librado & Rozas, 2009). To determine whether sampled whitefly populations were stable or expanding, Tajima's D and Fu's Fs were calculated using Dna-SP 6.12.03.

3. Results and Discussion

B. tabaci whiteflies samples were collected from sweet potato (*Ipomeoa batata* L.), cassava (*Manihot esculenta* Crantz), tomato (*Solanum lycopersicum* L.) and squash (*Cucurbita pepo* L. 'Zucchini') from 10 locations in Juba County, Central Equatoria State, South Sudan. The locations included Tokiman (TOK), Lologo 2 (LO2), Beitery (BET), Juba Na Bari-Jezira (JNB-JZ), Mouna Suk Hajer (MO-SH), Gudele 1 Block 5 (GU1-B5), Gudele 1 Block 7 (GU1-B7), Gudele 2 Jopa (GU2-JP), Lemon Gaba (LEG), and University of Juba (UOJ). *B. tabaci* colonizing sweet potato were collected from 8 locations except Tokiman and Gudele 1 Block 7. *B. tabaci* on cassava were sampled from 5 locations: Lologo 2, Juba Na Bari-Jezira, Gudele 1 Block 7, Gudele 2 Jopa, and Lemon Gaba. On tomato, *B. tabaci* were collected from three locations including Juba Na Bari-Jezira, Gudele 1 Block 5 and Gudele 1 Block 7, whereas whiteflies on squash were collected from Tokiman (Table 2). *B. tabaci* fourth instar nymphs were also collected from sweet potato in Lologo 2 and from cassava in Gudele 1 Block 7. Most of the whiteflies sampled were from sweet potato and cassava, the main targeted crops of this study. As a result, the whiteflies collected from tomato and squash were from fields adjacent to either sweet potato or cassava plantings.

In total 183 whitefly samples were sequenced, out of which 162 produced high quality mtCOI sequences. There was a high level of diversity among *B. tabaci* populations collected from the sampled crop plants. The sequences obtained from sweet potato, tomato and squash grouped into three phylogenetically distinct groups, which included (MED), Indian Ocean (IO) and Uganda. The sequences from cassava were grouped into three distinct groups, SSA1-SG1,

Table 2. Number of *B. tabaci* sequences obtained from sampled locations and host plants in Juba County, Central Equatoria State, South Sudan.

Location	Host Plant				
	Sweet potato (Sp)	Cassava (Ca)	Tomato (To)	Squash (Sq)	Total
Tokiman (TOK)	-	-	-	4	4
Logogo 2 (LO2)	20	3	-	-	23
Beitery (BET)	9	-	-	-	9
Juba Na Bari-Jezira (JNB-JZ)	3	6	2	-	11
Mouna-Suk Hager (MO-SH)	5	-	-	-	5
Gudele 1 Block 5 (GU1-B5)	6	-	11	-	17
Gudele 1 Block 7 (GU1-B7)	-	41	2	-	43
Gudele 2 Jopa (GU2-JP)	4	1	-	-	5
Lemon Gaba (LEG)	25	6	-	-	31
Univeristy of Juba (UOJ)	14	-	-	-	14
Total	**86**	**57**	**15**	**4**	**162**

SSA1-SG3, and SSA2 (Figure 2). These groups were identified based on the topology of the phylogenetic tree and the clustering of the sequences obtained from this study relative to the reference sequences retrieved from GenBank. The predominant haplotype MED had a total of 90 whiteflies which accounted for 55.5% of all the whiteflies collected from the four host plants. Of these, 72 whiteflies (44.4%) were found on sweet potato (Table 3). The second most abundant haplotype was SSA2 with 43 whiteflies (26.5%), all of them found on cassava. SSA1-SG1 was a second haplotype found only on cassava for which there were 13 whiteflies (8%). The other haplotype was Uganda which was present on sweet potato and had a total of 11 whiteflies (6.8%), Indian Ocean with 4 whiteflies (2.5% and SSA1-SG3 which was the least frequent haplotype with only 1 whitefly (0.6%) found on sweet potato (Table 3). A total of 45 selected sequences representing haplogroups found in this study have been submitted to GenBank under the following accession names (MN318379 - MN318423).

