Molecular Resolution of Genetic Variability of Major Sweetpotato Viruses and Improved Diagnosis of Potyviruses Co-infecting Sweetpotato

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Abstract

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The studies presented in this thesis contribute to improved resolution of genetic variability and diagnosis of potyviruses co-infecting sweetpotatoes.

The most prevalent viruses infecting sweetpotato in Tanzania were *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic stunt virus* (SPCSV) and Sweet potato chlorotic fleck virus (SPCFV). They were serologically detected and results confirmed by RT-PCR and sequencing. Nucleotide (nt) sequences of the 3´-proximal genomic part of SPFMV and SPMMV and partial *HSP70h* gene of SPCSV isolates from Tanzania and Uganda were determined. Results revealed limited genetic variability among East African (EA) isolates for SPFMV and SPCSV but high variability for SPMMV. Phylogenetic analysis grouped Tanzanian and Ugandan isolates into an EA strain for SPFMV and SPCSV. Phylogenetically, four strains of SPFMV were detected: EA, RC and O, and strain C which is distantly related to them. SPCSV isolates were grouped into two distantly related strains: EA group with only East African isolates, and a non-East African (NEA) group with isolates from West Africa and other parts of the world. No distinct strain groups could be inferred among the SPMMV isolates from East Africa

In Australia, Sweet potato virus Y (SPVY; synonymous to SPV-2) (family Potyviridae) was found to co-infect sweetpotatoes with strains RC and/or C of SPFMV. Five SPVY and SPFMV isolates were sequence-characterised for their 3'-proximal end of the genome and compared with isolates from Asia, Africa, Europe and North America. Phylogenetic analysis showed that Australian SPVY isolates formed a separate clade closest to the North American isolates.

Utilising findings of molecular resolutions of major viruses in East Africa and Australia, a simple and sensitive RT-PCR/RFLP based diagnostic procedure was devised to differentiate potyviruses in complexes which readily differentiated SPVY and strains C and RC of SPFMV.

Keywords: Genetic variation, *Sweet potato feathery mottle virus*, Sweet potato virus Y, *Sweet potato mild mottle virus*, *Sweet potato chlorotic stunt virus, Sweet potato chlorotic fleck virus*, virus complex, virus diagnosis, RT-PCR/RFLP, *Ipomoea batatas,* sweetpotato.

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To my beloved parents, relatives and friends

whose support and sacrifice I reached at this point of journey

and

everyone who supported me along the way.

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Tairo, F., Kullaya, A. & Valkonen, J.P.T. 2004. Incidence of viruses infecting sweetpotato in Tanzania. Plant Disease 88, 916-920.

II. Mukasa, S.B., Tairo, F., Kreuze, J.F., Kullaya, A., Rubaihayo, P.R. & Valkonen, J.P.T. 2003. Coat protein sequence analysis reveals occurrence of distinct strains of SPFMV in Uganda and Tanzania. Virus Genes 27, 49-56.

III. Tairo, F., Mukasa, S.B., Jones, R.A.C., Kullaya, A., Rubaihayo, P.R. & Valkonen, J.P.T. 2005. Unravelling the genetic diversity of the three main viruses involved in Sweet Potato Virus Disease (SPVD) and its implications. Molecular Plant Pathology 6, 199-211.

IV. Tairo, F., Jones, R.A.C. & Valkonen, J.P.T. 2005. Potyvirus complexes in sweetpotato: Occurrence in Australia, serological and molecular resolution, and phylogenetic analysis of the Sweet potato virus Y (SPV2) component (Submitted).

Abbreviations

aa	Amino acid
cDNA	Complementary DNA
CD	Chlorotic dwarf
CIP	Centro International de la Papa (International Potato Centre)
CP	Coat protein
cv.	Cultivar
DAS-ELISA	Double antibody sandwich-ELISA
EA	East Africa
ELISA	Enzyme-linked immunosorbent assay
Kb	Kilobase
kDa	Kilodalton
HC-Pro	Helper component proteinase
HSP70h	Heat shock protein 70 homolog
MAb	Monoclonal antibody
NCM	Nitrocellulose membrane
NEA	Non East African
nt	Nucleotide
ORF	Open reading frame
P-Pro	Papain-like proteinase
RdRp	RNA dependent RNA polymerase
RFLP	Restriction fragment length polymorphism
RT-PCR	Reverse transcription polymerase chain reaction
RNaseIII	Ribonuclease III
TAS-ELISA	Triple antibody sandwich-ELISA
SPCFV	*Sweet potato chlorotic fleck virus*
SPCSV	*Sweet potato chlorotic stunt virus*
SPFMV	*Sweet potato feathery mottle virus*
SPMMV	*Sweet potato mild mottle virus*
SPSMD	Sweet potato severe mosaic disease
SPVD	Sweet potato virus disease
SPVG	*Sweet potato virus G*
SPVY	Sweet potato virus Y
UTR	Untranslated region
VPg	Viral protein genome-linked

Introduction

The crop

Sweetpotato (*Ipomoea batatas* L.) is a dicotyledonous, perennial plant that produces edible tuberous roots. It belongs to the Morning glory family *Convolvulaceae* (genus *Ipomoea*) that is thought to contain over 500 species with ploidy levels ranging from 2x to 6x (Ozias-Akins and Jarret, 1994). Sweetpotato is the only *Ipomoea* species of economic importance as a food crop (Woolfe, 1992). It has both 4x and 6x forms (2n=4x=60 or 2n=6x=90) and probably originates from a cross between the ancestors of *I. trifida* and another wild *Ipomoea sp*., in central or northern South America, at least 5000 years ago (Huang and Sun, 2000). It is now believed to have a secondary centre of origin in the South West Pacific (Zhang *et al.,* 2004).

Production

Sweetpotato is among the world's most important versatile and adaptable food crops. With more than 127 million tons in annual production (FAO, 2004), sweetpotato currently ranks as the world's seventh most important food crop and fifth most important food crop on fresh-weight basis in developing countries after rice, wheat, maize, and cassava. Production is concentrated in East Asia, the Caribbean and tropical Africa. The bulk of the crop (82.7%) is grown in China (FAO, 2004). In Africa, sweetpotato is the second most important tuber crop after cassava. Production is concentrated in the East African countries around Lake Victoria. At an annual production of 2.6m tons, Uganda is the biggest producer of sweetpotato in Africa. Tanzania is the seventh biggest producer and second in East Africa with annual production of 0.97m tons. Rwanda ranks the third with 0.90m tons, followed by Kenya with an annual production of 0.58m tons (FAO, 2004).

Importance and utilisation

Sweetpotato plays an important role in East African farming systems. It performs well in relatively poor soils with few inputs and has a short growing season. Because of its hardy nature and broad adaptability it can be grown in several agro-ecological zones hence providing a sustainable food supply when other crops fail (Jana, 1982). Due to these qualities the crop has been in focus for global efforts steered by the International Potato Centre (CIP, Lima, Peru) to realise its full potential as a source of food, feed, processed products and source of income for millions of resource-poor farmers in developing countries. Sweetpotato is processed into candy, noodles, snacks, starch, liquor, flour and a variety of other industrial products. Its tubers are rich in vitamin C and essential mineral salts. The yellow and orange fleshed varieties represent the least expensive year-round source of dietary vitamin A available to poor families in Southern and Eastern Africa (CIP, 1999b). In addition to being used for human consumption, sweetpotato is also widely used as an animal feed (CIP, 2000b). Among the major starch staple crops, it has the largest rates of production per unit time (Woolfe, 1992): in some areas up to three harvests per year can be achieved (Karyeija *et al.,* 1998a). Despite its importance in food systems, production is mostly in developing countries by resource-poor farmers, mostly women. Due to its

enormous genetic diversity (Zhang *et al.,* 1998), and the accompanying diversity in phenotypic and morphological traits (Woolfe, 1992), the crop has great potential for further development to accommodate specific uses. It is in this respect that CIP with a mandate for research on sweetpotatoes in developing countries reviewed the general agronomic principles of sweetpotato in order to boost production. The results are increased areas planted with sweetpotato and output, e.g., in China and Africa after years of decline (CIP, 2000a). The crop is now a high priority commodity in the research and development agenda of national Agricultural Research Programmes of the East African countries.

Production constraints

In spite of intense global coordinated efforts on sweetpotato to realise its full potential as a source of food, feed and processed products through reviewed agronomic principles many constraints remain. One of these major constraints in increasing production of sweetpotato has been insect damage and plant diseases, of which viruses are by far the most serious problem causing substantial losses worldwide (CIP, 2000a). The most devastating syndrome of sweetpotato viruses are Sweet Potato Virus Disease (SPVD) caused by co-infection of *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV). Another severe disease is Chlorotic Dwarf (CD) caused by SPFMV, SPCSV and *Sweet potato mild speckling virus* (SPMSV) which occurs in South America (Di Feo *et al.,* 2000). SPVD can cause yield reduction of as high as 56-98% in Africa (Gibson *et al.,* 1998) and in numerous countries throughout the world (Carey *et al.,* 1999; Clark and Moyer 1998; Lenné 1991). The consequences are not only limited to reduction in crop yield but also undermine the ongoing efforts in genetic improvement for yield and quality, since farmers normally abandon susceptible but otherwise high yielding varieties (Aldrich, 1963), for instance recently promoted cultivars with high starch content and vitamin A levels.

The prevalence of SPVD in sweetpotato is, among other factors due to lack of rapid reliable routine techniques for detection and differentiation viruses involved. Furthermore, understanding of the epidemiology of SPVD and other complex diseases of sweetpotato has been complicated by limited knowledge of its causative viruses and their relative importance which are exacerbated by frequent mixed infections. Understanding of molecular characteristics of each component virus, their interactions with hosts and the mechanism by which viruses evolve and adapt to different hosts is a prerequisite for the control of virus diseases through quicker and accurate diagnosis.

Viruses, smart disease-causing pathogens

The virus is one of the simplest and most efficient replication machines to have evolved; instead of evolving its own machinery for making more copies of itself, it utilises a chosen host to do the task. A more refined definition by Hull (2002) seems to be best suited for defining the virus: "A set of one or more nucleic acid template molecules normally encased in a protective coat or coats of protein or lipoprotein that is able to organise its own replication only within suitable host cells. It can usually be horizontally transmitted between host protein-synthesising machinery, (2) organised from pools of the required materials rather than by binary fission, (3) located at sites that are not separated from the host cell contests by a lipoprotein bilayer membrane, and (4) continually gives rise to variants through various kinds of change in the viral nucleic acid''.

Viruses are to be blamed for the countless number of deaths resulted from infectious diseases such as polio, influenza and rabies. Human immunodefiency virus (HIV) causes AIDS that continue to cause human suffering throughout the world. The negative impacts of viruses are not only limited to direct loss of human life and livestock, but indirectly, many lives are also lost through hunger and starvation. It is estimated that viruses can cause crop losses worth up to 500 billion dollars each year to crops (Fermin *et al.,* 2000).

