DETECTION, DISTRIBUTION AND GENETIC DIVERSITY OF SWEET POTATO LEAF CURL VIRUS (SPLCV) FROM WESTERN, COASTAL AND CENTRAL REGIONS OF KENYA

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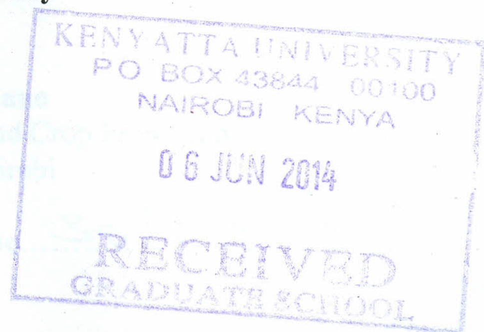
A research thesis submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science (Biotechnology) of the school of pure and applied sciences of Kenyatta University



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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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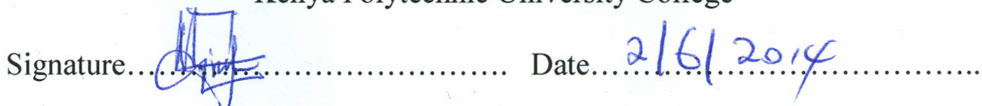

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DEDICATION

This work is dedicated to GOD of heaven and earth for his promises, mercy and love that endures forever. My aunt Loise, my parents and my late grandfather Maina wa Thuku.

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TABLE OF CONTENTS

CONTENTS
DECLARATION ii
DEDICATION iii
ACKNOWLEDGMENTS iv
TABLE OF CONTENTS v
TABLES vii
FIGURES viii
ABBREVIATIONS AND ACRONYMYS x
ABSTRACT xi

CHAPTER ONE 1
INTRODUCTION 1
1.1 Background information 1
1.2 Problem statement 2
1.3 Justification of the study 2
1.4 Research Questions 3
1.5 Hypotheses 3
1.6 Objectives 3
1.6.1 Broad Objective 3
1.6.2 Specific Objectives 4

CHAPTER TWO 5
LITERATURE REVIEW 5
2.1 Origin and distribution of sweet potato 5
2.2 Sweet potato production 5
2.3 Sweet potato virus diseases 6
2.4 Molecular biology of Geminiviruses 7
2.5 Molecular biology and morphology of genus begomoviruses 9
2.6 Sweet potato leaf curl virus 11
2.7 Whitefly vector for sweet potato leaf curl virus 12
2.8 Taxonomic criteria for species demarcation 13
2.8.1 Sweet potato begomoviruses an overlooked component 14
2.8.2 Begomovirus detection by polymerase chain reaction 15
2.8.3 Begomovirus detection by rolling circle amplification 16

CHAPTER THREE 19
MATERIALS AND METHODS 19
3.1 Optimization of Polymerase Chain Reaction for the Detection of Sweet Potato Leaf Curl Virus 19
3.3 Biological inoculation of sweet potato with sweet potato leaf curl virus 19
3.4 Analysis DNA sequence data 20

CHAPTER FOUR 25
RESULTS 25

4.1 Optimization of Polymerase Chain reaction protocol for the Detection of SPLCV 25
4.2 Distribution of Begomoviruses infecting sweet potato in Kenya 26
4.2.1 Result of surveys conducted in western, central and coastal regions 26
4.3 Virus diversity and variability studies 31
4.3.1 Genetic Diversity and variability in AC1 fragments 31
4.3.2 Genetic Diversity and variability in AV1 fragments 35

CHAPTER FIVE 46
DISCUSSION, CONCLUSIONS AND RECCOMENDATIONS 46
5.1 Discussion 46
5.2 Conclusion 51
5.3 Recommendation 52
REFERENCES 53
APPENDICES 59
APPENDIX 1:Buffers and stock solutions 59

LIST OF TABLES

Table 3.1: PCR sequences used in detection of SPLCV:..21

Table 3.2: Geminiviruses sequences obtained from the Gene Bank used in the analyses of the AV1, AC1, AC2 and AC4 fragment of sweet potato begomoviruses:............................24

Table 4.1: Percentage of sweet potato samples infected with SPLCV from Western region of Kenya:..29

Table 4.2: Percentage of sweet potato samples infected with SPLCV from Central region of Kenya. The sign +VE refer to positive and –VE refer negative when applied using PCR:........30

Table 4.3: Percentage of sweet potato samples infected with SPLCV from Central region of Kenya. The sign +VE refer to positive and –VE refer negative when applied using PCR:........30

Table 4.4: Pairwise percent nucleotide sequence identity (upper diagonal) and amino acid sequence identity (lower diagonal) among the partial ORF AC1 of begomovirus isolates as determined using ClustalX software...34

Table 4.5: Sweet potato leaf curl virus sequences for AV1 (coat protein).The virus isolates were collected from (W) western (C) Central and CO Coast region of Kenya.............................38

Table 4.6: Pairwise percent nucleotide sequence identity (upper diagonal) and amino acid sequence of identity (lower diagonal) among the partial ORF AV1 of begomovirus isolates as determined using ClustalX software:..40

Table 4.7: Samples sequenced for AC2 Gene 41

Table 4.8: Pair wise percent nucleotide sequence identity (upper diagonal) and amino acid sequence identity (lower diagonal) among the partial ORF AC1 of begomovirus 45

LIST OF FIGURES

Figure 2 1: Genome organization of the Sweet potato leaf curl virus (SPLCV) component with AV1 for infection, AC1 replication enhancer and AC2 for transcription (Lotrakul and Valverde 1999)..........9

Figure 2.2: Specific primers sequences used for the amplification of a fragment of the AC1 467 bp and the full length coat protein gene (AV1) 765 bp..........16

Figure 2.3: Schematics of the design used for peptide nucleic acid assisted rolling circle amplification reactions performed either on earring probe (A) or with employment of artificial nickase system (B). Both schemes are based on the formation of peptide D-loop consisting of the locally open short dsDNA segment, a pair of bisPNA openers and an oligodeoxyribonucleotide 18

Figure 4.1: Optimization of primer SPG 1/2 for use in PCR detection of *Sweet potato leaf curl virus* (SPLCV) in sweet potato 25

Figure 4.2: Optimization of primer SPB 1&2 for use in PCR detection of Sweet potato leaf curl virus (SPLCV). 26

Figure 4.3: Optimization of primer PW285-1/2 for use in PCR detection of Sweet potato leaf curl virus (SPLCV)..........24

Figure 4.4: Sweet potato samples collected from Central and Coastal regions of Kenya showing different different *Sweet potato leaf curl virus* (SPLCV) symptom: 27

Figure 4.5: Polymerase chain reaction amplified products from sweet potato samples infected with *Sweet potato leaf curl virus* (SPLCV) using.......... 28

Figure 4.6: Polymerase chain reaction amplified products from sweet potato samples infected with *Sweet potato leaf curl virus* (SPLCV) using Primers, SPB1/2 (A), Lane L, 1-kb DNA ladder; lane 5 positive control..........26

Figure 4.7: *Ipomoea setosa* plants showing *Sweet potato leaf curl virus* (SPLCV) symptoms after graft-inoculation with Central samples SPLCV infected scions from sweet potato.......... 31

Figure 4.8: Multiple alignment A 512 nucleotide fragment corresponding to the AC1 was amplified with primers PW285-1 and PW285-2 for Kenya collected isolates..........32

Figure 4.9: Multiple alignment A 512 nucleotide fragment corresponding to the AC1 was amplified with primers PW285-1 and PW285-2 with other sequences from gene bank..........33

Figure 4.10: Neighbor-joining tree based on the nucleotide sequence of the AC1 fragment showing the relationship among begomoviruses infecting sweet potato in Kenya and other begomoviruses. *Beet curly top virus* (BCTV), genus *Curtovirus*..33

Figure 4.11: Neighbor-joining tree based on the nucleotide sequence of the AC1 fragment showing the relationship among begomoviruses infecting sweet potato in Kenya. 34

Figure 4.12: Multiple alignment Isolates from Western, Central and coast were selected twenty nine samples for the sequence analysis of the CP gene (AV1) with others from the gene bank.. 36

Figure 4.13: Multiple alignment Isolates from Western, Central and coast were selected for the sequence analysis of the CP gene (AV1)..37

Figure 4.14: Neighbor-joining tree based on the nucleotide sequence of the AV1 fragment show the relationship among begomovirus infecting sweet potato in Kenya and other begomovirus. 37

Figure 4.15: Neighbor-joining tree based on the nucleotide sequence of the AV1 fragment show the relationship among begomoviruses infecting sweet potato in Kenya and other begomoviruses. Beet curly top virus (BCTV), genus Curtovirus, was used as outgroup............................39

Figure 4.16: Multiple sequence alignment of SPLCV nucleotide fragment corresponding to the AC1 and AC2 amplified with degenerate primers SPG1/2 and compared with other sequences from the Genbank.. 42

Figure 4.17: Multiple sequence alignment of nucleotide fragment corresponding to the AC1 and AC2 amplified with degenerate primers SPG1/2...42

Figure 4.18: Neighbor-joining tree based on the nucleotide sequence of the AC2 fragment showing the relationship among begomoviruses infecting sweet potato in Kenya and other begomoviruses. Beet curly top virus (BCTV), genus Curtovirus,..................................43

Figure 4.19: Neighbor-joining tree based on the nucleotide sequence of the AC2 fragment showing the relationship among begomovirus in Kenya...44

LIST OF ABBREVIATIONS AND ACRONYMYS

ANOVA:...Analysis of Variance

BCTV:...Beet curly top virus

Bp:...Base pairs

CP:...Coat protein

CR:...Common regions

CTAB:...Cetyltreimethyl Ammonium Bromide

EDTA:...Ethylenedeamine tetra acetate

ELISA:..Enzyme-Linked Immunosorbent Assay

ORF:..Open reading frame

PCR:..Polymerase chain reaction

PNA:..Peptide nucleic acid

PTGS:..Post Transcription Gene Silencing

PVP:...Polyvinylpyrrolidone

SDS:...Sodium Dodecyl Sulphate

SPLCGV..*Sweet potato leaf curl Georgia virus*

SPLCV CP...*Sweet potato leaf curl virus coat protein*

SPLCV..*Sweet potato leaf curl virus*

TYLCV...*Tomato yellow leaf curl virus*

USA:...United States of America

ABSTRACT

Leaf curling in sweet potato has been reported throughout the world. One of the causal agents is *Sweet potato leaf curl virus* (SPLCV) which belongs to the genus *Begomovirus* (family *Geminiviridae*). Since SPLCV could become an important constraint for sweet potato production in Kenya; detection, distribution and genetic diversity of sweet potato leaf curl virus from Western, Coastal, and Central regions of Kenya is important. Polymerase chain reaction (PCR) was done in 512 collected sweet potato samples, using degenerate and specific primers. Specific primers SPB, PW were used to amplify the coat protein (AV1), and the ORFs AC1 and AC4 fragments. Western region had infected samples with cumulative positive of 78% followed by Coast with 69.4% and Central 3.9%. To evaluate genetic diversity and variability among begomoviruses obtained from 45 sweet potato genotypes; analysis of the nucleotide sequence of a fragment of the protein gene (AC1), (AV1) and (AC2) was carried out. Phylogenetic analysis using the obtained nucleotide sequences of the AC1, the full length nucleotide sequences of the coat protein gene (AV1) and (AC2) clustered all sweet potato begomoviruses together. However in AC1, 3 were closely related to SPLCV with nucleotide sequence identities that varied from nearly 61 to 96 % and closely related to Asia samples. AV1 protein from Central samples was closely related with an over 92% nucleotide sequence identity. The diversity within the coat protein (AV1) was available but distributed in all regions. Results indicated that these isolates were closely related to SPLCV coat protein with amino sequence identities that ranged from 90 to 100 %. AC2 fragments from Western and Coast had a close relationship of 95 % nucleotide sequence identity and amino acid sequence identity of 96% showing that the AC2 protein may have the same ancestor. Samples from Western and Coast with 95% sequence identity and 96% amino acid sequence identity supports that the AC2 protein fragment was from the same ancestor. Several isolates from western clustered as subgroups with 86-85% nucleotide sequence identity. The research study reports the first PCR detection of begomovirus infecting sweet potato and the first genetic diversity and variability of begomovirus infecting sweet potato in Western, Central and coastal regions of Kenya. The study indicates that future development of sweet potato begomovirus management such as genomics strategies should focus on SPLCV as a threat in Kenya sweet potato growing regions.

CHAPTER ONE

INTRODUCTION

1.1 Background

Sweet potato *(Ipomoea batatas L)* is among the most important food crops in the world and is ranked seventh based on total production, and is one of the most important crops in developing countries. It is a valuable source of vitamins and other micronutrients, especially its storage roots, which contain carotenoids; the precursor for vitamin A. Sweet potato is a very important source of carbohydrates, often crucial during famine, due to its rapid production of storage roots following the onset of rains, making it a good food security crop in marginalized areas. The crop does well in less fertile land and demands few inputs, making it affordable for resource-poor farmers (Ndolo *et al.,* 1997). The crop has been an important staple food for consumption and income generation for smallholder farmers in Kenya (Horton, 1988; Carey, 1996).

Sweet potato production in Africa is concentrated around Lake Victoria and Uganda has the largest production in Africa and is second in the world after China (FAO, 2001). Despite its importance, sweet potato production is constrained by a number of factors key among them being pests and diseases. Among the major biotic production constraints are the sweet potato weevil and the sweet potato virus disease (SPVD). Abiotic stresses include low soil fertility, drought, poor agronomic practices and use of low yielding cultivars for planting. Rarely do adverse weather conditions cause a total crop failure. Among the biotic constrains, viral diseases are the second, weevils being the most devastating (Geddes, 1990) and reduces yield significantly (Mukasa *et al.,* 2003).