The clustering of the whiteflies SSA2, SSA1-SG1 and SSA1-SG3 into a distinct major clade separate from *B. tabaci* whiteflies that do not colonize cassava is consistent with what has been reported in other studies of *B. tabaci* from various cassava-growing countries in Africa (Sseruwagi et al., 2005; Tocko-Marabena et al., 2017; Wosula et al., 2017). The grouping of MED and Indian Ocean haplotypes is also consistent with what has been reported in previous

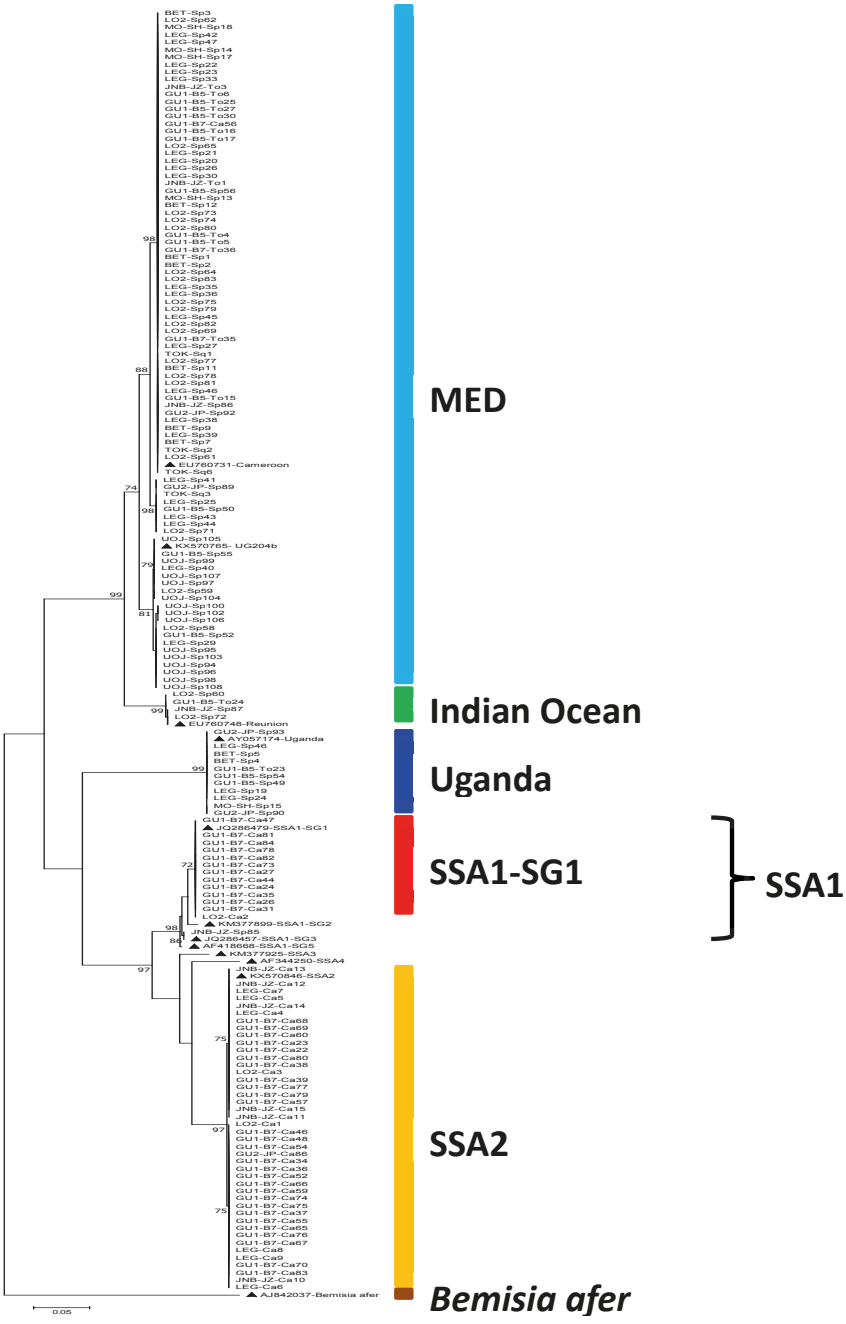

Figure. 2. Maximum likelihood phylogenetic tree constructed for mtCOI sequences obtained from *Bemisia tabaci* collected from 10 locations in Juba County, central Equatoria State, South Sudan between July and August 2018. Reference sequences from GenBank (▲) are included for comparison.