The persistence of viruses is influenced by their extreme evolutionary capacity that allow them to adapt and parasitise all known groups of organisms and rapidly adapt to numerous host species within a kingdom (Schneider & Roossinck *et al.,* 2001). One of their adaptive strategies is the efficient use of the limited amount of genome nucleic acids they possess. To cope with the large size of their preys (plant hosts), viruses have devised their gene products to adapt multifunctional roles, e.g., Hc-Pro and CP proteins for potyviruses (Hull, 2002). Their existence as quasispecies, an important biological characteristic (Domingo *et al.,* 1997), effects their adaptability within host cells in spite of environmental changes, probably conferring them freedom to move from one host to another (Rybicki, 1994; Kikkert *et al.,* 1999). For instance, RNA viruses often exploit mutation to achieve changes in host range and escape recognition by the host immune response e.g. HIV-1 thus making them masters of disguise (Mascola and Montefiori, 2003). RNA viruses due to their small genome size of an average 3 to 10 kb, generally tolerate higher levels of mutagenesis (Domingo, 1997) as compared to DNA viruses. Complex genetic information contained in DNA genomes relative to their large size genomes; compromises the maintenance of its complex information hence less tolerant to mutagenesis compared to RNA viruses. Despite increased evidence (Anandilakshmi *et al.,* 1998; Kasschau *et al.,* 1998) that hosts recruit RNA silencing as a cellular surveillance mechanism against virus invasion, also viruses retaliate by ensuring their successful invasion and colonisation by counteracting silencing by encoding silencing-suppressor proteins (Kreuze *et al.,* 2005; Moissiard *et al.,* 2004). It is believed that some of the viruses in the *Closteroviridae* family (Karasev, 2000; Kreuze *et al*., 2005) co-evolve with their hosts and may have taken up some important genes (*HSP70h*, *RNaseIII*) to strengthen their counter-defence systems.

Viruses infecting sweetpotato

Since early reports of suspected viral diseases of sweetpotato in the USA (Moyer & Salazar, 1989) and in Eastern Africa (Hansford, 1944), today more than 20 viruses are known to infect cultivated sweetpotato worldwide (Loebenstein *et al*., 2003). Traditionally, bioassays (host range, symptoms and vector transmission) were largely used to explain the etiology of the various diseases which is time consuming and challenging since extensive knowledge of taxonomy is a pre-requisite. Advancement of electron microscopy, serology and currently molecular biology techniques have improved detection and characterisation of viruses from different parts of the world. Of the 17 described viruses worldwide, 11 have been confirmed and recognised by the International Committee of Taxonomy for Viruses (Table 1).

Table 1. Viruses that have been reported from sweetpotatoes

Virus	Genus	Virus distribution	Reference
Approved species:			
Sweet potato feathery mottle virus (SPFMV)(74)[1]	*Potyvirus*	Worldwide	1, 2, 3, 4
Sweet potato latent virus (SPLV) (3)	*Potyvirus*	Taiwan, Peru, China, Japan	5, 6, 7
Sweet potato mild speckling virus (SPMSV) (1)	*Potyvirus*	Argentina, Brazil, Peru	8
Sweet potato virus G (SPVG) (5)	*Potyvirus*	China, Egypt, USA	9, 10, 11
Sweet potato mild mottle virus (SPMMV) (17)	*Ipomovirus*	East Africa	12, 5
Sweet potato chlorotic stunt virus (SPCSV) (29)	*Crinivirus*	Worldwide	13, 14 ,15, 16
Cucumber mosaic virus (CMV)	*Comovirus*	Israel, Kenya, Egypt,	17, 18
Tentative species:			
Ipomoea yellow vein virus (IYVV)(1)	Begomovirus	Spain	19
Sweet potato leaf curl virus (SPLCV)(3)	Begomovirus	USA, Sicily	20, 21
Sweet potato leaf curl Georgia *virus* (SPLCGV)(1)	Begomovirus	USA	22
Sweet potato yellow dwarf virus (SPYDV)(0)	Ipomovirus	Taiwan, Far East	5
Sweet potato virus Y (SPVY)(15)	Potyvirus*	USA,Taiwan, Spain S. Africa, Zambia	23, 24, 9
Sweet potato chlorotic fleck virus (SPCFV) (3)	Carlavirus*	S. America, East Africa, Uganda	25, 26, 27
Sweet potato vein mosaic virus (SPVMV)(0)	Potyvirus	Argentina	28
Sweet potato ringspots virus (SPRSV) (0)	Nepovirus	Papua New Guinea	29
Sweet potato caulimolike virus (SPCaLV)(0)	Caulimovirus	PuertoRico, Uganda	30, 26
Sweet potato leaf speckling virus (SPLSV) (0)	Luteovirus	Peru, Cuba	31

(For footnote, see the next page)

Footnote to Table 1

1) Abad *et al.,* 1992; 2) Colinet & Kummert *et al.,*1996; 3) Sakai *et al.,* 1997; 4) Kreuze *et al.*, 2000; 5) Liao *et al.,* 1979; 6) Colinet *et al.*, 1997; 7) Yu *et al.,* 2002; 8) Alvarez *et al.,* 1997; 9) Souto *et al.,* 2003; 10) IsHak *et al.,* 2003; 11) Colinet *et al.,* 1994; 12) Hollings *et al.,* 1976; 13) Winter *et al.,*1992, 14) Cohen *et al.,* 1996; 15) Hoyer *et al.,* 1996; 16) Alicai *et al.,* 1999; 17) Cohen & Loebenstein, 1991; 18) IsHak *et al.,* 2002; 19) Banks *et al.,* 1999; 20) Lotrakul *et al.,* 1998; 21) Briddon, R. W., unpubl; 22) Lotrakul, P., unpubl; 23) Ateka *et al.,* 2003; 24) Ateka, E., unpubl; 25) CIP, 1993; 26) Aritua and Adipala, 2004; 27) Mukasa, 2004; 28) Nome *et al.,* 1974; 29) Brown *et al.,* 1988; 30) Atkey & Brunt, 1987; 31) Fuentes *et al.,* 1996;

1= Number of isolates confirmed by sequence data
*= Genus has been determined by percent sequence identity with representative members of the respective genus.

Potyviruses

Potyvirus comprise the largest genus among the six genera of the family *Potyviridae*, the others being *Macluravirus, Bymovirus, Rymovirus, Tritimovirus* and *Ipomovirus*. These viruses have monopartite genomes, with the exception of the bipartite bymoviruses (van Regenmortel *et al*., 2000). Members of the genus *Potyvirus* are predominantly transmitted by aphids in a non-persistent manner. The virus is carried in the aphid stylet where it remains infectious only for a short period of time (minutes to a few hours). Rymoviruses and tritimovirus are transmitted by mites of genus *Abacarus* or *Aceria*, respectively (van Regenmortel *et al*., 2000). Ipomoviruses (SPMMV) may be transmitted by whiteflies to sweetpotato (Hollings *et al*., 1976) while bymoviruses are transmitted by plasmodisphorids (root infecting parasites once considered to be fungi).

Potyviruses with probably more than 200 members constitute the largest and economically most important group of plant viruses comprising nearly 25% of the known plant viruses (Khan & Dijkstra, 2002). Their virions are flexuous, non-enveloped, rod-shaped particles, 680-900 nm long and 11-15 nm wide (Urcuqui-Inchima, *et al*., 2001). One of their characteristic features is that they form virus–encoded cytoplasmic cylindrical inclusion (CI) bodies in the cytoplasm (Van Regermortel, 2000).

Table 2. Known functions of the mature potyviral proteins*

Protein	Functions	References
P1	Proteinase	1, 2
	Modulator of gene silencing	3
	Virus replication/virus propagation	4, 5
Hc-Pro	Proteinase	6
	Aphid transmission	7
	Seed transmission	8
	Suppressor of gene silencing	9
	Cell-to-cell and systemic movement	10, 11
	Virus replication /virus propagation	12
P3	Genome amplification	13, 14
6K1	Virus replication	15
CI	RNA Helicase	16
	Cell-to-cell movement	17, 18
6K2	Virus replication	19
	Long distance movement	20
	Symptom induction	21
NIa/Vpg	Binds to initiation factors eIF(iso)4E and eIF4E	22
	Cell-to-cell and systemic movement	23
	Virus replication	24
NIa/Pro	Protease	25
NIb	RNA-dependent RNA polymerase	26
CP	Encapsidation of RNA	27
	Cell-to-cell and systemic movement	28, 29
	Aphid transmission	30
	Seed transmission	8
	Virus replication/virus propagation	31

*= All coding regions as well as the 5´and 3´UTR are essential for virus propagation (Klein *et al.,* 1994; Kekarainen *et al.,* 2002). 1) Carrington, Freed, D.D & Sanders T.C 1989; 2) Carrington, Freed & Oh 1990; 3) Anandalakshmi *et al.,* 1998; 4) Verchot & Carrington, 1995; 5) Kekarainen, Savilahti & Valkonen, 2002; 6) Carrington *et al.,* 1989; 7) Atreya *et al.,* 1992; 8) Wang and Maule, 1994; 9) Kasschau & Carrington, 1998.; 10) Kasschau *et al.,* 1997, 11) Klein *et al.,* 1994; 12); Kasschau & Carrington, 1997, 13); Merits *et al.,* 1999; 14) Rodriguez-Cerezo *et al.,* 1993; 15) Reichmann *et al.,* 1992; 16) Lain *et al.,* 1990; 17) Carrington *et al.,* 1998; 18) Roberts *et al.,* 1998; 19) Schaad, Jense & Carrington, 1997; 20) Rajamäki and Valkonen, 1999; 21) Spetz and Valkonen, 2004; 22) Wittman *et al.,* 1997; 23) Schaad *et al.,* 1997; 24) Schaad *et al.,* 1996; 25) Dougherty *et al.,* 1989; 26) Hong and Hunt, 1996; 27) Jagadish *et al.,* 1993; 28) Dolja *et al.,* 1994; 30) Atreya *et al.,* 1995; 31) Haldeman-Cahill *et al.,* 1998.

SPFMV

SPFMV (genus *Potyvirus*, family *Potyviridae*) is the most common sweetpotato virus worldwide (Moyer and Salazar, 1989). SPFMV genome consists of a positive-sense single-stranded linear ssRNA of about 10.8 kb with a poly(A) region at the 3´end (SPFMV-S; Sakai *et al*., 1997). The genome is larger than the average (9.7kb) of a potyvirus genome (Shukla *et al*., 1994). SPFMV CP is exceptionally large (38 kDa) as compared to other potyviruses (Abad *et al*., 1992). Like other potyviruses the genome contains a single ORF, flanked by a UTR at both the 5´-end 3´-ends (Figure 1) and encodes a large polyprotein ca. (3490 aa) that is processed to mature proteins by virus-encoded proteases: P1, HC-Pro and NIa-Pro (Reichmann *et al*., 1992). P1 and HC-Pro catalyse their own cleavage from the polyprotein (Carrington *et al*., 1989) while NIa-Pro is responsible for the cleavage of the C-terminal two-thirds of the polyprotein (Dougherty and Carrington, 1998). The primary events are probably co-translational and autocatalytic, yielding precursors and mature products. The fully processed proteins are P1 proteinase (P1-Pro), helper component proteinase (Hc-Pro), the third protein (P3), 6kDa protein 1 (6K1), cylindrical inclusion protein that is an RNA helicase (CI), 6kDa protein 2 (6K2), nuclear inclusion protein a (NIa), which can be further processed into the viral protein genome-linked (VPg) and NIa proteinase (Pro). The last two proteins are the nuclear inclusion protein b (NIb) and the CP. The functions of each mature protein have been described in Table 2.