Viral diseases have been reported in all areas where the crop is grown (Carey, 1996). One of the viruses reported infecting sweet potato is the *Sweet potato leaf curl virus* (Lotrakul *et al.,* 1998). The virus has been reported in different parts of the world including Peru, Unites States of America and Kenya (Lotrakul *et al.*, 1998; Miano *et al.*, 2006). In Kenya, SPLCV was first reported in a germplasm collection plot (Miano *et al.,* 2006). However, no further studies were conducted to determine its distribution and diversity in the country. The aim of this study was to determine the distribution of begomoviruses infecting sweet potato in Kenya and determine their relationship with others that have been characterized from different parts of the world.

1.2 Problem statement and justification

One of the difficulties of managing Begomovirus is lack of information on genetic diversity and their geographical distribution. Little work has been done on Sweet potato leaf curl begomovirus in Kenya. It is therefore important to generate basic information on the virus distribution and genetic diversity. However, a rapid diagnostic protocol will help in the management of the virus. Sweet potato production is greatly constrained by different viruses. While most of the viruses have been extensively studied, the potential importance of sweet potato leaf curl diseases has been overlooked, in part, because leaf curl symptoms are not common and do not persist in sweet potato (Valverde *et al.,* 2007). Since SPLCV could become an important constraint for sweet potato production in Kenya, understanding its molecular diversity and distribution is important. Once genetic diversity of begomoviruses is elucidated, and determined it will enable evaluation of the prevalence of distinct species, strains, or variants to be identified as well.

Diagnosis, identification, and characterization are essential to develop an appropriate management strategy. This information will be helpful to breeding programs aimed at development of sweet potato cultivars resistance to *sweet potato leaf curl virus*.

1.4 Research Questions

1. Are Geminiviruses infecting sweet potato present and widely distributed in Kenya?
2. Is there variability between different isolates of *Sweet potato leaf curl virus* infecting sweet potato in different regions of Kenya?

1.5 Hypotheses

1. Geminiviruses infecting sweet potato are present and widely distributed in Kenya.
2. There is variability between different isolates of *sweet potato leaf curl virus* infecting sweet potato in different regions of Kenya

1.6 Objectives

1.6.1 Broad Objective

To determine distribution and genetic diversity of different isolates of begomoviruses infecting sweet potato in Kenya.

1.6.2 Specific Objectives

1. To optimize diagnostic protocol for the detection of *sweet potato leaf curl virus* based on nucleic acid amplification.
2. To determine the distribution of different begomoviruses infecting sweet potato in Kenya.
3. To determine variation among different isolates of begomoviruses collected from major sweet potato growing regions of Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and distribution of sweet potato

Sweet potato, *Ipomoea batatas* (*L.*) Lam belongs to family *Convolvulacea.* It is an important storage root crop cultivated throughout the tropical and warm temperate regions wherever there is sufficient water to support growth and important global food crop (Woolfe, 1992). Sweet potato is an extremely important food crop for subsistence farmers in the relatively humid areas of sub-Saharan Africa, from the coastal west to the central and southern regions (Anonymous, 2007).

2.2 Sweet potato production

Sweet potato is cultivated mainly for its edible storage roots and is generally grown as an annual (Kemper, 2009). Sweet potato contains appreciable amounts of zinc, potassium, sodium, manganese, calcium, magnesium, iron, vitamin C, and fiber (Antia, 2006). Due to its importance as a food, sweet potato is ranked fourth in importance in the developing world after rice, wheat, and corn (Kays, 2005). Worldwide productivity of sweet potato is greatly constrained by pests and diseases that cause yield reduction by up to 98% (Kapinga *et al.,* 2007). It is an important food crop in Kenya, the fifth largest producer in Africa after Uganda, Nigeria, Rwanda and Burundi (Anon, 1999).

The main sweet potato production areas in Kenya include Western, Nyanza, Central, Coast and Eastern Provinces with about 75% production (Ndolo *et al* 1997). The low average yield of about 9.8 t/ha realized in Kenya is partly due to disease constraints (Ndolo *et al.,* 1997). Over 20 viruses are known to affect sweet potato worldwide and these are thought to be responsible for more than 50% of the yield losses occurring in sweet potato production (Hahn, 1981).

2.3 Sweet potato virus diseases

The areas with the most detailed and updated knowledge on prevailing viruses in sweet potato include southern parts of the United States, Peru, Israel, Japan, Australia and East and South Africa (Loebenstein *et al.,* 2004; Tairo *et al.,* 2005; Clark and Hoy, 2006; Ateka *et al*., 2007; Clark and Hoy, 2007). Research to elucidate the etiology of viruses as the cause of some of the diseases has not yet been done. For example, in East Africa many sweet potato plants which exhibit virus-like disease symptoms in the field are found to be sero-negative when tested with antibodies to the common viruses (Mukasa *et al.,* 2003; Ateka *et al*., 2004; Tairo *et al.,* 2004). In the United States, virus symptoms are common in the field, but the effects on the crop are more moderate than in Africa. In USA the typical syndrome consists of chlorotic spotting, ring spotting, and vein banding with or without purple borders, depending on the pigmentation of the sweet potato cultivar. SPFMV is universal, but two other potyviruses. *Sweet potato virus* G (SPVG) and Sweet potato virus 2 (SPV2) synonymous with Ipomoea vein mosaic virus (IVMV) are also common. Sweet potato virus disease (SPVD) is one of the major constraints to sweet potato production.

Sweet potato virus disease (SPVD) is one of the major constraints to sweet potato production. Worldwide it causes a yield reduction of up to 90% (Schaefers and Terry, 1976; Gibson *et al.,* 1998; Carey *et al.,* 1999; Karyeija *et al.,* 2000; Gutierrez *et al.*, 2003; Loebenstein *et al.,* 2004). In Africa, infection by different viruses rank second to weevils in causing yield reduction (Geddes, 1990).

Some of the viruses reported to infect sweet potato include *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato latent virus* (SPLV), Sweet potato caulimolike virus (SPCa-LV), *Cucumber mosaic virus* (CMV), Sweet potato virus Y (SPVY), C-6 *Sweet potato virus G* (SPVG), *Sweet potato mild speckling virus* (SPMSV) and *Sweet potato leaf curl virus* (SPLCV) (Mukasa *et al.,* 2003; Untiveros *et al.,*2007). In East Africa a total of five viruses including SPCSV, SPMMV, SPCFV and SPLCV have been detected (Ateka *et al.*, 2004). The diversity and distribution of SPLCV in Kenya and the east Africa region has not been done.

2.4 Genome organization of Geminiviruses

The family Geminiviridae comprises a diversity of plant viruses that infect a broad variety of plants begomoviruses causing significant crop losses throughout the world (Hanley-Bowdoin *et al.,* 1999). They have circular single-stranded DNA genomes (2.5-3.0 kb) encapsidated in quasi-isometric virions of about 20-30 nm in diameter (Stanley *et al.,* 2005).

For efficient coding of proteins, the coat protein is conserved in its capacity to form these unique virions, but has diverged in terms of specificity for insect transmission (Briddon *et al.,* 1990).

The first geminivirus to be characterized at the sequence level, was the *African cassava mosaic virus* (ACMV) by Stanley and Gay (1983). It has typical genome arrangement of the majority of begomoviruses, consisting of two genomic components. Genomic component DNA A encodes all viral factors required for DNA replication, gene expression, and insect transmission (Figure 1), while the second (DNA B) encodes factors required for cell-to-cell movement in plants. A single viral coded protein is essential for replication: the replication associated protein that is conserved in sequence, position, and function (Gutierrez, 1999). All geminiviruses also carry one or more intergenic regions, one of which contains the origin of replication and the signature stem-loop structure containing an invariant nanonucleotide motif involved in rolling circle replication (Hanley-Bowdoin *et al.,* 1999).

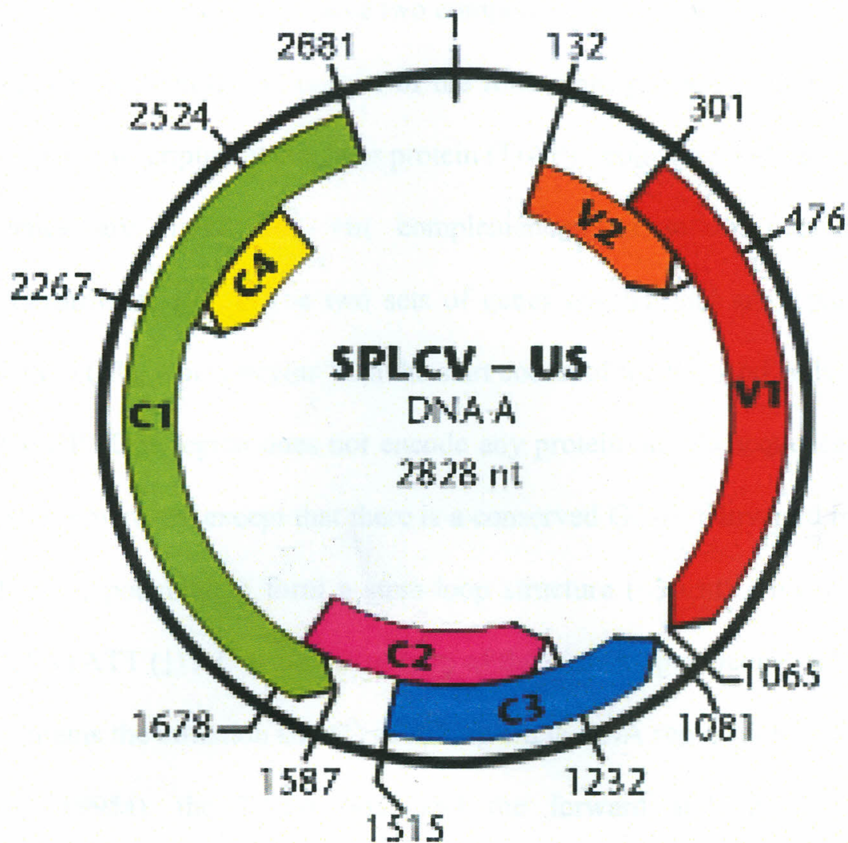


Figure 2.1: Genome organization of the Sweet potato leaf curl virus (SPLCV) component with AV1 for infection, AC1 replication enhancer and AC2 for transcription (Lotrakul and Valverde, 1999).

2.5 Genome organization and morphology of genus begomoviruses

The *Begomovirus* is the largest genus in the family geminiviridae and comprises whitefly transmitted geminiviruses which infect dicotyledonous plants (Fauquet *et al.,* 2003; 2008). They are small (ca. 18-30 nm) plant viruses with single-stranded circular DNA genomes that are encapsidated in twinned quasi-icosahedral particles. Begomoviruses are transmitted by whiteflies, and infect dicotyledonous plants; their genomes can be mono- or bipartite (Lazarowitz, 1992). Begomoviruses are mostly bipartite, but some old world begomoviruses are monopartite.

Bipartite begomoviruses have two components, designated A and B. Each component has ~2,600 nucleotide and consist of the following, genes — replication-associated protein (Rep), transcriptional activator protein (TrAP), replication enhancer (REn), and AC4. The genes are transcribed +in complementary sense or counterclockwise direction (Lazarowitz, 1992). The two sets of genes overlap and are separated by an intergenic region (IR), which begins with the start codon of the Rep and ends with the start codon of the CP. This region does not encode any protein and its sequence varies widely among begomoviruses, except that there is a conserved GC-rich inverted repeat sequence, which has the potential to form a stem-loop structure (~30 nt) with the invariant nanomeric TAATATT (↓) AC sequence or loop of the stem-loop structure. The nanomeric sequence contains the initiation site (↓) of rolling circle DNA replication (Gutierrez, 2000; Laufs *et al.,* 1995a), the TATA box, and the forward and inverted repeat. In bipartite begomoviruses, the IR also contains an identical sequence of ~200 nt in the A and the B components called the common region (CR) (Lazarowitz, 1992).

The CR sequence is different among different begomoviruses and is used to identify the A and B components of the same virus. The CP is required for encapsidation of progeny virions, vector transmission, virion structure, and host specificity (Figure1). For bipartite begomoviruses, the CP is not required for either local or systemic viral spread. In contrast, in all monoparticle begomoviruses, the CP is essential for viral spread (Gafni and Epel, 2002). The Rep is the only gene essential for replication, being required for transcription of both A and B components (Argüello-Astorga *et al.,* 1994).

Begomoviruses replicate in the nucleus of infected cells through a double-stranded DNA intermediate via a rolling circle mechanism. These inverted repeats or interactive sequences (iterons) have been reported to be specific binding sites for the Rep (Rep iteron-related domain), to initiate the rolling circle replication process (Argüello-Astorga and Ruiz-Medrano, 2001).

2.6 Sweet potato leaf curl virus

Different begomovirus have been reported infecting sweet potato. This include *ipomoea yellow vein virus* (IYVV), *sweet potato leaf curl virus*(SPLCV), *sweet potato leaf curl Georgia virus* (SPLCGV), *Ipomoea crinkle leaf curl virus* (ICLCV) (Argüello-Astorga and Ruiz-Medrano, 2001). All the viruses are transmitted by the same vector, the whitefly. Knowledge on the presence of different sweet potato leaf curl viruses in Kenya is still limited, though this is essential for crop protection. DNA-B component or DNA satellites have not been found associated with these begomoviruses (Lotrakul and Valverde, 1999; Lotrakul *et al.,* 2003; Briddon *et al.,* 2006).

They have satellite DNA-β molecules that may offer an advantage to begomoviruses, as they are thought to encode suppressor(s) of post transcription gene silencing (Mansoor *et al.*, 2001). The potential importance of some sweet potato begomoviruses has been overlooked mainly because, the virus do not show symptoms (Valverde *et al.,* 2007). SPLCV causes yellow vein symptoms in some Ipomoea species *(I. aquatica*, *I. fistulosa,* and *I. cordatotriloba*) and leaf curl symptoms in others (*I. alba, I. batatas* W-285, *I. lacunosa*, *I. lobata, I.* nil, *I. setosa* and *I. trifida*) (Lotrakul *et al.,* 2002).

