

EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF SPFMV, SPVG AND SPLCV INFECTING SWEETPOTATO IN SOUTH AFRICA

Mapula Julia Mulabisana

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, in
fulfilment of the requirements for the degree of Doctor of Philosophy in the School of
Molecular and Cell Biology.

Johannesburg, September 2018

DECLARATION

I, Mapula Julia Mulabisana (693857), am a registered student for the degree of Doctor of Philosophy (MCBG 9002) in the academic year 2018. I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where explicitly indicated otherwise and acknowledged.
- I have not submitted this work before for any other degree or examination at this or any other University.
- The information used in the Thesis has been obtained by me while employed by the Agricultural Research Council (ARC), Pretoria, South Africa.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signature



27 day of September 2018

ACKNOWLEDGEMENTS

- I would like to acknowledge my supervisors, Dr Michele Cloete (ARC) and Professor Chrissie Rey (University of the Witwatersrand) for guiding and training me throughout the duration of the study. Professor Rey, thank you for editing the manuscripts and the invaluable knowledge you shared with me.
- Dr Dean Oelofse (ARC) and Dr Sonja Venter, I am grateful for your support and encouragement throughout the study. Your guidance did not go unnoticed.
- I sincerely appreciate the help of Dr Sunette Laurie who took interest in the study and supported it to the end. For pushing me to go forward when things seemed impossible.
- Dr Mariette Truter who taught me how to analyse sequences with patience and it was through her that I developed interest in working with virus sequences.
- Many thanks are owed to Mr Kenneth Mabasa, Ms Mercy Maserumule, Ms Whelma Mphela, Ms Midana Mmboyi and the late Mr Elias Thobejane for their support with surveys and assisting in field trials and in the laboratory.
- Dr Martha Pofu, Dr Rene Sutherland, Dr Inge Gazendam and Ms Nokuthula Myeza, you were all very supportive. Your words of encouragement helped me to get through this process.
- The ARC for giving me the opportunity to study. The Gauteng Department of Agriculture and Rural Development (GDARD), the International Foundation for Science (IFS) (C/5312-1), the National Research Foundation-Technology and Human Resources for Industry Programme (NRF-THRIP) (UID 79983) and NRF-Thuthuka PhD track (Grant 99368) for funding the project.
- The Almighty God for his grace, wisdom and strength and seeing me through when my brains could not stretch any longer.

DEDICATIONS

- To my husband, Thomas, our children, Thendo Kaitlin, Zawadi Ndalamo and Thomani Ramaano Mulabisana for their understanding, support and encouragement throughout the study. Studies imposed that I neglect you while I was trying to make the best out of this and you all persevered.
- My late grandmother, Rahaba, who raised me with love to be the grand daughter that I am today.
- To my mother, Masindi Gloria Domola, Father, Simon Domola, younger sister Dakalo Happy and my late brother, Ndivhuho Ronald Domola. I know that you are all proud of me.

RESEARCH OUTPUTS

Manuscripts published in Conference Proceedings and peer reviewed scientific journals

- Mulabisana, M.J., Cloete, M., Mabasa, K.G., Laurie, S.M., Oelofse, D., Esterhuizen, L.L. & Rey, M.E.C. 2018. Surveys in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa reveal novel isolates of sweetpotato viruses. *South African Journal of Botany* 114: 280-294.
- Mulabisana, J., Cloete, M., Mabasa, K., Laurie, S.M., Oelofse, D. & Rey, M.E.C. 2015. Genetic characterization of viruses infecting sweetpotato in South Africa. *Acta Horticulturae*, 1105, 155-161. ISHS. DOI 10.17660/ActaHortic.2015.1105.22.
- Laurie, S.M., Myeza, P.N., Mulabisana, M.J., Mabasa, K.G., Thompson, A., Greyling, R., Cloete, M. & Adebola, P.O. 2016. In vitro propagation and disease testing as a means of producing healthy planting materials to support root and tuber crops production in South Africa. *Acta Horticulturae*, 1113. ISHS 2016. DOI 10.17660/ActaHortic.2016.1113.34.

National and international conference presentations and symposiums

- Mulabisana, J., Cloete, M., Mphela, W., Laurie, S.M., Oelofse, D & Rey, M.E.C. 2017. Effects of single and co-infections of SPFMV, SPVG, and SPLCV on the storage root yields of local sweetpotato varieties. A poster presented at the 50th Anniversary Congress of the Southern African Society for Plant Pathology, Champagne Sports Resort, Drakensberg, 15-18 January 2017.
- Mulabisana, M.J, Cloete, M., Mabasa, K., Laurie, S., Oelofse D. & Rey, M.E.C. 2014. Genetic characterization of sweetpotato viruses infecting sweetpotatoes in South Africa. An oral presentation presented at International Horticultural Conference (IHC), Brisbane Convention Centre, Brisbane, Australia, August 2014.

- Mulabisana J., Cloete M, Laurie S, Mabasa, S., Esterhuizen, L. & Rey, M.E.C. 2013. Control measures for virus in sweetpotato: screening for virus resistance and optimisation of rapid diagnostic nucleic acid based detection methods. A poster presented at the 48th congress of the Southern African Society for Plant pathology, ATKV Klein Kariba, Bela Bela, Limpopo Province.
- Mulabisana, J., Cloete, M., Mabasa, K., Rakuambo, J. & Netshifhefhe, S. 2012. The optimisation and implementation of a rapid diagnostic nucleic acid based test for the detection of sweetpotato feathery mottle virus. A poster presented at Second All Africa Horticulture Conference, Skukuza, January 2012.

ABSTRACT

Root crops such as sweetpotato [*Ipomoea batatas* (L.) Lam.] are highly regarded as alternative food sources to address food insecurities in South Africa and other African countries. Sweetpotato viruses, commonly occurring in mixed infections of viruses of different taxonomic groups are a threat to production of sweetpotato. Potyviruses, belonging to the *Potyviridae* family are virus species *Sweet potato feathery mottle virus*, *Sweet potato virus G*, *Sweet potato virus C* and *Sweet potato virus 2* and these have been reported to occur in South Africa. Crinivirus species *Sweet potato chlorotic stunt virus*, belonging to the *Closteroviridae* family was also reported. Monopartite, single stranded DNA begomoviruses are also a threat to sweetpotato production globally. All these viruses were detected in South Africa in previous studies. Symptoms associated with potyviruses include mild chlorotic spots and in severe cases, plants may develop some chlorotic spots with purple pigmentations, mild vein banding and in some cases, plants may be symptomless. Symptoms caused by crinivirus SPCSV alone are relatively mild on sweetpotato and plants may become mildly stunted. Plants infected with begomoviruses may exhibit mild upward curling of the leaves, however, it is reported that plants may also appear symptomless in the field. Begomovirus species *Sweet potato leaf curl Sao Paulo virus* and *Sweet potato mosaic virus* were reported to occur in the Limpopo Province of South Africa in 2012. This led to field surveys being conducted to determine the prevalence of potyviruses and begomoviruses in the three provinces of South Africa, namely, Limpopo, Gauteng and Mpumalanga. Furthermore, to characterize Sweet potato feathery mottle virus (SPFMV), Sweet potato virus C (SPVC), Sweet potato virus G (SPVG) and Sweet potato leaf curl virus (SPLCV) by means of partial genome cloning and sequencing, and full genome sequencing using next generation sequencing (NGS). Surveys were conducted over two seasons and a total sample number of 272 (2012/13) and 332 (2013/14) of sweetpotato samples were grafted onto the indicator plant *Ipomoea setosa* [*I. setosa* (Kerr.)] Kerr to observe for

virus related symptoms. Virus disease incidences were low and these were 18% (Limpopo Province), 19% (Mpumalanga Province) and 23% (Gauteng Province) in season 1, 4% (Limpopo Province), 21% (Gauteng Province) and 22% (Mpumalanga Province), however, this varied in areas within the three provinces surveyed. Infestations of adult aphids and whiteflies as virus vectors were low (1-5 insects score equivalent to low infestation). In spite of low virus disease incidences in the field, and low infestations of aphids and whiteflies, viruses were identified in both asymptomatic and symptomatic samples grafted onto *I. setosa* using nitrocellulose enzyme linked immunosorbent assay (NCM-ELISA), double antibody ELISA, reverse transcription polymerase chain reaction (RT-PCR) and PCR to verify the identity of viruses. The incidence of SPFMV, either as single or co-infection with SPVC was 52% and 16% in season 1 and 2, respectively. SPVG (1.5% and 35%), SPCSV (2% and 16%), Sweet potato mild mottle virus (SPMMV) (3% and 5%), SPV2 (3% and 8%) and Sweet potato mild speckling virus (SPMSV) (2% and 2.4%) were also detected, in season 1 and 2, respectively. Other viruses detected include Sweet potato chlorotic fleck virus (SPCFV) and Sweet potato latent virus (SwPLV) in low (0.6 to 1%) incidences in both seasons. Viruses were also detected in single infections with the most common single infection being that of SPFMV. Mixed infections of SPFMV and SPVG were common and those of SPFMV/SPVG and SPCSV were also detected.

There was a need to characterize and to determine the genetic variability of potyviruses SPFMV, SPVC and SPVG, and to investigate the possible occurrences of begomoviruses in the three provinces since such information was lacking. Genetic characterization was accomplished by partial genome sequencing for potyviruses (SPFMV, SPVC and SPVG) and full-length genome sequencing for begomovirus isolates. A total of 5 (SPVC), 4 (SPFMV), 6 (SPVG) and 9 (begomoviruses) isolates were sequenced and analyzed. Phylogeny, pairwise

comparison and analysis of the coat protein (CP) encoding amino acid (aa) sequences revealed that the russet crack (RC) strain of SPFMV in this study shared 97% to 99% nucleotide (nt) identity with isolates reported in other parts of the world and that it was the only strain of SPFMV detected in the three provinces surveyed. SPVC isolates in this study were also confirmed to share the nt identity of between 86% to 94% with isolates reported in South Africa (Kwazulu Natal Province, Western Cape Province) and other parts of the world. SPVG was also confirmed to occur in the three provinces surveyed and minor sequence variability at nt level were observed between isolates in this study and those reported in Kwazulu Natal Province. However, analysis of the CP encoding aa sequences confirmed that all analysed isolates are the same strain of SPVG. Rolling circle amplification (RCA) and full-length genome sequencing with next generation sequencing (NGS) detected begomoviruses. Sweet potato leaf curl SaoPaulo virus (SPLCSPV) and Sweet potato mosaic virus (SPMV) were confirmed to occur in the Gauteng Province. SPLCSPV isolate Tanz: ELLP_ZA (Limpopo Province) and SPLCSPV isolate Tanz: NMP-ZA (Mpumalanga Province) were related to SPLCSPV isolate TZ-SNG7:12 of Tanzania, with which they shared the nt identity of 94% and 95%, respectively. TMBLP-ZA was identified as a novel isolate of SPLCV and we propose that it is classified as SPLCV isolate South Africa (ZA): TMBLP-ZA. The effects of begomoviruses SPLCSPV/SPLCV and SPMV on the yield of sweetpotato varieties is not widely studied and this led to the study expanding to determine the effects of these viruses as mixed infections of two begomoviruses and potyviruses. Two field trials were planted in January 2014 and 2015 with eight locally bred sweetpotato varieties (Bophelo, Impilo, Ndou, Monate, Mvuvhelo, Blesbok, Bosbok and Ribbok), three from the United States of America (USA) (W-119, Hernandez and Resisto) and one (199062.1) from the International Potato Centre (CIP, Lima, Peru). Single and co-infections of potyviruses and begomoviruses in sources of virus combinations were confirmed by NCM-ELISA, RT-PCR, PCR and NGS.

Virus treatments (T) were as follows: T1= co-infections of SPFMV (RC strain) + SPVG + SPVC + begomoviruses (SPLCSV + SPMV), T2 = co-infections of SPFMV (RC strain) + begomoviruses, T3=single infection of SPFMV (RC strain), T4= co-infection of SPLCSV + SPMV. The total and marketable yield of all 12 varieties was significantly reduced by treatments T1 and T2, and the yield was severely decreased in trial 2 with all the treatments. Treatments T3 and 4 also reduced the total and marketable yield, however, varieties differed in their responses to these treatments. The total yield reduced with T3 were between 27% and 78% in trial 1, and varieties such as Bophelo, Hernandez, Impilo and Ribbok had yield reductions of less than 50%, when compared to the other eight varieties. However, the yields were severely reduced in trial 2 (61% to 99%). Total average yield were also reduced with begomovirus co-infections (T4) and yield losses were between 32% and 84% in trial 1, and varieties such as Bophelo, Bosbok, Mvuvhelo, Ndou and Resisto had yield reductions of less than 50%, when compared to the other seven varieties. Production of low and poor quality yield in sensitive sweetpotato varieties in South Africa was confirmed to be due to begomoviruses as mixed infection of SPLCSPV/SPLCV and SPMV, in mixed infections with potyviruses. The findings in this study of seven distinct virus species, is significant as it is associated with high yield losses. The quality of sweetpotato is therefore compromised, which ultimately affect the livelihoods of those who depend on the crop for household consumption and as a source of income. Stringent disease management strategies to prevent virus spread is urgently needed and farmers to be taught how prevent the spread. Furthermore, the government need to implement robust quarantine regulations to prevent new virus strains from entering the country.

LIST OF ABBREVIATIONS

aa	Amino acids
Anova	Analysis of variance
bp	Base pairs
ds	Double stranded
cDNA	Complementary DNA
cm	Centimeters
CP	Coat protein
CIP	International Potato Centre
DAS-ELISA	Double antibody ELISA
DEPC	Diethyl pyrocarbonate-treated
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EM	Electron microscopy
GPS	Global Positioning System
HC-Pro	Helper component-proteinase
IPTG	isopropyl B-D-1-thiogalactopyranoside
kb	Kilobase pairs
LAN	Limestone Ammonium Nitrate
LB Broth	Luria-Bertani Broth
LSD	Least significance difference
ml	Mililitres
M	Molar
Mm	Millimolar
min	Minutes
NCM-ELISA	Nitrocellulose membrane ELISA
nm	Nanometer
NaCl	Sodium chloride
nt	Nucleotides
NGS	Next generation sequencing

NPK	Nitrogen (N): Phosphorus (P): Potassium (K)
OD	Optical density
ORF	Open reading frames
PBS	phosphate buffered saline
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
REN	Replication enhancer protein
RCR	Rolling circle replication
ss	Single stranded
SPFMV	Sweetpotato feathery mottle virus
SPMMV	Sweetpotato mild mottle virus
SPVG	Sweetpotato virus G
SPV2	Sweetpotato virus 2
SPCSV	Sweetpotato chlorotic spot virus
SPLCV	Sweetpotato leaf curl virus
SPLCSPV	Sweetpotato leaf curl Sao Paulo virus
SPMV	Sweetpotato mosaic
ton/ha	Tones per hectare
TrAp	Transcriptional activator protein
TBS	Tris buffered saline
UTR	Untranslated region
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside
μ g	Microgram (s)
μ l	Microlitres

Table of Contents

Declaration.....	ii
Acknowledgement	iii
Dedication	iv
Research outputs.....	v
Abstract.....	vii
LIST OF ABBREVIATIONS.....	ix
LIST OF FIGURES	
Fig. 1.1. Genomic map of a typical member of the genus Potyvirus.	8
Fig. 1.2. Symptoms of sweetpotato viruses as observed on sweetpotato plants and grafted <i>I. setosa</i> plants.	11
Fig. 1.3. Genome organization of four genera belonging to the family Geminiviridae and satellites (beta-satellite and alpha-satellite) associated with begomovirus.....	19
Fig. 2.1. Map of South Africa showing Limpopo, Gauteng and Mpumalanga Provinces.....	75
Fig. 2.2. Systemic symptoms associated with potyviruses and begomoviruses as recorded on indicator plant <i>I. setosa</i> post graft inoculations.....	88
Fig. 2.3. Gel electrophoresis image of the PCR generated with the DNA of representative <i>I. setosa</i> tested.....	89
Fig. 2.4. Gel electrophoresis image showing the RCA products and the restriction digestion of selected samples with <i>Bam</i> HI.....	90
Fig. 2.5. Phylogenetic analysis [Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000)], of the NIb/coat protein/3' UTR encoding nt sequences.....	91
Fig. 2.6. Phylogenetic analysis [Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000)] of the NIb/coat protein/3' UTR encoding nt sequences.....	92
Fig. 2.7. Phylogenetic tree [Maximum Likelihood method [(1000 bootstrap replicates) based on the General Time Reversible model (Nei and Kumar, 2000)] based on the 5'-proximal part of the Nib and CP-encoding region nucleotide sequences of SPVG isolates (bold*).....	94
Fig. 2.8. Alignment of the CP N-terminal aa sequences of SPFMV (RC, O and EA strains) and SPVC samples from this current survey (illustrated by boxes with X crosses) with reference sequences.....	96

Fig. 2.9. Phylogenetic tree [Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000) with 1000 bootstrap replicates] of NCBI Genbank reference viruses compared with full length genome nt sequences of SPLCSPV and SPLCV isolates: TZ:ELLP-ZA (Limpopo Province), TZ:NMP-ZA (Mpumalanga Province); SSB1-ZA (Gauteng Province), RDGP-ZA (Gauteng Province) and ZA:TMBLP-ZA (Limpopo Province), Bootstrap values are shown next to the branches.....	100
Fig. 3.1. Indicator plant <i>I. setosa</i> (A) grafted with sweetpotato scion of a sample collected from Verena in Mpumalanga Province.....	123
Fig. 3.2. Symptoms of sweetpotato viruses observed during season 1 (2012/2013) and season 2 (2013/2014) surveys conducted in the three provinces of South Africa.....	125
Fig. 3.3. Symptoms of sweetpotato viruses as observed on <i>I. setosa</i> post-graft inoculations of representative samples collected from Gauteng, Limpopo and Mpumalanga provinces.....	127
Fig. 3.4. NCM-ELISA results conducted with samples collected from different regions in the Mpumalanga Province during season 1 (2012/2013) survey.....	128
NCM-ELISA of representative samples collected from Tshiombo in Limpopo Province during season 2 (2013/2014) surveys, which shows samples that tested positive or negative for (A) SPFMV, (B) SPCSV and (D) SPVG.....	129
Fig. 4.1. Molecular phylogenetic analysis inferred using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000) to determine relationships between the South African (bold) (A) SPFMV (RC strain) and SPVC, and (B) SPVG and SPV2 with the related full-length genome sequences retrieved from Genbank.....	157
Fig. 4.2. Symptoms recorded on representative sweetpotato varieties in field trials (2014 trial) planted to determine the effects of SPFMV-RC*SPVC*SPVG*BVs (T1), SPFMV-RC*BVs (T2), SPFMV-RC (T3) and BVs (T4) treatments on the yield of 12 sweetpotato varieties.....	158
Fig. 4.3. The effects of SPFMV-RC*SPVC*SPVG*BVs (T1), SPFMV-RC*BVs (T2) and BVs (T4) on the storage roots of variety Blesbok as recorded during trial 2 when field trials were planted to determine the effects of single, co- and multiple infections of potyviruses and begomoviruses treatments.....	163
Fig. 4.4. Average total (A) and marketable (B) yields produced by 12 sweetpotato varieties when subjected to four virus treatments during two seasons. Means with the same letter are not significantly different ($P<0.05$).....	167

Fig. 4.5. The average of the combined total yield (TY) (ton ha⁻¹) of 12 varieties infected with SPFMV-RC*SPVC*SPVG*BVs (T1), SPFMV-RC*BVs (T2), SPFMV-RC (T3) and SPLCSV+SPMV (T4) compared to the uninfected control (T5).....168

LIST OF TABLES

Table 1.1: Viruses reported to infect sweetpotato, genus, distribution, transmission and authors.....5

Table 2.1: Accession numbers and virus names of reference sequences (GenBank database) used to determine relationships between the South African sequence isolates of SPFMV, SPVC, SPVG and SPLCV from this study and isolates reported in other countries.....80

Table 3.1: Regions (GPS co-ordinates) surveyed during the field surveys conducted to determine disease symptom incidence and aphids and whiteflies infestation in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa in 2012/2013 (season 1) and 2013/2014 (season 2).....120

Table 3.2: The χ^2 probabilities (<0.05% level) showing the mean percentage infection by SPFMV, SPVG, SPMMV, SPV2, SPCSV, SwPLV, SPCFV, SPMSV and CMV in samples collected from Gauteng, Limpopo and Mpumalanga provinces in season 1 and season 2 surveys.....132

Table 4.1: Primers (primers sequences) used for the PCR amplification of the RC strain of SPFMV, SPVC, SPVG and SPLCV.....148

Table 4.2: Near full-length genome sequence isolates of SPFMV, SPVC, SPVG and SPV2 isolated from South African sweetpotato variety SSBles.....153

Table 4.3: Total yield (TY) (tha⁻¹) in field trials of twelve sweetpotato varieties following the four virus treatments.....163

LIST OF APPENDICES

Table 1: Mean percentages of virus disease incidences recorded during the epidemiology surveys conducted in season 1 in the Gauteng, Limpopo and Mpumalanga Provinces.....186

Table 2: Mean percentage of virus disease incidences recorded during the epidemiology surveys conducted in season 2 (2014/2015) in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa.....187

Table 3: Means scores of aphids and whitefly infestations recorded on sweetpotato plants during epidemiology surveys conducted in season 2 in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa.....188

Table 4: The χ^2 probabilities (<0.05% level) of single, co and multiple infection of viruses (mean percentage incidences) generated by detection of viruses by NCM and DAS ELISA in samples collected in season 1 and 2 epidemiology surveys conducted in Gauteng, Limpopo and Mpumalanga Provinces.....	189
Table 5: Pairwise comparison of the nt sequences generated with part of the NlB, CP and UTR encoding region of SPVG.....	190
Table 6: Alignment of the CP aa sequences of SPVC and the RC strain of SPFMV isolates showing the conserved motifs associated with potyviral CP region, which are indicated by a solid box.....	191
Table 7: Pairwise comparison of the full CP aa sequences generated with part of the CP-encoding region nt sequences of SPVC and the RC strain of SPFMV.....	192
Table 8: Pairwise comparison of the CP aa sequences generated with part of the CP-encoding region nt sequences of SPVG.....	194
Table 9: Pairwise comparison of the full-length nt sequences of SPLCSPV and SPLCV generated with TZ:ELLP-ZA, TZ:NMP-ZA, SSB1-ZA, RDGP-ZA and ZA:TMBLP-ZA isolates of this study collected from Limpopo, Gauteng and Mpumalanga provinces.....	195
Table 10: Pairwise comparison of the full-length nt sequences of SPMV generated with SPMV Isolate SSB1_ZA, SPMV isolate ELLP_ZA, SPMV Isolate RDGP_ZA and SPMV Isolate TsMBLP_ZA isolates of this study collected from Limpopo, Gauteng and Mpumalanga provinces.....	196
Table 11: The alignment of the of the C1 open reading frame encoded aa of SPLCSPV and SPLCV, showing the conserved motifs associated with begomoviruses. Isolates of this study are marked with an asterisk and conserved motifs indicated by a solid box.....	197
Table 12: Pairwise comparison of the full-length nt sequences of SPFMV and SPVC (A), and SPV2 and SPVG (B) generated with sample SSbles (indicated with a dot) used as a source of multiple infection for the field trials conducted to determine the effects of single infection of potyviruses and co-infections of potyviruses and begomoviruses.....	198
Table 13: Pairwise comparison of the CP aa sequences of SPVG (A) and SPV2 (B) generated with sequences of sample SSbles (indicated with a dot) used as a source of multiple infection for the field trials conducted to determine the effects of single infection of potyviruses and co-infections of potyviruses and begomoviruses.....	200
Table 14: Alignment of the CP encoding aa sequences of SPVG (A) and SPV2 (B) generated with sequences of sample SSbles (indicated with a dot) used as a source of multiple infection	

for the field trials conducted to determine the effects of single infection of potyviruses and co-infections of potyviruses and begomoviruses.....202

Table 15: Marketable yield (MY) (ton ha⁻¹) in field trials of twelve sweetpotato varieties following the four virus treatments.....203

CHAPTER 1: LITERATURE REVIEW

1. INTRODUCTION.....	1
1.1 Production of sweetpotato at a global scale.....	1
1.2 The importance and cultivation practices of sweetpotato.....	1
1.3 SWEETPOTATO VIRUSES.....	4
1.3.1 Potyviridae.....	7
1.3.1.1 Sweet potato feathery mottle virus (SPFMV).....	10
1.3.1.2 Sweet potato virus C (SPVC).....	12
1.3.1.3 Sweet potato mild mottle virus (SPMMV).....	12
1.3.1.4 Sweet potato virus G (SPVG) and Sweet potato virus 2 (SPV2).....	13
1.3.1.5 Sweet potato mild speckling virus (SPMSV).....	13
1.3.1.6 Sweet potato latent virus (SwPLV).....	14
1.3.2 Closteroviridae.....	14
1.3.2.1 Sweet potato chlorotic stunt virus (SPCSV), viral synergism and disease complexes.....	15
1.3.2.2 Genetic variability of RNA viruses.....	16
1.3.3 Geminiviridae.....	17
1.3.3.1 Sweet potato leaf curl virus (SPLCV).....	20
1.3.3.2 Genetic variability of DNA viruses.....	21
1.3.4 OTHER SWEETPOTATO VIRUSES.....	22
1.3.5 DETECTION OF SWEETPOTATO VIRUSES.....	23
1.3.5.1 Infectivity (Biological) assays.....	23
1.3.5.2 Serological analysis.....	24
1.3.5.3 Nucleic acid based analysis.....	25
1.3.5.3.1 Nucleic acid hybridization.....	26
1.3.5.3.2 Reverse transcription polymerase chain reaction (RT-PCR) and PCR.....	27
1.3.5.3.3 Sequencing and phylogenetic analysis.....	28
1.3.5.4 Next Generation Sequencing (NGS) and Metagenomics.....	29
1.3.5.5 Electron microscopy (EM) and immunosorbent electron microscopy (ISEM).....	30

1.3.6 MANAGEMENT OF SWEETPOTATO VIRUSES.....	31
1.4 RATIONAL AND MOTIVATION.....	34
1.4.1 Background to the project.....	34
1.4.2 Problem statement.....	35
1.4.3 PROJECT AIMS.....	36
1.5 REFERENCES.....	37
CHAPTER 2: SURVEYS IN THE GAUTENG, LIMPOPO AND MPUMALANGA PROVINCES OF SOUTH AFRICA REVEAL NOVEL ISOLATES OF SWEETPOTATOVIRUSES.....	70
2.1 Absract.....	70
2.2 Introduction.....	72
2.3. Materials and Methods.....	73
2.3.1 Field surveys of sweetpotato viruses in Gauteng, Mpumalanga and Limpopo Provinces.....	73
2.4 Viral Analysis.....	75
2.4.1 Indexing and serological analysis.....	75
2.5 Detection of SPFMV, SPVC and SPVG with RT-PCR.....	77
2.5.1 Total RNA extractions and RT-PCR amplification.....	77
2.5.2 Cloning and sequencing and phylogenetic analysis.....	77
2.5.3 Sequence analysis of the partial genomes.....	78
2.6 Detection of begomoviruses, confirmation by rolling circle amplification (RCA) and full-length genome sequencing.....	85
2.6.1 Total DNA extractions and PCR reactions.....	85
2.6.2 RCA and restriction fragment length polymorphism (RFLP).....	85
2.6.3 Full genome sequencing of begomoviruses.....	86
2.7. Results.....	87
2.7.1 Field surveys.....	87
2.7.2 Indexing and serological analysis.....	87
2.7.3 RT-PCR, PCR amplifications, RCA and RFLP.....	89
2.7.4 Sequence analysis (partial genome).....	90
2.7.4.1 Nucleotide sequence analysis of SPVC.....	90
2.7.4.2 Nucleotide sequence analysis of SPFMV.....	91
2.7.4.3 Nucleotide analysis of SPVG	93
2.7.4.4 Amino acid analysis (SPFMV, SPVC and SPVG).....	94

2.8 Full genome sequencing of begomoviruses.....	97
2.9 Discussion.....	101
2.10 Conflict of interest.....	106
2.11 Acknowledgements.....	106
2.12 References.....	107
CHAPTER 3: EVALUATION OF DISEASE INCIDENCE, APHIDS AND WHITEFLIES INFESTATION LEVELS ON SWEETPOTATO IN THE GAUTENG, MPUMALANGA AND LIMPOPO PROVINCES OF SOUTH AFRICA.....	
3.1 Abstract.....	117
3.2 Introduction.....	119
3.3 Material and methods.....	120
3.3.1 Areas surveyed.....	120
3.3.2 Field surveys and sample collections.....	121
3.3.3 Viral analysis.....	122
3.3.3.1 Indexing.....	122
3.3.3.2 Serological Analysis.....	123
3.3.3.2.1 Nitrocellulose based enzyme linked immunosorbent assay (NCM-ELISA).....	123
3.3.3.2.1 Double antibody sandwich ELISA.....	123
3.3.3.2.3 Detection of samples showing symptoms associated with begomoviruses.....	124
3.3.4. Analysis of data generated with serological tests.....	124
3.4. Results.....	124
3.4.1 Field surveys and sample collections.....	124
3.4.2 Virus disease incidences.....	125
3.4.3 Aphid and whitefly evaluations.....	126
3.4.4 Biological indexing.....	127
3.4.5 Serological analysis.....	128
3.4.6 Virus single, co- and multiple infections.....	133
3.5 Discussion.....	134
3.6 References.....	138
CHAPTER 4: YIELD EVALUATION OF BEGOMOVIRUS AND POTYVIRUS MIXED INFECTIONS ON SWEETPOTATO VARIETIES UNDER FIELD CONDITIONS.....	
4.1 Abstract.....	144

4.2. Introduction.....	145
4.3. Material and methods.....	157
4.3.1 Confirmation of viruses in source sweetpotato material by NCM-ELISA, RT-PCR and PCR.....	157
4.3.2 Confirmation of virus co-infection by NGS.....	149
4.3.3 Field trials to determine the effects of begomoviruses and potyviruses on the yield of sweetpotato varieties.....	150
4.3.3.1 Inoculation of varieties with virus treatments and confirmation of infection.....	150
4.3.3.2 Experimental design of field trials planted to determine the effects of potyviruses and begomoviruses on the total and marketable yields of 12 varieties.....	151
4.4. Results and discussion.....	152
4.4.1. Confirmation of virus combinations and identities in sweetpotato source material (used for inoculations in the field trials).....	152
4.4.2 The impact of virus treatments on total and marketable yields (tones ha ⁻¹).....	158
4.4.2.1 Symptoms assessment during field trials.....	158
4.4.2.2 Total yield of 12 varieties subjected to different virus treatments.....	159
4.4.2.3 Marketable yieldsof 12 varieties subjected to different virus treatments.....	165
4.4.2.4 Average total and marketable yields produced by 12 varieties treated with four virus treatments over two seasons.....	166
4.4.2.5 Average of the combined total yield (TY) (tha ⁻¹) of 12 varieties infected with different virus combinations (treatments).....	167
4.5 Conclusion.....	168
4.6 Conflict of interest.....	169
4.7 Acknowledgements.....	170
4.8 References.....	170
CHAPTER 5: CONCLUDING REMARKS.....	180
References.....	185

CHAPTER 1

LITERATURE REVIEW

1. INTRODUCTION

1.1 Production of sweetpotato at a global scale

Sweetpotato (*Ipomoea batatas* Lam.) is a hexaploid dicot plant, belonging to the family *Convolvulaceae* (Wang et al., 2011). The crop originated in the central and tropical parts of South America, Panama, Peru and Mexico [(Steinbauer and Kushman, 1971; Department of Agriculture, Forestry and Fisheries (DAFF), 2011a)]. It ranks as the seventh most important staple crop in the world, and the third most important tuber crop in sub-Saharan Africa after wheat (*Triticum aestivum*), maize (*Zea mays*), rice (*Oryza sativa*), potato (*Solanum tuberosum*), barley (*Hordeum vulgare*), cassava (*Manihot esculenta* Crantz) and yam (*Dioscorea* spp. L.) [(Karyeija et al., 1998; Kreuze and Fuentes, 2008)]. Nigeria and Uganda are listed as the largest producer of sweetpotato in Africa and production of 2,126,989 3,478,270 metric tons was recorded, respectively [(Food and Agriculture Organization of the United Nations (FAOSTAT), 2015; FAOSTAT, 2017; World Atlas, 2017)]. Asia produces about 76% to 85% of sweetpotato and America is the 10th largest producer (Lucier et al., 2002; FAOSTAT, 2017). According to vegetable production statistics, South Africa produces about 60 to 63 tons per year, selling about 29 to 32 tons on the major fresh produce market, at an average price of R699/ ton in 2014/2015 (DAFF, 2011a; b; DAFF, 2016).

1.2 The importance and cultivation practices of sweetpotato

Sweetpotato is grown by both smallholder and commercial farmers in all the provinces of South Africa and it is a major staple food crop that feeds millions of people on the African continent.

The main production areas in South Africa are Limpopo, Mpumalanga and Western Cape (DAFF, 2010; DAFF, 2011a) and, in the recent years, the crop has been introduced to the Eastern Cape, KwaZulu Natal, Free State and Gauteng provinces (DAFF, 2010). Food insecurity is a term that describes lack of physical, social or economic access to food (FAO, 2010). Sweetpotato is one of the traditional crops that plays an important role in addressing food insecurities in rural households (Grüneberg et al., 2015). The crop has been used as a relief for hunger in war-torn areas in Uganda and other African countries (Kapinga et al., 2005). Sweetpotato is propagated as an annual crop and it can be used in all farming sectors since it is adaptable to a broad range of agro-ecological conditions and can be cultivated by farmers with low input cropping systems (Mukhopadhyay et al., 2011; Motsa et al., 2015). Sweetpotato is a good source of nutrients and 100 g of sweetpotato contains vitamin C (2.4 mg), fiber (20.12 g), calcium (30 mg) and vitamin A (709 µg) (USDA National Nutrient Database for Standard Reference, 2016). It is also known to contain a significant amount of protein, manganese, antioxidants, ascorbic acid and riboflavin (Ewell and Mutuura, 1991; USDA National Nutrient Database for Standard Reference, 2016). Leaves of sweetpotato can be used as vegetable relish and vines as fodder for animals (Clark et al., 2012). New products such as sweetpotato bread, chips and frozen products are developed for the retail markets (van den Berg et al., 2004; Engoru et al., 2005; Clark et al., 2012). Faber and co-researchers (2017) showed that food-secure households are more likely to consume a more varied diet, which is likely to contain vitamin A whereas the food insecure households are at a risk of suffering from nutritional deficiencies. Recently, breeding efforts are focused on the production and improvements of orange-fleshed sweetpotato (OFSP) which is reported to be rich in β -carotene, a relevant source of pro-vitamin A, as a means to address vitamin A deficiency in affected food insecure households (van Jaarsveld et al., 2005; Grüneberg et al., 2015; Kurabachew, 2015). The Mozambique government gives priorities to multiplication and distribution of OFSP planting

material to address problems of nutritional deficiencies (Kapinga et al., 2005; Brito et al., 2012). Laurie and co-researchers (2012) confirmed the ability of OFSP varieties to contribute $\pm 100\%$ of the recommended dietary allowance of provitamin A, depending on the variety planted. South Africa was one of the partner countries of the Vitamin A for Africa (VITAA) initiative and has implemented a national vitamin A supplementation (VAS) programme that promotes food fortification programs, the production and consumption of OFSP varieties to alleviate vitamin A deficiency (Faber et al., 2001; Labadarios et al., 2007; Laurie et al., 2015a).

Sweetpotato is grown in sandy-loam and clay loam soils [van den Berg and Laurie, 2004; DAAF, 2011a; Agricultural Research Council (ARC), 2011, <http://www.arc.agric.za>]. A soil pH of 6.0 is recommended for root development (DAAF, 2011b). The crop is propagated from vine cuttings of 30 cm to 40 cm long, on ridges of about 30 to 40 cm apart (ARC, 2011). A spacing of 25 cm to 35 cm apart between plants is recommended for the cultivation of sweetpotato (ARC, 2011). The crop requires warm climates and is very sensitive to frost. The standard for water requirements is between 450 mm to 600 mm per planting season, however, the plant is capable of developing some level of draught tolerance when storage roots have developed (ARC, 2011). Soil fertilization is recommended during planting and an optimum level of Nitrogen, Phosphorus and Potassium (NPK) before planting is required and this can be 800 kg/ha⁻¹, which contain 53.3 kg of N, 80 kg of P and 106.7 kg of K for high input production (a yield of 70 to 80 t/ha⁻¹) (Allemann, 2004). High nitrogen and potassium content is required for top dressing, and this should consist of lime with ammonium sulphate and nitrogen (LAN) of about 150 kg/ha⁻¹, 3 weeks and 6 weeks after planting (Allemann, 2004; ARC, 2011). Locally developed sweetpotato varieties include Blesbok, Bosbok, Ribbok, Koedoe and Mafutha, which all originated from the ARC, Vegetable and Ornamental Plants (ARC-VOP) breeding program (du Plooy, 1986; Laurie et al., 2015b).

Sweetpotato is prone to production losses due to biotic factors such as pests and diseases. The most common fungal diseases reported to affect sweetpotato production is *Fusarium* wilt, which is characterized by yellowing of older leaves, wilting of the leaves and stunting (Moyer and Clark, 2013). Other fungal diseases include *Alternaria* leaf spot, which is caused by different species of *Alternaria* (such as *Alternaria bataticola*, *Alternaria alternata*) (Moyer and Clark, 2013). Some of the bacterial diseases reported to affect sweetpotato production are bacterial wilt (*Pseudomonas solanacearum*) which is characterized by greyish brown water soaked discolorations, soil rot (*Streptomyces ipomoea*), which is the appearance of necrotic lesions on storage roots (Clark, 2013). Among the diseases reported, those caused by viruses are the most problematic, and production losses due to sweetpotato viruses have been reported (Ames et al., 1996). Furthermore, while several RNA and DNA viruses are reported to infect sweetpotato, sweetpotato viruses often occur in mixed virus complexes (Gibson and Kreuze, 2015).

1.3 SWEETPOTATO VIRUSES

One of the major limitations to sweetpotato production is high yield losses due to the cumulative effects of virus infection (Clark et al., 2002), which is a concern to the commercial and emerging farmers globally (Gutierrez et al., 2003). More than 30 sweetpotato viruses have been identified world-wide (Lozano et al., 2009), including RNA and DNA viruses from several families and genera (Table 1.1). In South Africa, sweetpotato viruses have been associated with high yield losses since the mid 1940's (McClean and Klessner, 1947). Sweet potato feathery mottle virus (SPFMV) was identified as the most important virus affecting sweetpotato production in South Africa (Jericho and Thompson, 2000; Domola et al., 2008) and other parts of the world (Moyer and Salazar, 1989). Other viruses known to affect

sweetpotato production includes potyvirus Sweet potato virus 2 (SPV2), potyvirus Sweet potato virus C (SPVC), ipomovirus Sweet potato mild mottle virus (SPMMV), potyvirus Sweet potato latent virus (SwPLV), potyvirus Sweet potato mild speckling virus (SPMSV), carlavirus Sweet potato chlorotic fleck virus (SPCFV) and begomovirus Sweet potato leaf curl virus (SPLCV) (Moyer and Salazar, 1989; Winter et al., 1992; Chavi et al., 1997; Di Feo et al., 2000; Ateka et al., 2007; Lozano et al., 2009; Tugume et al., 2010). In addition to these, viruses such as Sweet potato collusive virus (SPCV), previously known as Sweetpotato caulimovirus (SPCaLV) and Sweet potato ringspot virus (SPRSV) are reported to infect Sweetpotatoes (Winter et al., 1992; Brunt et al., 1996). Over 32 viruses infecting sweetpotato have been reported (Clark et al., 2012). Table 1.1 shows, in summary, some of the viruses commonly known to infect sweetpotato worl-wide. A detailed review on the most important viruses is described in the following sections.

Table 1.1: Viruses reported to infect sweetpotato, genus, distribution, transmission and authors.

VIRUSES	GENUS	REPORTED DISTRIBUTION	TRANSMISSION	REFERENCES
SPFMV	<i>Potyvirus</i>	Worldwide	Aphids	Clark and Moyer, 1988; Moyer and Salazar, 1989; Clark and Valverde, 2013
SPVC	<i>Potyvirus</i>	Worldwide	Aphids	Clark and Moyer, 1988; Moyer and Salazar, 1989; Untiveros et al., 2010; Clark and Valverde, 2013
SPMMV	<i>Ipomovirus</i>	Eastern and Southern Africa, China, India, Indonesia, New Zealand, Papua New Guinea, Peru and Philippines	Whiteflies	Hollings et al., 1976; Jericho and Thompson, 2000; Mukasa et al., 2006; Tugume et al., 2010; Clark and Valverde, 2013
SPVG	<i>Potyvirus</i>	Africa, Australia, United State of America, Asia, South America and China	Aphids	Colinet et al., 1998; Clark and Valverde, 2013
SPV2	<i>Potyvirus</i>	Zambia, Zimbabwe, South Africa, China, Australia, Portugal, Peru etc.	Aphids	Ateka et al., 2006; Ateka et al., 2007; Tairo et al., 2006; Untiveros et al., 2008; Clark and Valverde, 2013
SPCSV	<i>Crinivirus</i>	Worldwide	Whiteflies	Winter et al., 1997; Gibson et al., 1998; Clark and Valverde, 2013

SwPLV	<i>Potyvirus</i>	Africa, Taiwan, China, Korea, Peru, Brazil and Indonesia	Unknown	Colinet et al., 1998; Kreuze and Fuentes, 2008; Clark and Valverde, 2013
SPCFV	<i>Carlavirus</i>	Australia, Bolivia, Brazil, China, Columbia, India, Indonesia, Japan, Korea, Panama, Kenya, Tanzania, Rwanda, South Africa, Taiwan and Uganda	Unknown	Ateka et al., 2004a; Aritua et al., 2007; Jones and Dwyer, 2007; Clark and Valverde, 2013; Tugume et al., 2016
SPLCV	<i>Begomovirus</i>	Spain, China, Japan, USA, Uganda and South Africa	Whiteflies	Clark and Hoy, 2006; Lozano et al., 2009; Trenado et al., 2011; Wasswa et al., 2011; Bi and Zhang, 2012; Liu et al., 2014
SPMSV	<i>Potyvirus</i>	Brazil, China, Egypt, India, Indonesia, New Zealand, Argentina, Nigeria, Peru and Philippines and South Africa	Aphids	Kreuze and Fuentes, 2008; Alvarez et al., 1997; Domola et al., 2008; Clark and Valverde, 2013
SPCV	<i>Cavemovirus</i>	China, Puerto Rico, Australia, Egypt, Kenya, Madeira, New Zealand, Nigeria, Papua New Guinea, Philippines, Tanzania, Togo, Uganda and the United State of America	Unknown	Cuellar et al., 2011; Clark and Valverde, 2013
C-6 virus	<i>Potyvirus</i>	Uganda, Egypt, India, Cuba, Indonesia, New Zealand, Argentina, Nigeria, Peru and Philippines, United State of America, South Africa and Kenya	Unknown	
SPRSV	<i>Nepovirus</i>	Papua New Guinea and Kenya	Unknown	Clark and Valverde, 2013
CMV	<i>Cucumovirus</i>	Israel, Egypt and Kenya	Aphids	Clark and Valverde, 2013
Ipomoea yellow vein virus (IYVV)	<i>Begomovirus</i>		Whiteflies	Moyer et al., 1989; Clark and Valverde, 2013
Sweet potato Pakakuy virus: badnavirus A and B	<i>Badnavirus</i>	Spain		
Sweet potato golden vein virus (SPGVaV)	<i>Begomovirus</i>	Peru, South Africa, Tanzania, Guatemala	Meallybugs	Lozano et al., 2009; Kreuze et al., 2009; Kashif et al., 2012; Mbanzibwa et al., 2014; Bhat et al., 2006; Nhlapo et al., 2018
Sweet potato yellow dwarf virus (SPYDV)	<i>Ipomovirus</i>	South Korea	Whiteflies	Paprotka et al., 2010; Kil et al., 2014
Sweet potato mosaic virus (SPMV)	<i>Begomovirus</i>	Taiwan	Whiteflies	Green and Lo, 1989; Loebestein, 2009
Sweet potato leaf curl Sao Paulo virus (SPLCSPV)	<i>Begomovirus</i>	Brazil, South Africa	Whiteflies	Paprotka et al., 2010; Esterhuizen et al., 2012b
Sweet potato leaf curl Uganda (SPLCU)	<i>Begomovirus</i>	Brazil, Tanzania, South Africa	Whiteflies	Esterhuizen et al., 2012b; Mbazimbwa et al., 2014
SPLC South Carolina virus	<i>Begomovirus</i>	Uganda	Whiteflies	
SPLC Georgia virus	<i>Begomovirus</i>	United State of America	Whiteflies	Wasswa et al., 2011
SPLC China virus	<i>Begomovirus</i>	United State of America	Whiteflies	Ling et al., 2010; Zhang and Ling, 2011
SPLC Lanzarote virus (SPLCLaV)	<i>Begomovirus</i>	China	Whiteflies	Ling et al., 2010
SPLC Spain virus (SPLCSV)	<i>Begomovirus</i>	Spain	Whiteflies	Luan et al., 2007
	<i>Begomovirus</i>	Spain	Whiteflies	Lozano et al., 2009; Trenado et al., 2011
	<i>Begomovirus</i>	Spain Central	Whiteflies	Lozano et al., 2009
Sweet potato vein clearing virus (SPVCV)	<i>Solendovirus</i>	America, Dominican Republic, East Africa, Guatemala, Jamaica, Kenya, Panama and Uganda.	Seed borne	
Sweet potato C-3 virus (SPC3V)	<i>Phlebovirus?</i>	Brazil	Grafting	Cuellar et al., 2011; Dennien, 2015
				Loebestein et al., 2009

Sweet potato symptomless virus-1 (SPSMV-1)	<i>Mastrevirus</i>	Peru, Tanzania, Korea	Not known	Kwak et al., 2014; Dennien, 2015; Kim et al., 2017
Sweet potato leaf speckling virus (SPLSV)	<i>Polerovirus</i>	Cuba and Peru	Aphids	Dennien, 2015

1.3.1 *Potyviridae*

The family *Potyviridae*, genus *Potyvirus* constitute one of the largest group of plant viruses (Brunt et al., 1996; Valkonen et al., 2002; Mishra et al., 2013), and a large number of sweetpotato viruses fall into this family (Table 1.2). The genome particles of potyviruses are non-enveloped, flexuous filamentous shaped rods ranging from 680-900 nanometers (nm) in length and 12 nm in width (Brunt et al., 1996; Pappu et al., 1993; Sakai et al., 1997; Lima et al., 2014). The group consists of positive sense, single-stranded RNA (ssRNA) genomes, which are expressed via the production of a large polyprotein that is made up of 12 functional proteins (Sakai et al., 1997; Mishra et al., 2013; Lima et al., 2014). Some of the major potyviruses known to infect plants include the Potato virus Y (PVY) (Boonham et al., 2002; Syller and Grupa, 2014, Mascia and Gallitelli, 2014), Tobacco etch virus (TEV) (Verchot et al., 1991), Zucchini yellow mosaic virus (ZYMV) (Coutts et al., 2011), Watermelon mosaic virus (WMMV) (Desbiez et al., 1997). The genome of potyviruses consist of 10 mature proteins, one large open reading frame (ORF) starting from 118 nucleotides (nt), encoding a large polyprotein [(3493 base pairs (bp)] at 10599 nt and amino acids (aa), followed by the untranslated region (UTR) (Sakai et al., 1997). Fig. 1.1 illustrates the functional genome structure of the polyproteins of potyviruses, consisting of the following genes, namely, the first protein (P1), the helper component-proteinase (HC-Pro), the third protein helper component (P3), first 6 kDa protein (6K1), cytoplasmic inclusion protein (CI), second 6 kDa protein (6K2), small nuclear inclusions proteins such as the Nia-Pro and VPg, the large nuclear inclusion body (NIb) and coat protein (CP) (Sakai et al., 1997; Kasschau et al., 1997; Mascia and Gallitelli, 2014). Two short ORFs, PISPO and PIPO are also found located in the P1 and P3 regions,

respectively, of the *Potyviridae* genome, whereby PISPO is found in sweetpotato viruses, whereas PIPO is found in all potyvirids (Mingot et al., 2016). Through the process of polymerase slippage mechanism, the two ORFs become P1N-PISPO and P3N-PIPO (Rodamilans et al., 2015).

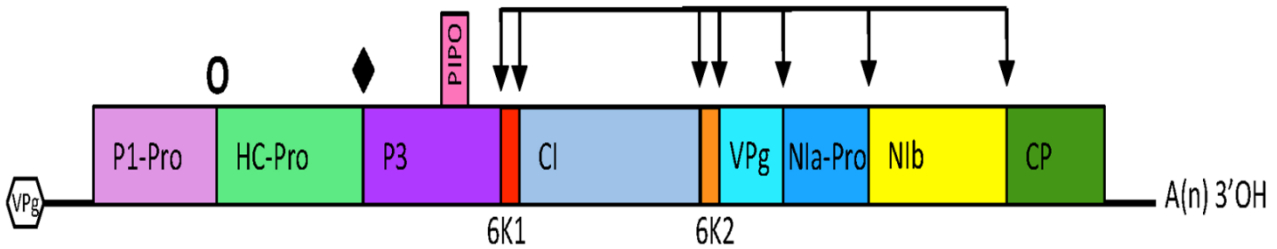


Fig. 1.1. Genomic map of a typical member of the genus Potyvirus. The ssRNA genome is represented by a line and an open box representing the ORF translated into a polyprotein. Names associated with the mature proteins proteolytically processed from the polyprotein are shown. Wylie et al., 2017. https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/potyviridae.

The functions of the HC-Pro protein is complex, however, it is known to be involved in the aphid-mediated transmission, genome amplification, polyprotein processing and the long distance movement of the viral RNA (Kasschau et al., 1997; Rojas et al., 1997). It is also suggested that the HC-Pro may interact with the CP in the cell-to-cell movement (Rojas et al., 1997) due to the interaction of the CP with the plasmodesmatal proteins to facilitate the movement of the RNA and genome amplification (Mishra et al., 2013). Dolja and co-researchers (1994) also showed that, other than the cell-to-cell movement function, the CP also possess some distinct activities required for virion assembly and long-distance transportation of the virions. The CP encoding polyprotein is also suspected to be involved in the vector transmission (Mishra et al, 2013). Other proteins include the P3 and the C1, which are speculated to function in the cell-to-cell movement (P1 and C1) and genome replication (C1 and P3) (Mishra et al., 2013). Verchot and Carrington (1995) suggested that the function of the

P1 proteinase could extend to genome amplification. The NIa-VPg, NIa-Pro and NIb proteins contribute to genome replication (Mishra et al., 2013). The proteinase of P3-6K1, C1-6K2 and the NIa-VPg, NIa-Pro also play a role in the viral infection cycles (Merits et al., 2002). Potyviruses such as TEV requires the interaction between the N-terminal VPg domain and the C-terminal NIa-Pro protease to be infective (Schaad et al., 1997). P1N-PISPO is produced by SPFMV and like the HC-Pro in other potyvirids, it acts as an RNA silencing suppressor, limiting the accumulation of viral RNAs in host plants (Mingot et al., 2016). It is also reported that recombination among potyviruses occurs, which is the biggest contributor of their ever evolving nature (Clark et al., 2012). Table 1.2 is the summary of the functions of major polyproteins of the potyviruses and potyviruses reported to infect sweetpotatoes are reported below.

Table 1.2: Summary of the major potyvirus polyproteins and their functions

Polyproteins	Functions	References
P1	Cell-to-cell movement Genome replication Genome amplification	Mishra et al., 2013 Verchot and Carrington, 1995 Mishra et al., 2013
HC-Pro	Aphids mediated transmission Genome replication Long distance movement of the viral RNA Suppression of si-RNA-based antiviral defence of plants Proteolysis of the polyproteins	Kasschau et al., 1997 Rojas et al., 1997 Kasschau et al., 1997 Kokkinos, 2006 OH and Carrington, 1989
P3	Virus propagation Genome replication	Verchot and Carrington, 1995 Verchot and Carrington, 1995
6k1	Viral infection cycles, genome stabilization, regulation of proteolytic processing	Merits et al., 2002; Ivanov et al., 2014
C1	Genome replication, membrane attachment, cell to cell movement	Mishra et al., 2014
6k2	RNA replication, membrane anchoring of replication machinery.	Mishra et al., 2014
Nia-VPg	Viral infection cycles, genome stabilization, regulation of proteolytic processing	Merits et al., 2002; Ivanov et al., 2014

Nia-Pro	Genome replication	Merits et al., 2002
	Viral infection cycles	Merits et al., 2002
Nlb	Genome replication	Merits et al., 2002
	Viral infection cycles	Merits et al., 2002
P1N-PISPO	RNA silencing supressor	Mingot et al., 2016
P3N-PIPO	Cell to cell propagation and movement	Wei et al., 2010; Vijayapalani et al., 2012

1.3.1.1 Sweet potato feathery mottle virus (SPFMV)

SPFMV is recognized as the most important of all sweetpotato viruses in South Africa and worldwide (Moyer and Salazar, 1989; Domola et al., 2008). It is a member of the family *Potyviridae*, genus *Potyvirus*, which is one of the largest family of plant viruses (Clark and Moyer, 1988; Moyer and Salazar, 1989). The virus is widely distributed wherever sweetpotato is grown (Clark and Valverde, 2013). A study conducted in South Africa (2001 to 2003) revealed the prevalence of about 73% (SPFMV). Co-infection of SPFMV with other potyviruses was confirmed to reduce total yield (17%) and marketable (30%) yield by $\pm 30\%$ in field trials with South African varieties (Domola et al., 2008). SPFMV consists of elongated particles of about 810 to 865 nm (Campbell et al., 1973) and the RNA genome is 10 820 nt long, excluding the poly(A) tail (Sakai et al., 1997). SPFMV is transmitted non-persistently by the green peach aphids (*Myzus persicae* Sulzer) and cotton aphids (*Aphis gossypii* Glover) (Clark and Moyer, 1988; Clark and Valverde, 2013). Non-persistent transmission is a phenomenon in which aphids acquire the virus from the infected plants while feeding, retain it in the mouthparts and immediately transmit it to other plants through brief feedings of only 20-30 seconds, and if the virus is not transmitted within that time period, it loses its viability (Clark and Moyer, 1988). Different strains of SPFMV, namely, the russet crack (RC), the ordinary (O) and the East African (EA) strains have been identified (Campbell et al., 1973; Cali and Moyer, 1981; Hammond et al., 1992; Tairo et al., 2005; Tairo et al., 2006; Untiveros et al., 2008; Rännäli et al., 2009; Clark and Valverde, 2013). The RC strain of SPFMV is reported to

occur in countries such as Japan (Sakai et al., 1997), United States of America (USA) (Cali and Moyer, 1981) and China (Colinet and Kummert, 1993). Only the RC and O strains of SPFMV are prevalent in South Africa (Rännäli et al., 2009; Sivparsad and Gubba, 2012; Mulabisana et al., 2015). Strain RC of SPFMV affect the storage roots by causing severe cracking, and sometimes necrotic lesions of the roots, thus leading to a drastic reduction in marketable yield and the quality of the storage roots (Campbell et al., 1973; Clark and Valverde, 2013). Strain EA of SPFMV is highly distributed in the East African regions, whereas others can be found wherever sweetpotato is grown (Tairo et al., 2005). The host range of SPFMV is narrow, and it is mostly limited to the family *Convolvulaceae*, especially the genus *Ipomoea* (Mukasa et al., 2003a). Symptoms associated with SPFMV infection are characterized by general chlorotic spots and chlorotic vein banding (vein clearing) on indicator plants *Ipomoea setosa* (*I. setosa*) and sweetpotato plants, ring spotting (sweetpotato) with or without the purple pigmentation (Fig. 1.2), and this is dependent on the genetic characteristics of the plant and the environmental conditions (Clark and Valverde, 2013).

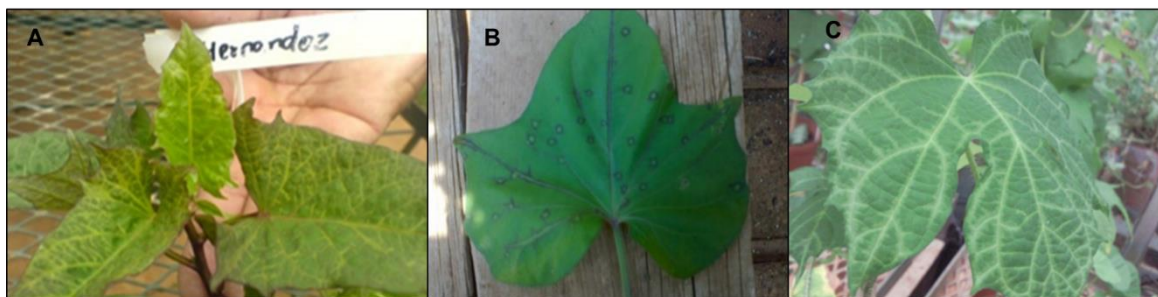


Fig. 1.2. Symptoms of sweetpotato viruses as observed on sweetpotato plants and grafted *I. setosa* plants. (a) Yellowing of the mid veins (vein banding/vein clearing) on variety Hernandez, (b) purple ring spots and (c) yellowing of the mid vein/vein clearing on indicator plant *I. setosa*. Picture by M.J. Mulabisana, Agricultural Research Council, Vegetable and Ornamental Plants.

1.3.1.2 Sweet potato virus C (SPVC)

SPVC was previously referred as the common (C) strain of SPFMV (Clark and Valverde, 2013). Sequence (CP aa) comparison of strains of SPFMV and SPVC revealed that SPVC is distinctly different from other strains of SPFMV due the missing CP aa sequences at position 62 and 63 (Unitiveros et al., 2008; Tairo et al., 2005), a unique feature which differentiate SPVC from SPFMV. The distinction is suspected to have originated from the interviral recombination that occurred between the different strains of SPFMV (Tairo et al., 2005; Untiveros et al., 2010; Clark et al., 2012). The host range of SPVC is also limited to the family *Convolvulaceae*, especially the genus *Ipomoea* (Mukasa et al., 2003a). It is also a member of the *Potyviridae* family, transmitted by aphids and widely distributed wherever sweetpotato is grown (Clark and Valverde, 2013). Symptoms associated with SPVC are similar to those induced by SPFMV.