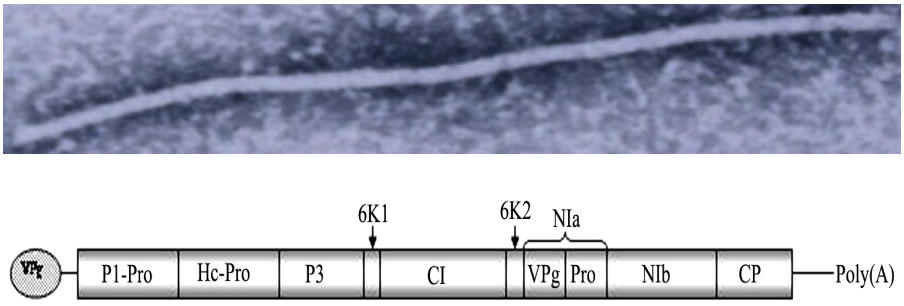

Figure 1. Particle morphology (courtesy J. Valkonen) and genome organisation of a potyvirus. The particle (above) is flexuous filamentous rod that encapsidates a single positive stranded RNA molecule. The genome (below) contains one open reading frame and names of the final protein products are indicated as boxes, separated by lines that indicate the putative cleavage sites of the polyprotein. The 5´-end and 3´-untranslated regions are indicated by single lines. The RNA genome is 3´-polyadenylated and has a viral protein (VPg) linked to the 5´-end.

SPFMV enters the host cell via a stylet of several aphid species (e.g *Aphis gossypii, A. craccivora, Myzus persicae*) in a non-persistent manner. The transmission by aphids depends on the HC-Pro and an N-terminal amino acid motif Asp-Ala-Gly in the CP (DAG; Atreya *et al*., 1992). Their host range is narrow, limited to plants of the family *Convolvulaceae* (genus *Ipomoea*). Some strains have been reported to infect *Nicotiana benthamiana* and *Chenopodium amaranticolor* (Campbell *et al*., 1974; Moyer *et al*., 1980). As other potyviruses, traditional criteria to discriminate between species and isolates are predominantly

based on serology and biological criteria such as host range, cross-protection and symptomatology (Shukla *et al*., 1994). SPFMV can routinely be diagnosed by indexing on a sensitive indicator host *I. setosa* (Green *et al*., 1988) and serology with available antibodies from CIP, Lima, Peru.

However, the use of serology for SPFMV requires confirmation with RT-PCR and/or cloning and sequencing as conflicting/incomplete results are normally encountered due to serological relationships between virus species (cross-reactions) or lack of detection of all strains of SPFMV. As for the case of SPFMV, cross-reaction has also been experienced between strains EA and RC (and probably O) using antibodies raised against the viral CP that is highly similar between these strains. Moreover, lack of detection of some strains is perhaps due to the variability of the N-terminus of CP that may be lost due to proteolysis during virus extraction from plant tissues. This has been reported from SPFMV (Shukla *et al*., 1994). Comparing nucleic acid sequences especially the 3´-end UTR and CP gene sequences (Shukla *et al*., 1994; Berger *et al*., 1997; Van Regenmortel *et al*., 2000, Adams *et al*., 2005b) has become a powerful tool not only for studying the taxonomy of the viruses belonging to potyvirus genus but also to differentiate closely related strains or isolates (Berger *et al*., 1997; Adams *et al* 2005).

The common strain (SPFMV-C) and russet crack strain (SPFMV-RC) of SPFMV were originally described based on serological differences and the different types of symptoms induced in sweetpotato (Moyer, Kennedy & Abou-Ghadir, 1980; Moyer & Kennedy, 1978, Cali & Moyer 1981). Though SPFMV alone generally causes only minor damage to sweetpotato cultivars, the RC strain is associated with russet cracking of the tuberous roots in certain cultivars and has been reported from China (Chen *et al*., 2001), Japan (Sakai *et al*., 1997), Egypt (IsHak *et al*., 2003), Korea (Ryu *et al*., 1998) and the USA (Cali and Moyer, 1981; Abad *et al*., 1992). Isolates of strain C deviate from RC by 82% aa and have been reported from Argentina, China and the USA (Abad *et al*., 1992; Colinet *et al*., 1998). Additional molecular data on SPFMV isolates (Kreuze *et al*., 2000) further revealed two additional phylogenetic lineages: ordinary strain (O) and the East African strain (EA), relatively closely related to RC group but clearly distant from C strain by 75.8-78.3% nt identity. The O strain isolates have been reported from Niger, Nigeria, Japan, Korea, China and Argentina while the EA strain isolates have only been found in Uganda and Madagascar (Kreuze *et al*., 2000).

SPVY

Sweet potato virus Y (synonymous to SPV-2) was for the first time detected in Taiwan from the sweetpotato plants showing mild virus-like symptoms, such as mottle, vein yellowing and/or ring spots (Rossel and Thottappilly, 1988). Based on the observed first symptoms in the sweetpotato plants, the virus was thought to be an SPFMV isolate (Rossel and Thottappilly, 1988). However, in the indicator host *Nicotiana benthamiana*, the virus induced mild yellowing and chlorotic mottles which were unusual symptoms for SPFMV (Rossel and Thottappilly, 1988). A subsequent attempt to transmit the virus to some sweetpotato clones resulted in no symptoms. Moreover, in serological characterisation the virus appeared to be

different from SPFMV. Therefore based on these biological and serological distinctions from SPFMV, the virus was later designated as SPV-2 (Rossel and Thottappilly, 1988; Ateka *et al*., 2004b).

SPV-2 has filamentous particles with an approximate length of 850 nm and induces cylindrical inclusions comprising pinwheels and scrolls characteristic of potyviruses (Ateka *et al*., 2004b). The virus is aphid-transmitted and mechanically can only be transmitted to *Ipomoea* and *Nicotiana* spp. (Loebenstein *et al*., 2003). SPV-2 can systematically infect *Ipomoea* and *Nicotiana* spp. and induce symptoms ranging from vein clearing to mosaic and leaf deformations (Ateka *et al*., 2004a). However, SPV-2 can systematically infect sweetpotato cv.Tanzania to a detectable level only when the plant is pre-infected with SPCSV (Ateka *et al*., 2004b). Serologically, SPV-2 has no close relationship with any presently known potyviruses. Its detection is possible using the antibodies available. Since its first description 1980s (Rossel and Thottappilly, 1988), it is only recent that molecular information available shows that SPV-2 is closely related to the tentatively named Ipomoea vein mosaic virus (IPMV) isolates LSU2 and LSU 5 (Souto *et al*., 2003) from the USA and distantly related to SPFMV (Ateka *et al*., 2004b). Strong synergistic symptoms distinct from SPVD have been reported in sweetpotato plants of cv. Beauregard co-infected with LSU2 or LSU5 and SPCSV (Souto *et al*., 2003). SPV-2 was confirmed to be a member of potyvirus by characterisation of the 3´proximal genomic sequence. Owing to its failure to induce any obvious symptoms on sweetpotato, the virus was proposed to be named SPVY (Ateka *et al*., 2004b).

SPMMV

SPMMV (genus *Ipomovirus*, family *Potyviridae*) was originally described from Kenya, Uganda and Tanzania (Hollings *et al.,* 1957) and has been serologically detected in South Africa, Egypt, Indonesia, New Zealand and Peru (Carey *et al.,* 1998; Fletcher *et al.,* 2000; Thompson *et al.,* 2003). The virus has flexuous filamentous particles between 830-850nm in length. It contains a (+)ssRNA genome of about 10.8kb (Colinet *et al.,* 1998b) similar to those of other *Potyviridae* except the bymoviruses (van Regenmortel *et al.,* 2000). SPMMV is a type member in the genus *Ipomovirus*, including also *Cassava brown streak virus* (CBSV) (Monger *et al.,* 2001), *Cucumber vein yellowing virus* (CVYV) (Lecoq *et al.,* 2000) and *Tomato mild mottle virus* (ToMMV) (Monger *et al.*, 2001; Adams *et al.,* 2005a). SPMMV was once reported to be transmitted by whiteflies (*Bemisia tabaci*) in a persistent manner (Hollings *et al.,* 1976). The virus has a wide host range and has been successfully transmitted to 14 plant families (Hollings *et al.,* 1976). It induces leaf mottling, vein chlorosis, dwarfing and poor growth on sweetpotato plants. Morphologically and in size, the virion is similar to potyviruses. Cytoplasmic inclusions are also induced in SPMMV-infected cells (Moyer and Salazar, 1989). The difference between SPMMV and potyviruses is the DAG tripeptide that is associated with aphid-transmibility of potyviruses (Atreya *et al.,* 1992) but is missing from SPMMV (Mukasa *et al.,* 2003b). Serologically, SPMMV has no relationship with potyviruses. In spite of a wide genetic variability (82-100% aa) in the sequence of the CP-encoding region of SPMMV isolates from East Africa (Mukasa *et al.,* 2003b), diagnosis is fairly

reliable using serology with available antibodies. Little is known about genetic diversity of this virus from outside EA though there have been reports on its occurrence in different countries outside Africa.

SPCSV

SPCSV is a member of the genus *Crinivirus* under the family *Closteroviridae* (Kreuze *et al.,* 2002). The family *Closteroviridae* is composed of mainly three taxa (Van Regenmortel *et al.,* 2000). *Ampelovirus* includes mealybug-transmitted viruses with monopartite genomes. Criniviruses have a bipartite genome and are transmitted by whiteflies. Closteroviruses are mostly aphid-transmitted and have monopartite genomes. Members of the *Closteroviridae* have the largest genomes of all plant (+)ssRNA viruses approaching 20 kb (Martelli *et al.,* 2002).

SPCSV is an agriculturally important pathogen of sweetpotato transmitted by whiteflies (e.g. *Bemisia tabaci* and *Trialeurodes abutilonea*) in a semi-persistent non-circulative manner (Sim *et al.,* 2000). SPCSV hosts are limited mainly to the genus *Ipomoea,* some species of *Nicotiana* and *Amaranthus palmeri* (Cohen *et al.,* 1992) and wild species of lisiantus (*Eustoma grandiflorium*) (Cohen *et al.,* 2001). SPCSV is phloem-limited. None of the criniviruses can be sap-transmitted (Martelli *et al.,* 2002). SPCSV has flexuous particles of 850-950 nm in length and 12 nm in diameter. The genome consists of two RNA molecules (Kreuze *et al.,* 2002). RNA1 (9407 nt) contains five putative ORFs for replication–related proteins and RNA2 (8223 nt) seven putative ORFs typical of the family (Figure 2). The virus encodes two types of CP proteins the major CP of 33kDa and a minor CP. These proteins are analogous to other *Criniviruses* (Cohen *et al.,* 1992).