studies (Sseruwagi et al., 2005; Tocko-Marabena et al., 2017). Uganda, which was depicted by a clearly defined monophyletic grouping in our mtCOI sequence analysis has previously been identified as a genetically distinct haplotype occurring in East Africa (Hadjistylli et al., 2016; Legg et al., 2002).

Table 3. *Bemisia tabaci* haplotype groups (numbers and percentages) on four host plants from Juba County, Central Equatoria State, South Sudan.

B. tabaci	Host plants				
haplotypes	Sweet potato	Cassava	Tomato	Squash	Total
MED	72 (44.4%)	1 (0.6%)	13 (8.0%)	4 (2.5%)	90 (55.5%)
Indian Ocean	3 (1.9%)	-	1 (0.6%)	-	4 (2.5%)
Uganda	10 (6.2%)	-	1 (0.6%)	-	11 (6.8%)
SSA1-SG1	-	13 (8.0%)	-	-	13 (8.0%)
SSA1-SG3	1(0.6%)				1(0.6%)
SSA2	-	43 (26.5%)	-	-	43 (26.5%)
Total	86	57	15	4	162

We found that *B. tabaci* MED was predominant on sweet potato, tomato and squash in all sampled locations. MED is a globally important *B. tabaci* haplotype group which is thought to have originated from Africa. Consequently, there are numerous other reports of its prevalence on a wide range of crop and weed hosts (Sseruwagi et al., 2005; Gnankine et al., 2013; Romba et al., 2018). *B. tabaci* MED has been reported to be extremely polyphagous and invasive (De Barro et al., 2011), causing damage to both field and greenhouse crops (Horowitz et al., 2003). It has also developed resistance to various insecticides under intensive production systems (Horowitz & Ishaaya, 2014; Roditakis et al., 2009; Wang et al., 2010). The presence of *B. tabaci* MED in all locations and on all sampled crop plants in our study in South Sudan suggests that this haplotype is an important pest of sweet potato and other crops in Juba County and likely also in other parts of South Sudan. Moreover, since SPCSV transmitted by *B. tabaci* is one of the most important viruses affecting sweet potato in this region of East Africa (Mukasa et al 2006), it is likely that this is the main vector of this virus in South Sudan. Future investigations, however, should determine the relative abilities to transmit SPCSV of each of the three *B. tabaci* haplotypes occurring on sweet potato. Since no similar studies have been conducted anywhere else in sub-Saharan Africa, this represents an important gap in the existing

understanding of the relationship between *B. tabaci* haplotype groups and the viruses that they vector.

MED haplotype analyses revealed six haplotypes amongst the samples collected from South Sudan (Table 4). Two of these are previously described African MED haplotypes, whilst the other four are new unique haplotypes falling within the MED group. Haplotype diversity (0.51), nucleotide diversity (0.012) and a positive significant Tajima's D (2.07283: $P < 0.05$) suggest that the population is undergoing balancing selection and has not undergone rapid recent expansion. Sixty-two of the 90 MED sequences (Haplotype 1) represent an important African MED haplotype for which there are a further 17 sequences in GenBank from Cameroon, Uganda and Nigeria. The samples in this haplotype were predominantly from sweet potato, although there were also individuals from tomato, squash and cassava, indicating that it could be sharing host plants. Haplotype 2 had 8 sequences from sweet potato which were identical to 13 sequences from GenBank originating from Sudan, Cameroon, Uganda and Burkina Faso. Haplotype 3 had 8 sequences from sweet potato and squash. These were most closely matched (99.7%) with a GenBank sequence from Uganda KX570768. Haplotypes 4 and 5 occurred on sweet potato and had 8 and 3 sequences respectively. These were most closely related (99.7%) to a sequence from China (MH908653). Haplotype 6, recorded from sweet potato, had 1 sequence sharing 99.9% homology with MH908653 from China. Currently GenBank hosts 944 MED sequences that comprise 168 haplotypes. 673 (71%) of the sequences cluster in three major haplotypes that are spread worldwide. Of the 168 haplotypes, 137 (81%) have only one sequence in GenBank, although it is possible that some of these may erroneously be considered as unique haplotypes due to the frequent occurrence of sequencing errors in mtCOI data submitted to this database.