1.3.1.3 Sweet potato mild mottle virus (SPMMV)

SPMMV belongs to the family *Potyviridae*, genus *Ipomovirus* (Hollings et al., 1976; Hammond et al., 1992; Kreuze and Fuentes, 2008). It is transmitted by whiteflies *Bemisia tabaci* (*B. tabaci*) species in a non-persistent manner (Hollings et al., 1976; Clark and Valverde, 2013). In Africa, SPMMV is reported to occur in South Africa and Uganda (Jericho and Thompson, 2000; Domola et al., 2008; Mukasa et al., 2006; Tugume et al., 2010). Sensitive varieties induces symptoms such as leaf spots, mild vein chlorosis, mild mottling and some mosaic when infected with SPMMV as a single infection (Hollings et al., 1976; Mukasa et al., 2003b; Mukasa et al., 2006). The genome size is about 1.8 to 2.1 kilo base pairs (kb) (Colinet et al., 1996; Colinet et al. 1998). SPMMV infect about 21 wild *Ipomoea* species which makes the transmissibility to be easy and faster (Tugume et al., 2010). Recombination is reported to exist

in the 5' end of the P1- and HC-Pro-encoding regions of SPMMV genome, which is suspected to assist the virus to adapt to a wide host range plants (Tugume et al., 2010).

1.3.1.4 Sweet potato virus G (SPVG) and Sweet potato virus 2 (SPV2)

Other potyviruses of sweetpotato, belonging to the family *Potyviridae* are SPV2, also known as Sweet potato virus Y (SPVY) (Ateka et al., 2006; Li et al., 2012a) and SPVG (Colinet et al., 1994). The name SPV2 is the one recognized by the ICTV (Kreuze and Fuentes, 2008). SPVG is reported to occur in South Africa and other African countries, Australia, United State of America, Asia and South America (Colinet et al., 1998; Sivparsad and Gubba, 2012; Clark and Valverde, 2013). SPV2 is reported to occur in African countries such as Zambia, Zimbabwe, South Africa, China, Australia, Portugal, Peru and other parts of the world (Ateka et al., 2006; Ateka et al., 2007; Tairo et al., 2006; Untiveros et al., 2008; Clark and Valverde, 2013). The genome sizes of SPV2 and SPVG are about 10800 and 10731 bp, respectively (Li et al., 2012). They are both transmitted by green peach aphids (*M. persicae* Sulzer) and cotton aphids (*A. gossypii*) (Clark and Valverde, 2013). The two viruses are frequently detected in plants that are also infected with SPFMV and co-infection of these viruses is associated with yield losses (Domola et al., 2008). Tairo and co-researchers (2006) showed that plants infected with SPV2 might display obvious ring symptoms with red margins, slightly different from those induced by SPFMV. Hosts plants of SPV2 include goosefoot (*Chenopodium* L.), sonweed (*Datura stramonium* L.) and tobacco (*Nicotiana* spp.) plants (Clark and Valverde, 2013).

1.3.1.5 Sweet potato mild speckling virus (SPMSV)

SPMSV is also a member of the genus *Potyvirus* (Alvarez et al., 1997). SPMSV has flexuous rod particle which are about 800 nm long and is transmitted by aphid species (Kreuze and

Fuentes, 2008; Clark and Valverde, 2013). Serology and nucleic acid based molecular techniques detected SPMSV in countries such as Brazil, China, Egypt, India, Indonesia, New Zealand, Argentina and South Africa (Kreuze and Fuentes, 2008; Alvarez et al., 1997; Domola et al., 2008; Clark and Valverde, 2013). SPMSV was previously described as one of the viruses that are responsible for causing Sweet potato chlorotic dwarf virus (SPCDV) disease complex (Clark and Valverde et al., 2013).

1.3.1.6 Sweet potato latent virus (SwPLV)

SwPLV is also a member of the genus *Potyvirus* and is transmitted by aphid species (Kreuze and Fuentes, 2008; Clark and Valverde, 2013). SwPLV is reported to be distributed in China, Japan, Korea, Brazil, and low incidences of this virus have been reported in other parts of Africa such as Rwanda and South Africa (Kreuze and Fuentes, 2008; Domola et al., 2008; Clark and Valverde, 2013). Symptoms associated with SwPLV are very mild, sometimes absent and its biology is not well studied (Clark and Valverde, 2013).

1.3.2 Closteroviridae

The genome of viruses in the *Closteroviridae* family consists of ssRNA. Three genera in this family include the *Closterovirus* (*Beet yellows virus*), *Ampelovirus* (*Grapevine leaf roll virus* 3) and *Crinivirus* (*Lettuce infectious yellows virus*) (Martelli et al., 2002; Martelli et al., 2012; Cueller et al., 2015). Closteroviruses are transmitted by aphids while ampeloviruses are transmitted by mealy bugs (Martelli et al., 2002). The genome of criniviruses is bipartite, positive sense, ssRNA which is divided into two RNA molecules (RNA 1 and RNA 2). The virus particles are flexuous rod of about 850 nm long with a genome size of about 15.3 to 19 kb (Martelli et al., 2002; Clark and Valverde, 2013; Kiss et al., 2013; Rubio et al., 2013). Criniviruses are transmitted by whitefly species.

1.3.2.1 Sweet potato chlorotic stunt virus (SPCSV), viral synergism and disease complexes

SPCSV is a whitefly (*B. tabaci* biotype B) transmitted virus, belonging to the family Closteroviridae, genus *Crinivirus* (Aritua et al., 1998a; Fauquet and Mayo, 1999; Karyeija et al., 2000). The genome size of both RNA 1 and RNA 2 is about 8.1 kb long (Qin et al., 2010). The RNA 1 encodes the protein that is responsible for replication (Kiss et al., 2013). Based on the serological analysis, East African (EA) and West African (WA) strains of SPCSV have been reported, however, the EA strain is prevalent in East Africa, whereas, the WA strain has been detected in many countries where sweetpotato is grown (Gibson and Aritua, 2002; Gutierrez et al., 2003; Tairo et al., 2005). SPCSV was also detected in Egypt (Winter et al., 1997; IsHak et al., 2003). Triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA), using monoclonal antibodies and nitrocellulose membrane ELISA (NCM-ELISA kit, CIP, Lima, Peru) are useful tools in detecting SPCSV. However, it is of utmost importance to confirm infection with nucleic acid based techniques such as the reverse transcription polymerase chain reaction (RT-PCR) with primers targeting the HSP70 homologue and the CP genes in infected sweetpotato material (Winter et al., 1997; Alicai et al., 1999; IsHak et al., 2003). SPCSV is known to occur in mixed infections with SPFMV, resulting in synergistic infection, which causes sweetpotato virus disease (SPVD) syndrome (Gibson et al., 1998; Gibson and Aritua, 2002; Mwanga et al., 2002a). Co-infection of SPFMV and SPCSV is reported to cause symptoms which are typically of potyviruses and the difference is that these are more intense and characterized by severe leaf distortion, including narrowing (strap-like) of leaves, yellowing of the mid veins or vein banding, chlorotic spotting (Gibson and Aritua, 2002; Clark and Valverde, 2013). However, a single infection of SPFMV or SPCSV will induce very mild symptoms which are characterized by chlorosis, slight reduction in the growth, purpling of the older leaves and in some cases, plants may appear symptomless (Gibson and Aritua, 2002; Clark and Valverde, 2013). Yield loss of 80 to 90% has been

recorded in areas affected by SPVD (Di Feo et al., 2000; Gutierrez et al., 2003). Although both SPFMV and SPCSV have been reported to occur in South Africa (Domola et al., 2008), co-infection of the two viruses have not been studied thoroughly to confirm the presence of SPVD. A study conducted by Mukasa and co-researchers (2006) suggests that SPCSV may synergize with ipomovirus SPMMV, producing symptoms similar to those recorded from plants affected by SPVD. SPCSV is also reported to have the ability to synergize SPCV, a member of the Caulimoviridae, belonging to the genus *Cavemovirus* (Cuellar et al., 2011). Ateka and co-researchers (2007) observed and reported synergism that occurs between the crinivirus SPCSV and potyvirus SPV2 on *I. setosa* plants. This was confirmed by high virus titers and severe symptoms of SPV2 infection on sweetpotato plants. However, when in single infections, SPV2 induces mild vein clearing or yellowing on the veins and mosaic symptoms on few leaves (Ateka et al., 2007; Clark and Valverde, 2013).

1.3.2.2 Genetic variability of RNA viruses

RNA viruses consist of a genetic diverse populations and are susceptible to genetic variation more than DNA viruses (Schneider and Roossinck, 2001). Evolution with RNA viruses occur through a collection of closely related mutant spectra/cloud termed quasispecies (Schneider and Roossinck, 2001). Replication with RNA viruses is highly error-prone due to lack of proof reading enzyme RNA-dependent RNA polymerase (RdRp), therefore, this results in large, highly diverse replicating population (Holland et al., 1982). Mechanism leading to genetic diversity and evolution are factors such as mutation, negative selection and long distance migration (human transport and propagative plant material) and recombination between divergent sequence variants, which generate new viruses (Holmes, 2009; Rubio et al., 2013). Tugume and co-researchers (2010) showed that high nucleotide diversity is observed in the P1 gene region (i.e SPMMV), which is associated with host-adaptation of potyviruses (Adams et

al., 2005; Mbewe et al., 2017). The N-terminal sequences of the CP gene region of potyviruses is reported to be variable (Untiveros et al., 2008), with the exception of the Asp-Ala-gly (DAG) amino acid triplet motif which is associated with aphid transmissibility and provide the basis for immunospecificity (Atreya et al., 1995). For potyviruses, phylogenetic analysis based on the CP nt and aa sequences has led to the classification and establishment of strain relatedness and has proven to be the region used to establish evolutionary relationships (Shukla and Ward, 1989). Untiveros and co-researchers (2010) also showed that the N-terminal part of the P1 region of SPFMV is the largest region associated with high genetic diversity compared to that of the other potyviruses.

1.3.3 *Geminiviridae*

Viruses in the *Geminiviridae* family have been associated with viral infection of most vegetable crops for many years (Briddon et al., 2002; Lozano et al., 2009). These are grouped into more than nine genera which includes *Begomovirus*, *Curtovirus*, *Mastrevirus*, *Topocurvirus*, *Becurtovirus*, *Grablovirus*, *Turncurtovirus*, *Capulavirus* and *Eragrovirus* (Zerbini et al., 2017; Varsani et al., 2017). Several begomoviruses have been found to infect sweetpotato worldwide, namely, Sweet potato leaf curl Uganda virus (SPLCUV), Sweetpotato leaf curl South Carolina virus (SPLCSCV) and SPLCSPV (Wasswa et al., 2011; Zhang and Ling, 2011; Albuquerque et al., 2011). In South Africa, two begomoviruses were recently reported in sweetpotato in the Limpopo Province. The viruses are SPLCSPV and Sweet potato mosaic virus (SPMV) (Esterhuizen et al., 2012b). Viruses in the genus *Begomovirus* consist of a geminate particle morphology which is about 18 to 38 nm in length (Brunt et al., 1996; Gutierrez, 2000). Begomoviruses consist of a single-stranded (ss) circular DNA genome of ± 3000 bp in size (Mayo, 2002; Pakkianathan and Murad, 2014; Rizvi et al., 2015). Begomoviruses exclusively infect dicotyledonous plants (Gutierrez, 2000; Lozano et al., 2009). The genome structure of

most begomoviruses (Fig. 1.3) can either be monopartite (single DNA component) or bipartite (two DNA components, namely, the DNA-A and DNA-B) (Gutierrez, 2000; Lozano et al., 2009; Pakkianathan and Murad, 2014; Rizvi et al., 2015). The DNA-A component encodes the virus functional structures such as the capsid protein (CP) and 4 other proteins which include replication initiator protein (Rep) which is also described as C1 (Rizvi et al., 2015), viral transcriptional activator protein (TrAp), replication enhancer protein (REn), C4 and V2 (Gutierrez, 2000; Lozano et al., 2009). The DNA-B encodes proteins responsible for viral movement (BC1 and BV1) (Gutierrez, 2000). Proteins C4 and V2 of DNA-A are not well defined, however, they could be responsible for viral movement (V2), pathogenicity and suppression of post-transcriptional gene silencing (AC4/C4) (Lozano et al., 2009). The *Ipomoea* infecting begomoviruses are monopartite and the Rep gene is the only factor responsible for initiating replication (Nash et al., 2011; Rizvi et al., 2015). Replication is by means of rolling circle replication (RCR) and it occurs in two stages (Gutierrez, 2000; Yadava et al., 2010). The first stage is where the genomic ssDNA is converted into double stranded DNA (dsDNA) or replicative forms (RF) to form viral minichromosomes and this is made possible by the complementary open reading frames (ORF's) which encode Rep, REn (AC3/C3) and TrAp (AC2/C2) (Gutierrez, 2000; Yadava et al., 2010). The Rep binds to the iterons, oligomerizes and cleaves within the nonanucleotide sequence within the viral strand stem loop (Gutierrez, 2000; Rizvi et al., 2015). The second stage is to generate additional replicative forms of dsDNA, which will act as a template to synthesize ssDNA through the RCR mode (Yadava et al., 2010). The synthesized ssDNA provides foundation for viral assembly and/or viral spread (Yadava et al., 2010).

Host proteins are involved in the rolling circle mechanism to enhance replication and the proteins involved include the proliferating cell nuclear antigen (PCNA), DNA polymerases, minichromosome maintenance protein 2 (MCM2), replication protein a32 (RPA32),

replication factor C (RFC), which will bind to the 3'-OH (Rizvi et al., 2015). The 3' end of the virus is extended by the host's DNA polymerase, binding the 5' end on the Rep gene (Rizvi et al., 2015). Nonanucleotides sequences are generated, ligated to the parent virion strand by Rep, resulting in the generation of the viral genome size (Rizvi et al., 2015). Nonanucleotides sequences are the identical sequences of nine nucleotides that are involved in the priming of DNA replication (Osinga et al., 1982). For accurate determination of whether a begomovirus is a monopartite or bipartite genome, scientists to date have developed degenerate primers which are able to amplify viral DNA fragments (DNA-A and DNA-B) (Rojas et al., 1993; Marwal et al., 2013). Recombinant events are common with begomoviruses, including those that infect sweetpotato (Paprotka et al., 2010).

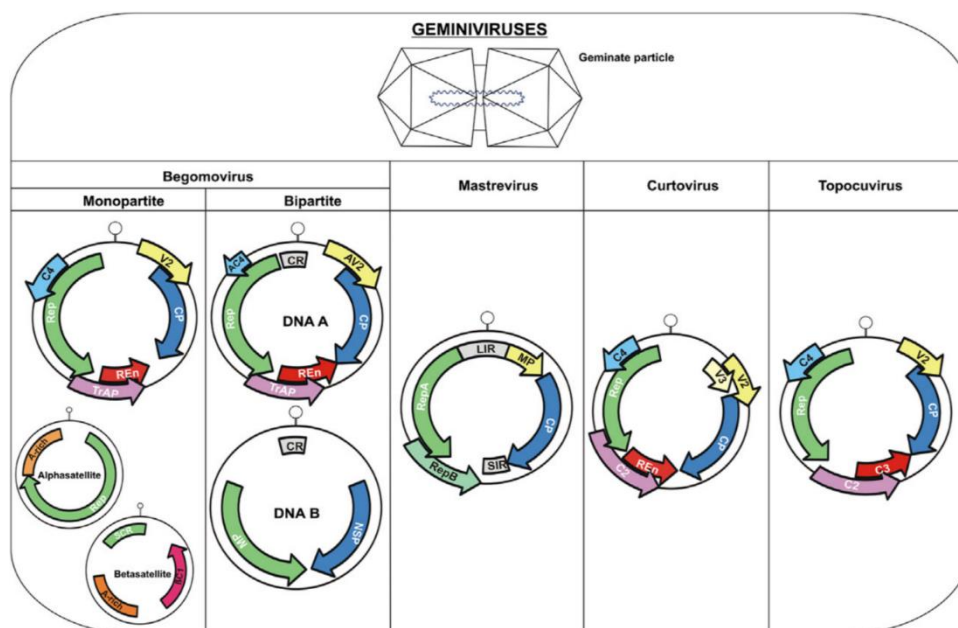


Fig. 1.3. Genome organization of four genera belonging to the family *Geminiviridae* and satellites (beta-satellite and alpha-satellite) associated with Begomovirus (Adapted from Khan et al., 2014). The position, orientation of virion-sense (V), complementary-sense (C) genes, and the predicted hairpin structure containing the TAATATTAC sequences are shown. Mastreviruses contain two non-coding regions, referred to as the large and small intergenic regions (LIR and SIR), respectively. The DNA-A and DNA-B components of bipartite Begomoviruses are of the same size, but DNA-A encodes six ORFs and DNA-B two ORFs.

Viruses belonging to the *Geminiviridae* family are transmitted by the whitefly species *B. tabaci* (Lozano et al., 2009; Simmons et al., 2009), and of all the whitefly-transmitted viruses and strains, begomoviruses are the largest (90%) (Jones, 2013; Rizvi et al., 2015). Whiteflies belong to the *Aleyrodidae* family and are mostly found in the warm climates (Jones, 2013). Whiteflies have been recognized as vectors of begomoviruses since the 1980's, and different biotype species have been described, with the B-biotype being the serious pest due to its wide geographic distribution (Jones, 2013). The B-biotype of *B. tabaci* comprise the majority of the whiteflies identified as pests of *Solanum lycopersicum* (*S. lycopersicum*), *I. batatas* and *Curcubita* species (Esterhuizen et al., 2012a).

1.3.3.1 Sweet potato leaf curl virus (SPLCV)

SPLCV is a member of the family *Geminiviridae*, genus *Begomovirus* (Trenado et al., 2011). Begomoviruses are circular, ssDNA viruses that cause economic losses in a wide range of crops worldwide (Gutierrez, 2000). High yield losses due to SPLCV were recorded in the United States of America (USA), where single infection of SPLCV resulted in drastic yield losses of between 26% (Louisiana) and 63% (South Carolina), affecting sensitive varieties (Clark and Hoy, 2006; Ling et al., 2010; Zhang and Ling, 2011). Begomoviruses SPLCSV and SPMV were detected for the first time in the Limpopo Province of South Africa (Esterhuizen et al., 2012b). SPLCSV originated from South America while SPLCV and related begomoviruses have been reported to occur in countries such as the USA, Spain, Asia and Uganda (Paprotka et al., 2010; Albuquerque et al., 2011; Trenado et al., 2011; Lotrakul et al., 2003; Luan et al., 2007; Wasswa et al., 2011; Zulfiqar et al., 2011; Bi and Zhang, 2012; Liu et al., 2014). Begomoviruses of sweetpotato are referred to as sweepoviruses (Wasswa et al., 2011; Bi and Zhang, 2012). SLCUV is reported to be distinct from other SPLCV isolates (Wasswa et al., 2011). Arkorful and Addae-Frimpomaah, (2015) also reported the presence of SPLCV in

Ghana. Symptoms induced by SPLCV can be mild and the plants can be symptomless and this is dependent on the variety's genetic characteristics (Simmons et al., 2009). Symptoms are characterized by typical leaf curling or rolling and yellowing of the veins (Valverde et al., 2007; Clark and Hoy, 2007; Wasswa et al., 2011; Zulfiqar et al., 2011). The DNA B component of SPLCV has not been identified in sweetpotato, suggesting that begomoviruses of sweetpotato are monopartite viruses (Kreuze and Fuentes, 2008). SPLCV is transmitted by whitefly species (*B. tabaci* biotype B) (Luan et al., 2007). Adult *B. tabaci* acquire the virus while feeding and this can result in 100% infection (Simmons et al., 2009). The spread of begomoviruses is perpetuated by increase in the population of whiteflies (Ling et al., 2010) and the fact that SPLCV is found to be seed transmissible in sweetpotato seeds (Kim et al., 2015), which suggests the possibility of begomovirus SPLCSPV and SPMV to be seed transmissible in future.

1.3.3.2 Genetic variability of DNA viruses

Genetic variation also exists with begomoviruses due to the fact that all viral populations contain a genetic pool of sequences that are prone to mutation. Factors such as mutation and interspecific recombination are responsible for generating novel viruses (Harrison and Robinson, 1999; Vadivukarasi et al., 2007; Albuquerque et al., 2012). Genetic evolution occurs in order for viruses to manipulate the plant cell machinery in order to maximize DNA replication and the number of cells viable for infection in order to circumvent the host antiviral defense systems (Rojas et al., 2005).

1.3.4 OTHER SWEETPOTATO VIRUSES

SPCFV belongs to the family *Betaflexiviridae*, genus *Carlavirus* and was detected and confirmed (by molecular methods) in countries such as Australia (Jones and Dwyer, 2007) and parts of East Africa (Aritua et al., 2007; Tugume et al., 2016). SPCFV was reported to infect wild species of the *Convolvulaceae* and it is known to co-infect sweetpotato plants with SPFMV, SPCSV and SPMMV (Tugume et al., 2016). Other viruses detected in sweetpotato include SPCV, a member of the *Cavemovirus*, belonging to the family *Caulimoviridae* (De Souza and Cuellar, 2011). Virions of *Caulimoviridae* consist of dsDNA which is 7.7-8.2 kilobase pairs (kb). It is not known if cavemoviruses are transmitted by vectors and their dissemination is linked to vegetative planting material (Geering and Hull, 2017). Viruses belonging to the genus *Badnaviruses* in the family *Caulimoviridae* family are reported to infect root crops such as taro, yam and sweetpotato (Bousalem et al., 2008; Kreuze et al., 2017). Badnaviruses have a monopartite genome of circular dsDNA of about 7.2-9.2 kb (Geering and Hull, 2017). They are transmitted semi-persistently by mealybugs, aphids or lace bugs. Symptoms caused by badnaviruses are variable and they depend on host plants, environmental conditions and the virus species (Geering and Hull, 2017). New technologies such as next generation sequencing (NGS) made it possible for other viruses to be discovered and viruses such as Sweet potato pakakuy virus (SPPV) A and B were reported (Clark and Valverde, 2013; Kreuze et al., 2017). SPPV has a very low titer in sweetpotato and does not show obvious symptoms on sweetpotato and indicator plant *I. setosa* and is considered as viruses that do not pose any threat to sweetpotato production (Kreuze et al., 2017).

1.3.5 DETECTION OF SWEETPOTATO VIRUSES

Identification of viruses, including those infecting sweetpotato, involve physical observation of symptoms on the host plants and indicator plants. The application of methods such as infectivity assays, serological and nucleic acid based detection methods are also used in the identification process.

1.3.5.1 Infectivity (biological) assays

Infectivity assays play an important role in plant virus identification and have been used to study virus symptoms in different host plants (Walkey, 1991). Biological assays include grafting and mechanical transmission, which are commonly used as techniques to conduct virus transmission experiments and for symptoms identification (Lima et al., 2014). Mechanical or sap transmission is the use of plant sap as an inoculum and it is accomplished by rubbing the infectious sap onto the leaf of a healthy plant (Gibbs and Harrison, 1976; Walkey, 1991). Different species of *Nicotiana tabacum* have been used for many years as host plants for the identification of PVY symptoms (Walkey, 1991). Grafting is when a union is established between two tissues of different plants and is used to carry out experimental transmission of viruses that cannot be transmitted by mechanical transmission (Walkey, 1991). Grafting of sweetpotato plant cuttings onto the indicator plant, *I. setosa* Kerr. is an initial step towards studying sweetpotato viruses (Valverde et al., 2007). Other *Ipomoea* species, such as *I. nil* (L) Roth, *I. aquatica* and *I. purpurea* (L) Roth have also been used to determine the host range of newly identified sweetpotato viruses (Cali and Moyer, 1981; Kreuze and Fuentes, 2008). Ateka and co-researchers (2006) showed that SPV2 could not be readily detected from sweetpotato plant material with serology unless the infected plants were prior grafted onto *I. setosa*.

1.3.5.2 Serological analysis

Immunodiagnostic assays such as ELISA have been the mainstay of plant virology since the 1970's (Voller et al., 1976). Voller and co-researchers (1976) successfully detected *Arabidopsis mosaic virus* and *Plum pox virus* species using ELISA. ELISA utilizes antibodies that are produced in animals to recognize the antigen proteins (Clark and Adams, 1977), and these are attached to the enzymes that facilitate the binding of the virus antigen and the antibodies to the solid phase of the polystyrene microtitre plates (Ehlers and Paul, 1984). A substrate solution interacts with enzyme labelled antibody to give a colour reaction that enables the virus to be qualitatively or quantitatively measured (Walkey, 1991). Clark and Adams (1977) adapted the ELISA procedure and since then, it has been used as a routine diagnostic technique to detect plant viruses. Different types of ELISA methods have been reported, and these include the double antibody sandwich ELISA (DAS-ELISA) and the direct and indirect ELISA, which differs, based on whether the antigen or the virus specific antibody is loaded first on the microtitre plates (Converse and Martin, 1990). Nitrocellulose membrane enzyme linked immunosorbent assay (NCM-ELISA) uses the principle of loading the virus antigen directly onto the membrane, which is incubated in a solution containing the specific antibody and the enzyme conjugated antibody to detect viruses (Lazarovits, 1990). NCM-ELISA together with DAS-ELISA, have been used successfully to confirm the presence of sweetpotato viruses, potato viruses such as tuber necrotic strain of PVY^{NTN} and Tomato spotted wilt virus (TSWV) (Aritua et al., 1998a, 1998b; Domola et al., 2008; Cloete et al., 2013; Sivparsad and Gubba, 2012). The use of polyclonal and monoclonal antibodies assisted in unravelling the relationships between SPFMV, SPMV and SwPLV (Hammond et al., 1992). Serological analysis was used successfully to detect sweetpotato viruses in Ethiopia (Tesfaye et al., 2011), Kenya (Ateka et al., 2004a; 2004b), Tanzania (Ndunguru et al., 2009), Uganda (Aritua et al., 1998a) and South Africa (Jericho and Thompson, 2000; Domola et al., 2008; Sivparsad and

Gubba, 2012). Biological assays, host range studies, virus/vector relationships and serological analysis has led to the discovery and confirmation of sweetpotato virus etiology, especially in cases where symptom identification alone was not sufficient. The ELISA technique has the ability to detect virus antigens of different size and morphology, it can be used in testing a large number of samples and it allows the provision of quantitative data to be available for further interpretation when ELISA plate reader is used (Jones, 2013; Lima et al., 2014). ELISA is a useful testing tool in virus resistance trials and in quarantine facilities where screening imported plant material and certification is crucial (Boonham et al., 2014). The limitation with ELISA technique is that it requires good quality and commercially available antibodies and some of the serological methods are not sensitive enough to detect antigens at low concentrations and those viruses occurring in complexes (Chavi et al., 1997; Boonham et al., 2014). Therefore, nucleic acid based methods become an alternative solution (Kreuze et al., 2009).

1.3.5.3 Nucleic acid based analysis

Nucleic acid methods are used together with biological and serological assays to circumvent false positive results, especially where there is mixed infections of viruses. Nucleic acid methods include the RT-PCR, PCR, quantitative real-time RT-PCR (qRT-PCR), nucleic acid hybridization (Abad and Moyer, 1992; Gibson et al., 1998; Kokkinos and Clark, 2006; Rännäli et al., 2009; Lima et al., 2014). Nucleic acid based techniques (PCR and RT-PCR) targets the genetic component of the virus genome and relies on the specific oligonucleotide primers that bind to the virus genome (Abad and Moyer, 1992; Gibson et al., 1998; Kokkinos and Clark, 2006; Rännäli et al., 2009; Lima et al., 2014). The extension process is enabled by the thermostable DNA polymerase in three different cycles, namely, the denaturation of the template, annealing of the oligonucleotides primers and the extension of the annealed primers

that extend the targeted DNA in order for it to be detectable by electrophoresis on an agarose gel (Sambrook and Russel, 2001; Webster et al., 2004). RT-PCR is utilized to detect RNA viruses by converting the RNA into a complementary DNA (cDNA) strand with reverse transcriptase enzymes (Webster et al., 2004).

1.3.5.3.1 Nucleic acid hybridization

The procedure of nucleic acid hybridization involves the immobilization of the viral genetic material, which are denatured on the membrane, followed by the virus specific probes, which are labelled by radioactive or non-radioactive methods, which are then used to detect the viral genetic material on the membrane (Valverde et al., 2007). Radioactive techniques involves labelling of membranes with ^{32}P or ^{35}S probes (Karcher, 1994). Following hybridization, autoradiography will then be used to visualize the results (Abad et al., 1992). The disadvantage of nucleic acid hybridization is that it requires licensing, waste disposal management and there are also safety concerns associated with the use of radioactive material (Karcher, 1994). The non-radioactive method utilizes different enzyme labelling such as the horseradish peroxidase, the digoxigenin and the biotin-streptavidin systems, which are attached to the probe that detects the DNA by chemiluminescent substrates (Karcher, 1994). Nucleic acid hybridization with both the radioactive and non-radioactive methods were successfully used in the past to detect SPFMV (Abad and Moyer, 1992; Abad et al., 1992). The advantage of nucleic acid hybridization is its ability to detect viruses as either a single stranded or a double stranded form (Webster et al., 2004).

1.3.5.3.2 Reverse transcription polymerase chain reaction (RT-PCR) and PCR

Degenerate primers that target the conserved regions of the virus genome have been developed and proven useful in detecting viruses of sweetpotato. This has led to the identification and differentiation of the RC, EA and O strains of SPFMV and SPVC (Colinet and Kummert, 1993; Mori et al., 1995; Souto et al., 2003; Tairo et al., 2006; Rännäli et al., 2009), and SPVG (Rännäli et al., 2008). Multiplex PCR (mPCR) is used to detect multiple virus infection in a single reaction by combining the oligonucleotides primers targeting the specific viruses and strains (Webster et al., 2004; Boonham et al., 2014). The benefits of this technique is based on the fact that it lessens the time and the costs of reagents as compared to the routine diagnosis with conventional PCR methods (Li et al., 2012b; Boonham et al., 2014). Researchers developed primers for the simultaneous detection of SPFMV, SPVC, SPVG and SPV2, and co-infection of SPFMV and SPCSV (Opiyo et al., 2010; Li et al., 2012b). The disadvantage of mPCR is that it requires robust optimization of primer concentration and thermal cycling conditions (Li et al., 2012b). Quantitative RT-PCR (qRT-PCR) is also a useful tool in quantifying virus loads especially in virus resistant studies (Bustin and Mueller, 2005) and this utilizes the probe base methods (TaqManTM) and non-probe based (SYBR green) (Boonham et al., 2014). The probe based method utilizes three oligonucleotides (the primers and probe), which will produce a signal when the target gene is present in the reaction, avoiding the productions of signals at unspecific genes (Boonham et al., 2014). Kokkinos and Clark (2006) used qRT-PCR for the detection of SPFMV, SPVG, SPCSV and SPLCV. Barkley and co-researchers (2012) also used qRT-PCR to identify SPLCV from sweetpotato germplasm in the United State Department of Agriculture, which therefore, prevented the spread of SPLCV to farmers. Oligonucleotides primers were also developed to detect SPLCV with conventional PCR (Li et al., 2004; Wasswa et al., 2011; Bi and Zhang, 2012) and are used together with the rolling circle amplification (RCA) to detect begomoviruses of sweetpotato (Li et al., 2004;

Haible et al., 2006; Wasswa et al., 2011; Bi and Zhang, 2012). Rolling circle amplification is achieved by using the circular phage DNA (from bacteriophage), which produces tandem repeats by rolling circle replications (RCR) (Reagin et al., 2003). The RCA technique utilizes the RCR to amplify circular DNA using sequence specific primers (random hexamer) and the DNA polymerase (Phi29 DNA polymerase) that is responsible for strand displacements to produce replicative events (Reagin et al., 2003).

1.3.5.3.3 Sequencing and phylogenetic analysis

Cloning part of the genome, sequencing and phylogenetic analysis is used successfully to determine virus relationships and diversity within viruses and their strains. Cloning of the CP gene of SPFMV, followed by sequencing and phylogenetic analysis aided the identification of different strains of SPFMV in Uganda and Tanzania which were not previously identified in Africa at the time (Mukasa et al., 2003a). Sakai and co-researchers (1997) were able to determine the size (10820 bp) of the full genome sequence of SPFMV by means of cloning and sequencing of the entire RNA genome. This was also shown by Li and co-researchers (2012a) who were able to confirm that SPVG and SPV2 were different virus species. RT-PCR, cloning and sequencing part of the genome also led to the confirmation of SPV2 from South African isolates, making partial genome sequences of SPV2 available in the database (Ateka et al., 2006). Confirmation of virus infection with molecular techniques is crucial since detection with serological techniques alone may yield inaccurate results due to serological relationships that may exist between virus species (cross reaction) or lack of detection of all strains of viruses (i.e. SPFMV) (Mori et al., 1995; Sakai et al., 1997; Tairo, 2006; Li et al., 2012a). One of the advantages of nucleic acid based detection is that viruses at a very low concentration can be detected (Valverde et al., 2007; Kreuze et al., 2009).

1.3.5.4 Next Generation Sequencing (NGS) and Metagenomics

The term “metagenomics” is used to refer to the study of the genome of a micro-organism from its environment, based on the cloning of the DNA fragments and sequence analyses (<https://www.everythingbio.com>). The entire microbial communities are sequenced in order to determine the diversity of all organisms living in that specific population (Saeed et al., 2012). NGS is used to generate full genome sequences without the requirement of DNA cloning (Marguerat et al., 2008; Metzker, 2010; Boonham et al., 2014), thus saving time and costs which could be incurred with commercial vectors used in cloning. NGS is able to determine variations in multiple strains of highly related genomes of pathogens, and it can be used to analyze samples containing mixed virus infections (Metzker, 2010; Liu et al., 2011; Patowary et al., 2012). NGS can be used in diseases outbreaks investigation (Patowary et al., 2012). Coetzee and co-researchers (2010) sequenced the entire dsRNA of grapevine viruses and were able to identify new and novel viruses. Sequencing of small RNAs (sRNAs) from symptomatic and asymptomatic sweetpotato plants with NGS identified novel viruses belonging to the genera potyvirus, badnavirus and mastrevirus (Kreuze et al., 2009), attesting to the fact that NGS can be used to identify both known and unknown viruses, even viruses with low titers (Kobayashi et al., 2012; Hadidi et al., 2016). The advantage of NGS is the large number of data that is generated from the ribo-depleted RNA and sRNAs library using the *de novo* approach, which yields high genome coverage to cover the whole virus genome. This is better than the data generated with the transcriptome data generated from poly(A)-selected RNA (Visser et al., 2016). The use of sRNAs, deep sequencing, coupled with Sanger sequencing has also led to the generation of genomes for sweetpotato viruses belonging to the family *Caulimoviridae*, *Betaflexiviridae* and *Potyviridae* (Mbanzibwa et al., 2014). DNA viruses require enrichment of the circular, ssDNA using the RCA method and the use of the RCA product as a template (Idris et al., 2014). Kobayashi and co-researchers (2012) further analyzed the efficiency of NGS as a

routine diagnostic tool, and they indicated that the prices of the sequencing machines are still expensive and that the nucleotide sequences generated requires high performance computers and skills in bioinformatics to analyze large amounts of sequence data generated. The need to align NGS generated sequence data to known viruses as references is also a limitation since undiscovered viruses will escape detection (Wang, 2015). The challenges listed above make NGS not yet suitable for routine diagnosis of plant viruses compared to sequences obtained with the Sanger method (Kumar et al., 2012). However, scientist are continuously developing easy to follow protocols and cost effective ways to circumvent the challenges experienced. This was shown by the development of high-throughput RNA library (HTR) preparation protocols, which has been proven to be better for Illumina (Kumar et al., 2012). Despite the good attributes that are associated with the NGS technology, it is reported that new virus information generated from the NGS data will require validation with other methods such as standard PCR, Sanger sequencing and biological analysis with indicator plants (Hadidi et al., 2016).

1.3.5.5 Electron microscopy (EM) and immunosorbent electron microscopy (ISEM)

Electron microscopy (EM) is a rapid method used to identify the group to which an unknown virus belongs to (Walkey, 1991). Immunosorbent electron microscopy (ISEM) involves the mixing of virus particles with antisera for EM analysis (Walkey, 1991). Negative stains, such as uranyl acetate, are then used to observe long filamentous, rod-shaped virus-like particles, to confirm the presence of viruses (Winter et al., 1992; Jericho and Thompson, 2000). Sivparsad (2006) used EM to observe the viral particles and to determine the length of TSWV in pepper leaf samples. The advantage of EM is that it provides detailed characterization of the virus and allows the testing of samples which cannot be identified using molecular methods (Liu et al.,

2011). The disadvantage to EM and ISEM is the fact that it requires expensive equipment, therefore, it cannot be used as a routine diagnostic tool (Feng et al., 2000).

1.3.6 MANAGEMENT OF SWEETPOTATO VIRUSES

The vegetative nature of propagation of sweetpotato and cultural practices, such as sharing of planting material among neighbours and friends provides a highly efficient mechanism for virus dissemination and spread (Terry, 1982). The exchange of germplasm for research purposes can also contribute to the introduction of new viruses into a country (Paproka et al., 2010), especially in countries where quarantine programs are not stringent. Management of plant viruses is dependent on good agricultural and cultural practices, and accurate identification with different techniques (Gibson et al., 1998; Clark et al., 2012). The incidence of sweetpotato viruses can be minimized by integrated control strategies such as the use of virus-indexed planting material, early rouging of plants that exhibits virus symptoms, chemical control of insect vectors and the use of resistant varieties (Karyeija et al., 1998; Feng et al., 2000; Orr, 2003; Gibson et al., 2004). Integrated pest management (IPM) is regarded as the best approach in controlling begomoviruses (Leke et al., 2015). In addition to these control strategies, government legislations such as the phytosanitary and quarantine regulations also play a crucial role in preventing the introduction of unwanted pathogens into a country (Maddox, 1998). Subsistence farmers in the East African regions (Uganda) select planting material from symptomless plants as starting planting materials (Karyeija et al., 1998; Gibson et al., 2000; Adam et al., 2015). The disadvantage of this strategy is that symptomless plants harbour virus diseases and become a carrier to infect more uninfected plants. Yield loss in sweetpotato is due to continuous planting with virus infected planting material. The use of virus-free planting material from virus-indexed schemes is believed to be the answer in

restoring the yielding ability of sweetpotato varieties (Feng et al., 2000). The virus indexed schemes are referred to as the use and multiplication of virus-free planting material under insect proof glasshouses, coupled with assessment of symptoms using the indicator plant, *I. setosa* (Feng et al., 2000). Producing planting material from the apical meristem tip of the healthy appearing sweetpotato stem is also a good control strategy and this is more effective when it is in combination with thermotherapy, chemotherapy and confirmation with serology and nucleic acid methods (Clark et al., 2012; Mashilo et al., 2013). An alternative method of virus elimination is the use of cryotherapy that involves brief exposure of shoot to liquid nitrogen and results in extermination of viruses (Wang et al., 2008).

SPVD is a devastating disease in most African countries and the development of varieties with increased resistant to SPVD is seen as the only effective control strategy against this virus complex (Mwanga et al., 2002a). Mwanga and co-researchers (2003) indicated that resistant varieties can be obtained through evaluation and selection for agronomic characteristics and assessing reactions of varieties to the most common pests and diseases. Resistance is based on the fact that plants are characterized by less severe symptoms and low virus titers and the yield loss is also minimized (Mwanga et al., 2002a; Mwanga et al., 2002b). Moodley and co-researchers (2015) used symptoms evaluation and molecular techniques to identify varieties that are resistant to PVY affecting pepper production in Kwazulu Natal and the same techniques can be employed in identifying sweetpotato varieties that have potential resistance towards sweetpotato viruses under local conditions. The advantage of breeding for virus resistance is that there is no need for farmers to apply any control measures since there is a direct link to the integrated control strategies which helps in preventing yield and quality losses (Khetarpal et al., 1998). Different methods of resistance engineering are continually being investigated and the danger is that resistance can be broken if virus strains are different from those used when resistant varieties were developed. Karyeija and co-researchers (2000) showed

that it is possible to identify a variety that is resistant to SPFMV but such resistance can be broken down when the same variety is infected with SPCSV, most probably due to the fact that SPCSV enhances the virus titer of SPFMV. Kreuze and co-researchers (2008) successfully used RNA silencing approach to generate resistance to SPFMV and SPCSV using a transcript that generates a dsRNA hairpin generating siRNAs homologous to the polymerase genes of each virus, inducing RNA silencing defense against the two viruses. Sivparsad and Gubba (2014) also demonstrated and confirmed that gene silencing using the CP encoding regions of SPFMV, SPMMV, SPVG and SPCSV can reduce virus infection when symptoms on the transgenic plants were delayed and milder than those of the non-transgenic plants.

Marker assisted selection for resistance to SPFMV and SPCSV was reported to have a potential in identifying resistance genes against SPVD and it can be achieved using the amplified fragment length polymorphism (AFLP) and randomly amplified polymorphism (RADP) (Mwanga et al., 2002b; Mcharo et al., 2005; Miano et al., 2008). Mwanga and co-researchers (2002b) identified two major recessive genes, namely, e41m33.a (*spcsv1*) and s13.1130 (*spfmv1*) responsible for mediating resistance to SPCSV and SPFMV. Virus-tested programs and seed multiplication systems have not been well established in the most SPVD stricken countries and is dominated by the informal seed systems, which still utilize the retained planting material from previous seasons (Clark et al., 2012; Clark and Valverde et al., 2013). This challenge emphasizes the need for a sustainable seed multiplication system in Africa in general. In South Africa, the Agriculture Research Council (ARC-VOP, Roodeplaat, Pretoria) has a sweetpotato virus-indexing scheme which provides virus-free planting material to sweetpotato vine cuttings distributors, smallholder and commercial farmers and this system can contribute to a larger extent, in reducing the spread of viruses (Laurie et al., 2016).

1.4 RATIONAL AND MOTIVATION

1.4.1 Background to the project

Sweetpotato is grown mostly by smallholder farmers in rural areas as a food security crop that is harvested piecemeal when there is a need for food to feed the family. Sweetpotato viruses negatively affect production and food supply to rural people and smallholder farmers who depend on the crop as their food source. Viruses are one of the main causes of yield reduction in sweetpotato production and this is due to the vegetative nature of its propagation, which increases the accumulation of viruses from previous seasons, thereby increasing the spread from one area to the other (Clark et al., 2012). Jericho and Thompson (2000), followed by Domola and co-researchers (2008) conducted field surveys in the Limpopo, Mpumalanga, Kwazulu Natal, Gauteng, Eastern Cape and North West provinces in the 1998 and 2001/2003, respectively and viruses such as SPFMV and SPVG were detected using serological methods. Low levels of SPCSV, SPV2, SPMMV, SPCFV and SPLV were also detected in multiple virus infections with SPFMV and SPVG. Sivparsad and Gubba (2012) confirmed the occurrence of SPFMV, SPVC, previously known as the C strain of SPFMV and SPVG in Kwazulu Natal by molecular methods. Begomoviruses, SPLCSPV and SPMAY were later reported to occur in the Waterpoort area of Limpopo Province of South Africa (Esterhuizen et al., 2012b). Mulabisana and co-researchers (2015) also confirmed the cocurrence of SPFMV and SPVC in samples collected from Gauteng Province by molecular methods. Domola and co-researchers reported that both total and marketable yield is negatively affected by multiple infections of potyviruses. Viruses such as SPFMV, SPVG, SPLCSPV and SPMAY are currently the main viruses threatening the production of sweetpotato in South Africa.

1.4.2 Problem statement

Potyvirus SPFMV and SPVG have been found to be the most important and commonly detected viruses in some provinces of the country. Following the detection of begomoviruses SPLCSPV and SPMV in the Limpopo Province, there is also a need to investigate the possible occurrence of begomoviruses in other provinces of the country. Therefore, these viruses were selected for this study. Detection of viruses using serological methods is challenged due to virus titers that are often low and undetectable, and the presence of internal inhibitors in the sweetpotato leaf samples that interferes with detection methods (Clark et al., 2012). Such a disadvantage may affect virus identification, including that of sweetpotato. Furthermore, genetic characterization of SPFMV, SPVG, SPLCSPV and SPMV with molecular techniques has not been widely studied throughout the different provinces of South Africa. It is therefore, of utmost importance to confirm the occurrence of the selected viruses by molecular techniques and to conduct genetic variability studies with more virus isolates to understand genetic relationships with those from other geographic regions of the world. Additionally, the effects of begomovirus and potyvirus co- and multiple infections on the yield of South African varieties need to be determined. The genetic characterization at the sequence level will enable the development of accurate and sensitive PCR-based detection techniques for routine diagnosis. This will allow future development of integrated control measures for sweetpotato viruses. Moreover, it is critical that yield losses due to viruses is reduced and this will be achieved through field trials to determine the effects of single and multiple virus infection on the yield of different sweetpotato varieties in order to select for virus resistance. Identification of resistant varieties will assist rural and smallholder farmers to produce more sweetpotato for household consumption, and for commercial farmers to get a return on their investment on input costs (Clark et al., 2012).

1.4.3 PROJECT AIMS

Based on the problems and challenges discussed above, the current study was aimed at investigating the incidence of sweetpotato viruses through epidemiological surveys conducted in Gauteng, Limpopo and Mpumalanga provinces of South Africa. The following were the specific objectives of the project,

- To determine the occurrence of sweetpotato viruses with more emphasis on potyviruses (SPFMV and SPVG) and begomovirus (SPLCV) by conducting epidemiological surveys of symptomatic and asymptomatic plants in the three sweetpotato production provinces of South Africa.
- To identify sweetpotato viruses using infectivity assays and serological techniques and to further characterize SPFMV, SPVG and SPLCV by means of partial genome sequencing followed by phylogenetic analyses.
- To conduct full genome NGS of SPLCV isolates that showed clear differences with restriction fragment length polymorphism (RFLP) in order to accurately determine the species and strains identified.
- To conduct field trials to determine the effects of begomoviruses (SPLSPV and SPMV) and potyviruses (SPFMV, SPVG) on the yield of locally bred sweetpotato varieties.

1.5 REFERENCES

1. Abad, J.A., Moyer, J.W., 1992. Detection and distribution of Sweetpotato feathery mottle virus (SPFMV) in sweetpotato by *in vitro* transcribed RNA probes (Rhiboprobes), membrane immunobinding assay (MIBA), and direct blotting. *Phytopathology* 82, 300-305.
2. Abad, J.A., Conkling, M.A, Moyer, J.W., 1992. Comparison of the capsid protein cistron from serologically distinct strains of *Sweet potato feathery mottle virus*. *Archives of Virology* 126,147-157.
3. Adam, R.I., Sindi, K. Badstue, L., 2015. Farmers' knowledge, perceptions and management of diseases affecting Sweetpotatoes in the Lake Victoria Zone region, Tanzania. *Crop Protection* 72, 97-107.
4. Adams, M.J., Antoniow, J.F, Beaudoin, F., 2005. Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. *Molecular Plant Pathology* 6 (4), 471-487.
5. Albuquerque, L.C., Inoue-Nagata, A.K., Pinheiro, B., Ribeiro, S.G., Resende, R.O., Moriones, E., Navas-Castillo, J.N., 2011. A novel mopopartite Begomovirus infecting sweetpotato in Brazil. *Archives of Virology* 156, 1291-1294. Doi 10.1007/s00705-011-1016-x.
6. Albuquerque, L.C., Inoue-Nagata, A.K., Pinheiro, B., Resende, R.O., Moriones, E, Navas-Castillo, J.N., 2012. Genetic diversity and recombination analysis of sweepoviruses. *Virology Journal* 9 (241), 1-13. <http://www.virologyj.com/content/9/1/241>.
7. Alicai, T., Fenby, N.S., Gibson, R.W., Adipala, E., Vetten, H.J., Foster, G.D., Seal, S.E., 1999. Occurrence of two serotypes of *Sweet potato chlorotic stunt virus* in East Africa and their associated differences in coat protein and HSP70 homologue gene sequences. *Plant Pathology* 48, 718-726.

8. Allemann, J., 2004. Fertilization, irrigation and weed control. In, Guide to sweetpotato production in South Africa. Niederwieser J.G. (Eds.). ARC-Roodeplaat Vegetable and Ornamental Plant Institute. ISBN 1-86849-293-3. pp. 27-38.
9. Ames, T., Smit, N.E.J.M., Braun, A.R., O'Sullivan, J.N, Skoglund, L.G., 1996. Sweetpotato, Major Pests, Diseases, and Nutritional Disorders. International Potato Center (CIP). Lima, Peru. pp 152.
10. Alvarez, A., D.A., Biderbost, E., Nome, S.F., 1997. Sequencing and characterization of the coat protein and 3' non-coding region of a new sweetpotato Potyvirus. Archives of Virology 142, 1635-1644.
11. ARC, 2011. Growing vegetables. Published by the Agricultural Research Council. ISBN 978-186849-408-8. <http://www.arc.agric.za>.
12. Aritua, V., Alicai, T., Adipala, E., Carey, E.E. , Gibson, R.W., 1998a. Aspects of sweetpotato virus disease in sweetpotato. Annals of Applied Biology 132, 387-398.
13. Aritua, V., Adipala, E., Carey, E.E. , Gibson, R.W., 1998b. The incidence of sweetpotato virus disease and virus resistance of sweetpotato grown in Uganda. Annals of Applied Biology 132, 399-411.
14. Aritua, V., Barg, E., Adipala, E., Vetten, H.J., 2007. Sequence analysis of the entire RNA genome of *Sweet potato chlorotic fleck virus* isolate reveals that it belongs to a distinct carlavirus species. Archives of Virology 152, 813-818.
15. Arkorful, E., Addae-Frimpomaah, F., 2015. Detection of Sweetpotato (*Ipomoea batatas* L.) leaf curl virus (SPLCV) in Ghana using visual symptomatology and PCR technique. International Journal of Plant Breeding and Genetics 9 (3), 106-115. ISSN 1819-3595/ doi 10.3923/ijpbg.2015.106.115.

16. Ateka, E.M., Njeru, R.W., Kibaru, A.G., Kimenju, J.W., Barg, E., Gibson, R.W., Vetten, H.J., 2004a. Identification and distribution of viruses infecting sweetpotato in Kenya. *Annals of Applied Biology* 144, 371-379.
17. Ateka, E.M., Barg, E., Njeru, R.W., Lesemann, D.-E., Vetten, H.J., 2004b. Further characterization of *Sweet potato virus 2*, a distinct species of the genus Potyvirus. *Archives of Virology* 149, 225-239.
18. Ateka, E.M., Barg, E., Njeru, R.W., Thompson G., Vetten, H.J., 2006. Biological and molecular variability among geographically diverse isolates of *Sweet potato virus 2*. *Archives of Virology* 52 (3), 479-488.
19. Ateka, E.M., Barg, E., Njeru, R.W., Thompson, G., Vetten, H.J., 2007. Biological and molecular variability among geographically diverse isolates of *Sweet potato virus 2*. *Archives of Virology* 152, 479-488.
20. Atreya, P.L., Lopez-Moya, J.J., Chu, M., Atreya, C.D., Pirone, T.P., 1995. Mutational analysis of the coat protein N-terminal amino acids involved in Potyvirus transmission by aphids. *Journal of General Virology*, 76, 265-270.
21. Barkley, N.A., Pinnow, D.L., Wang, M.L., Ling, K.S., Jarret, R.L., 2012. Detection and classification of SPLCV isolates in the U.S. Sweetpotato germplasm collection via a Real-Time PCR assay and phylogenetic analysis. *Plant Disease* 95 (11), 1385-1391.
22. Bhat, A.I., Hohn, T., Selvarajan, R., 2016. Badnaviruses: The Current Global Scenario. Ploss A (eds.). *Viruses* 8(6), 177. Doi:10.3390/v8060177.
23. Bi, H., Zhang, P., 2012. Molecular characterization of two sweepoviruses from China and evaluation of the infectivity of the cloned SPLCV-JS in *Nicotiana benthamiana*. *Archives of Virology*, 157, 441-454.

24. Boonham, N., Walsh, K., Hims, M., Preston, S., North, J., Barker, I., 2002. Biological and sequence comparisons of *Potato virus Y* isolates associated with potato tuber necrotic ringspot disease. *Plant Pathology* 51, 117-126.
25. Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., Mumford, R., 2014. Methods in virus diagnostics, From ELISA to next generation sequencing. *Virus Research* 186, 20-31.
26. Bousalem, M., Douzery, E., Seal, S., 2008. Taxonomy, molecular phylogeny and evolution of plant reverse transcribing viruses (family *Caulimoviridae*) inferred from full-length genome and reverse transcriptase sequences. *Archives of Virology* 153, 1085-1102.
27. Briddon, R.W., Bull, S.E., Mansoor, S., Amin, I., Markham, P.G., 2002. Universal primers for the PCR-mediated amplification of DNA β . *Molecular Biotechnology* 20, 315-318.
28. Brito, L., Brouwer, R., Falcão, M., 2012. Sweetpotato-Biotechnology in different guises on broad range of scales. *Technological Forecasting and Social change* 79, 204-212.
29. Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L., 1996. *Viruses of Plants. Descriptions and Lists from VIDE Database*. C.A.B International. Cambridge, United Kingdom. Pages 1223-1224.
30. Bustin, S.A., Mueller, R., 2005. Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. *Clinical Science* 109, 365-379.
31. Cali, B.B., Moyer, J.W., 1981. Purification and particle morphology of two russet crack strains of *Sweet potato feathery mottle virus*. *Phytopathology* 71, 302-305.
32. Campbell, R.N., Hall, D.H., Mielimis N.M., 1973. Aetiology of sweetpotato russet crack disease. *Phytopathology* 64, 120-218.
33. Chavi, F., Robertson, A.I., Verduin, B.J.M., 1997. Survey characterization of viruses in sweetpotato from Zimbabwe. *Plant Disease* 81, 1115-1122.

34. Clark, C.A., Hoy, M.W., 2006. Effect of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. *Plant Disease* 90, 83-88.
35. Clark, C.A., Moyer, J.W., 1988. *Compendium of sweetpotato Diseases*. American Pathological Society. St Paul, USA, APS Press.
36. Clark, C.A., Valverde, R.A., 2013. Diseases caused by viruses. In, *Compendium of sweetpotato diseases, pests and disorders*, second edition. Clark, C.A., Ferrin, D.M., Smith, T.P., Holmes, G.J. (Eds.). American Phytopathological Society.
37. Clark, C.A., 2013. Diseases caused by bacteria. In, *Compendium of sweetpotato diseases, pests and disorders*, second edition. Clark, C.A., Ferrin, D.M., Smith, T.P., Holmes, G.J. (Eds.). American Phytopathological Society, pp 8-11.
38. Clark, C.A., Davis, J.A., Abad, A., Cuellar, W.J., Fuentes, S., Kreuze, J.F., Gibson, R.W., Mukasa, S.B., Tugume, A.K., Tairo, F.D. Valkonen, J.P.T., 2012. Sweetpotato viruses, 15 years of progress on understanding and managing complex diseases. *Plant Disease* 96 (2), 168-185.
39. Clark, C.A., Valverde, R.A., Fuentes, S., Salazar, L.F., Moyer, J.W., 2002. Research for improved management of sweetpotato pests and diseases, variety decline. Ames, T. (Eds). *Proceedings of the 1st international conference on sweetpotato, food and health for the future*. Lima, Peru, pp 103-112. *Acta Horticulturae* 583.
40. Clark, M.F., Adams, A.N., 1977. Characteristics of the micro plate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34, 475-483.
41. Cloete, M., Mabasa, K., Mulabisana, J., Visser, A., Rakuambo, J., Netshifhehe, S., 2013. Evaluation of commercial potato varieties for resistance to the tuber necrotic strain of

- Potato Virus Y*. Proceedings of the second All Africa Horticulture Conference. Acta Horticulturae 1007, 255-260.
42. Coetzee, B., Freeborough, M.J., Maree, H.J., Celton, J.M., Rees, D.J., Burger, J.T., 2010. Deep sequencing analysis infecting grapevines, virome of a vineyard. *Virology* 400 (2), 157-163.
43. Colinet, D., Kummert, J., 1993. Identification of a *Sweet potato feathery mottle virus* isolate from China (SPFMV-CH) by polymerase chain reaction with degenerate primers. *Journal of Virological Methods* 45, 149-159.
44. Colinet, D., Kummert, J., Lepoivre, P., 1996. Molecular evidence that the whitefly-transmitted sweetpotato mild mottle virus belongs to a distinct genus of the *Potyviridae*. *Archives of Virology* 141, 125-135.
45. Colinet, D., Nguyen, M., Kummert, J., Lepoivre, P., 1998. Differentiation among Potyviruses infecting sweetpotato based on genus and virus-specific reverse transcription polymerase chain reaction. *Plant Disease* 82, 223-229.
46. Colinet, J., Kummert, P., Semal, J., 1994. Identification of distinct Potyviruses mixedly infected sweetpotato by the polymerase chain reaction with degenerate primers. *Phytopathology* 84, 65-69.
47. Converse, R.H., Martin, R.R., 1990. ELISA methods for plant viruses. In, *Serological methods for detection and identification of viral and bacterial plant pathogens*. Hampton, R., Ball, E., De Boer, S. (Eds.). A laboratory manual. APS Press, Minnesota, USA. pp. 179-196.
48. Coutts, B.A., Kehoe, M.A., Webster, C.G., Wylie, S.J., Jones, R.A.C., 2011. *Zucchini yellow mosaic virus*, biological properties, detection procedures and comparison of coat protein gene sequences. *Archives of Virology* 156, 2119-2131.