The genomic organisation of all criniviruses shares two distinct features. The genome organisation of SPCSV is shown in Figure 2. The key genomic features include RNA1 which includes polyprotein encoding viral replication proteins such as papain-like protease, methlytransferase and helicase domain (ORF1) and an RdRp encoded from ORF1b by a +1 frameshift (Martelli *et al.,* 2002, Coutts *et al.,* 2003; Tzanetakis *et al.,* 2005). The 3´ proximal half of the RNA2 (Figure 2) encodes an array of four genes (*HSP70h, CPh, CP* and *CPm*) that are expressed via subgenomic RNAs characteristic of all members of the family *Closteroviridae (*Karasev *et al.,* 2000). These genes are believed to be associated with movement and virion assembly in all *Closteroviridae* (Peremyslov *et al.,* 2002; Napuli *et al.,* 2003).

The remarkable feature of the genus *Crinivirus* is its significant divergence in ORFs downstream of the replication proteins. Unlike *Potyviridae* with similar genome organisation and a standard set of genes for all members, the crinivirus genome organisation is variable among its members regardless of similar general layout of the genome. For instance the occurrence of ORFs that encode an RNase III-like protein, a small hydrophobic protein (p7) and a 3´-terminal protein (p22) have only been described downstream of RdRp of SPCSV RNA1 (Figure 2) (Kreuze *et al* 2002) and absent in other members of the genus (Klassen *et al.,* 1996; Livieratos *et al.,* 2004; Wintermantel *et al.,* 2005; Tzanetakis *et al.,* 2005).

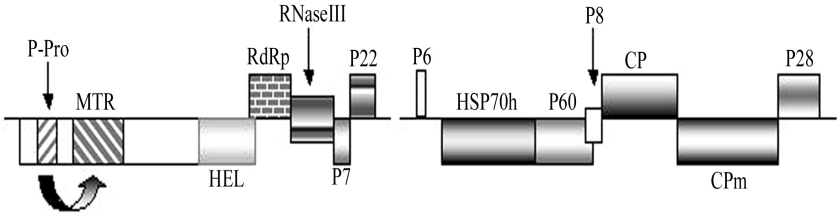

Figure 2. Schematic representation of the genome organisation of SPCSV (Kreuze *et al.,* 2002). The functional domains in each ORF are indicated above/below the boxes. The boxes correspond to ORFs and the putative proteins and their functions: *P-Pro*; Papain-like protease including an arrow to indicate the predicted auto catalytic cleavage site; *MTR*: methyltransferase domain; *HEL*: helicase domain; *RdRp*: RNA dependant RNA polymerase domain; *Hsp70h:* heat shock 70 family protein homologue; *CP*: coat protein, *CPm*: coat protein minor. Additional two ORFs: absent in other some members of *Crinivirus* in RNA1: RNaseIII: (ribonuclease III) and p22. The lines at the 3´-ends of SPCSV RNA1 and RNA2 indicate the near–identical 3´sequences.

Analyses of the genome sequence of SPCSV revealed several new features as compared to other *Closteroviridae*. Distinctively, SPCSV encodes proteins that are not found in any viruses. For instance RNA1 contains an ORF for a putative RNaseIII and also 22 kDa protein (p22) (Figure 2) that shows no significant similarity to any known proteins from any organism (Kreuze *et al.,* 2002). The most striking is the evidence shown (Kreuze *et al.,* 2005) that the two novel proteins (RNase III & p22) cooperatively control the RNA silencing suppressor function, which may elucidate the mechanisms employed by SPSCV to breakdown host resistance in favour of unrelated virus during dual infection on sweetpotato which causes SPVD (Gibson *et al.,* 1998; Karyeija *et al.,* 200b).

The detection of SPCSV is fairly reliable; combination of a biological assay using sweetpotato cv. TIb 8 pre-infected by SPFMV and strain specific monoclonal antibodies (MAbs) (Hoyer *et al.,* 1996; Cohen *et al.,* 1992) has been routinely used in many countries including Africa. The highly conserved heat shock protein 70 family homologue (*Hsp70h*) gene unique to *Closteroviridae* viruses (Agranovsky *et al.,* 1997) can be used as a target for primers designed to the conserved phosphate domains encoded in the N-terminal part of the *Hsp70h* gene and reliably used for detection of *Closterovirus* infections in plants (Tian *et al.,* 1996; Fenby *et al.,* 2002).

Based on partial *HSP70h* (nt) sequences and characterisation with monoclonal antibodies, SPCSV exists as two serotypes and phylogenetic groups: SPCSV-NEA with isolates from many different geographic origins (IsHak *et al.,* 2003) and SPCSV-EA, a distinct group with isolates from East Africa (Kreuze *et al.,* 2002; Fenby *et al.,* 2002). However, the resolution of the SPCSV diversity is likely to change when more sequences from other regions are available (Carey *et al.,* 1998; Gutierrez *et al.,* 2003; Valvarde *et al.,* 2004).

SPCFV

SPCFV is one of the less characterised viruses of sweetpotato. The virus was first detected in the sweetpotato accession DLP942 from the CIP´s germplasm collection showing chlorotic spots (Fuentes and Salazar, 1992). SPCFV has a wide geographic distribution and serologically has been reported from different countries: Colombia, Brazil, Philippines, India, Cuba, Panama, Bolivia, Peru, China, Japan, Uganda, Kenya, India, Indonesia and Austria (Fuentes and Salazar, 1992; Usugi *et al.,* 1991; Ateka *et al.,* 2004).

SPCFV is mostly symptomless in its natural host and no known vector has been reported for its transmission. The virus can be transmitted mechanically to *Convolvulacea*e and *Chenopodaceae* species (Fuentes and Salazar, 1992). Although SPCFV can be mechanically transmitted onto *Ipomoea* species, no clear symptoms are induced on the universal indicator host *I.setosa*. Previous morphological characterisation of SPCFV revealed a flexuous rods ca. 750-800 nm length with a CP of Mr 34.5 KDa (Fuentes and Salazar, 1992). Due to close similarity of its filamentous particle to that of potyviruses, SPCFV was first thought to be a *potyvirus* (Fuentes & Salazar, 1992). However, no serological relationship with any potyvirus-infecting sweetpotatoes has been reported.

Detection is fairly good with available antibodies from CIP. Serologically, SPCFV relates with an uncharacterised Japanese isolate (Sweet potato symptomless virus: SPSV) (Loebenstein *et al.,* 2003) although morphologically they are distinct from each other. A complete nucleotide sequence of SPCFV (Aritua and Adipala*,* 2004) has recently been determined and shown to represent a distinct species in the genus *Carlavirus* under the family *Flexiviridae.*

Viral synergism and disease complexes

Infection of plants by multiple viruses is a common phenomenon. Double or mixed infection of plants often results in one virus assisting a second, co-infecting virus leading to increased titre and intensified symptom severity, a phenomenon known as synergism (Hull, 2002). Synergism has been reported from viruses and their satellite virus or RNA (Rodriguez-Alvarado *et al.,* 1994; Sanger *et al.,* 1994; Scholthof, 1999), or between viruses and viroids (Valkonen, 1992). The mechanism behind synergism occurs in diverse ways. For instance, unilateral synergism by the helper virus may aid another virus in movement (Barker, 1989), and thereby enable it to invade tissues it otherwise could not. In some cases, viral replication and accumulation (Savenkov and Valkonen, 2001) or transmissions by vector are enhanced (Falk & Duffs, 1981; Zhang *et al.,* 2000).

The most studied synergism cases are those where potyvirus induces an increase in the titres of a second, unrelated virus (Rochow and Ross, 1995; Goodman and Ross, 1974a; Vance, 1991). Unexpectedly, SPCSV, a crinivirus enhances the titres of potyvirus (Karyeija *et al.,* 2000b) and the ipomovirus (Mukasa *et al.,* 2006) in co-infected plants. The economical importance of viral synergism in sweetpotato production is the complex diseases they induce when co-infecting sweetpotatoes. Sweet potato virus disease (SPVD) is the most commonly known complex disease of sweetpotato. The disease was first described in 1939 in Ituri province in the

Democratic Republic of Congo, then 1944 in Uganda (Hansford, 1944) and later in Kenya, Tanzania, Rwanda, Burundi, Malawi and thence Transvaal Province in South Africa (Sheffield, 1957). The disease is caused by synergistic interaction between SPFMV and SPCSV (Schaefers & Terry, 1976; Winter *et al.,* 1992; Gibson *et al.,* 1998). The disease can reduce tuber yield of infected plants by up to 80% (Mukiibi, 1977). Yield reduction is the consequence of reduced photosynthesis (Njeru *et al.,* 2004) associated by stunting and leaf distortions, characteristic symptoms of the disease (Hahn, 1979). Sweet potato chlorotic dwarf (CD) is another recognised complex disease described in Argentina (Di Feo *et al.,* 2000) and is caused by interaction between SPMSV, SPFMV and SPCSV. In addition, in Israel *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*) was found to infect sweetpotato together with SPCSV and usually also SPFMV, producing similar symptoms to SPVD and up to 80% in yield loss (Milgram *et al.,* 1996).

Following the discovery of more complex diseases with more or less similar symptoms to SPVD, the name SPVD has been retained specifically to the association involving SPCSV and SPFMV. To avoid confusion it was proposed (Salazar & Fuentes, 2000) that the new complex diseases should be named differently. For example, the recently studied complex disease caused by co-infection of SPCSV and SPMMV is proposed to be named Sweet Potato Severe Mosaic Disease (SPSMD) (Mukasa *et al.,* 2006). More commonly, in all recognisable viral synergistic diseases, infection with each virus separately causes only mild or no symptoms in sweetpotatoes while severe disease is induced in the presence of SPCSV. Moreover, symptoms are even more severe in a triple infection involving SPCSV (Di Feo *et al.,* 2000; Mukasa *et al.,* 2006). In contrast to potyvirus-associated synergism (Vance *et al.,* 1995; Pruss *et al.*, 1997), in the SPCSV-associated synergism, the level of SPCSV titres are reduced (Mukasa *et al.,* 2006). Although types and nature of synergism and their resulting diseases have largely been reported from other crops (Fondong *et al.,* 2000; Wang *et al.,* 2004; Andika *et al.,* 2005), it was only recently that SPCSV was evidently shown to break host resistance in sweetpotato in favour of other co-infecting viruses (Karyeija *et al.,* 2000b).

Sweetpotato virus detection

The detection and identification of sweetpotato viruses remains a cumbersome procedure. This is complicated by frequent occurrence of mixed infections and synergistic complexes such as SPVD (Moyer & Kennedy, 1978). Traditional methods such as symptom diagnosis based on biological indexing, serology and PCR are mostly used despite their limited reliability and sensitivity. Thus, it requires several tests to certainly identify the viruses/strains present in a diseased plant.

Biological indexing on susceptible indicator hosts, which entails graft-inoculation onto *Ipomoea* species, is widely used to assay many sweetpotato viruses (Green *et al.,* 1988; Loebenstein *et al.,* 2003). However, the plants produce nearly similar symptoms which are difficult to interpret or rule as diagnostic symptoms for particular virus species/strains. For example, the indexing method for SPFMV/SPCSV has been graft-inoculation onto a sweetpotato tester clone Tib 8

(Thottappilly and Rossel, 1988) pre-inoculated by SPCSV/ SPFMV, respectively. The resulting SPVD-like symptoms are taken as conclusive diagnosis for presence of SPFMV/SPCSV. The true identity of the virus strain present requires subsequent confirmatory assays based on serology and/or RT-PCR.