SPLCGV causes leaf curl symptoms in several Ipomoea species, but unlike SPLCV, SPLCGV does not cause yellow vein symptoms on *I. aquatica* and *I. cordatotriloba* (Lotrakul *et al.,* 2003).

Genotypes that do develop characteristic upward curling symptoms generally do this only during warm periods of the year and may require the presence of other viruses for symptom development (Clark *et al.,* 2002). In the US, SPLCV was found in mixed infection with SPFMV and SPLCGV (Lotrakul *et al.,* 1998; 2003). Experiments with single and mixed infections with russet crack strain of SPFMV and SPLCV resulted in higher titers of SPLCV in mixed infections, while that of SPFMV remained the same (Kokkinos, 2006). It is possible that higher SPLCV titers could result in a more efficient transmission by whiteflies and therefore natural spread of the virus to uninfected plants. More research is needed to get information on the prevalence, economic impact and effects of mixed infection with SPLCV.

2.7 Whitefly vector for sweet potato leaf curl virus transmission

Bemisia tabaci is a plant sap-sucking insect in the family *Aleyrodidae* of superfamily *whitefly. Bemisia. tabaci* was first described in 1889 as a pest of tobacco in Greece, as *Aleurodes tabaci* (Gennadius, 1889). It is believed to have originated in India (Fishpool& Burban, 1994) and as a result of widespread dispersal, it is now distributed worldwide. Whitefly is a vector of *Begomovirus (Geminiviridae),Crinivirus (Closteroviridae) and Carlavirus or Ipomovirus (Potyviridae)* (Jones, 2003).

Whitefly-transmitted viruses cause important losses in many commercial crops throughout the tropics, and also in warmer temperate regions such as the Mediterranean basin (Morales, 2007). Begomoviruses are the most numerous of the *B. tabaci* transmitted viruses and can cause crop yield losses of between 20% and 100% (Brown and Bird, 1992). SPLCV is transmitted by *B. tabaci*, but not by the banded winged whitefly, *Trialeurodes abutilonea* (Valverde *et al.,* 2004).

2.8 Taxonomic criteria for species demarcation in Geminiviridae

Taxonomic criteria for demarcating species of Geminiviruses have been proposed by the International Committee on Taxonomy of Viruses (ICTV) based on the reliability and applicability of these criteria to the large number of characterized begomoviruses (Fauquet *et al.,* 2003). Nucleotide sequence comparison plays a much greater role in determining taxonomic status. Thus, for comparative analyses, only full-length DNA A sequences were considered based on recombination events that readily occur among begomoviruses (Pita *et al*., 2001; Fauquet *et al.,* 2003). A cut-off value of 89% of nucleotide sequence identity (NSI) of the A component was established to distinguish different species from strains (Fauquet *et al*., 2003). However, the coat protein region of this virus is fairly conserved between different species and is used to detect species and determine diversity in different virus groups, including begomoviruses.

2.8.1 Sweet potato begomoviruses an overlooked component

Until recently, most surveys of sweet potato viruses did not mention begomoviruses, even though leaf curl symptoms caused by unknown viruses have been observed for many years (Clark and Moyer 1988). SPLCV was first reported in the USA by Lotrakul *et al.*, (1998) and in Japan by Onuki and Hanada (1998). A molecular characterization of the US isolate of SPLCV was conducted by Lotrakul and Valverde (1999). An apparently distinct geminivirus *(Ipomoea crinkle leaf curl virus*), was also found in sweet potato in Israel (Cohen *et al.*, 1997). *Ipomoea yellow vein virus* formerly known as SPLCV-Ipo, has been found in *Ipomoea indica* in Spain and Sicily (Briddon *et al.*, 2006).

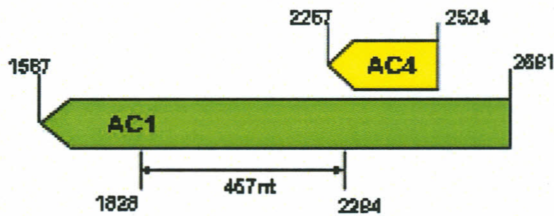
Although these reports are recent, there is evidence that there is considerable variability among the strains of begomovirus represented in these reports (Lotrakul *et al.*, 2002) and prior observations of leaf curl symptoms suggest that these viruses were present long before they were reported. Some of the strains either do not induce symptoms or induce very mild, transient symptoms in the standard indicator host. Some genotypes of sweet potato, such as cv. 'Beauregard', do not show any symptoms when infected with SPLCV (Clark and Hoy, 2006). Genotypes that do develop the characteristic upward curling at the margins of young leaves generally do so only during warm periods of the year and may require the presence of other viruses for symptom development (Clark *et al.*, 2002). The potential importance of begomoviruses is indicated in part by the study of Clark and Hoy (2006) in which they found that yields of 'Beauregard' sweet potato were reduced by 25-30% by SPLCV despite the fact that no symptoms were observed on the plants.

The development of sensitive PCR (Li *et al.,* 2004) and real-time PCR (Kokkinos and Clark, 2006) assays have helped recognize that SPLCV and related begomoviruses have sometimes escaped detection prior to the advent of these technologies.

2.8.2 Begomovirus detection by polymerase chain reaction

To detect sweet potato Begomoviruses, vine segments from sweet potato are graft inoculated onto an indicator host, *I. setosa,* which develops symptoms if the source material was infected (Cohen *et al*., 1997). To ensure accuracy, the grafting assay has to be done twice. Nucleic acid-based techniques, including polymerase chain reaction (PCR), offer the potential of great savings in time, greenhouse space, efficiency, and cost.

Genomic sequences of geminiviruses are available and have been utilized for designing primers for detection of geminiviruses in sweet potato. Different primers have been designed for the detection of SPLCV (Li *et al.,* 2004; Gutierez, 2008), some of which were degenerate in nature and very effective in detecting different begomoviruses. Gutierez (2008) designed specific primers to amplify the AC1 region and the full coat protein gene. Degenerate primers have broader detection range than virus-specific primers; therefore, they can be used to detect geminiviruses in invitro plantlets and greenhouse-grown sweet potato plants, and in several *Ipomoea* hosts.



Primers

PW285-1: 5'-TAATTCGAACTGCAGTTCCGTATTTCAGTT-3'
PW285-2: 5'-GCTAGAGGAGGCCTGCAGACTGCTAACGACG-3'

Primers

SPB-1: 5'-CAGAGTCGGTACCTATGACAGGGCGAA-3'
SPB-2: 5'-TACTCTGCAGTTAATTGTTGTGCGAATC-3'

Figure 2.2: Specific primers sequences used for the amplification of a fragment of the AC1 467 bp and the full length coat protein gene (AV1) 765 bp. Adapted from (Gutierrez, 2008).

2.8.3 Detection of Begomovirus by rolling circle amplification

The first task in virus research is the detection of virus or its viral nucleic acid in a host. The prevailing techniques, based on PCR and serology, are constrained by availability of prior sequence information and virus-specific antibodies. Moreover, these techniques are getting handicapped in the wake of fast evolving characteristics of the single-stranded viral genomes. To overcome these problems, many laboratories are employing a rolling circle amplification (RCA) technique, which uses a high fidelity ϕ29 DNA polymerase along with random hexamers to detect the genomes of various begomoviruses.

Owing to its simplicity, high specificity, sensitivity and multiplexity, rolling-circle amplification (RCA) has attracted significant attention in basic and applied research and is now turning into a customary technique for molecular diagnostics (Schweitzer and Kingsmore, 2001; Demidov, 2002, Demidov, 2004a; Kingsmore and Patel, 2003; Zhang and Liu, 2003). As analytical tool, RCA is generally based on the isothermal enzymatic rolling replication of DNA minicircles hybridized to single-stranded (ss) DNA or RNA targets. Since DNA samples are normally obtained in the double-stranded (ds) form, a denaturation is needed to separate the DNA complementary strands for probe hybridization. Peptide nucleic acid (PNA) oligomers can be employed as site-specific openers of the DNA double helix to locally expose a designated marker sequence inside duplex DNA (Figure 3). The opened DNA site is then hybridized to a circularizable oligonucleotide probe, which is subsequently closed by DNA ligase. This way, the marker sequence from the DNA duplex of interest can be isothermally amplified by a variety of DNA polymerases via the (RCA) mechanism (Zhang and Liu, 2003).

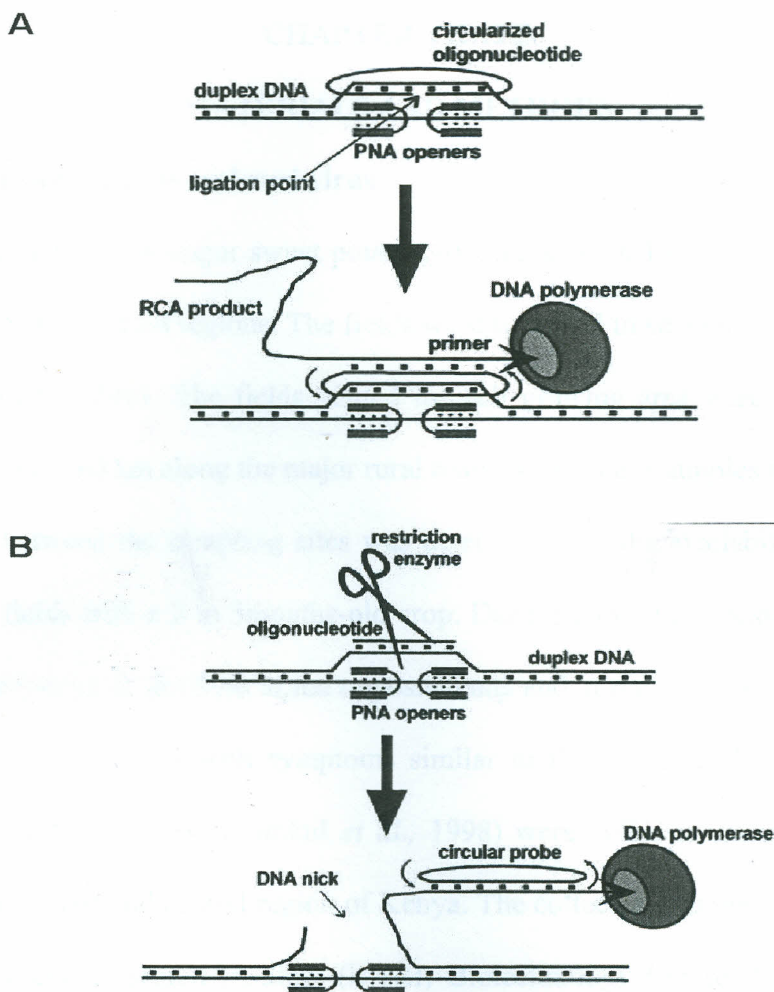


Figure 2.3: Schematics of the design used for peptide nucleic acid assisted rolling circle amplification reactions performed either on earring probe (A) or with employment of artificial nickase system (B). Both schemes are based on the formation of peptide D-loop consisting of the locally open short dsDNA segment, a pair of bisPNA openers and an oligodeoxyribonucleotide (Bukanov *et al.,* 1998). Another approach developed for the PNA-assisted RCA of dsDNA sequences employs the design of site-specific artificial nickase system (Kuhn *et al.,* 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Survey of sweet potato leaf curl virus

Survey was conducted in major sweet potato growing areas in Kenya, namely Western, Coast, Eastern and Central regions. The fields were surveyed in several counties between March and August 2011. The fields visited in each growing area were sampled at an interval of about 6-10 km along the major rural roads from where samples were collected. The interval between the sampling sites was determined by the availability of suitable sweet potato fields with a 2 to 3months-old crop. During sampling, plants were selected along representatives of the field at the opposite ends and at the centre of two diagonals of the fields. Sweet potato with symptoms similar to those observed in sweet potato infected with leaf curl virus (Lotrakul *et al.,* 1998) were collected from three regions namely western coast and central region of Kenya. The collected samples were planted at Kenya Agricultural Research Institute (KARI) Biotechnology Centre, Nairobi. Leaves from the sweet potato plants were collected for DNA extraction and PCR analysis using the three primer pairs. The PCR products from the positive samples were sent to Macrogen Amsterdam for sequencing using Sanger sequencing platform.

3.2 Graft inoculation of sweet potato with sweet potato leaf curl virus

Central region sweet potato cuttings positive with specific primers PW285-1/2 and SPB 1/2 were respectively grafted to a one week old *Ipomoea setosa* test plant in greenhouse. The infected stock as the virus donor was used to transfer virus and make sure the new sprouts had a greater viral titer compared with the mother plant.

It involved cutting the infected plant with at least two nodes, the leaves were trimmed and a wedge shape cut made on both side of the plant. A clean slit was made using a flame sterilized blade. The scion was inserted while ensuring the xylem and phloem of both donor and recipient *I. setosa* were in contact and wrapped with parafilm gently to avoid breaking. Plant were maintained at low temperature in the green house and left for 8-10 days for symptom expressions and assessment done for 8 days. The *Ipomoea setosa* were observed for any symptom variations that may occur due to infection by different *Sweet potato leaf curl virus* isolates

3.3 Optimization of Polymerase Chain Reaction for the Detection of *Sweet Potato Leaf Curl Virus*

Three primer pairs were tested for their ability to detect SPLCV in the sweet potato samples suspected to be infected with the virus. The samples were obtained from a germplasm collected from different parts of the country and maintained in a greenhouse at KARI Biotechnology Centre, Nairobi, Kenya. The primer pairs tested included primer PW1/2, SPB 1/2 and SPG 1/2. Primer pair PW1/2 was expected to amplify a 500 base pair fragment (Li *et al.*, 2004), primer SPB 1/2 expected to amplify a 700 base pair fragment while SPG was expected to amplify 900 base pair fragment (Gutierrez, 2008) (Table 3.1).