In this study, *B. tabaci* Indian Ocean were collected from sweet potato and tomato, and their sequences were most closely related to Reunion 1 from Spain (Gueguen et al., 2010), although *B. tabaci* Indian Ocean has also been widely reported from sub-Saharan Africa and the surrounding islands (Delatte et al., 2011; Mugerwa et al., 2012; Sseruwagi et al., 2005). Haplotype analysis revealed the existence of two Indian Ocean haplotypes. Uganda haplotype sequences were obtained from several whiteflies collected from sweet potato and one individual from tomato. The South Sudan 'Uganda' haplotype sequences were identical to the original Uganda haplotype sequence also obtained from a whitefly adult collected from sweet

Table 4. Population genetic analysis of *Bemisia tabaci* groups from Juba county, Central Equatoria state, South Sudan.

Parameter	All	MED	SSA2	SSA1-SG1	Uganda	Indian Ocean	SSA1-SG3
Sample size	162	90	43	13	11	4	1
Number of haplotypes	13	6	2	1	1	2	1
Polymorphic sites (S)	212	28	3	0	0	1	-
Average number of nucleotide differences (k)	73.45702	9.26367	1.52824	0.000	0.000	0.66667	-
Nucleotide diversity (Pi)	0.09873	0.01245	0.00205	0.000	0.000	0.00090	-
Haplotype diversity (Hd)	0.804	0.506	0.509	0.000	0.000	0.667	-
Variance of Hd	0.00054	0.00356	0.00039	0.000	0.000	0.04167	-
Standard deviation of Hd	0.023	0.060	0.020	0.000	0.000	0.204	-
Theta per sequence	45.21592	5.52109	0.69336	-	-	0.54545	-
Theta per site	0.06077	0.00742	0.00093	0.000	0.000	0.00073	-
Fu's Fs statistic	96.201	16.737	5.448	-	-	0.540	-
Tajima's D	2.01806	2.07283	2.57824	-	-	1.63299	-
P	0.10 > P > 0.05	P < 0.05	P < 0.05			P > 0.10	

potato in Uganda (33NamSP-AY057174) (Legg et al., 2002). Sseruwagi et al. (2005), however, reported the occurrence of sweet potato Uganda haplotype on crop plants other than sweet potato, and Wainana (2019) made similar observations from western Kenya, noting the presence of the Uganda haplotype on common bean as well as sweet potato. These data suggest that this haplotype is confined to East Africa and has a relatively narrow host range, specializing on sweet potato. Our results represent the northernmost record of haplotype 'Uganda' and the third country report.

In the cassava group of *B. tabaci* studied, the largest number of samples were SSA2, and these were distributed through all locations. Two SSA2 haplotypes were identified (Table 4). Haplotype diversity (0.509), nucleotide diversity (0.00205) and a positive significant Tajima's D (257824: $P < 0.05$) suggest that the population has not undergone recent expansion but is instead experiencing balancing selection. SSA1-SG1 was less frequent as it was only detected at two locations and comprised only one haplotype. These results differ from other recent findings from East and Central Africa which have shown SSA1-SG1 to be the predominant *B. tabaci* haplotype on cassava (Legg et al., 2014; Tocko-Marabena et al., 2017; Wosula et al., 2017). SSA2, which was previously associated with the severe CMD epidemic in Uganda (Legg et al., 2002), has been reported to be absent in more recent whitefly collections from cassava in Uganda and western Kenya (Mugerwa et al., 2012; Sseruwagi et al., 2006), and replaced by SSA1-SG1 (Legg et al., 2014), although low frequencies of this haplotype were reported from Uganda and Kenya between 2004 and 2010. Recent studies have noted the occurrence of SSA2 on cassava in western Kenya and weedy hosts in Uganda (Manani et al., 2017; Mugerwa et al., 2018; Chen et al., 2019). The detection of SSA1-SG1 and SSA2 fourth instar nymphs of *B. tabaci* confirms that both haplotypes colonize cassava in South Sudan. A recent continent-wide assessment of cassava-colonizing *B. tabaci* in sub-Saharan Africa noted that SSA2 was the most widely distributed of the haplotypes recorded (Chen et al., 2019). Significantly, this haplotype co-occurs with others throughout its geographic range (stretching from Sierra Leone in West Africa to Kenya in the East), but in all cases it appears to be less frequent than SSA1 haplotypes. Our data suggest that South Sudan could be an exception to this pattern, since there were more than three times as many SSA2 individuals recorded compared to those of SSA1-SG1.