Table 3. Predominant symptoms on different plant hosts for indexing different sweetpotato virus species.

Virus	Indicator host*	Symptoms	Reference
SPFMV	*I. batatas*	Transient Chlspt	1, 2, 3
	I. setosa	SI VC	
	I. incarnata	SI VC, VB	
	I. nil	SI VC, VB	
SPVY	*I. batatas*	SL	4, 5,6
	I. setosa	VC, ChlMos	
	N. benthamiana	SI VC, LD, Mos	
	C.quinoa	ChL	
SPMMV	*I. batatas*	MdVChl/diffuse Mot,	7, 8, 9, 10
	I. setosa	VC,	
	N. glutinosa	VC, LC, LD	
	C. quinoa	LL	
SPCFV	*I. batatas*	SL	10, 11
	I. setosa	ns	
	I. nil	Fine Chlspt	
	C. quinoa	LL	
SPCSV	*I. batatas*	MdChlspt, Pup	12, 13, 4
	I. setosa	ChlSt	

*, Standard indicator hosts by ICTV VIDE database
C, Chenopodium; I, Ipomoea; N, Nicotiana; ChL, chlorotic lesion; ChlMos, chlorotic mosaic; Chlspt, chlorotic spot; Chlst, chlorotic stunting; LC, leaf curling; LD, leaf deformation LL; local lesion; MdVChl, mild vein chlorosis; Mos, mosaic; Mot, mottling; ns, no clear symptom; Pup, purpling; SL, symptomless; SI, systemic infection;; VB, vein banding; VC, vein clearing.
1) Moyer *et al,* 1980; 2) Campbell, 1974; 3) Karyeija *et al.,* 1998a; 4) Rossel and Thottappily, 1988; 5) Souto *et al.,* 2003; 6) Ateka *et al.,* 2004b; 7) Mukasa *et al.,* 2003a; 8) Hollings, *et al.,* 1976; 9) Mukasa *et al.,* 2006; 10) Loebenstein, 2003; 11) Fuentes and Salazar, 2000; 12) Gibson *et al.,* 1998; 13) Gibson and Aritua, 2002.

Serological tests represent a more practical method in routine diagnostics due to convenience and possibility of standardisation. To date, a kit containing antibodies for 10 sweetpotato viruses is available from CIP together with a standardised NCM-ELISA protocol. The sensitivity and specificity can be attained utilising different forms of ELISA techniques and specific antisera. For example two serotypes of SPCSV (Alicia *et al.,* 1999) can be detected by TAS-ELISA using specific monoclonal antibodies (Hoyer *et al.,* 1996; IsHak *et al.,* 2003). However, presence of interfering phenolic substances and inhibitors (Abad and Moyer 1992, Gibbs and Padovan, 1993; Karyeija *et al.,* 1998a), low concentration and erratic distribution of viruses in infected sweetpotato plants (Green *et al.,* 1998, Esbenshade *et al.,* 1993, Gutierrez *et al.,* 2003) and multiple infections of different viruses or strains hampers the accuracy of serology. Thus subsequent tests are needed to clear the discrepancies between assays and confirm the results. This is

particularly important for potyviruses due to the nature of close serological relationship (Shukla *et al.,* 1994), lack of detection of all strains of a given virus (Spetz *et al.,* 2003).

Combining molecular techniques and serology with immuno capture-reverse transcription polymerase chain reaction (IC-RT PCR) (Padovan *et al.,* 1993) improves sensitivity and detection of low virus titre often encountered in virus-infected samples. Application of PCR with universal degenerate primers (Gibbs *et al.,* 1997; Chen *et al.,* 2001; Winter *et al.,* 1997; Colinet *et al.,* 1998b; Ateka *et al.,* 2004a) followed by cloning and sequencing has provided sequence information of many new or uncharacterised viruses infecting sweetpotato (Colinet *et al.,* 1994) including the poorly/partially characterised viruses SPV-2 (Ateka *et al.,* 2004a), and SPCFV (Aritua and Adipala, 2004). Availability of CP-encoding and UTR nucleotide sequences has helped to clarify the taxonomy, establish criteria that distinguish viruses and assign the unknown viruses into the appropriate groups (Adams *et al.,* 2005b). By comparison to the available reference species/strain, the identity of the virus in question can be revealed based on threshold levels of CP sequence similarities. Isolates belonging to distinct potyvirus species exhibit CP sequences similarities ranging from 31-71 % nt whereas closely related species share 76-77% nt (<82% aa) sequence identity (Adams *et al.,* 2004b). Strains of the same species share 90-99% identity (Khan and Dijkstra, 2002). UTR nt sequence is another useful genetic marker for which close species share 83-99% and different species show as low as 39-53% identity (Bousalem *et al.,* 2000). Between different genera of ipomoviruses, the CP nt sequence identity is <31% while different species are 55-75% identical. Identity of of 90-99% (Schubert *et al.,* 1999; Shukla *et al.,* 1994) indicates that the isolates are of the same species. In criniviruses, a difference of >10% nt identity of any of the two frequently used genetic marker major *CP* or *HSP70h* gene (Martelli *et al.,* 2002) is used to differentiate species. Nucleotide identity of <72% (82% aa) of CP or replicase is used as genetic marker to discriminate between species in the genus *Carlavirus* (Adams *et al.*, 2004).

Sequence information has also simplified the design of specific primers and probes that enable specific detection of viruses and virus strains (Moyer & Abad, 1992a & b; Colinet *et al.,* 1998; Souto *et al.,* 2003). However, though molecular based-techniques have helped to alleviate some challenges often encountered by bioassays and serology, their practical application in routine work has not yet universally been accepted because of the need to validate techniques and determine their limitations related to specificity and reproducibility (Martins *et al.,* 2000). Standardisation of the assays is perhaps the most important concern for use of PCR for the routine diagnosis of sweetpotato viruses. Advancement in detection methods like use of a multiplex system for simultaneous detection of many virus targets has reduced cost (Menzel *et al.,* 2002). Costly and complex nucleic acid extraction protocols remain another hurdle. Presently, no convenient methods exist to allow an accurate identification and distinction of potyviruses co-infecting sweetpotatoes. It remains that the most important way of combating viral diseases is the use of resistant material and virus-free propagules. This strategy needs efficient and reliable virus detection systems.

Aims of the study

This study was part of the East African Regional Network for Biotechnology, Biosafety and Biotechnology Policy Development programme (BIO-EARN) that aims to build capacity in biotechnology in Ethiopia, Kenya, Tanzania, and Uganda. Since accurate identification and early detection of pathogens is the cornerstone of disease management in many crops, this thesis focuses on understanding incidences and genetic variability of the major viruses of sweetpotato in Tanzania and also Australia and utilises the information to develop a simple and sensitive diagnostic procedure for the identification and differentiation of potyviruses co-infecting sweetpotatoes.

The specific objectives of this study were:

- To establish the incidences and distribution patterns of viruses affecting sweetpotato in Tanzania
- To determine the genetic variability of four viruses: SPFMV, SPCSV, SPMMV, and SPVY.
- To develop a specific and sensitive diagnostic procedure for rapid identification and differentiation of sweetpotato viruses.

Results and discussion

Occurrence of sweetpotato viruses in Tanzania.

Sweetpotato viruses were for the first time surveyed systematically in two major sweetpotato growing zones in Tanzania. The two major zones as described in (**I**) include the main sweetpotato-growing the zone in the Lake Victoria basin with three districts: Misungwi, Tarime, and Bukoba. The second zone includes the Eastern coastal zone with Bagamoyo on the mainland and Unguja on the island of Zanzibar in the Indian Ocean. Four viruses namely SPFMV, SPCSV, SPMMV and SPCFV were serologically detected.

Viruses were detected directly from the symptomatic sweetpotato plants and on I. setosa grafted with asymptomatic plants. The detection was achieved by the NCM-ELISA using a kit containing antibodies for SPFMV, SPCSV, SPMMV, SPCFV, SPVG1 and SPVG2 kindly donated by CIP, Lima, Peru. Identity of a few isolates in sero-positive plants from each virus species were confirmed by RT-PCR (**III**), sequencing and BLAST search from the EMBL database. A high incidence of viruses was found in the Lake zone districts compared to the Eastern coastal zone (I). SPFMV, the most widely spread and characterised virus in all major sweetpotato growing areas (Moyer *et al*., 1992a), was the most prevalent virus detected in 85% of all sero-positive plants and 56% of all the symptomatic plants. It was found in all districts surveyed. SPCSV was the second most prevalent virus detected in relatively high frequency (23%) in four districts. The least frequently detected viruses were SPMMV followed by SPCFV, all from the Lake Victoria districts. SPMMV was mostly detected in co-infection with SPFMV (12-29%) while SPCFV was only detected in five samples from two districts in the Lake Victoria zone.

Though a high incidence of sweetpotato virus disease (SPVD) was found in both major sweetpotato producing zones, there was a higher incidence in the Lake Victoria basin than Eastern coastal zone (Figure 3 in **I**). Higher incidence in the Lake Victoria basin was consistent to the prevalence of SPCSV in the Lake zone districts which corresponds to similar incidences observed in the neighbouring country of Kenya (Ateka *et al*., 2004a). Most East African sweetpotato landraces are tolerant to single infection of SPFMV or SPMMV (Aritua *et al*., 1998) but co-infection with SPCSV (Karyeija *et al*., 2000) overcomes their resistance and results in a severe disease (Njeru *et al.,* 2004; Mukasa *et al*., 2006). In general the contrasting differences of viruses and virus diseases between the two major sweetpotato producing zones in Tanzania is accounted for by differences in cropping systems between these zones. In the Eastern coastal zone sweetpotato is grown as a monocrop. The season is closed completely and plant debris is destroyed after the harvest, which destroys inoculum sources. In major producing zone at the Lake districts, sweetpotato is continuously cultivated throughout the year combining a new and old crop in the same field, which enhances virus movement between crops. In addition, a shortage of planting materials experienced during a drought period in the districts of Misungwi and Tarime away from the Lake shore (**I**) is another limitation for proper selection of clean planting materials. It forces farmers to choose within the available materials, even those that are apparently diseased.

A variety of symptoms were displayed influenced not only by infection with the viruses but also cultivar differences (Figure 4 in **I**), which confuses them with physiological disorders.

All SPVD-infected samples displayed severe symptoms within 2-3 weeks of re-establishment in the screen-house, mostly those with dual infection of SPCSV and SPFMV or SPMMV, which are also easily recognisable by farmers. The symptoms simplify early control through roguing normally done by farmers, which limits the spread of disease. In contrast, variable symptoms displayed by single-virus infected plants require a trained eye to identify them. Despite different cultivars are used in the two production zones, common virus-like symptoms were observed, including vein clearing, mottling, chlorotic spot, leaf distortion, stunting, purpling and general yellowing. However, following grafting on *I. setosa*, a universal indicator host, SPFMV-infected samples exhibited clear uniform symptoms which were mainly vein clearing, leaf epinasty and leaf chlorosis. For SPCSV-infected samples the whole *I.setosa* plant was dominated by general chlorosis. SPMMV induced mottles on sweetpotato leaves and excessive epinasty on *I.setosa* (Figure 4 in **I**). On the other hand SPCFV did not produce any diagnostic symptoms either on its natural host sweetpotato or on *I. setosa*, which is consistent with an earlier description of this virus as Sweet potato symptomless virus in Japan (CIP, 1992). Furthermore, SPCFV co-infecting with SPCSV-EA caused no severe disease symptoms. Serologically, SPCFV did not react with antibodies to any other virus. Weak NCM-ELISA signals were observed on sweetpotato, which is consistent with symptomless appearance of infected sweetpotato plants and suggests sweetpotato cultivars being rather resistant to multiplication of this virus.

