Molecular Characterization of Tomato Leaf Curl Disease-Causing Viruses in Uganda

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In this study, Tomato Leaf Curl Disease symptom-bearing samples were collected from dry savannah (Eastern and Northern) and wet equatorial/tall grass savannah (Central and Western) agro-climatic zones of Uganda. Their total DNA was extracted using a modified Dellaporta protocol. Virus DNA was amplified with five different primer pairs for *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV-Sic) and *Tomato leaf curl Uganda virus* (TLCUV) as well as their combinations. The results indicated that there is wide variation in the tomato leaf curl viruses in the Country. The viruses were grouped into 24 categories and three (3) distinct virus genomes, namely, *Tomato leaf curl Uganda virus*-Soroti, *Tomato leaf curl Uganda virus*- Iganga (Pallisa) and *Tomato leaf curl Arusha virus*-Mubende, were characterized. Partial results indicated occurrence of viruses related to TYLCV and TYLCSV-Sic in wet agro-climatic zones. Genomic variation was detected within isolates and recombination was suspected.

Key words: Tomato; Begomoviruses; Genome; Co-Evolution; Recombination

Introduction

Tomato (*Solanum lycoperscicum*) is a fruiting vegetable. It originated in Latin America and has become one of the most widely grown vegetables, with ability to produce in diverse environmental conditions (Rice *et al.,* 1987). The tomato fruit is fairly rich in vitamins A and C; is of high cash value; and has much potential for value-adding processing. In Uganda, about 5-30% of the farming households grow tomato, not only as a source of vitamins and food security, but also as a source of income (Ssekyewa, 2006). Tomato grows best in fertile, well-drained soils, with pH6 and ambient temperatures of about 25^0C (Villareal, 1979; Rice *et al*., 1987), which are common in Uganda (Mukiibi, 2001). Thus, National Agricultural Research Organization (NARO, 1999) prioritizes it among the vegetables to be promoted in the country.

With proper management practices, a smallholder commercial farmer is expected to reap at least 100t/ha (AVRDC, 1994). In Uganda, however, average yield is 10t/ha (Ssekyewa, 2006). This is due to a number of factors, including the use of unimproved varieties, occurrence of pests and diseases and ignorance about sustainable agronomical practices (Akemo et al., 2001). Caribe, Magrobe, Pakmor and Tropic tomato varieties are reported to be acceptable to farmers and consumers in Uganda while VF 6203 and Peto-C-8100 159, which have resistance to *Verticilium* and *Fusarium*, are recommended for processing (Mwaule, 1995). Mwaule (1995) and Akemo *et al.* (2001) also confirmed the World Vegetable Centre's MT 40, 41, 55, 56 and 57 tomato lines to be resistant to bacterial wilt (*Ralstonia solanacearum*). It is also

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reported that sustainable agronomic practices, like plant spacing of 45x90cm, mulching, staking and pruning to two leader vines, enhance fruit quality and optimize yields (Mwaule, 1995; Rice *et al*., 1987). On the other hand, pests and diseases like Blight (*Phytophthora infestans* and *Alternaria solani*), Bacterial wilt (*R. solanacearum*), Root nematodes (*Meloidogyne* spp.), African bollworm (*Helicoverpa armigera*), Thrips (*Thrips tabaci*), Whitefly (*Bemisia tabaci*) and Aphids (*Myzus persicae*) are reported to affect tomato (Kagezi *et al*., 2001). There are also a number of viral diseases whose symptoms have been encountered in several smallholder farmers' fields in Uganda. These include *Alfalfa mosaic virus* (AMV), *Chili veinal mottle virus* (ChiVMV), *Cucumber mosaic virus* (CMV), *Pepper veinal mottle virus* (PVMV), *Potato virus* X (PVX), *Potato virus* Y (PVY),*Tomato mosaic virus* (ToMV) and *Tomato spotted wilt virus* (TSWV) (Ssekyewa, 2006).

Virus-infected plants are typically stunted, crinkled and bear small, if any, fruits. In some cases, infected plants show leaf curl, marginal yellowing and dieback symptoms before flowering. Viruses of family *Geminiviridae* cause similar symptoms. They are insect-transmitted plant pathogens that have small geminate, circular, quasi-icosahedra particles with a single-stranded DNA genome. The most economically important, and numerous, are *Begomoviruses*, which are transmitted by whiteflies (*Bemisia tabaci*). The number of sequenced and characterized *Begomoviruses* is increasing and they differ in genome organization and components (Fauquet *et al*, 2003). These include Tomato Yellow Leaf Curl Viruses (Van Regenmortel *et al*., 1997), a species of genus *Begomovirus* and family *Geminiviridae* (Czosnek *et al*., 1988). Yassin (1989) reported tomato leaf curl symptoms to be caused by *Tomato Leaf Curl Virus* (ToLCV) in Sudan; Nono-Womdim *et al*. (1996) identified *Tomato yellow leaf curl virus*-Israel strain (TYLCV-IL) in tomato yellow leaf curl symptom-bearing samples collected from Tanzania; while Chiang *et al*. (1996) reported *African tomato leaf curl virus* (ATLCV), a new geminivirus, in Arusha region of Tanzania.