49. Cuellar, W.J., De Souza, J., Barrantes, I., Fuentes, S., Kreuze, J.F., 2011. Distinct *Cavemoviruses* interact synergistically with *Sweet potato chlorotic stunt virus* (genus Crinivirus). *Journal of General Virology* 92 (5), 1233-1243.
50. 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 sweetpotato (*Ipomoea batatas* L.). *Molecular plant pathology* 16 (5), 459-471.
51. DAFF. 2010. A profile of the South African sweetpotato market value chain. http://www.DAFF.gov.za/docs/AMCP/sweetpotatovcp2010_11.PDF. Department of Agriculture, Forestry and Fisheries.
52. DAFF. 2011a. Abstracts of Agricultural statistics, 2011. http://www.DAFF.gov.za/docs/statisticsinfo/Abstract_2011. Department of Agriculture, Forestry and Fisheries.
53. DAFF. 2011b. Sweetpotato (*Ipomoea batatas* L.) production. http://www.nda.agric.za/docs/Brochures/PG_sweetpotato.pdf. Department of Agriculture, Forestry and Fisheries.
54. DAFF. 2016. Abstracts of Agricultural statistics, 2016. http://www.DAFF.gov.za/docs/statisticsinfo/Abstract_2016.
55. Dennien, S., 2015. Sweetpotato viruses and phytoplasmas in Australia: Literature review March 2015. Horticulture innovation Australia. <https://www.aspg.com.au/wp-content/uploads/2015/03/Managing-sweetpotato-viruses-in-Australia-Literature-review-March-2015-.pdf>.
56. De Souza, J., Cuellar, W., 2011. Sequence analysis of the replicase gene of ‘*Sweetpotato caulimo-like virus*’ suggests that this virus is a distinct member of the genus *Cavemovirus*. *Archives of Virology*, 156, 535-537.

57. Desbiez, C., Costa, C., Wipf-Scheibel, C., Girard, M., Lecoq, H., 1997. Serological and molecular variability of *Watermelon mosaic virus* (genus Potyvirus). Archives of Virology 152, 775-781. doi 10.1007/s00705-006-0899-4.
58. Di Feo, L., Nome, S.F., Biderbost, E., Fuentes, S., Salazar, L.F., 2000. Etiology of sweetpotato chlorotic dwarf disease in Argentina. Plant Disease 84, 35-39.
59. Dolja, V.V., Haldeman, R., Robertson, N.L., Dougherty, W.G., Carrington, J.C., 1994. Distinct functions of capsid protein in assembly and movement of Tobacco etch Potyvirus in plants. The EMBO Journal 13 (6), 1482-1491.
60. Domingo, E., Martín, V., Perales, C., Grande-Pérez, A., García-Arriaza, J., Arias, A., 2006. Viruses as quasi species, Biological implications. In, Quasi species, Concept and Implications for Virology. Domingo, E. (Eds.). Berlin, Heidelberg, Springer-Verlag. pp. 51-82.
61. Domola, M.J., Thompson, G. J., Aveling T.A.V., Laurie, S.M., Strydom, H., van Den Berg, A.A., 2008. Sweetpotato viruses in South Africa and the effect of viral infection on storage yield. African Plant Protection 14, 15-23.
62. du Plooy, C.P., 1986. Progress and limitations in breeding of the sweetpotato (*Ipomoea batatas*) in South Africa. Acta Horticulturae 194, 77-82.
63. Ehlers, U., Paul, H.L., 1984. Binding of viruses from crude plant extracts to glutaraldehyde-treated plates for indirect ELISA. Journal of Virological Methods 8, 217-224.
64. Engoru, P., Mugisha, J., Bashaasha, B., 2005. A characterization of tuber utilization among sweetpotato producers in Eastern Uganda. African Crop Science Conference Proceedings 6, 726-731. ISSN 1023-070X/2005.
65. Esterhuizen, L.L., Mabasa, K.G., van Heerden, S.W., Czosnek, H., Brown, J.K., van Heerden, H., Rey, M.E.C., 2012a. Genetic identification of members of *Bemisia tabaci*

- cryptic species complex from South Africa reveals native and introduced haplotypes. *Journal of Applied Entomology*. Doi 10.1111/j.1439-0418.2012.01720.x.
66. Esterhuizen, L.L., van Heerden, S.W., Rey, M.E.C., van Heerden, H., 2012b. Genetic identification of two sweet-potato-infecting Begomoviruses in South Africa. *Archives of Virology*. Doi 10.1007/s00705-012-1398-4.
 67. Ewell, P.T., Mutuura, J., 1991. Sweetpotato in the food system of eastern and southern Africa. In, *Proceedings of the Ninth symposium of the International Society for Tropical Root Crops. Tropical root crops in a developing economy*. Ofori, F., Hahn, S.K. (Eds.). Accra, Ghana.
 68. Faber, M., Venter, S., Phungula, M.A.S., Benadé, A.J.S., 2001. An intergrated primary helth-care and provitamin A household food-production program, Impact on food-consumption patterns. *Food and Nutrition Bulletin*, vol. 22, no. 4. The United Nations University.
 69. Faber, M., Wenholdb, F.A.M., Laurie, S.M., 2017. Dietary diversity and vegetable and fruit consumption of households in a resource-poor peri-urban South Africa. *Ecology of Food and Nutrion* Vol 56 (1), 62-80. <http://dx.doi.org/10.1080/03670244.2016.1261024>.
 70. Fauquet, M.C., Mayo, M.A., 1999. Abbreviations for plant virus names. *Archives of Virology* 144, 6. *Virology division news*. pp. 1267-1268.
 71. Feng, G., Yifu, G., Pinbo, Z., 2000. Production and deployment of virus-free sweetpotato in China. *Crop Protection* 19, 105-111.
 72. Food and Agriculture Organization of the United Nations, Rome, 2010. The state of food security in the world, addressing food insecurity in protracted crises. www.fao.org/docrep/013/i1683e/i1683e.pdf.

73. Food and Agriculture Organization of the United Nations (FAOSTAT), 2015.
<http://faostat3.fao.org/browse/Q/QC/E>.
74. Food and Agriculture Organization of the United Nations (FAOSTAT), 2017.
<http://faostat.fao.org/>.
75. Geering, A.D.W., Hull, R., 2017. Descriptions of Plant Viruses Online,
<http://www.dpvweb.net/index.php>.
76. Gibbs, A., Harrison, B., 1976. Plant Virology, The principles. Printed by Butler and Tanner, LTD., Frome, Great Britain.
77. Gibson, R.W., Aritua, V., 2002. The perspective of *Sweet potato chlorotic stunt virus* in sweetpotato production in Africa, A review. African Crop Science Journal 10, 281-310.
78. Gibson, R.W., Aritua, V., Byamukama, E., Mpembe, I., Kayongo, J., 2004. Control strategies for sweetpotato virus disease in Africa. Virus Research 100, 115-122.
79. Gibson, R.W., Mpembe, I., Alicai T., Carey, E.E., Mwanga, R.O.M., Seal, S.E., Vetten, H. J., 1998. Symptoms, etiology and serological analysis of sweetpotato virus disease in Uganda. Plant Pathology 47, 95-102.
80. Gibson, R.W., Kreuze, J.F., 2015. Degeneration in sweetpotato due to viruses, virus cleaned planting material and reversion, a review. Plant Pathology 64, 1-15.
81. Grüneberg, W.J., Ma, D., Mwanga, R.O.M, Carey, E.E., Huamani, K., Diaz, F., Eyzaguirre, R., Guaf, E., Jusuf, M., Karuniawan, A., Tjintokohadi, K., Song, Y.-S., Anil, S.R., Hossain, M., Shofiur Rahaman, E.H.M., Attaluri, S., Some, K., Afuape, S., Adofo, K., Lukonge, E., Karanja L., Ndirigwe, J., Ssemakula, G., Agili, S., Randrianaivoarivony, J.-M, Chiona, M., Chipungu, F., Laurie, S., Ricardo, J., Andrade, M., Rausch Fernandes, F., Mello, A.S., Khan, A., Labonte, D.R., Yencho, G.C., 2015. Advances in sweetpotato breeding from 1993 to 2012. In, Potato and Sweetpotato in Africa, transforming the value chains for food

- and nutrition security. Low, J., Nyongesa, M., Quinn, S., Parker, M. (Eds.). www.cabi.org.za. pp 1-77.
82. Gutierrez, C., 2000. *Geminiviruses* and plant cell cycle. *Plant Molecular Biology* 43, 763-772.
83. Gutierrez, D.L., Fuentes, S., Salazaar, L.F., 2003. Sweetpotato virus disease (SPVD), Distribution, incidence and effect on sweetpotato yield in Peru. *Plant Disease* 87, 297-302.
84. Green, S., Lo, C., 1989. 1989., Elimination of sweetpotato yellow dwarf virus (SPYDV) by meristem tip culture and by heat treatment. *Journal of Plant Diseases and Protection* 96 (5), 464-469. Retrieved from <http://www.jstor.org/stable/43385752>.
85. Haible, D., Kober, S., Jeske, H., 2006. Rolling Circle Amplification revolutionizes diagnosis and genomics of *Geminiviruses*. *Journal of Virological Methods* 135, 9-16.
86. Hadidi, A., Flores, R., Candresse, T., Barba, M., 2016. Next-generation sequencing and genome editing in plant virology. *Frontiers in Microbiology*, Volume 7. doi 10.3389/fmicb.2016.01325.
87. Hammond, J., Jordan, L.R., Larsen, C.R., Moyer, J.W., 1992. Use of polyclonal antisera and monoclonal antibodies to examine serological relationships among three filamentous viruses of sweetpotato. *Phytopathology* 82, 713-717.
88. Harrison, B.D, Robinson, D.J., 1999. Natural genomic and antigenic variation in whitefly-transmitted *Geminiviruses* (Begomoviruses). *Annual Review of Phytopathology* 37 (1), 369-398.
89. Holland, J., Spindler, K., Horodyski, F., Grabau, E., Nichol, S., Vande Pol, S., 1982. Rapid evolution of RNA genomes. *Science* (215), 1577-1585.

90. Holmes, E.C., 2009. The Evolutionary Genetics of Emerging Viruses. The Annual Review of Ecology, Evolution and Systematics 40, 353-372. 10.1146/annurev.ecosys.110308.120248.
91. Hollings, M., Stone, O.M., Bock, K.R., 1976. Sweetpotato mild mottle virus. Description of plant viruses (162). Commonwealth Agricultural Bureaux and the Association of Applied Biologists. United Kingdom.
92. Idris, A., Al-Saleh, M., Piatek, M.J., Al-Shahwan, I., Ali, S., Brown, J.K., 2014. Viral Metagenomics, Analysis of Begomoviruses by Illumina high-throughput sequencing. Viruses 6, 1219-1236. doi 10.3390/v6031219.
93. IsHak, J.A., Kreuze, J.F., Johansson, A., Mukasa, S.B., Tairo, F., Abo El-Abbas, F.M., Valkonen, J.P.T., 2003. Some molecular characteristics of three viruses from SPVD-affected sweetpotato plants in Egypt. Archives of Virology 148, 2449-2460.
94. Ivanov, K.I., Eskelin, K., Lohmus, A., Mäkinen, K., 2014. Molecular and cellular mechanisms underlying Potyvirus infection. Journal of General Virology 95, 1415-1429.
95. Jericho, C. Jr., Thompson, G.J., 2000. Detection of sweetpotato viruses in S.A. African Potato Association Proceedings 73, 379-391.
96. Jones, R.A.C., Dwyer, G.I., 2007. Detection of *Sweet potato chlorotic fleck virus* and *Sweet potato feathery mottle virus*-strain O in Australia. Australian Plant Pathology 36, 591-594.
97. Jones, R.C.A., 2013. Trends in plant virus epidemiology, opportunities from new or improved technologies. Virus Research. doi.org/10.1016/j.viruses.2013.11.003.
98. Kapinga, R., Andrade, M., Lemaga, B., Gani, A., Crissman, C., Mwanga, R., 2005. Role of orange fleshed-sweetpotato in disaster mitigation, experiences from the East and Southern Africa. African Crop Science Conference Proceedings (7), 1321-1329. ISSN 1023-070X/2005.

99. Karcher, S.J., 1994. Non-radioactive nucleic acid detection systems. In, Plant Molecular Biology Manual, second edition. Gelvin, S., B., Schilperoort, R. (Eds.). Kluwer Academic Publishers. F3, pp. 1-25.
100. Karyeija, R.F., Gibson, R.W., Valkonen, J.P.T., 1998. The significance of *Sweet potato feathery mottle virus* in subsistence sweetpotato production in Africa. Plant Disease 82, 4-15.
101. Karyeija, R.F., Kreuze, J.F., Gibson, R.W., Valkonen, J.P.T., 2000. Synergistic interaction of a Potyvirus and a phloem-limited Crinivirus in sweetpotato plants. Virology 269, 26-36.
102. Kashif, M., Artola, K., Jones, R.A.C., Tugume, A.K., Mäkinen, K., Valkonen, J.P.T., 2012. Detection of viruses in sweetpotato from Honduras and Guatemala augmented by deep-sequencing of small-RNAs. Plant Disease 96 (10), 1430-1437.
103. Kasschau, K.K., Cronin, S., Carrington, J.C., 1997. Genome amplification of long-distance movement functions associated with the central domain of Tobacco etch Potyvirus helper component-proteinase. Virology 228, 251-262.
104. Khetarpal, K.R., Maisonneuve, B., Maury, Y., Chalhoub, B., Dinant, S., Lecoq, H., Varma, A., 1998. Breeding for resistance to plant viruses. In, Plant virus disease control. Hadidi, A., Khetarpal, R.K., Konganezwa, H. (Eds). APS Press. The American Phytopathological Society, St. Paul, Minnesota. pp. 14-32.
105. Kim, J., Kil, E.-J., Kim, S., Seo, H., Byun, H.-S., Park, J., Chung, M.-N., Kwak, H.-R., Kim, M.-K., Kim, C.-S., Yang, J.-W., Lee, K.-Y., Choi, H.-S., Lee, S., 2015. Seed transmission of sweet potato leaf curl virus in sweetpotato (*Ipomoea batatas*). Plant Pathology 64, 1284-1291.

106. Kil, E.-J., Kim, J., Byun, H.-S., Kim, S., Kwak, H.-R., Kim, M.-K., Choi, H.-S., Chung, M.-N., Lee, S., 2014. First Report of sweet potato golden vein associated virus infecting sweetpotato in Korea. *Plant Disease* 98 (8) 1163-1163.
107. Kim, J., Yang, J. W., Kwak, H.-R., Kim, M.-K., Seo, J.-K., Chung, M.-N., Lee, H.U., Lee, K.B., Nam, S.S., Kim, C.S., Lee, G.S., Kim, J.S., Lee, S., Choi, H.-S., 2017. Virus Incidence of sweetpotato in Korea from 2011 to 2014. *The Plant Pathology Journal*, 33(5), 467-477. <http://doi.org/10.5423/PPJ.OA.08.2016.0167>.
108. Khan, A.J., Akhtar, S., Mansoor, S., Amin, I., 2014. Engineering crops for resistance to *Geminiviruses*. In, *Plant Virus-Host Interaction, molecular approaches and viral evolution*. Gaur, R.K., Hohn, T., Sharma, P. (Eds.). Elsevier Inc. pp 291-323.
109. Kiss, Z.A., Medina, V., Falk, B.W., 2013. Crinivirus replication and host interactions. *Frontiers in Microbiology*. doi 10.3389/fmicb.2013.00099.
110. Kobayashi, K., Atsumi, G., Yamaoka, N., Sekine, K., 2012. Sequencing-based virus hunting and virus detection. *Japan Agricultural Research Quarterly* 46 (2), 123-128. <http://www.jircas.affrc.go.jp>.
111. Kokkinos, C.D., 2006. Assessment of interactions among viruses infecting sweetpotato. PhD Thesis, Louisiana State University, pp 1-134, <http://etd.lsu.edu/docs/available/etd-03092006-163704/>.
112. Kokkinos, C.D., Clark, C.A., 2006. Real-Time PCR assays for the detection and quantification of sweetpotato viruses. *Plant Disease* 90, 783-788.
113. Kreuze, J., Fuentes, S., 2008. Sweetpotato viruses. International Potato Centre (CIP), Lima, Peru. Elsevier LTD. pp 659-669.
114. Kreuze, J.F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., Simon, R., 2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing

- of small RNAs, A generic method for diagnosis, discovery and sequencing of viruses. Virology 388, 1-7.
115. Kreuze, J.F., Perez, A., Galveza, M., Cuellar, W.J., 2017. *Badnaviruses* of sweetpotato, symptomless co-inhabitants on a global scale. bioRxiv 140517. <https://doi.org/10.1101/140517>.
 116. Kumar, R., Ichihashi, Y., Kimura, S., Chitwood, D.H., Headland, L.R., Peng, J., Maloof, J.N., Sinha, N.R., 2012. A high-throughput method for Illumina RNA-Seq library preparation. Frontiers in Plant Science. <http://genomics.xprize.org/doi>, 10.3389/fpls.2012.00202.
 117. Kurabachew, H., 2015. The Role of orange-fleshed sweetpotato (*Ipomea batatas*) for Combating Vitamin A Deficiency in Ethiopia, A Review. International Journal of Food Science and Nutrition Engineering 5 (3), 141-146. doi, 10.5923/j.food.20150503.05.
 118. Kwak, H.R., Kim, M.K., Chung, M.N., Lee, S.H., Park, J.W., Kim, K.H., Choi, H.S., 2006. Virus disease incidences of Sweetpotatoes in Korea. Plant Pathology Journal 22, 239-247. Doi: 10.5423/PPJ.2006.22.3.239.
 119. Labadarios, D. Moodie, I.M., Van Rensburg, A., 2007. Selected micronutrient status, vitamin A. In, National Food Consumption Survey-Fortification Baseline (NFCS-FB), South Africa, 2005. Labadarios, D. (Eds.). pp. 409-446. Directorate Nutrition, Department of Health, Pretoria.
 120. Laurie, S., van Jaarsveld, P.J., Faber, M., Philpott, M.F., Labuschagne, M.T., 2012. Trans- β -carotene, selected mineral content and potential nutritional contribution of 12 sweetpotato varieties. Journal of food composition and Analysis 27, 151-159.
 121. Laurie, S., Faber, M., Adebola, P., Beletse, A., 2015b. Biofortification of sweetpotato for food and nutrition security in South Africa. Food Research International. <http://dx.doi.org/10.1016/j.foodres.2015.2015.06.001>.

122. Laurie, S.M., Tjale, S.S., van den Berg, A.A., Mtileni, M.M., Labuschagne, M.T., 2015a. Agronomic performance of new cream to yellow-orange sweetpotato varieties in diverse environments across South Africa. *South African Journal of Plant and Soil*, 1-9. ISSN 0257-1862, EISNN 2167-034x.
123. Laurie, S.M., Myeza, P.N., Mulabisana, M.J., Mabasa, K.G., Thompson, A., Greyling, R., Cloete, M., Adebola, P.O., 2016. In vitro propagation and disease testing as a means of producing healthy planting materials to support root and tuber crops production in South Africa. *Acta Horticulturae*, 1113. ISHS 2016. doi 10.17660/ActaHortic.2016.1113.34.
124. Lazarovits, G., 1990. The dot immunobinding assay. In, *Serological methods for detection and identification of viral and bacterial plant pathogens. A laboratory manual*. USA. Hampton, R., Ball, E., De Boer, S. (Eds.). APS Press, Minnesota. pp. 179-196.
125. Loebenstein, G., Thottappilly, G., Fuentes, S., Cohen, J., 2009. Virus and phytoplasma diseases. In: *The sweetpotato*. Loebenstein, G., Thottappilly, G. (eds). Springer Sciences Business Media BV, Dordrecht, The Netherlands. pp 105-134.
126. Leke, W.N., Mignouna, D.B., Brown, J.K., Kvarnheden, A., 2015. Begomovirus disease complex, emerging threat to vegetable production systems of West and Central Africa. *Agriculture and Food Security* 4, (1), 1-14. doi 10.1186/s40066-014-0020-2
127. Li, R., Salih, S., Hurtt, S., 2004. Detection of *Geminiviruses* in sweetpotato by polymerase chain reaction. *Plant Disease* 88, 1347-1351.
128. Li, F., Xu, D., Abad, J., Li, R., 2012a. Phylogenetic relationships of closely related Potyviruses infecting sweetpotato determined by genomic characterization of *Sweet potato virus G* and *Sweet potato virus 2*. *Virus Genes*. doi 10.1007/s11262-012-0749-2.

129. Li, F., Ruijuan, Z., Abad, J., Donglin, X, Gaili, B. , Li, R., 2012b. Simultaneous detection and differentiation of four closely related sweetpotato Potyviruses by multiplex one-step RT-PCR. *Journal of Virological Methods*. <http://dx.org/10.1016/j.jviromet.2012.07.021>.
130. Lima, J.A.A., Nascimento, A.K.Q., Lima, R.C.A., Oliveira, V.C., Anselmo, G.C., 2014. Methods of diagnosis, stability, transmission, and host interaction of *Papaya lethal yellowing virus* in papaya. In, *Plant Virus-Host Interaction, molecular approaches and viral evolution*. Gaur, R.K, Hohn, T., Sharma, P (Eds.). Elsevier Inc. pp 207-227.
131. Ling, K., Jackson, D.M., Harrison, H., Simmons, Pesic-VanEsbroek, Z., 2010. Field evaluation of yield effects on the U.S.A heirloom sweetpotato varieties infected by *Sweet potato leaf curl virus*. *Crop Protection*, 757-765.
132. Liu, S., Vijayendran, D., Bonning, B.C., 2011. Next generation sequencing technologies for insect virus discovery. *Viruses* 3, 1849-1869. doi 10.3390/v3101849. ISSN. 1999-4915. www.mdpi.com/journal/viruses.
133. Liu, Q., Zhang, Z., Li, J., Qiao, Q., Qin, Y., Zhang, D., Tiang, Y., Wang, S., Wang, Y., 2014. Complete genome of novel monopartite Begomovirus infecting sweetpotato in China. *Archives of Virology* 159, 1537-1540.
134. Lotrakul, P., Valverde, R.A., Clark, C.A., Fauquet, C.M., 2003. Properties of a Begomovirus isolated from sweetpotato [*Ipomoea batatas* (L.) Lam.] infected with Sweetpotato leaf curl virus. *Revista Mexicana de Fitopatología* 21, 128-136.
135. Lozano, G., Trenado, H.P., Valverde, R.A., and Navas-Castillo, J., 2009. Novel Begomovirus species of recombinant nature in sweetpotato (*Ipomoea batatas*) and *Ipomoea indica*, taxonomic and phylogenetic implications. *Journal of General Virology* 90, 2550-2562.

136. Luan, Y.S., Zhang, J., Lui, D M., 2007. Molecular characterization of *Sweet potato leaf curl virus* isolate from China (SPLCV-CN) and its phylogenetic relationship with other members of the *Geminiviridae*. *Virus Genes* 35, 379-385.
137. Lucas, J., 2006. Plant viral movement proteins, agents for cell-to-cell trafficking of viral genomes. *Virology* 344, 169-184.
138. Lucier, G., Allhouse, J., Hwan Lin, B., 2002. Sweetpotato, Getting to the root of demand. Agricultural Outlook in, Economic Research Service, USDA. www.ers.usda.gov/publications/aib777.
139. Maddox, D., 1998. Implications of new technologies for seed health testing and the worldwide movement of seed. *Seed Science Research* 9, 277-284.
140. Marguerat, S., Wilhelm, B.T., Bähler, J., 2008. Next generation sequencing, applications beyond genomes. *Biochemical Society Transactions* 36, 1091-1096. doi, 10.1042/BST0361091.
141. Martelli, G.P., Agranovsky, A.A., Bar-Joseph, M., Boscia, D., Candresse, T., Coutts, R.H.A., Dolja, V.V., Falk, B.W., Gonsalves, D., Jelkmann, W., Karasev, A.V., Minafra, A., Namba, S., Vetten, H.J., Wisler, G.C., Yoshikawa, N., 2002. The family *Closteroviridae* revised. *Archives of Virology* 147, 10.
142. Martelli, G.P., Ghanem-Sabanadzovic, N.A., Agranovsky, A.A., Alrwah- nih, M., Dolja, V.V., Dova, C.I., Fuchs, M., Gugerli, P., Hu, J.S., Jelkmann, W., Katis, N.I., Maliogka, V.I., Melzer, M.J., Menzel, W., Minafra, A., Rott, M.E., Rowhani, A., Sabanadzovic, S., Saldarelli, P., 2012. Taxonomic revision of the family *Closteroviridae*, with special reference to the grapevine leaf roll-associated members of the genus *Ampelovirus* and the putative species unassigned to the family. *Journal of Plant Pathology* 94, 7-19.

143. Marwal, A., Sahu, A.K., Gaur, R.K., 2013. Molecular Characterization of Begomoviruses and DNA satellites associated with a new host Spanish Flag (*Lantana camara*) in India. ISRN Virology. <http://dx.doi.org/10.5402/2013/915703>.
144. Mascia, T., Gallitelli, D., 2014. Synergism in plant-virus interactions, a case study of CMV and PVY in mixed infection in tomato. In, Plant Virus-Host Interaction, molecular approaches and viral Evolution. Gaur, R.K, Hohn, T., Sharma, P (Eds.). Elsevier Inc. pp 196-206.
145. Mashilo, J., van Niekerk, R., Shanahan, P., 2013. Combined thermotherapy and meristem tip culture for efficient elimination of feathery mottle virus in sweetpotato (*Ipomoea batatas*). Proceedings of the 2nd All Africa Horticulture Congress. Acta Horticulturae, 1007. ISHS.
146. Mayo, M.A., 2002. Plant viruses as molecular pathogens. In, Principles and current practice of plant virus taxonomy. Khan, A.J., Dijkstra, J. (Eds.). Food Products Press®, New York, London.
147. Mbanzibwa, D.R., Tugume, A.K., Chiunga, E., Mark, D., Tairo, F.D., 2014. Small RNA deep sequencing-based detection and further evidence of DNA viruses infecting sweetpotato plants in Tanzania. Annals of Applied Biology 165, 329-339. doi 10.1111/aab.12136.
148. Mbewe, W., Tairo, F., Sseruwagi, P., Ndunguru, J., Duffy, S., Mukasa, S., Benesi, I., Sheat, S., Koerbler, M., Winter, S., 2017. Variability in P1 gene redefines phylogenetic relationships among cassava brown streak viruses. Virology Journal 14, 118. doi 10.1186/s12985-017-0790-9.
149. McClean, A.P.D., Klessner, P.J., 1947. Mottle-leaf disease of sweetpotato. In, *Farming in South Africa*, 76. Horticultural Research Institute, Pretoria. pp. 1-4.

150. Mcharo, M., LaBonte, D., Mwanga, R.O.M., Kreigner, A., 2005. Associated molecular markers with virus resistance to classify sweetpotato genotypes. *Journal of the American Society of Horticultural Science* 130, 355-359.
151. Merits, A., Rajamäki, M., Lindholm, P., Runeberg-Roos, P., Kekarainen, T., Puustinen, P., Katri Mäkeläinen, K., Valkonen, J.P., Saarma, M., 2002. Proteolytic processing of potyviral proteins and polyprotein processing intermediates in insect and plant cells. *Journal of General Virology* 8, 1211-1221.
152. Metzker, M., 2010. Sequencing technologies-the next generation. *Nature Reviews*, volume 11. <http://nature.com/reviews/genetics>.
153. Miano, D.W., LaBonte, D.R., Clark, C.A., 2008. Identification of molecular markers associated with sweetpotato resistance to sweetpotato virus in Kenya. *Euphytica* 160, 15-24.
154. Mingot, A., Valli, A., Rodamilans, B., San León, D., Baulcombe, D.C., García, J.A., López-Moya, J.J., 2016. The P1N-PISPO trans-frame gene of Sweet potato feathery mottle potyvirus is produced during virus infection and functions as an RNA silencing suppressor. *Journal of Virology* 90, 3543-3557. doi:10.1128/JVI.02360-15.
155. Mishra, R., Verma, R.K., Sharma, P., Choudhary, D.K., Gaur, R.K., 2013. Interaction between viral proteins with transmission of Potyvirus. *Archives of Phytopathology and Plant Protection*. <http://dx.doi.org/10.1080/03235408.2013.807659>.
156. Mishra, R., Verma, R.K., Sharma, P., Choudhary, D.K., Gaur, R.K. 2014. Interaction between viral proteins with the transmission of Potyvirus. *Archives of Phytopathology and Plant Protection* 47 (2), 240-253. DOI: 10.1080/03235408.2013.807659.
157. Mori, M., Sakai, J., Kimura, T., Usugi, T., Hayashi, T., Hanada, K., Nishiguchi, M., 1995. Nucleotide sequence analysis of two nuclear inclusion body and coat protein genes of *Sweet*

- potato feathery mottle virus* severe strains (SPFMV) genomic RNA. Archives of Virology 140, 1473-1482.
158. Motsa, N.M., Modi, A.T., Mabhaudhi, T., 2015. Sweetpotato response to low-input agriculture and varying environments of KwaZulu-Natal, South Africa, implications for food security strategies. Acta Agriculturae Scandinavica, Section B- Soil, Plant Science, 65 (4), 329-340. doi 10.1080/09064710.2015.1011222.
 159. Moyer, J.W., Salazar, L.F., 1989. Virus and virus-like disease of sweetpotato. Plant Disease 73, 451-445.
 160. Moyer, J.W., Clark, C.A., 2013. Alternaria leaf spot and leaf petiole and stem blight. In, Compendium of sweetpotato diseases, pests and disorders, second edition. Clark, C.A., Ferrin, D.M., Smith, T.P., Holmes, G.J. (Eds.). American Phytopathological Society, pp 21-22.
 161. Moyer, J.W., Clark, C.A., 2013. Fusarium wilt. In, Compendium of sweetpotato diseases, pests and disorders, second edition. Clark, C.A., Ferrin, D.M., Smith, T.P., Holmes, G.J. (Eds.). American Phytopathological Society, pp 40-42.
 162. Moodley, V., Naidoo, R., Gubba A., 2015. Screening of pepper (*Capsicum annuum* L.) lines for resistance to an isolate of Potato virus Y (PVY) occurring in KwaZulu-Natal (KZN), Republic of South Africa. Crop Protection 68, 36-40.
 163. Mukasa, S.B., Tairo, F., Kreuze, J.F., Kullaya, A., Rubaihayo, P.R., Valkonen, J.P.T., 2003a. Coat protein sequence analysis reveals occurrence of new strains of *Sweet potato feathery mottle virus* in Uganda and Tanzania. Virus Genes 27, 49-56.
 164. Mukasa, S.B., Rubaihayo, P.R., Valkonen, J.P.T., 2003b. Sequence variability within the 3'-proximal part of *Sweet potato mild mottle virus* genome. Archives of Virology 148, 487-496.

165. Mukasa, S.B., Rubaihayo, P.R., Valkonen, J.P.T., 2006. Interactions between a Crinivirus, an Ipomovirus and a Potyvirus in co-infected sweetpotato. *Plant Pathology* 55, 458-467.
166. Mukhopadhyay, S.K., Chattopadhyay, A., Chakraborty, I., Bhattacharya, I., 2011. Crops that feed the world 5. Sweetpotato. Sweetpotatoes for income and food security. *Food Security* 3, 283-305. doi 10.1007/s12571-011-0134-3.
167. Mulabisana, J., Cloete, M., Mabasa, K., Laurie, S.M., Oelofse, D, Rey, M.E.C., 2015. Genetic characterization of viruses infecting sweetpotato in South Africa. *Acta Horticulturae* 1105. ISHS. doi 10.17660/ActaHortic.2015.1105.22.
168. Mwanga, R.O.M., Moyer, J.W., Zhang, D.P., Carey, E.E., Yencho, G.C., 2002a. Nature of resistance to Sweetpotato virus disease. *Acta Horticulturae* 583, 113-119.
169. Mwanga, R.O.M., Kriegner, A., Cervantes-Flores, J.C., Zhang, D.P., Moyer, J.W., Yencho, G.C., 2002b. Resistance to *Sweetpotato chlorotic stunt* and *Sweetpotato feathery mottle virus* is mediated by two separate recessive genes in sweetpotato. *Journal of American Horticultural Science* 127 (95), 798-806.
170. Mwanga, R.O.M., Odongo, B., Turyamureeba, G., Ajalo, A., Yencho, G.C., Gibson, R.W., Smit, N.E.J.M., Carey, E.E., 2003. Release of six sweetpotato varieties ('Naspot 1' to 'Naspot 6') in Uganda. *Horticultural Science* 38, 475-476.
171. Nash, T.E., Dallas, M.B., Reyes, M.I., Buhrman, G.K., Ascencio-Ibanez, J.T., Hanley-Bowdoin, L., 2011. Functional Analysis of a novel motif conserved across *Geminivirus* Rep proteins. *Journal of Virology*, 1182-1192.
172. Ndunguru, J., Kapinga, R., Sseruwagi P., Bulili, S., Mwanga, R., Tumwegamire, T., Rugutu, C., 2009. Assessing the sweetpotato virus disease and its associated vectors in

- Northwest Tanzania and Central Uganda. *African Journal of Agricultural Research* 4 (4), 334-343.
173. Nhlapo, T.F., Mulabisana, J.M., Odeny, D.A., Rey, M.E.C., Rees, D.J.G., 2018. First report of Sweetpotato badnavirus A and Sweetpotato badnavirus B in South Africa. *Plant Disease*. <http://dx.doi.org/10.1094/PDIS-08-17-1235-PDN>.
 174. OH, C., Carrington, J.C., 1989. Identification of Essential Residues in Potyvirus Proteinase HC-Pro by Site-Directed Mutagenesis. *Virology* 173, 692-699.
 175. Opiyo, S.A., Ateka, E.M., Owour, P.P., Manguro, L.O., Miano, O.W., 2010. Development of a multiplex PCR technique for simultaneous detection of Sweetpotato feathery mottle virus and Sweetpotato chlorotic stunt virus. *Journal of Plant Pathology* 92 (2), 363-366.
 176. Orr, A., 2003. Integrated pest management for resource-poor African Farmers, Is the emperor naked? *World Development* 31 (5), 831-845.
 177. Osinga, K.A., De Haan, M., Christianson, T., Tabak, H.F., 1982. A nonanucleotide sequence involved in promotion of ribosomal RNA synthesis and RNA priming of DNA replication in yeast mitochondria. *Nucleic acid Research* 10 (24), 7993-8006.
 178. Pakkianathan, B.C., Murad, G., 2014. Recent advances on interactions between the whitefly *Bemisia tabaci* and Begomoviruses, with emphasis on *Tomato yellow leaf curl virus*. In, *Plant Virus-Host Interaction, molecular approaches and viral evolution*. Gaur, R.K., Hohn, T., Sharma, P. (Eds.). Elsevier Inc. pp 79-103.
 179. Pappu, S.S., Brand, R., Pappu, H.R., Rybicki, E.P., Gough, K.H., Frenkel, M.J., Niblett, C.L., 1993. A polymerase chain reaction method adapted for selective amplification and cloning of 3' sequences of potyviral genomes, application to *Dasheen mosaic virus*. *Journal of Virological Methods* 41, 9-20.

180. Paprotka, T., Bioteux, L.S., Fonseca, M.E.N., Resende, R.O., Jeske, H., Faria, J.C., Ribeiro, S.G., 2010. Genomic diversity of Sweetpotato *Geminiviruses* in a Brazilian germplasm bank. *Virus Research* 149, 224-233.
181. Patowary, A., Chauhan, R.K., Shamsudheen, K.V., Periwal, V., Kushwaha, K.P., Sapkal, G.N., Bondre, V.P., Gore, M.M., Sivasubbu, S., Scaria, V., 2012. De novo identification of viral pathogens from cell culture hologenomes. *BMC Research Notes* 5, 11. Short Report.
182. Qin, Z., DeFee, M., Isaacs, J.S., Parsons, C., 2010. Extracellular Hsp90 serves as a co-factor for MAPK activation and latent viral gene expression during de novo infection by KSHV. *Virology* 403, 92-102.
183. Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis, R.I., Mu, L., Dwyer, G.I., Coutts, B.A., Valkonen, J. P. T., 2008. Molecular characterization of Sweet potato virus G (SPVG) isolates from areas of the Pacific Ocean and Southern Africa. *Plant Disease* 92, 1313-1320.
184. Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis, R.I., Mu, L., Valkonen, J. P.T., 2009. Molecular characterization of Sweetpotato feathery mottle virus (SPFMV) isolates from Easter Island, French Polynesia, New Zealand, and Southern Africa. *Plant Disease* 93, 933-939.
185. Reagin, M.J., Giesler, T.I., Meria, A.I., Resetar-Gerke, J.M., Kapolka, K.M., Mamone, J.A., 2003. TempliPhi, A sequencing template preparation procedure that eliminates overnight cultures and DNA purification. *Journal of Biomolecular Techniques* 14, 143-148.
186. Rizvi, I., Choudhury, N.R., Tuteja, N., 2015. Insights into the functional characteristics of *Geminivirus* rolling-circle replication initiator protein and its interaction with host factors affecting viral DNA replication. *Archives of Virology* 160, 375-387. doi 10.1007/s00705-014-2297-7.

187. Rodamilans, B., Valli, A., Mingot, A., San León, D., Baulcombe, D., López-Moya, J.J., García, J.A., 2015. RNA polymerase slippage as a mechanism for the production of frameshift gene products in plant viruses of the Potyviridae family. *Journal of Virology* 89, 6965-6967. doi:10.1128/JVI.00337-15.
188. Rojas, M.R., Gilbertson, R.L., Russel, D.R., Maxwell, D.P., 1993. Use of degenerate primers in the polymerase chain reaction to detect whiteflies-transmitted *Geminiviruses*. *Plant Disease* 77, 340-347.
189. Rojas, M.R., Zerbini, F.M., Allison, R.F., Gilbertson, R.L., Lucas, W.J., 1997. Capsid protein and helper component-proteinase function as Potyvirus cell-to-cell movement proteins. *Virology* 237, 283-295.
190. Rojas, M.R., Hagen, C., Lucas, W.J., Robert, L., Gilbertson, R.L. 2005. Exploiting Chinks in the Plant's Armor, Evolution and emergence of *Geminiviruses*. *Annual Review of Phytopathology* 43 (1), 361-394.
191. Rubio, L., Guerri, J., Moreno, P., 2013. Genetic variability and evolutionary dynamics of viruses of the family *Closteroviridae*. *Frontiers in microbiology* 4 (151), 1-15. doi 10.3389/fmicb.2013.00151.
192. Saeed, I., Tang, S., Halgamuge, S.K., 2012. Unsupervised discovery of microbial population structure within metagenomes using nucleotide base composition. *Nucleic Acids Research* 40 (5). doi 10.1093/nar/gkr1204.
193. Sakai, J., Mori, M., Morishita, T., Takana, M., Hananda, K., Usugi, T., Nishiguchi M., 1997. Complete nucleotide sequence and genome organization of *Sweet potato feathery mottle virus* (S strain) genomic RNA, The large coding region of P1 gene. *Archives of Virology* 142, 1553-1562.

194. Sambrook, J., Russel, D.W., 2001. Molecular cloning, A laboratory manual. Third edition. Cold Spring Harbour Laboratory Press, Cold Spring Harbor, New York.
195. Schaad, M. C., Jensen, P. E., Carrington, J. C., 1997. Formation of plant RNA virus replication complexes on membranes, role of an endoplasmic reticulum-targeted viral protein. EMBO Journal 16, 4049-4059.
196. Simmons, A.M., Ling, K., Harrison, H.F., Jackson, D.M., 2009. Sweetpotato leaf curl virus, efficiency of acquisition, retention and transmission by *Bemisia tabaci* (Hemiptera, Aleyrdidae). Crop Protection 28, 1007-11011.
197. Sorel, M., Garcia, J. A., German-Retana, S., 2014. The Potyviridae cylindrical inclusion helicase, a key multipartner and multifunctional protein. Molecular Plant Microbe Interactions 27, 215-226.
198. Shukla, D.D., Ward, C.W., 1989. Identification and classification of Potyviruses on the basis of capsid protein sequence and serology. Archives of Virology 106, 171-200.
199. Schneider, W.Y., Roossinck, M., 2001. Genetic diversity in RNA virus quasispecies is controlled by host-virus interactions. Journal of Virology 75 (14), 6566-6571. doi 10.1128/JVI.75.14.6566–6571.2001.
200. Sivparsad, B.J., 2006. Isolation and molecular characterisation of Tomato spotted wilt virus (TSWV) isolates occurring in South Africa. A Thesis submitted to the partial fulfilment of the requirements for the degree of Master of Science. University of KwaZulu-Natal.
201. Sivparsad, B.J., Gubba, A., 2012. Molecular resolution of the genetic variability of major viruses infecting sweetpotato (*Ipomoea batatas* L.) in the province of KwaZulu-Natal in the Republic of South Africa. Crop Protection 41, 49-56.

202. Sivparsad, B.J., Gubba, A., 2014. Development of transgenic sweetpotato with multiple virus resistance in South Africa. *Transgenic Research* 23, 377-388.
203. Souto, E.R., Sim, J., Chen, J., Valverde, R.A., Clark, C.A., 2003. Properties of strains of Sweetpotato feathery mottle virus and two newly recognized Potyvirus infecting sweetpotato in the United States. *Plant Disease* 87, 1226-1232.
204. Steinbauer, C.E., Kushman, L.J., 1971. Sweetpotato culture and diseases. *Agriculture Handbook* no. 388. Agricultural Research Service. United States Department of Agriculture, Washington, D.C.
205. Syller, J., Grupa, A., 2014. The effects of co-infection by different Potato virus Y (PVY) isolates on virus concentration in Solanaceous hosts and efficiency of transmission. *Plant Pathology* 63, 466-475.
206. Tairo, F., Mukasa, S.B., Jones, R.C.J., Kullaya, A., Rhubaihayo, P.R., Valkonen, J.P., 2005. Unravelling the genetic diversity of the three main viruses involved in sweetpotato virus disease (SPVD), and its practical amplifications. *Molecular Plant Pathology* 6 (2), 199-211.
207. Tairo, F., Jones, R.A.C., Valkonen, J. P.T., 2006. Potyvirus complexes in sweetpotato, Occurrence in Australia, serological and molecular resolution, and analysis of the *Sweetpotato virus 2* (SPV2) components. *Plant Disease* 90, 1120-1128.
208. Terry, E.R., 1982. Sweetpotato (*Ipomoea batatas*) virus diseases and their control. In, Sweetpotato. Proceedings of the First International Symposium. Villareal, R.L., Griggs, T.D. (Eds.). Asian Vegetable Research and Development Center. Shanhua, Tainan, Taiwan, China. pp. 161-168.

209. Tesfaye, T., Feyissa, T., Abraham, A., 2011. Survey and serological detection of sweetpotato (*Ipomoea batatas* (L.) Lam.) viruses in Ethiopia. *Journal of Applied Biosciences*, 41, 2746-2756.
210. Trenado, H.P., Orílio A.F., Márquez-Martin B., Moriones E., Navas-Castillo, J., 2011. Sweepoviruses cause disease in sweetpotato and related *Ipomoea* spp., fulfilling Koch's postulates for a divergent group in the genus Begomovirus. *PloS ONE* 6 (11), e27329. doi,10.1371/journal.pone.0027329.
211. Tugume, A.K., Mukasa, S.B., Kalkiinen, N, Valkonen, J.P., 2010. Recombination and selection pressure in the Ipomovirus *Sweet potato mild mottle virus* (Potyviridae) in wild species and cultivated sweetpotato in the centre of evolution in East Africa. *Journal of General Virology* 91 (4), 1092-1108.
212. Tugume, A.K., Mukasa, S.B., Valkonen, Y., 2016. Mixed infections of four viruses, the incidence and phylogenetic relationships of *Sweet potato chlorotic fleck virus* (Betaflexiviridae) isolates in wild species and Sweetpotatoes in Uganda and evidence of distinct isolates in East Africa. *PLoS ONE* 11 (12), e0167769. Doi 10.1371/journal.pone.0167769.
213. Untiveros, M., Fuentes, S., Kreuze, J., 2008. Molecular variability of *Sweet potato feathery mottle virus* and other Potyviruses infecting sweetpotato in Peru. *Archives of Virology* 153, 473-483.
214. Untiveros, M., Quispe, D., Kreuze, J., 2010. Analysis of complete genomic sequences of isolates of the *Sweet potato feathery mottle virus* strain C and EA, molecular evidence for two distinct Potyvirus species and two P1 protein domains. *Archives of Virology* 155, 2059-2063.

215. USDA National Nutrient Database for Standard Reference, 2016. Basic report 11507, Sweetpotato, raw, unprepared. <http://www.ncSweetpotatoes.com/usda-sweet-potato-nutritional-analysis/benefits-of-Sweetpotato/>. Basic Report 11507.
216. Vadivukarasi, T., Girish, K.R., Usha, R., 2007. Sequence and recombination analyses of the *Geminivirus* replication initiator protein. *Journal of Bioscience* 32 (1), 17-29.
217. Valkonen, J.P.T., Rajamäki, M., Kekarainen, T., 2002. Mapping of the viral genomic regions important in cross-protection between strains of a Potyvirus. *MPMI* 15 (7), 683-692. Publication no. M-2002-0510-01R. The American Phytopathological Society.
218. Valverde, R.A., Clark, C.A., Valkonen, J.P.T., 2007. Viruses and virus disease complexes of sweetpotato. *Plant Viruses* 1 (1), 116-126.
219. van den Berg, A.A., Laurie, S.M., 2004. Cultivation. In, *Guide to sweetpotato production in South Africa*. Niederwieser J.G. (Eds.). ARC-Roodeplaat Vegetable and Ornamental Plant Institute. pp. 15-26. ISBN 1-86849-293-3.
220. van den Berg, A.A., Laurie, S.M., Niederwieser, J.G., 2004. Harvesting, storage and marketing. In, *Guide to sweetpotato production in South Africa*. Niederwieser J.G. (Eds.). ARC-Roodeplaat Vegetable and Ornamental Plants. pp. 39-56. ISBN 1-86849-293-3.
221. van Jaarsveld, P.J., Faber, M., Tanumihardjo, S.A., Nestel, P., Lombard, C.J., Sprinler Benadé, A.J., 2005. B-Carotene-rich orange-fleshed sweetpotato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test ¹⁻³. *American Society of Clinical Nutrition*, 81, 1080-1087.
222. Varsani, A., Roumagnac, P., Fuchs, M., Navas-Castillo, J., Moriones, E., Idris, A., Briddon, R.W., Rivera-Bustamante, R., Zerbini, F.M., Martin, D.P., 2017. Capulavirus and Grablovirus: two new genera in the family Geminiviridae. *Archives of Virology*, doi 10.1007/s00705-017-3268-6. *Virology Division News*.

223. Verchot, J., Carrington, J.C., 1995. Evidence that the Potyvirus P1 Proteinase functions in trans as an accessory factor for genome amplification. *Journal of Virology*, 3668-3674.
224. Verchot, J. Koonin, E.V., Carrington, J.C., 1991. The 35-kDa Protein from the N-Terminus of the Potyviral Polyprotein as a Third Virus-Encoded Proteinase. *Virology* 18, 527-535.
225. Visser, M., Bester, R., Burger, J.T., Maree, H.J., 2016. Next-generation sequencing for virus detection, covering all the bases. *Virology Journal* 13, 85. doi 10.1186/s12985-016-0539-x.
226. Vijayapalani, P., Maeshima, M., Nagasaki-Takekuchi, N., Miller, W.A., 2012. Interaction of the Trans-Frame Potyvirus Protein P3N-PIPO with Host Protein PCaP1 Facilitates Potyvirus Movement. *PLoS Pathogens*, 8(4), e1002639. <http://doi.org/10.1371/journal.ppat.1002639>.
227. Voller, A., Bartlett, A., Bidwell, D.E., Clark, M.F., Adams, A.N., 1976. The detection of viruses by enzyme-linked immunosorbent assays (ELISA). *Journal of General Virology* 33, 165-167.
228. Walkey, D.G.A., 1991. *Applied Plant Virology*. Second Edition. Chapman and Hall, London, New York. Printed by St Edmundsbury Press.
229. Wang, Z., Li, Z., Huang, L., Chen, X., Fang, B., Li, Y., Chen, J., Zhang, X., 2011. Characterization and development of EST-derived SSR markers in cultivated sweetpotato (*Ipomoea batatas*). *BioMed Central Biology* 11, 139.
230. Wang, D., 2015. Fruits of virus discovery, new pathogens and new experimental models. *Journal of Virology* 89 (3), 1486-1488. doi, 10.1128/JVI.01194-14.
231. Wang, Q.C., Cuellar, W.J., Rajamaki, M.L., Hiraka, Y., Valkonen, J.P.T., 2008. Combined thermotherapy and cryotherapy for virus eradication: relation of virus

- distribution, subcellular changes, cell survival and viral RNA degradation in shoot tips o efficient production of virus-free plants. *Molecular Plant Pathology* 9, 237-250.
232. Wasswa, P., Otto, B., Maruthi, M.N., Mukasa, S.B., Monger, W., Gibson, R.W., 2011. First identification of sweetpotato Begomovirus (sweepovirus) in Uganda, characterization, detection and distribution. *Plant Pathology*. doi 10.1111/1365-3059.2011.02464.x.
 233. Webster, C.G., Wylie, S.J., Jones, M.G.K., 2004. Diagnosis of plant viral pathogens. *Current Science* volume 86, no. 12. Review Article.
 234. Wei, T., Zhang, C., Hong, J., Xiong, R., Kasschau, K.D., 2010. Formation of complexes at plasmodesmata for potyvirus intercellular movement is mediated by the viral protein P3N-PIPO. *PLOS* 6 (6), e1000962. <https://doi.org/10.1371/journal.ppat.1000962>.
 235. Winter, S., Purac, A., Leggett, F., Frison, E.A., Rossell, H.W., Hamilton, R.I., 1992. Partial characterization and molecular cloning of a closterovirus from sweetpotato infected with the sweetpotato virus disease complex from Nigeria. *Phytopathology* 82, 869-875.
 236. World Atlsas, 2017. Top Sweet Potato Growing Countries. <https://www.worldatlas.com/articles/top-sweet-potato-growing-countries.html>
 237. Winter, S., Körbler, M., Köllner, U., 1997. Detection and differentiation of sweetpotato *Closteroviruses* by RT-PCR and single-strand conformation polymorphism. *Diagnosis and Identification of plant pathogens*. pp 473-475. Kluwer academic publishers, Netherlands.
 238. Wylie, S., Adams, M., Chalam, C., Kreuze, J., López-Moya, J., Ohshima, K., Praveen, S., Rabenstein, F., Stenger, D., Wang, A., Zerbini, F., ICTV Report Consortium. 2017. ICTV Virus Taxonomy Profile: Potyviridae. *Journal of General Virology* 98 (3), 352-354. Doi:10.1099/jgv.0.000740

239. Zerbini, F.M., Briddon, R.W., Idris, A., Martin, D.P., Moriones, E., Navas-Castillo, J., Rivera-Bustamante, R., Roumagnac, P., Varsani, A. 2017. ICTV Virus Taxonomy Profile: Geminiviridae. *Journal of General Virology* 98, 131-133. Doi 10.1099/jgv.0.000738.
240. Zhang, S.C.Z., Ling, K. S., 2011. Genetic diversity of sweetpotato begomoviruses in the United States and identification of a natural recombinant between Sweet potato leaf curl virus and Sweetpotato leaf curl Georgia virus. *Archives of Virology*. doi 10.1007/s00705-011-0930-2.
241. Zulfiqar, A., Ren, H., Zhang, J., Li, J., Cui, X., Xie, Y., 2011. Molecular characterization of two isolates of Sweet potato leaf curl virus infecting *Ipomoea indica* in China. *African Journal of Biotechnology* 10 (45), 9061-9067. doi 10.5897/AJB11.1223.

CHAPTER 2

SURVEYS IN THE GAUTENG, LIMPOPO AND MPUMALANGA PROVINCES OF SOUTH AFRICA REVEAL NOVEL ISOLATES OF SWEETPOTATO VIRUSES

Published in the South African Journal of Botany

Authors: Mulabisana, M.J., Cloete, M., Mabasa, K.G., Laurie, S.M., Oelofse, D., Esterhuizen, L.L. & Rey, M.E.C. 2018. Surveys in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa reveal novel isolates of sweetpotato viruses. South African Journal of Botany 114, 280-294.

Author contributions: M.J Mulabisana conducted all experimental work and wrote the manuscript. Mr K.G Mabasa and Dr S.M Laurie assisted during field surveys. Dr L.L Esterhuizen provided guidance on the detection of begomoviruses. Dr D. Oelofse provided guidance and oversaw the overall progress of the project. Prof M.E.C Rey and Dr M. Cloete supervised the study and assisted with data analysis, interpretation and manuscript editing.

Surveys in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa reveal novel isolates of sweetpotato viruses

J. Mulabisana^{a,*} M. Cloete^a K.G. Mabasa^a S.M. Laurie^a D. Oelofse^a L.L. Esterhuizen^b
M. E.C. Rey^c

^aAgricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP), Private Bag X293, Roodeplaat, Pretoria, 0001, South Africa, ^bDepartment of Biochemistry, University of Johannesburg, PO Box 524, Auckland Park, 2006, Johannesburg, South Africa, ^cSchool of Molecular and Cell biology, University of the Witwatersrand, Private Bag X 3, P.O. Box Wits, 2050, Johannesburg, South Africa

*corresponding author: *E-mail address:* mjmulabisana@arc.agric.za; Tel: +27-12-808-8000.

2.1 Abstract

Previous surveys carried out between 2001 and 2003 in the Limpopo, Mpumalanga, Gauteng, Western Cape, Eastern Cape, Kwazulu Natal and North West Provinces of South Africa reported virus species *Sweet potato feathery mottle virus* and *Sweet potato virus G* as the most common viruses of sweetpotato. The two potyviruses were detected by indexing onto the indicator plant *Ipomoea setosa* (*I. setosa* Kerr.) and by nitrocellulose enzyme linked immunosorbent assays (NCM-ELISA). In this study, surveys (2012/2013 and 2013/2014) were conducted in the three provinces in South Africa, Gauteng, Limpopo and Mpumalanga. Results showed the frequent occurrence of the russet crack (RC) strain of virus species *Sweet potato feathery mottle virus*, *Sweet potato virus G*, *Sweet potato virus C*, and begomoviruses *Sweet potato leaf curl Sao Paulo virus*, *Sweet potato leaf curl virus* and *Sweet potato mosaic virus* in the three Provinces surveyed. Potyvirus identities were confirmed by partial sequencing of the part of nuclear inclusion body (NIb) protein, coat protein (CP) gene and the 3'-non-translated region (3' NTR) of *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato virus C* (SPVC), and the NIb and CP-encoding region of *Sweet potato virus G* (SPVG). The RC strain

of SPFMV from this study shared nucleotide (nt) identity of between 97% to 99% with the RC strains from Australia, Peru and China, and 94% to 98% with isolates from New Zealand and United States of America. SPVC isolates from this study shared 91% to 94% (VER-7_SPVC and REF-26_SPVC) and 86% to 91% (GBD-12_SPVC and MAM-15_SPVC) nt identity with the previously reported SPVC isolates reported in South Africa and other parts of the world. Similarly, SPVG samples from the current study shared 94% to 97% nt identity with the South African CTB3-3 and isolates from other countries, and genetic diversity was observed when samples of the current study shared the nt identity of 85% to 87% with the Kwazulu Natal isolates. The presence of begomoviruses was confirmed by observing for typical symptoms such as the upward curling and rolling of leaves on *I. setosa*. Rolling circle amplification (RCA) and next generation sequencing (NGS) of the full-length genome revealed novel begomoviruses isolates, which were identified as Sweet potato leaf curl Sao Paulo virus (SPLCSPV) isolate Tanz: ELLP_ZA and SPLCSPV isolate Tanz: NMP-ZA since the two shared 94% and 95% nt identity with SPLCSPV isolate TZ-SNG7:12 of Tanzania, respectively. TMBLP-ZA appears to be a novel isolate of Sweet potato leaf curl virus (SPLCV) since it shared 91% nt identity with SPLCV isolates from different geographic regions of the world. Therefore, we propose that it is classified as SPLCV isolate South Africa (ZA): TMBLP-ZA. Detection of potyviruses and begomoviruses in co- and mixed infections was confirmed to occur in the three provinces surveyed. This is an indication that yield is likely to be severely affected since all these viruses are reported to reduce storage root yield of sensitive sweetpotato varieties.

Key words: Sweet potato feathery mottle virus (SPFMV), Sweet potato virus C (SPVC), Sweet potato virus G (SPVG), Sweet potato leaf curl Sao Paulo virus (SPLCSPV), Sweet potato leaf curl virus (SPLCV), Sweet potato mosaic virus (SPMV), next generation sequencing, South Africa.

2.2 Introduction

Sweetpotato (*Ipomoea batatas* Lam.), a hexaploid dicot plant belonging to the family *Convolvulaceae*, is a major staple food crop in Africa, and a cash crop on the African continent (Kurabachew et al., 2015). Faber and co-workers (2017) showed that resource-poor families in peri-urban South Africa have less access to vitamin A-rich foods compared with food secured households. Health issues associated with vitamin A deficiency (VAD) have increased the planting of orange fleshed sweetpotato (OFSP) varieties globally and in South Africa to address nutritional deficiencies (Faber et al., 2001; Faber et al., 2017). Potyviruses reported to threaten sweetpotato production in South Africa include SPFMV, SPVC and SPVG (Domola et al., 2008; Sivparsad and Gubba, 2012; Mulabisana et al., 2015). These are members of the family *Potyviridae* and are transmitted non-persistently by green peach aphids (*Myzus persicae* Sulzer) and cotton aphids (*Aphis gossypii*) (Clark and Valverde, 2013), and are the most widespread of all the viruses affecting sweetpotato. Different strains of SPFMV have been reported and these include the russet crack (RC), the ordinary (O) and the East African (EA) strains (Tairo et al., 2005; Tairo et al., 2006; Untiveros et al., 2008; Clark and Valverde, 2013). Only the RC and O strains of SPFMV were detected in Western Cape (Rännäli et al., 2009) and in Kwazulu Natal Provinces in South Africa (Sivparsad and Gubba, 2012) during previous surveys. SPVC (Untiveros et al., 2010), previously known as strain C of SPFMV has also been reported in Western Cape and Kwazulu Natal Provinces, and recently in Gauteng Province (Mulabisana et al., 2015). Co-infections with other potyviruses affect sweetpotato production and total yield (17%) and marketable yield (30%) losses have been reported for South African varieties (Domola et al., 2008). Begomoviruses, including isolates of SPLCSV and SPMV, have recently been identified from samples collected from Limpopo Province of South Africa (Esterhuizen et al., 2012). SPLCSPV was first described in South America while SPLCV and related begomoviruses have been reported to occur in countries such as the United

States of America (USA), Asia and Africa (Albuquerque et al., 2011; Lotrakul et al., 2003; Luan et al., 2007; Wasswa et al., 2011). Begomoviruses are a group of viruses that belongs to the family *Geminiviridae* (Brunt et al., 1996; Lozano et al., 2009). The viral genome consist of single-stranded circular DNA which is converted into double stranded DNA during replication and this process occurs though rolling circle replication (RCR) mechanism within the replication-enhancer protein (REn) (Gutierrez, 2000; Rizvi et al., 2015; Lozano et al., 2009). They are transmitted by whiteflies (*Bemisia tabaci*) (Luan et al., 2007). Yield reduction of between 30% to 50% (Louisiana) and 60% (South Carolina) in USA due to SPLCV was reported (Clark and Hoy, 2006; Ling et al., 2010; Zhang and Ling, 2011). The objective of this study was to characterize and establish the genetic diversity of potyviruses (SPFMV, SPVC and SPVG), and begomoviruses infecting sweetpotato in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa.