In the 1990s, CMD was reported to be highly destructive in the Western Equatoria Province of pre-independence southern Sudan (Dafalla & Ahmed, 2005). Furthermore, in a baseline survey

conducted on cassava in 2005 in Eastern and Western Equatoria states, African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), and East African cassava mosaic virus-Uganda (EACMV-UG) were found to be the viruses affecting cassava (Ntawuruhunga et al., 2007). SSA2 was shown to be the most abundant *B. tabaci* haplotype in areas affected by the severe CMD epidemic which spread through Uganda in the 1990s. It is quite likely that there may have been a similar association between virus and vector in southern Sudan during this period. However, whilst SSA1-SG1 subsequently displaced SSA2 as the predominant *B. tabaci* haplotype on cassava in Uganda, this change may not have happened further north in southern Sudan, with the result that SSA2 is currently the main cassava-colonizing *B. tabaci* haplotype in present day South Sudan. The reasons behind these contrasting patterns of population change in Uganda and South Sudan are not currently apparent but would be a useful topic for future study.

In this study, a single individual of non-cassava *B. tabaci* haplotype MED was collected from cassava. These rare occurrences have been reported elsewhere (Berry et al., 2004; Sseruwagi et al., 2006; Tocko-Marabena et al., 2017). However, previous studies have demonstrated that non-cassava *B. tabaci* whiteflies are unable to reproduce on and colonize cassava (Legg, 1996) partly since they are unable to feed effectively on cassava plant hosts (Milenovic et al., 2019). In each of these instances, it has been concluded that whiteflies of non-cassava *B. tabaci* haplotypes occurring on cassava are present as visitors and not colonizing the crop.

4. Conclusions

This study presents the first report on the genetic diversity of *B. tabaci* whitefly populations collected from South Sudan. Six *B. tabaci* haplotype groups, including three non-cassava groups (MED, Indian Ocean and Uganda) and three cassava groups (SSA1-SG1, SSA1-SG3 and SSA2) were identified. MED and SSA2 were the most prevalent and most widely distributed amongst the sampled locations. The Uganda haplotype is also widespread and was identified from five of the locations. The discovery of six *B. tabaci* haplotype groups from the relatively small portion of South Sudan that was sampled does suggest that, like Uganda, this part of East Africa has a high level of whitefly diversity. This provides a strong indication that this part of Africa may have been a source for MED whiteflies that have had devastating global impacts as an invasive pest (Ramos et al., 2018). It is also significant that the MED species group of *B. tabaci* includes some of the most insecticide-resistant populations of whiteflies. Therefore, although *Bemisia* whiteflies may not be present on sweet potato and other host

plants at high abundance levels, any future management efforts will need to apply extreme caution in the application of chemical insecticides in order to preclude the development of whitefly resistance. Whitefly populations observed in South Sudan were associated with transmission of viruses causing damaging disease in cassava and sweet potato. Improving understanding of the dynamic interactions between vector and virus will be important for each of these crop-virus-vector pathosystems. An essential first step in this task will be conducting a comprehensive assessment of the genetic diversity, geographical distribution, population dynamics and host range of *B. tabaci* species in South Sudan. This new knowledge will then provide the basis for the development of effective whitefly management strategies.

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