Table 3.1: PCR sequences used in detection of SPLCV

PRIMER NAME	PRIMER SEQUENCE	REFERENCE
SPG1/2	5′-CCCCKGTGCGWRAATCCAT-3′	
	5′-ATCCVAAYWTYCAGGGAGCTAA-3′	Li et *al.,* 2004
SPB1/2	5-CAGAGTCGGTACCTATGACAGGGCGAA-3’	Gutierez,2008
	5-TACTCTGCAGTTAATTGTTGTGCGAATC-3’	Gutierez,2008
PW285-1/2	5-TAATTCGAACTGCAGTTCCGTATTTCAGTT-3′	Li et *al.,* 2004
	5-GCTAGAGGAGGCCTGCAGACTGCTAACGAC-3′	Li et *al.,* 2004

After primer optimization, leaves from all samples were collected from the *plants* in the screen house by cutting a small portion into a polythene bag. DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) extraction method (Li *et al.,* 2004). Extraction buffer (450 µl) constituted (3% CTAB, 1% polyvinylpyrrolidone, 100 mM Tris- HCl, pH 8.0, 1.4 M NaCl, 0.5M EDTA, and 1% mercaptoethanol).

Thirty milligrams of leaves were homogenized in a mortar and pestle; and the homogenate was incubated at 65°C for 15 min and centrifuged at 10,000rpm for 10 min. The supernatant was transferred to a 1.5-ml micro centrifuge tube and mixed with an equal volume of chloroform: isoamyl alcohol (24:1). The mixture was centrifuged at 12,000 rpm for 10 min and the supernatant (500 µl) transferred to a 1.5-ml micro centrifuge tube before adding 700 µl of isopropanol. The mixture was incubated on ice for 10 min and centrifuged at 12,000 rpm for 15 min. The pellet was washed with 70% ethanol and centrifuged at 12,000rpm for 5 min. Pellet was air dried and dissolved in 50 µl free molecular grade water.

The polymerase chain reaction (PCR) amplifications were performed on a Gene Amp PCR system 9700 (APPLIED BIOSYSTEM) in 25-μl reaction mixtures containing 2 μl of the DNA extract, 0.5 μl of each primer (10 μM), 0.5 μl of 10 mM dNTP mix, 1.6 μl of *Taq* DNA polymerase (BIOLABS USA), 2.5 μl reaction buffer, 2.5 μl of 25 mM $MgCl_2$, 0.2 μl, and 16.34 μl of molecular water. PCR conditions used were as follows: 94°C for 1min, 55°C for 1 min, 72°C for 3 min), 45 cycles and 72°C for 10 min. PCR products were assessed by electrophoresis in 1% agarose gels in TBE (Tris, boric acid, EDTA) buffer (0.9M Tris, 20 mM EDTA, 0.9M boric acid, and pH 8.0), stained with ethidium bromide, and viewed under ultraviolet light.

DNA purification was subsequently done using Qiagen kit by Adding 5 volumes of Buffer PBI to 1 volume of the PCR sample and mix. For example, add 500 μl of Buffer PBI to 100 μl PCR sample (not including oil, check that the color of the mixture is yellow (similar to Buffer PBI without the PCR sample). If the color of the mixture is orange or violet, add 10 μl of 3 M sodium acetate, pH and mix. The color of the mixture will turn to yellow. Place a QIAquick spin column in a provided 2 ml collection tube.

To bind DNA, apply the sample to the QIAquick column and centrifuge for 30 – 60s. Discard flow-through. Place the QIAquick column back into the same tube Collection tubes are re-used to reduce plastic waste. To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 30–60 s, discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min and the purified PCR products sent to Macrogen Amsterdam for sequencing.

3.4 Analysis DNA sequence data

Nucleotide sequence comparison and phylogenetic analysis were done using multiple sequence alignment of CLUSTALW program (Thompson *et al.,* 1994). Phylogenetic trees were generated using MEGA5 program (Tamura *et al.,* 2011).

Nucleotide and derived amino acid sequences of the AC1, AC2, AC4 fragments and the full length of the CP gene (AV1) were compared to the corresponding sequences of sweet potato Begomovirus isolates available in the Genbank using pair wise and multiple alignments. Two sequences of SPLCV isolates SPLGCV and SPLCV-USA, previously reported in the US (Lotrakul *et al*., 2002), were included in the analysis.

Nucleotide sequences of four SPLCV isolates from Japan, SPLCV-Japan (Kumamoto), SPLCV-Japan (Miyazaki), China-SPLCV, *Tomato yellow leaf curl China virus*, SPAIN, KOREA, two from Kenya (Kenya 43 and Kenya 4), SPLCV-Puerto Rico I and II and *Ipomoea yellow vein virus* were used. *Beet curly top virus* was also included as an out-group for analysis and phylogenetic trees (Table2). Neighbor-joining trees with bootstrap analysis (1000 replicates) were constructed from the multiple alignments and drawn using MEGA5 computer software.

Table 3.2: Geminiviruses sequences obtained from the Gene Bank used in the analyses of the AV1, AC1, AC2 and AC4 fragment of sweet potato Begomoviruses

VIRUS NAME	ASSIGNED ABBREVIATION	ACCESION NUMBER
SPLCV-Kenya 43	KENYA 43	DQ361005
Beet curly top virus	BCTV	NC_001412
Tomato yellow leaf curl China virus	TYLCCNV	NC_004044
Ipomoea yellow vein virus	IYVV	AJ586885
Sweet potato leaf curl Georgia virus	SPLCGV	AF326775
SPLCV US-	AL-PRAKASH	AY679764
SPLCV-China	CHINA	DQ512731
SPLCV-Japan (Miyazaki)	JAPAN-MI	AB433786
SPLCV-Japan (Kumamoto)	JAPAN-KU	AB433787
SPLCV-Puerto Rico I	P.RICO I	AY679766
SPLCV-Puerto Rico II	P.RICO II	AY679767
SPAIN	SPAIN	EF456743
KOREA	KOREA	AY679765
KENYA 4	KENYA 4	DQ361004

CHAPTER FOUR

RESULTS

4.1 Optimization of Polymerase Chain reaction protocol for the Detection of *sweet potato leaf curl virus*

A total of 6 cassava infected samples showing leaf curl symptoms were identified from germplasm maintained at the KARI Biotechnology Centre screen house. Primer pair SPG1/2 amplified the expected 900bp fragment (Figure 4). The other primer pairs SPB1/2 and PW285-1/2 were also subjected to amplification and gave the expected 500bp and 700bp fragments, respectively (Figure 5 and 6).

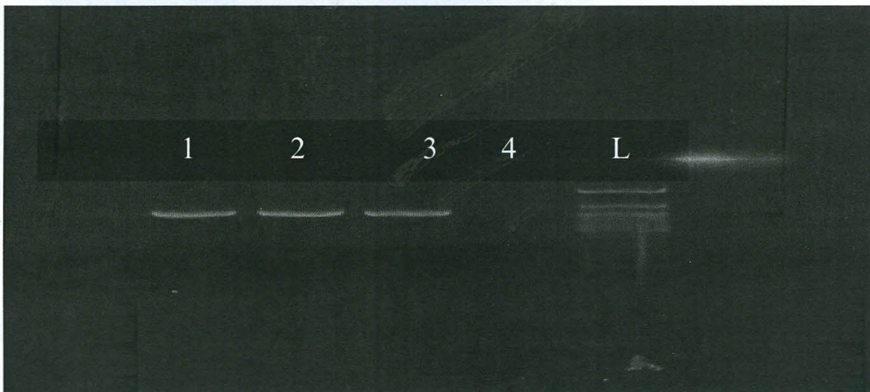


Figure 4.1: Optimization of primer SPG 1/2 for use in PCR detection of *Sweet potato leaf curl virus* (SPLCV) in sweet potato. Lane marked L is DNA ladder while PCR product from sweet potato infected with SPLCV are shown in lanes 1to 4.

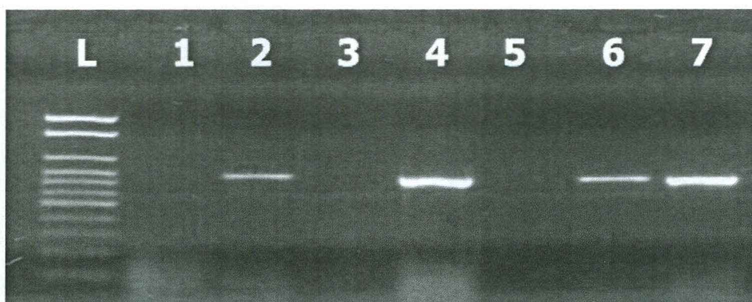


Figure 4.2: Optimization of primer SPB 1&2 for use in PCR detection of Sweet potato leaf curl virus (SPLCV). Lane marked L is DNA ladder while PCR product from sweet potato infected with SPLCV are shown in lanes 1to 7.

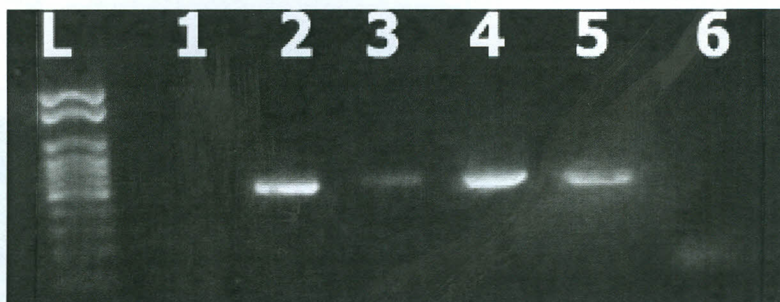


Figure 4.3: Optimization of primer PW285-1/2 for use in PCR detection of Sweet potato leaf curl virus (SPLCV). Lane marked L is DNA ladder while PCR product from sweet potato infected with SPLCV are shown in lanes 1to 6.

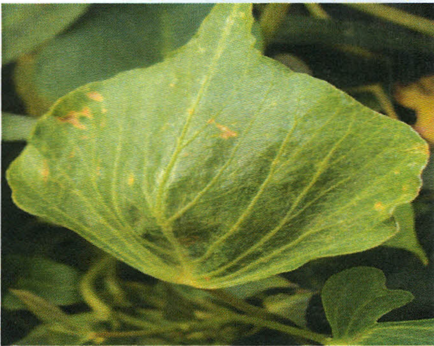
4.2 Distribution of Begomoviruses infecting sweet potato in Kenya

4.2.1 Result of surveys conducted in western, central and coastal regions

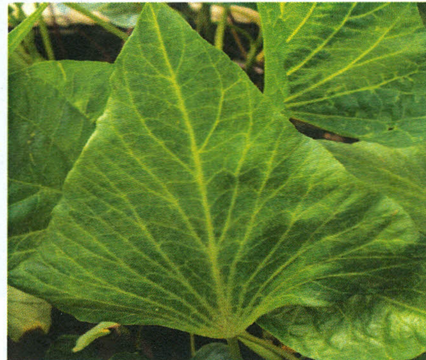
A total of 512 samples were collected from western, central and coastal regions of Kenya (Table 3). Samples collected exhibited varied symptoms which included mosaic, leaf curl, yellowing, vein clearing, stunting, upward cupping, reduced leaf size, and chlorosis after greenhouse establishment (Figure 7).

A)Vein clearing

B)Stunting and chrolosis



C) Backward folding of the leaf



D) Vein Banding

Figure 4.4: Sweet potato samples collected from Central and Coastal regions of Kenya showing different different *Sweet potato leaf curl virus* (SPLCV) symptoms; (A) Vein clearing (B) Stunting and chrolosis (C) Backward folding of the leaf (D) Vein Banding.

All the 512 samples were tested for the presence of SPLCV using the 3 primer pairs described in section 2.1. The results are summarized in table 3.

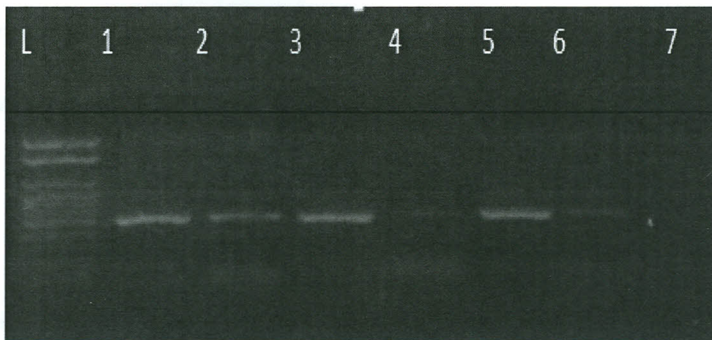


Figure 4.5: Polymerase chain reaction amplified products from sweet potato samples infected with *Sweet potato leaf curl virus* (SPLCV) using Primers, PW285-1/2 (A), Lane L, 1-kb DNA ladder; lane 1 positive control lane 7 negative control.

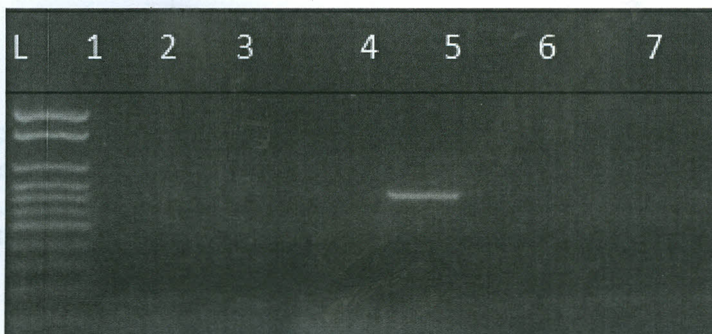


Figure 4.6: Polymerase chain reaction amplified products from sweet potato samples infected with *Sweet potato leaf curl virus* (SPLCV) using Primers, SPB1/2 (A), Lane L, 1-kb DNA ladder; lane 5 positive control.