2.3. Materials and Methods

2.3.1 Field surveys of sweetpotato viruses in Gauteng, Mpumalanga and Limpopo Provinces

Fields surveys were conducted in the mid to late planting seasons (2012/2013 and 2013/2014) and plants were examined and sampled following a W-pattern walking transect. Surveys were conducted from both smallholder and commercial farms of Vhembe District (Lwamondo, Tshiombo, Dzimauli, Rammbuda, Maungani and Elim) situated around Makhado and Thohoyandou, and two commercial farms in the Waterberg District (Groblersbridge and Wynberg) in Lephalale in Limpopo Province. Samples were also collected from nine locations in the Enhlanzeni District [Tonga, Naas, Mzinti, Kamhlushwa, Tjakastad, Mbejeka, Snymansbelt (Barberton), New Forest, and Thulamahashe]] and one in Nkangala (Verena) near Witbank in Mpumalanga Province. In Gauteng Province, samples were collected in several

locations in the Metsweding (Refilwe near Cullinan and Dzithobeni), Tshwane district (Soshanguve L extension, Soshanguve S extension, Hamanskraal, Mamelodi and Roodeplaat) and Ekurhuleni (Daveyton) Districts. Fig. 2.1 shows the map of South Africa indicating the three Provinces (Limpopo, Gauteng and Mpumalanga) where samples were collected. Visual evaluation of symptoms was conducted using a scoring scale of 0-5, where 0 represented no symptoms, 1-2 mild symptoms, 3 moderate symptoms, 4 severe and 5 very severe symptoms (Domola et al., 2008; Ndunguru et al., 2009). Data for the two seasons (2012/2013 and 2013/2014) surveys were analyzed using SAS/Statistical Software Version 9.3, Volume 2, 1999. A one-way analysis of variance (ANOVA) was conducted with season 1 (2012/2013) data. Fisher's unprotected least significant difference (LSD) test was used to determine significant differences at $P \leq 0.05$, $P \leq 0.05$, and $P \leq 0.01$ for Gauteng, Mpumalanga and Limpopo Province's data, respectively. A combined ANOVA was conducted with season 2 data (2013/2014). The standardized residuals were tested for deviations for normality using Shapiro-Wilk's test (Shapiro and Wilk, 1965). The residuals were symmetrical or normally distributed, therefore the data was considered reliable. Means of significant effects were compared using Fisher's unprotected t-LSD (least significant difference) at a 5% level of significance (Snedecor and Cochran, 1980). Twenty to 30 samples were collected from each location depending on the size of the field. Both symptomatic and asymptomatic sweetpotato samples were collected as vines at each sampling points and this was due to the fact that some varieties did not exhibit virus symptoms in the field (Domola et al., 2008).

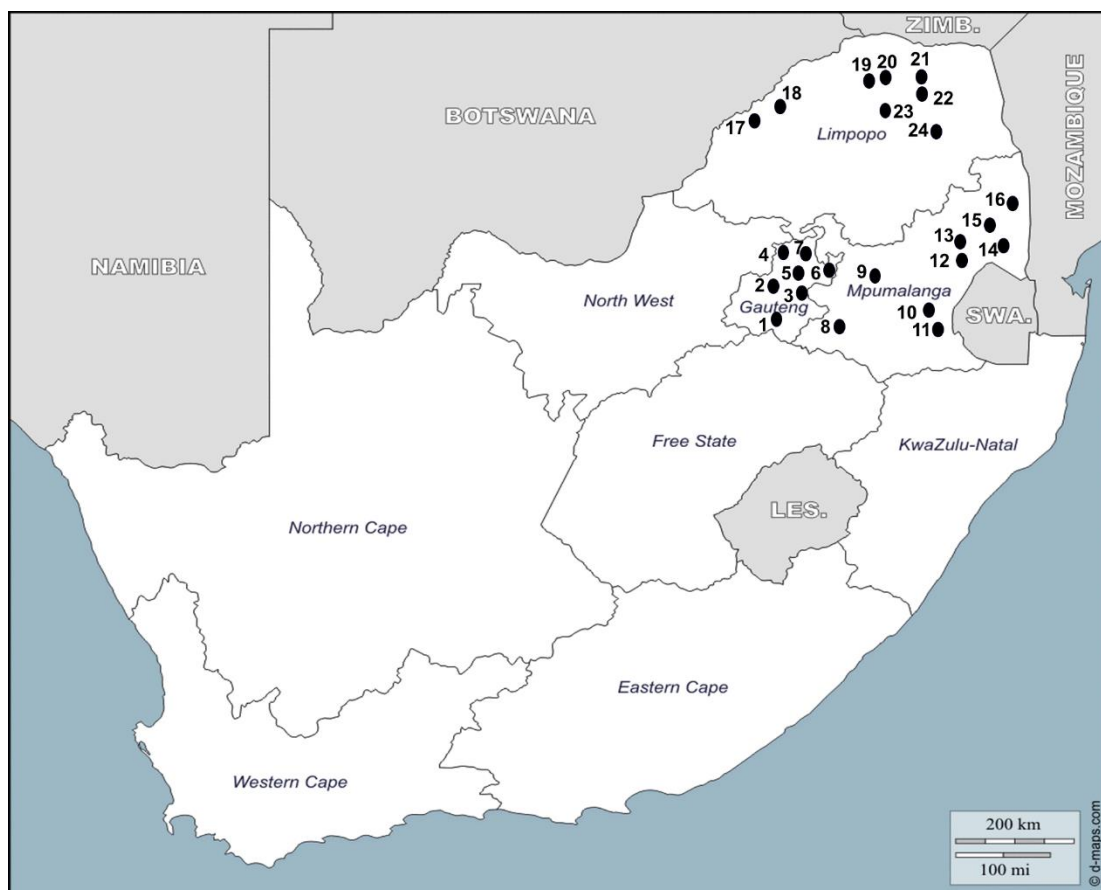


Fig. 2.1. Map of South Africa showing Limpopo, Gauteng and Mpumalanga Provinces. Areas where field surveys were conducted are indicated with a black dot. Map source: http://d-maps.com/carte.php?num_car=4414&lang=en. Areas are as follows: (1) Daveyton, (2) Roodeplaat, (3) Dzithobeni, (4) Hamanskraal, (5) Refilwe (Cullinan), (6) Mamelodi, (7) Soshanguve, (8) Verena, (9) Baberton, (10) Tjakastad, (11) Kamhushwa, (12) Mzinti, (13) Naas, (14) Tonga, (15) Thulamahashe, (16) New Forest, (17) Groblersbridge, (18) Wynberg, (19) Tshiombo, (20) Dzimauli, (21) Rammbuda, (22) Maungani, (23) Lwamondo and (24) Elim.

2.4 Viral Analysis

2.4.1 Indexing and serological analysis

Cuttings from the collected sweetpotato samples were side-grafted onto the indicator plant *I. setosa* to evaluate development of different symptoms, which were subsequently matched to known virus infections. Detection of SPFMV, SPVC and SPVG was conducted six weeks post graft inoculations using polyclonal antibodies in nitrocellulose membrane enzyme linked

immunosorbent assays (NCM-ELISA) according to the method described by Gibb and Padovan (1993). The NCM-ELISA kit was supplied by International Potato Centre (CIP, Lima, Peru). Antibodies for SPFMV also detect SPVC, and the two viruses can only be differentiated by reverse transcription polymerase chain reaction (RT-PCR). Symptomatic leaf discs of individual *I. setosa* samples were homogenized in microfuge tubes in 0.02 M Tris buffer and 0.50 M sodium chloride (NaCl) (TBS, pH 7.5), containing 1.0 g sodium sulphite as an extraction buffer. Fifteen to 20 µl of sample sap was spotted onto the centre of the nitrocellulose membranes marked squares and allowed to dry for 15 to 30 min. Membranes containing samples, together with positive control strips (provided by CIP) were subsequently processed to detect viruses according to the instructions provided. The NCM-ELISA kit also contains antibodies that allows for the detection of other known viruses: Sweet potato mild mottle virus (SPMMV), Sweet potato latent virus (SPLV), Sweet potato chlorotic stunt virus (SPCSV), Sweet potato chlorotic fleck virus (SPCFV), Sweet potato mild speckling virus (SPMSV), Sweet potato collusive virus (SPCV), Cucumber mosaic virus (CMV) and Sweet potato C6 virus (SPC6V). Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) was used to detect Sweet potato virus 2 (SPV2) (Polyclonal antibody (PAb), supplied by DSMZ GmbH Sammlung Pflanzenviren, Inhoffenstraße 7 B, D-38124 Braunschweig, Germany). Samples that induced typical begomovirus symptoms (upward curling and rolling of leaves) on sweetpotato plants and indicator plant *I. setosa* were tested for the presence of SPLCV by PCR according to the methods provided by Li et al. (2004). NCM and DASELISA data was combined and processed in Microsoft Office Professional Plus 2013 Excel (64 bit) as individual counts of single, co- and triple infections of each virus tested. Individual counts for each variable were classified into $r \times c$ frequency tables (contingency) (Kele and Mouton, 2011) and processed in SAS/Statistical Software Version 9.3, Volume 2, 1999). χ^2 tests were used to show the dependence for each variables and χ^2 probabilities for dependencies were obtained.

2.5 Detection of SPFMV, SPVC and SPVG with RT-PCR

2.5.1 Total RNA extractions and RT-PCR amplification

Samples that tested positive with NCM-ELISA were selected for RT-PCR to confirm the presence of SPFMV and strains, SPVC and SPVG. Leaf tissues of *I. setosa* and sweetpotato (0.1 g) were frozen in liquid nitrogen and ground using a mortar and pestle. Total RNA was extracted using the RNEASY® Plant mini extraction kit (QIAGEN) as per manufacturer's instructions. cDNA synthesis was carried out using the high capacity RNA-to-cDNA Master Mix (Applied Biosystems). Reaction components consisted of 4 µl complete master mix, 2 µl (10 pg-1 µg) total RNA and Diethyl pyrocarbonate-treated (DEPC) water up to a total volume of 20 µl. The cycling conditions were performed as per manufacturer's instructions. Forward primers FMV1360-F, SPVC474-F (Tairo et al., 2006), and reverse primer 10820-R (Tairo et al., 2005), which differentiate between the RC/O and EA strains of SPFMV and SPVC, were used. The primers are reported to anneal to the NIb protein, the entire CP gene and the 3' NTR (Rännäli et al., 2009), yielding PCR product sizes of 1356 bp and 1335 bp for the RC strain of SPFMV and SPVC, respectively. Forward primer PMB-9 and reverse primer PMB-10 (Rännäli et al., 2008), which amplify the 5'-proximal part of the NIb and the CP-encoding gene region, yielding a PCR product size of 719 bp, were used to detect SPVG. All PCR reactions were performed using the KAPA® Taq Ready Mix DNA polymerase (KAPA BIOSYSTEMS) containing Mg⁺. Taq Ready Mix (10 µl) was mixed with 10 µM of each primer and 2-5 µl of the total cDNA in a total reaction volume of 20 µl. The cycling conditions for SPFMV, SPVC and SPVG were performed as described by Rännäli and co-workers (2008; 2009).

2.5.2 Cloning and sequencing and phylogenetic analysis

The PCR products of the selected isolates of SPFMV (RC strain), SPVC and SPVG were excised from a 1% low melting agarose gel and purified using the Zymoclean™ (Zymo

Research CORP) gel DNA recovery kit. These were cloned into the pGEM[®]-T Easy vector (Promega Corporation[™], Anatech, South Africa), according to the manufacturers' instructions. The ligated products were transformed into 50 µl of *Escherichia coli* (*E. coli*), strain JM109 high efficiency competent cells (Promega Corporation[™], Anatech, South Africa). Transformed cells (100 µl) were plated out in duplicate on Luria-Bertani Broth (LB) media supplemented with ampicillin (100 µg/ml), 0.5 mM IPTG and X-Gal (80 µg/ml), and incubated overnight at 37 °C. Single white bacterial colonies were picked and verified by colony-PCR using specific primers that detects SPFMV, SPVC and SPVG as described by Tairo and co-workers (2005; 2006) and Rännäli and co-workers (2008). Clones were sent to Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa, for nucleotide sequencing.

2.5.3 Sequence analysis of the partial genomes

Table 1.1 is a list of sequenced clones of SPFMV, SPVC, SPVG and full genome sequences of begomoviruses from this study and reference sequences downloaded from the National Centre for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov/GENBANK). Partial genome sequences of SPFMV, SPVC and SPVG were edited using BioEdit v 7.2.3 and aligned with reference sequences using ClustalW program embedded in CLC Genomic Workbench 8 (<http://www.clcbio.com>). The species demarcation criteria for potyviruses were based on the cut-off point of 76% to 77% for nucleotide (nt) identity for species and 83% to 99% as same strains (Adams et al., 2005; Frenkel et al., 1989). Aligned sequences were used to conduct phylogenetic analysis in MEGA6 (Tamura et al., 2013). Maximum Likelihood trees with 1000 replicates (bootstraps) were constructed and isolate CTB3 (SPVG) was used to root the SPVG and SPVC tree. Likewise, isolate CTB 3-1 (SPFMV) was used to root SPVG tree. Generated nt sequences of potyviruses were translated into amino acids (aa) (<https://seqtool.sdsc.edu/CGI/BW.cgi#>) to determine the putative coding open reading frames

(ORFs). Conserved domains were determined using standard aa BLAST (<https://blast.ncbi.nlm.nih.gov>) and SMART BLAST (<https://blast.ncbi.nlm.nih.gov/smartblast/smartBlast.cgi>). Multiple sequence alignments and pairwise identity matrices of the entire CP encoding sequences of SPFMV, SPVC and part of the CP of SPVG were conducted with reference aa sequences (NCBI GenBank database) using ClustalW (CLC genomic workbench 8). The CP aa homology cut-off point of 90% to 99% was used to determine same strain relationships of potyviruses (Shukla and Ward, 1989).

Table 2.1: Accession numbers and virus names of reference sequences (GenBank database) used to determine relationships between the South African sequence isolates of SPFMV, SPVC, SPVG and SPLCV from this study and isolates reported in other countries.

Nucleotides Accession no.	Isolates names	*Viruses and strains	^aProvince and/country of origin	References
AY459601	MD 1/1	SPVC	KZN, South Africa	Rännäli et al., 2009
EU809485	CTB3.1	SPVC	WC, South Africa	Rännäli et al., 2009
JQ073713	Umfume2	SPVC	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073714	UMP1-C	SPVC	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073716	UMP3-C	SPVC	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073717	UMBUM-C	SPVC	KZN, South Africa	Sivparsad and Gubba, 2012
EU809510	ZIMB 1-2	SPVC	Zimbabwe	Rännäli et al., 2009
EU809499	PS1B-4	SPVC	Easter Island	Rännäli et al., 2009
EU809486	OR1-2	SPVC	Moorea	Rännäli et al., 2009
AY459596	Nam12	SPVC	Namibia	Ateka et al., 2003, unpublished
MF359257	Daveyton 3-C (DVT-3_SPVC)	SPVC	Daveyton, GP, South Africa Groblersbridge, LP, South Africa	Mulabisana et al., 2015
MF359254	GBD-12_SPVC	SPVC	Verena, MP, South Africa	This study
MF359256	VER-7_SPVC	SPVC	Mamelodi, GP, South Africa	This study
MF359255	MAM-15_SPVC	SPVC	Refilwe (Cullinan), GP, South Africa	This study
MF359253	REF-26_SPVC	SPVC	WC, South Africa	Rännäli et al., 2009
EU809503	CTB3.2	SPFMV-RC	Australia	Rännäli et al., 2009
AJ781777	Aus 6	SPFMV-RC	Peru	Rännäli et al., 2009
EU021066	KmtMil	SPFMV-RC	Korea	Kim et al., 1998
AF015540	K1	SPFMV-RC	New Zealand	Rännäli et al., 2009
EU809505	NZ4-1	SPFMV-RC	USA	Abad et al., 1992
S43050	C (RC)	SPFMV-RC	China	Chen et al., 2001, unpublished
AJ310201	SPFMV virus partial genomic RNA	SPFMV-RC		

MF359252	RDPLT11-04_SPFMV	SPFMV-RC	Roodeplaat, GP, South Africa	Mulabisana et al., 2015
MF359249	GBD-10_SPFMV	SPFMV-RC	Groblersbridge, LP, South Africa	This study
MF359250	REF-26_SPFMV	SPFMV-RC	Refilwe (Cullinan), GP, South Africa	This study
MF359251	SSBles_SPFMV	SPFMV-RC	Roodeplaat, GP, South Africa	This study
EU809509	ZIMB 1-1	SPFMV-EA	Zimbabwe	Rännäli et al., 2009
FJ795748	KBNYL	SPFMV-EA	Uganda	Tugume et al., 2010
EU809504	CTB3.3	SPFMV-O	WC, South Africa	Rännäli et al., 2009
JQ073708	Umfume.O	SPFMV-O	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073709	UMP1.O	SPFMV-O	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073710	UMP2-0	SPFMV-O	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073711	UMP3-0	SPFMV-O	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073712	UMBUM-O	SPFMV-O	KZN, South Africa	Sivparsad and Gubba, 2012
EU220756	CTB3	SPVG	WC, South Africa	Rännäli et al., 2008
EU220755	JAAC	SPVG	Java	Rännäli et al., 2008
EU220754	PS1B	SPVG	Easter Island	Rännäli et al., 2008
EU220753	HawA2	SPVG	Hawaii	Rännäli et al., 2008
EU220752	Zimb1C	SPVG	Zimbabwe	Rännäli et al., 2008
EU220751	Zimb1H	SPVG	Zimbabwe	Rännäli et al., 2008
EU220757	3817	SPVG	Tahiti	Rännäli et al., 2008
EU220758	NZ-4	SPVG	New Zealand	Rännäli et al., 2008
EU220759	3900A	SPVG	Tubuai	Rännäli et al., 2008
AJ515380	SPVG partial Egypt 1	SPVG	Egypt	IsHak et al., 2003
KC962218	SPVG-NC	SPVG	USA	Almeyda et al., 2013
JQ073707	UMFUME-VG	SPVG	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073706	UMP2-VG	SPVG	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073705	UMP1-VG	SPVG	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073704	UMBUM-VG	SPVG	KZN, South Africa	Sivparsad and Gubba, 2012
JQ073703	UMP3-VG	SPVG	KZN, South Africa	Sivparsad and Gubba, 2012

Z83314	Sweetpotato virus G	SPVG	North Carolina	Colinet et al., 1998
MF359258	GBD14_SPVG	SPVG	Groblersbridge, LP, South Africa	This study
MF359259	Naas4_SPVG	SPVG	Naas, MP, South Africa	This study
MF359260	RDPLTBles_SPVG	SPVG	Roodeplaat, GP, South Africa	This study
MF359261	SSBles_SPVG	SPVG	Roodeplaat, GP, South Africa	This study
MF359262	TsMB1_SPVG	SPVG	Tshiombo, LP, South Africa	This study
MF359263	SPLCSPV isolate SSB1_ZA	SPLCSPV	Roodeplaat, GP, South Africa	This study
MF359264	SPLCSPV isolate RDGP_ZA	SPLCSPV	Roodeplaat, GP, South Africa	This study
MF359265	SPLCSPV isolate Tanz: ELLP_ZA	SPLCSPV	Elim, LP, South Africa	This study
MF359266	SPLCSPV isolate Tanz: NMP_ZA	SPLCSPV	Naas, MP, South Africa	This study
MF359267	SPLCV isolate TMBLP_ZA	SPLCV	Tshiombo, LP, South Africa	This study
MF359268	SPMV isolate SSB1_ZA	SPMV	Roodeplaat, GP, South Africa	This study
MF359269	SPMV isolate RDGP_ZA	SPMV	Roodeplaat, GP, South Africa	This study
MF359270	SPMV isolat ELLP_ZA	SPMV	Elim, LP, South Africa	This study
MF359271	SPMV isolate TMBLP_ZA	SPMV	Tshiombo, LP, South Africa	This study
KX611145	SPLC Georgia virus Isolate ARMDZ	SPLC Georgia virus	Argentina	Saldago et al., 2016, unpublished
AF326775	SPLC Georgia virus (16)	SPLC Georgia virus	Argentina	Lotrakul et al., 2003
JX286653	SPLCV Isolate GZ01 Segment-DNA A	SPLCV isolate China	China	Tang and He, 2012, unpublished
KF040466	SPLCV China Shandong 11:2012	SPLC China	China	Liu et al., 2013, unpublished
HQ393473	SPLCV-SP BR:AlvM:09	SPLCV	Brazil	Albuquerque et al., 2011
HQ393455	SPLCV isolate: SPLCVPR[BR:OPO:08]	SPLCV	Brazil	Albuquerque et al., 2011
HQ393445	SPLCV isolate SPLCV-PR[BR-CA:08]	SPLCV	Brazil	Albuquerque et al., 2011
KX859238	SPLCSPV KTOA11	SPLCSPV	South Africa	Nhlapo et al., 2018 unpublished

FR751068	SPLC Uganda: Kampala: 2008	SPLC Uganda	Uganda	Wasswa et al., 2011
KF836891	SPLCSPV TZ:SNG7:12	SPLCSPV	Tanzania	Mbazimbwa et al., 2014
JQ621844	SPLCSPV- ZA:WP:2011 isolate PstI-02	SPLCV	South Africa	Esterhuizen et al., 2012
JQ621843	SPMV- ZA:WP:2011 isolate PstI-01	SPMV	South Africa	Esterhuizen et al., 2012
KX859239	SPMV KTOB11	SPMV	South Africa	Nhlapo et al., 2016, unpublished
FJ969831	SPMV - [Brazil:Brasilia1:2007] isolate BR:BSB1	SPMV	Brazil	Paprotka et al., 2010
NC-003803	<i>South African cassava mosaic virus</i>	SACMV	South Africa	Berrie et al. 2001
Amino acids Accession no.	Isolates names	Viruses and strains	Province and/Country of Origin	References
AFD22452	HN:5RC:08	SPFMV-RC	Honduras	Kashif et al., 2012
NP-734318	Polyprotein SPFMV	SPFMV-RC	Japan	Sakai et al., 1997
AAC59320	CP partial SPFMV	SPFMV-RC	Korea	Ryu et al., 1998
AGO86051	SC2 CP	SPFMV-RC	China	Deng et al., 2013, unpublished
CAB53558	Isolate MBL	SPFMV-RC	East Africa	Kreuze et al.2000
ACJO1838	Isolate 3817-1	SPFMV-RC	Tahiti	Rännäli et al., 2009
AET79524	Jiangsu14	SPFMV-RC	China	Qin et al., 2011, unpublished
CAH03772	Aus2	SPFMV-RC	Australia	Rännäli et al., 2009
AAB23253	SPFMV_RC	SPFMV-RC	USA	Abad et al., 1992
CAJ19387	Aust142	SPFMV-RC	Australia	Tairo et al., 2005
AG01854	Zimb 1-2	SPFMV-EA	Zimbabwe	Rännäli et al., 2009
ACY74480	KBNYL	SPFMV-EA	Uganda	Tugume et al., 2010
AET79520	Fujan3	SPVC	Fuji	Qin et al., 2013
AEG79935	Hunan 4	SPVC	China	Qin et al., 2013
CAA04764	Strain CH2	SPVC	China	Colinet et al., 1998
AAS88730	Spain 1C	SPVC	Spain	Ateka et al., 2004a, unpublished
ACJ01843	PS1B-4	SPVC	Easter Island	Rännäli et al., 2009
ACJ01829	CTB3-1	SPVC	WC, South Africa	Rännäli et al., 2009

AAS38447	MD1/1	SPVC	KZN, South Africa	Ateka et al., 2004a, unpublished
AEU17211	UMBUM-C	SPVC	KZN, South Africa	Sivparsad and Gubba, 2012
AAS99567	SPVC polyprotein	SPVC	Unknown	Ateka et al., 2004a, unpublished
ACJ01843	CTB3-2	SPVC	WC, South Africa	Rännäli et al., 2009
AEU17209	UMP2-C	SPVC	KZN, South Africa	Sivparsad and Gubba, 2012
AEG79997	Hainan	SPVG	China	Qin et al., 2013
AEG80025	Shandong 5	SPVG	China	Qin et al., 2013
AEG80011	Hunan 4	SPVG	China	Qin et al., 2013
AGO86063	Isolate SPVGSC2	SPVG	China	Deng et al., 2013, unpublished
AEU17204	Umfume-VG	SPVG	KZN, South Africa	Sivparsad and Gubba, 2012
YP-006493343	Jesus Maria	SPVG	Argentina	Rodriguez et al., 2012
AEG79999	Henan coat protein gene	SPVG	China	Qin et al., 2013
AEU17202	Ump3-VG	SPVG	KZN, South Africa	Sivparsad and Gubba, 2012
AGO86062	SPVG-SC1	SPVG	China	Deng et al., 2013, unpublished

*Sweet potato feathery mottle virus russet crack (SPFMV-RC), SPVC = Sweet potato virus C, SPVG = Sweet potato virus G and SPLCV = Sweet potato leaf curl virus. **KZN= Kwazulu Natal, WC= Western Cape, GP= Gauteng, LP= Limpopo and MP= Mpumalanga.

2.6 Detection of begomoviruses, confirmation by rolling circle amplification (RCA) and full-length genome sequencing

2.6.1 Total DNA extractions and PCR reactions

Total DNA was extracted from selected sweetpotato and *I. setosa* leaf samples. Leaf tissues (0.1 g) were frozen in liquid nitrogen and ground using a mortar and pestle. Total DNA was extracted using the DNEASY® Plant mini extraction kit (QIAGEN). Forward primer SPG1 and reverse primer SPG2 were used to test samples for the presence of SPLCV (Li et al., 2004). Primers are reported to amplify the conserved regions in the open reading frames (ORF) AC2 and AC1 (Li et al., 2004), yielding the PCR product size of 912 bp. All PCR reactions were performed using the KAPA® Taq Ready Mix DNA polymerase (KAPA BIOSYSTEMS) as described in section 2.3.1. Total DNA was used as a template in a total reaction volume of 20 µl. The PCR cycling conditions described by Wasswa and co-workers (2011) were used to amplify the DNA. All the PCR reactions were carried out on an Applied Biosystems Veriti® thermal cycler. The amplified products were analyzed on 1% agarose gel electrophoresis and gel images were recorded on an Alpha Innotech AlphaImager® HP gel imaging system.

2.6.2 RCA and restriction fragment length polymorphism (RFLP)

RCA as described by Haible et al. (2006) and RFLP were conducted to determine the presence of the circular DNA and to identify potential begomoviruses in selected samples that tested positive for SPLCV with PCR. Amplification of the circular DNA (RCA) was performed using the bacteriophage phi (ϕ) 29 DNA polymerase [Illustra™ Templiphi 100 Amplification kit (GE Healthcare Life Sciences)] according to the manufacturer's instructions. One to 200 ng of total DNA was dissolved in 5 µl of sample buffer and denatured at 95 °C for 3 min in the thermal cycler (Applied Biosystems Veriti® thermal cycler), followed by subsequent cooling at room temperature. The reaction buffer (5 µl) and enzyme mix (0.2 µl) were added to the denatured

DNA and incubated at 30 °C for 4 to 18 h, and the DNA polymerase was inactivated at 65 °C for 10 min. The RCA products were digested with BamHI (BioLabs®) to release the full-length genome size (± 3000 bp) of begomoviruses DNA fragments (Paprotka et al., 2010) and this was conducted according to the manufacturers' instructions. Products of restriction digestions were analyzed by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and gel images were recorded as described above.

2.6.3 Full genome sequencing of begomoviruses

The RCA products were used to generate a DNA library using the Nextera DNA sample preparation kit (Illumina), according to the manufacturer's instructions. The DNA amplicons were sequenced using Illumina MiSeq Benchtop Sequencer (Illumina Inc., San Diego, CA, USA) at the Agricultural Research Council's Biotechnology Platform, Pretoria, South Africa. Data was analyzed in CLC Genomic Workbench 8 (<http://www.clcbio.com>). Generated data was trimmed for quality and adaptor sequences that were added during the library preparations. Sequence reads were subsequently mapped to known virus reference sequences (NCBI GenBank) at a stringency of between 0.95 and 0.99 to generate the consensus sequences. Trimmed reads were also analyzed by de novo assembly to make provision of reads that did not map with known references. Generated contigs sequences (± 3000 bp) were selected and subjected to nt BLAST at NCBI GenBank to retrieve reference sequences closely related to the full genome contigs generated. Reads were again mapped to contigs, from which, consensus sequences were generated. These were aligned (multiple sequence alignment) to the closely related references with ClustalW program (CLC Bio Genomic Workbench 8). Sequence alignment was used to conduct phylogenetic analysis in MEGA6 (Tamura et al., 2013) using Maximum Likelihood (1000 bootstraps replicates). Pairwise identity matrix was conducted to confirm species and strain identity using ClustalW embedded in the Species Demarcation Tool

(SDT)- Version 1.2, which utilizes the Needleman-Wunsch algorithm (Muhire et al., 2013), ideal for species belonging to the Geminiviridae family. The threshold of 91% nt sequence identity between full-length DNA viruses was used for species demarcation and 93% to 94% for strains within the virus group/species (Brown et al., 2015). Generated nt sequences were translated into aa using ORF finder program (<http://www.ncbi.nlm.nih.gov/orffinder/>). The generated replication-associated protein (Rep) and the CP protein encoding sequences were aligned with reference aa sequences using ClustalW (CLC genomic workbench 8). The Rep amino acid sequences were also aligned and analyzed for the conserved motifs associated with begomoviruses.

2.7. Results

2.7.1 Field surveys

A total of 272 (season 1) and 332 (season 2) samples were maintained and analyzed for the presence of viruses. The average means of virus incidence in season 1 were 23%, 18% and 19% in Gauteng, Limpopo and Mpumalanga provinces, respectively. There were no significant difference between the three provinces in season 1. In season 2, the virus incidence recorded for Mpumalanga and Gauteng provinces had average means of 21% and 22%, respectively, higher than the virus incidence observed in Limpopo Province (4%). Significant differences existed between Limpopo Province and Mpumalanga and Gauteng provinces due to the low virus incidence (4%) observed in Limpopo Province.

2.7.2 Indexing and serological analysis

Symptoms observed on *I. setosa* post grafting were mild to severe and were described as vein clearing/banding, leaf mottling and chlorosis (Fig. 2.2), which are typical potyvirus symptoms as described by Clark and Valverde (2013). NCM-ELISA confirmed that symptomatic *I. setosa*

were infected with either a single infection of SPFMV/SPVC, which were 52% and 16% in season 1 and season 2, respectively. Single infection of SPVG was low (0 to 3%) in all seasons. Co-infections of SPFMV/SPVC with SPVG was 0.74% in season 1 and 12.7% in season 2 and this was identified as the most common co-infection. Co-infection of SPFMV/SPVC with ipomovirus SPMMV was 3% and 2.4% in season 1 and season 2, respectively. Co-infection of SPFMV/SPVC with crinivirus, SPCSV was also observed in few samples (1% to 6%). Typical symptoms (Clark and Valverde, 2013) associated with begomoviruses, such as the upward curling of leaves, severe yellowing of the main veins (sweet potato) and greening of the main veins were observed (Fig. 2.2).

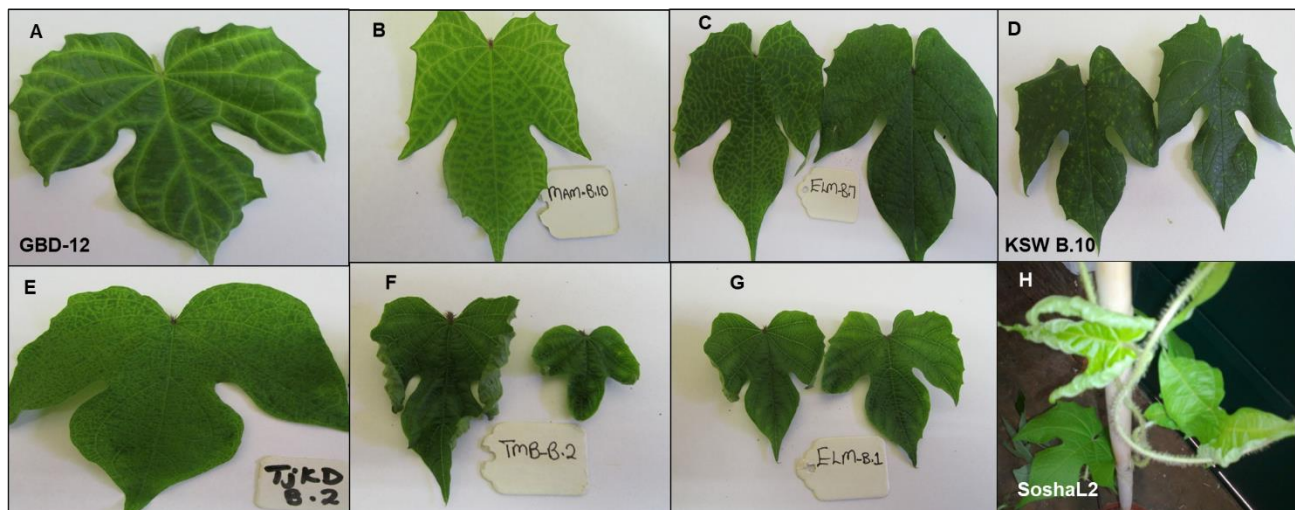


Fig. 2.2. Systemic symptoms associated with potyviruses and begomoviruses as recorded on indicator plant *I. setosa* post graft inoculations. (A) Vein clearing/banding on the main veins of the leaves (single infection of SPVC), (B and C) Severe yellowing of the leaf veins (co-infections of SPFMV with SPVG), (D) Chlorotic spotting (single infection of SPVG), (E) Severe yellowing of the leaf veins and chlorosis (co-infection of SPVG with SPMMV), (F, G and H) Upward curling/rolling of leaves and greening of the main veins (typical begomovirus symptoms). Samples were collected from Groblersbdridge (GBD-12), Elim (ELMB.7 and ELM-B.1), Tshiombo (TMB-B.2), Kamhlushwa (KSWB.10), Tjakastad (TJKD B.2), Mamelodi (MAM-B.10) and Soshanguve (Soshal2) in the Limpopo, Mpumalanga and Gauteng provinces.

2.7.3 RT-PCR, PCR amplifications, RCA and RFLP

RT-PCR confirmed the presence of the RC strain of SPFMV and SPVC in tested samples. Amplicons with band sizes of 1356 bp and 1335 bp were generated for the two viruses, respectively. Viruses were detected either as a single strain infection of the RC strain of SPFMV or SPVC, or as co-infections of both in RT-PCR tests. SPVG was also detected as a single infection or as co-infections with the RC strain of SPFMV and SPVC. Degenerate primers SPG1 and SPG2 successfully detected SPLCV (Fig. 2.3) in samples that showed symptoms associated with begomoviruses as described above (Fig. 2.2) and in some cases in symptomless sweetpotato leaf samples.

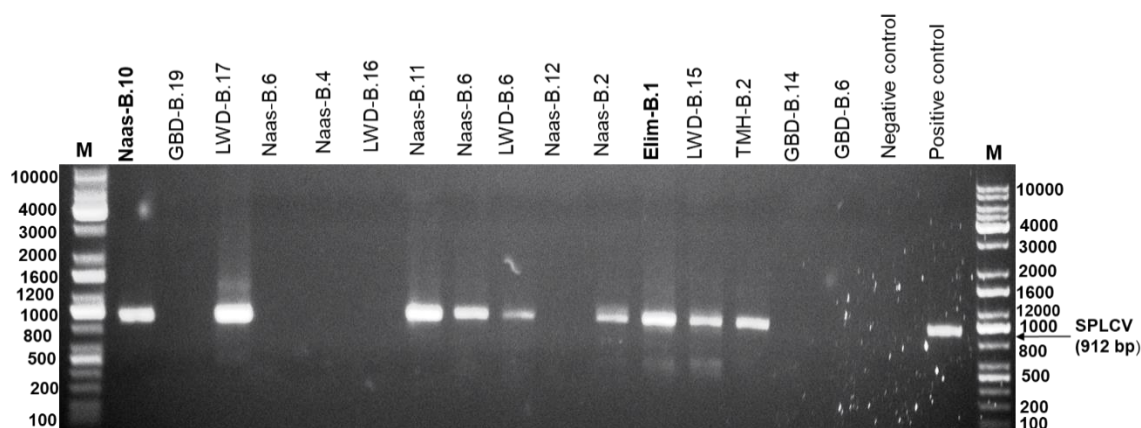


Fig. 2.3. Gel electrophoresis image of the PCR generated with the DNA of representative *I. setosa* tested. (M): KAPA™ Universal 1 KB DNA ladder size marker. The 912 bp bands of SPLCV amplification products are indicated. Samples codes denote areas from which they were collected. Full-length genome sequences were obtained for the samples indicated in bold letters.

RCA and restriction digestions with *Bam*HI confirmed the presence of the full-length genome size (± 3000 bp) of begomoviruses in samples Naas 10, Dzith 10, RDPLT-13-6, Sosha L13, Elim-B1, SoshaL2 and REF 6 (Fig. 2.4, lanes 2, 3, 4, 5, 6, 8 and 9). Samples were collected from Dzithobeni (Dzith 11, 10, and 2), Soshanguve (Sosha L13 and SoshaL2), Roodeplaat (RDPLT 13-6) and Refilwe near Cullinan (REF 6) in Gauteng Province, Naas (Naas 10) in Mpumalanga Province and Elim (Elim-B.1) in Limpopo Province.

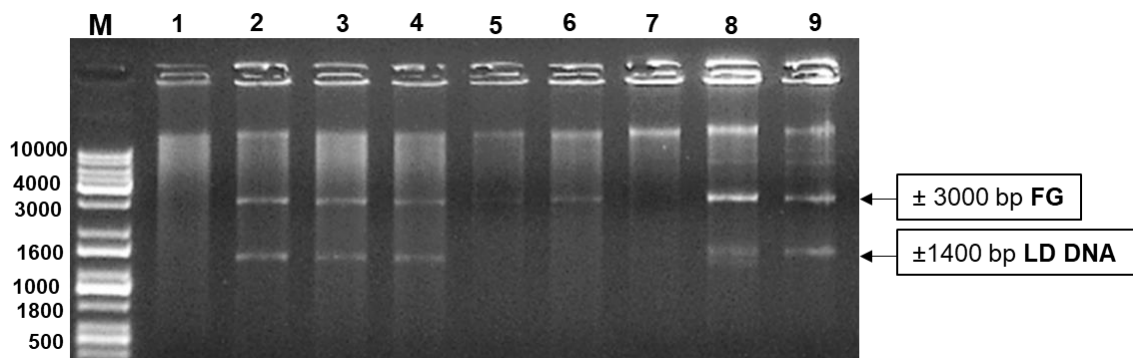


Fig. 2.4. Gel electrophoresis image showing the RCA products and the restriction digestion of selected samples with *Bam*HI. (M) KAPA™ Universal 1 KB DNA ladder size marker. Lanes (1) Dzith 11, (2) **Naas 10**, (3) Dzith 10, (4) **RDPLT-13-6**, (5) Sosha Ll3, (6) **Elim B.1** (7) Dzith 2, (8) Sosha L2 and (9) REF 6. *Bam*HI revealed the full-length genome (**FG**) size (± 3000 bp) of begomoviruses with samples in lanes 2, 3, 4, 5, 6, 8 and 9. Linearized defective (**LD**) DNA of ± 1400 bp was also obtained with some samples (lanes 2, 3, 4, 8 and 9). Full-length genome sequences were obtained with samples in lanes 2, 4 and 6.

2.7.4 Sequence analysis (partial genome)

2.7.4.1 Nucleotide sequence analysis of SPVC

VER-7_SPVC and REF-26_SPVC shared 91% nt identity on their own and 91% to 94% nt identity with the South African CTB3-1, MD1/1 and DVT-3_SPVC (Daveyton 3-C), and OR1-2, PSB1-4 and PS3A-3, Zimb1-2 from Morea, Easter Island and Zimbabwe, respectively (Rännäli et al., 2009; Mulabisana et al., 2015). GBD-12_SPVC and MAM-15_SPVC of this study also shared 86% to 91% nt identity with CTB3-1, OR1-2, PSB1-4 and PS3A-3, MD 1/1, Zimb 1-2. VER-7_SPVC and REF-26_SPVC also shared 82% nt identity with the South African DVT-3_SPVC, UMBUMB_C, Ump3-C and UMFUME2-C, and 75% with UMP1-C (Sivparsad and Gubba, 2012; Mulabisana et al., 2015). Isolates of from this study also shared the nt identity of 94% with each other. All sequences of SPVC clustered together in a single species clade within the *Potyviridae* family tree as expected (Fig. 2.5). It was observed that VER-7_SPVC and REF-26_SPVC grouped closely with DVT-3_SPVC, CTB3-1, OR1-2, PSB1-4 and PS3A-3 (Rännäli et al., 2009; Mulabisana et al., 2015), and these were supported

by the bootstrap value of 98%. Genetic variability was also observed when GBD-12_SPVC and MAM-15_SPVC formed a sub-cluster of their own with a strong bootstrap value of 97%, with their main branch connected to the SPVC group, including the South African MD 1/1 and Zimbabwean Zimb 1-2 (Rännäli et al., 2009).

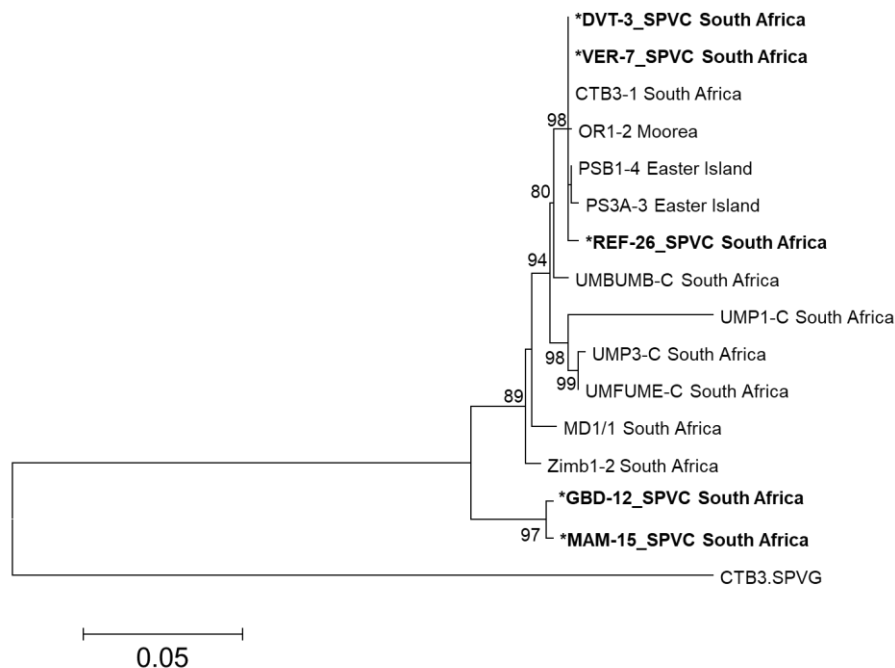


Fig. 2.5. Phylogenetic analysis [Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000)], of the N1b/coat protein/3' UTR encoding nt sequences. SPVC clades are depicted. Relationships between the SPVC sequences from this study (**bold***) with those with those from around the world and previously reported isolates from South Africa are shown. The bootstrap values of 1000 replicates, with which the associated taxa clustered together, are shown next to the branches. The viruses identified in this study in the three Provinces, reference sequences and their Genbank accession numbers are listed in Table 2.1.

2.7.4.2 Nucleotide sequence analysis of SPFMV

Pairwise comparison revealed that SSBles-RC shared 99% to 99.6% nt identity with the Australian (Aust 6), the Peruvian (KmtMil) and SPFMV-RC isolate reported in China. Similarly, GBD-10_SPFMV, REF-26_SPFMV, and RDPLT11-04_SPFMV also shared nt identity of 98.5% to 99% with Aust 6, KmtMil and SPFMV-RC isolate. These also shared the

nt identity with NZ4-1 (94%) and C(RC) (98%). CTB2-3, RC strain of SPFMV, shared nt identity with the EA strain Zimb 1-1 (95%) and KBNYL (91%), and also shared the nt identity of 96% with the South African O strain of SPFMV, isolate CTB3-3 (Rännäli et al., 2009). Similarly, CTB2-3 also shared the nt identity of 91% to 92% with some of the clones belonging to the RC strain of SPFMV, making it very difficult to assign it to a strain group. Phylogenetic analysis demonstrated that samples fell into one of three strain clusters, namely, RC, O and the EA of SPFMV (Fig. 2.6). Clones from this study (SSBles_SPFMV, GBD-10_SPFMV and REF-26_SPFMV) grouped with RDPLT11-04_SPFMV (South Africa), kmtMil (Peru), Aust 6 (Australia), SPFMV-RC (China), C (RC) USA, K1 (Korea) and NZ4-1 (New Zealand) isolates (Abad et al., 1992; Kim et al., 1998; Rännäli et al., 2009; Almeyda et al., 2013; Mulabisana et al., 2015), with a bootstrap value of 87% to 89%. Strains O and EA of SPFMV were not identified with the sequences from the current study and the EA strains of SPFMV observed were those of references KBNYL and Zimb 1-1 (Tugume et al., 2010; Rännäli et al., 2009).

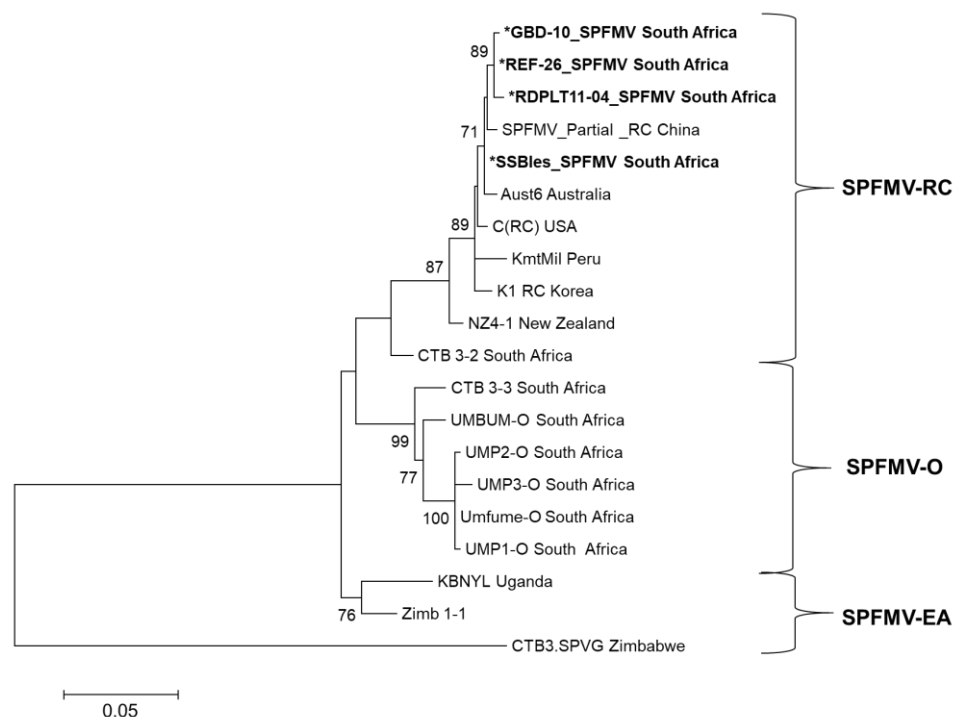


Fig. 2.6. Phylogenetic analysis [Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000)] of the NIB/coat protein/3' UTR encoding nt

sequences. SPFMV clades comprised the RC, O and EA strains are depicted. Relationships between the virus sequences from this study (**bold***) with those from around the world and other South African virus isolates are shown. The bootstrap values of 1000 replicates, with which the associated taxa clustered together, are shown next to the branches. The viruses identified in this study in the three provinces, reference sequences and their Genbank accession numbers are listed in Table 2.1.

2.7.4.3 Nucleotide analysis of SPVG

Samples GBD14_SPVG shared the nt identity of 93% to 95% with other isolates from this study. Isolates SSBles_SPVG and Naas_SPVG, and RDPLT_BlesSPVG and TsMB1_SPVG also shared the nt identity of 99% and 99.6%, respectively (Table 5, Appendices). All isolates from this study also shared the nt identity of 94% (RDPLT_BlesSPVG and TsMB1_SPVG) to 98% (SSBles_SPVG) with reference sequences CTB3, 3900A, HawA2, JAAC, Zimb-H, NZ-4, 3817, PS13, Zimb1C, SPVG isolate Egypt-1 and SPVG NC (Table 5, Appendices). Genetic variability was observed between virus isolates in this study and those detected previously from the Kwazulu Natal Province (UMP1-VG, UMP3-VG; UMFUME-VG, UMBUMB-VG and UMP2-VG) (Sivparsad and Gubba, 2012), demonstrating the nt identity of 85% and 87% (Table 5, Appendices). Phylogenetic analysis of SPVG sequences showed that GBD14_SPVG grouped with UMP1_VG and UMP3_SPVG detected in Kwazulu Natal Province with 99% bootstrap support (Fig. 2.7). TsMB1_VG and RDPLTBles-SPVG grouped together with 100% bootstrap with a branch extending to the Kwazulu Natal Province UMFUME-VG. SSBles_SPVG and Naas4_SPVG grouped on their own from a branch that grouped UMBUMB-VG and UMP2-VG together (Sivparsad and Gubba, 2012).

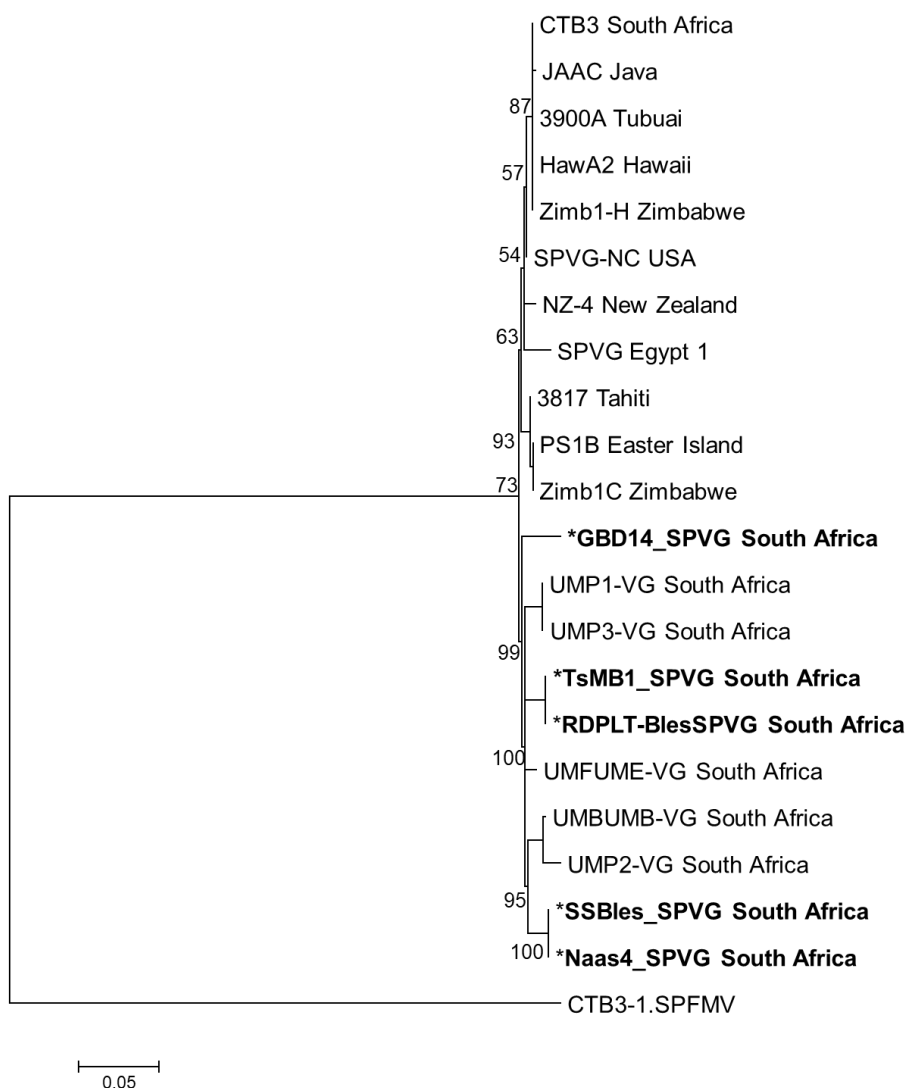


Fig. 2.7. Phylogenetic tree [Maximum Likelihood method [(1000 bootstrap replicates) based on the General Time Reversible model (Nei and Kumar, 2000)] based on the 5'-proximal part of the Nib and CP-encoding region nucleotide sequences of SPVG isolates (**bold***) from Gauteng (RDPLT Bles_SPVG, SSBles-SPVG), Limpopo (TsMB1_SPVG and GB14_SPVG) and Mpumalanga (Naas4_SPVG) Provinces. The viruses identified in this study in the three provinces, reference sequences and their Genbank accession numbers are listed in Table 2.1.

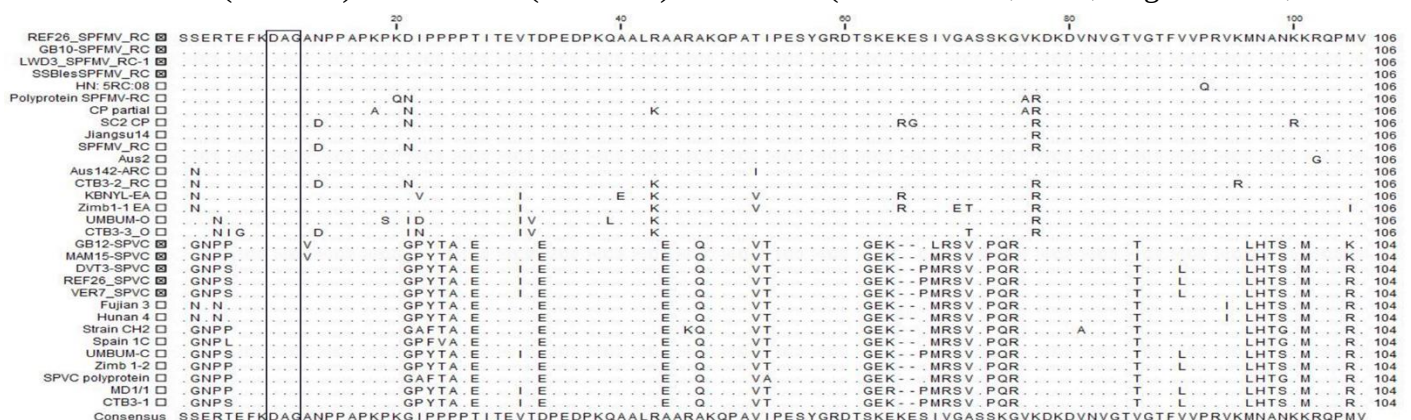
2.7.4.4 Amino acid analysis (SPFMV, SPVC and SPVG)

The translation of the nt sequences of the full CP gene of SPFMV was 315 (aa) except for RDPLT 11-04-RC, which translated into a 207 aa polypeptide. The CP coding regions of SPVC

encoded 313 aa. The CP aa sequences of SPFMV and SPVC were aligned with reference sequences (NCBI Genbank) (Fig. 2.8) and conserved motifs, typical of potyviral CP regions were observed (Table 6, Appendices). The RQ (Arg-Gln) at position 204 to 205 and AFDF (Ala-Phe-Asp-Phe) at position 246 to 249 associated with potyviruses were observed. The Asp-Ala-gly (DAG) aa triplet motif, associated with transmission of potyviruses by aphid vectors (Atreya et al., 1995; Jacquet et al., 1998) was identified at the N-terminus position of the CP (position 9-11) and this was present in both the SPFMV and SPVC clones. Other identified conserved motifs, associated with potyviruses included the WCIENG (position 165-170) and QMKAAAI (position 266-272) in the core centre of the CP gene region (Pappu et al., 1993). The cleavage site between the NIb and the CP encoding region were VYHQ/S for both SPFMV and SPVC (Wang et al., 2007). All CP aa sequences of SPVC of this study and reference sequences had two aa bases missing at position 65 to 66 when compared with the SPFMV (Table 6, Appendices), which is attributed to deletion of 6 nt (2 aa) from position 190 to 195 of the CP gene (Tairo et al., 2005; Untiveros et al., 2008). The two missing bases are considered a unique feature of SPVC, when compared to other potyviruses of sweetpotato (Tairo *et al.*, 2005). The CP aa sequences of SPVC from the current study were variable at position 309 to 313, and these ended with MRGVQ (GBD-12_SPVC and MAM-15_SPVC) and MRGVH (DVT-3_SPVC, VER-7_SPVC and REF-26_SPVC). The CP encoding aa sequences of the RC strain of SPFMV terminated with MRGVQ.