In 2005, a new tomato leaf curl begomovirus named *Tomato leaf curl Uganda virus*-Iganga (TLCUV) was identified, from a few samples collected from Iganga District, in Eastern Uganda (Shih *et al*., 2005). Tomato yellow leaf curl virus disease isolates were later collected from seven other districts of the Country. These were characterized, partially sequenced and identified to be either tomato yellow leaf curl viruses or tomato leaf curl viruses (Ssekyewa, 2006). However, the tomato leaf curl diseases found elsewhere in the Country were not characterized. Thus, their relationship with *Tomato leaf curl Uganda virus*–Iganga, and other begomoviruses, whose sequences exist in the Genbank was not established. In other areas, the high genomic variation and rapid evolution of begomoviruses has been studied and the role of changing cropping systems, introduction of susceptible varieties and geographical barriers recognized (Seal *et al*., 2006; Ssekyewa, 2006). These events, which sometimes evolve into recombination and increased virulence, are responsible for the increasing global significance of begomoviruses (Fauquet and Stanley, 2003). The smallholder tomato production situation, the complex farming system and ecological variation in Uganda reflects the above concerns (Ssekyewa, 2006). Therefore, this paper reports the findings of a study that delved into the character of the viruses causing tomato yellow leaf curl and tomato leaf curl diseases in different ecological zones of Uganda and their relationship with the begomoviruses whose sequences exist in the Genbank.

Materials and Methods

Location grids were recorded using the geographical information systems programme (GPIS). Two hundred seventy-six (276) leaf curl symptom bearing samples were collected from the short grass savannah, tall grass savannah and bimodal equatorial agro-climatic zones of Uganda as indicated in Table 1.

Table 1: Source of Tomato Leaf Curl Symptom Bearing Samples and Incidence of the Disease in March and April 2007

Location		Agro-climatic zone	Incidence (%)
KAS	00.236N, 30.116E	Western tall savannah	1
FORT-E	00.616N, 30.219E	Highland ranges	5
FORT-D	00.622N, 30.214E	Highland ranges	20
FORT-C	00.702N, 30.321E	Highland ranges	15
FORT-B	00.693N, 30.331E	Highland ranges	10
FORT-A	00.693N, 30.331E	Highland ranges	5
KYEN-C	00.509N, 30.847E	Tall grass western savannah	3
KYEN-B	00.693N, 30.331E	Tall grass western savannah	1
KYEN-A	00.542N, 30.859E	Tall grass western savannah	4
MUB-I	00.406N, 32.109E	Tall grass western savannah	20
MUB-H	00.513N, 31.319E	Tall grass western savannah	7
MUB-G	00.515N, 31.319E	Tall grass western savannah	14
MUB-F	00.546N, 31.370E	Tall grass western savannah	4
MUB-E	00.546N, 31.370E	Tall grass western savannah	10
MUB-D	00.487N, 31.829E	Tall grass western savannah	4
MUB-C	00.424N, 31.942E	Tall grass western savannah	25
MUB-B	00.400N, 31.953E	Lake Victoria Crescent	50
MUB-A	00.401N, 31.953E	Lake Victoria Crescent	30
KIB-B	00.773N, 31.922E	Tall grass western savannah	7
KIB-A	00.925N, 31.430E	Tall grass western savannah	10
HOI-C	01.362N, 31.427E	Tall grass western savannah	4
HOI-B	01.393N, 31.340E	Tall grass western savannah	5
HOI-A	01.393N, 31.340E	Tall grass western savannah	10
MAS-B	01.720N, 31.690E	Tall grass western savannah	3
MAS-A	01.730N, 31.690E	Tall grass western savannah	4
LIRA-B	02.250N, 32.920E	Short grass North Eastern savannah	5
LIRA-A	02.240N, 32.980E	Short grass North Eastern savannah	10
SORO-B	01.533N, 33.442E	Dry savannah Kyoga plains	2
SORO-A	01.700N, 33.733E	Dry savannah Kyoga plains	2
PSA	01.020N, 33.840E	Dry savannah Kyoga plains	10

Stratified smart sampling was used, to the end that only yellow leaf curl symptom bearing tomato plants were selected. The leaf samples were dried, between blotting paper, packed and labelled. The number of symptom-bearing plants was noted, per field, and the incidence of the disease calculated as:

$$\text{Incidence of Disease}\,(\%) = \frac{\text{Number of infected plants}}{\text{Number of plants in the field}} \times 100 \qquad (1)$$