In the western region, a total of 76 samples out of the 97 collected were positive to either one, two or all of the 3 primers, giving a the highest virus occurrence of 78.4% among the three sweet potato growing regions of Kenya (Table 4.1). A total of 38 samples were positive with degenerate primer SPG1/2, 33 samples were positive with SPB1/22 and 40 samples were positive with 285-1/2. Kakamega was the most affected while Siaya and Emuhaya counties had the least occurrence. In Central region, no sample was positive with degenerate primer SPG1/2, 6 samples were positive with SPB1/2, and 4 samples were positive with PW285-1/2.

A total of 9 samples out of the 232 collected were positive to either one or two of the 3 primers, giving a virus occurence of 3.9%. Some of the counties such as central Imenti, Gichugu south, Emuhaya, Kirinyaga central, Muranga, Mwea, South Imenti, and Tetu had no SPLCV being detected (Table 4.2). The Coastal region had the second highest SPLCV occurence at 69.4% with 127 out of 183 samples being positive to either one two or all the three primers. A total of 110 samples were positive with SPG1/2, 31 samples positive with SPB1/2 and 21 samples were positive with PW285-1/2 (Table 4.3). Wundanyi was the most affected area and Taita Taveta being the least affected.

Table 4.1: Percentage of sweet potato samples infected with SPLCV from Western region of Kenya.

Western region	TOTALS	+VE SPG	+VESPB	+VEPW	Cumulative Total
Kakamega	54	21	19	25	44
Siaya	12	8	5	9	12
Busia	17	6	7	8	10
Bungoma	14	2	1	8	10
Total	**97**	**37 (38.1%)**	**32 (33%)**	**39 (40.20)**	**76 (78.4%)**

The sign +VE refer to positive and –VE refer negative when applied using PCR

Table 4.2: Percentage of sweet potato samples infected with SPLCV from Central region of Kenya. The sign +VE refer to positive and –VE refer negative when applied using PCR.

Central region	TOTALS	+VE SPG	+VESPB	+VEPW	Cumulative Total
Murang'a	35	0	0	1	1
Nyeri	54	0	0	0	0
Kirinyaga	33	0	0	1	1
Embu	20	0	0	1	1
Meru south	11	0	0	0	0
Tharaka Nithi	14	0	0	0	0
Meru	47	0	6	1	6
Total	**232**	**0**	**6(2.6%**	**3 (1.3%)**	**9 (3.9 %)**

Table 4.3: Percentage of sweet potato samples infected with SPLCV from Central region of Kenya. The sign +VE refer to positive and –VE refer negative when applied using PCR.

Coast region	TOTALS	+VE SPG	+VESPB	+VEPW	Cumulative Total
Kilifi	57	34	8	6	41
Taita taveta	67	45	11	7	47
Kwale	59	31	12	8	39
Total	**183**	**110 (60.1%)**	**31 (17.0%)**	**21 (11.5%)**	**127(9.4%)**

4.2.2 Symptom expression of SPLCV in *I. setosa*

The different SPLCV isolates collected from the different region were subjected to biological characterization using the near universal indicator plants, *Ipomoea setosa*, to determine if there was a difference in symptom expression. The samples were selected on the basis of being positives using PCR. Leaf rolling, chlorosis and leaf necrosis were the common symptoms observed in *I. setosa* (Figure 4.7). No distinct symptom expression was associated with specific samples.

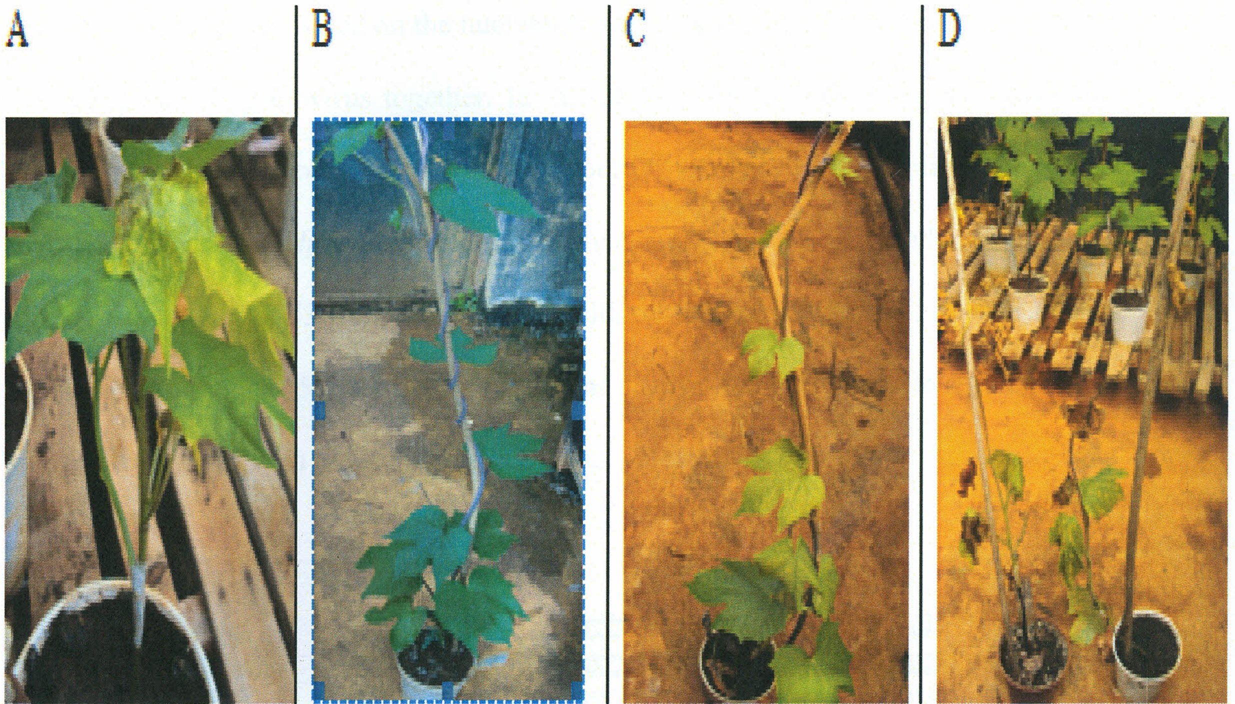


Figure 4.7: ***Ipomoea setosa*** **plants showing** ***Sweet potato leaf curl virus*** **(SPLCV) symptoms after graft-inoculation with Central samples SPLCV infected scions from sweet potato. A, shows an inoculated plant, B shows leaf curling, C shows yellowing, while D shows leaf death.**

4.3 Virus diversity and variability studies

4.3.1 Genetic Diversity and variability in AC1 fragments

Among the samples that tested positive with PW285-1/2, five were sequenced and their nucleotide and amino acid sequences compared with others reported from different parts of the world (Figure 4.8 and 4.9). The five samples were Kenya 4, 10 Western, 50 Western, 60 Western, Kenya. The other three were closely related to PRICO 1 US isolate with nucleotide sequence identities that varied from 61 to 96%, while one (50-Coast) was related to SPLCV Kenya 4 previously reported in Kenya (Miano *et al.,* 2006) with 94% nucleotide sequence identity (Table 4.4).

A neighbor-joining tree based on the nucleotide sequence of the AC1 fragments clustered all sweet potato begomovirus together. In this cluster, sweet potato begomovirus were divided in two major groups (Figure 4.10). Group I included SPLCV isolate from USA, Japan-KY, Japan-KU, *Ipomoea yellow vein virus* (IYVV), Korea and a previously reported isolate Kenya 43 from Kenya along with 60-Western (Figure 4.11). The second group included isolates 50-coast, 10-western and Kenya-4 which were closely related to Prico1 and 11 along with the isolate from China.

```
Kenya4       CGGCATCCATGACATTTTCAGCGGCCCAATCGCTGATAATGTCAGGGACAGCATTGAAAG
10Western    CGGCATCCATGACATTTTCAGCGGCCCAATCGCTGATAATGTCAGGGACAGCATTGAAAG
50           CGGCATCCATGACATTTTCAGCGGCCCAATCGCTGATAATGTCAGGGACAGCATTGAAAG
Kenya        CGGAATCCATGACATTTTCAGCAGCCCAGTCGCTGATAATGTCAGGAACGGCATTGAAAG
60Western    CGGAATCCATGACATTTTCAGCGGCCCAGTCGCTGATAATGTCAGGAACGGCGTTGAAAG
             *** ****************** ***** **************** ** ** *******

Kenya4       AAGAAGATAAAAAAGGTGAAGAATATACAGATGGGGGAGGAGAAAATATCCTATCTAAAT
10Western    AAGAAGATAAAAAAGGTGAAGAATATACAGATGGGGGAGGAGAAAATATCCTATCTAAAT
50           AAGAAGATAAAAAAGGTGAAGAATATACAGATGGGGGAGGAGAAAATATCCTATCTAAAT
Kenya        AAGAAGAATAAAAAGGAGAAGAATATACAGATGGTGGAGGAGAAAATATCCTATCTAAAT
60Western    AAGACGAAGAAAAAGGAGAAGAATATACAGAAGGTGGAGGAGAAAAGATCCTATCTAAAT
             **** **  ******* ************** ** *********** *************
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Figure 4.8: Multiple alignment A 512 nucleotide fragment corresponding to the AC1 was amplified with primers PW285-1 and PW285-2 for Kenya collected isolates.

```
CLUSTAL 2.0.11 multiple sequence alignment ORFS of AC1 Kenyan isolates compared with other countries.

PRICO               --TGGATTGCCAGTCCTTCTGGGCCCCCATGAAT--TCTTTAAAGTGCTTTAGGTA--TT 54
China               --TGGCTTCCCGTACTTTGTGTTTGATTGCCAGTC-TCTTTGGGCCCCAATGAATTCTTT 57
PRICO1              --TGGATTGCCAGTCCTTCTGGGCCCCCATGAAT--TCCTTAAAGTGCTTTAGGTA--TT 54
Japan-Ky            --TGGATTGCCAGTCCTTCTGGGCCCCCATGTAT--TCCTTAAAGTGCTTTAGGTA--TT 54
Japan-KU            --TGGATTGCCAGTCCTTCTGGGCCCCCATGAAT--TCCTTAAAGTGCTTTAGGTA--TT 54
IYVV                --TGGATTGCCAGTCCTTCTGGGCCCCCATAAAT--TCCTTAAAGTGCTTTAGGTA--TT 54
Kenya               --TAGATTGCCAGTCCTTCTGGGCCCCCATGAAT--TCCTTAAAGTGCTTTAGGTA--TT 54
Korea               --TGGATTGCCAGTCCTTCTGGGCCCCCATGAAT--TCCTTAAAGTGCTTTAGGTA--TC 54
50                  -AATCGTTGT--AGTCTAGCAGGCCTCCTCTACA--CCTTAAAAGTGCTTTAAGTA--TT 53
60Western           TAACAGTCG---ATACTAGTGTGCCGTACCTTCA--CTTAGACAGTGCTTTAGGTA--TT 53
10western           -GTTGGGTGC--GTCTTTCTGGGGACCCATGA-C--TCTTTAAAGTGTTTTAGGTA--TT 52
Kenya4              --TGGATTGCCAGTCCTTCTGGGCCCCCATGAAC--TCTTTAAAGTGCTTTAGGTA--TT 54
BCTV                --TTGTCTGCCACTCCTTTTGTGCTCCAATAAGGTGTTTCCAGTGTTTCTTAAGTAAGT- 57
                                    *                                 *   *   *