The pairwise comparison of the CP aa residues of SPVC isolates (Table 7, Appendices) showed that GBD-12_SPVC and MAM 15_SPVC shared 99% identity on their own. Similarly, the aa residues of SPVC clones of VER-7_SPVC and REF-26_SPVC shared 100% identity on their own and 96.5% aa identity with GBD-12_SPVC and MAM 15_SPVC of this study. The aa residues of the full CP genes of SPVC from this survey (VER-7_SPVC and REF-26_SPVC) were also identical to the South African DVT-3_SPVC (100%), CTB3-1 (100%), UMBUM-C

(100%), MD1/1 (100%) and Zimbabwean Zimb1 (99%) isolates (Rännäli et al., 2009; Sivparsad and Gubba, 2012; Mulabisana et al., 2015). Similarly, the full CP genes of GBD-12_SPVC and MAM 15_SPVC were also 96% to 97% identical to the above reference sequences. All clones from this survey shared 96% to 97% nt identity with Fujian 3, Hunan 4, StrainCH2, SPVC-polyprotein and Spain 1C (Colinet et al., 1998; Qin et al., 2013). The pairwise comparison of the deduced CP aa residues of the RC strain of SPFMV (Table 7, Appendices) revealed that clones SSBles_SPFMV, GBD-10_SPFMV and REF-26_SPFMV from the current study shared CP aa identity of between 99.68% to 100% with RDPLT11-04_SPFMV (Mulabisana et al., 2015). The CP aa residues of the RC strain of SPFMV from the current study and RDPLT11-04_SPFMV shared the CP aa identities of 99% (average) with the CP aa sequences of references HN:5RC:08, Polyprotein SPFMV, CP partial, Jiangsu14, SPFMV-RC, Aust2, SC2 and Aust142-ARC (Sakai et al., 1997; Kashif et al., 2012; Ryu et al., 1998). CTB3-2 (Rännäli et al., 2009) shared the CP aa identity of 97% with the RC strains of SPFMV from the current study and that of reference sequences (HN:5RC:08, Polyprotein SPFMV, CP partial, Jiangsu14, SPFMV-RC, aust2, SC2 and Aust142-ARC) and 98% with the RC strains of SPFMV of China. However, CTB 3-2 also shared 97% CP aa identity with the EA strain (KBNYL) and O strain (Zimb 1-2) of SPFMV (Rännäli et al., 2009; Tugume et al.,



2010).

Fig. 2.8. Alignment of the CP N-terminal aa sequences of SPFMV (RC, O and EA strains) and SPVC samples from this current survey (illustrated by boxes with X crosses) with reference

sequences. The DAG (associated with aphid transmissibility) is indicated by a solid box. Out of all the SPVC and SPFMV (RC strain) isolates, only REF26_RC and SPFMV-SC2 CPs have two amino acids (KE and RG, respectively) at positions 65 and 66. Changes in aa residues are shown and dots denote similar aa residues.

The translation of the nucleotide sequences of the part of the NIb/CP gene regions of SPVG yielded 210 (GBD14_SPVG and SSBles_SPVG), 206 (TsMB1_SPVG and RDPLT-Bles_SPVG) and 203 (Naas4) aa polypeptides, instead of the full 355 aa CP residues since primers that targets the 5'-proximal part of the NIb and CP-encoding gene regions were used. The identified CP-encoding motifs associated with potyviruses included the DAG box at the N-terminus (position 7 to 9), the KR (Lys/Arg) (position 41 to 42) and WCIENG at position 205 to 210. Other CP-encoding motifs associated with potyviruses (AFDF, QMKAAAL) (Pappu et al., 1993) could not be identified due to the shorter CP ORF generated with the nt sequences from this survey. The cleavage site between the NIb and the CP encoding region was VHHQ/S. Pairwise comparison with the deduced CP aa sequences of SPVG (Table 8, Appendices) showed that clones from this study (TsMB1_SPVG, RDPLT-BlesSPVG and SSBles_SPVG) were between 91% and 100% identical. The CP aa sequences of SPVG also shared an identity of between 92.25% (Naas4-SPVG) to 98.7% (TsMB1-SPVG, SSBles-SPVG) with Umfume-VG from Kwazulu Natal Province. Clones from the current survey also shared CP aa identity of 96% (TsMB1-SPVG, SSBles-SPVG), 98% (SSbles_SPVG), 95% to 98% (GBD14_SPVG) and 90% to 92% (Naas 4) with reference virus isolates from NCBI Genbank.

2.8 Full genome sequencing of begomoviruses

Five dsDNA samples generated with RCA (Naas B.10, Elim-B.1, TMB-B.2, SSB1 and RDPLT 13-6), identified to be infected with SPLCV by biological indexing (Fig. 2.2) and PCR (Fig. 2.3), were sequenced using Illumina NGS. Different read numbers were generated with

different samples, and these ranged between 33,640 (SSB1) and 15,847,584 (TMB-B.2). Samples will be referred to as NMP-ZA (Naas 10), ELLP-ZA (Elim-B1), TMBLP-ZA (TMB-B.2), SSB1-ZA (SSB1) and RDGP-ZA (RDPLT 13-6) going forward. A consensus length of 2780 bp and 2769 bp were generated with isolates SSB1-ZA and RDGP-ZA, respectively, which mapped closely to SPLCSPV-ZA:WP:2011 isolate PstI-012 (JQ621843), and a consensus length of 2781 bp (SSB1-ZA) and 2783 bp (RDGP-ZA) were generated with samples that mapped with SPMV. Reads of sample NMP-ZA and the contig (± 3000) of ELLP-ZA mapped to SPLCSPV isolate TZ-SNG7:12 (KF836891) (Mbanzibwa et al., 2014) and produced consensus lengths of 2751 bp and 2769 bp, respectively.

Sequence reads of ELLP-ZA also mapped closely to isolates of SPMV and a consensus length of 2780 bp was produced. Reads of sample TMBLP-ZA mapped with SPLCV and generated a consensus sequence of 2820 bp. Pairwise identity analysis of the full-length nt sequences of begomoviruses was performed (Table 9, Appendices). Among the SPLCSPV isolates, SSB1-ZA shared 95% nt identity with the South African isolate KT10A11 (KX859238). Isolate RDGP-ZA shared 96% nt identity with SPLCSPV isolates KT10A11 and SPLCSPV-ZA:WP:2011 isolate PstI-012 (JQ621843). Isolates of SPLCSPV (SSB1-ZA and RDGP-ZA) from this study shared 96% nt identity with each other. Isolates ELLP-ZA and NMP-ZA shared 94% and 95% nt identity with the Tanzanian SPLCSPV isolate TZ-SNG7:12 (KF836891), respectively. The two (ELLP-ZA and NMP-ZA) only shared 90% to 92% nt sequence identity with the South African SPLCSPV of isolates KT10A11 (KX859238) and SPLCSPV-ZA:WP:2011 isolate PstI-012 (JQ621843) and isolates from this study (SSB1-ZA and RDGP-ZA). TMBLP-ZA shared 91% nt identity with SPLCV isolate GZ01, SPLCV isolate SPLCV-PR[BR:OPO:08], SPLCV isolate SPLCV-PR[BR:CA:08] and SPLCV isolate China:Shandong11:2012. Therefore, TMBLP-ZA appears to be a strain of SPLCV. In accordance with the demarcation thresholds of same species (91%) and strains (94%) for

begomoviruses, we propose that ELLP-ZA, NMP-ZA, TMBLP-ZA are classified as SPLCSPV isolate Tanz: ELLP-ZA, SPLCSPV isolate Tanz: NMP_ZA and SPLCV isolate South Africa (ZA): TMBLP-ZA. Similarly, SSB1-ZA and RDGP-ZA can be classified as SPLCSPV isolate SSB1-ZA and SPLCSPV isolate RDGP-ZA.

Phylogenetic tree of SPLCSPV and SPLCV isolates generated 4 clusters (Fig. 2.9). Cluster I consisted of SPLCSPV isolates TZ:ELLP-ZA and TZ:NMP-ZA, which grouped with the Tanzanian isolate TZ:SNG7:12 (KF836891) (Mbanzibwa et al., 2014) with 98% to 99% bootstrap support. Cluster II comprised of *Sweetpotato leaf curl Georgia virus* (SPLCGV) isolates only. SPLCSPV isolates SSB1-ZA and RDGP-ZA (cluster III) grouped with the South African SPLCSPV-ZA:WP:2011 isolate PstI-012 (JQ621843) and KT10AII (KX859238) with 99% to 100% branch support. SPLCV isolate TMBLP-ZA grouped in the IV cluster with isolates reported in China and Brazil.

The pairwise analysis with SPMV sequences (Table 10, Appendices) confirmed that SSB1-ZA shared nt identity (98% to 99%) with three South African isolates, namely, SPMV isolate KT10B11 (KX859239), SPMV [ZA:WP:2011]-Isolate-PstI01 (JQ621843) and the Brazil SPMV-[Brazil:Brasilia1:2007]-Isolate BR:BSB1 (FJ969831), indicating that they are related strains. Similarly, SPMV isolate SPMV-ELLP-ZA also shared the nt identity (97% to 98%) with SSB1-ZA. SPMV isolates RDGP-ZA and TMBLP-ZA also shared 94% to 95% nt identity with SPMV isolates from this study and reference sequences in the database. SPMV isolate RDGP-ZA however was 93% identical to SPMV isolate ELLP-ZA, an indication that these might be different strains within the SPMV group (Brown et al., 2015).

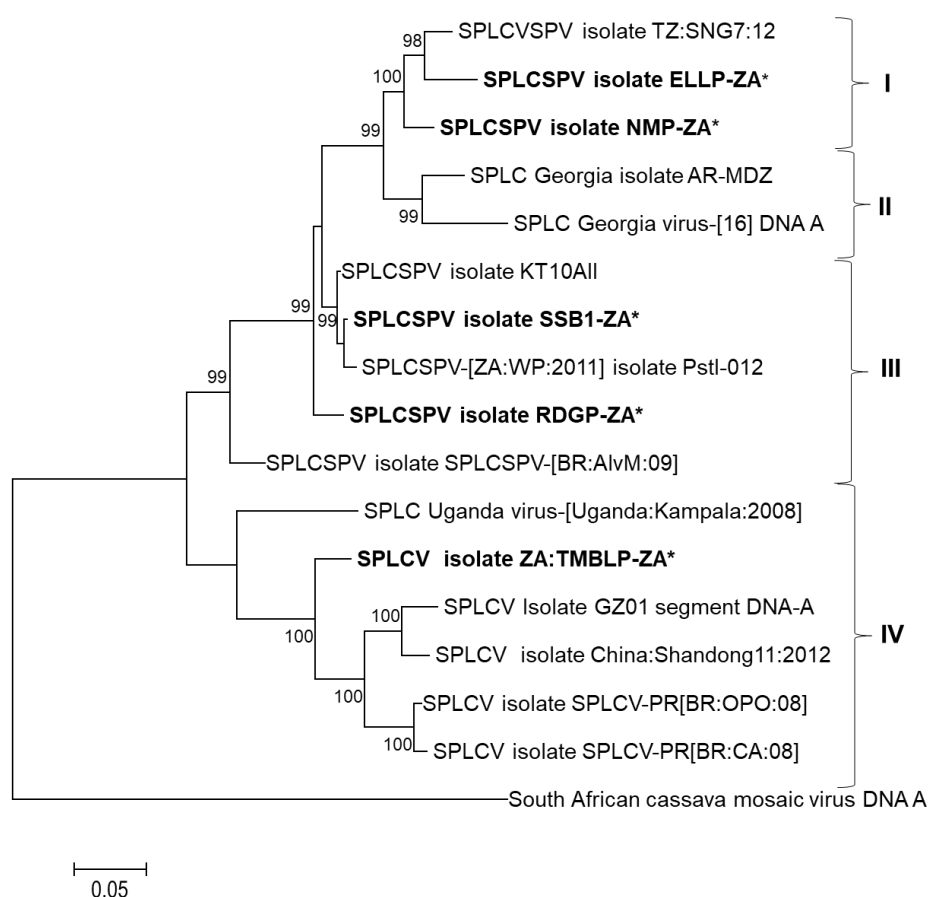


Fig. 2.9. Phylogenetic tree [Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000) with 1000 bootstrap replicates] of NCBI Genbank reference viruses compared with full length genome nt sequences of SPLCSPV and SPLCV isolates: TZ:ELLP-ZA (Limpopo Province), TZ:NMP-ZA (Mpumalanga Province); SSB1-ZA (Gauteng Province), RDGP-ZA (Gauteng Province) and ZA:TMBLP-ZA (Limpopo Province), Bootstrap values are shown next to the branches. The isolates of this study are indicated in bold letters and an asterix. Begomoviruses identified in this study, in the three Provinces, reference sequences and their Genbank accession numbers are listed in Table 2.1.

BLASTp (NCBI Genbank) analysis of the begomoviruses *C1* ORF-encoded aa sequences was undertaken. Begomovirus *C1* codes for Rep protein (*C1*) which is essential for viral replication initiation (Gutierrez, 2000; Lozano et al., 2009). The Rep conserved motif (IRD) in the N-terminus region was identified as **M**PRQPGFRVS in SPLCSPV isolates SSB1-ZA, RDGP-ZA and NMP-ZA (Table 11, Appendices) as described by Lozano et al. (2009); Zhang and Ling

(2011); Bi and Zhang (2012). The Rep IRD of isolate ELLP-ZA (MAARQPGFRVS) differed from the ones previously described in the literature (Lozano et al., 2009; Zhang and Ling, 2011; Bi and Zhang, 2012). SPLCV isolate ZA:TMBLP-ZA Rep IRD also differed (MAPPKRFRIS) from the South African SPLCSPV isolates (Table 11, Appendices). However, it was similar with the SPLCV-Spain isolate ES:CI:BG1:02 (EF456741) and SPLCV-Spain isolate ES:CI:BG6:02 (EF456744) (Lozano et al., 2009). The Rep IRD motif in the *C1* ORF of South African SPMV isolates were identified as MAPPKRFFKIQ in isolates SSB1-ZA, TMBLP-ZA and ELLP-ZA from this study. These are similar with those of begomoviruses described in the literature (Lozano et al., 2009; Bi and Zhang, 2012). Rep (*C1* ORF) of SPLCV, SPLCSPV and SPMV isolates from this study demonstrated the three conserved motifs I (FLTYPQC/FITYPRC), II (HLHALI or HLHVLV), and III (YVDK/YIDK/YIEK) (Table 11, Appendices) which are characteristic of geminiviruses (Orozco et al., 1996; Orozco et al., 1998; Vadivukarasi et al., 2007). Motif YVDK is essential for DNA binding site for cleavage (Orozco et al., 1998). The conserved nonanucleotide region 5'-TAATATTAC-3' of the intergenic region (IR) (Vadivukarasi et al., 2007; Bi and Zhang, 2012) was also identified in sequences from this study.

2.9 Discussion

Previous studies detected potyvirus SPFMV (SPFMV: RC and O strain), SPVC and SPVG in Kwazulu Natal and Western Cape Provinces of South Africa (Rännäli et al., 2009; Sivparsad and Gubba, 2012). Begomoviruses SPLCSPV and SPMV were confirmed to occur in the Limpopo Province of South Africa (Esterhuizen et al., 2012). In this survey, it was shown that RNA potyviruses and DNA begomoviruses occur in sweetpotato cultivation districts in Gauteng, Mpumalanga and Limpopo Provinces of South Africa. Genetic differences between sweetpotato virus strains of potyviruses and clustering of isolates were validated by nt

sequencing of the CP/NIb/3' NTR gene region, phylogenetic analyses, and alignment of aa sequences. Clones from this study (SSBles_SPFMV GBD-10_SPFMV, REF-26_SPFMV), including RDPLT11-04_SPFMV (Mulabisana et al., 2015), shared both the nt (99% to 99.5%) and the full CP aa (99%) homology with the RC strains of SPFMV. Phylogenetic analysis based on the CP/NIb/3' NTR encoding sequences could not detect strains O and EA of SPFMV in the current surveys. However, strain O was detected in Kwazulu Natal Province in a previous study (Sivparsad and Gubba, 2012), therefore, the introduction of new strains into different regions in Gauteng, Limpopo and Mpumalanga provinces in the future cannot be ruled out. A large number of samples in a wider geographical area need to be sampled. Pairwise comparison of the nt sequences showed that SPFMV strains RC, O and EA share a close identity, as shown by CTB3-2 (SPFMV-RC strain), which shared the nt identity of 94% with Zimb 1-1 (SPFMV-EA strain) and 95% with CTB3-3 (SPFMV-strain), a common occurrence since strains within the same virus group are known to be closely related (Shukla and Ward, 1989; Mori et al., 1995). It is suggested from our study that aa similarity should be included in virus identification as this dictates functionality, which is more important for virus pathogenicity, host range and other biological features during plant host-virus interactions. Clones of SPVC (GBD-12_SPVC, MAM-15_SPVC, DVT-3_SPVC, VER-7_SPVC and REF-26_SPVC) also shared the nt identity of 75% to 91%, and full CP aa homology of 96% to 100% with the previously reported South African isolates (Rännäli et al., 2009; Sivparsad and Gubba, 2012) and reference isolates from other geographic regions of the world.

SPVG nt sequences of the current study grouped closely with sequences reported in the Kwazulu Natal Province in based on phylogenic analysis and these also shared nt identity of 85% to 87% with each other, and according to the strain homology cut-off point of (83% to 99%) of potyviruses, these can be regarded as the same strain. Comparison of the CP encoding

aa confirmed that all isolates from this study are strains of other reported SPVG. The South African strains of SPVG are also related to SPVG strains reported in other geographic regions of the world. SPVG was described for the first time in China and later in African countries such as Egypt and South Africa (Colinet et al., 1994; Colinet et al., 1998; Ishak et al., 2003; Rännäli et al., 2008). Minor sequence variability between SPVG nt sequences from the current study with previously reported South African sequences and reference sequences was observed. This is an indication that closely related variants of SPVG were introduced into South Africa in contaminated vegetative propagating material. Genetic variability across ORF's, in particular the CP, of RNA viruses within a single replicating population (termed quasispecies), which is associated with minor variations to allow viruses to evolve and adapt in response to host immune selection pressure and new conditions (Domingo et al., 1998; Rubio et al., 2013). The DAG and other motifs of structural functionalities in the CP gene region of SPFMV, SPVC and SPVG isolates were identified in this study, and these did not differ from those reported in the literature (Atreya et al., 1995; Jacquet et al., 1998). These motifs are important for aphid transmissibility and are usually under strict evolutionary constraints (Atreya et al., 1995; López-Moya et al., 1999). Atreya and co-workers (1995) showed that substitution of this motif with aa residues with minimal side chains (alanine) can affect transmissibility of potyviruses by aphid vectors. Virus co-infections are common in sweetpotato and other vegetatively propagated crops (Clark et al., 2012; Hillocks and Thresh, 2000). Indexing on indicator plant *I. setosa*, RT-PCR and PCR confirmed the occurrence co-infections of potyviruses on their own and co-infections with begomoviruses, criniviruses, and ipomoviruses. Co-infections of samples with the RC strain of SPFMV and SPVC (REF-26_SPFMV and REF-26 SPVC), and SPFMV with SPVG (SSBles_SPFMV and SSBles_SPVG) was also confirmed by cloning and sequencing.

Indexing on *I. setosa* and sample analysis with PCR with degenerate primers SPG1 and SPG2 (Li et al., 2004) successfully detected SPLCV in both symptomatic and asymptomatic samples. While PCR was found to be useful and cost effective in identification, however, in order to establish species, strain and isolate identity, and to distinguish meaningful genetic variability, full-length genome sequencing of begomoviruses is necessary. SPLCSPV isolates NMP-ZA and ELLP-ZA from Mpumalanga (Naas) and Limpopo (Elim) Provinces were found to share 94% nt identity, and 97% and 95% aa identity, respectively, with SPLCSPV isolate TZ-SNG7:12, confirming that the two isolates are closely related to the Tanzanian isolate. We thus propose the classification of the two isolates as SPLCSPV isolate Tanz: NMP_ZA (MF359266) and SPLCSPV isolate Tanz: ELLP_ZA (MF359265), respectively. Phylogenetic analysis grouped TMBLP-ZA as a distinct strain (91%-92% nt identity) of SPLCV from China and Brazil isolates. In accordance with the strain demarcation threshold (94%) for begomoviruses (Brown et al., 2015), we therefore suggest that TMBLP-ZA be classified as SPLCV isolate TMBLP_ZA (MF359267). To our knowledge, this is the first report of the detection of the SPLCSPV Tanzania strains and SPLCV in South Africa. Previous studies identified strains of SPLCSPV and SPMV in South Africa (Esterhuizen et al., 2012). Detection of *Begomoviruses* not native to South Africa or Tanzania is an indication of the global spread of *Begomoviruses* (Rey et al., 2012) due to introduction and exchange of vegetative sweetpotato germplasm globally. This perpetuates dissemination of different viruses and strains, which are then also subjected to natural genetic variation and mutations in their new geographical locations.

Begomoviruses were detected as co-infections of SPLCSPV/SPLCV and SPMV. Severe curling and rolling symptoms on both indicator plant *I. setosa* and sweetpotato plants infected with these viruses is an indication that they may cause significant yield losses in susceptible varieties (Clark and Valverde, 2013). Furthermore, the presence of begomoviruses in different provinces of South Africa, and their natural tendency to co-infect with *Potyvirus*es and viruses

of other taxonomic groups such as SPCSV (Cuellar et al., 2015), is a concern to the sweetpotato industry and for the small-holder farmers in South Africa. This requires robust management strategies at national level to prevent loss of quality and yield. More studies to determine the effects of begomoviruses on the yield of local sweetpotato varieties are required, and future breeding initiatives should involve screening and development of varieties resistant to multiple viruses. Available sweetpotato multiplication schemes should adopt an effective system of indexing of mother varieties to indicator plants, testing for the presence of viruses with both serological screening methods, and confirmation with more accurate and sensitive molecular diagnostic tools such as RT-PCR and PCR to ensure distribution of virus free planting material to farmers and sweetpotato vine cuttings distributors. In the past, only indexing and testing of samples with serological techniques was practiced and the unavailability of serological tests for the detection of begomoviruses contributed in masking earlier detection of these viruses. Low virus incidences of between 18% (Limpopo Province) and 23% (Gauteng Province) were recorded in season 1. Similarly, low virus incidences of between 4% (Limpopo Province) and 22% (Gauteng Province) were recorded in season 2. Sweetpotato samples collected from the fields in Mpumalanga, Kwazulu Natal and Limpopo Province during the 1990's were symptomless and only developed symptoms after one month of maintenance in the glasshouse (Jericho and Thompson, 2000). Domola and co-workers (2008) also recorded low virus incidences in most of the Provinces of South Africa, however, indexing onto *I. setosa* and subsequent testing with NCM-ELISA revealed 81% of infection with SPFMV. This suggests that sweetpotato plants do not always exhibit virus symptoms under South African field conditions, which poses as a challenge since farmers may not be able to remove virus-infected plants from the field.

The maximum temperature in some areas in the Limpopo Province (i.e Lephalale, and Thohoyandou) can be as high as 30°C to 31°C in summer planting season (<https://en.climate->

data.org/location/26819/https://www.worldweatheronline.com/thohoyandou-weather-averages/limpopo/za.aspx). This could contribute to masking virus symptoms as indicated by Ssremba and co-workers (2017) who showed that high temperatures (30° C to 39° C) produces less virus disease severities in sweetpotato crops as compared with low temperatures (20° C to 29° C). SPFMV is the most common virus and the most widespread throughout South Africa and co-infection of SPFMV with viruses such as SPVG, SPCSV is the most prevalent (Ateka et al., 2004b; Domola et al., 2008). In this study, analysis with PCR also showed that begomoviruses are also in mixed infections with these viruses.

2.10 Conflict of interest

Authors declare that there is no conflict of interests regarding the publication of this work.

2.11 Acknowledgements

The authors would like to acknowledge the Gauteng Department of Agriculture and Rural Development (GDARD), the International Foundation for Science (IFS) (C/5312-1), the Agricultural Research Council (ARC), the National Research Foundation-Technology and Human Resources for Industry Programme (NRF-THRIP) (UID 79983) and the NRF-Thuthuka PhD track (Grant 99368) for funding the project. We also acknowledge Dr Segundo Fuentes (CIP, Lima, Peru) for his willingness to share his knowledge on viruses of sweetpotato. Dr Inge Gazendam (ARC-VOP) is also acknowledge for her willingness to share her knowledge where she could and her contribution made this study possible. Ms Mercy Maserumule, the late Mr Elias Thobejane, Ms Midana Mmboyi and Mr Thakhani Ramathavhana are greatly acknowledged for their dedicated technical assistance throughout the study. Ms Nicolene Thiebaut of the Agricultural Research Council, Biometry Services, for statistical analysis of symptoms data.

2.12 References

1. Abad, J.A., Conkling, M.A., Moyer, J.W., 1992. Comparison of the capsid protein cistron from serologically distinct strains of Sweet potato feathery mottle virus (SPFMV). *Archives of Virology* 126, 147-157.
2. Adams, M.J., Antoniw, J.F., Fauquet, C.M., 2005. Molecular criteria for genus and species discrimination within the family Potyviridae. *Archives of Virology* 150, 459-479.
3. Albuquerque, L.C., Inoue-Nagata, A.K., Pinheiro, B., Resende, R.O., Moriones, E., NavasCastillo, J.N., 2011. Genetic diversity and recombination analysis of sweepoviruses. *Virology Journal* 9, 1-13.
4. Almeyda, C.V., Abad, J.A., Pesic-VanEsbroeck, Z., 2013. First report of sweet potato virus G and Sweet potato virus 2 infecting sweetpotato in North Carolina. *Plant Disease* 97, 1516.
5. Ateka, E.M., Njeru, R.W., Kibaru, A.G., Kimenju, J.W., Barg, E., Gibson, R.W., Vetten, H.J., 2004b. Identification and distribution of viruses infecting sweetpotato in Kenya. *Annals of Applied Biology* 144, 371-379.
6. Atreya, P.L., Lopez-Moya, J.J., Chu, M., Atreya, C.D., Pirone, T.P., 1995. Mutational analysis of the coat protein N-terminal amino acids involved in potyvirus transmission by aphids. *Journal of General Virology* 76, 265-270.
7. Berrie, L.C., Rybicki, E.P., Rey, M.E., 2001. Complete nucleotide sequence and host range of South African cassava mosaic virus: further evidence for recombination amongst begomoviruses. *Journal of General Virology* 82, 53-58.
8. Bi, H., Zhang, P., 2012. Molecular characterization of two sweepoviruses from China and evaluation of the infectivity of the cloned SPLCV-JS in *Nicotiana benthamiana*. *Archives of Virology* 157, 441-454.
9. Brown, J.K., Zerbini, F.M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva, J.C.F., Fiallo-Olive, E., Briddon, R.W., Hernández-Zepeda, C., Idris, A., Malathi, V.G.,

- Martin, D.P., Rivera-Bustamante, R., Ueda, S., Varsani, A., 2015. Revision of begomovirus taxonomy based on pairwise sequence comparisons. *Archives of Virology* 160, 1593-1619.
10. Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L., 1996. *Viruses of Plants, Description and Lists from the VIDE Database*. C.A.B International, Cambridge, United Kingdom, pp. 1223-1224.
11. Clark, C.A., Hoy, M.W., 2006. Effect of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. *Plant Disease* 90, 83-88.
12. Clark, C.A., Valverde, R.A., 2013. Diseases caused by viruses. In: Clark, C.A.D., Ferrin, M., Smith, T.P., Holmes, G.J. (Eds.), *Compendium of Sweetpotato Diseases, Pests and Disorders*, 2nd edn American Phytopathological Society, pp. 73-93.
13. Clark, C.A., Davis, J.A., Abad, A., Cuellar, W.J., Fuentes, S., Kreuze, J.F., Gibson, R.W., Mukasa, S.B., Tugume, A.K., Tairo, F.D., Valkonen, J.P.T., 2012. Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Disease* 96, 168-185.
14. Colinet, D., Kummert, J., Lepoivre, P., 1994. The complete nucleotide sequences of the coat protein cistron and the 3' non-coding region of a newly-identified potyvirus infecting sweetpotato, as compared to those of Sweet potato feathery mottle virus. *Archives of Virology* 139, 27.
16. Colinet, D., Nguyen, M., Kummert, J., Lepoivre, P., Xia, F.Z., 1998. Differentiation among potyvirus infecting sweetpotato based on genus- and virus-specific reverse transcription polymerase chain reaction. *Plant Disease* 82, 223-229.
16. 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, 459-471.

17. Domingo, E., Baranowski, E., Ruiz-Jarabo, C.M., Martín-Hernández, A.M., Sáiz, C.J., Escarmís, C., 1998. Quasispecies structure and persistence of RNA viruses. *Emerging Infectious Diseases* 4 (Perspectives).
18. Domola, M.J., Thompson, G.J., Aveling, T.A.V., Laurie, S.M., Strydom, H., van Den Berg, A.A., 2008. Sweetpotato viruses in South Africa and the effect of viral infection on storage yield. *African Plant Protection* 14, 15-23.
19. Esterhuizen, L.L., van Heerden, S.W., Rey, M.E.C., van Heerden, H., 2012. Genetic identification of two sweetpotato infecting begomoviruses in South Africa. *Archives of Virology* 157, 2241-2245.
20. Faber, M., Venter, S., Phungula, M.A.S., Benadé, A.J.S., 2001. An integrated primary healthcare and provitamin A household food-production program: impact on foodconsumption patterns. *Food and Nutrition Bulletin* 22 (The United Nations University).
21. Faber, M., Wenholdb, F.A.M., Laurie, S.M., 2017. Dietary diversity and vegetable and fruit consumption of households in a resource-poor peri-urban South Africa. *Ecology of Food and Nutrition* 56:62–80. <https://doi.org/10.1080/03670244.2016.1261024>.
22. Frenkel, M.J., Ward, C.W., Shukla, D.D., 1989. The use of 3' non-coding nucleotide sequences in the taxonomy of potyviruses: application to watermelon mosaic virus 2 and soybean mosaic virus-N. *Journal of General Virology* 70, 2775-2783.
23. Gibb, K.S., Padovan, A.C., 1993. Detection of Sweetpotato feathery mottle virus in sweet potato grown in Northern Australia using an efficient and simple assay. *International Journal of Pest Management* 39, 223-228.
24. Gutierrez, C., 2000. Geminiviruses and plant cell cycle. *Plant Molecular Biology* 43, 763–772.
25. Haible, D., Kober, S., Jeske, H., 2006. Rolling circle amplification revolutionizes diagnosis and genomics of gemini viruses. *Journal of Virological Methods* 135, 9-16.

26. Hillocks, R.J., Thresh, J.M., 2000. Cassava Mosaic Virus and Cassava Brown Streak Virus Diseases in Africa. *Roots* 7 (1) Special Issue. Newsletter (SARRNET and EARRNET).
27. IsHak, J.A., Kreuze, J.F., Johansson, A., Mukasa, S.B., Tairo, F., Abo El-Abbas, F.M., Valkonen, J.P., 2003. Some molecular characteristics of three viruses from SPVD-affected sweet potato plants in Egypt. *Archives of Virology* 148, 2449-2460.
28. Jacquet, C., Delecolle, B., Raccach, B., Lecoq, H., Dunez, J., Ravelonandro, M., 1998. Use of modified plum pox virus coat protein genes developed to limit heteroencapsidation associated risks in transgenic plants. *Journal of General Virology* 79, 1509-1517.
29. Jericho Jr., C., Thompson, G.J., 2000. Detection of sweetpotato viruses in S.A. African Potato Association Proceedings 73, 379-391.
30. Kashif, N., Pietila, S., Artola, K., Jones, R.A.C., Tugume, A.K., Makinen, V., Valkonen, J.P.T., 2012. Detection of viruses in sweetpotato from Honduras and Guatemala augmented by deep-sequencing of small-RNAs. *Plant Disease* 96, 1430-1437.
31. Kele, T., Mouton, S., 2011. In: Lombaard, C., van der Merwe, R., Kele, T., Mouton, S. (Eds.), *Elementary statistics for business and economics*, revised edition. Pearson Education South-Africa (Pty), p. 352.
32. Kim, S.J., Ryu, K.H., Choi, J.K., Park, W.M., 1998. Sequence analysis of the coat protein and 3'-noncoding region for Korean and other strains of Sweetpotato feathery mottle virus. *Molecules and Cells* 8, 777-785.
33. Kreuze, J.F., Karyeija, R.F., Gibson, R.W., Valkonen, J.P., 2000. Comparisons of coat protein gene sequences show that east African isolates of Sweet potato feathery mottle virus form a genetically distinct group. *Archives of Virology* 145, 567-574.
34. Kurabachew, H., 2015. The role of orange-fleshed sweetpotato (*Ipomea batatas*) for combating vitamin A deficiency in Ethiopia: a review. *International Journal of Food and*

Nutrition and Nutrition Engineering 5:141-146. <https://doi.org/10.5923/j.food.20150503.05>.

35. Li, R., Salih, S., Hurtt, S., 2004. Detection of geminiviruses in sweetpotato by polymerase chain reaction. *Plant Disease* 88, 1347-1351.
36. Ling, K., Jackson, D.M., Harrison, H., Simmons, A.M., Pesic-VanEsbroek, Z., 2010. Field evaluation of yield effects on the U.S.A heirloom sweetpotato varieties infected by sweet potato leaf curl virus. *Crop Protection* 29, 757-765.
37. López-Moya, J.J., Wang, R.Y., Pirone, T.P., 1999. Context of the coat protein DAG motif affects potyvirus transmissibility by aphids. *Journal of General Virology* 80, 3281-3288.
38. Lotrakul, P., Valverde, R.A., Clark, C.A., Fauquet, C.M., 2003. Properties of a Begomovirus isolated from sweetpotato [*Ipomoea batatas* (L.) Lam] infected with Sweet potato leaf curl virus. *Revista Mexicana de Fitopatología* 21, 128-136.
39. Lozano, G., Trenado, H.P., Valverde, R.A., Navas-Castillo, J., 2009. Novel Begomovirus species of recombinant nature in sweetpotato (*Ipomoea batatas*) and *Ipomoea indica*: taxonomic and phylogenetic implications. *Journal of General Virology* 90, 2550-2562.
40. Luan, Y.S., Zhang, J., Lui, D.M., 2007. Molecular characterization of Sweet potato leaf curl virus isolate from China (SPLCV-CN) and its phylogenetic relationship with other members of the Geminiviridae. *Virus Genes* 35, 379-385.
41. Mbanzibwa, D.R., Tugume, A.K., Chiunga, E., Mark, D., Tairo, F.D., 2014. Small RNA deep sequencing-based detection and further evidence of DNA viruses infecting sweet potato plants in Tanzania. *Annals of Applied Biology* 163, 329-339.
42. Mori, M., Sakai, J., Kimura, T., Usugi, T., Hayashi, T., Hanada, K., Nishiguchi, M., 1995. Nucleotide sequence analysis of two nuclear inclusion body and coat protein genes of Sweet potato feathery mottle virus severe strains (SPFMV) genomic RNA. *Archives of Virology* 140, 1473-1482.

43. Muhire, B., Martin, D.P., Brown, J.K., Navas-Castillo, J., Moriones, N., Zerbini, F.M., Rivera Bustamante, R., Malathi, V.G., Briddon, R.W., Varsani, A., 2013. A genome-wide pairwise-identity-based proposal for the classification of viruses in the genus Mastrevirus (family Geminiviridae). *Archives of Virology* 158, 1411-1424.
44. Mulabisana, J., Cloete, M., Mabasa, K., Laurie, S.M., Oelofse, D., Rey, M.E.C., 2015. Genetic characterization of viruses infecting sweetpotato in South Africa. *Acta Horticulturae* 1105:155–161. <https://doi.org/10.17660/ActaHortic.2015.1105> (ISHS).
45. Ndunguru, J., Kapinga, R., Sseruwagi, P., Bulili, S., Mwanga, R., Tumwegamire, T., Rugutu, C., 2009. Assessing the sweetpotato virus disease and its associated vectors in Northwest Tanzania and Central Uganda. *African Journal of Agricultural Research* 4, 334-343.
46. Nei, M., Kumar, S., 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
47. Orozco, B.M., Hanley-Bowdoin, L., 1996. A DNA structure is required for geminivirus replication origin function. *Journal of Virology* 70, 148-158.
48. Orozco, B.M., Hanley-Bowdoin, L., 1998. Conserved sequence and structural motifs contribute to the DNA binding and cleavage activities of geminivirus replication protein. *The Journal of Biological Chemistry* 273, 24448-24456.
49. Pappu, S.S., Brand, R., Pappu, H.R., Rybicki, E.P., Gough, K.H., Frenkel, M.J., Niblett, C.L., 1993. A polymerase chain reaction method adapted for selective amplification and cloning of 3' sequences of potyviral genomes: application to dasheen mosaic virus. *Journal of Virological Methods* 41, 9-20.
50. Paprotka, T., Boiteux, L.S., Fonseca, M.E.N., Resende, R.O., Jeske, H., Faria, J.C., Ribeiro, S.G., 2010. Genomic diversity of sweetpotato geminiviruses in a Brazilian germplasm bank. *Virus Research* 149, 224-233.

51. Qin, Y., Zhang, Z., Qiao, Q., Zhang, D., Tian, Y., Wang, Y., 2013. Molecular variability of Sweet potato chlorotic stunt virus (SPCSV) and five potyviruses infecting sweet potato in China. *Archives of Virology* 158, 491-495.
52. Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis, R.I., Mu, L., Dwyer, G.I., Coutts, B.A., Valkonen, J.P.T., 2008. Molecular characterization of Sweetpotato virus G (SPVG) isolates from areas of the Pacific Ocean and southern Africa. *Plant Disease* 92, 1313-1320.
53. Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis, R.I., Mu, L., Valkonen, J.P.T., 2009. Molecular characterization of Sweet potato feathery mottle virus (SPFMV) isolates from Easter Island, French Polynesia, New Zealand, and southern Africa. *Plant Disease* 93, 933-939.
54. Rey, M.E.C., Ndunguru, J., Berrie, L.C., Paximadis, M., Berry, S., Cossa, N., Nuaila, V.N., Mabasa, K.G., Abraham, N., Rybicki, E.P., Martin, D., Pietersen, G., Esterhuizen, L.L., 2012. Diversity of dicotyledenous-infecting geminiviruses and their associated DNA molecules in Southern Africa, Including the South-West Indian Ocean Islands. *Virus* 4:1753-1791. <https://doi.org/10.3390/v4091753>.
55. Rizvi, I., Choudhury, N.R., Tuteja, N., 2015. Insights into the functional characteristics of geminivirus rolling-circle replication initiator protein and its interaction with host factors affecting viral DNA replication. *Archives of Virology* 160:375–387. <https://doi.org/10.1007/s00705-014-2297-7>.
56. Rodriguez, P., Pardina, P.E., Bejerman, N., Luque, A.V., Di Feo, L., 2012. Complete nucleotide sequence of an Argentinean isolate of Sweetpotato virus G. *Virus Genes* 45, 593-595.
57. Rubio, L., Guerri, J., Moreno, P., 2013. Genetic variability and evolutionary dynamics of viruses of the family Closteroviridae. *Frontiers in Microbiology*. <https://doi.org/10.3389/fmicb.2013.00151>.

58. Ryu, K.H., Kim, S.J., Park, W.M., 1998. Nucleotide sequence analysis of the coat protein genes of two Korean isolates of Sweet potato feathery mottle potyvirus. *Archives of Virology* 143, 557-562.
59. Sakai, J., Mori, M., Morishita, T., Tanaka, M., Hanada, K., Usugi, T., Nishiguchi, M., 1997. Complete nucleotide sequence and genome organization of Sweet potato feathery mottle virus (S strain) genomic RNA: the large coding region of the P1 gene. *Archives of Virology* 142, 1553-1562.
60. SAS Institute, Inc., 1999. SAS/STAT User's Guide, Version 9, 1st Printing, Volume 2. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513.
61. Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52, 591-611 (Non-Normality test).
62. Shukla, D.D., Ward, C.W., 1989. Structure of potyvirus coat proteins and its application in the taxonomy of the potyvirus group. *Advances in Virus Research* 36, 273-314.
63. Sivparsad, B.J., Gubba, A., 2012. Molecular resolution of the genetic variability of major viruses infecting sweetpotato (*Ipomoea batatas* L.) in the province of KwaZulu-Natal in the Republic of South Africa. *Crop Protection* 41, 49-56.
64. Snedecor, G.W., Cochran, W.G., 1980. *Statistical Methods*, Sixth Edition. The Iowa State University Press, AMES, IOWA, USA, pp. 11-12 (Chapter 4).
65. Sseremba, G., Mukasa, S., Kyamanywa, S., 2017. Effect of temperature on sweetpotato virus disease symptom expression. *African Journal of Agricultural Research* 12, 2295-2309.
66. Tairo, F., Mukasa, S.B., Jones, R.C.J., Kullaya, A., Rhubaihayo, P.R., Valkonen, J.P., 2005. Unravelling the genetic diversity of the three main viruses involved in sweetpotato virus disease (SPVD), and its practical amplifications. *Molecular Plant Pathology* 6, 199-211.

67. Tairo, F., Jones, R.A.C., Valkonen, J.P.T., 2006. Potyvirus complexes in sweetpotato: occurrence in Australia, serological and molecular resolution, and analysis of the Sweet potato virus 2 (SPV2) components. *Plant Disease* 90, 1120-1128.
68. Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30, 2725-2729.
69. Tugume, A.K., Mukasa, S.B., Kalkiinen, N., Valkonen, J.P., 2010. Recombination and selection pressure in the ipomovirus Sweet potato mild mottle virus (Potyviridae) in wild species and cultivated sweetpotato in the centre of evolution in East Africa. *Journal of General Virology* 91, 1092-1108.
70. Untiveros, M., Fuentes, S., Kreuze, J., 2008. Molecular variability of Sweet potato feathery mottle virus and other potyviruses infecting sweetpotato in Peru. *Archives of Virology* 153, 473-483.
71. Untiveros, M., Quispe, D., Kreuze, J., 2010. Analysis of complete genomic sequences of isolates of the Sweet potato feathery mottle virus strain C and EA: molecular evidence for two distinct potyvirus species and two P1 protein domains. *Archives of Virology* 155:2059-2063. <https://doi.org/10.1007/s00705-010-0805-y>.
72. Vadivukarasi, T., Girish, K.R., Usha, R., 2007. Sequence and recombination analyses of the geminivirus replication initiator protein. *Journal of Biosciences* 32, 17-29.
73. Wang, L.Y., Chen, K.C., Chen, T.C., Yeh, S.D., 2007. Nucleotide sequence analysis of the coat protein genes of two isolates of Sweet potato feathery mottle virus from central Taiwan. *Plant Pathology Bulletin* 16, 203-213.
74. Wasswa, P., Otto, B., Maruthi, M.N., Mukasa, S.B., Monger, W., Gibson, R.W., 2011. First identification of Sweetpotato begomovirus (sweepovirus) in Uganda: characterization, detection and distribution. *Plant Pathology* 60:1030–1039. <https://doi.org/10.1111/1365-3059.2011.02464.x>.

75. Zhang, S.C.Z., Ling, K.S., 2011. Genetic diversity of sweetpotato begomoviruses in the United States and identification of a natural recombinant between Sweet potato leaf curl virus and Sweet potato leaf curl Georgia virus. *Archives of Virology* 156, 955-968. <https://doi.org/10.1007/s00705-011-0930-2>.

CHAPTER 3

Evaluation of disease incidence, aphids and whiteflies infestation levels on sweetpotato in the Gauteng, Mpumalanga and Limpopo provinces of South Africa

3.1 Abstract

Visuses of sweetpotato are associated with production losses in both smallholder and commercial farmers in South Africa. Due to that, field surveys were conducted following a W-pattern walking transect during two planting seasons (2012/2013 and 2013/2014) to determine virus incidences on sweetpotato fields in the Gauteng, Limpopo and Mpumalanga provinces of South Africa. Virus incidences were recorded using a scale (0-5) where 0 represented no symptoms and 5 severe symptoms. Both symptomatic and asymptomatic samples were collected for virus verification and identification. In season 2 (2013/2014), aphid and whitefly infestations levels were also assessed using a scale (0 to 4) where no infestation and 4 represented high infestation. Sweetpotato samples were grafted onto *Ipomoea setosa* (Kerr.) (*I. setosa*) to observe for virus symptoms and leaf samples from *I. setosa* were tested with nitrocellulose membrane-based enzyme linked immunosorbent assays (NCM-ELISA) and double antibody sandwich enzyme linked immunosorbent assays (DAS-ELISA). Among others, viruses such as Sweet potato feathery mottle virus (SPFMV), Sweet potato virus G (SPVG), Sweet potato virus virus 2 (SPV2) and Sweet potato chlorotic stunt virus (SPCSV) were tested with serological methods. Data for the two seasons (2012/2013 and 2013/2014) surveys were analyzed using SAS/Statistical Software Version 9.3, Volume 2, 1999. Disease incidence varied in different areas within the different provinces in both season 1 and season 2. High disease incidence was recorded in Maungani (62%) and Lwamondo (31%) in the Limpopo Province, Thulamahashe (41%), Verena (32%) and Tonga (31%) in the Mpumalanga Province, and Hamanskraal (32%) to Soshanguve M extension (60%) in the Gauteng Province

in season 1. Similarly, high disease incidences were also recorded in season 2 in Verena (36%) in Mpumalanga Province and 31% (Daveyton) to 50% (Soshanguve S extension) in Gauteng Province. Aphids and whiteflies were observed at low frequencies in all the provinces surveyed. The mean incidence score of aphid infestations was between 0 (Mpumalanga Province) and 0.44 (Gauteng Province), and the mean score of whitefly infestations was between 0 (Mpumalanga Province) and 0.3 (Limpopo Province). NCM-ELISA detected SPFMV in 45% (Gauteng Province) to 80% (Limpopo Province) of samples in season 1 and, 49% (Limpopo Province) to 79% (Gauteng Province) in season 2 from a total of 272 (season 1) and 332 (season 2) of samples collected in the three provinces surveyed. SPFMV is still the most prevalent virus in South Africa as it was reported in previous studies. Other viruses detected included SPVG and the highest (36%) infection of SPVG was in Gauteng Province in season 2. SPV2 and SPCSV were also detected in 3% and 8%, and 2% and 16% of samples in season 1 and season 2, respectively. Sweet potato mild mottle virus (SPMMV) was detected in 0 (Gauteng Province) to 11% (Mpumalanga Province) in season 1 and infection of 1% (Gauteng Province) to 8% (Limpopo Province) was also recorded in season 2. Other viruses detected in the two seasons include Sweet potato latent virus (SwPLV) (1%), Sweet potato chlorotic fleck virus (SPCFV) (1%), Sweet potato mild speckling virus (SPMSV) (2% to 2.4%) and Cucumber mosaic virus (CMV) (0-0.7%). Single infection of SPFMV was high in different provinces and this ranged from 29% (Mpumalanga Province) to 35% (Gauteng Province). Single infections of SPVG, SPCSV and SPV2 were detected in less than 5% of samples in all the three provinces in both the two seasons of surveys. The study shows that virus incidences were recorded in all the three provinces surveyed regardless of the low infestation of aphids and whiteflies in the field, which is an indication that infected planting material contributes to virus dissemination.

3.2 Introduction

Sweetpotato (*Ipomoea batatas* Lam.) plays an important role as a commercial and food security crop grown by both smallholder and commercial farmers in all the Provinces of South Africa. Smallholder farmers prefer sweetpotato because it requires low inputs to grow (Mukhopadhyay et al., 2011; Motsa et al., 2015). Sweetpotato is prone to fungal and bacterial diseases, however, viral diseases are the most damaging due to high yield losses attributed to co- and multiple viral infections (Clark and Valverde, 2013). Comprehensive surveys were conducted in 1998/1999, and 2001/2003 to determine the incidence of sweetpotato viruses in the Limpopo, Mpumalanga, Gauteng and KwaZulu Natal provinces of South Africa (Jericho and Thompson, 2000; Domola et al., 2008). NCM-ELISA and DAS-ELISA detected potyviruses SPFMV, SPVG, SPV2 and SwPLV, and luteovirus SPMSV, ipomovirus SPMMV and crinivirus SPCSV (Jericho and Thompson, 2000; Domola et al., 2008). SPFMV was the most prevalent and was detected in 73% of the samples tested (Domola et al., 2008). Co-infection of SPFMV with SPCSV is associated with the development of severe symptoms causing sweetpotato virus disease (SPVD) (Ngeve and Bouwkamp, 1991; Gibson and Aritua, 2002; Clark and Valverde, 2013). Disease surveys are used to obtain disease incidence information taking into consideration factors such as the geographic distribution, farming practices and factors contributing to disease dissemination (Creelman, 1968). Surveys serve to evaluate losses, forecast disease outbreaks, and are used to acquire knowledge on the etiology and epidemiology of major diseases within a geographic area (Creelman, 1968; Braithwaite et al., 2006). Field surveys, and detection of sweetpotato viruses by NCM and DAS ELISA techniques, were also conducted in Zimbabwe (Chavi et al., 1997), Uganda (Aritua et al., 1998a, b), Tanzania (Ndunguru and Aloyce, 2000; Ndunguru et al., 2009), Kenya (Ateka et al., 2004a;b) and in Ethiopia (Tewodros et al., 2011). This study was conducted to determine the virus disease incidences in the Gauteng, Limpopo and Mpumalanga provinces of South Africa.

Furthermore, evaluation of the prevalence of common vectors of sweetpotato viruses, namely, aphids (potyviruses) and whitefly (begomoviruses), was also undertaken. The the frequency or presence of single or mixed virus infections was also determined.

3.3 Material and methods

3.3.1 Areas surveyed

Fields surveys were conducted in two planting seasons (2012/2013 and 2013/2014) in Limpopo, Mpumalanga and Gauteng provinces. Fig. 1 in Chapter 2 is the map of South Africa indicating the study sites in the provinces of Limpopo, Gauteng and Mpumalanga. The surveyed regions, districts and their Global Positioning System (GPS) coordinates are listed in Table 3.1.

Table 3.1: Regions (GPS co-ordinates) surveyed during the field surveys conducted to determine disease symptom incidence and aphids and whiteflies infestation in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa in 2012/2013 (season 1) and 2013/2014 (season 2).

Provinces	District	Regions surveyed	GPS co-ordinates
Limpopo	Vhembe	Lwamondo	22°58'01.3"S 30°26'41.0"E
	Vhembe	Tshiombo	22°48'33.9"S 30°29'52.0"E
	Vhembe	Dzimauli	22°35'23.1"S 30°26'16.6"E
	Vhembe	Rambuda	22°47'42.4"S 30°23'55.4"E
	Vhembe	Maungani	22°57'49.7"S 30°25'19.5"E
	Waterberg district	Groblersbridge	22°59'44.8"S 27°57'03.7"E
	Vhembe	Elim	23°08'44.8"S 30°04'02.8"E
	Waterberg district	Wynberg	22°59'44.8"S 27°57'03.7"E
Mpumalanga	Enhlanzeni	Tonga	25°41'40.3"S 31°47'09.4"E
	Enhlanzeni	Naas	25°40'21.4"S 31°52'06.6"E
	Enhlanzeni	Mzinti	25°41'46.1"S 31°44'40.5"E
	Enhlanzeni	Kamhlushwa	25°39'58.0"S 31°40'41.8"E

Gauteng	Enhlanzeni	Tjakastad (Khuzulwandle)	25°59'17.2"S 30°48'54.3"E
	Enhlanzeni	Mbhejeka	25°58'26.3"S 30°51'19.8"E
	Nkangala	Verena	25°28'05.0"S 29°01'06.0"E
	Enhlanzeni	Snymansbelt (Baberton)	25°46'08.0"S 31°01'15.2"E
	Enhlanzeni	Thulamahashe (Bushbuckridge)	24°43'11.7"S 31°12'23.7"E
	Enhlanzeni	Witbank	25°52'23.6"S 28°58'06.0"E
		Elukwatini	25°44'32.1"S 30°41'26.6"E
	Tshwane district	Soshanguve L extension	25°32'09.5"S 28°06'13.1"E
	Tshwane district	Soshanguve S extension	25°31'45.4"S 28°05'29.7"E
	Tshwane district	Soshanguve M extension	25°32'50.4"S 28°05'42.1"E
	Metsweding	Cullinan (Refilwe)	25°39'00.2"S 28°31'29.3"E
	Metsweding	Dzithobeni (Bronkorspruit)	25°47'00.3"S 28°43'17.0"E
	Ekurhuleni	Daveyton	26°08'12.4"S 28°30'11.1"E
	Tshwane district	Mamelodi	25°43'35.0"S 28°23'07.6"E
	Tshwane district	Roodeplaat	25°36'53.2"S 28°21'18.8"E

3.3.2 Field surveys and sample collections

Fields surveys were conducted and both asymptomatic and symptomatic plants were assessed and the presence or absence of symptoms was recorded using a scoring scale of 0-5 for both plants as explained in Chapter 2, Section 2.1. The percentage virus incidence was calculated by translating the scoring rates (0-5) into percentages as follows: 0 was equivalent to 0%, 1 to 20%, 2 to 40%, 3 to 60, 4 to 80% and 5 to 100%. Samples were collected as vine cuttings of about 15 cm in length at each sampling point (Chapter 2, 2.1) for further analysis to determine the identity of single, co or mixed virus infections. The presence or absence of visible adult aphids and whiteflies on the upper and the bottom sides of the leaves were observed and recorded in the morning before 11h 00 am. The infestation levels were estimated by direct counting of adult insects feeding on the upper and the lower sides of the young leaves. A scoring scale of 0 to 4 was used, where 0 is equivalent to no infestation; 1 (1-5 insects) equivalent to low infestation; 2 (6 to 10 insects) equivalent to medium infestation; 3 (11 to 20

insects) equivalent to high infestation and 4 (20 and higher) equivalent to very high infestation. Scores of 3 or 4 usually result in 100% virus infection (Esterhuizen, University of Johannesburg, South Africa, pers. comm.). These were also translating into percentages as explained above. Three shoots per plant were selected as representative samples and the number of aphids or whiteflies observed on sweetpotato plants were counted and recorded as described by Ndunguru et al. (2009). Field survey data was analysed as reported in Chapter 2, Section 2.1. Data on aphids and whiteflies infestation level was analysed using SAS/Statistical Software Version 9.3, Volume 2, 1999. A combined ANOVA was conducted and the means of significant effects were compared using Fisher's unprotected t-LSD (least significant difference) at a 5 % level of significance (Snedecor & Cochran, 1980).

3.3.3 Viral analysis

3.3.3.1 Indexing

Scions of about 15 cm of sweetpotato cuttings were side-grafted onto the indicator plant *I. setosa* (Fig. 3.1) to determine the presence of viruses in samples collected from the field. A downward cleft was created on the stem of a 3 to 4 weeks old *I. setosa* plants (3 leaf stage) with a sharp blade. The upward cleft was also created on the scion of sweetpotato stem and the two clefts were joined tightly together and sealed with a latex bandage (Stericrepe) or Parafilm (American National CanTM) at the graft union. Plants were transferred to a glasshouse and maintained at temperatures of 25 to 28°C for 16 hours (day cycle) and 15°C for 8 hours (night cycle). Plants were watered once a day and supplemented with soluble nutrient fertilization (Multifeed[®] P 5:2:4 (43), Nulandis[®]) on a weekly basis. Insect pests such as aphids, whiteflies and mites were monitored and controlled by spraying with recommended insecticides to prevent virus cross contaminations.



Fig. 3.1. Indicator plant *I. setosa* (A) grafted with sweetpotato scion of a sample collected from Verena in Mpumalanga Province (B); (C) union that joins the sweetpotato scion and the *I. setosa* cleft (indicated by the red arrow) to prevent infection by opportunistic pathogens; (D) is *I. setosa* plants at 2 to three leaf stage.

3.3.3.2 Serological Analysis

3.3.3.2.1 Nitrocellulose based enzyme linked immunosorbent assay (NCM-ELISA)

NCM-ELISA was used to analyse the samples for the presence of viruses commonly known to infect sweetpotato viruses. These viruses include SPFMV, SPMMV, SPVG, SPCSV, SPCFV, SPMSV, CMV, *Sweetpotato latent virus* (SPLV), *Sweetpotato collusive virus* (SPCV) and *Sweetpotato C6 virus* (SPC6V). Sources of antibody and the methodology used in processing of the samples was as described in Chapter 2, Section 2.2.1.

3.3.3.2.2 Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA)

DAS-ELISA was used to detect SPV2 and information on the sources of antibody used is provided in Chapter 2, Section 2.2.1.

3.3.3.2.3 Detection of samples showing symptoms associated with begomoviruses

Sweetpotato and *I. setosa* plants showing leaf curling and rolling symptoms associated with begomoviruses (Clark and Valverde, 2013) were analyzed with nucleic-acid based methods as already described (Chapter 2, 2.4).

3.3.4. Analysis of data generated with serological tests

Data generated with NCM and DAS-ELISA were combined and analysed using the statistical methodology illustrated in Chapter 2, Section 2.2.1.

3.4. Results

3.4.1 Field surveys and sample collections

Smallholder farmers in rural areas of Limpopo and Mpumalanga generally grow landraces with no formal variety descriptions. Commercial farmers in Groblersbridge/Wynberg in Limpopo Province grow both white fleshed (Blesbok, Bosbok, Ribbok) and the orange-fleshed variety Beauregard for local and international markets. However, OFSP varieties such as Bophelo, Ndou, Impilo, Mvuvhelo and Monate were introduced to smallholder farmers in Gauteng and other parts of Limpopo and Mpumalanga provinces as part of the food diversification programs to address nutrient deficiency challenges (Laurie and Magoro, 2008; Laurie et al., 2015). Virus related symptoms which are a result of a possible mix of potyviruses and viruses of other taxonomic groups were observed in all the three provinces surveyed and the severity of symptoms varied in each Province. Typical potyvirus symptoms were characterized by purple ring spots, vein banding, while symptoms associated with co-infections with begomoviruses

consisted of the leaf curling, rolling and stunting of the plants (Fig. 3.2); (Clark and Valverde, 2013; Wasswa et al., 2011).



Fig. 3.2. Symptoms of sweetpotato viruses observed during season 1 (2012/2013) and season 2 (2013/2014) surveys conducted in the three provinces of South Africa. (A) Vein yellowing and mild upward curling of leaves observed in Maungani in Limpopo Province, a possible co-infection of potyviruses and begomoviruses, (B) purple ring spots on older leaves observed in Verena in Mpumalanga Province, a possible single or co-infections of potyviruses (C) and (D) severe leaf curling/rolling and stunting of sweetpotato plants observed in the field of Wagendrift (Moloto) in Gauteng Province, a possible co-infections of begomoviruses.