The occurrence of SPCSV, SPFMV, SPMMV and SPCFV observed in Tanzania (I) was consistent with other independent surveys (Aritua *et al*., 1998, Mukasa *et al*., 2003 and Ateka *et al*., 2004a) done within the region (EA). They all indicate that these four viruses are the major ones in sweetpotato in East Africa. The incidence and severity of SPVD in EA appeared to depend on the ecological factors, cropping system and probably abundance of SPCSV vectors. Hitherto, no responsible vector for the transmission of SPMMV or SPCFV has been confirmed experimentally. Therefore, in one way, their low prevalence in EA in contrast to SPFMV and SPCSV can may be connected to poor transmission due to lack of efficient vectors, which requires further studies. For SPCFV, possible limited vector transmission and inability to synergise with SPCSV, which normally boosts titres of other viruses (Karyeija *et al*., 2000; Mukasa *et al*., 2006), may also account for its low prevalence in EA.

Molecular characterisation of viruses

SPFMV

Following our first sequence information on two SPFMV isolates from Tanzania (**II**), we decided to enhance the resolution of genetic variability of these major viruses of sweetpotato in East Africa. Previously, molecular information on major sweetpotato viruses of EA was exclusively based on isolates from Uganda and Madagascar (Kreuze *et al*., 2002). Therefore, we sequenced more isolates of

SPFMV, SPCSV and SPMMV from two major sweetpotato growing zones of Tanzania (EA) (**I**) and for the first time SPFMV and SPVY from the geographically diverse region, Australia. The 3´terminal regions ca. (1.8kb) of the additional SPFMV isolates from Tanzania and Australia were cloned and sequenced as described in (**III**). Computer translation of the sequences revealed an open reading frame ending up to a stop codon (UAG) followed by 3´UTR of 221nt. The major difference between C strain and other strains was a deletion of six nt within the N terminal part of the CP-encoding region (**II**, **III**).

Sequence analysis using complete CP-encoding nt sequences of Tanzanian isolates showed 95.4-99.8% identity to those of EA strain and those from Australia shared 97.8-99.4% identity to RC isolates described by Abad *et al*., (1992) from the USA. Strain C from Australia shared 93.8-99.0% identity with other strain C isolates and both deviated from the other three strains by 75.4%-78.1%. Phylogenetic analysis of the CP nt sequences of the Tanzanian and Australian isolates grouped all Tanzanian isolates together with EA isolates and those from Australia in RC and C strain groups. Four strain groups previously shown (Kreuze *et al*., 2000) were revealed (Figure 3). Improved resolution of geographical grouping was enhanced by combining new SPFMV isolates from the database. Strain EA previously described as restricted to EA (Kreuze *et al*., 2000) was grouped together with isolates from Portugal [Portugal (AY459599)] and Spain [Canary Island (AY459600), Spain (AY518939)] with similarity > 94% (Figure 3) thus confirming previously reported occurrence of SPFMV-EA serotype in Spain (Valvade *et al*., 2004). Strain C distributed worldwide was found to occur also in EA in Uganda (**II**). It also seems to occur in Kenya. The O strain seems to occur in Kenya, Tanzania and Uganda (EMBL database; Ateka *et al*., 2003 unpublished) (Figure 3). The RC strain, though distributed worldwide, has not yet been reported from EA. RC strain isolates from Australia grouped together with RC isolates from America (Figure 3). Based on different serological properties and a low level of CP sequence (aa) similarity between C strain and other SPFMV strains (**II**), strain C has been proposed to be considered as a distinct species and the name Sweet potato virus C (SPVC) has been proposed (**III**).

Table 4. Coat protein-encoding (nt) sequences of SPFMV[1] isolates used in this study

Virus isolates	Strain	Country, origin	Accession no.	Reference
Bag	EA	Bagamoyo, Tanzania	AJ81780	**II**
Bkb1	EA	Bukoba, Tanzania	AJ81781	**II**
Bkb2	EA	Bukoba, Tanzania	AJ81782	**II**
Bny	EA	Bushenyi, Uganda	AJ539130	**III**
Canary3	EA	Canary island, Spain	AY459600	E.M. Ateka 2003, unpublished
Mis1	EA	Misungwi, Tanzania	AJ81783	**II**
Portugal	EA	Portugal	AY459599	E.M. Ateka 2003, unpublished
Tar1	EA	Tarime, Tanzania	AJ81784	**II**
Tar2	EA	Tarime, Tanzania	AJ81785	**II**
Tz1	EA	Bagamoyo, Tanzania	AJ539131	**III**
Tz2	EA	Bagamoyo, Tanzania	AJ539132	**III**
Unj1	EA	Unguja, Tanzania	AJ781786	**II**
Aus5RC	RC	Perth, W-Australia	AJ781776	**II**
Aus6	RC	Perth, W-Australia	AJ781777	**II**
Aus120-7	RC	East Kimberley, Australia	AM050889	**IV**, F. Tairo 2006, unpublished
Aus142-ARC	RC	East Kimberley, Australia	AM050890	**IV**, F. Tairo 2006, unpublished
RC	RC	North Carolina, USA	S43450	Abad *et al*., 1992
Arua	O	Arua, Uganda	AY459595	E.M. Ateka 2003, unpublished
Ken115/1S	O	Kenya	AY523540	E.M. Ateka 2003, unpublished
Tz4	O	Tanzania	AY459598	E.M. Ateka 2003, unpublished
Strain5	O	Cordoba, Argentina	U96624	V. Alvarez 1997, Unpublished
Aus4	C	Perth, W-Australia	AJ 781778	**II**
Aus5C	C	Perth, W-Australia	AJ 781779	**II**
Aus55-C	C	Broom W.Kimberlay, Australia	AM050892	**IV**, F. Tairo 2006, unpublished
Aus142A	C	East Kimberley, Australia	AM050891	**IV**, F. Tairo 2006, unpublished
Ken51/9S	C	Kenya,	AY459591	E.M. Ateka 2003, unpublished
Pink-2C	C	Broom W.Kimberlay, Australia	AM050893	**IV**, F. Tairo 2006, unpublished
Spain	C	Spain	AY518937	E.M. Ateka 2003, unpublished

1. All the sequences used in this study are available in the EMBL database

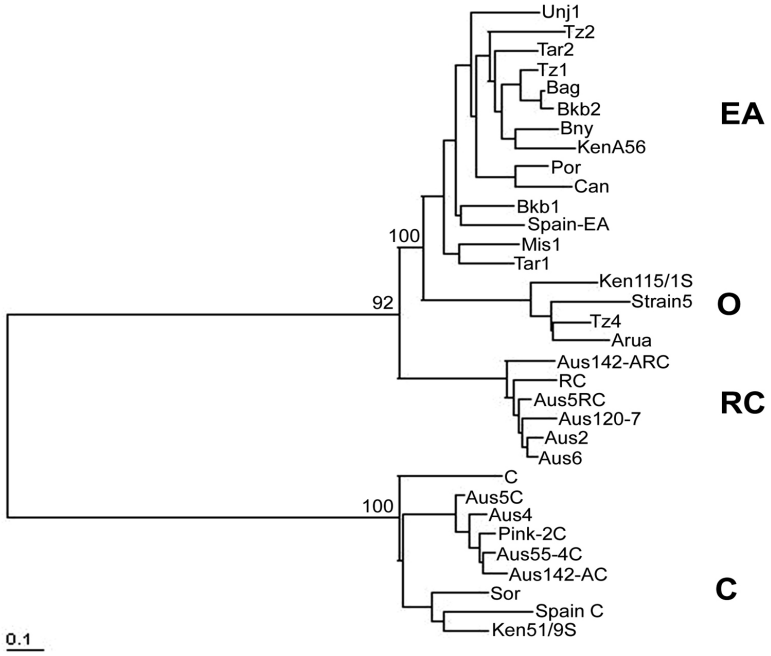

Figure 3. Phylogenetic tree based on the CP-encoding (nt) sequences of SPFMV isolates showing the taxonomic relationship between Tanzanian and Australian isolates with other SPFMV. Only bootstrap values higher than 80% are presented after 1000 replicates.

Interestingly, enhanced molecular resolution of SPFMV in this thesis revealed a significant correlation between clustering of the viruses and geographical origin. The clustering pattern of the isolates may provide indication of the results of the introduction of virus isolates from one geographical locality to another (Gibson *et al*., 2002). The phylogenetic relationship between EA strain isolates from East Africa with those from Portugal (Figure 3) suggest possible introduction from either side during colonial interaction between EA and Portugal in the 16th century. A similar situation could explain the clustering of Australian RC and C strain isolates with those of the USA. These strains were isolated from the sweetpotato cv. Beauregard, both host and viruses being originally described in the USA (**III**).

SPVY

Following the first report on molecular characterisation of SPFMV in Australia (**III**), we extended our understanding on diversity of sweetpotato viruses in the state of Western Australia. From the survey, five SPVY sero-positive plants we studied more closely. Their 3´-terminal regions corresponding to 1381-1856 nt excluding poly(A) tail were cloned by RT-PCR and sequenced from five SPVY isolates (**IV**). Sequence analysis showed Australian SPVY isolates to have a conserved DAG motif common for aphid-transmitted potyviruses (Atreya, *et al*., 1992) and a CP (332 aa) the size at which is the third largest of all known potyviruses infecting sweetpotatoes. Pair-wise comparison of the CP-encoding (nt) sequences revealed that Australian isolates shared the highest identity (99.8% nt, 98% aa) among each other and in average an identity of 93% nt in comparison with other isolates of the species. The closest matches within the genus were with SPFMV-Zw (65.7%), SPVG (55% nt) and SPFMV (45% nt). Although SPVY was first detected serologically in the 1980s (Rossel & Thottapilly, 1988), its description at sequence level has been recent (Souto *et al*., 2003; Ateka *et al*., 2003). Prior to this thesis there is no published comparison or phylogenetic analysis on SPVY isolates. The partial CP-encoding nt sequences of the five Australian isolates in this study (**IV**) were phylogenetically analysed together with SPVY isolates available in the database.