PRICO               GGGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTATACACTTTTGGACTCA 114
China               GGGGGTCGACGTCATCAATGACGTTGTACCAAGCACTATTACTGTACACTCTTGGACTCA 117
PRICO1              GGGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTATACACTTTTGGGCTCA 114
Japan-ky            GGGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTGTACACTTTAGGGCTTA 114
Japan-KU            GGGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTGTATACTTTAGGGCTTA 114
IYVV                GGGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTGTACACCTTTGGGCTTA 114
Kenya               GCGGGTTGACGTCATCGGTGACGTTATACCAAGCACTGTTGCTGTATACTTTTGGGCTTA 114
Korea               GGGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTGTACACTTTTGGGCTTA 114
50                  GCGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTGTACACTTTTGGGCTTA 113
60Western           GGGGATTGACGTCATCAATGACGTTGTACCAAGCACTGTTGCTGTAAACTTTTGGGCTTA 113
10western           GGGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTGTACACTTTTGGGCTTA 112
Kenya4              GCGGGTTGACGTCATCAATGACGTTATACCAAGCACTGTTGCTGTACACTTTTGGGCTTA 114
BCTV                -GGGATCTACGTCATCAATGACGTTGTATTCCACTTCATCATAATACGTTCTAGGGCTAA 116
                      ** *  ********  ******* **     *    *     **     * ** ** *
*-Conserved region
```

Figure 4.9: Multiple alignment A 512 nucleotide fragment corresponding to the AC1 was amplified with primers PW285-1 and PW285-2 with other sequences from gene bank.

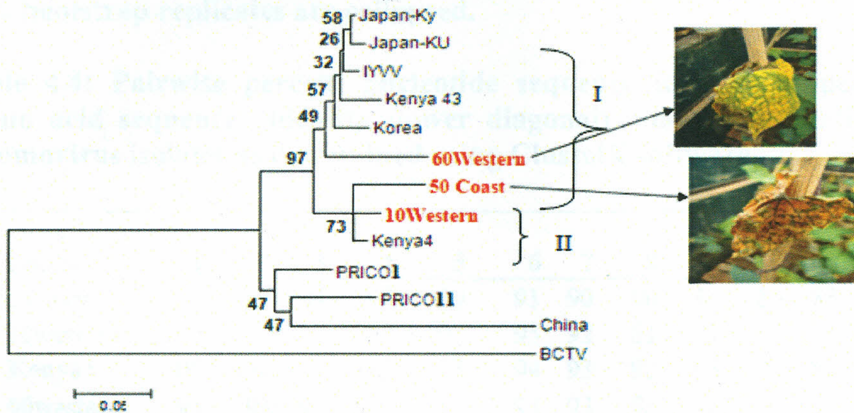

Figure 4.10: Neighbor-joining tree based on the nucleotide sequence of the AC1 fragment showing the relationship among begomoviruses infecting sweet potato in Kenya and other begomoviruses. *Beet curly top virus* (BCTV), genus *Curtovirus*, was used as outgroup. The tree was generated using MEGA5 (Tamura *et al.*, 2011). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed.

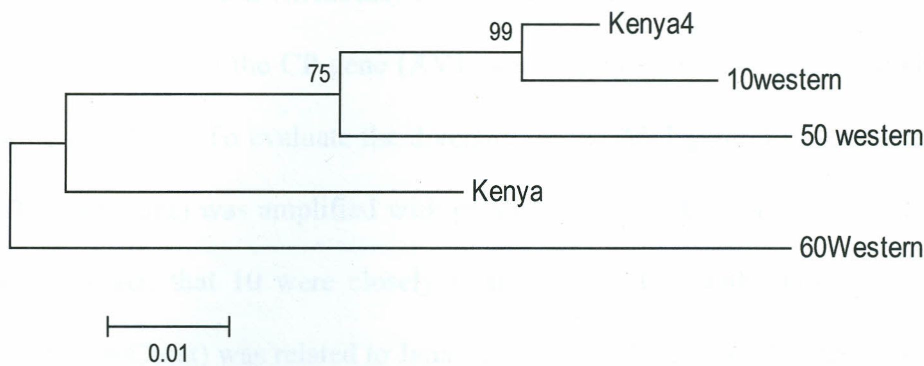

Figure 4.11: Neighbor-joining tree based on the nucleotide sequence of the AC1 fragment showing the relationship among begomoviruses infecting sweet potato in Kenya. *Beet curly top virus* (BCTV), genus *Curtovirus*, was used as out group. The tree was generated using MEGA5 (Tamura *et al.*, 2011). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed.

Table 4.4: Pairwise percent nucleotide sequence identity (upper diagonal) and amino acid sequence identity (lower diagonal) among the partial ORF AC1 of begomovirus isolates as determined using ClustalX software.

	virus isolate	1	2	3	4	5	6	7	8	9	10	11	12	13
1	50coast		94	94	90	89	91	90	90	89	86	83	78	61
2	10western	100		97	89	92	93	92	81	92	89	86	80	62
3	Kenya4	100	100		89	93	94	93	93	93	90	88	81	62
4	60western	95	95	95		91	93	93	91	92	85	83	78	62
5	Korea	97	97	97	96		96	95	94	96	90	87	81	61
6	Japan-Ky	94	94	94	94	94		97	95	98	90	88	81	62
7	IYVV	92	92	92	90	92	92		96	97	90	88	80	62
8	Kenya	97	97	97	96	98	94	92		96	88	86	79	63
9	Japan-KU	98	98	98	96	98	94	92	99		90	87	81	62
10	PRICO1	96	96	96	94	96	92	91	96	97		90	83	62
11	PRICO	97	97	97	96	98	94	92	98	99	96		63	62
12	China	78	78	78	76	79	80	78	78	78	76	78		
13	BCTV	54	54	54	52	52	53	50	52	52	52	52	46	

4.3.2 Genetic Diversity and variability in AV1 fragments

The sequence analysis of the CP gene (AV1) was done by multiple sequence alignment (Figure 4.12 and 4.13). To evaluate the diversity on the AV1 gene, full length of the CP gene (793 nucleotides) was amplified with primers SPB-1/2. Out of fifteen sweet potato genotypes indicated that 10 were closely related to SPLCV with nucleotide sequence identities. One (6-Coast) was related to Japan-KY with 99% nucleotide sequence identity. Nucleotide sequence of the AV1 fragment of (17-Central) was related to SPLCV- Spain isolate with 95% nucleotide sequence identity and 94% amino acid sequence similarity with India isolate. According to the nucleotide sequence of the AV1 fragments, (15-Central, 13-Coast, 30-Western, 15-Coast) the isolates were closely related to SPLCV with over 95 % nucleotide sequence.

Isolates 30 and 3 were closely related with 98 % nucleotide sequence. A neighbor-joining tree based on the nucleotide sequence of the AV1 fragments clustered all sweet potato begomoviruses together. In this cluster, sweet potato begomoviruses were divided in three major groups (Figure 4.14 and 4.15). Group one included Kenya collected isolates (23-Central, 19-Central, 26-Central and 25-Western). Group two included isolates from USA, Japan and China. Group three had 19-coast and SPLGCV were related with 93 % nucleotide sequence identity and an amino acid sequence identity of 96%. Isolates (17-central), (10-Coast) were closely related to isolates from Spain and India. Results indicated that isolates were closely related to SPLCV with amino sequence identities that ranged from 90 to 100 % (Table 8). The CP gene sequence of the (6-Coast) isolate, was related to 13-Western).

```
CLUSTAL 2.0.11 multiple sequence alignment ORFS of AV1 Kenyan isolates compared with other countries.


SPAIN           ---ATGGGGGACTC--CGGCAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCTTA 55
17central       --TTAGGGGGACT---CGGACAAACGGAGGCTGAACTTCGAGACCGCTATCGTGCCTTA 55
INDIA           -ATGGGGGAGAC----CGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
10Coast         ---ATGGGGGACTC--CGGCAAGACGGAGGCTGAACTTCGAGACCGCTATCGTGCCTTA 55
15central       -ATAGGGGAGAC----AGGTAAGACGGAGACTGAACTTCGAGACAGCTATCGTGCCTTA 55
13coast         -ATGGGGGAGAC----AGGTAAGACGGAGACTGAACTTCGAGACAGCTATCGTGCCTTA 55
30western       -TAAGGGGAGAC----AGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCTTA 55
3Western        -ATGGGGGAGAC----AGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCTTA 55
15Coast         -ATGGGGGAGAC----AGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
26central       -ATGGGGGAGAC----CGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
19Central       -ACGGGGGAAGTA---CGGTAAAA-GGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
23central       -ATGGGGGAGAC----CGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
25western       -ATGGGGGAGAC----CGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
USA             -ATGGGGGAGAC----CGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
JAPAN           -ATGGGGGAGAC----CGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
CHINA           -ATGGGGGAGAC----CGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
19coast         -ATAGGGGGAGA----AGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
SPLGCV          -ATGGGGGAGAC----CGGTAAGACGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTA 55
14Western       -ATGGGGGACAC----CGGTAAGACGGAGGCTGAACTTCGAGACCGCTATCGTGCCCTA 55
13Western       AGTAGTAGGGATC----AGGACATACGGAGGCTGAACTTCGAGACCGCTATCGTGCCCTA 56
6coast          -GTAAGGGGGACTC--CGGCTAAACGGAGGCTGAACTTCGAGACCGCTATCGTGCCTTA 57
BCTV            ---ATGAGGAAATATACAAGAAATACGTATAC-AATGTCCAGAAAAGAAAGGTGA---A 53
                       **         *  * * * *  *  *  * * ***     *  ***    *
```

***-Conserved region**

Figure 4.12: Multiple alignment Isolates from Western, Central and coast were selected twenty nine samples for the sequence analysis of the CP gene (AV1) with others from the gene bank.

```
15central     CGGAGACTGAACTTCGAGACAGCTATCGTGCCTTACACTGGGAATGCTGTCCCAATTGCT
30western     CGGAGGCTGAACTTCGAGACAGCTATCGTGCCTTACACTGGGAATGCTGTCCCAATTGCT
3Western      CGGAGGCTGAACTTCGAGACAGCTATCGTGCCTTACACTGGGAATGCTGTCCCAATTGCT
13coast       CGGAGACTGAACTTCGAGACAGCTATCGTGCCTTACACTGGGAATGCTGTCCCAATTGCT
15Coast       CGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTACACTGGGAATGCTGTCCCAATTGCT
14Western     CGGAGGCTGAACTTCGAGACCGCTATCGTGCCCTACACTGGGAATGCTGTCCCAATTGCT
13Western     CGGAGGCTGAACTTCGAGACCGCTATCGTGCCCTACACTGGGAATGCTGTCCCAATTGCT
23central     CGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTACACTGGGAATGCTGTCCCAATTGCT
26central     CGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTACACTGGGAATGCTGTCCCAATTGCT
25western     CGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTACACTGGGAATGCTGTCCCAATTGCT
19Central     -GGAGGCTGAACTTCGAGACAGCTATCGTGCCCTACACTGGGAATGCTGTCCCAATTGCT
19coast       CGGAGGCTGAACTTCGAGACAGCTATCGTGCCCTACACTGGGAATGCTGTCCCAATTGCT
10Coast       CGGAGGCTGAACTTCGAGACCGCTATCGTGCCTTACACTGGGAATGCTGTCCCAATTGCT
17central     CGGAGGCTGAACTTCGAGACCGCTATCGTGCCTTACACTGGGAATGCTGTCCCAATTGCT
6coast        CGGAGGCTGAACTTCGAGACCGCTATCGTGCCTTACACTGGGAATGCTGTCCCAATTGCT
               **** ************** *********** ***************************
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Figure 4.13: Multiple alignment Isolates from Western, Central and coast were selected for the sequence analysis of the CP gene (AV1)

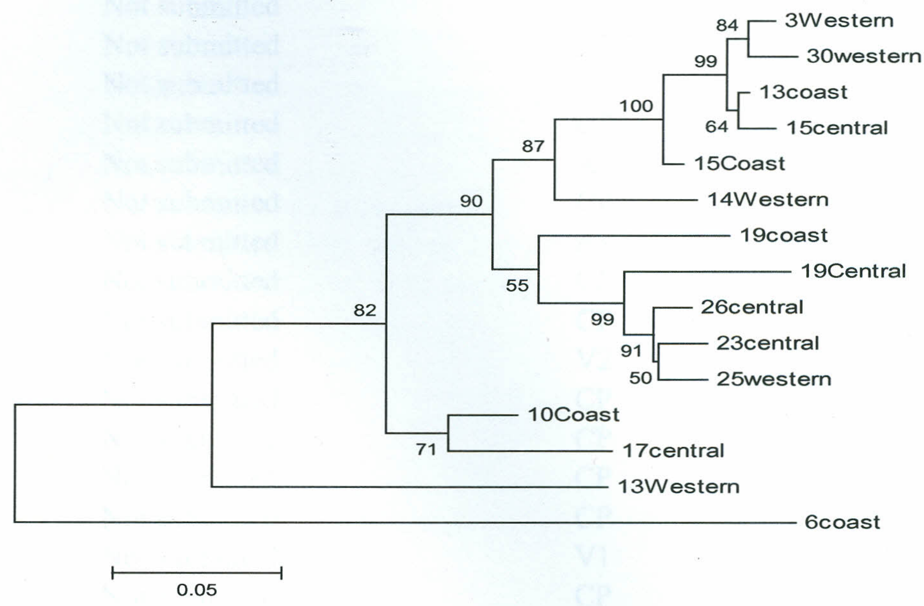

Figure 4.14: Neighbor-joining tree based on the nucleotide sequence of the AV1 fragment show the relationship among begomovirus infecting sweet potato in Kenya and other begomovirus.

Table 4.5: Sweet potato leaf curl virus sequences for AV1 (coat protein).The virus isolates were collected from (W) western (C) Central and CO Coast region of Kenya.

SAMPLE NO	GENE BANK ACCESSION NUMBER	GENE
11CO	Not submitted	AV1
38 CO	Not submitted	CP
24 CO	Not submitted	CP
6W	Not submitted	CP
8W	Not submitted	AV1
10CO	Not submitted	CP
82CO	Not submitted	CP
13CO	Not submitted	CP
13W	Not submitted	AV1
14CO	Not submitted	CP
15W	Not submitted	CP
15CO	Not submitted	AV1
17CO	Not submitted	AV1
19CO	Not submitted	CP
25W	Not submitted	AV1
19CO	Not submitted	CP
23CO	Not submitted	AV1
30W	Not submitted	CP
40CO	Not submitted	CP
14W	Not submitted	V2
45W	Not submitted	CP
13CO	Not submitted	CP
26CO	Not submitted	CP
C212	Not submitted	CP
C222	Not submitted	V1
38W	Not submitted	CP
1C	Not submitted	V2
2CO	Not submitted	V2

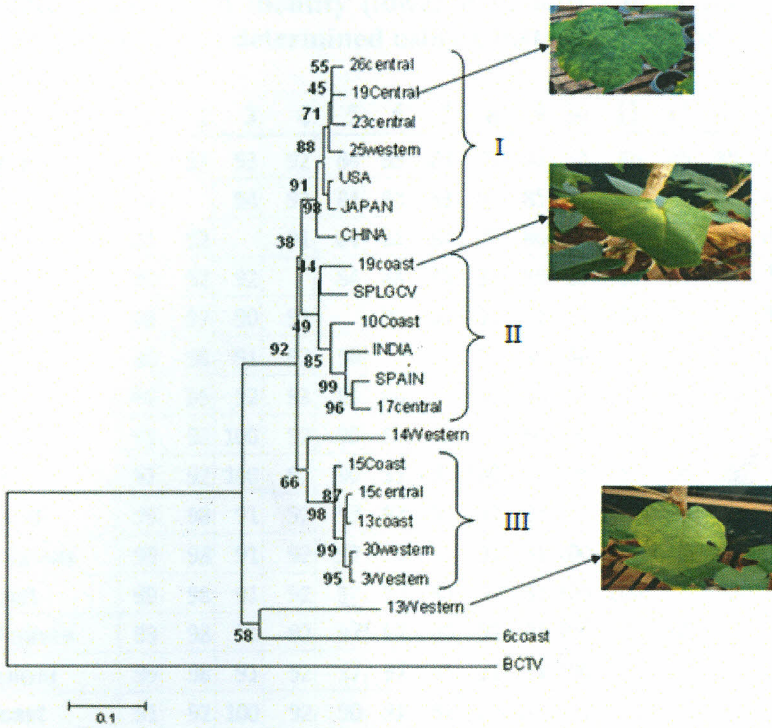

Figure 4.15: Neighbor-joining tree based on the nucleotide sequence of the AV1 fragment show the relationship among begomoviruses infecting sweet potato in Kenya and other begomoviruses. Beet curly top virus (BCTV), genus Curtovirus, was used as outgroup.