3.4.2 Virus disease incidences

The means for overall virus disease incidences for each Province recorded in season 1 and season 2 are shown in Table 1 and 2 (Appendices) and already reported in Chapter 2, Section 3.1. When comparing virus disease incidences within different districts and regions within the provinces, Maungani (62%) had the highest incidence, which was significantly higher compared with regions such as Lwamondo (31%), Wynberg (23%) and Dzimauli (21%) in Limpopo Province, during season 1 surveys. In Mpumalanga Province, virus disease incidences ranged from 21% (Elekhwathini) to 41% (Thulamahashe) and the incidence in Thulamahashe was significantly higher than the incidences recorded in other regions of the

province. In Gauteng Province, virus disease incidences ranged from 16% (Cullinan and Daveyton) to 60% (Soshanguve extension M). Virus disease incidences in Soshanguve extension M were significantly higher than the incidences recorded in other areas in Gauteng Province.

Similarly, Soshanguve S extension (Gauteng Province) had the highest virus disease incidence (50%) which was significantly higher than the incidences recorded in the other 23 areas surveyed in season 2. This was followed by Verena (Mpumalanga Province), Mamelodi (Gauteng) and Daveyton (Gauteng), which had incidences of 36%, 34% and 31%, respectively. These incidences were higher than those observed in other areas. Virus disease were recorded in all areas of Mpumalanga Province and incidences ranged from 17% (Mbejeka) to 36% (Verena). In Gauteng Province, virus disease incidences ranged between 4% (Soshanguve L extension) and 50% (Soshanguve S extension). In Limpopo Province, virus disease incidences were between 0% (Tshiombo, Rammbuda, Maungani and Elim) and 11% (Wynberg).

3.4.3 Aphid and whitefly evaluations

Table 3 (Appendices) shows the mean scores of the aphid and whitefly infestation level in the areas surveyed. Low infestation scores of aphids were observed on sweetpotato plants in Soshanguve S extension (0.1), Dzithobeni (0.8), Mamelodi (0.3). Medium infestation levels were recorded in Cullinan (2), which was significantly higher than the infestation levels in other regions in the three provinces surveyed. Very low to medium infestation levels of whiteflies were observed in Wynberg (1.6), Tshiombo (0.6) and Rammbuda (0.5) and the infestation levels in Wynberg were significantly higher than those observed in other areas in the three provinces surveyed. Aphids and whiteflies were not observed on sweetpotato in Mpumalanga Province despite observation of symptoms.

3.4.4 Biological indexing

Symptoms observed on *I. setosa* varied from moderate to severe, and the most common symptoms vein clearing/banding, leaf mottling, mosaic, leaf shrinking (deforming), chlorosis and shoe stringing (Fig. 3.3). Symptoms associated with sweetpotato begomoviruses such as leaf rolling, upward curling and greening of the main veins of leaves were also observed on *I. setosa* samples even on samples that did not exhibit begomovirus-like symptoms in the field (Fig. 3.3D). Severe shoe stringing symptoms, an indication of the possible presence of multiple virus infections of different taxonomic groups were observed on samples LWD-B.9 and TMH-B.8 collected from Lwamondo in Limpopo Province and Thulamahashe in the Mpumalanga Province (Fig. 3.3A and B). Typical potyvirus symptoms such as vein banding and yellowing of the main veins were observed on samples MNG-B.8 and MAM-B.15, collected from Maungani in Limpopo Province and Mamelodi in Gauteng Province, respectively, (Fig. 3.3C and E).

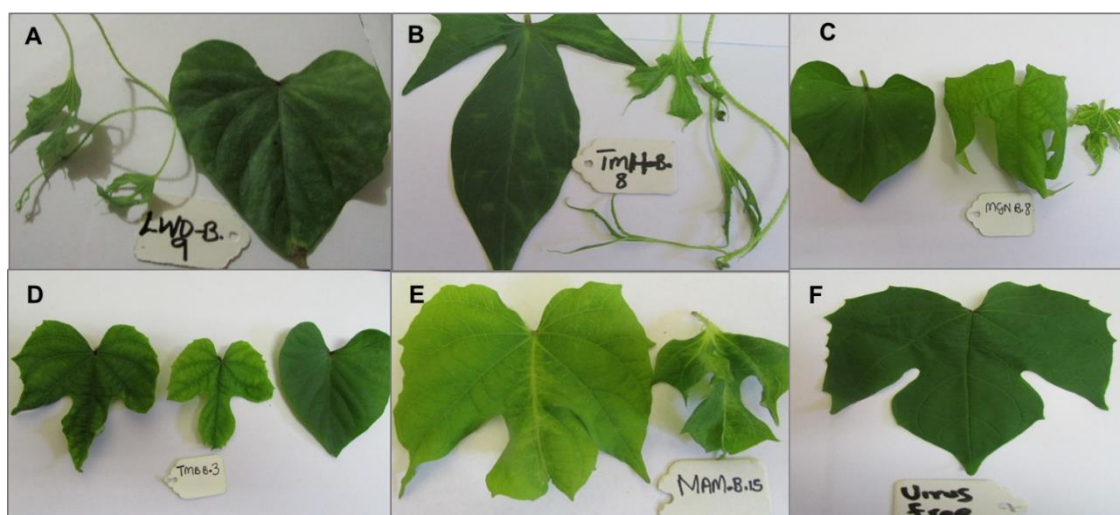


Fig. 3.3. Symptoms of sweetpotato viruses as observed on *I. setosa* post-graft inoculations of representative samples collected from Gauteng, Limpopo and Mpumalanga provinces. (A) and (B) Severe shoe stringing symptoms on *I. setosa* leaves and mild chlorotic spots on sweetpotato leaf, (C) Leaf rolling and vein clearing on *I. setosa* and mild chlorotic spots on sweetpotato leaf, (D) Mild upward curling and greening of the main veins on *I. setosa* and sweetpotato leaf with no viral symptoms, (E) Mosaic symptoms on the main veins and (F) uninfected *I. setosa*.

3.4.5 Serological analysis

The number of samples collected and analyzed with NCM and DAS-ELISA were reported in Chapter 2, Section 3.1. NCM-ELISA detected different sweetpotato viruses in representative samples from Baberton (BBT), Verena (VRN) and New Forest (NF) (Fig. 3.4) in Mpumalanga Province. The purple colour on samples no. 2 (BBT -A.10), 3 (BBT-A.11), 4 (BBT-A.6), 5 (BBT-A.9), 6 (BBT-A.12), 7 (BBT-A.15), 8 (BBT-A.8), 9 (BBT-A.13), 11 (VRN-A.1), 12 (VRN-A.9), 14 (VRN-A.7), 15 (VRN-A.13), 17 (VRN-A.15), 18 (VRN-A.2), 19 (NF-A. 2) denotes that samples tested positive for SPFMV (Fig. 3.4A). Samples no 1 (BBT-A.14), 10 (VRN-A.12), 13 (VRN-A.3) and 16 (NF-A.3) were also regarded as positive for SPFMV (Fig. 3.4A). Samples 21 (NF-A.1) and 22 (MZN-A.1) had a very faint colour to no colour and were regarded as negative for SPFMV (Fig. 3.5A). Sample VRN-A.7 tested positive for SPMMV (Fig. 3.4B, no 14) and sample VRN-A.15 tested positive for SPMSV (Fig. 3.4C, no 17). SPVG was not detected in this batch of samples.

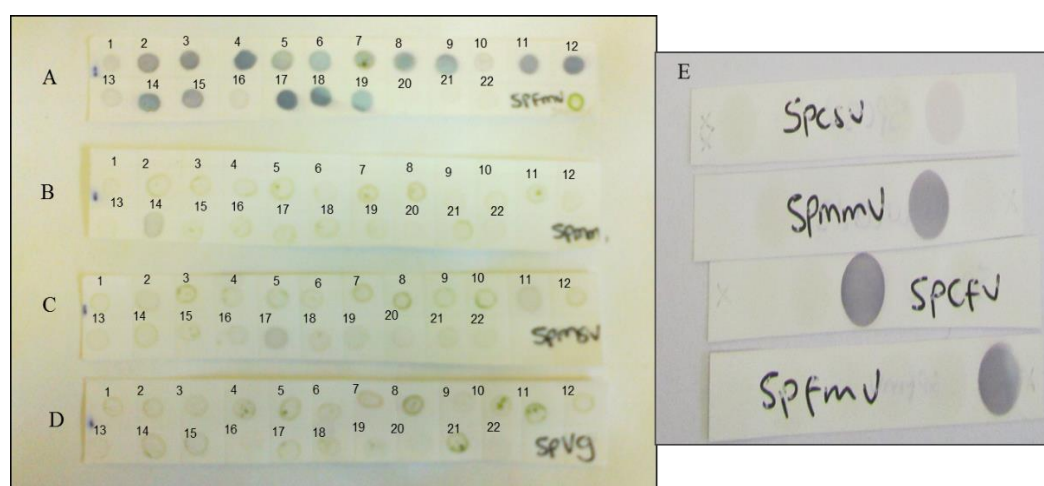


Fig. 3.4. NCM-ELISA results conducted with samples collected from different regions in the Mpumalanga Province during season 1 (2012/2013) surveys. (A) SPFMV, (B) SPMMV, (C) SPMSV, (D) SPVG and (E) is the representative of control strips of SPFMV, SPMMV, SPCSV and SPFMV, which were included in the tests. Samples were loaded as follows: (1) BBT-A.14, (2) BBT-A.10, (3) BBT-A.11, (4) BBT-A.6, (5) BBT-A.9, (6) BBT-A.12, (7) BBT-A.15, (8) BBT-A.8, (9) BBT-A.13, (10) VRN-A.12, (11) VRN-A.1, (12) VRN-A.9, (13) VRN-A.4, (14)

VRN-A.7, (15) VRN-A.13, (16) VRN-A.3, (17) VRN-A.15, (18) VRN-A.2, (19) NF-A. 2, (20) NF-A. 3, (21) NF-A.1 and (22) MZN-A.1. The purple colour is an indication of a sample that tested positive for a virus and no colour indicates an uninfected sample (i.e. negative).

Representative samples collected from Tshiombo village in Limpopo Province were positive for SPFMV, SPCSV and SPVG by NCM ELISA (Fig. 3.5). Samples TMB-B.4, TMB-B.8, TMB-B.10, TMB-B.13, TMB-B.14, TMB-B.18 and TMB-B.19 tested positive for SPFMV (Fig. 3.5A, no., 4, 8, 10, 12, 13, 15 and 16). Samples TMB-B.2, TMB-B.6, TMB-B.7, TMB-B.12, TMB-B.14, TMB-B.16 and TMB-B.18 tested positive for SPCSV (Fig. 3.5 B, no., 2, 6, 7, 11, 13, 14 and 15). SPVG was detected in samples TMB-B.7, TMB-B.8, TMB-B.10, TMB-B.13, TMB-B.14, TMB-B.18 (Fig. 3.5 C, no., 7, 8, 10, 12, 13 and 15). Multiple infections SPFMV, SPVG and SPCSV were detected in sample TMB-B.14 (Fig. 3.5A, B and C, no 13 and 15). Co-infection of SPVG and SPCSV was detected in sample TMB-B.7 (Fig. 3.5, B7 and C7) and co-infection of SPFMV and SPVG in sample TMB-B.13 (Fig. 3.5, B12 and C12).

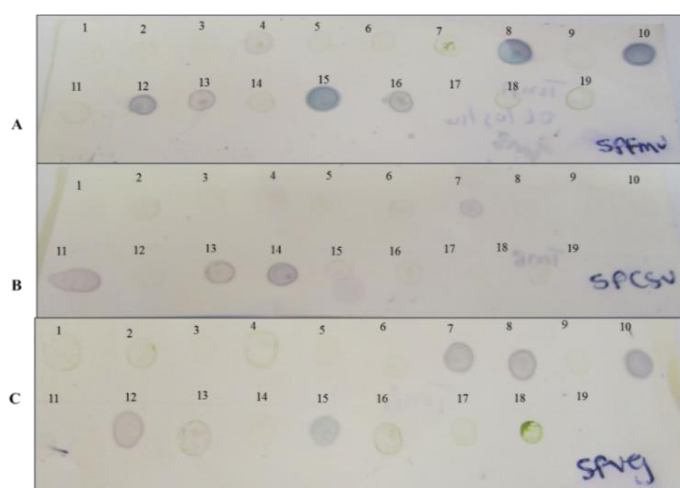


Fig. 3.5. NCM-ELISA of representative samples collected from Tshiombo in Limpopo Province during season 2 (2013/2014) surveys, which shows samples that tested positive or negative for (A) SPFMV, (B) SPCSV and (D) SPVG. Samples were loaded as follows: (1) TMB-B.1, (2) TMB-B.2, (3) TMB-B.3, (4) TMB-B.4, (5) TMB-B.5, (6) TMB-B.6, (7) TMB-B.7, (8) TMB-B.8, (9) TMB-B.9, (10) TMB-B.10, (11) TMB-B.12, (12) TMB-B.13, (13) TMB-B.14, (14) TMB-B.16, (15) TMB-B.18, (16) TMB-B.19, (17) TMB-B.20, (18) TMB-

B.15 and (19) Negative control. The purple colour is an indication of a sample that tested positive for a virus and no colour indicates an uninfected sample (i.e. negative).

Sample LWD-B.9 tested positive for co-infection of SPFMV, SPCSV and SPV2 while sample TMH-B.8 tested positive for co-infection of SPFMV and SPCSV. These are the samples that exhibited shoe-stringing symptoms on *I. setosa* plant (Fig. 3.3A and B). Sample TMB-B.3, which induced the upward curling of leaves and greening of the main veins (Fig. 3.3D), tested negative for all 12 viruses that are detected by NCM and DAS-ELISA. This sample tested positive by PCR for Sweetpotato leaf curl virus (SPLCV) (results not shown). Sample MNG B.8 and MA-B.15 that exhibited typical symptoms of potyviruses (Fig. 3.3C and D) tested positive for SPFMV (MNG B.8) and co-infection of SPFMV and SPVG (MA-B.15).

The table of means and the chi-square of probabilities (<0.05% level) obtained with the NCM and DAS-ELISA tests is shown in Table 3.2. SPFMV was detected in 60% and 56% (average) of samples tested in both season 1 and season 2, respectively. When Provinces were compared, SPFMV was detected in 45% (Gauteng Province), 72% (Mpumalanga Province) and 80% (Limpopo Province) of samples in season 1 and significance differences existed between the three provinces. In season 2, SPFMV was detected in 49% (Limpopo Province), 50% (Mpumalanga Province) and 79% (Gauteng Province) of samples and significant differences existed between the three provinces. The average infection by SPVG was low in season 1 (1.5%) and was high in season 2 (25%). Infection by SPVG ranged between 0% (Gauteng Province) and 4% (Mpumalanga Province) in season 1. SPVG infection was high in season 2 and was detected in 13% (Limpopo Province), 27% (Mpumalanga Province) and 36% (Gauteng Province) of samples. Furthermore, SPV2 was also detected and the average means were 3% and 8% in season 1 and season 2, respectively. In the three provinces surveyed, SPV2 was detected in Gauteng Province (5%) and Mpumalanga Province (4%) and was not detected

in Limpopo Province in season 1. Infection by SPV2 was detected in season 2 in 9% (Gauteng Province), 4% (Limpopo Province) and 9% (Mpumalanga Province) of samples. SPMMV was detected in 3% and 5% of samples in season 1 and season 2, respectively, and was more prevalent in Mpumalanga Province (11%) in season 1 and in Limpopo Province (8%) in season 2. SPCSV was also detected in 2% (season 1) to 16% (season 2) on average, and significant differences were observed between the two seasons. Infection by SPCSV was between 0% (Mpumalanga Province) and 2% (Limpopo and Gauteng Province) in season 1 and infection was high in season 2 and SPCSV was detected in 10% (Mpumalanga Province) to 29% (Gauteng Province) of samples. Other viruses detected include SwPLV (between 0.6% and 1%), SPCFV (between 0.9% to 1%), SPMSV (between 2% and 2.4%) and CMV (between 0% and 0.7%) in the three Provinces surveyed in season 1 and season 2. Sweet potato caulimo-like virus (SPCaLV) and Sweet potato collusive virus (SPCV) were not detected and Sweet potato C-6 virus (SPC-6V) was detected in only one sample collected in Roodeplaat in Gauteng Province.

Table 3.2: The χ^2 probabilities (<0.05% level) showing the mean percentage infection by SPFMV, SPVG, SPMMV, SPV2, SPCSV, SwPLV, SPCFV, SPMSV and CMV is samples collected from Gauteng, Limpopo and Mpumalanga provinces in season 1 and season 2 surveys.

	SPFMV	SPVG	SPMMV	SPV2	SPCSV	SwPLV	SPCFV	SPMSV	CMV
Mean Season 1	60	1,5	3	3	2	1	1,1	2	0,7
Chi-Square P	<,0001	<,0001	<,0001	0,0251	<,0001	0,4994	0,8059	0,6302	0,1176
Means for Provinces									
Gauteng	45	0	0	5	2	2	1	0	1
Limpopo	80	2	2	0	2	0	1	2	0
Mpumalanga	72	4	11	4	0	0	0	6	0
Chi-Square P (Provinces)	<,0001	0,078	<,0001	0,1216	0,5137	0,2443	0,7194	0,0169	0,3922
Likelihood Ratio Chi-Square	<,0001	0,044	0,08	0,033	0,3092	0,1372	0,5581	0,0132	0,2669
	SPFMV	SPVG	SPMMV	SPV2	SPCSV	SwPLV	SPCFV	SPMSV	CMV
Mean season 2	56	25	5	8	16	0,6	0,9	2,41	0
Chi-Square P	<,0001	<,0001	0,1538	0,0251	<,0001	0,4994	0,8059	0,6302	0,1176
Means for Provinces									
Gauteng	79	36	1	9	29	0	3	1	0
Limpopo	49	13	8	4	15	2	0	5	0
Mpumalanga	50	27	4	9	10	0	1	1	0
Chi-Square Provinces	<,0001	0,001	0,0714	0,195	0,0015	0,1206	0,1713	0,1791	0
Likelihood Ratio Chi-Square	<,0001	0,0007	0,0613	0,1599	0,0025	0,1026	0,1593	0,2063	0

Chi-square P= chi-square probability. Sweet potato feathery mottle virus (SPFMV), Sweet potato virus 2 (SPV2), Sweet potato virus G (SPVG), Sweet potato latent virus (SwPLV), Sweet potato chlorotic stunt virus (SPCSV), Sweet potato mild mottle virus (SPMMV), Sweet potato chlorotic fleck virus (SPCFV), Sweet potato mild speckling virus (SPMSV), Sweet potato collusive virus (SPCV), Cucumber mosaic virus (CMV) and Sweet potato C-6 virus (SPC-6V).

3.4.6 Virus single, co- and multiple infections

The table of means (averages of Season 1 and 2) and the chi-square probabilities of single, co- and multiple infections of viruses as detected by NCM and DAS-ELISA are listed in Table 4 (Appendices) and were also reported in Chapter 2, Section 3.2. The results of single virus infections recorded in different provinces are reported in this section (Table 5, appendices). Single infection of SPFMV was detected in 34.8% (Gauteng Province), 32.5% (Limpopo Province) and 29% (Mpumalanga Province) of samples tested. Single infection of SPCSV ranged from 2.3% (Gauteng Province) to 3.6% (Mpumalanga Province). Single infection of SPVG was low and was detected in 0.9% (Gauteng Province) to 3.05% (Mpumalanga Province) of samples tested. Single infection of SPV2 was also observed in few samples, and was between 0% (Gauteng Province) to 3.08% (Mpumalanga Province). Low percentages of single infection of SPMMV, SwPLV or SPMSV were also observed. Provinces varied with regard to virus co-infections and co-infection of SPFMV with SPVG was detected in 7.44% (Gauteng), 3.66% (Limpopo Province) and 10.77% (Mpumalanga Province) of samples. Co-infection of SPFMV with SPV2 occurred in 1% (Season 1) to 1.8% (Season 2) of samples tested. Co-infection of SPFMV and SPV2 was low and was only detected in 1.86% and 2.56% of samples in Gauteng and Mpumalanga provinces, respectively. Co-infection of SPFMV and SPV2 was not detected in Limpopo Province. Detection of co-infection of SPFMV with SPCSV ranged from 2.6% (Limpopo and Mpumalanga Provinces) and 5.6% (Gauteng Province). Multiple infection of SPFMV with SPVG and SPV2 were also detected in few samples (0 to 1.8%) in both the two seasons.

3.5 Discussion

Sweetpotato viral disease symptoms were observed in both the two seasons in the three provinces surveyed. However, symptoms varied in different areas within the three provinces surveyed. High virus incidences were recorded in Maungani and Lwamondo in Limpopo Province; Thulamahashe, Verena and Tonga in Mpumalanga Province, and Soshanguve extension S and M, Daveyton and Hamanskraal in Gauteng Province in both season 1 and 2. In Gauteng Province, high virus disease incidences were recorded in Soshanguve S extension, Verena, Mamelodi and Daveyton only, and all other areas showed low virus incidence levels. Virus incidences are reported to vary in different seasons in different production regions and may increase or decrease, depending on the virus/viruses present in the samples and other factors associated with symptom development (Sseremba et al., 2017). Ndunguru and co-researchers (2009) and Gibson and co-researchers (1998) demonstrated that sweetpotato virus symptoms, including that of SPVD, are variable in different regions of African countries. Furthermore, smallholder farmers grow mostly local landraces, which probably have developed the natural ability to mask virus symptoms, resulting in low virus symptoms incidences when field surveys are conducted. This is supported by the results of this research, which showed that when representative asymptomatic samples collected from all the regions were grafted onto *I. setosa*, typical potyvirus symptoms (severe shoe stringing symptoms, vein clearing/banding and chlorotic spots) were observed. Similarly, begomovirus symptoms such as the greening of the main veins, leaf rolling and the upward curling of leaves were also observed post graft inoculation on *I. setosa* plants. This confirms previous reports which showed that disease symptoms were less prevalent or absent in most areas in different provinces of South Africa and viruses associated with symptoms could only be revealed by grafting of collected asymptomatic samples onto *I. setosa* and subsequent testing with different detection methods (Domola et al., 2008; Mulabisana et al., 2018). However, the ability of some

varieties to mask virus symptoms pose as a challenge because this makes rouging of virus-infected plants impossible for farmers.

Low (scoring rate of 1) infestations of aphids and whiteflies were recorded in the Gauteng and Limpopo Provinces, but not in Mpumalanga Province, in season 2. The absence of aphids and whiteflies in Mpumalanga Province could be attributed to rainfall [50 mm to 100 mm (<http://www.weathersa.co.za/climate/historical-rain-maps>) recorded in Mpumalanga during the week of the surveys (21 to 31 January 2014). Alicai and co-workers (1999) showed that high rainfall decreases whitefly populations, which could also be the case with low (scoring rate of 1) aphid populations. This suggests that viruses (potyviruses such as SPFMV, SPVG, SPV2; and begomoviruses) associated with symptoms in sweetpotato during these surveys were likely to already be present in vegetative planting material used for plantings in seasons 1 and 2. High whitefly populations may contribute to transmission of viruses, but are not the only factor associated with high virus disease incidences, such as that of SPVD (Alicai et al., 1999). Many of these viruses do not manifest as symptoms in sweetpotato (Karyeija et al., 1998a; Clark et al., 2012), and vegetative propagation contributes to transfer of infected material (Karyeija et al., 1998a). Furthermore, brief or non-persistent transmission by vectors is known for potyviruses of sweetpotato and natural wild host plants growing in sweetpotato fields or in close proximity of crops as weeds also harbour viral diseases and serve as sources for virus transmission by vectors (Tugume et al., 2008; 2016). Tugume and co-researchers (2008; 2016) detected viruses such as SPFMV, SPCSV, SPMNV and SPCFV in about 26 *Ipomoea* species such as *I. sinensis*, *I. cairica*, *I. acuminata*, *Hewittia sublobata* (*H. sublobata*). All these indicate that sweetpotato viruses will remain a challenge to production if farmers continue to use infected sweetpotato plants as planting material, not taking into consideration sanitation processes such as weeding, rouging of infected plants, and most importantly, the use of virus tested sweetpotato planting material obtained from reputable vine growers..

From the grafting assays and serological/sequencing identification tests (Chapter 2), SPFMV was detected in 60% and 56% of samples in season 1 and 2, respectively. A survey in Korea (2011 to 2014) revealed the incidence of SPFMV to vary (87% in 2012 and 35% in 2014) in different growing seasons (Kim et al., 2017). Other viruses detected in the three provinces surveyed are SPVG, SPV2, SPCSV, SPMSV and these occurs in mixed infection with SPFMV. Co-infections of SPFMV and SPVG (0.74% and 12.7%), SPFMV and SPCSV (1% and 5.74%) were recorded in season 1 and 2 (Chapter 2). Co-infections of SPFMV and SPVG were recorded in both Limpopo (4%), Gauteng (7.4%) and Mpumalanga (11%) provinces. Co-infection of SPFMV and SPCSV was recorded to be between 3% (Limpopo and Mpumalanga provinces) and 5.6% (Gauteng Province). Irregular occurrence of co-infections of SPFMV and SPV2, and SPFMV and SPMMV were also recorded in the three provinces surveyed. Although virus disease incidences were between only 18% and 23% (season 1) and 4 and 22% (season 2) (Chapter 2), viruses such as SPFMV as single or in co-infections with viruses of other taxonomic group were still detected in 45% (Gauteng Province) and 80% (Limpopo Province) of samples tested in season 1 alone. This indicates that virus symptoms incidences in the field cannot be used as the only determining factor of whether sweetpotato viruses are prevalent or not in an area or a region.

Although the infection by SPV2 (between 2 and 4%) and crinivirus SPCSV (0 and 3.1%) appeared to be low when compared to that of SPFMV and SPVG in the three provinces, the presence of the crinivirus SPCSV, in co-infection with SPFMV and SPV2 is a concern to the production of sweetpotato in South Africa. This is due to the fact that the two viruses have been reported to have synergistic effects that can results in the expression of severe symptoms on sensitive varieties, causing SPVD (Aritua et al., 1998a; Karyeija et al., 1998a; Valverde et al., 2007). In fact, leaf samples taken, in this study, from grafted *I. setosa* with severe mosaic and shoe stringing symptoms associated with SPVD, were found to have co-infections of potyvirus

SPFMV/SPV2/SPVG and crinivirus SPCSV. Therefore, there is a need to determine the effects of co-infection of crinivirus SPCSV and potyviruses on sweetpotato varieties, to determine if co-infections causes SPVD under field conditions. Begomovirus related symptoms were also observed in some areas within the three provinces surveyed, and these have been confirmed in Chapter 2 to occur frequently in co-infections with potyviruses. These can also have a negative impact on sweetpotato production in South Africa.

Virus distribution, most importantly, SPFMV, SPVG, SPCSV, SPV2, in sweetpotato material may be attributed to poor agricultural practices such as poor sanitation, which involves cutting of vines with unsterilized pruning scissors carried out at farm level, or the inflow of infected planting material from neighbouring borders and the climatic conditions (Gasura et al., 2008). Farmers (smallholder) in some areas indicated that they retain plants from previous seasons for use as planting material in the next seasons and this is one possible reason for the apparent continued infection due to propagation of infected material. The availability of virus inoculum in the form of sources of planting material retained from previous seasons plays an important role in the dissemination of sweetpotato viruses, and this has been shown in countries such as Uganda (Karyeija et al., 1998b).

The information from this study continues to demonstrate that SPFMV remains the most prevalent detected virus in South Africa, confirming the findings of the study conducted by Jericho and Thompson (2000) and Domola and co-workers (2008). This highlights the importance of governmental facilities in South Africa to implement strict rules on virus testing of sweetpotato varieties to be disseminated to farmers. Informing and training farmers and extension officials on the importance of viruses and their effects is also crucial and can minimize dissemination of viruses through planting material. Sweet potato virus C (SPVC) was previously called the common strain of SPFMV (Tairo et al., 2005) and in this study, it should be noted that detection of SPFMV with an antibody specific for SPFMV does not differentiate

between SPFMV and SPVC, which suggests the possible presence of SPVC in SPFMV infected samples. Further characterization with RT-PCR, cloning and sequencing part of the genome detected SPVC in some of the SPFMV infected samples (Chapter 2).

3.6 References

1. Alicai, T., Adipala, E., Gibson, R.W., 1999. Seasonal changes in whitefly numbers and their influence on incidence of Sweetpotato chlorotic stunt virus and Sweetpotato virus disease in sweetpotato in Uganda. *International Journal of Pest Management* 45 (1), 51-55.
2. Aritua, V., Alicai, T., Adipala, E., Carey, E.E., Gibson, R.W., 1998a. Aspects of sweetpotato virus disease in sweetpotato. *Annals of Applied Biology* 132, 387-398.
3. Aritua, V., Adipala, E., Carey, E.E., Gibson, R.W., 1998b. The incidence of sweetpotato virus disease and virus resistance of sweetpotato grown in Uganda. *Annals of Applied Biology* 132, 399-411.
4. Ateka, E.M., Njeru, R.W., Kibaru, A.G., Kimenju, J.W., Barg, E., Gibson, R.W., Vetten, H.J., 2004a. Identification and distribution of viruses infecting sweetpotato in Kenya. *Annals of Applied Biology* 144, 371-379.
5. Ateka, E.M., Barg, E., Njeru, R.W., Lesemann, D., -E., Vetten, H.J., 2004b. Further characterization of Sweet potato virus 2: a distinct species of the genus *Potyvirus*. *Archives of Virology* 149, 225-239.
6. Braithwaite, M., Hill, C.F., Ganev, S., Pay, J.M., Pearson, H.G., Alexander, B.J.R. 2006. A survey of sub-tropical nursery plants for fungal diseases in Northland.
7. Clark, C.A., Davis, J.A., Abad, A., Cuellar, W.J., Fuentes, S., Kreuze, J.F., Gibson, R.W., Mukasa, S.B., Tugume, A.K., Tairo, F.D., Valkonen, J.P.T., 2012. Sweetpotato viruses: 15

years of progress on understanding and managing complex diseases. *Plant Disease* 96, 168-185.

8. Clark, C.A., Valverde, R.A., 2013. Diseases caused by viruses. In: *Compendium of Sweetpotato diseases, pests and disorders*, second edition. Clark, C.A., Ferrin, D.M., Smith, T.P., Holmes, G.J. (Eds.). American Phytopathological Society.

9. Chavi, F., Robertson, A.I., Verduin, B.J.M., 1997. Survey characterization of viruses in sweetpotato from Zimbabwe. *Plant Disease* 81, 1115-1122.

10. Creelman, D.W., 1968. Surveys to assess plant disease losses. *Canadian Plant Disease Survey* 48 (2), 58-60.

11. Domola, M.J., Thompson, G. J., Aveling T.A.V., Laurie, S.M., Strydom, H., van Den Berg, A.A., 2008. Sweetpotato viruses in South Africa and the effect of viral infection on storage yield. *African Plant Protection* 14, 15-23.

12. Gasura, E., A.B. Mashingaidze, A.B., Mukasa, S.B., 2008. Genetic variability for tuber yield, quality, and virus disease complex traits in Uganda sweetpotato germplasms. *African Crop Science Journal* 16 (2), 147-160.

13. Gibson, R.W., Aritua, V., 2002. The perspective of *Sweet potato chlorotic stunt virus* in sweetpotato production in Africa: A review. *African Crop Science Journal* 10 (4), 281-310.

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

15. Jericho, C. Jr., Thompson, G.J., 2000. Detection of sweetpotato viruses in South Africa. *African Potato Association Proceedings* 73, 379-391.

16. Karyeija, R.F., Gibson, R.W., Valkonen, J.P.T., 1998a. Resistance to Sweet potato virus diseases (SPVD) in wild East African *Ipomoea*. *Annals of Applied Biology* 133, 39-44.
17. Karyeija, R.F., Gibson, R.W., Valkonen, J.P.T., 1998b. Significance of *Sweet potato feathery mottle virus* in subsistence sweetpotato production in Africa. *Plant Disease* 82 (1), 4-15.
18. Laurie, S.M., Magoro, M.D., 2008. Evaluation and release of new sweetpotato varieties through farmer participatory selection. *African Journal of Agricultural Research* 3, 672-676.
19. Laurie, S.M., Tjale, S.S., van den Berg, A.A. Mtileni, M.M., Labuschagne, M.T., 2015. Agronomic performance of new cream to yellow-orange sweetpotato varieties in diverse environments across South Africa. *South African Journal of Plant and Soil* 2015, 1-9. ISSN 0257-1862, EISNN 2167-034x.
20. Kim, J., Yang, J., Kwak, H., Kim, M., Seo, J., Chung, M., Lee, H., Lee, K.B., Nam, S.K., Kim, C.S., Lee, G.S., Kim, J.S., Lee, S., Choi, H.S., 2017. Virus Incidence of sweetpotato in Korea from 2011 to 2014. *Plant Pathology Journal* 33 (5), 467-477.
21. Motsa, N.M., Modi, A.T., Mabhaudhi, T., 2015. Sweetpotato response to low-input agriculture and varying environments of KwaZulu-Natal, South Africa: implications for food security strategies. *Acta Agriculturae Scandinavica, Section B- Soil & Plant Science*, 65 (4), 329-340. DOI: 10.1080/09064710.2015.1011222.
22. Mukhopadhyay, S.K., Chattopadhyay, A., Chakraborty, I., Bhattacharya, I., 2011. Crops that feed the world 5. Sweetpotato. Sweetpotatoes for income and food security. *Food Security* 3, 283-305. DOI 10.1007/s12571-011-0134-3.
23. Mulabisana, M.J., Cloete, M., Mabasa, K.G., Laurie, S.M., Oelofse, D., Esterhuizen, L.L., Rey, M.E.C., 2018. Surveys in the Gauteng, Limpopo and Mpumalanga Provinces of South

Africa reveal novel isolates of sweetpotato viruses. *South African Journal of Botany* 114, 280-294.

24. Ndunguru, J., Aloyce, R.C., 2000. Incidence of sweetpotato virus disease in sweetpotato grown under different cropping systems in the Lake Victoria zone of Tanzania. *African Potato Association Conference Proceedings* 5, 405-408.

25. Ndunguru, J., Kapinga, R., Sseruwagi P., Bulili, S., Mwanga, R., Tumwegamire, T., Rugutu, C., 2009. Assessing the sweetpotato virus disease and its associated vectors in Northwest Tanzania and Central Uganda. *African Journal of Agricultural Research* 4 (4), 334-343.

26. Ngeve, J.M., Bouwkamp, J.C., 1991. Effects of sweetpotato virus disease (SPVD) on the yield of sweetpotato genotypes in Cameroon. *Explanatory Agriculture* 27, 221-225.

27. SAS Institute, Inc., 1999. *SAS/STAT User's Guide, Version 9, 3, Volume 2*. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513. *Elementary Statistics for Business and Economics*. In Lombaard, C., van der Merwe, L., Kele, T., Mouton, S. (Eds.), Pearson Education South-Africa (Pty) 2011, pp 352.

28. Snedecor, G.W., Cochran, W.G., 1980. *Statistical Methods, Sixth Edition*. The Iowa State University Press, AMES, IOWA, USA. Chapter 4, pp. 11-12.

29. Ssremba, G., Mukasa, S., Kyamanywa, S., 2017. Effect of temperature on sweetpotato virus disease symptom expression. *African Journal of Agricultural Research* 12 (27), 2295-2309.

30. Tairo, F, Mukasa, S.B., Jones, R.C.J., Kullaya, A., Rhubaihayo, P.R., Valkonen, J.P., 2005. Unravelling the genetic diversity of the three main viruses involved in sweetpotato virus disease (SPVD), and its practical amplifications. *Molecular Plant Pathology* 6 (2), 199-211.

31. Tewodros, T., Tileye, F., Adane, A., 2011. Survey and serological detection of sweetpotato (*Ipomoea batatas* (L.) Lam.) viruses in Ethiopia. *Journal of Applied Biosciences* 41, 2746-2756. ISSN 1997-5902.
32. Valverde, R.A., Clark, C.A., Valkonen, J.P.T., 2007. Viruses and virus disease complexes of sweetpotato. *Plant Viruses* 1 (1), 116-126.
33. Wasswa, P., Otto, B., Maruthi, M.N., Mukasa, S.B., Monger, W., Gibson, R.W., 2011. First identification of sweetpotato *Begomovirus* (sweetpotato virus) in Uganda: characterization, detection and distribution. *Plant Pathology* Doi 10.1111/1365-3059.2011.02464.x.

CHAPTER 4

YIELD EVALUATION OF BEGOMOVIRUS AND POTYVIRUS MIXED INFECTIONS ON SWEETPOTATO VARIETIES UNDER FIELD CONDITIONS

M.J. Mulabisana^{a,*}, M. Cloete^a, S.M. Laurie^a, W. Mphela^a, M.M. Maserumule^a, T.F. Nhlapo^b, N.M. Cochrane^c, D. Oelofse^a, M. E.C. Rey^d

Yield evaluation of begomovirus and potyvirus mixed infections on sweetpotato varieties under field conditions. Manuscript submitted to Crop Protection Journal.

^aAgricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP), Private Bag X293, Roodeplaat, Pretoria, 0001, South Africa, ^bAgricultural Research Council (ARC)-Biotechnology Platform, Private Bag X5, Onderstepoort 0110, South Africa; ^cAgricultural Research Council, Biometry Services, P.O Box 8783, Pretoria, 0001, South Africa; ^dUniversity of the Witwatersrand, School of Molecular and Cell biology, Private Bag X 3, P.O. Box Wits, 2050, Johannesburg, South Africa

*corresponding author: *E-mail address:* mjmulabisana@arc.agric.za; Tel: +27-12-808-8000.

Author contributions: Ms M.J Mulabisana conducted all experimental work and wrote the manuscript draft. Dr S.M Laurie is a sweetpotato breeder who provided sweetpotato varieties to evaluate, provided guidance on the agronomic aspects of planting sweetpotato and assisted with the interpretation of the data. Ms W. Mphela is a technician who assisted with the laying out of the field trials and ensuring that all agronomic practices for sweetpotato production are adhered to. Ms T.F Nhlapo provided guidance with regard to NGS data analysis. Mrs N.M Cochrane is the Biometrician who designed the field trial layouts, analyzed and assisted with the interpretation of the field trial data. Dr. D. Oelofse provided guidance on the overall

progress of the project. Prof. M.E.C Rey and Dr. M. Cloete supervised the study and assisted in data analysis, manuscript editing, interpretation, and provided guidance throughout the study.

4.1 Abstract

Potviruses and begomoviruses are regarded as the most common viruses affecting sweetpotato production in South Africa. The impact of single and mixed virus infections on the yield of sweetpotato varieties have not been established in South Africa. The objective of this study was to determine the effects of potviruses and begomoviruses on sweetpotato total and marketable yields. Twelve sweetpotato varieties; 8 locally bred and four imported, were selected for evaluations and were graft-inoculated with different virus combinations: 1) co-infections of the russet crack (RC) strain of Sweet potato feathery mottle virus (SPFMV) + Sweet potato virus G (SPVG) + Sweet potato virus C (SPVC) + begomoviruses [Sweet potato leaf curl Sao Paulo virus (SPLCSPV) + Sweet potato mosaic virus (SPMV)] [(treatment (T) 1)], 2) co-infections of SPFMV (RC strain) + begomoviruses (T2), 3) single infection of the RC strain of SPFMV (T3); and 4) co-infection of the begomoviruses SPLCSPV and SPMV (T4). Next generation sequencing (NGS), reverse transcription polymerase chain reaction (RT-PCR) or PCR confirmed the identification of the viruses in sweetpotato plants used as sources of virus treatments. Two consecutive field trials were planted in January 2014 and 2015. Varieties produced significantly reduced total [(F11, 236 = 5.17); $P < 0.0001$]) and marketable [(F11, 236 = 3.65); $P < 0.0001$]) yield with all the treatments in both the trials. T1 reduced the total yield by between 66% and 93% in trial 1, and by between 66% and 99.5% in trial 2. The loss in marketable yield ranged between 67% and 98% in trial 1, and between 58% and 100% in trial 2 with T1. T2 also led to reduced average total yield of varieties by between 28% and

94% in trial 1 and the total yield was severely reduced by between 74% and 99% in trial 2. Similar trends were recorded for the marketable yield of varieties treated with T2. The total yield reduced with T3 was between 27% and 78% in trial 1 and varieties such as Bophelo, Hernandez, Impilo and Ribbok had yield reduction of less than 50% when compared to the other eight varieties. However, the total yield was severely reduced in trial 2 (61% to 99%). Similar trends were also observed with marketable yield of varieties treated with T3. Total average yield was also reduced with begomovirus co-infections (T4) and yield loss was between 32% and 84% in trial 1 and varieties such as Bophelo, Bosbok, Mvuvhelo, Ndou and Resisto had yield reduction of less than 50% when compared to the other seven varieties. Total yield was severely reduced in trial 2 with T4 and only varieties 199062.1 and Hernandez had yield reduction of less than 50%. Marketable yield was also reduced with T4 and only varieties Bophelo, Bosbok, Mvuvhelo and Ndou had yield reduction of less than 50% in trial 1. The results demonstrate that single or co-infections of potyviruses and begomoviruses have variable negative effects on the yield of sweetpotato varieties.

Key words

Potviruses, begomoviruses, single and co-infections of viruses, total and marketable yields, Next-generation sequencing (NGS)

4.2. Introduction

Sweetpotato (*Ipomoea batatas* L.) is grown by both smallholder and commercial farmers in seven provinces of South Africa (Department of Agriculture, Forestry and Fisheries (DAFF), 2016), and it is one of the traditional crops that play an important role in addressing food insecurity in rural households of South Africa (Bester and Louw, 1992) and other sub-Saharan

African (SSA) countries (Grüneberg et al., 2015). Cream and orange-fleshed sweetpotato (OFSP) are grown for both home consumption and commercial production (Laurie et al., 2013). However, due to their richness in β -carotene, a source of pro-vitamin A, OFSP varieties are currently used in food and dietary initiative programs to address vitamin A deficiency, a global challenge in SSA (Kurabachew, 2015). Total yields (17%) and marketable yield losses (30%) were recorded over two seasons due to co-infections of potyviruses (Domola et al., 2008). Marketable yield is considered to be the storage roots that are between 100-300g (small) and 1kg (large), which are of good quality according to the standards sets out at local markets (DAFF, 2011; van den Berg et al., 2004). Unmarketable storage roots are those that are undersized (less than 100g), oversized (more than 1kg), cracked, damaged by rodents, and those that consist of defects such as thick veins, greening and sprouting (DAFF, 2011; van den Berg et al., 2004).

Sweetpotato production is constrained by sweetpotato viral diseases globally, and drastic yield losses have been reported in the Eastern and Western Africa due to synergistic co-infections of crinivirus Sweet potato chlorotic stunt virus (SPCSV), and potyvirus, SPFMV (Gibson et al., 1998). Single and co-infections of viruses such as SPFMV, SPVC, Sweet potato mild mottle virus (SPMMV), Sweet potato virus 2 (SPV2) and SPVG have previously been reported to affect sweetpotato production in South Africa (Domola et al., 2008; Sivparsad and Gubba, 2012). Potyviruses belong to the *Potyviridae* family and are transmitted by green peach aphids (*Myzus persicae* Sulzer) and cotton aphids (*Aphis gossypii* Glover) (Clark and Valverde, 2013). Sweepoviruses such as SPLCSPV and SPMV, now re-assigned to the genus *Begomovirus* (Brown et al., 2015), have been reported to occur in the Limpopo Province of South Africa and recently in Gauteng and Mpumalanga provinces (Esterhuizen et al., 2012; Mulabisana et al., 2018). Begomoviruses belong to the *Geminiviridae* family and are transmitted by whitefly species *Bemisia tabaci* Genn. (Lozano et al., 2009).

Different detection methods are available to identify sweetpotato viruses, amongst which are nitrocellulose based enzyme linked immunosorbent assay (NCM-ELISA), double antibody sandwich ELISA (DAS-ELISA) and nucleic acid methods such as the reverse transcription polymerase chain reaction (RT-PCR), PCR, cloning and partial virus nucleic acid sequencing (Li et al., 2004; Kokkinos & Clark, 2006; Rännäli et al., 2008; 2009). Full-length virus genome sequencing with high-throughput NGS provides more accurate identification and additional characterization by providing large sequence data which allows generation of full-length RNA virus genomes (Patowary et al., 2012; Nhlapo et al., 2018). The first objective of the study was to confirm the identity of the viruses in sweetpotato used as sources of viruses in yield trials. Secondly, we aimed to determine the impact of begomoviruses (SPLCSPV and SPMV) and potyviruses (SPFMV-RC strain, SPVG and SPVC) in various combinations, on the total and marketable yields of twelve imported or South African sweetpotato varieties in consecutive season field trials.

4.3. Material and methods

4.3.1 Confirmation of viruses in source sweetpotato material by NCM-ELISA, RT-PCR and PCR

Viruses present in source sweetpotato material, to be used in field trials, were identified by grafting onto indicator plant *Ipomoea setosa* (*I. setosa*), and analysed by NCM-ELISA (Gibb and Padovan, 1993) six weeks post graft inoculation. Polyclonal antibodies [(NCM-ELISA kit supplied by International Potato Center (CIP, Lima, Peru)], were used to confirm identity of SPFMV, SPVG, SPMV, SPCSV, Sweet potato chlorotic fleck virus (SPCFV), Sweet potato latent virus (SwPLV), Sweet potato mild speckling virus (SPMSV), Sweet potato caulimo virus (SPCaLV), Sweet potato collusive virus (SPCV) and Cucumber mosaic virus (CMV). DAS-

ELISA was used to detect SPV2 [(polyclonal antibody supplied by DSMZ GmbH Sammlung Pflanzenviren, Inhoffenstraße 7 B, D-38124 Braunschweig, Germany)]. RT-PCR, using forward primers FMV1360-F (Tairo et al., 2006) and reverse primer 10820-R (Tairo et al., 2005), were used to detect the RC strain of SPFMV (Table 4.1). Forward primer SPVC474-F (Tairo et al., 2006) paired with reverse primer 10820-R (Tairo et al., 2005) were used to detect SPVC (Table 4.1). SPVG was detected using forward primer PMB-9 and reverse primer PMB-10 (Rännäli et al., 2008). The presence of begomoviruses was confirmed in PCR reactions using primers SPG1 and SPG2 (Li et al., 2004) (Table 4.1). Total RNA and DNA were extracted using RNEASY® and DNEASY® Plant mini-extraction kits (QIAGEN) as per manufacturer's instructions. PCR reactions were performed using the KAPA® Taq Ready Mix DNA polymerase (KAPA BIOSYSTEMS) containing Mg⁺. Taq Ready Mix (10 µl) was mixed with 10 µM of each primer and 2-5 µl of the total cDNA in a total reaction volume of 20 µl. The cycling conditions for SPFMV, SPVC, SPVG and begomoviruses were performed as described by Rännäli et al. (2008; 2009) and Li et al. (2004).

Table 4.1: Primers (primers sequences) used for the PCR amplification of the RC strain of SPFMV, SPVC, SPVG and SPLCV.

Primer names	Primer sequences	Fragment size (bp)	Virus detected	References
FMV1360-Forward	5'-GACCAAGCCCCATACAATGA-3'	1356	SPFMV-RC strain	Tairo et al., 2006
10820-Reverse	5'-GGCTCGATCACGAACCAAAA-3'			
SPVC474-Forward	5'-CAGTGAAGTAGCACGAAC-3'	1335	SPVC	Tairo et al., 2006
10820-Reverse	5'-GGCTCGATCACGAACCAAAA-3'			Tairo et al., 2005
PMB-9-Forward	GGAAGTATCAGAGTATGCACGAG-3'	719	SPVG	Rännäli et al., 2008
PMB-10 Reverse	5'-GTTCCATTCTCGATGCAC-3'			
SPG1_Foward	5'-CCCCCKGTGCGWRAATCCAT-3'	912	SPLCV	Li et al., 2004
SPG2_Reverse	5'-ATCCVAAYWTYCAGGGAGCTAA-3'			

4.3.2 Confirmation of virus co-infection by NGS

Mixed infections of potyviruses (RC strain of SPFMV, SPVC and SPVG) were confirmed by NGS in sample SSBles collected from Roodeplaat in Gauteng Province. Total RNA was extracted as described in 4.2.1 and treated with the Ribo-Zero™ Magnetic Kit (Plant Leaf, Epicentre) to remove the cytoplasmic ribosomal RNA (rRNA). The generated library DNA was subsequently sequenced using Illumina MiSeq Benchtop Sequencer (Illumina Inc., San Diego, CA, USA). The full genome data was analysed using CLC Genomic Workbench 9 (<https://www.qiagenbioinformatics.com/products/clc-genomics-workbench>). Generated reads were subsequently mapped to known reference sequences obtained from the National Centre for Biotechnology Information (NCBI) GenBank database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) at a stringency of between 0.95 and 0.99 to generate the consensus sequences. Trimmed reads were subjected to *de novo* assembly to generate contig sequences, which also, were mapped (nBLAST) to closely related sequences in NCBI GenBank. Clustal W program in CLC Genomic Workbench 9 was used to align (multiple sequence alignment) consensus sequences with closely related references (NCBI GenBank). Sequences were trimmed at both ends to remove excess unaligned sequences. Subsequently, phylogenetic analysis was conducted in MEGA6 (Tamura et al., 2013) using Maximum Likelihood (1000 bootstraps replicates). Pairwise identity matrices were conducted to confirm species and strain identity using Clustal W in CLC Genomic Workbench 9. The species demarcation criteria for potyviruses were based on the cut-off point of 76% to 77% nucleotide (nt) identity for same species and 83% to 99% for strains (Adams et al., 2005; Frenkel et al., 1989). An amino acid (aa) similarity of 90% to 99% (average 95%) for the coat protein (CP) was applied to demarcate strains (Shukla and Ward, 1989). NGS was also used to confirm the presence of begomoviruses SPLCSPV and SPMV in sample SSBles (Mulabisana et al., 2018).

4.3.3 Field trials to determine the effects of begomoviruses and potyviruses on the yield of sweetpotato varieties

4.3.3.1 Inoculation of varieties with virus treatments and confirmation of infection

Field trials were planted with 8 locally bred sweetpotato varieties (Bophelo, Impilo, Ndou, Monate, Mvuvhelo, Blesbok, Bosbok and Ribbok), 3 from the United States of America (USA) (W-119, Hernandez and Resisto) and one (199062.1) from the International Potato Centre (CIP, Lima, Peru) (Tumwegamire et al., 2014). Virus treatments (T) were as follows: T1= mixed infections of SPFMV (RC strain) + SPVG + SPVC + begomoviruses (SPLCSV + SPMV), T2 = mixed infections of SPFMV (RC strain) + begomoviruses, T3=single infection of SPFMV (RC strain), T4= co-infection of SPLCSV + SPMV. Treatments will be referred to as SPFMV-RC*SPVC*SPVG*BVs (T1), SPFMV-RC*BVs (T2), SPFMV-RC (T3) and SPLCSV+ SPMV (T4). Sample SSBles collected from Gauteng Province was used as a source of inoculum for T1. Varieties were graft-infected onto *I. setosa* plants harboring the different virus combinations (T1 to T4). T5 was the 12 uninfected/ virus-free sweetpotato varieties that were used as controls, and these were obtained from the ARC-Vegetable and Ornamental Plants (ARC-VOP) (Pretoria, South Africa) sweetpotato scheme. Plants were maintained in an insect proof glasshouse with a temperature of 25°C to 28°C for 16 h (day cycle) and 15°C for 8 h (night cycle). Twelve weeks post-graft inoculation, leaf samples were collected and samples were tested with RT-PCR and PCR as described in section 2.1 to confirm infection with all virus treatments. Sixteen weeks post-inoculation, plants were multiplied in seedling trays to generate enough planting material for the field trials. Virus infected (T1-T4) varieties were retained and maintained in the insect proof glasshouse and were used as planting material for the second trial in the following season.

4.3.3.2 Experimental design of field trials planted to determine the effects of potyviruses and begomoviruses on the total and marketable yields of 12 varieties

Twelve months post graft-inoculation and multiplication of plants, the first field trial (January 2014) was established, and 24 months post graft-inoculation, the second trial (January 2015) was planted at ARC-VOP (25.6014° S, 28.3603° E). The experimental design was a randomized complete block with 3 replicates. Cuttings of 300 mm long were planted on ridges (1 m apart) of 300 mm height and 300 mm wide at a spacing of 30 cm between plants. Plots consisted of five plants per variety for both treated and control plants and two border rows were planted to act as barriers to prevent or reduce aphids and whiteflies infesting the experimental plants. The trials received recommended supplementary fertilisation [(500 kg/ha of NPK (1:0:1) and 600 kg/ha of Calsiphos, which contains Phosphorus)] a day before planting and top dressing [(183 kg/ha of LAN and 200 kg/ha of potassium chloride, respectively)] 16 days and 21 days post planting (recommendation from soil analyses provided by the ARC-Institute for Soil, Climate and Water, South Africa). Plants received 634 mm of water (irrigation) for the period of 5 months. Synthetic chemicals such Silicron (Profenofos) were applied using a Boom sprayer, at a dose of 500g /litre once a month for the first three months to control aphids and whiteflies. Plants had 5- months growing period, and thereafter were harvested in June 2014 and 2015. Symptoms induced by the sweetpotato varieties infected with different virus treatments were observed and recorded. Data on the total and marketable yields (tones/ha⁻¹) were recorded. The storage roots produced by each variety per treatment, in each replicate block, were dug out with forks and all roots were weighed (fresh weight) with a digital floor platform scale (JADEVER JWI-700S) as the total yield (tons/ha⁻¹) produced by each variety. Thereafter, storage roots were sorted and graded according to marketable and unmarketable yield. Storage roots considered marketable were between 100g to 300g (small), 301g to 600g (medium) and 601 to 1000g (large), with no defects and these were weighed separately, per

each variety per treatment and yields of individual varieties were combined to get the average of the 12 varieties. Data was analysed using SAS 9.4 statistical software (SAS/ STAS 14.1 Userguide). A combined analysis of variance (ANOVA) (John and Quenouille, 1977) over the two trials (2014 and 2015) was performed after testing for homogeneity of the trial variances (Leverne's test at $p > 0.01$) (Leverne, 1960), which indicated that the F probability (Fprob) for the total and marketable yield (ton/ha^{-1}) were 0.031 and 0.1009 ($p > 0.01$), respectively. The standardized residuals were acceptable, exhibiting a normal distribution (Shapiro-Wilks test) (Shapiro and Wilk, 1965), and therefore the means of the significant effects derived from 12 varieties were separated using Fisher's Unprotected t-test least significant difference (LSD) at the 5% level of significance (Snedecor & Cochran, 1967). Total and marketable yields were further categorized according to low (0 to 9 ton/ha^{-1}), moderate (10 to 20 ton/ha^{-1}) and high (20 to 30 ton/ha^{-1}) yields. The percentage (%) reductions in total and marketable yields, due to different virus treatments, were determined as follows:

$$\text{Reduction (\%)} = \frac{\text{Mean uninfected control (T5)} - \text{Mean Virus Treatment}}{\text{Mean uninfected control (T5)}} \times \frac{100}{1}$$

4.4. Results and discussion

4.4.1. Confirmation of virus combinations and identities in sweetpotato source material (used for inoculations in the field trials)

NCM-ELISA and DAS-ELISA detected single and mixed infections of three potyviruses in sweetpotato plants, used for inoculations in this study, collected from Limpopo Province of South Africa during the 2012/13 and 2013/14 surveys (Mulabisana et al., 2018). Virus identities were further confirmed by RT-PCR and PCR. RT-PCR, PCR and NGS also confirmed the presence of mixed infections of potyviruses, SPFMV (RC strain), SPVC, SPVG together with begomoviruses SPLCSPV (MF359263) and SPMV (MF359268) in sample

SSBles selected as a source of virus mixed infections (RC*SPVC*SPVG*BVs) (Mulabisana et al., 2018). SPV2 was not successfully detected by DAS-ELISA in sweetpotato variety SSBles initially selected for this study as a source of virus mixed infections (RC*SPVC*SPVG*BVs).

A total of 31 260 contigs were generated with NGS data and contigs 125, 72, 74, and 111 (>10 000 bp) were selected and designated SPVG isolate SSBles-125_ZA (10781 bp), SPVC isolate SSBles-72_ZA (10793 bp), SPV2 isolate SSBles-74_ZA (10731 bp) and SPFMV isolate SSBles-111_ZA (10803 bp) based on the BLAST (NCBI) results (Table 4.2).

Table 4.2: Near full-length genome sequence isolates of SPFMV, SPVC, SPVG and SPV2 isolated from South African sweetpotato variety SSBles.