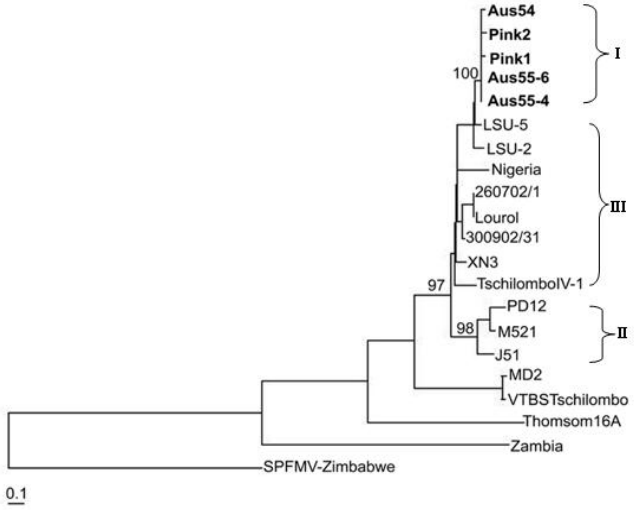

Figure 4. Phylogram of the partial (543nt) encoding sequences of SPV-2 isolates showing taxonomic relationship between five Australian isolates (in bold) and other SPVY isolates from China, Portugal, Nigeria, South Africa, the USA, and Zambia. Bootstrap values higher than 80% are presented after 1000 replicates. The origins of isolates are shown in Table 4 in **IV.**

Partial CP-encoding (543nt) sequence grouped all Australian isolates into one phylogenetic group (Figure 4) and revealed two other groups. Two isolates from the USA (Souto *et al*., 2003) were closer to Australian isolates than those from other regions. Three of the eight South African isolates (Table 4 in **IV**) formed the second group. The third group combined the two American isolates, three Portuguese isolates and one isolate each from South Africa, Nigeria and China. Three South African and Zambian isolates did not cluster to any of the three groups. However, the Zambian isolate was the most distant from the rest of the isolates with the average similarity of 79% and 76% at nt and aa level respectively. The similarity is below the species demarcation for potyviruses (76-77%) (Adams *et al*., 2005b) suggesting Zambian isolate to be a distinct species. Our results on SPFMV and SPVY imply that Australian isolates are more close related to those in the USA than other regions, which may indicate consequences of reciprocal introduction of plant materials/food products (**III**) due to the geographical interactions.

SPMMV

SPMMV has been reported serologically from different parts of the world but sequence information exists mainly from Ugandan isolates (Mukasa *et al*., 2003b) and a Kenyan isolate (Colinet *et al*., 1998). Phylogenetic analysis of these isolates and four new isolates from Tanzania was considered in order to improve the resolution of SPMMV variability in EA. The 3´terminal (ca.1.8 Kb) region of four SPMMV isolates were sequenced as described (**III**). Sequence analysis revealed variable sequence lengths resulting from the variable 3´-UTR that ranged from 305 to 314 nt (**III**).

The CP-encoding (nt) sequences of the four isolates showed identities of 88.2-100 % nt (94.0-100 % aa), while isolates Tar3 and Bkb3 (Lake Victoria zone) were identical. Identities among isolates were 89.9-98.1 % for 3'-UTR sequences. In comparison to all available SPMMV isolates in the database, shared identity of 88.0 and 89.9 % was found for the CP-encoding region and the 3'-UTR respectively. Search for the possible NIb/CP proteolytic cleavage site using the deduced polyproteins sequences of the Tanzanian isolates could not reveal a clear site. The proposed first site by Colinet *et al*., (1998b) VVQ/RE and the second VYVE/P (Mukasa *et al*., 2003b) were present at the respective locations of all isolates. However, with additional information from this study, it is likely that the proposed second cleavage site (VYVE/P) is the actual cleavage site for SPMMV. This is because it is conserved in all SPMMV isolates whereas the first site which was found to be different in isolate Tar2 (**III**) with substitution of arginine (R) for lysine (K) (data not shown). Furthermore, the proposed second cleavage site by Mukasa *et al*., (2003b) is consistent with the cleavage site VYVEPH/A proposed in another member of *Ipomovirus; Cucumber vein yellow virus* (Lecoq *et al*., 2000; Adams *et al*., 2005a). The aa motif H/A is conserved in all 16 SPMMV isolates studied. As previously mentioned (Mukasa, 2004) knowledge of the NIb/CP cleavage site is important in production of bacterially expressed CPs that can be used for production of antibodies for routine sero-diagnosis. For SPMMV, the determination of the exact cleavage site is exacerbated by the absence of CP

N-terminal DAG motif required for aphid-transmissibility of potyviruses and which is normally present close to the N-terminus of the CP (Atreya *et al*., 1992, Shukla *et al*., 1994). It simplifies the determination of the correct position of the CP N-terminus in aphid-transmitted viruses.

Inspite of the high level of genetic variability shown by SPMMV isolates, (**III**) they had similar serological properties and could be detected by the same antibodies (**I**). Phylogenetic analysis using the CP-encoding (nt) sequences of our four Tanzanian isolates (**III**) with previously reported ones did not help to structure the resolution of the genetic variability of SPMMV isolates, as no phylogenetically indistinguishable groups were observed (Figure 5).

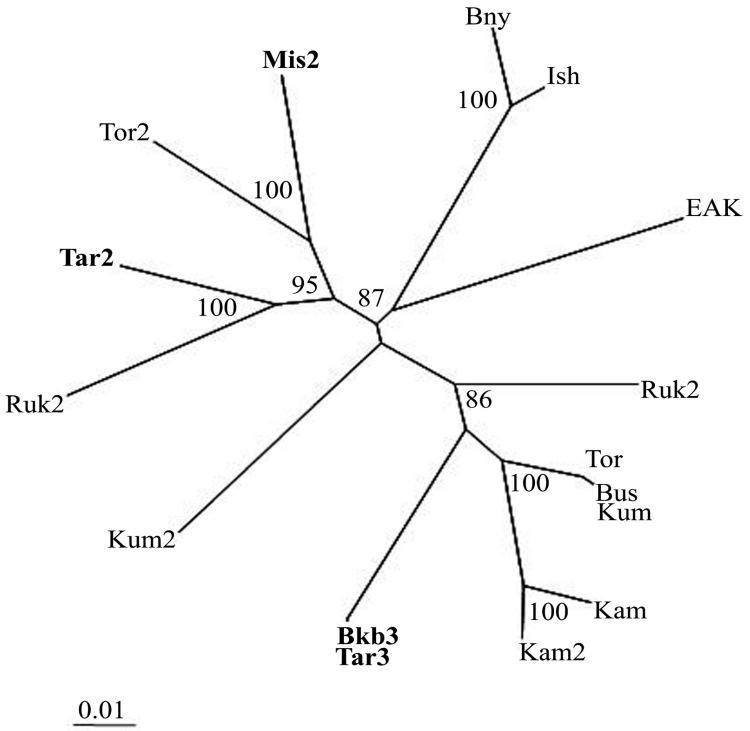

Figure 5. The phylogram based on 904 nt sequences of the CP-encoding regions of SPMMV from Tanzania (in bold), EAK from Kenya and the remaining isolates from Uganda. All the sequences are available from the EMBL database. Their accession numbers are given in Table 1 in (**III**). The horizontal distances are proportional to the genetic distances. Bootstrap values are shown as percentage and only nodes over 80% are labelled after 1000 replication.

These data suggest the existence of a less differentiated virus population influenced possibly by the host factor (Garcia-Arenal *et al*., 2001). This may be supported by the fact that SPMMV has a wide host range of more than 14 plant families (Hollings *et al*., 1976). Its lower prevalence in sweetpotato in one way may be due to an inefficient vector but on the other hand this may indicate that sweetpotato is not a favourable host for SPMMV, thus inhibiting further differentiation of this virus in sweetpotato plants.

SPCSV

SPCSV besides SPFMV is the major pathogen in sweetpotatoes and the most economically the most important virus in terms of losses in sweetpotato production (Gutierrez *et al*., 2003) due to its ability to synergise other less capable viruses like SPFMV, SPMMV and SPMSV and thereby cause very severe diseases (Karyeija, *et al*., 1998; Mukasa *et al*., 2006; Di Feo *et al*, 2000). It can also cause diseases on its own (Gibson and Aritua, 2002). Any control strategies directed toward it may be an effective control against SPVD, CD and probably SPSMD. Our data from Tanzania improved the resolution on intra-specific phylogeny of SPCSV. Nucleotide sequence comparison using five *HSP70h* genes obtained in this study and 16 HSP70h nt sequences from the database retained two clusters previously described by Kreuze (2002). Five Tanzanian isolates were grouped together with other isolates described from East Africa (**III**) including Uganda (Alicai *et al*., 1999) and Madagascar, with high sequence identity (>98% and 100% aa). However, among EA-strains, isolate Unj2 from Tanzania and Mad from Madagascar, both Islands in the Indian Ocean (Figure 2 in **III**), were phylogenetically distinguishable from each other and the rest of EA isolates. The second cluster comprising isolates from different geographic origins designated as (NEA) were placed distantly from EA cluster (Figure 6). Biologically, these two strains induced similar chlorotic stunt symptoms on *I. setosa* and purpling on sweetpotato plants (**III**). SPVD-characteristic symptoms (Gibson *et al*., 1998) are also induced on sweetpotato cultivars with either strain co-infecting with SPFMV (IsHak *et al*., 2003, Fenby *et al*., 2000). Serologically they are clearly different: no SPCSV-infected samples from Tanzania (**I**) reacted with the MAb (mix2) detecting NEA strains (Alicia *et al*., 1999).

Intraspecific phylogeny using *HSP70h* gene sequence showed low sequence identity of <77% (nt) between the EA and NEA strains, as previously reported (Alicia *et al*., 1999; Fenby *et al* 2002) which has also been shown using *CP* and *p60* gene sequences (Barg *et al*., 2003). These accumulated data using different genes and serological differences indicate these two strains to be different species of crinivirus and the name East African sweetpotato stunt virus (EASpSV) was proposed (**III**). Increasing diversity within the NEA strain group was revealed when an isolate from Argentina [(SPSCV-Cor (AY729021), Nome *et al*., 2004] was compared to NEA isolates (Figure 6), resulting in two NEA subgroups. The Argentinean isolate grouped distantly with identity of 88% (nt) with the rest. Apart from the report on serological detection of EA strain in Peru (Gutierez *et al*., 2003), the uniqueness of the East African SPCSV has become apparent following this study having most of the East African region surveyed and characterised for SPCSV. The uniqueness is supported by the low genetic variability within SPCSV genome of the EA group, suggesting the stable population structure in contrast to that of NEA group.

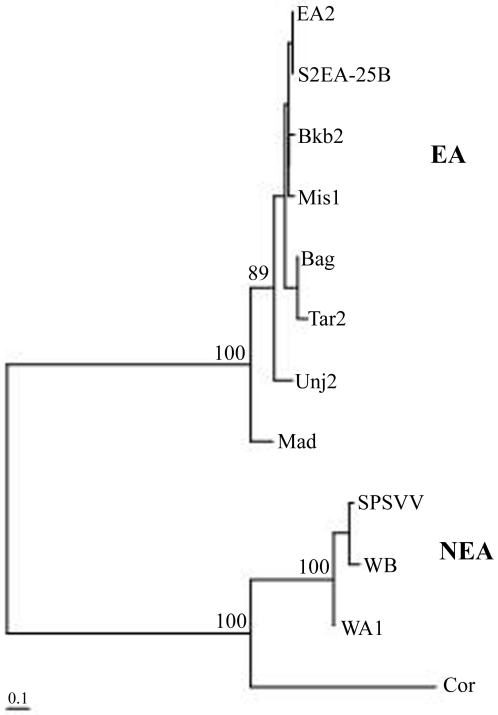

Figure 6. Phylogram of the partial *HSP70h* (446nt) encoding sequences of SPCSV isolates showing taxonomic relationship between Tanzanian isolates and other SPCSV. Only bootstrap values higher than 80% are presented after 1000 replicates. The origins of isolates are shown in Table 4 in (**III**)

Our enhanced population structure (**III**) provided substantial evidence for the genetic stability of SPCSV-EA (<4% divergence), which indicates that engineered resistance against this strain using the SPCSV-EA genes would be able to alleviate the SPVD in EA. However, in criniviruses the genome organisation, especially in RNA1, is not conserved despite similar layout (Coutts *et al*., 2003; Livieratos *et al*., 2004; Wintermantel *et al*., 2005; Tzanetakis *et al*., 2005). Recently, elucidation of a mechanism employed by SPCSV to synergise SPFMV-EA, two novel proteins (RNaseIII & p22) (Kreuze *et al*., 2005) were experimentally shown to be responsible for suppressing the host defensive mechanism. Therefore further genetic variability studies on these two genes from more SPCSV-EA isolates will be particularly important to ensure that the engineered resistance will cover the EA strain.