Table 4.6: Pairwise percent nucleotide sequence identity (upper diagonal) and amino acid sequence of identity (lower diagonal) among the partial ORF AV1 of begomovirus isolates as determined using ClustalX software.

VIRUS ISOLATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1:Western		95	93	92	84	85	84	85	85	84	86	85	86	86	87	88	90	82	72	74	51
2:USA	97		94	93	84	83	83	84	85	85	85	85	87	86	86	90	90	83	73	73	49
3:SPAIN	91	92		91	84	84	84	85	86	84	86	86	87	87	87	90	90	82	75	71	48
4:SPAIN	92	92	92		84	84	85	85	89	87	89	89	89	89	89	92	90	84	75	70	51
5:INDIA	96	97	90	91		98	97	95	87	87	87	87	90	89	89	86	86	86	71	67	51
6:CHINA	98	98	91	92	96		97	97	88	88	88	88	90	89	89	86	86	86	73	66	51
7:BCTV	96	95	92	93	95	96		95	88	87	87	88	89	88	89	86	86	86	73	67	50
8:SPLGCV	91	92	100	92	90	91	92		90	90	89	90	91	91	91	87	88	88	74	67	50
9:6coast	91	92	100	92	90	91	92	100		96	97	96	95	95	94	91	90	85	72	66	50
10:10Coast	99	98	91	92	97	97	95	91	91		95	95	94	95	93	90	89	88	81	74	56
11:13Western	99	98	91	92	97	97	95	91	91	100		96	95	95	94	91	90	84	73	67	51
12:13coast	99	98	91	92	97	97	95	91	91	100	100		95	96	94	91	90	84	72	75	49
13:14Western	99	98	91	92	97	97	95	91	91	100	100	100		98	94	91	90	85	73	67	51
14:15central	99	98	91	92	97	97	95	91	91	100	100	100	100		94	91	90	85	72	67	49
15:15Coast	91	92	100	92	90	91	92	100	100	91	91	91	91	91		91	91	85	74	69	49
16:17central	98	98	91	93	97	99	97	91	91	97	97	97	97	97	91		93	84	70	67	51
17:19Central	95	95	94	92	95	96	97	94	94	95	95	95	95	95	94	96		84	72	70	52
18:19coast	96	96	90	92	95	98	96	90	90	96	96	96	96	96	90	98	95		77	66	48
19:23central	98	98	91	93	97	99	97	91	91	97	97	97	97	97	91	100	96	98		70	50
20:25western	100	97	91	92	96	98	96	91	91	99	99	99	99	99	91	98	95	96	98		
21:26central	98	97	90	91	96	96	94	90	90	99	99	99	99	99	90	96	94	95	96	98	

4.3.3 Genetic Diversity and variability of SPLCV in AC2 fragments

A 912 nucleotide fragment corresponding to the AC1 and AC2 was amplified with degenerate primers SPG1/2. Sequence analyses based on the 912 nucleotides from the 10 sweet potato samples (Table 4.7) were aligned with other sequences from gene bank (Figure 4.16 and 4.17).

A neighbor-joining tree based on the nucleotide sequence of the AC2 fragments clustered all sweet potato begomoviruses together. In this cluster, sweet potato begomoviruses were divided in three major groups (Figure 4.18). Group one included all the SPLCV isolates collected Kenya (68-Western) and (80-Coast) which were closely related with over 95% nucleotide sequence identity and amino acid sequence identity of 96%. Group two included two isolates, 52 and 82, from Coast with a close relationship of 97% nucleotide sequence identity and amino acid sequence identity of 100% showing that the two isolates were the same (Table 4.8). Group three included sample from the gene bank SPLCGV and SPLGVV with a 91% nucleotide sequence identity. Asian isolates clustered together with Japan-Ku, India and China with 94% nucleotide sequence identity and less than 80% amino acid sequence identity (Table 4.8). AC2 samples had most of the Kenyan isolate grouping together but there was a geographical based diversity seen on the Asian isolates. There was a close relationship between Western and Coastal isolates. No samples from Central Kenya were amplified by the degenerate primers.

Table 4.7: Samples sequenced for AC2 Gene

SAMPLE NO	GENE BANK ACCESSION NUMBER	GENE
80CO	Not submitted	REP
90CO	Not submitted	REP
86CO	Not submitted	REP
52CO	Not submitted	REP
82CO	Not submitted	REP
74CO	Not submitted	REP
68W	Not submitted	REP
67W	Not submitted	REP
79W	Not submitted	REP
777W	Not submitted	REP

```
CLUSTAL 2.0.11 multiple sequence alignment of ORFS of AC2 Kenyan isolates compared with other countries.

SPLCGV           TAAGACGGAGGCTGAACTTCG--AGACAGCTATCGTGCCCT--ACACTG-GGAATGCTGT 169


SPGVV            TAAGACGGAGGCTGAACTTCG--AGACAGCTATCGTGCCCT--ACACTG-GGAATGCTGT 169
INDIA            TAAGACGGAGGCTGAACTTCG--AGACAGCTATCGTGCCCT--ACACTG-GGAATGCTGT 169
Japan            TAAGACGGAGGCTGAACTTCG--AGACAGCTATCGTGCCCT--ACACTT-GGAATGCTGT 169
china            TAAGACGGAGGCTGAACTTCG--AGACAGCTATCGTGCCCT--ACACTG-GGAATGCTGT 169
spain            CAAGACGGAGGCTGAACTTCG--AGACAGCTATCGTGCCTT--ACACTG-GGAATGCTGT 169
                       ***  *      * *          *   *     *  *      ***     *

52Coast          GTTCTTCACAGCCCAGTTCTTGAGTGCTTCTTGTTCTGGCTTGTCCAGCCAGAGTTTAAA 228
82coast          GTTCTTCACAGCCCAGTTCTTGAGTGCTTCTTGTTCTGGCTTGTCCAGCCAGAGTTTAAA 230
74western        GTTTGCTGTTGCCCAATTTTTGAGTGCCTCTTGTTCTGGTTTGTCTAGCCAGATTTTAAA 228
90coast          GTTCTTCACAGCCCAATTTTTGAGTGCTCCTTGCTCTGGCTTGTCCAACCAGAGTTTAAA 227
79western        GTTCTTCACAGCCCAATTTTTGAGTGCTCCTTGCTCTGGCTTGTCCAACCAGAGTTTAAA 230
68Western        GTTCTTCACAGCCCAATTCTTGAGTGCTTCTTGCTCTGGCTTGTCCAGCCAGAGTTTAAA 227
80coast          GTTCTTCACAGCCCAATTCTTGAGTGCTTCTTGATCTGGCTTGTCCAGCCAGAGTTTAAA 225
86coast          GTTCGTTGCTGCCCAATTTTTGAGTGCTCCTTGCTCTGGCTTGTCCAACCAGAGTTTAAA 227
67Western        GTTCGTCGCAGCCCAATTCTTGAGTGCTCCTTGTTCTGGCTTGTCCAACCAGAGTTTAAA 225
77western        GTTCGCCGTTGCCCAATTCTTGAGTGCTCCTTGTTCTGGTTTGTCCAACCAGAGTTTAAA 226
BCTV             ATTATGTAATGTCCAGGACTTAAGGGCTTCATTTCTGGTTTTTCAAGAAATTGTTGGTA 232
SPLCGV           CCCAATTGCTGCCCGAAGCTATGTCCCGGTTTCAAGAGGCGTCCGGATGAAGAGAAAGAG 229
SPGVV            CCCAATTGCTGCCCGAAGCTATGTCCCGGTTTCAAGAGGCGTCCGGATGAAGAGAAAGAG 229
INDIA            CCCAATTGCTGCCCGAAGCTATGTCCCGGTTTCAAGAAGCGTCCAGATGAAGAGAAGGAA 229
Japan            CCCAATTGCTGCCCGAAGCTATGTCCCGGTTTCAAGAGGCGTCCGGATGAAGAGAAAGAG 229
china            CCCAATTGCTGCCCGAAGCTATGTCCCGGTTTCAAGAGGCGTTCGGATGAAGAGAAAGAG 229
spain            CCCAATTGCTGCCCGAAGCTATGTCCCGATTTCAAGAAGCGCCCAGATGAAGAGAAGGAA 229
                           * **      *      *    *      *       *   * *

-Conserved region
```

Figure 4.16: Multiple sequence alignment of SPLCV nucleotide fragment corresponding to the AC1 and AC2 amplified with degenerate primers SPG1/2 and compared with other sequences from the Genbank.

```
60Western        CAATTTAGATAGGATT---TTTTCTCCTCCCC--CTTCTGTATATTCTTCTCCCTATTCT
52Coast          GACATCAGAGAAAATTGCGTTCTTCACAGCCCAGTTCTTGAGTGCTTCTTGTTCTGGCTT
67Western        GACATCACAGAAAACTGCGTTCGTCGCAGCCCAATTCTTGAGTGCTCCTTGTTCTGGCTT
68Western        GACATCACAGAAGATTGCGTTCTTCACAGCCCAATTCTTGAGTGCTTCTTGCTCTGGCTT
74western        GACATCGCAGAATATGGCGTTTGCTGTTGCCCAATTTTTGAGTGCCTCTTGTTCTGGTTT
82coast          GACATCAGAGAAAATTGCGTTCTTCACAGCCCAGTTCTTGAGTGCTTCTTGTTCTGGCTT
86coast          GACATCACAGAATATTGCGTTCGTTGCTGCCCAATTTTTGAGTGCTCCTTGCTCTGGCTT
90coast          GACATCATAGAAAACTGCGTTCTTCACAGCCCAATTTTTGAGTGCTCCTTGCTCTGGCTT
77western        GACATCACAGAAAACGGCGTTCGCCGTTGCCCAATTCTTGAGTGCTCCTTGTTCTGGTTT
79western        GACATCATAGAAAACTGCGTTCTTCACAGCCCAATTTTTGAGTGCTCCTTGCTCTGGCTT
80coast          GACATCACAGAAGATTGCGTTCTTCACAGCCCAATTCTTGAGTGCTTCTTGATCTGGCTT
                  *  *   * *  *     **        ***   *  **  *     *    **    *
```

Figure 4.17: Multiple sequence alignment of nucleotide fragment corresponding to the AC1 and AC2 amplified with degenerate primers SPG1/2.

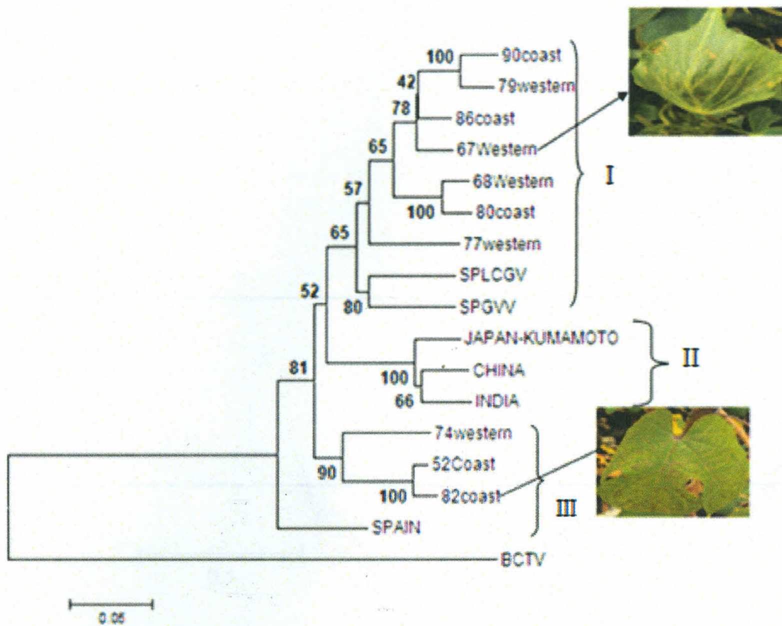

Figure 4.18: Neighbor-joining tree based on the nucleotide sequence of the AC2 fragment showing the relationship among begomoviruses infecting sweet potato in Kenya and other begomoviruses. Beet curly top virus (BCTV), genus Curtovirus, was used as outgroup. The tree was generated using MEGA5 (Tamura *et al.,* 2011). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed.

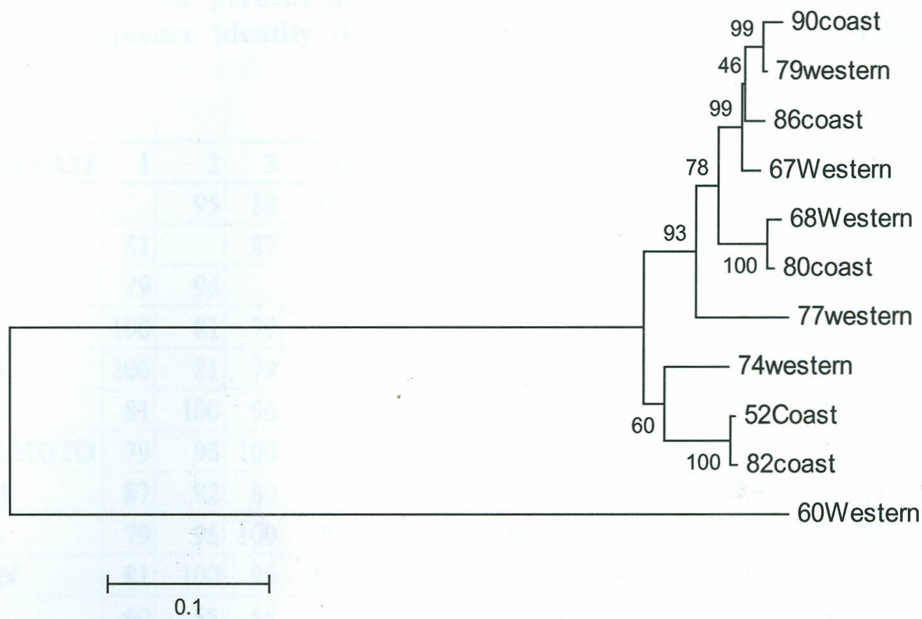

Figure 4.19: Neighbor-joining tree based on the nucleotide sequence of the AC2 fragment showing the relationship among begomovirus infecting sweet potato in Kenya.