Nucleotides Accession no.	Isolates names	Viruses and strains	Origin
MH023308	SPFMV isolate SSBles-111_ZA	SPFMV	Roodeplaat, South Africa
MH023309	SPVC isolate SSBles-72_ZA	SPVC	Roodeplaat, South Africa
MH023310	SPVG isolate SSB1-125_ZA	SPVG	Roodeplaat, South Africa
MH023311	SPV2 isolate SSBles-74_ZA	SPV2	Roodeplaat, South Africa

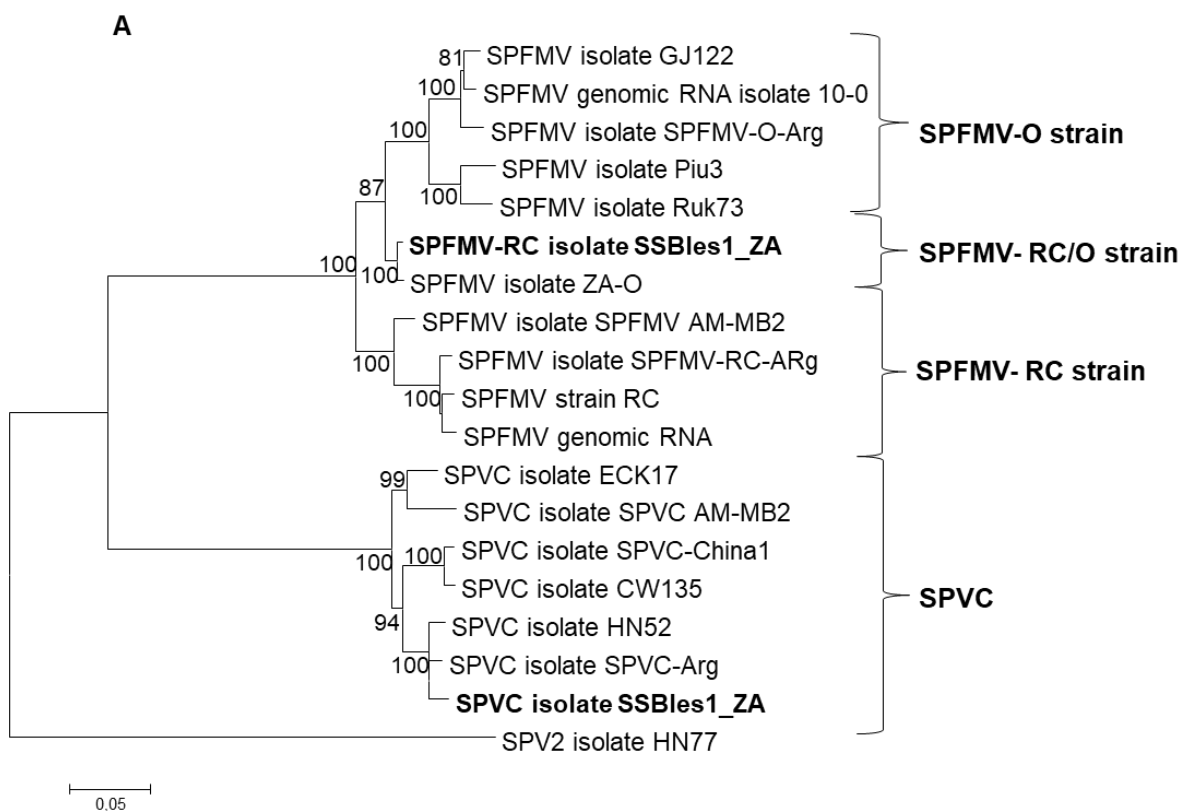
Phylogenetic analyses demonstrated that SPFMV isolate SSBles-111_ZA was closely related to the South Africa SPFMV isolate ZA-O (KT069222) (Nhlapo et al., 2018), and the two clustered in sub-clade, away from isolates reported in other countries (Fig. 4.1A). However, SPFMV isolate SSBles-111_ZA shared 99% nucleotide (nt) identity with the previously sequenced South Africa SPFMV isolate ZA-O (KT069222) and 92% to 93% with other RC strains of SPFMV (SPFMV AM-MB2, SPFMV-RC-Arg, SPFMV RC strain and SPFMV genomic RNA) (Bejerman et al., 2014, unpublished; Mingot et al., 2016; Qin et al., 2016,

unpublished; Sakai et al., 1997) (Table 12A, Appendices). This specific isolate was also confirmed to be the RC strain of SPFMV by cloning of the CP gene region and DNA sequencing (Mulabisana et al., 2018), therefore, the results indicate that the South African RC or O strain of SPFMV are closely related and distinct from other strains reported in other countries. SPVC isolate SSBles-72_ZA identified from this study grouped closely with the SPVC-Arg (KF386015) and SPVC isolate HN52 (KP115621) of Argentina and Korea (Bejerman et al., 2014, unpublished; Kwak et al., 2015), respectively (Fig. 4.1A). SPVC isolate SSBles-72_ZA shared 97% nt identity with SPVC-Arg and SPVC isolate HN52, and 93% with other isolates of SPVC, including the South African ECK17 (KT069223) isolate (Nhlapo et al., 2018) (Table 12A, Appendices).

SPVG isolate SSBles-125_ZA grouped within the SPVG clade with 100% bootstrap support and shared 83% nt identity with SPVG isolates GWB-G (JN613805) (Li et al., 2012), IS103 (KM014815) (Kwak et al., 2015) and Jesus Maria (JQ824374) (Rodriguez Pardina et al., 2012) used as reference sequences (Fig. 4.1B). However, the nt sequence identity of SPVG isolate SSBles-125_ZA in this study shared only 78% nt similarity with WT325 (KF790759) reported in Taiwan (Wang et al., 2014, unpublished) (Table 12B, Appendices). The nt identity between SPVG isolate SSBles-125_ZA with the South African SPVG isolate WCFR11 (KT069224) (Nhlapo et al., 2018) was also 82.4%, indicating the diversity of 17.6% between the two isolates (Table 12B, Appendices). Comparison at CP level showed that SPVG isolate SSBles-125_ZA of this study shared 97% to 97.5% amino acid (aa) homology with SPVG isolates GWB-G, IS103, Jesus Maria and the South African WCFR11 isolate (Table 13A, Appendices). The SPVG isolate of this study also shared 94.4% CP aa homology with WT325 reported in Taiwan (Table 13A, Appendices). According to the CP aa homology cut-off point of 90% to 99% for potyviruses (Shukla and Ward, 1989), the isolates were considered as the same strains of SPVG.

However, while SPV2 was not successfully detected by DAS-ELISA in sweetpotato variety SSBles initially selected for this study as a source of virus co-infections (RC*SPVC*SPVG*BVs), subsequently it was detected by NGS. Phylogenetic analysis showed that the clade of SPV2 isolates, including SPV2 isolate SSBles-74_ZA formed a separate branch (100% bootstrap support) from the other reference sequences (Fig. 4.1B). SPV2 isolate SSBles-74_ZA shared only 80% to 81% nt identity with reference sequences (Table 12B, Appendices), however, comparison of the CP encoding aa sequences of this isolate with reference sequences SPV2 isolate GWB-2 (JN613807) (Li et al., 2012), SPV2 isolate TM37 (KX017447) (Maina et al., 2016) and SPV2 isolate HN77 (KP115617) (Kwak et al., 2015) revealed that all these isolates share CP aa identity of 91% to 95% (Table 13B, Appendices). SPV2 isolate SSBles-74_ZA also shared CP aa homology of 100% with SPV2 isolate Thomas16A (AY459608) and SPV2 isolate TschilomboIV1-1 (AY459612) (Table 13B, Appendices) from South Africa (Ateka et al., 2007). Therefore, based on the demarcation criteria (the CP aa homology) of 90% to 99% of potyvirus species (Shukla and Ward, 1989), all these isolates are closely related as same strains of SPV2. Multiple sequence alignment of the CP gene encoding aa sequences of the RC strain of SPFMV and SPVC revealed the presence of the DAG aa triplets motif with SPFMV isolate SSBles-111_ZA and SPVC isolate SSBles-72_ZA at position 9 to 11. The DAG aa triplets motif was identified at position 8 to 10 with SPV2 and position 7 to 9 with SPVG (Table 14A and B, Appendices). The nt diversity observed with SPVG and SPV2 suggests that single nt variation occurred but no change in aa which suggests silent or non-synonymous mutations which did not affect the CP function, symptom phenotype or transmission (Edwards et al., 2012). This supports the notion that the CP is highly conserved in these viruses due to its involvement in transmission and insect recognition (Atreya et al., 1995; Mishra et al., 2013). Moreover, genetic diversity is common with RNA viruses, and is attributed to viral evolutionary processes that occur due to mutation-

selection balances, which are essential for their adaptation in host plants (Holmes, 2009). The inability to detect SPV2 in source of virus combinations and sweetpotato varieties selected for the experiments is attributed to mixed infections, diverse viral strains, uneven virus distribution within the plant, and the presence of SPFMV which masks the presence of other viruses (Valverde et al., 2007). This was also shown by Ateka et al. (2004) who failed to inoculate sweetpotato varieties by mechanical or graft inoculations unless varieties were pre-inoculated with the crinivirus SPCSV.



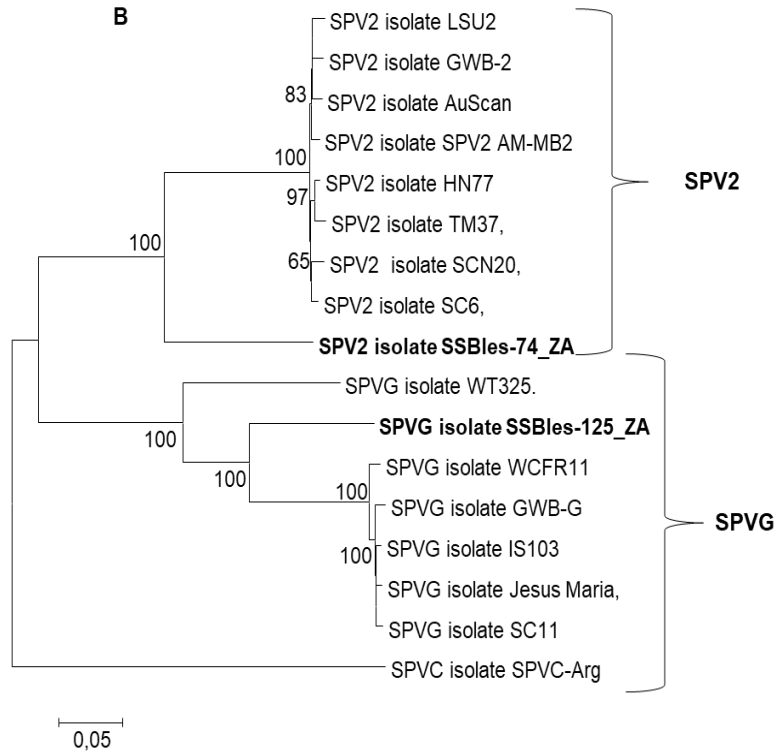


Fig. 4.1. Molecular phylogenetic analysis inferred using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000) to determine relationships between the South African (**bold**) (A) SPFMV (RC strain) and SPVC, and (B) SPVG and SPV2 with the related full-length genome sequences retrieved from Genbank. The bootstrap values (1000 replicates), with which the associated taxa clustered together are shown next to the branches. The isolates and Genbank accession numbers used as reference sequences are as follows: **SPFMV**: SPFMV isolate ZA-O (KT069222), SPFMV isolate GJ122 (KP115609), SPFMV isolate AM-MB2 (KU511268), SPFMV isolate RC (KY296451), SPFMV isolate Piu3 (FJ155666), SPFMV-O-Arg (KF386013), SPFMV genomic RNA (D86371), Ruk73 (KP729265), SPFMV-RC-Arg (KF386014), SPFMV isolate 10-0 (AB439206). **SPVC**: SPVC-Arg (KF386015), HN52 (KP115621), ECK17 (KT069223), SPVC-China1 (KU877879), CW135 (KP115620) and SPVC-AM-MB2 (KU511269). **SPVG**: SPVG isolate WCFR11 (KT069224), SPVG IS103 (KM014815), SPVG isolate Jesus Maria (JQ824374), SPVG isolate GWB-G (JN613805), SPVG isolate WT325 (KF790759) and SPVG isolate UMP3-VG (JQ073703). **SPV2**: **SPV2** isolate AM-MB2 (KU511270) SPV2 isolate LSU-2 (KP29268), SPV2 isolate GWB-2 (JN613807), SPV2 isolate HN77 (KP115617), SPV2 isolate TM37 (KX017447), SPV2 isolate SCN20 (KP115619), SPV2 isolate SC6 (KP115618), SPV2 isolate Thomas 16A (AY459608) and SPV2 isolate Tschilombo IV1-1 (AY459612).

4.4.2 The impact of virus treatments on total and marketable yields (tones ha⁻¹)

4.4.2.1 Symptoms assessment during field trials

Symptoms induced by the four different virus treatments varied, depending on the variety and virus combinations. Variety 199062.1, treated with SPFMV-RC*SPVC*SPVG*BV_s (T1) exhibited severe purple ring spots and mild upward curling of leaves (Fig. 4.2A). Symptoms observed on variety 199062.1 inoculated with SPFMV-RC*BV_s (T2) and SPFMV-RC (T3) were characterized by very mild chlorotic spots to no symptoms (Fig. 4.2B) and this variety did not exhibit any virus symptoms with T3 and T4 (Fig. 4.2C and D). Variety Impilo was sensitive to T1, T2 and T3, and symptoms such as chlorosis and vein banding (T1), mild to severe chlorotic spots with (T2) and (T3), and mild chlorotic spots to no symptoms (T4) were recorded (Fig. 4.2 E, F, G and H). Variety Blesbok was also sensitive to all 4 virus treatments and symptoms such as severe chlorosis and upward curling of leaves (T1), severe chlorosis (T2), chlorosis and purple ring spots (T3) and mild upward curling of leaves (T4) were recorded (Fig. 4.2 I, J, K and L).

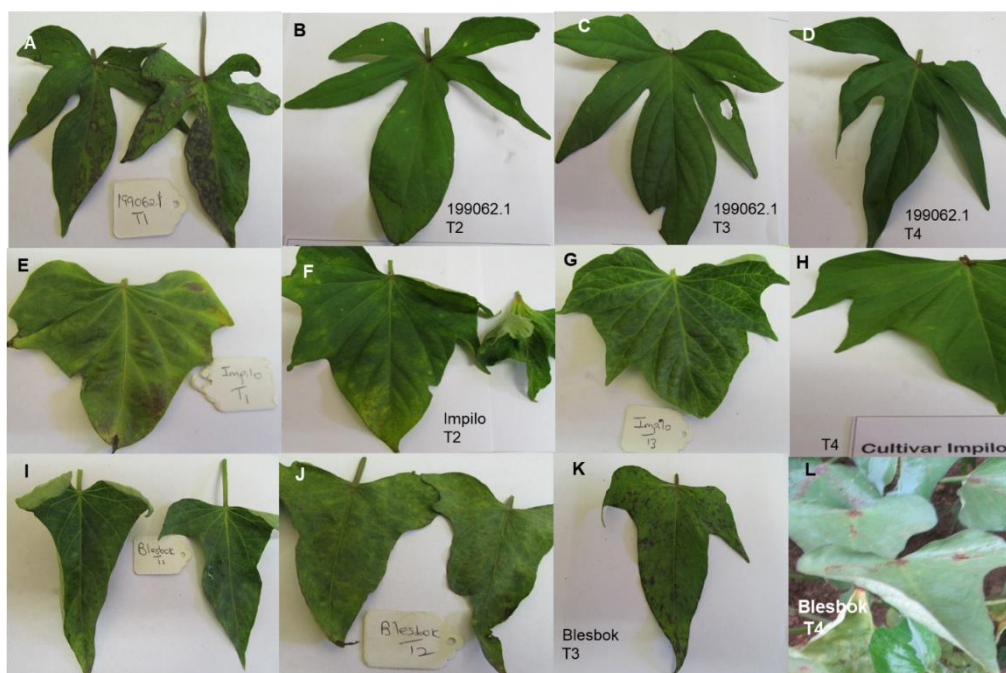


Fig. 4.2. Symptoms recorded on representative sweetpotato varieties in field trials (2014 trial) planted to determine the effects of SPFMV-RC*SPVC*SPVG*BV_s (T1), SPFMV-RC*BV_s (T2), SPFMV-RC (T3) and BV_s (T4) treatments on the yield of 12 sweetpotato varieties.

4.4.2.2 Total yield of 12 varieties subjected to different virus treatments

The results of the combined ANOVA and the mean values performed on the total yield data to determine the effects of the four virus treatments (T1 to T4) on the total of the 12 sweetpotato varieties and the uninfected control (T5) is shown in Table 4.3. Significant differences ($p < 0.05$) were observed between trial 1 and 2 [(F11, 236 = 3.65); $P = < 0.0001$]. Significant difference was also observed between the trial, variety and treatment interactions [(F11, 236 = 3.65); $P = 0.003$].

Results demonstrated that the 12 varieties responded differently towards the different virus treatments in relation to yield, in both trial 1 and trial 2. Although varieties differed in their responses to virus treatments, the yield of almost all varieties was affected by SPFMV-RC*SPVC*SPVG*BVs (T1) (Table 4.2) and the total yield recorded was between 1.46 tones (t) ha^{-1} (W-119) and 7.100 tha^{-1} (199062.1) in trial 1, and 0.200 tha^{-1} (Bophelo, Blesbok, Bosbok) and 6 tha^{-1} (Ribbok) in trial 2. Therefore, the percentage yield reduction was between 66% (Ribbok) and 93% (Blesbok) for the 12 varieties tested in trial 1 and between 66% (Ribbok) and 99% (Blesbok, Bophelo and Bosbok) in trial 2.

The yield produced by varieties treated with SPFMV-RC*BVs (T2) was between 0.533 tha^{-1} (W-119) and 10.167 tha^{-1} (Hernandez) in trial 1 (Table 4.2). The reduction in yield was between 28% (Bophelo and Hernandez) and 94% (W-119). Similar to trial 1, the total yield produced by varieties in trial 2 was reduced and the recorded yield was between 0.2 tha^{-1} (Hernandez) and 8 tha^{-1} (Blesbok) and the total yield loss was between 74% (Ribbok and Resisto) and 99 % (Hernandez) (Table 4.2).

Variety 199602-1 was identified as a high yielding variety, however, it was sensitive to virus infections, and most importantly virus mixed infections. Yield reduction was recorded in variety 199602-1 with all treatments regardless of the absence or presence of mild symptoms

(Fig. 4.2) observed during field assessment. Symptomless plants may therefore still harbour viruses and suffer yield loss, and additionally be able to infect other uninfected plants if mother plants were not tested before planting material were distributed. Moreover, although moderate yield was recorded with some varieties, the study revealed that mixed infections of potyviruses with begomoviruses (T1) and (T2) severely reduced the total yield of sensitive sweetpotato varieties. The results are in agreement with previous reports that showed that virus complexes of potyvirus SPFMV and crinivirus SPCSV reduce the yields by $\pm 70\%$ (Aritua et al., 1998; Gibson and Kreuze, 2015). Bryan et al. (2003) showed that viruses accumulate rapidly, in sweetpotato leading to variety decline, and virus complexes (SPFMV, SPVC, SPVG and SPV2) have the ability to affect the development of the adventitious roots by 51%, resulting in poor or lack of storage roots initiation (Villordon and Clark, 2014). Variety 199062.1 (Peru) and Impilo (South Africa) were reported to have moderate to high resistance to sweetpotato viruses (Tumwegamire et al., 2014). The reduction in yield by 50% and higher was recorded with 199062.1 (T1) and Impilo (T2) in this study, which is an indication that the two varieties are sensitive to mixed infections of potyvirus and begomoviruses. Resistance breakdown has been reported to occur, which is attributed to different virus strains or highly virulent viruses, and co-infections of viruses of different taxonomic group (Winter et al., 1992; Miano et al., 2008). Ngailo et al. (2013) also indicated that the resistant ability of varieties bred in different agro-ecological zones may be lost in different countries due to genetic differences in virus strains, or the environment. Varieties Resisto and W-119 were reported to have low resistance to viruses (Tumwegamire et al., 2014) and this is in agreement with our findings. Enhanced reduction in the yield due to co- and mixed infections of potyviruses and begomoviruses in sweetpotato production fields of South Africa could be attributed to the increased virus titer when begomoviruses are co-infected with SPFMV, since the *HC-Pro* (all potyviruses) and

P1N-PISPO gene of SPFMV acts as suppressors of host RNA silencing, a plant's defence against viral infection (Kokkinos, 2006; Mingot et al., 2016).

Treatment 3 was a single infection of the RC strain of SPFMV) led to reduction in total yield of all 12 varieties by between 27% (Bophelo) and 78% (199602_1) in trial 1 (Table 4.2). Varieties such as Blesbok (from 26 to 12.03 tha^{-1}), Impilo (21 to 13 tha^{-1}), Monate (26 to 10.63 tha^{-1}), Mvuvhelo (25 to 10.4 tha^{-1}) and Ribbok (17.6 to 11.5 tha^{-1}) were affected and these had a yield reduction of between 35% (Ribbok) and 58% (Mvuvhelo and Monate) in trial 1. Varieties such as 199062.1, Bosbok, Resisto, Ndou, Hernandez, Bophelo and W-119 produced severely reduced total yield of between 3 tha^{-1} (W-119) and 9.20 tha^{-1} (Bophelo) in trial 1 and the yield reduction of between 27% (Bophelo) and 75% (Bosbok) was recorded for these varieties. This treatment affected all varieties in trial 2 and the recorded yield was between 0.3 tha^{-1} (Bosbok) and 7.2 tha^{-1} (Mvuvhelo) and the yield reduction for all 12 varieties was between 61% (Hernandez) and 99% (Bosbok). The results obtained are in agreement with the findings by Gibson and Kreuze (2015) who showed that a single infection with SPFMV has the ability to reduce yield by 50% (Gibson and Kreuze, 2015). Single infection of SPCSV is also associated with the reduction of the lateral roots (LR) length, number and density, which also prevents the storage root from initiating (Villordon and Clark, 2014).

Begomoviruses treatment SPLCSV+SPMV (T4) led to reduction in total yields of all 12 varieties in trial 1 (Table 4.2). The total yield of varieties Impilo (from 21 to 9.4 tha^{-1}), 199062.1 (33 to 10.3 tha^{-1}), Bosbok (23 to 15 tha^{-1}), Monate (26 to 12 tha^{-1}), Mvuvhelo (25 to 17 tha^{-1}), Ndou (22 to 14 tha^{-1}) and Resisto (17 to 10.6 tha^{-1}) was reduced with T4 in trial 1. The reduction in total yield for these varieties was between 37% (Resisto and Ndou) and 69% (199062.1). The total yield of varieties Blesbok (26 to 6 tha^{-1}), Bophelo (12.6 to 6.8 tha^{-1}), Hernandez (14 to 6.6 tha^{-1}), W-119 (9.3 to 1.5 tha^{-1}) and Ribbok (17.6 to 7 tha^{-1}) was severely reduced by this treatment in trial 1 and the reduction in yield was between 46% (Bophelo) and

84% (W-119) for these varieties. In trial 2, varieties that produced moderate total yield with T4 were only 199062.1 (16.9 tha^{-1}) and Hernandez (10.4 tha^{-1}), which led to a yield reduction of 23% and 24%, respectively. All other varieties produced the total yield of between 2 tones ha^{-1} (Mvuvhelo) and 7.4 tha^{-1} (Monate) in trial 2, translating into a yield reduction of between 67% (Resisto) and 94% (Mvuvhelo) for all 12 varieties. Variety Hernandez is reported to be high yielding (Loader et al., 2000), however, in this study its total yield was not consistent with T4 since it produced low yield in trial 1 and moderate yield in trial 2. Nonetheless, it is clear that variety Hernandez is also sensitive to T4. The reduction in the yield by co-infection of begomoviruses is also in agreement with the findings by Clark and Hoy (2006), and Ling et al. (2010) who showed that begomovirus Sweet potato leaf curl virus (SPLCV) is capable of reducing the storage root yield by 26% to over 60%. This study indicated that single infections of SPFMV and multiple infections of begomoviruses have negative effects on the total yield of sweetpotato varieties, and the longer the period viruses are retained in the vegetative plants, the higher the yield reductions will be experienced.

Figure 4.3 shows the quantity and quality of the storage roots produced by variety Blesbok with multiple virus infection of potyviruses and begomoviruses. It was undoubtedly clear that variety Blesbok treated with T1, T2 and T3 produced fewer or thinly developed storage roots, which were also small in diameter as compared to those produced by the uninfected controls.



Fig. 4.3. The effects of SPFMV-RC*SPVC*SPVG*BV_s (T1), SPFMV-RC*BV_s (T2) and BV_s (T4) on the storage roots of variety Blesbok as recorded during trial 2 when field trials were planted to determine the effects of single, co- and multiple infections of potyviruses and begomoviruses treatments. (A) and (B) storage roots produced by variety Blesbok with T1 and T2, (C) storage roots produced by variety Blesbok treated with T4 and (F) storage roots produced by the uninfected controls (T5) of variety Blesbok.

Table 4.3: Total yield (TY) (tha⁻¹) in field trials of twelve sweetpotato varieties following the four virus treatments.

Trial 1	Varieties	TY_tmt1	% yield reduction	TY_tmt2	% yield reduction	TY_tmt3	% yield reduction	TY_tmt4	% yield reduction	TY_tmt5
	199602_1	7,100	78,5	9,167	72,2	7,200	78,16	10,267	68,9	32,967
	Blesbok	1,900	92,6	2,400	90,7	12,033	53,24	6,033	76,6	25,733
	Bophelo	2,000	84,1	9,033	28,3	9,200	26,98	6,767	46,3	12,600
	Bosbok	3,300	85,5	8,600	62,2	5,767	74,67	15,133	33,5	22,767
	Hernandez	1,800	87,2	10,167	27,7	8,967	36,26	6,567	53,3	14,067
	Impilo	6,500	69,2	10,6	49,8	12,767	39,59	9,400	55,5	21,133
	Monate	3,267	87,3	4,233	83,5	10,633	58,52	12,100	52,8	25,633
	Mvuvhelo	4,367	82,6	7,033	71,9	10,433	58,38	17,133	31,6	25,067
	Ndou	2,900	87,0	4,733	78,8	6,867	69,25	14,100	36,9	22,333
	Resisto	3,167	81,3	5,133	69,7	4,567	73,03	10,600	37,4	16,933
	Ribbok	6,000	65,9	7,900	55,1	11,467	34,85	6,933	60,6	17,600
	W-119	1,467	84,2	0,533	94,2	2,733	70,50	1,533	83,5	9,267
Trial 2	Varieties	TY_tmt1	% yield reduction	TY_tmt2	% yield reduction	TY_tmt3	% yield reduction	TY_tmt4	% yield reduction	TY_tmt5
	199602_1	0,800	96,4	1,567	92,9	4,933	77,6	16,867	23,3	22,000
	Blesbok	0,200	99,5	7,733	79,9	1,900	95,1	4,400	88,6	38,500
	Bophelo	0,233	99,1	2,267	90,8	1,767	92,8	3,033	87,7	24,567
	Bosbok	0,200	99,2	3,333	86,9	0,300	98,8	2,067	91,9	25,467
	Hernandez	1,233	90,9	0,200	98,5	5,267	61,3	10,400	23,5	13,600
	Impilo	0,967	96,6	1,167	95,8	1,733	93,8	6,500	76,8	28,033
	Monate	0,967	96,8	4,733	84,1	4,433	85,1	7,400	75,1	29,767
	Mvuvhelo	3,133	87,2	5,867	76,1	7,200	70,7	1,533	93,8	24,567
	Ndou	0,933	94,4	0,700	95,8	2,167	87,1	2,433	85,5	16,733
	Resisto	0,767	96,0	4,933	74,0	4,733	75,0	6,333	66,6	18,967
	Ribbok	5,833	66,4	4,533	73,9	3,733	78,5	2,933	83,1	17,367
	W-119	1,100	94,4	0,93	95,3	7,100	64,0	2,133	89,2	19,733
	Statistics for the combined trials					ANOVA				
	Mean: Trials combined 8,6	F Prob (model) <,0001	LSD (p=0.05) 7,4585	CV% 53,75	SEM 2.67	F Prob: Trial*Var*Tmt (tmt 5 included) 0,003			F Prob: Trial*Var*Tmt (tmt 5 removed) 0,001	

Tmt= treatments, CV= Coefficient of Variance, SEM=Standard error of means, LSD= least significant difference, var= varieties.

4.4.2.3 Marketable yieldsof 12 varieties subjected to different virus treatments

The marketable yield of almost all varieties were severely reduced by virus treatments SPFMV-RC*SPVC*SPVG*BVs (T1), SPFMV-RC*BVs (T2), SPFMV-RC (T3), and SPLCSV+SPMV (T4) (Table 15, Appendices) in both trial 1 and trial 2. Significant differences [(F11, 236 = 3.65); P <0.0001]] were observed between trial 1 and 2. Significant differences was also observed between the trial, variety and treatment interactions [(F11, 236 = 3.65); P =0.009]]. The marketable yield in trial 1 with T1 was between 0.2 tha⁻¹ (Bophelo) and 6.1 tha⁻¹ (199062.1). The yield loss for 12 varieties was between 67% (Ribbok) and 98% (Bophelo). There was an increase in the reduction of the marketable yield in trial 2 with T1 and the yield recorded was between 0 (Blesbok, Resisto, Bosbok, Bophelo and W-119) and 4.7 tha⁻¹ (Ribbok). The recorded loss for all 12 varieties was between 58% and 100%. In South Africa, Blesbok is a high yielding commercial variety, which is capable of producing the marketable yield of 48 tha⁻¹ (Laurie et al., 2015). The reduction in the marketable yield of variety Blesbok from 22.5 to 1.1 tha⁻¹ (95%) and 33.6 to 0 tha⁻¹ (100%) with T1 in trial 1 and 2, respectively, indicates that Blesbok is highly sensitive to virus complexes of potyviruses and begomoviruses. The effects of T2 was almost similar to that of T1 and the reduction in marketable yields was between 32% (Hernandez) and 100% (W-119) in trial 1, and the marketable yield was severely decreased in trial 2 and this was between 71% (Ribbok) and 100% (Hernandez, Impilo and W-119).

The marketable yield recorded with T3 was between 2.3 tha⁻¹ (W-119) and 11.6 tha⁻¹ (Impilo) in trial 1, and 0 (Bosbok) and 5.7 tha⁻¹ (W-119) in trial 2. The reduction in marketable yield of varieties Bophelo (24%), Impilo (34%), Ribbok (30%) and Hernandez (41%) was below 50% with T3, however, the reduction in yield of other eight varieties was between 53% (Blesbok) and 82% (199602_1) in trial 1. The marketable yield of all 12 varieties was low and the reduction in yield was between 62% (W-119) and 100% (Bosbok) in trial 2.

Mixed infections of begomoviruses (T4) also affected the marketable yield of different varieties and the yield of between 1.2 tha^{-1} (W-119) and 15.6 tha^{-1} (Mvuvhelo) in trial 1 was recorded, and the reduction in yield was between 29% (Mvuvhelo) and 86% (W-119). However, the reduction in marketable yield for varieties such as Mvuvhelo, Ndou, Bosbok and Resisto was less than 40% with T4. In trial 2, the marketable yield recorded was between 0 (Mvuvhelo and Ndou) and 14 ton ha^{-1} (199062.1). The reduction in the marketable yield of varieties 199062.1 and Hernandez was less than 40% and the reduction in yield of other 10 varieties was between 71% and 100% in trial 2. It is unclear why the reduction in marketable yield of variety 199062.1 was low with T4 in trial 2, however, we speculate that defects and mechanical damage affected the yield of the uninfected control (T5), which led to a reduced loss in the marketable yield of this variety.

4.4.2.4 Average total and marketable yields produced by 12 varieties treated with four virus treatments over two seasons

Varieties varied with regard to the total and marketable yield produced with virus treatments [SPFMV-RC*SPVC*SPVG*BVs (T1), SPFMV-RC*BVs (T2), SPFMV-RC (T3), and SPLCSV+SPMV (T4)] in both trial 1 and 2 (Fig. 4.4A and B). Varieties Ribbok, Monate, Bosbok, 199062.1, Impilo and Mvuvhelo produced low to moderate mean total yield of between 8 and 10 tha^{-1} in trial 1 and all other 6 varieties produced severely reduced total yields of between 1.6 tha^{-1} and 7 tha^{-1} . All 12 varieties produced low mean total yield of between 2.8 tha^{-1} (W-119) and 6 tha^{-1} (199062.1) in trial 2. The average marketable yield produced by varieties in trial 1 was low ($<10 \text{ tha}^{-1}$) and the marketable yield of all 12 varieties were severely reduced in trial 2. Variety W-119 was identified as highly sensitive to virus treatments in both trial 1 and 2.

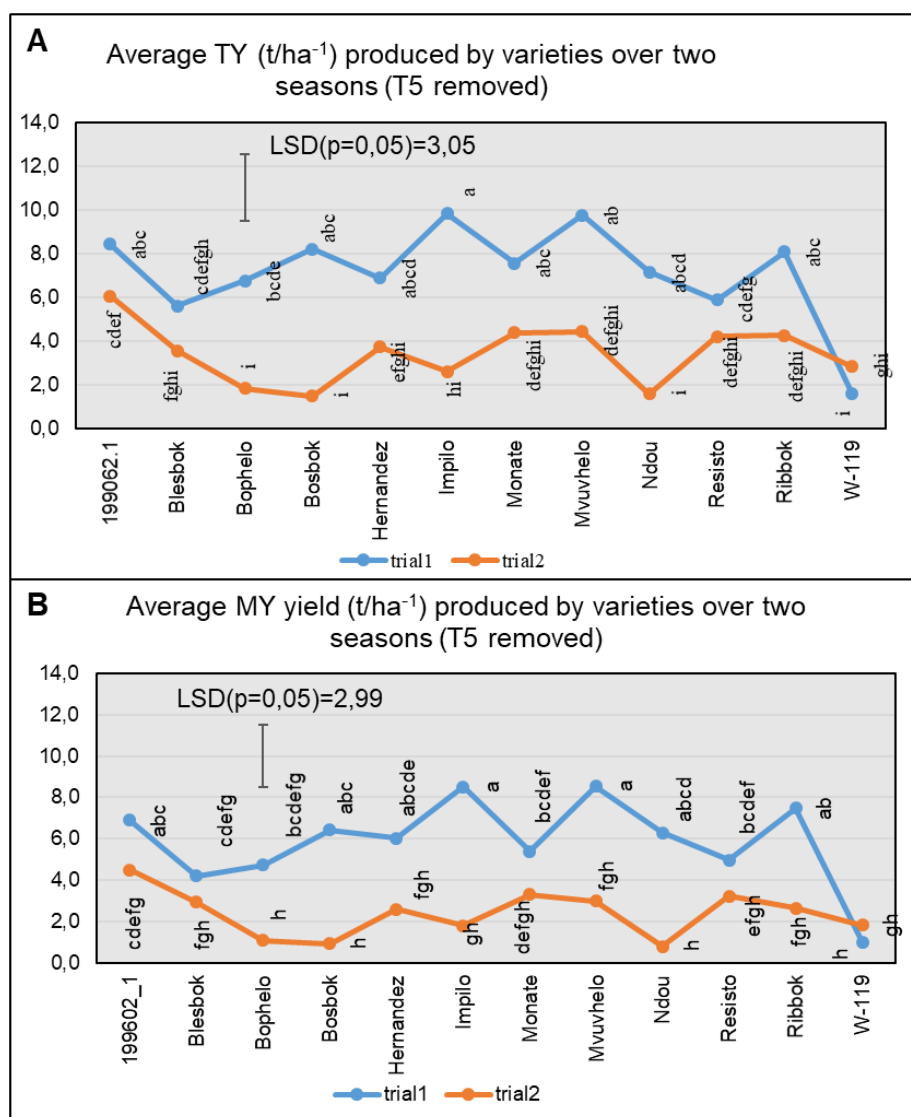


Fig. 4.4. Average total (A) and marketable (B) yields produced by 12 sweetpotato varieties when subjected to four virus treatments during two seasons. Means with the same letter are not significantly different ($P < 0.05$). TY= total yield and MY= marketable yield.

4.4.2.5 Average of the combined total yield (TY) (tha⁻¹) of 12 varieties infected with different virus combinations (treatments)

The average total yield (tha⁻¹) recorded with all four treatments (T1-4) were 3.6 tha⁻¹, 6.6 tones ha⁻¹, 8.6 tha⁻¹ and 9.7 tha⁻¹, respectively and the yield produced by the uninfected control was 20.5 tha⁻¹ in trial 1. This translated into the reduction in the total yield by 82%, 68%, 58% and 52% with T1, T2, T3 and T4, respectively (Fig. 4.5). Yield losses increased in trial 2 and the

average total yield recorded was 1.4 tha⁻¹ (T1), 3.2 tha⁻¹ (T2), 3.8 tha⁻¹ (T3), 5.4 tha⁻¹ (T4) and 23.5 tha⁻¹ (T5), also indicating the reduction in the average total yield by 94% (T1), 86% (T2), 84% (T3) and 77% (T4), respectively (Fig. 4.4). Significant differences were observed between T1 with T2, T3 and T4 due to low yield produced with T1 in trial 1, however, there were no significant differences between T2, T3 and T4. The average total yield of uninfected control varieties was significantly higher than the yield resulting from the four virus treatments.

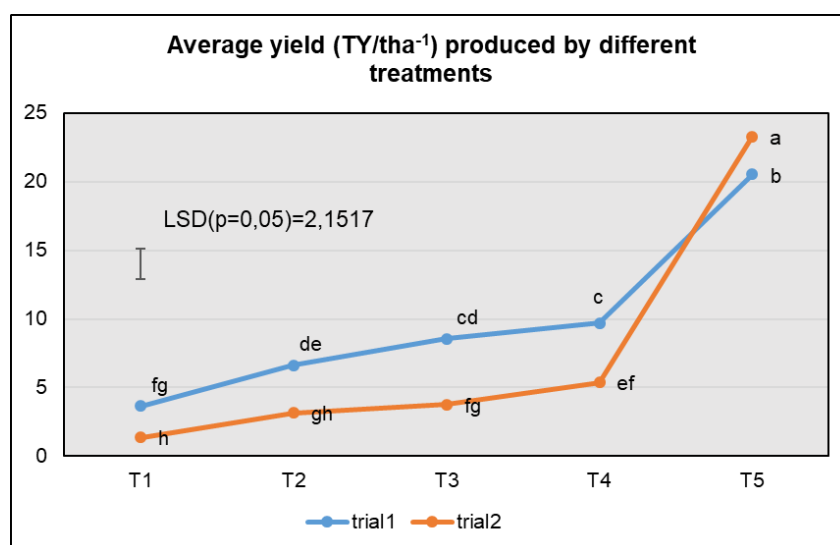


Fig. 4.5. The average of the combined total yield (TY) (ton ha⁻¹) of 12 varieties infected with SPFMV-RC*SPVC*SPVG*BVs (T1), SPFMV-RC*BVs (T2), SPFMV-RC (T3) and SPLCSV+SPMV (T4) compared to the uninfected control (T5). Means with the same letter are not significantly different ($P < 0.05$).

4.5 Conclusion

Sweetpotato viruses are systematic pathogens and are readily transferrable in planting material, which ultimately cause infection and high yield losses (Clark et al., 2012). Additionally, the transfer of sweetpotato vegetative material worldwide, despite diagnostic screening, has led to the distribution of many co-infecting viruses in vegetative material, including South Africa (Thresh and Cooter, 2005; Rey et al., 2012). Previous studies world-wide have concentrated

on SPVD as the most important disease of sweetpotato (Gutiérrez et al., 2003), but in this study, virus complexes of potyviruses and begomoviruses were widespread in South Africa, and were confirmed to have detrimental effects on the yield of sensitive sweetpotato. The total and marketable yield of all 12 varieties was severely reduced by a complex of potyviruses SPFMV (RC strain), SPVC and SPVG, in co-infection with begomoviruses. Only varieties such as Hernandez and Bophelo had a decrease in the total yield by less than 30% with co-infection of the RC strain of SPFMV and begomoviruses SPLCSV and SPMV. Furthermore, the field trials herein were designed to represent mixed or co-infections as reflected in several provinces in South Africa (Mulabisana et al, 2018), and results in this study demonstrate for the first time, the severe impact these viruses have, singly or in combination, on storage roots yields. This impacts small-scale and subsistence farmers, not only not only in South Africa but elsewhere in SSA, and solutions need to be implemented to reduce losses. It is also critical that future studies investigate the potential interaction of begomovirus and potyvirus expressed genes in moderating host defence responses, or to determine if synergy influences symptoms, virus titers and yield loss, as was reported with SPVD, a complex of potyvirus SPFMV and crinivirus SPCSV (Gutiérrez et al., 2003). Evaluating varieties for virus resistance appears to be the long-term solution towards addressing sweetpotato virus complexes. Laurie et al. (2013) evaluated sweetpotato landraces for drought and heat tolerance and three landraces were identified. This suggests that landraces may provide a gene pool to evaluate for other desired traits, including virus resistance. Landraces are believed to be exposed to different conditions, including disease pressures, and may contain natural genes associated with virus resistance (Nwankwo et al., 2016; Low et al., 2017). Mwanga et al. (2002) also showed that genetic resistance can be achieved with selection and breeding techniques, and this should also be explored in future.

4.6 Conflict of interest

Authors declare that there is no conflict of interests regarding the publication of this work.

4.7 Acknowledgements

The authors acknowledge the International Foundation for Science (IFS) (C/5312-1) and the National Research Foundation (NRF)-Thuthuka PhD track (Grant 99368) for funding the project. The late Mr Elias Thobejane, Ms Midana Mmboyi and Ms Kate Phetla are greatly acknowledged for their dedicated technical assistance throughout the study.

4.8 References

1. Adams, M.J., Antoniw, J.F., Fauquet, C.M., 2005. Molecular criteria for genus and species discrimination within the family Potyviridae. *Archives of Virology* 50 (3), 459-479.
2. Aritua, V., Alicai, T., Adipala, E., Carey, E.E., Gibson, R.W., 1998. Aspects of resistance to sweetpotato virus disease in weetpotato. *Annals of Applied Biology* 132, 387-398.
3. Ateka, E. M., Barg, E., Njeru, RW, Lesemann, D.-E., Vetten, H. J., 2004. Further characterization of 'Sweetpotato virus 2': a distinct species of the genus potyvirus. *Archives of Virology* 149, 225-239.
4. Ateka, E.M., Barg, E., Njeru, R.W., Thompson, G., Vetten, H.J., 2007. Biological and molecular variability among geographically diverse isolates of Sweet potato virus 2. *Archives of Virology* 152 (3), 479-488.
5. Atreya, P.L., Lopez-Moya, J.J., Chu, M., Atreya, C.D., Pirone, T.P., 1995. Mutational analysis of the coat protein N-terminal amino acids involved in potyvirus transmission by aphids. *Journal of General Virology* 76, 265-270.
6. Bester, C., Louw, J.H., 1992. Sweetpotato breeding in South Africa. *Journal of the South African Society of Horticulture* 2 (2), 96-98.
7. Bryan, A.D., Pesic-Van Esbroeck, Z., Schoeltheis, J.R., Pecota, K.V., Swallow, W.H., Yencho, G.C, 2003. Variety decline in sweetpotato: Impact of micro propagation,

- storage root quality and virus incidence on Beauregard. *Journal of American Society of Horticultural Science* 128 (6), 846-855.
8. Brown, J.K., Zerbini, F.M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva, J.C.F., Fiallo-Olive, E., Briddon, R.W., Hernández-Zepeda, C., Idris, A., Malathi, V.G., Martin, D.P., Rivera-Bustamante, R.R., Ueda, S., Varsani, A., 2015. Revision of Begomovirus taxonomy based on pairwise sequence comparison. *Archives of Virology* 160, 1593-1619.
 9. Clark, C.A., Hoy, M.W., 2006. Effect of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. *Plant Disease* 90, 83-88.
 10. Clark, C.A., Davis, J.A., Abad, A., Cuellar, W.J., Fuentes, S., Kreuze, J.F., Gibson, R.W., Mukasa, S.B., Tugume, A.K., Tairo, F.D., Valkonen, J.P.T., 2012. Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Disease* 96 (2), 168-185.
 11. Clark, C.A., Valverde, R.A., 2013. Diseases caused by viruses. In: Clark, C.A., Ferrin, D.M., Smith, T.P., Holmes, G.J. (Eds.), *Compendium of Sweetpotato diseases, pests and disorders*, second ed., American Phytopathological Society, pp. 80-81.
 12. DAFF., 2011. Sweetpotato (*Ipomoea batatas* L.) production. http://www.nda.agric.za/docs/Brochures/PG_Sweetpotato.pdf. Department of Agriculture, Forestry and Fisheries.
 13. DAFF., 2016. A profile of the South African sweetpotato market value chain.. <http://www.nda.agric.za/daDev/sideMenu/Marketing/Annual%20Publications/Commodity%20Profiles/field%20crops/SWEET%20POTATO%20MARKET%20VALUE%20CHAIN%20PROFILE%202016.pdf>. Department of Agriculture, Forestry and Fisheries.

14. Domola, M.J., Thompson, G. J., Aveling T.A.V., Laurie, S.M., Strydom, H., van Den Berg, A.A., 2008. Sweetpotato viruses in South Africa and the effect of viral infection on storage yield. *African Plant Protection* 14, 15-23.
15. Edwards, N.C., Hing, Z.A., Perry, A., Blaisdell, A., Kopelman, D.B., Fathke, R., 2012. Characterization of coding synonymous and non-synonymous variants in ADAMTS13 using Ex Vivo and In Silico Approaches. *PLoS ONE* 7 (6), e38864. <https://doi.org/10.1371/journal.pone.0038864>.
16. Esterhuizen, L.L., van Heerden, S.W., Rey, M.E.C., van Heerden, H., 2012. Genetic identification of two sweet-potato-infecting begomoviruses in South Africa. *Archives of Virology*. DOI: 10.1007/s00705-012-1398-4.
17. Frenkel, M.J., Ward, C.W., Shukla, D. D., 1989. The Use of 3' Non-coding Nucleotide Sequences in the Taxonomy of potyviruses: Application to Watermelon Mosaic Virus 2 and Soybean Mosaic Virus-N. *Journal General Virology* 70, 2775-2783.
18. Gibb, K. S., Padovan, A.C., 1993. Detection of Sweet potato feathery mottle virus in sweet potato grown in Northern Australia using an efficient and simple assay. *International Journal of Pest Management* 39 (2), 223-228.
19. Gibson, R.W., Mpembe, I., Alicai T., Carey, E.E., Mwanga, R.O.M., Seal, S.E., Vetten, H. J., 1998. Symptoms, etiology and serological analysis of sweetpotato virus disease in Uganda. *Plant Pathology* 47, 95-102.
20. Gibson, R.W., Kreuze, J.F., 2015. Degeneration in sweetpotato due to viruses, virus cleaned planting material and reversions: a review. *Plant Pathology* 64, 1-15.
21. Grüneberg, W.J., Ma, D, Mwanga, R.O.M, Carey, E.E., Huamani, Diaz, F, Eyzaguirre, R, Guaf, E., Jusuf, M., Karuniawan, A., Tjintokohadi, K., Song, Y.-S., Anil, S.R., Hossain, M., Shofiur Rahaman, E.H.M., Attaluri, S., Some, K., Afuape, S., Adofo, K., Lukonge, E.,

- Karanja, L., Ndirigwe, J., Ssemakula, G., Agili, S., Randrianaivoarivony, J.-M, Chiona, M., Chipungu, F., Laurie, S., Ricardo, J., Andrade, M., Rausch Fernandes, F., Mello, A.S., Khan, A., Labonte, D.R., Yencho, G.C., 2015. Advances in sweetpotato breeding from 1993 to 2012. In: Potato and Sweetpotato in Africa, transforming the value chains for food and nutrition security. Low, J., Nyongesa, M., Quinn, S., Parker, M. (Eds.). www.cabi.org.za. pp 1-77.
22. Gutiérrez, D. L., Fuentes, S., Salazar, L. F., 2003. Sweetpotato Virus Disease (SPVD): Distribution, incidence, and effect on sweetpotato yield in Peru. *Plant Disease* 87, 297-302.
23. Holmes, E.C., 2009. The evolutionary genetics of emerging viruses. *Annual Review of Ecology, Evolution and Systematics* 40, 353-372.
24. John, J.A., Quenouille, M.H., 1977. Experiments: Design and Analysis, Charles Griffin & Company LTD, London and High Wycombe. Combined experiments, Chapter 13, pp 232-248.
25. Kokkinos, C.D., 2006. Assessment of interactions among viruses infecting sweetpotato. PhD Thesis, Louisiana State University, pp 1-134, <http://etd.lsu.edu/docs/available/etd-03092006-163704/>.
26. Kokkinos, C.D., Clark, C.A., 2006. Real-Time PCR assays for the detection and quantification of sweetpotato viruses. *Plant Disease* 90, 783-788.
27. Kurabachew, H., 2015. The Role of Orange Fleshed Sweetpotato (*Ipomea batatas*) for Combating Vitamin A Deficiency in Ethiopia: A Review. *International Journal of Food Science and Nutrition Engineering* 5 (3), 141-146. DOI: 10.5923/j.food.20150503.05.
28. Kwak, H.R., Kim, J., Kim, M.K., Seo, J.K., Jung, M.N., Kim, J.S., Lee, S., Choi, H.S., 2015. Molecular Characterization of five potyviruses infecting Korean Sweetpotatoes based on analyses of complete genome sequences. *Plant Pathology* 31 (4), 388-401.

29. Laurie, S.M., Calitz, F.J., Adebola, P.O., Lezar, A., 2013. Characterization and evaluation of South African sweetpotato (*Ipomoea batatas* (L.) LAM) land races. South African Journal of Botany 85, 10-16.
30. Laurie, S.M., Tjale, S.S., van den Berg, A.A., Mtileni, M.M., Labuschagne, M.T., 2015. Agronomic performance of new cream to yellow-orange sweetpotato varieties in diverse environments across South Africa. South African Journal of Plant and Soil 1-9. ISSN 0257-1862, EISNN 2167-034x.
31. Levene, H., 1960. "Robust test in the equality of variance" in Contributions to Probability and Statistics. Olkin, I., Palo Alto, CA (Eds.). Stanford University Press, pp 278-292.
32. Li, R., Salih, S., Hurtt, S., 2004. Detection of begomoviruses in sweetpotato by polymerase chain reaction. Plant Disease 88, 1347-1351.
33. Li, F., Xu, D., Abad, J., Li, R., 2012. Phylogenetic relationships of closely related potyviruses infecting sweetpotato determined by genomic characterization of Sweet potato virus G and Sweet potato virus 2. Virus Genes 45 (1), 118-125.
34. Ling, K., Jackson, D.M., Harrison, H., Simmons, Pesic-VanEsbroek, Z., 2010. Field evaluation of yield effects on the U.S.A heirloom sweetpotato varieties infected by Sweet potato leaf curl virus. Crop Protection 29, 757-765.
35. Loader, L., Harper, S., Amante, S., 2000. Sweetpotato variet improvement. Queensland Horticulture Institute. Horticultural Research and Development Cooperation. <https://ausveg.com.au/app/data/technical-insights/docs/VG97023.pdf>.
36. Lozano, G., Trenado, H.P., Valverde, R.A., Navas-Castillo, J., 2009. Novel begomovirus species of recombinant nature in sweetpotato (*Ipomoea batatas*) and *Ipomoea indica*: taxonomic and phylogenetic implications. Journal of General Virology 90, 2550-2562.

37. Low, J.W., Mwanga, R.O.M., Andrade, M., Carey, E., Ball, A., 2017. Tackling vitamin A deficiency with biofortified sweetpotato in sub-Saharan Africa. *Global Food Security* 14, 23-30.
38. Maina, S., Edwards, O.R., de Almeida, L., Ximenes, A., Jones, R.A., 2016. Complete genome sequences of the potyvirus Sweetpotato virus 2 from East Timor and Australia. *Genome Announcement* 4 (3).
39. Miano, D., Labonte, D., Clark, C., 2008. Identification of molecular markers associated with sweetpotato resistance to sweetpotato virus disease in Kenya. *Euphytica* 160, 15-24.
40. Mishra, R., Verma, R.K., Sharma, P., Choudhary, D.K., Gaur, R.K., 2013. Interaction between viral proteins with transmission of Potyvirus. *Archives of Phytopathology and Plant Protection*. <http://dx.doi.org/10.1080/03235408.2013.807659>.
41. Mingot, A., Valli, A., Rodamilans, B., San Leon, D., Baulcombe, D.C., Garcia, J.A., Lopez-Moya, J.J., 2016. The P1N-PISPO Trans-frame Gene of Sweet potato feathery mottle potyvirus is produced during virus infection and functions as RNA silencing suppressor. *Journal of Virology*. Doi:10.1128/JVI.02360-15.
42. Mwanga, O.M., Yencho, G.C., Moyer, J.W., 2002. Diallel analysis of Sweetpotatoes for resistance to sweetpotato virus disease. *Euphytica* 128, 237-248.
43. Mulabisana, M.J., Cloete, M., Mabasa, K.G., Laurie, S.M., Oelofse, D., Esterhuizen, L.L., Rey, M.E.C., 2018. Surveys in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa reveal novel isolates of sweetpotato viruses. *South African Journal of Botany* 114, 280-294.
44. Nei, M., Kumar, S., 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

45. Ngailo, S., Shimelis, H., Sibiya, J., Mtunda, K., 2013. Sweetpotato breeding for resistance to sweetpotato virus disease and improved yield: progress and challenges. *African Journal of Agricultural Research* 8 (25), 3202-3215.
46. Nhlapo, T.F., Rees, D.J.G., Odeny, D.A., Mulabisana, J.M., Rey, M.E.C., 2018. Viral metagenomics reveals sweetpotato virus diversity in the Eastern and Western Cape provinces of South Africa. *South African Journal of Botany* 117, 256-267.
47. Nwankwo, I.I.M., Nwaigwe, G.O., Eluagu, J.C., 2016. Potentials of selected sweetpotato landraces for crop improvement, biodiversity conservation and for official registration in Nigeria. *W.J. Research and Review* 2 (50), 74-82.
48. Patowary, A., Chauhan, R.K., Shamsudheen, K.V., Periwal, V., Kushwaha, K.P., Sapkal, G.N., Bondre, V.P., Gore, M.M., Sivasubbu, S., Scaria, V., 2012. De novo identification of viral pathogens from cell culture hologenomes. *BMC Research Notes* 5, 11. Short Report.
49. Rodriguez Pardina, P.E., Bejerman, N., Luque, A.V., Di Feo, L., 2012. Complete nucleotide sequence of an Argentinean isolate of Sweet potato virus G. *Virus Genes* 45 (3), 593-595.
50. Rey, M.E.C., Ndunguru, J., Berrie, L.C., Paximadis, M., Berry, S., Cossa, N., Nuaila, V.N., Mabasa, K.G., Abraham, N., Rybicki, E.P., Martin, D., Pietersen, G., Esterhuizen, L.L., 2012. Diversity of dicotyledenous-infecting geminiviruses and their associated DNA molecules in Southern Africa, including the South-West Indian Ocean Islands. *Viruses* 4, 1753-1791. Doi:10.3390/v4091753.
51. Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis, R.I., Mu, L., Dwyer, G.I., Coutts, B.A., Valkonen, J. P. T., 2008. Molecular characterization of Sweet potato virus G (SPVG) isolates from areas of the Pacific Ocean and Southern Africa. *Plant Disease* 92, 1313-1320.

52. Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis R.I., Mu, L., Valkonen, J.P.T., 2009. Molecular characterization of Sweet potato feathery mottle virus (SPFMV) isolates from Easter Island, French Polynesia, New Zealand, and Southern Africa. *Plant Disease* 93, 933-939.
53. SAS/STAT User's Guide, Version 2014. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.
54. Sakai, J., Mori, M., Morishita, T., Tanaka, M., Hanada, K., Usugi, T., Nishiguchi, M., 1997. Complete nucleotide sequence and genome organization of Sweet potato feathery mottle virus (S strain) genomic RNA: the large coding region of the P1 gene. *Archives of Virology* 142 (8), 1553-1562.
55. Shapiro, S.S., Wilk, M.B., 1965. An Analysis of Variance Test for Normality (complete samples), *Biometrika*, 52, 591-611.
56. Shukla, D.D., Ward, C.W., 1989. Structure of potyvirus coat proteins and its application in the taxonomy of the potyvirus group. *Advance in Virus Research* 36, 273-314.
57. Sivparsad, B.J., Gubba, A., 2012. Molecular resolution of the genetic variability of major viruses infecting sweetpotato (*Ipomoea batatas* L.) in the province of KwaZulu-Natal in the Republic of South Africa. *Crop Protection* 41, 49-56.
58. Snedecor, G.W., Cochran, W.G., 1967. *Statistical Methods*, Sixth Edition, The Iowa State University Press, AMES, IOWA USA.
59. Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Molecular Biology and Evolution* 30, 2725-2729.

60. Tairo, F., Mukasa, S.B., Jones, R.C.J., Kullaya, A., Rhubaihayo, P.R., Valkonen, J.P., 2005. Unravelling the genetic diversity of the three main viruses involved in Sweet potato virus disease (SPVD), and its practical amplifications. *Molecular Plant Pathology* 6 (2), 199-211.
61. Tairo, F., Jones, R.A.C., Valkonen, J. P.T., 2006. Potyvirus complexes in sweetpotato: Occurrence in Australia, serological and molecular resolution, and analysis of the Sweet potato virus 2 (SPV2) components. *Plant Disease* 90, 1120-1128.
62. Thresh, J.M., Cooter, R. J., 2005. Strategies for controlling cassava mosaic virus disease in Africa. *Plant Pathology* 54, 587-614. Doi: 10.1111/j.1365-3059.2005.01282.x.
63. Tumwegamire, S., Mwanga, R.O.M, Andrade, M.I., Low, J.W., Ssemakula, G.N., Laurie, S.M., Chipungu, F.P., Ndirigue, J., Agili, S., Karanja, L., Chiona, M., Njoku, J.C., Mtunda, K., Ricardo, J., Adofo, K., Carey, E, Grüneberg, W.J., 2014. Orange-fleshed sweetpotato for Africa. Catalogue, Second Ed.). International Potato Center (CIP) Lima, Peru. <https://cgspace.cgiar.org/handle/10568/65113>.
64. van den Berg, A.A., Laurie, S.M., Niederwieser, J.G., 2004. Harvesting, storage and marketing. In: Guide to sweetpotato production in South Africa. Niederwieser J.G. (Eds.). ARC-Roodeplaat Vegetable and Ornamental Plant Institute. ISBN 1-86849-293-3. pp. 39-56.
65. Valverde, R.A., Clark, C.A., Valkonen, J.P.T., 2007. Viruses and virus disease complexes of sweetpotato. *Plant Viruses* 1 (1), 116-126.
66. Villordon, A.Q., Clark, C.A., 2014. Variation in virus symptom development and root architecture attributes at the onset of storage root initiation in ‘Beauregard’ sweetpotato plants grown with or without nitrogen. *PLoS ONE* 9 (9), e107384. doi:10.1371/journal.pone.0107384.

67. Winter, S., Purac, A., Leggett, F., Frison, E.A., Rossel, H.W., Hamilton, R.I., 1992. Partial characterization and molecular cloning of a closterovirus from sweetpotato infected with sweetpotato virus disease complex from Nigeria. *Phytopathology* 82, 896-875.