SPCFV

In line with molecular characterisation of other viruses, we later managed to confirm the occurrence of SPCFV in Tanzania by partial cloning and sequencing of one of the five isolates of SPCFV sampled from Tanzania (**I**). A fragment of about 1.6 kb was amplified using a combination of specific degenerate primers from the SPCFV-infected plant that had previously been identified serologically (**I**). Computer-assisted translation of the nucleotide sequence revealed two overlapping ORFs. The first identified ORF from our sequence encoded a deduced 299 aa protein significantly similar to CP sizes of *Carlavirus* (family *Flexiviridae*) (Adams *et al*., 2004). The second ORF encoded a 133 aa protein equivalent to carlavirus nucleic acid binding (NB) protein followed by a stretch of 52 nt of the 3´-UTR. Pair-wise comparison using CP and NB genes showed our sequence was 93.9-98.7% and 97.7-99.1% identical to the two Ugandan SPCFV isolates; [Mas (AY81295) Mukasa, 2004; and Hoima4 (AY461421) Aritua and Adipala, 2004]. The closest member within the genus *Carlavirus* was Melon yellow associated virus (MaYV) (Nagata *et al*., 2005) which shared an aa identity of 60.5% for CP and slightly lower (47 %) for NB aa. However, phylogenetic analysis could not be done since sequences of only three SPCFV isolates were available. Based on the criteria for species demarcation (Van Regenmortal *et al*., 2000, Adam *et al*., 2004) using CP similarity between SPCFV-Tar and two Ugandan isolates, these qualify to be considered isolates of the same strain. The sequence obtained in this study is available in the EMBL database (Acc no. AJ781296).

RT-PCR/RFLP method for detection differentiation of potyvirus-infecting sweetpotato

In study (**III**), we reported molecular occurrence of SPFMV-RC and C strains in Australia phylogenetically similar to the USA isolates. In two sweetpotato disease complexes, SPVD (Gibson *et al*., 1998; Karyeija *et al* 2000b) and CD (Di Feo *et al*., 2001), SPFMV is reported as one of the key players. In study (**II**) we showed that SPFMV-C strain that commonly co-infects with other SPFMV strains is grossly deviated from other strains, thus escaping detection with the available antibodies for SPFMV. In study (**IV**) we found that SPVY, another potyvirus in Australia, co-infects sweetpotatoes with SPFMV-RC and/or C strain. One requirement for understanding of the epidemiology of sweetpotato complex diseases is to unravel the role played by each strain in the disease which is posed by difficulty in detecting the aforementioned viruses in mixed infection especially the lack of reliable and specific diagnostic tools that differentiate the strains of SPFMV in co-infected samples. Therefore, this study (**IV**) made use of information generated in (**I**, **II**, & **III**) and available published information to develop a sensitive accurate method that reliably detects and differentiates strains of SPFMV and also SPVY in single and mixed infected samples from Australia.

Symptoms were indistinguishable when single strains were grafted onto *I. setosa* indicating that bioassay is an inadequate diagnostic tool for differentiating mixed infection. Thus grafting on *I. setosa* is only useful for indication of the presence of virus and to boost virus titres but not for diagnostic (Karyeija *et al*., 2000a). The

interpretation is rather difficult for the case of co-infection of two potyviruses species (Figure 2 in **IV**)

Specificity of ELISA normally is limited by the availability of the antibodies specific to a particular virus/strain thus making it also an inadequate method. For instance some of the symptomatic samples we collected from the field (**IV**) tested sero-negative using our antibodies. This indicates that the symptomatic sero-negative samples had been infected by other viruses not detected with our antibodies. Low specificity due to high CP sequence homology > 94 % between strains of SPFMV or with other potyviruses results in cross-reaction (Shukla *et al*., 1994; Spetz *et al*., 2003) and also leads to inability to discriminate between closely related strains. These limitations indicate that serology alone is not a sufficient method for routine distinction of SPFMV strains and that available antibodies can not detect strain C due to low CP homology level < 85% aa (II). Application of RT-PCR with degenerate genus specific primers (Gibbs *et al*., 1997) has been a useful tool for characterisation of potyviruses. However, despite its sensitivity in amplifying low virus concentration into a detectable level, its acceptance as a routine diagnostic tool for SPFMV is still in question due to poor reproducibility (Souto *et al*., 2003) if used alone. The sensitivity in detecting different viruses simultaneously is low in multiple infections since the one in higher concentration is readily amplified over those in lower titres due to antagonistic interaction between closely related strains (Bos, 1999, Zhang *et al*., 2001) but especially because the similar amplification products from different potyviruses hinders detection of the presence of mixed infection. Therefore, the identities of species and strains in sero-positive plants (**IV**) were revealed by the inclusion of the RFLP analysis of the amplification products (Figure 7).

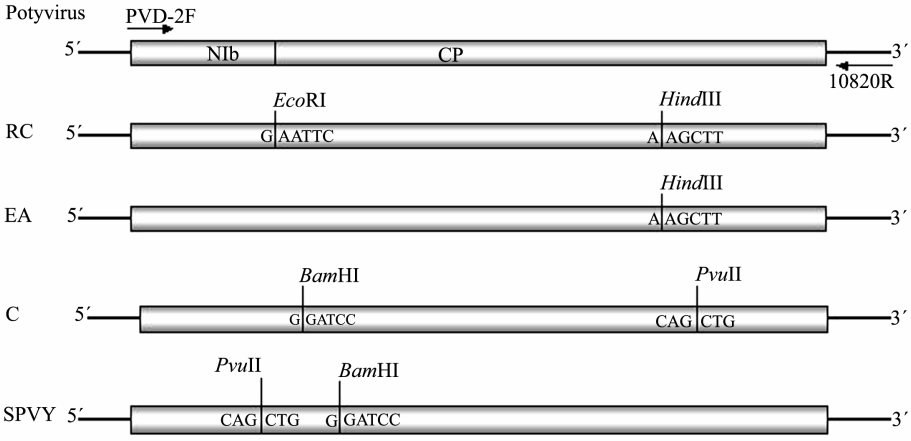

Figure 7. Schematic representation of the 3´terminal part (1.8kb) of the *Potyvirus* genome, showing predicted restriction sites revealed by the computer programme vector NTI suite8 (Informax, Wisconsin, USA) in SPFMV strains RC, EA and C and SPVY. The genome fragments were amplified by RT-PCR using a combination of *Potyviridae* forward degenerate primer with potyvirus genus specific reverse primer (**IV**).

Simultaneous detection and differentiation between strains RC and C of SPFMV and SPVY that commonly co-infect sweetpotatoes in Australia was achieved. A single RT-PCR amplification followed by digestion with *Pvu*II endonuclease (Figure 4; in **IV**) was enough to differentiate SPVY from SPFMV. Furthermore, the same PCR amplicon digested separately with *Hind*III, differentiated RC from C, which is not possible serologically. Interestingly, according to restriction map (Figure 7), using *Eco*RI it is possible to separate between RC and EA strain which is not possible serologically even by PCR due to close similarities of the CP nt sequence (**II**). Similarly, the C strain can further be differentiated from the three strains by using *Bam*HI (Figure 7). These results showed a significant reduction of costs as compared to individual strain-specific PCRs. Also the risk of contaminations is minimised considerably as one universal PCR followed by RFLP revealed identities of the species/strains in all seven samples we tested (Figure 4 in **IV**).

The increase of sequence information in the database on viruses from different regions has tremendously improved the diagnosis of sweetpotato viruses. This implies that it is now possible to circumvent the process of cloning and sequencing for identification purposes and instead search for suitable endonucleases using many respective sequences and identify the virus species and strain by RT-PCR followed by RFLP. This procedure can reduce the time needed for certain diagnosis of infected samples in medium level routine diagnosis laboratories.

The necessity of comprehensive diagnosis of the viruses involved in any complex disease is based on the fact that the current promising control approach for sweetpotato viruses is the application of the engineered RNA silencing based transgenic resistance which is highly sequence specific (Jones *et al*., 1998). Therefore, development of durable strain-specific resistance will only be successful following a comprehensive and accurate detection and identification of the virus species and strains prevalent in that particular locality.

Conclusions

The main conclusions from the results presented in this thesis are:

- Our surveys for sweetpotato viruses in Tanzania revealed the four major sweetpotato viruses commonly occurring in EA: SPFMV, SPCSV, SPMMV and SPCFV. Distribution patterns showed occurrences in varying incidences in two main sweetpotato producing zones of Tanzania.

- Our improved resolutions of genetic variability of SPFMV and SPCSV revealed narrow genetic diversity with organised population structures in EA. Strong relationship of SPFMV isolates from East Africa and Portugal, and those of Australia with the USA in a way indicate the consequence of interaction between regions.

- SPMMV showed a high genetic variability with unorganised population structure. Possibly more sequence information from other regions outside EA would enhance the resolution of genetic diversity of this virus.

- SPVY isolates from Australia showed a high similarity to each other and formed a unique phylogenetic group together with isolates from the USA. The other two groups contained isolates from different geographic regions.

- Molecular characterisation of prevalent viruses in Australia improved our knowledge of their genetic variability thus enabling us to develop a less costly and sensitive technique for specific detection and differentiation of potyviruses co-infecting sweetpotatoes.

- From the virus disease management point of view, results generated by this thesis showed low nucleotide sequence variability (< 8%) for EA strain of SPFMV and SPCSV and a similar uniqueness for Australian SPVY and SPFMV within the strains RC and C. Additionally, with added knowledge on the viruses occurring in mixtures in sweetpotato in these regions is essential for the durability of engineered resistance as part of integrated disease management (IDM) because of the ability of viruses to suppress RNA silencing based engineered resistance.

Future perspectives

- More studies are necessary for the determination of the responsible vector for the transmission of SPMMV which would help to resolve the factors that govern the diversity of SPMMV. The vector of SPCFV should also be determined.
- Currently, most studies on sweetpotato viruses have targeted East African region. Similar efforts at the other sweetpotato production areas will enhance the knowledge on the evolution of the viruses/strains and their spread to different geographical areas.
- Resolving potyviruses that infect sweetpotato in complexes n East Africa needs to be tested experimentally using the RT-PCR/RFLP method.

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