Table 4.8: Pair wise percent nucleotide sequence identity (upper diagonal) and amino acid sequence identity (lower diagonal) among the partial ORF AC1 of begomovirus.

VIRUS ISOLATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1:68W		95	88	88	90	88	84	85	85	85	87	92	88	91	90	91	65
2:80C	81		87	87	89	88	83	84	84	84	87	91	88	92	90	91	65
3:52Co	79	96		97	87	85	86	85	84	89	91	87	86	87	85	87	64
4:82Co	100	81	79		86	85	85	85	85	89	91	87	87	87	85	87	65
5:SPLGV	100	81	79	100		91	86	85	85	86	85	89	86	89	87	88	63
6:SPGVV	81	100	96	81	81		86	87	87	86	88	89	86	90	86	86	64
7:KUMAMOTO	79	96	100	79	79	96		94	94	86	84	85	89	86	83	84	64
8:CHINA	87	82	80	87	87	82	80		94	84	86	85	89	86	82	83	66
9:INDIA	79	96	100	79	79	96	100	80		84	86	85	88	85	83	83	65
10:SPAIN	81	100	96	81	81	100	96	82	96		89	84	83	84	84	84	65
11:74W	60	55	55	60	60	55	55	60	55	55		87	86	88	84	85	65
12:67W	100	81	79	100	100	81	79	87	79	81	60		92	95	92	93	65
13:77W	79	96	100	79	79	96	100	80	100	96	55	79		91	88	89	63
14:86CO	88	79	80	88	88	79	80	85	80	79	63	88	80		93	94	64
15:90CO	70	70	67	70	70	70	67	73	67	70	42	70	67	68		95	64
16:79W	87	81	80	87	87	81	80	96	80	81	60	87	80	85	73		64
17:BCTV	87	82	80	87	87	82	80	98	80	82	60	87	80	84	72	97	

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECCOMENDATIONS

5.1 Discussion

This is the first report and findings on the distribution of SPLCV in the main sweet potato growing regions in Kenya indicating its significance importance in sweet potato production and of economic importance to sweet potato production. Western region seem to have the high occurrence, confirming previous report that sweet potato leaf curl virus had been detected in western region at Kakamega (Miano *et al.*, 2006). Western region high occurrence could also be associated from lack of good phytosanitary measures for instance obtaining clean planting material which keeps on spreading on the existing fields. Coast region had also a high occurrence of SPLCV with Wundanyi being the highly affected.

Central region had the lowest occurrence of 4%. This could be associated with geographical location of the region being centrally and not neighboring any country such as Tanzania and Uganda compared to Coast and Western hence no incoming planting materials. Climatic condition which is cool and wet may be unfavorable for the presence of White flies which are key factors in spread of SPLCV. Low SPLCV occurrence in central could be probably because of climate influence. Central region cool climate lie in high altitude 1000-2000 m with annual rainfall of over 1400 mm while most of the areas in Coast and Western is lowland. Coast had wide plantation of sweet potato, and the hot climatic conditions may be associated with spreading of white flies which are the vectors of SPLCV.

The potential importance of SPLCV in Kenya may have been overlooked because sometimes it does not show symptoms; this has also been shown in other countries where some places leaf curl virus had not shown any symptoms, (Valverde *et al.,* 2007). This may be one of the justification of the SPLCV evenly distribution in Coast and Western Kenya. Since SPLCV, is prevalent in the great potential sweet potato growing areas, there is a possibility that the strains in Kenya have co-infection with other viruses as it has been reported in other places due to different symptoms observed in green house and fields. In other places such as the US, SPLCV has been found in mixed infection with SPFMV and SPLCGV (Lotrakul *et al.,* 1998). Experiments with single and mixed infections with russet crack Strain of SPFMV and SPLCV resulted in higher titers of SPLCV in mixed infections, while that of SPFMV remained the same (Kokkinos, 2006). There is urgent need to research on the same and find out if there are co-infections.

Methods available to detect SPLCV include graft inoculations to indicator hosts (*I. setosa*, *I. nil*, *I. aquatica*), and molecular hybridization (Valverde *et al*., 2008). Serological detection of SPLCV from crude sap extracts, which can be great use to diagnosticians in developing countries, is not currently available due to the lack of an antiserum specific for SPLCV. Attempts to purify the virus for antiserum production have not been successful. PCR using both degenerate and virus-specific primers also has been used for virus detection and identification in indicator plants (Lotrakul *et al*., 1998). Despite the use of the above techniques for virus detection in infected plants, they have not been used in Kenya. In this study, we report the successful application of PCR to detect samples collected in sweet potato growing regions in Kenya.

Primers amplified PCR products of different base pairs infecting sweet potato, regardless of geographic origin of the plant material. Variation at the primer regions among these isolates was low except the Central region where no samples were detected by degenerate primer but a few responded to specific primers. Degenerate primers SPG1/SPG2 were more sensitive than the other primer pairs tested in the PCR assays. Degenerate primers SPG1/SPG2 anneal to regions of ORFs AC2 and AC1 which are highly conserved in geminiviruses.

They amplified PCR products not only from geminiviruses infecting sweet potato, but also from other geminiviruses. According to our findings the degenerate primer amplified other products despite the SPLCV hence shows presence of other begomovirus; this is seen in Coastal region where 110 begomovirus were detected. The high sensitivity and broad detection range of these primers make them the best choice for general use in PCR-based detection at genus level.

To specify the presence of SPLCV, the specific primers SPB1&2, PW 2851&2, were used. To come up with good sweet potato crop management in Kenya, variability and genetic diversity was evaluated. The specific primer SPB was used to anneal to nucleotide sequences in coat protein gene AV1and ORFs. In this study, Nucleotide sequence identities of the AC1 fragment indicated that 3 were closely related to SPLCV. Most isolates of different geographical regions (Japan-Ky, Puerto Rico isolate I and 11, Korea, China, and Kenya) were closely related to SPLCV isolates collected from Western and Coastal region.

As was expected, isolates from the same region were more closely related to each other. The isolate II from Puerto Rico was far way closely related to SPLCV-China. This may be because the AC1 of this particular isolate from China is shorter than those of other SPLCV strains as previously reported (Luan *et al*., 2007). In other places has been reported that the AC1 (Rep) sequences of SPLCV, SPLCGV, IYVV and the SPLCV isolate from China were more conserved than the CP gene (AV1) sequences when they were compared to those of non-sweet potato begomoviruses (Lotrakul *et al*., 2003).

According to Padidam *et al.* (1995), the 5' end is the most variable region of the begomovirus CP gene, and is representative of the nucleotide sequence variability of the entire viral genome. Therefore, a phylogenetic analysis based on this region is usually sufficient to establish the taxonomic position of a given begomovirus isolate. In order to confirm the variability in the AV1 fragments from our samples, sequences of the CP gene (AV1) were obtained from selected SPLCV infected samples that were sequenced. Results of phylogenetic analysis based on CP sequences were different from the AC1 with 5 groups. The fifth group had two samples from Western, two from Coastal and one from Central. Nucleotide sequence of AV1 from western and coast were related over (94%). Sample 19-coast and SPLGCV were related with 93% nucleotide sequence identity. Group two isolates were closely related with over (94%), the highest having the coat protein similarity of (97%) hence shows that isolates were closely related In order to confirm the variability further in the AC2 fragments, sequences of the AC2 were obtained from selected SPLCV infected samples.

In this case, sweet potato begomoviruses clustered in five groups instead of three like the AC1. Group one contained SPLCV isolate from Kenya, Western region and Coastal region. In Group two, SPLCV isolates from Coast were clustered together. Group three were SPGVV and SPLCGV, group four was isolates from Asia and group five from Coast and Western. The nucleotide sequence of the AC2 fragment from the 68-Western isolate was more related to 80-Coast (95%). Among the AC2 the highest similarity was shown in 52-Coast and 82-Coast (97%). The results from this study confirm that the CP gene sequence is the most conserved among the sweet potato begomoviruses but distinct from those of other begomoviruses included in our analysis as reported by Luan *et al.* (2007).

A threshold of 89 % nucleotide sequence identity between the full length genome sequences of the A component has been established previously to demarcate between distinct species of geminiviruses, and 93 % nucleotide sequence identity to distinguish between strains (Fauquet *et al.*, 2003). The analysis of the AC1 fragment and the CP gene (AV1) confirmed previous suggestions regarding the variability of begomoviruses infecting sweet potato. However, it should be emphasized that partial sequences reported in this study are not enough to distinguish new viral species.

There is an urgent need for whole characterization on host range, virus transmission, and complete genome sequence in order to classify a virus strain or a new species. According to this study, genetic diversity and molecular variability of sweet potato begomoviruses may have important implications in host range, disease, and whitefly transmissions.

In contrast to the IR, the most conserved region among begomoviruses is the Coat Protein gene (AV1). However, CP plays a key role in processes that are involved in virus infection, survival and spread (Harrison *et al*., 2002). Coat protein has been reported as the most conserved protein among sweet potato begomoviruses, it is very different from the CP of begomoviruses that infect other plant species (Lotrakul and Valverde, 1999).

This could be a justification of the previous study reports of the low rate of transmission by the sweet potato whiteflies. Genetic diversity may also interfere with the reliability of molecular detection tools, such as PCR and nucleic acid hybridization. PCR amplification of a fragment of the AC1 and AV1 by SPLCV-specific primers (PW285/2), (SPB-1/2) required high quality DNA samples from sweet potato leaves to determine real variations. The variability among sweet potato leaf curl virus in Kenya may be likely because of recombination. Finally, the genetic diversity of sweet potato begomovirus in East Africa must be evaluated and the prevalence of distinct species, strains, or variants must be identified as well in their relationship to the germplasm present.

5.2 Conclusion

The research study reports the first PCR detection of begomovirus infecting sweet potato in Central, Western and Coastal regions of Kenya. The study also reports the first genetic diversity of begomovirus infecting sweet potato in Western, Central and Coastal regions of Kenya. Further results of this work has given the first genetic variations of begomovirus infecting sweet potato in Western, Central and Coastal regions of Kenya.

The first evaluation in distribution of sweet potato leaf curl virus gives a better move for sweet potato crop management practices. The finding shows the first relationship of Kenya SPLCV isolates with other from old world and new world begomovirus, where Kenya Isolates are closely related with other isolates from other countries. Since previously no work had been conducted on sweet potato begomovirus, from this work we also report the first protocol for PCR detection using degenerate and specific primers in Kenya. The PCR detection can be used in future for screening begomovirus in other crops.

5.3 Recommendation

Future development of sweet potato begomovirus management such as genetic engineering should focus on SPLCV as the greatest option, development of an antiserum to SPLCV may allow the use of less expensive serological method for SPLCV detection in Kenya. More research is needed to get information on the prevalence, economic impact and effects of mixed infection with SPLCV with other viruses and find out their effects in sweet potato yields in Kenya. There is also need for deep sequencing complete genome and sequences analysis of sweet potato begomovirus from different geographical regions of Kenya to allow for a better understanding of their evolution and which will lead to better management of the disease, production of SPLCV infectious clones will be helpful to evaluate the presence of either DNA or B component to develop resistant cultivars.

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APPENDICES

APPENDIX 1: Buffers and stock solutions

1% SDS

1g sodium dodecyl sulphate SDS was dissolved in distilled water to a final volume of 100ml and stored in a container at room temperature.

0.5M EDTA

Add 186.1 disodium ethylenedeamine tetraacetate EDTA to 800 ml f distilled water. Add sodium hydroxide pellets while stirring to bring the pH 8.0. Adjust volume to 1 liter with distilled water and sterilized by autoclaving and store at room temperature.

2% Polyvinylpyrrolidone {PVP-40}

2g of polyvinylpyrrolidone {PVP-40} was dissolved in 100 ml of water and stored at room temperature.

1M tris HCL Ph 8.0

121.1g Tris-HCL dissolved in about 700 ml distilled water. Adjust PH down 8.0 by adding concentrated HCL and top up the total volume to 1l with ddH2O.

75M Nacl

292.2 g of Nacl dissolved in 700ml 0f water the salt was dissolved gradually final volume topped to 1 liter.

7.5M ammonium acetate

57.81g of ammonium acetate were dissolved in 50 ml of water final volume topped to 100 ml.

5X TBE ELECTROPHORESISI BUFFER

For 500 ml stock solution of 0.5 M EDTA, 93.05 grams EDTA disodium salt was weighed and dissolved in 400 ml distilled water and the solution topped up to final volume of 500ml. 54g Tris bas 27.5 g boric were weighed and both dissolved in approximately 900 ml water 20 ml 0.5m EDTA PH 8.0 added the solution topped to a final volume of 1Litre. The solution was stored at room temperature and diluted to 1x prior to use in electrophoresis.

1L CTAB extraction buffer

100ml 1M Tris HCL Ph 8.0, 280ML 5 m Nacl, 40ml of 0.5 EDTA AND 20g of CTAB (cetyltreimethyl ammonium bromide). Top up the final volume to 1litre with distilled water.