CHAPTER 5

CONCLUDING REMARKS

Sweetpotato is one of the most important food crops in Sub-Saharan Africa, particularly to smallholder farmers in rural areas. One of the most critical factors affecting sweetpotato production is virus prevalence and the global distribution of sweetpotato viruses due to exchange of planting material among countries (Gasura et al., 2008). Some key studies have shown that mixed infections of viruses of different taxonomic groups have a negative impact on sweetpotato production. The most significant finding of this study was the molecular characterization of viruses, which confirmed the occurrence of the russet crack (RC) strain of *Sweet potato feathery mottle virus*, *Sweet potato virus G*, *Sweet potato virus C*, *Sweet potato leaf curl Sao Paulo virus*, *Sweet potato mosaic virus* and *Sweet potato leaf curl virus* species in the Gauteng, Limpopo and Mpumalanga Provinces. Genetic characterization of potyviruses *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus C* (SPVC) and *Sweet potato virus G* (SPVG) was not conducted in the previous studies in these provinces and the recent discovery of *Sweet potato leaf curl Sao Paulo virus* (SPLCSPV) in Limpopo Province prompted the study to investigate the prevalence of begomoviruses in Gauteng and Mpumalanga provinces.

Field surveys revealed low virus disease incidences in the three provinces surveyed. Low infestations of aphids and whiteflies were also observed. However, viruses were detected from both symptomatic and asymptomatic samples, which indicates that plants were already infected when field surveys were conducted. This is attributed to the fact that some varieties may not always produce symptoms under field conditions.

Grafting of sweetpotato cuttings on *I. setosa* and serological analyses detected SPFMV as the most prevalent virus, however, SPVG, Sweet potato virus 2 (SPV2), Sweet potato mild speckling virus (SPMSV), Sweet potato mild mottle virus (SPMMV) and Sweet potato chlorotic stunt virus (SPCSV) were detected in a substantial number of samples collected from the three provinces surveyed. Other researchers also reported these viruses in South Africa and globally, which highlights their importance in sweetpotato production, most importantly because they occur in mixed infections. The findings of the current study show that the five viruses identified have the potential of spreading to other parts of the country through vegetative propagative planting material. This poses a threat to sweetpotato production since synergistic effects between potyviruses and the crinivirus SPCSV have been reported to severely reduce the storage root yields in other African countries. Detection of SPCSV in mixed infection with potyviruses compels future studies to determine if such co-infections have a negative effect on the yield of locally grown sweetpotato varieties. More studies also need to be conducted with a large number of samples to determine the prevalence of SPV2, SPMMV and SPMSV countrywide and their genetic characteristics.

Further characterization of potyviruses SPFMV, SPVC and SPVG were conducted by partial sequencing, which confirmed the occurrence of these viruses in the three provinces surveyed. The RC strain of SPFMV was confirmed to occur in all the three provinces and the O and EA strain of SPFMV were not detected in the current study. Previous studies confirmed the occurrence of the O strain of SPFMV in the Kwazulu Natal and Western Cape provinces of South Africa. Therefore, further studies need to be conducted to determine if the O strain of SPFMV is only confined to the coastal regions of the country or not, and to determine the possible occurrences of the RC strain in other regions. Genetic variability was observed between SPVG isolates in this study and those reported in the Kwazulu Natal Province of South Africa at nt level, however, analyses of the CP encoding aa sequences confirmed that all

isolates analyzed were the same strain of SPVG. This evidently indicates that SPVG is prone to evolve, resulting in minor variations to allow the virus to adapt to new conditions, as it is known with most RNA viruses. We recommend that primers that amplify part of the NIb protein, the full CP gene and the 3' UTR be used in future to characterize SPVG isolates.

The presence of begomoviruses was determined by rolling circle amplification method (RCA), polymerase chain reaction (PCR) and next generation sequencing (NGS) sequencing, which confirmed the frequent occurrence of SPLCSPV, SPLCV and Sweet potato mosaic virus (SPMV). These viruses were confirmed based on their full genome characteristics compared to other begomoviruses since part of the genome could not provide accurate species and strain identification. Two isolates of SPLCSPV from Limpopo and Mpumalanga provinces shared a high level of nt identity with isolates reported in Tanzania and a novel SPLCV isolate South Africa (ZA) was identified on the basis of strain demarcation threshold of begomovirus, which confirmed that it was a different strain from SPLCV reported in other countries. However, more studies with large number of samples from different provinces of South Africa will confirm how widespread begomoviruses are in the country, most importantly SPLCV isolate South Africa (ZA).

Mixed infections of viruses is a common phenomenon with viruses of sweetpotato and this was also confirmed by full-length genome sequences of SPFMV, SPVC, SPVG, SPV2, and begomoviruses SPLCSPV and SPMV using NGS, which confirmed a possible occurrence of multiple infections of potyviruses and begomoviruses in sweetpotato growing areas of South Africa.

Limitations of using full-length nt genome sequences to assign different strains of SPFMV were experienced. An example of this was when an isolate of this study (SSBles-111_ZA) shared 99% nt identity with the reference isolate ZA-O (O strain of SPFMV) reported in South

Africa. However, analysis of the CP encoding aa sequences obtained by cloning and sequencing of the CP gene region of isolate SSBles_SPFMV (MF359251) (Chapter 2) positively identified isolate SSBles-111_ZA of this study as the RC strain of SPFMV. The experience highlights the importance of analyzing the CP encoding aa sequences of SPFMV and other potyviruses to confirm strain relatedness since the CP encoding aa sequences are associated with functionality and virus pathogenicity.

The discovery of SPV2 infection with NGS in a sample that tested negative for SPV2 with DAS-ELISA point to the need for more studies to be conducted with large number of samples and confirmation of infection must be conducted with RT-PCR to determine if there are certain strains of SPV2 which may escape detection with serology. Cloning and sequencing of the CP gene region will also determine genetic variability within different isolates of SPV2.

The total and marketable yield of sweetpotato varieties tested in this study were negatively affected by virus treatments, which varied between different treatments and the trials. The study showed that different virus treatments used have the ability to reduce both total and the marketable yield. Single infection of the RC strain of SPFMV and mixed infections of the two begomoviruses was also confirmed to reduce the total yield by 58% to 84% (RC strain of SPFMV) and 52% to 77% (begomoviruses) on average, in trial 1 and 2, respectively. Virus treatments reduced marketable yield by 60% to 86% (RC strain of SPFMV) and 57% to 79% (begomoviruses) in trial 1 and 2, respectively. Varieties such as Ribbok, Monate, Bosbok, 199062.1, Impilo and Mvuvhelo produced low to moderate mean total yields of between 8 and 10 tones ha⁻¹ in the first planting season. However, it is advisable that farmers and sweetpotato vine cuttings distributors obtain their planting material from formal facilities or organizations that produce and supply virus-tested planting material. Sweetpotato vine cuttings distributors should also regularly inspect mother plants for symptoms and subsequent grafting of mother material onto the indicator plant *I. setosa* to ensure that mother materials are not harbouring

virus infections. Further testing with RT-PCR and PCR may be conducted on symptomless *I. setosa* plants to rule out viral infections. Farmers are encouraged to refrain from using planting materials retained from three previous planting seasons and are encouraged to change the planting material after every 3 years to maintain good production. The findings emphasize the importance of the availability of formal virus free schemes to ensure that plants are virus free before they are distributed to farmers and sweetpotato vine cuttings distributors as planting material.

Future research endeavors should focus on the selection and breeding for resistance to both begomoviruses and potyviruses as a long-term solution to management of virus disease complexes. Some landraces have heat and drought resistance potential and selection of virus-free plants in landraces and evaluation of possible virus resistance needs to be determined. Landraces are repeatedly planted by smallholder farmers and are overly exposed to virus complexes and potential virus resistance landraces may be identified for crossing in future breeding programs. Varieties with some level of tolerance to potyviruses-begomoviruses complexes will provide a sustainable virus management strategy for rural and smallholder farmers.

There is also a need to investigate tissue culture technologies, such as chemotherapy, thermotherapy, cryotherapy and meristem-tip culture as alternative means to develop virus-free planting material from virus infected sweetpotato mother plants, due to recent discoveries of begomoviruses since this will ensure preservation of varieties with good traits. Future studies should also focus on investigating botanical/medicinal extracts for possible antiviral activities.

Viruses such as SPVG and SPV2 were first reported in Asia and Taiwan (Rossel and Thottappilly, 1988; Colinet et al., 1994). This compels that stringent quarantine regulations should be imposed at national level, which should involve a thorough screening of both

symptomatic and asymptomatic and tissue culture plants at borders. Regular monitoring of illegal influx of vegetative materials and storage roots at borders will also prevent new viruses and strains entering the country. Kenya Plant Health Inspectorate Service (KEPHIS) (www.sweetpotatoknowledge.org) is one of the organization that practice quarantine regulations by maintaining, observing and testing imported plants for pests and diseases before they are released. It will benefit society if same principles are adhered to in South Africa and other countries since new virus strains and species will be intercepted and prevented from entering countries where they are not known to occur.

References

1. Colinet, D., Kummert, J., Lepoivre, P., 1994. The complete nucleotide sequences of the coat protein cistron and the 3' non-coding region of a newly-identified potyvirus infecting sweetpotato, as compared to those of sweetpotato feathery mottle virus. *Archives of Virology* 139 (3-4), 27.
2. Gasura, E., A.B. Mashingaidze, A.B., Mukasa, S.B., 2008. Genetic variability for tuber yield, quality, and virus disease complex traits in Uganda sweetpotato germplasms. *African Crop Science Journal* 16 (2), 147-160.
3. Rossel, H.W, Thottappilly, G., 1988. Complex virus diseases of sweetpotato. In: *Exploration, Maintenance, and Utilization of Sweetpotato Genetic Resources. Report of the First Sweetpotato Planning Conference 1987*. International Potato Center, Lima, Peru, pp 291-302.

APPENDICES

Table 1: Mean percentages of virus disease incidences recorded during the epidemiology surveys conducted in season 1 in the Gauteng, Limpopo and Mpumalanga Provinces

Limpopo	Mean (%)	t-grouping	Mpumalanga	Mean (%)	t-grouping	Gauteng	Mean (%)	t-grouping
Maungani	62	a	Thulamahashe	41	a	Soshanguve M	60	a
Lwamondo	31	b	Verena	32	ab	Soshanguve L	47	ba
Wynberg	23	b	Tonga	31	ab	Hamanskraal	32	bc
Dzimauli	21	bc	Naas	26	abc	EnkangalaB	24	dce
Tshiombo	10	cd	Khuzulwandle	24	bc	SoshanguveS	20	dce
Rambuda	0	d	Elekhwatini	21	bc	Daveyton	16	dce
Kwaggahoek	0	d	Leeuport	13	cd	Cullinan	16	dce
Groblersbridge	0	d	Tjakastad	12	cd	EnkangalaD	7	de
			Mbejeka	3	d	Mamelodi	7	de
			Mzinti	2	d	Roodeplaat	1	e
			Snymansbelt	0	d			
Grand mean	18		Grand mean	19		Grand mean	23	
SEM	3.47		SEM	4.45		SEM	678.0143	
F. Pr>F	0.001		F. Pr>F	<.001		F. Pr>F	10.1	
LSD (1%)	13		LSD (5%)	16		LSD (5%)	19	
CV%	84.3		CV%	106.7		CV%	111.2508	

SEM= Standard errors of means, F. Pr>F= F probability, LSD= least significant difference and CV= coefficients of variants. Means with the same letter/letters do not differ significantly at the 1% or 5% significant level. * based on the bracket scoring scale

Table 2: Mean percentage of virus disease incidences recorded during the epidemiology surveys conducted in season 2 (2014/2015) in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa

Area	Number of sampling points	Mean: bracket scoring of symptoms	t-grouping
SoshanguveS	20	50	a
Verena	20	36	b
Mamelodi	20	34	bc
Daveyton	20	31	bcd
Naas	20	25	cde
Thulamahashe	20	22	def
Tonga	20	22	def
Kamhlushwa	20	20	efg
Mzinti	20	19	efgh
Snymansbelt	20	18	efghi
Tjakastad	19	17	efghi
Mbejeka	20	17	efghi
Cullinan	20	15	fghij
Dzimauli	20	12	ghijk
Wynberg	20	11	ghijk
Dzithobeni	20	10	hijk
Lwamondo	20	9	ijkl
Roodeplaat	20	6	jkl
SoshanguveL	20	4	kl
Groblersbridge	20	3	kl
Rammbuda	20	0	l
Maungani	20	0	l
Elim	20	0	l
Tshiombo	20	0	l
Means			
Gauteng	21,43a		
Limpopo	4,375b		
Mpumalanga	21,9a		
Anova	DF	MS	F. Pr>F
	23	3352	<.0001
Error	455	247,20	
	CV	LSD	
	98,83354	9,7815	
Shapiro-Wilk (P>W)		P= 0,981258	
Kurtosis		0,23	

DF= degree of freedom, MS=Mean square, F. Pr>F= F probability, LSD= least significant difference and CV= coefficients of variants. Means with the same letter indicates that there was no significant difference between areas at 5% level.

Table 3: Means scores of aphids and whitefly infestations recorded on sweetpotato plants during epidemiology surveys conducted in season 2 in the Gauteng, Limpopo and Mpumalanga Provinces of South Africa.

Area	Sampling points	Mean score**	t-grouping	Area	Sampling points	Mean score: Whitefly infestations	t-grouping
Cullinan	20	2,0	a	Wynberg	20	1,6	a
Rambuda	20	1,0	b	Tshiombo	20	0,6	b
Tshiombo	20	0,9	b	Rambuda	20	0,5	b
Dzithobeni	20	0,8	b	Daveyton	20	0	c
Lwamondo	20	0,4	c	Dzithobeni	20	0	c
Wynberg	20	0,4	c	Groblersbridge	20	0	c
Mamelodi	20	0,3	cd	Cullinan	20	0	c
Soshanguve S	20	0,1	cd	Kamhlushwa	20	0	c
Groblersbridge	20	0	d	Elim	20	0	c
Kamhlushwa	20	0	d	Mamelodi	20	0	c
Daveyton	20	0	d	Lwamondo	20	0	c
Mbejeka	20	0	d	Mbejeka	20	0	c
Elim	20	0	d	Mzinti	20	0	c
Naas	20	0	d	Naas	20	0	c
Mzinto	20	0	d	Dzimaui	20	0	c
Roodeplaat	20	0	d	Roodeplaat	20	0	c
Snymansbelt	20	0	d	Snymansbelt	20	0	c
Soshanguve L	20	0	d	Soshanguve L	20	0	c
Maungani	20	0	d	Maungani	20	0	c
Thulamahash	20	0	d	Thulamahashe	20	0	c
Tjakastad	19	0	d	Tjakastad	19	0	c
Tonga	20	0	d	Tonga	20	0	c
Dzimaui	20	0	d	Soshanguve S	20	0	c
Verena	20	0	d	Verena	20	0	c
Means				Means			
Gauteng	0,44a			Gauteng	0,00b		
Limpopo	0,34a			Limpopo	0,30a		
Mpumalanga	0,0b			Mpumalanga	0,0b		
Anova	DF	MS	Pr>F	Anova	DF	MS	Pr>F
	23	4,661	<.0001		23	2,364	<.0001
Error	455	0,274		Error	455	0,1467	
	CV	LSD			CV	LSD	
	216,174	0,3257			346,162	0,2383	
Shapiro-Wilk (P>W)		P= 0.645777		Shapiro-Wilk (P>W)		P= 0,44179	
Kurtosis		11,80		Kurtosis		11,04	

**Scoring rate: 1 (1-5 insects) equivalent to low infestation; 2 (6 to 10 insects) equivalent to medium infestation; 3 (11 to 20 insects) equivalent to high infestation and 4 (20 and higher) equivalent to very high infestation.

Table 4: The χ^2 probabilities (<0.05% level) of single, co and multiple infection of viruses (mean percentage incidences) generated by detection of viruses by NCM and DAS ELISA in samples collected in season 1 and 2 epidemiology surveys conducted in Gauteng, Limpopo and Mpumalanga Provinces.

ANOVA	SI SPFMV	SI SPCSV	SI SPVG	SI SPV2	SI SPMSV	SI SPLV	SI SPMMV	SPFMV+SPVG	SPFMV+SPV2
Mean Season 1	52.22	0.00	0.00	1.85	0.37	0.37	0.37	0.74	1.11
Mean season 2	16.01	5.14	3.02	1.21	0.60	0.00	0.30	12.69	1.81
Chi-Square P	<.0001	0.0002	0.0040	0.5183	0.6857	0.2678	0.8851	<.0001	0.4812
Likelihood Ratio Chi-Square P	<.0001	<.0001	0.0005	0.5197	0.6819	0.2056	0.8854	<.0001	0.4756
Means for Provinces (seasons combined)									
Gauteng	34.88	2.33	0.93	1.40	0.00	0.47	0.00	7.44	1.86
Limpopo	32.46	2.62	1.05	0.00	1.57	0.00	0.52	3.66	0.00
Mpumalanga	29.23	3.59	3.08	3.08	0.00	0.00	0.51	10.77	2.56
Chi-Square P-Provinces	0.4726	0.7262	0.1711	0.0447	0.0393	0.4069	0.5718	0.0275	0.1003
Likelihood Ratio Chi-Square	0.4712	0.7329	0.1962	0.0157	0.0316	0.3572	0.4117	0.0227	0.0276

	SPFMV+SPCSV	SPFMV+SPMMV	SPFMV+SPMSV	SPFMV+SPV2+SPVG	SPFMV+SPV2+SPCSV	SPFMV+SPV2+SPMSV	SPFMV+SPV2+SPVG+SPCSV	SPFMV+SPV2+SPVG+SPMSV	SPFMV+SPV2+SPVG+SPCSV+SPMSV
Mean Season 1	1.11	2.59	1.11	0.00	0.00	0.00	0.00	0.00	0.00
Mean season 2	5.74	2.42	0.91	1.81	0.30	2.42	0.30	1.51	0.91
Chi-Square P	0.0026	0.8908	0.8017	0.0262	0.3660	0.0101	0.3660	0.0426	0.1168
Likelihood Ratio Chi-Square	0.0014	0.8909	0.8022	0.0073	0.2745	0.0019	0.2745	0.0143	0.0581
Means for Provinces (seasons combined)									
Gauteng	5.58	0.47	0.47	1.40	0.00	1.86	0.47	0.00	0.00
Limpopo	2.62	4.19	1.05	1.05	0.52	1.05	0.00	0.00	0.00
Mpumalanga	2.56	3.08	1.54	0.51	0.00	1.03	0.00	2.56	1.54
Chi-Square P-Provinces	0.1735	0.0459	0.5492	0.6661	0.3413	0.6996	0.4069	0.0053	0.0433
Likelihood Ratio Chi-Square	0.1883	0.0225	0.5300	0.6455	0.3172	0.7099	0.3572	0.0034	0.0336

Chi-square P= chi-square probability. Means within each column with the same letter denotes that areas did not differ significantly at the 5% significant level. SI= Single infection.

Table 5: Pairwise comparison of the nt sequences generated with part of the N1b, CP and UTR encoding region of SPVG.

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
SPVG isolates of this study	CTB3.	1		100.00	100.00	99.86	100.00	99.28	99.00	98.85	98.85	97.99	95.56	86.96	86.82	86.96	86.96	85.82	96.72	97.13	96.28	94.85	94.44	
	3900A	2	100.00		100.00	99.86	100.00	99.28	99.00	98.85	98.85	97.99	95.56	86.96	86.82	86.96	86.96	85.82	96.72	97.13	96.28	94.85	94.44	
	HawA2	3	100.00	100.00		99.86	100.00	99.28	99.00	98.85	98.85	97.99	95.56	86.96	86.82	86.96	86.96	85.82	96.72	97.13	96.28	94.85	94.44	
	JAAC	4	99.86	99.86	99.86		99.86	99.14	98.85	98.71	98.71	97.85	95.42	86.82	86.68	86.82	86.82	85.67	96.58	96.99	96.14	94.71	94.29	
	Zimb1-H	5	100.00	100.00	100.00	99.86		99.28	99.00	98.85	98.85	97.99	95.56	86.96	86.82	86.96	86.96	85.82	96.72	97.13	96.28	94.85	94.44	
	NZ-4	6	99.28	99.28	99.28	99.14	99.28		98.85	98.71	98.71	97.85	95.13	86.82	86.68	86.82	86.82	85.67	96.58	96.99	96.14	94.71	94.29	
	3817	7	99.00	99.00	99.00	98.85	99.00	98.85		99.86	99.86	97.85	95.13	87.11	86.96	87.11	87.11	85.96	96.86	97.28	96.42	94.99	94.58	
	PS1B	8	98.85	98.85	98.85	98.71	98.85	98.71	99.86		100.00	97.71	94.99	86.96	86.82	86.96	86.96	85.82	96.72	97.42	96.57	94.85	94.44	
	Zimb1C	9	98.85	98.85	98.85	98.71	98.85	98.71	99.86	100.00		97.71	94.99	86.96	86.82	86.96	86.96	85.82	96.72	97.42	96.57	94.85	94.44	
	SPVG_isolate_Egypt_1	10	97.99	97.99	97.99	97.85	97.99	97.85	97.85	97.71	97.71		94.41	86.25	85.96	86.10	86.39	85.10	95.58	96.13	95.28	93.99	93.58	
	SPVG-NC	11	95.56	95.56	95.56	95.42	95.56	95.13	95.13	94.99	94.99	94.41		91.03	90.88	91.03	91.03	91.03	89.84	92.87	93.27	93.67	91.42	91.01
	UMBUMB-VG	12	86.96	86.96	86.96	86.82	86.96	86.82	87.11	86.96	86.96	86.25	91.03		97.58	97.74	97.90	98.71	85.45	86.39	86.76	86.53	86.41	
	UMP1-VG	13	86.82	86.82	86.82	86.68	86.82	86.68	86.96	86.82	86.82	85.96	90.88	97.58		99.84	97.90	96.61	85.59	85.96	86.33	86.68	86.55	
	UMP3-VG	14	86.96	86.96	86.96	86.82	86.96	86.82	87.11	86.96	86.96	86.10	91.03	97.74	99.84		98.06	96.77	85.73	86.10	86.47	86.82	86.70	
	UMFUME-VG	15	86.96	86.96	86.96	86.82	86.96	86.82	87.11	86.96	86.96	86.39	91.03	97.90	97.90	98.06		96.94	85.73	86.25	86.62	86.82	86.70	
	UMP2-VG	16	85.82	85.82	85.82	85.67	85.82	85.67	85.96	85.82	85.82	85.10	89.84	98.71	96.61	96.77	96.94		84.31	85.53	85.90	85.67	85.55	
	GBD14_SPVG	17	96.72	96.72	96.72	96.58	96.72	96.58	96.86	96.72	96.72	95.58	92.87	85.45	85.59	85.73	85.73	84.31		95.86	95.01	93.58	93.30	
	SSBles_SPVG	18	97.13	97.13	97.13	96.99	97.13	96.99	97.28	97.42	97.42	96.13	93.27	86.39	85.96	86.10	86.25	85.53	95.86		99.14	93.99	93.58	
	Naas4_SPVG	19	96.28	96.28	96.28	96.14	96.28	96.14	96.42	96.57	96.57	95.28	93.67	86.76	86.33	86.47	86.62	85.90	95.01	99.14		93.14	92.74	
	TsMB1_SPVG1	20	94.85	94.85	94.85	94.71	94.85	94.71	94.99	94.85	94.85	93.99	91.42	86.53	86.68	86.82	86.82	85.67	93.58	93.99	93.14		99.57	
	RDPLT-BlesSPVG	21	94.44	94.44	94.44	94.29	94.44	94.29	94.58	94.44	94.44	93.58	91.01	86.41	86.55	86.70	86.70	85.55	93.30	93.58	92.74	99.57		

Table 6: Alignment of the CP aa sequences of SPVC and the RC strain of SPFMV isolates showing the conserved motifs associated with potyviral CP region, which are indicated by a solid box.

SPFMV-RC	REF26_RC	☒	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALRAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVKDKD	VNVGTVGTFV	90
	GB10_RC	☒	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALRAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVKDKD	VNVGTVGTFV	90
	RDPLT 11-04_RC	☒	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALRAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVKDKD	VNVGTVGTFV	90
	SSBlesSPFMV_RC	☒	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALRAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVKDKD	VNVGTVGTFV	90
	IsolateMBL	☐	SDERTEFKDA	GANPPAPKPK	NVPPPTITE	ITDPEDPKQE	ALKAARAKQP	AVIPESYGRD	TSKEKESIVG	ASSKGVKDKD	VNVGTVGTFV	90
	Isolate 3817-1	☐	SSESTEFKDA	GANPPAPKSK	IDPPPTITE	IVDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	TSSKGVKDKD	VNVGTVGTFV	90
	HN: 5RC:08	☐	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALRAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVKDKD	VNVGTVGTFV	90
	Polyprotein SPFMV	☐	SSERTEFKDA	GANPPAPKPK	NIPPPPTITE	VTDPEDPKQA	ALRAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVKDKD	VNVGTVGTFV	90
	CP partial	☐	SSERTEFKDA	GANPPAPAPK	NIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVKDKD	VNVGTVGTFV	90
	SC2 CP	☐	SSERTEFKDA	GADPPAPKPK	NIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKERGSIVG	ASSKGVKDKD	VNVGTVGTFV	90
SPVC	Fujian 3	☐	SNENTEFKDA	GANPPAPKPK	GPYTAPEITE	VTEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - SMRS	VSPQRVKDKD	VNVGTTGTFV	88
	Hunan 4	☐	SNENTEFKDA	GANPPAPKPK	GPYTAPEITE	VTEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - SMRS	VSPQRVKDKD	VNVGTTGTFV	88
	Strain CH2	☐	SGNPPEFKDA	GANPPAPKPK	GAFTAPEITE	VTEPEDPKQA	ALREAKQKQP	AVTPESYGRD	TGEK - - SMRS	VSPQRVKDKD	ANVGTTGTFV	88
	Spain 1C	☐	SGNPPEFKDA	GANPPAPKPK	GPYTAPEITE	VTEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - SMRS	VSPQRVKDKD	VNVGTTGTFV	88
	GB12-C	☒	SGNPPEFKDA	GVPNPAPKPK	GPYTAPEITE	VTEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - SLRS	VSPQRVKDKD	VNVGTTGTFV	88
	DVT3-C	☒	SGNPSEFKDA	GANPPAPKPK	GPYTAPEITE	ITEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - PMRS	VSPQRVKDKD	VNVGTTGTFV	88
	VER7_C	☒	SGNPSEFKDA	GANPPAPKPK	GPYTAPEITE	ITEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - PMRS	VSPQRVKDKD	VNVGTTGTFV	88
	REF26_C	☒	SGNPSEFKDA	GANPPAPKPK	GPYTAPEITE	ITEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - PMRS	VSPQRVKDKD	VNVGTTGTFV	88
	MAM15-C	☒	SGNPPEFKDA	GVPNPAPKPK	GPYTAPEITE	VTEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - SMRS	VSPQRVKDKD	VNVGTTGTFV	88
	CTB3-1	☐	SGNPSEFKDA	GANPPAPKPK	GPYTAPEITE	ITEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - PMRS	VSPQRVKDKD	VNVGTTGTFV	88
PS1B-4	☐	SGNPSEFKDA	GANPPAPKPK	GPYTAPEITE	ITEPEDPKQA	ALREARQKQP	AVTPESYGRD	TGEK - - PMRS	VSPQRVKDKD	VNVGTTGTFV	88	
SPFMV-RC	REF26_RC	☒	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	GB10_RC	☒	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	RDPLT 11-04_RC	☒	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	SSBlesSPFMV_RC	☒	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	IsolateMBL	☐	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	Isolate 3817-1	☐	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	HN: 5RC:08	☐	VQPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	Polyprotein SPFMV	☐	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	CP partial	☐	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
	SC2 CP	☐	VPRVKMNANK	KRQPMVNGRA	IINFQHLSTY	EPEQFEVANT	RSTQEQFQAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	180
SPVC	Fujian 3	☐	VPRIKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	Hunan 4	☐	VPRIKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	Strain CH2	☐	VPRVKLHTGK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	Spain 1C	☐	VPRVKLHTGK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	GB12-C	☒	VPRVKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	DVT3-C	☒	VPRVKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	VER7_C	☒	VPRVKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	REF26_C	☒	VPRVKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	MAM15-C	☒	VPRVKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDTGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
	CTB3-1	☐	VPRVKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178
PS1B-4	☐	VPRVKLHTSK	MRQPRVNGIS	VVNQLHLATY	EPEQHNIGNT	RSTQEQFRAW	YEGVKGDYGV	DDAGMIGILLN	GLMVWC IENG	TSPN INGVWT	178	

			230		240		250		260		270
SPFMV-RC	REF26_RC	REF26_RC	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	GB10_RC	GB10_RC	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	RDPLT 11-04_RC	RDPLT 11-04_RC	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	SSBiesSPFMV_RC	SSBiesSPFMV_RC	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	IsolateMBL	IsolateMBL	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	Isolate 3817-1	Isolate 3817-1	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	HN: SRC:08	HN: SRC:08	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	Polyprotein SPFMV	Polyprotein SPFMV	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	CP partial	CP partial	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	SC2 CP	SC2 CP	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
SPVC	Fujian 3	Fujian 3	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	Hunan 4	Hunan 4	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	Strain CH2	Strain CH2	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	Spain 1C	Spain 1C	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	GB12-C	GB12-C	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	DVT3-C	DVT3-C	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	VER7_C	VER7_C	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	REF26_C	REF26_C	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	MAM15-C	MAM15-C	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
	CTB3-1	CTB3-1	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA
PS1B-4	PS1B-4	MMDGDEQV	PIKPLLDHAV	PTRFQIMTHF	SDVAEAYIEM	RNRKAYMPR	YGLQRNLTDM	SLARYAFDFY	ELHSTTPARA	KEAHLQMKAA	
SPFMV-RC	REF26_RC	REF26_RC	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	GB10_RC	GB10_RC	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	RDPLT 11-04_RC	RDPLT 11-04_RC	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	SSBiesSPFMV_RC	SSBiesSPFMV_RC	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	IsolateMBL	IsolateMBL	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	Isolate 3817-1	Isolate 3817-1	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	HN: SRC:08	HN: SRC:08	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	Polyprotein SPFMV	Polyprotein SPFMV	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	CP partial	CP partial	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
	SC2 CP	SC2 CP	ALKNNAKNNRLF	GLDGNVSTQE	EDTERHTTTD	VTRNIHNNLLG	MRGVQ	315			
SPVC	Fujian 3	Fujian 3	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVQ	313			
	Hunan 4	Hunan 4	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVQ	313			
	Strain CH2	Strain CH2	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVQ	313			
	Spain 1C	Spain 1C	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVH	313			
	GB12-C	GB12-C	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVH	313			
	DVT3-C	DVT3-C	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVH	313			
	VER7_C	VER7_C	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVH	313			
	REF26_C	REF26_C	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVH	313			
	MAM15-C	MAM15-C	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVH	313			
	CTB3-1	CTB3-1	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVH	313			
PS1B-4	PS1B-4	ALKNNAHNNRLF	GLDGNVSTQE	EDTERHTATD	VTRNIHNNLLG	MRGVH	313				

Table 7: Pairwise comparison of the full CP aa sequences generated with part of the CP-encoding region nt sequences of SPVC and the RC strain of SPFMV.

				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Reference SPFMV-RC	SPFMV-RC isolates of this study	REF26_RC	1		100.00	100.00	99.68	96.51	96.19	99.37	98.41	98.10	97.78	84.76	84.44	83.81	84.13	83.81	83.17	83.17	83.17	83.81	83.17	83.17
		GB10_RC	2	100.00		100.00	99.68	96.51	96.19	99.37	98.41	98.10	97.78	84.76	84.44	83.81	84.13	83.81	83.17	83.17	83.17	83.81	83.17	83.17
		RDPLT 11-04_RC	3	100.00	100.00		99.68	96.51	96.19	99.37	98.41	98.10	97.78	84.76	84.44	83.81	84.13	83.81	83.17	83.17	83.17	83.81	83.17	83.17
		SSBlesSPFMV_RC	4	99.68	99.68	99.68		96.83	96.51	99.68	98.73	98.41	98.10	84.44	84.13	83.49	83.81	84.13	82.86	82.86	82.86	84.13	82.86	82.86
	Reference SPVC	IsolateMBL	5	96.51	96.51	96.51	96.83		96.51	96.51	96.83	97.14	96.19	83.17	82.86	82.22	82.54	82.86	82.22	82.22	82.22	82.86	82.22	82.22
		Isolate3817-1	6	96.19	96.19	96.19	96.51	96.51		96.19	96.19	96.51	95.56	82.86	82.54	81.90	82.22	82.54	81.90	81.90	81.90	82.54	81.90	81.90
		HN: 5RC:08	7	99.37	99.37	99.37	99.68	96.51	96.19		98.41	98.10	97.78	84.13	83.81	83.17	83.49	83.81	82.54	82.54	82.54	83.81	82.54	82.54
		Polyprotein SPFMV	8	98.41	98.41	98.41	98.73	96.83	96.19	98.41		99.05	98.10	83.49	83.17	82.54	82.86	83.17	81.90	81.90	81.90	83.17	81.90	81.90
		CP partial	9	98.10	98.10	98.10	98.41	97.14	96.51	98.10	99.05		97.78	83.17	82.86	82.22	82.54	82.86	81.59	81.59	81.59	82.86	81.59	81.59
		SPVC isolates of this study	Reference SPVC	SC2 CP	10	97.78	97.78	97.78	98.10	96.19	95.56	97.78	98.10	97.78		83.49	83.17	82.54	82.86	83.17	81.90	81.90	81.90	83.17
Fujian 3	11			84.76	84.76	84.76	84.44	83.17	82.86	84.13	83.49	83.17	83.49		99.68	96.81	97.12	97.12	96.49	96.49	96.49	97.12	96.49	96.49
Hunan 4	12			84.44	84.44	84.44	84.13	82.86	82.54	83.81	83.17	82.86	83.17	99.68		96.49	96.81	96.81	96.17	96.17	96.17	96.81	96.17	96.17
Strain CH2	13			83.81	83.81	83.81	83.49	82.22	81.90	83.17	82.54	82.22	82.54	96.81	96.49		98.08	97.12	96.17	96.17	96.17	97.12	96.17	96.17
Spain 1C	14			84.13	84.13	84.13	83.81	82.54	82.22	83.49	82.86	82.54	82.86	97.12	96.81	98.08		97.12	97.12	97.12	97.12	97.12	97.12	97.12
SPVC isolates of this study	GB12-SPVC			15	83.81	83.81	83.81	84.13	82.86	82.54	83.81	83.17	82.86	83.17	97.12	96.81	97.12	97.12		96.49	96.49	96.49	99.36	96.49
	DVT3-SPVC		16	83.17	83.17	83.17	82.86	82.22	81.90	82.54	81.90	81.59	81.90	96.49	96.17	96.17	97.12	96.49		100.00	100.00	96.49	100.00	100.00
	VER7_SPVC		17	83.17	83.17	83.17	82.86	82.22	81.90	82.54	81.90	81.59	81.90	96.49	96.17	96.17	97.12	96.49	100.00		100.00	96.49	100.00	100.00
	REF26_SPVC		18	83.17	83.17	83.17	82.86	82.22	81.90	82.54	81.90	81.59	81.90	96.49	96.17	96.17	97.12	96.49	100.00	100.00		96.49	100.00	100.00
	MAM15-SPVC		19	83.81	83.81	83.81	84.13	82.86	82.54	83.81	83.17	82.86	83.17	97.12	96.81	97.12	97.12	99.36	96.49	96.49	96.49		96.49	96.49
SPVC isolates of this study	CTB3-1		20	83.17	83.17	83.17	82.86	82.22	81.90	82.54	81.90	81.59	81.90	96.49	96.17	96.17	97.12	96.49	100.00	100.00	100.00	96.49		100.00
	PS1B-4	21	83.17	83.17	83.17	82.86	82.22	81.90	82.54	81.90	81.59	81.90	96.49	96.17	96.17	97.12	96.49	100.00	100.00	100.00	96.49	100.00		

Table 8: Pairwise comparison of the CP aa sequences generated with part of the CP-encoding region nt sequences of SPVG.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
SPVG isolates of this study	Shandong 5	1		98.10	99.05	97.62	96.67	97.14	97.14	95.71	99.05	95.71	95.71	97.62	99.05	94.76	91.90
	Hunan 4	2	98.10		98.10	97.62	96.67	97.14	97.14	95.71	99.05	95.71	95.71	97.62	99.05	94.76	90.95
	Isolate SC2	3	99.05	98.10		97.62	96.67	97.14	97.14	95.71	99.05	95.71	95.71	97.62	99.05	94.76	90.95
	Isolate SC6	4	97.62	97.62	97.62		97.14	97.62	97.14	96.19	98.57	96.19	96.19	98.10	98.57	94.76	91.90
	Umfume–VG	5	96.67	96.67	96.67	97.14		97.62	96.19	96.19	97.62	96.19	96.19	98.10	97.62	94.76	91.90
	Shaamxi4	6	97.14	97.14	97.14	97.62	97.62		96.67	95.71	98.10	95.71	95.71	97.62	98.10	94.29	90.95
	Jesus Maria	7	97.14	97.14	97.14	97.14	96.19	96.67		95.24	98.10	95.71	95.71	97.62	98.10	94.76	90.48
	AEU17202.1	8	95.71	95.71	95.71	96.19	96.19	95.71	95.24		96.67	96.19	96.19	97.14	96.67	94.76	91.43
	SPVG SC1	9	99.05	99.05	99.05	98.57	97.62	98.10	98.10	96.67		96.67	96.67	98.57	100.00	95.71	91.90
	TsMB1_SPVG	10	95.71	95.71	95.71	96.19	96.19	95.71	95.71	96.19	96.67		100.00	98.10	96.67	93.81	94.17
	RDPLT–BlesSPVG	11	95.71	95.71	95.71	96.19	96.19	95.71	95.71	96.19	96.67	100.00		98.10	96.67	93.81	94.17
	SSBlesSPVG	12	97.62	97.62	97.62	98.10	98.10	97.62	97.62	97.14	98.57	98.10	98.10		98.57	95.71	92.38
	Hainan	13	99.05	99.05	99.05	98.57	97.62	98.10	98.10	96.67	100.00	96.67	96.67	98.57		95.71	91.90
	GBD14_SPVG	14	94.76	94.76	94.76	94.76	94.76	94.29	94.76	94.76	95.71	93.81	93.81	95.71	95.71		90.95
	Naas4	15	91.90	90.95	90.95	91.90	91.90	90.95	90.48	91.43	91.90	94.17	94.17	92.38	91.90	90.95	

Table 9: Pairwise comparison of the full-length nt sequences of SPLCSPV and SPLCV generated with TZ:ELLP-ZA, TZ:NMP-ZA, SSB1-ZA, RDGP-ZA and ZA:TMBLP-ZA isolates of this study collected from Limpopo, Gauteng and Mpumalanga provinces.

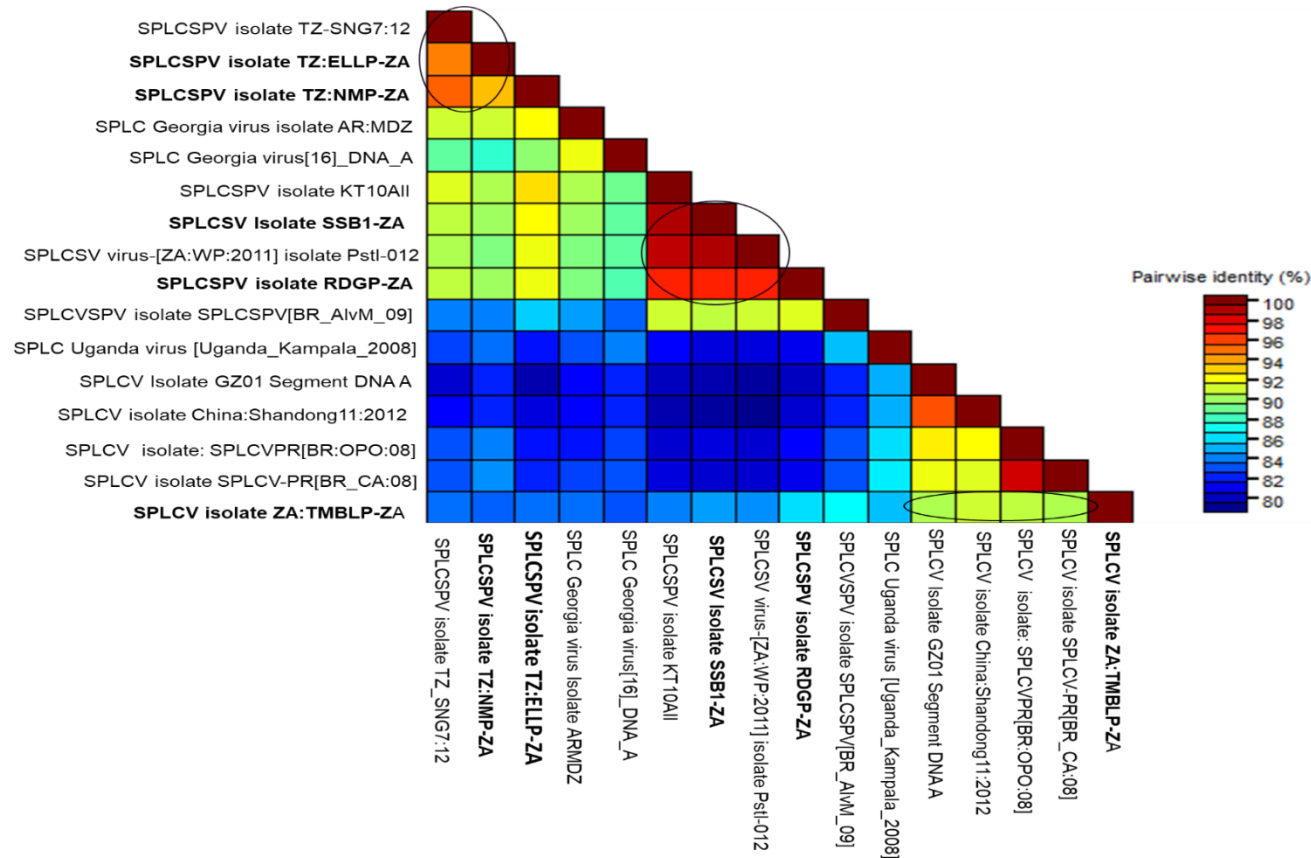


Table 10: Pairwise comparison of the full-length nt sequences of SPMV generated with SPMV Isolate SSB1_ZA, SPMV isolate ELLP_ZA, SPMV Isolate RDGP_ZA and SPMV Isolate TsMBLP_ZA isolates of this study collected from Limpopo, Gauteng and Mpumalanga provinces.

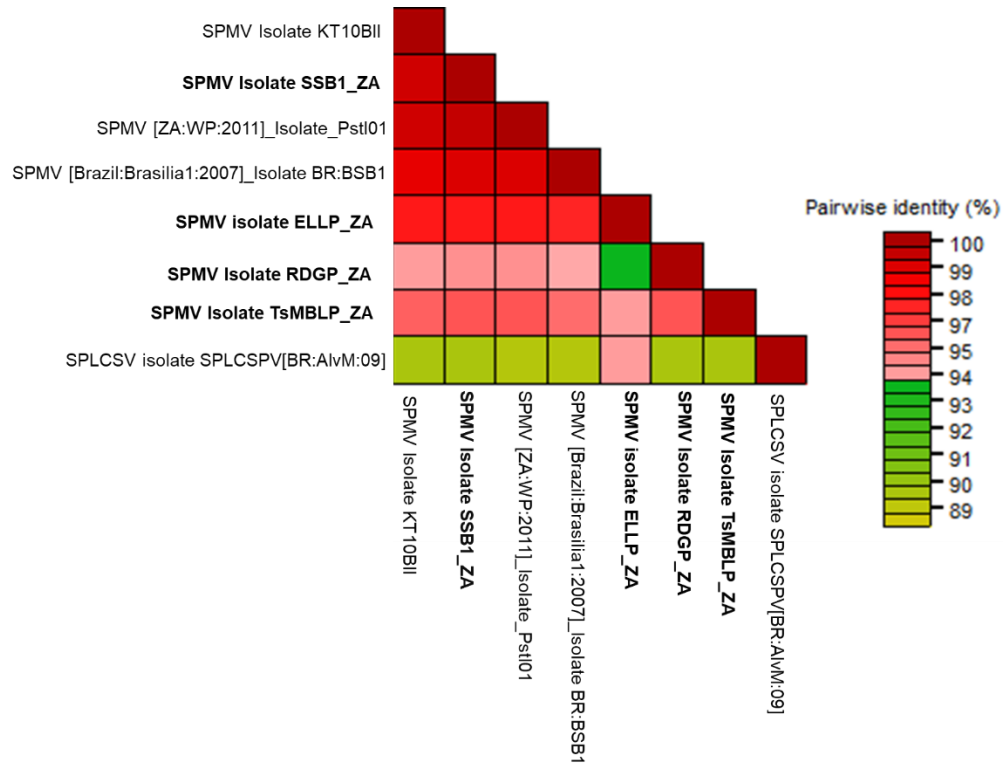


Table 11: The alignment of the of the C1 open reading frame encoded aa of SPLCSPV and SPLCV, showing the conserved motifs associated with begomoviruses. Isolates of this study are marked with an asterisk and conserved motifs indicated by a solid box.



Table 12: Pairwise comparison of the full-length nt sequences of SPFMV and SPVC (A), and SPV2 and SPVG (B) generated with sample SSbles (indicated with a dot) used as a source of multiple infection for the field trials conducted to determine the effects of single infection of potyviruses and co-infections of potyviruses and begomoviruses.

A		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	SPFMV isolate GJ122	1	98.25	96.59	92.74	93.06	94.02	93.94	89.38	89.30	89.49	90.65	71.41	71.28	71.39	71.42	71.52	71.34	71.32
	SPFMV isolate 10-0	2	98.25		96.89	92.66	92.96	93.91	93.83	89.29	89.21	89.49	90.68	71.28	71.14	71.23	71.30	71.40	71.26
	SPFMV isolate SPFMV O-Arg	3	96.59	96.89		93.74	94.31	92.70	92.67	87.61	87.62	87.70	89.00	71.02	70.90	70.98	70.94	71.02	71.00
	SPFMV isolate Piu3	4	92.74	92.66	93.74		95.51	91.93	91.93	88.45	88.49	88.52	89.86	71.27	71.13	71.15	71.20	71.25	71.18
	SPFMV isolate Ruk73	5	93.06	92.96	94.31	95.51		91.96	91.87	88.15	88.08	88.18	90.09	70.97	70.85	70.94	70.98	71.06	71.09
●	SPFMV-RC isolate SSbles1_ZA	6	94.02	93.91	92.70	91.93	91.96		99.32	92.17	92.20	92.10	93.56	71.43	71.46	71.50	71.47	71.58	71.48
	SPFMV isolate_ZA-O	7	93.94	93.83	92.67	91.93	91.87	99.32		92.07	92.14	92.03	93.50	71.50	71.50	71.54	71.52	71.62	71.53
	SPFMV RC_strain	8	89.38	89.29	87.61	88.45	88.15	92.17	92.07		98.33	98.25	94.93	71.21	71.19	71.19	71.20	71.16	71.25
	SPFMV isolate SPFMV-RC-ARg	9	89.30	89.21	87.62	88.49	88.08	92.20	92.14	98.33		98.28	95.10	71.14	71.07	71.16	71.11	71.03	71.26
	SPFMV genomic_DNA	10	89.49	89.49	87.70	88.52	88.18	92.10	92.03	98.25	98.28		94.89	71.16	71.12	71.20	71.10	71.05	71.27
	SPFMV isolate SPFMV AM-MB2	11	90.65	90.68	89.00	89.86	90.09	93.56	93.50	94.93	95.10	94.89		70.90	70.93	71.00	70.86	70.85	70.98
	SPVC isolate SPVC Arg	12	71.41	71.28	71.02	71.27	70.97	71.43	71.50	71.21	71.14	71.16	70.90		98.13	97.98	94.63	94.53	94.74
	SPVC isolate HNS2	13	71.28	71.14	70.90	71.13	70.85	71.46	71.50	71.19	71.07	71.12	70.93	98.13		97.64	94.47	94.38	94.51
●	SPVC isolate SSbles1_ZA	14	71.39	71.23	70.98	71.15	70.94	71.50	71.54	71.19	71.16	71.20	71.00	97.98	97.64		94.31	94.17	94.37
	SPVC isolate SPVC China_1	15	71.42	71.30	70.94	71.20	70.98	71.47	71.52	71.20	71.11	71.10	70.86	94.63	94.47	94.31		98.76	93.80
	SPVC isolate CW135	16	71.52	71.40	71.02	71.25	71.06	71.58	71.62	71.16	71.03	71.05	70.85	94.53	94.38	94.17	98.76		93.86
	SPVC isolate ECK17	17	71.34	71.26	71.00	71.18	71.09	71.48	71.53	71.25	71.26	71.27	70.98	94.74	94.51	94.37	93.80	93.86	
	SPVC isolate SPVC AM-MB2	18	71.32	71.26	70.93	71.17	70.89	71.37	71.44	71.33	71.27	71.32	71.02	93.62	93.28	93.35	93.27	93.30	95.25

B			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	SPV2 isolate AM-MB2	1		98.09	98.39	98.57	98.29	97.84	97.94	98.43	80.24	69.14	69.05	69.06	69.05	68.88	68.42	69.58
	SPV2 isolate LSU	2	98.09		97.96	97.96	97.83	97.37	97.47	98.02	80.04	69.04	69.01	68.97	68.96	68.94	68.27	69.67
	SPV2 isolate GWB-2	3	98.39	97.96		98.24	98.06	97.59	97.73	98.22	80.48	69.15	69.06	69.08	69.06	68.88	68.40	69.59
	SPV2 isolate AuScan	4	98.57	97.96	98.24		98.07	97.63	97.76	98.21	80.18	69.05	68.98	69.01	68.97	68.83	68.53	69.45
	SPV2 isolate HN77	5	98.29	97.83	98.06	98.07		98.69	98.18	98.65	80.17	69.40	69.30	69.29	69.27	69.10	68.74	69.68
	SPV2 isolate TM37	6	97.84	97.37	97.59	97.63	98.69		97.75	98.23	80.17	69.21	69.14	69.14	69.13	68.92	68.42	69.57
	SPV2 isolate SCN20	7	97.94	97.47	97.73	97.76	98.18	97.75		98.30	80.07	69.37	69.30	69.34	69.30	69.09	68.51	69.67
	SPV2 isolate SC6	8	98.43	98.02	98.22	98.21	98.65	98.23	98.30		80.42	69.23	69.15	69.16	69.14	68.93	68.57	69.59
●	SPV2 isolate SSBles-74_ZA	9	80.24	80.04	80.48	80.18	80.17	80.17	80.07	80.42		69.30	69.07	69.14	69.18	68.92	68.50	68.93
	SPVG isolate IS103	10	69.14	69.04	69.15	69.05	69.40	69.21	69.37	69.23	69.30		98.80	98.75	99.03	97.58	77.98	82.74
	SPVG isolate Jesus Maria	11	69.05	69.01	69.06	68.98	69.30	69.14	69.30	69.15	69.07	98.80		98.45	98.73	97.57	77.97	82.53
	SPVG isolate GWB-G	12	69.06	68.97	69.08	69.01	69.29	69.14	69.34	69.16	69.14	98.75	98.45		98.67	97.25	78.05	82.71
	SPVG isolate SC11	13	69.05	68.96	69.06	68.97	69.27	69.13	69.30	69.14	69.18	99.03	98.73	98.67		97.45	78.01	82.70
	SPVG isolate WCFR11	14	68.88	68.94	68.88	68.83	69.10	68.92	69.09	68.93	68.92	97.58	97.57	97.25	97.45		77.63	82.40
	SPVG isolate WT325	15	68.42	68.27	68.40	68.53	68.74	68.42	68.51	68.57	68.50	77.98	77.97	78.05	78.01	77.63		77.67
●	SPVG isolate SSBles-125_ZA	16	69.58	69.67	69.59	69.45	69.68	69.57	69.67	69.59	68.93	82.74	82.53	82.71	82.70	82.40	77.67	

Table 13: Pairwise comparison of the CP aa sequences of SPVG (A) and SPV2 (B) generated with sequences of sample SSbles (indicated with a dot) used as a source of multiple infection for the field trials conducted to determine the effects of single infection of potyviruses and co-infections of potyviruses and begomoviruses.

A			1	2	3	4	5	6	7
	SPVG isolate GWB-G	1		98.31	97.46	97.18	96.90	96.62	93.80
	SPVG isolate IS103	2	98.31		98.03	97.75	97.46	97.18	94.93
	SPVG isolate Jesus Maria	3	97.46	98.03		96.90	97.46	97.18	93.80
	WCFR11	4	97.18	97.75	96.90		97.18	96.90	94.37
●	SPVG isolate SSbles-125_ZA	5	96.90	97.46	97.46	97.18		97.46	94.37
	SPVG isolate UMP3	6	96.62	97.18	97.18	96.90	97.46		93.80
	SPVG isolate WT325	7	93.80	94.93	93.80	94.37	94.37	93.80	

B			1	2	3	4	5	6	7	8	9	10
	SPV2 isolate HN77	1		98.49	97.89	97.28	96.37	96.98	94.56	94.26	94.26	94.26
	SPV2 isolate TM37	2	98.49		98.19	97.28	96.37	96.98	94.56	94.26	94.26	94.26
	SPV2 isolate SC6	3	97.89	98.19		96.68	96.37	96.98	94.56	93.96	93.96	93.66
	SPV2 isolate SCN20	4	97.28	97.28	96.68		95.77	96.07	93.66	93.35	93.35	94.56
	SPV2 isolate LSU2	5	96.37	96.37	96.37	95.77		97.58	95.17	92.75	92.75	92.75
	SPV2 isolate AM-MB2	6	96.98	96.98	96.98	96.07	97.58		96.68	94.26	94.26	93.96
	SPV2 isolate GWB2	7	94.56	94.56	94.56	93.66	95.17	96.68		90.94	90.94	90.63
	SPV2 isolate Thomas16A	8	94.26	94.26	93.96	93.35	92.75	94.26	90.94		100.00	96.37
	SPV2 isolate Tchilomboiv1-1	9	94.26	94.26	93.96	93.35	92.75	94.26	90.94	100.00		96.37
●	SPV2 isolate SSbles-61_ZA	10	94.26	94.26	93.66	94.56	92.75	93.96	90.63	96.37	96.37	

Table 14: Alignment of the CP encoding aa sequences of SPVG (A) and SPV2 (B) generated with sequences of sample SSbles (indicated with a dot) used as a source of multiple infection for the field trials conducted to determine the effects of single infection of potyviruses and co-infections of potyviruses and begomoviruses.

[illegible]

The DAG motif is indicated by a solid box. Changes in the aa are shown dots denotes similar aa residues.

The DAG motif is indicated by a solid box. Changes in the aa are shown dots denotes similar aa residues.

Table 15: Marketable yield (MY) (ton ha⁻¹) in field trials of twelve sweetpotato varieties following the four virus treatments.

Trial 1	Varieties	TY_tmt1	% yield reduction	TY_tmt2	% yield reduction	TY_tmt3	% yield reduction	TY_tmt4	% yield reduction	TY_tmt5
	199602_1	6,100	80,1	7,533	75,4	5,433	82,28	8,5	72,2	30,667
	Blesbok	1,100	95,1	1,700	92,5	10,667	52,66	3,367	85,1	22,533
	Bophelo	0,167	98,2	6,200	34,3	7,200	23,67	5,333	43,5	9,433
	Bosbok	2,700	86,9	5,333	74,1	4,767	76,82	12,833	37,6	20,567
	Hernandez	1,067	92,0	9,133	31,5	7,833	41,25	6,033	54,8	13,333
	Impilo	5,667	67,8	9,433	46,4	11,600	34,09	7,300	58,5	17,600
	Monate	2,433	88,6	3,733	82,5	8,333	60,88	7,067	66,8	21,300
	Mvuvhelo	3,833	82,6	6,067	72,4	8,567	61,06	15,633	28,9	22,000
	Ndou	2,167	89,4	4,133	79,8	6,100	70,20	12,767	37,6	20,467
	Resisto	2,400	84,6	4,733	69,7	3,233	79,32	9,433	39,7	15,633
	Ribbok	5,500	66,6	7,233	56,1	11,467	30,36	5,700	65,4	16,467
	W-119	0,400	95,3	0,000	100,0	2,333	72,87	1,200	86,0	8,600
Trial 2	Varieties	TY_tmt1	% yield reduction	TY_tmt2	% yield reduction	TY_tmt3	% yield reduction	TY_tmt4	% yield reduction	TY_tmt5
	199602_1	0,367	97,6	0,500	96,8	3,133	79,7	13,933	10	15,400
	Blesbok	0,000	100,0	7,000	79,2	1,200	96,4	3,500	89,6	33,667
	Bophelo	0,000	100,0	1,300	93,3	0,733	96,2	2,233	88,4	19,267
	Bosbok	0,000	100,0	2,233	88,6	0,000	100,0	1,400	92,8	19,567
	Hernandez	0,967	90,0	0,000	100,0	3,500	63,9	5,833	39,9	9,700
	Impilo	0,333	98,6	0,000	100,0	0,900	96,2	5,867	75,0	23,467
	Monate	0,800	96,8	4,067	83,5	3,200	87,0	5,167	79,1	24,667
	Mvuvhelo	2,200	88,6	4,533	76,4	5,167	73,1	0,000	100,0	19,233
	Ndou	0,700	94,1	0,500	95,8	1,967	83,4	0,000	100,0	11,833
	Resisto	0,000	100,0	4,167	76,3	3,700	78,9	4,967	71,7	17,567
	Ribbok	4,700	58,4	3,233	71,4	1,333	88,2	1,267	88,8	11,300
	W-119	0,000	100,0	0,000	100,0	5,733	62,4	1,567	89,7	15,267
	Statistics for the combined trials					ANOVA				
	Mean: Trials combined	F Prob (model)	LSD (p=0.05)	CV%	SEM	F Prob: Trial*Var*Tmt (T5 included)			F Prob: Trial*Var*Tmt (T5 removed)	
	7,0	0.0012	3,4389	68,71	2,759	0,009			0,002	

MY=marketable yields, tmt= treatments, CV= Coefficient of Variance, SEM=Standard error of means, LSD= least significant difference, var= varieties.