Dry leaf samples, 2cm² each, were put in safe-lock eppendorf tubes and lysed in a Tissue Lyser (Retsch® GmbH, Haan Rheinische, Germany). Total DNA was extracted from dry tomato leaf samples using Dellaporta extraction protocol (Dellaporta *et al.*, 1983). However, Chloroform Isomyl (24:1) was used instead of Phenol Chloroform Isomyl (25:24:1, v/v/v), used by Rojas *et al.* (1993). Total DNA pellets were dried at room temperature. After 30 minutes, dry pellets were re-suspended in 100 µl of sterile molecular grade water. Total DNA was separated into two equal volumes and stored, in a refrigerator,

at 4ºC, for immediate use, or 20ºC, for mid-future use. Primers were selected to amplify *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV) or *Tomato leaf curl virus* (TLCV). As is shown in Table 2, five (5) primer pairs for TYLCV (Nakhla *et al*., 1993), *Tomato yellow leaf curl Sardinia virus* (TYLCSV-Sic) (Gorsane *et al*., 2004), and *Tomato leaf curl Uganda virus* (TLCUV) isolate from Eastern Uganda were used.

Table 2: Primers used to Test for *Begomovirus* Species of Tomato Yellow Leaf Curl Virus and Tomato Leaf Curl Virus

Primer Code[1]	5'Carbon end (Donor)–Sequence –3'Carbon end (Receptor)	Annealing region
Degenerate		
PAR1c 715	CATTTCTGCAGTTDATRTTYTCRTCCATCCA	715-745nt
PAL1v 1978	GCATCTGCAGGCCCACATYGTCTTYCCNGT	1978-2006nt
CPv	ACGCCCG(T/C)CTCGAAGGTTCG	-
CPc	GTACA(T/A)GCCATATACAATAACAAGGC	-
Specific		
PARIAc 1048	GGATTAGAGGCATGTGTGTACATG	1048-1071nt
PTYC 2v 1499	ATTTGTGGATCCTGATTACCTTCCTGATGTTGTGG	1499-1533nt
PTYAL1c 2196	AAATCTGCAGATGAACTAGAAGAGTGGG	2196-2223nt
PTLCVUGrep2r	GAGAATGTCATGAGTTCCGCTGCG	2437-2467nt
PTLCVUGcp1f	GTATTACATAGGGTTGGCAAGAGG	641-664nt

[1] P (Primer); TY (specific to TYLCV); TLCVUG (specific to ToLCV-UG); V (viral sense primer); C (complementary sense primer); f (forward); r (reverse)

The primers available could not amplify the lower region (part of C1, C2, C3 and V1) of isolate MUBA6. Therefore, a new primer pair, PMUBA6L, for 5'ACTCATAGAGTAGGAAAACGG3' and PMUBA6Lrev (5'CGGCCATTTAGACCCATGAG3') was used. Three PCR optimum conditions were applied. For reactions with degenerate primers CPv and CPc, as well as CPv and PARIAc, 1048 PCR conditions used were 3min of denaturation at 95ºC followed by 35 cycles at 95ºC for 50s, 55ºC for 50s, 72ºC for 1min and a final extension step of 72ºC for 10min as described by Gorsane *et al.* (2004). For the TYLCV primer pair PTYC 2v 1499 and PTYAL1c 2196, PCR conditions applied were 30 cycles of 1min of denaturation at 94ºC followed by 55ºC for 1min, 72ºC for 4min and one cycle of 94ºC for 1min, 55ºC for 2min and 72ºC for 4min, with an extension of 5min at 18ºC.

In the case of primer pairs PAR1c 715 and PAL1v 1978, as well as tomato leaf curl viruses primer pair PTLCVUGrep2r and PTLCVUGcp1f, PCR conditions included 30cycles of 94ºC for 1min, 55ºC for 2min, 72ºC for 2min, followed by 3min of extension at 72ºC and the experiment was held at 4ºC (Nakhla *et al*., 1993). The Laboratory PTC-100 Programmable Thermal Controller, MJ Research, Inc., UK, at the University of Wales, Bangor was used. Amplified DNA was run on 1% electrophoresis agarose gel, stained with 2.5 µl of ethidium bromide, and run at 85V current for about 15 min. Gels were viewed under UV light (Teare, 1996). The isolates were then grouped into categories based on amplification results.

The isolates whose total DNA was amplified were selected for sequencing. PCR amplicons were purified using the QIAquick® Gel Extraction Kit protocol (Qiagen, 2006). Purified DNA was subjected to sequencing, using the Beckman Coulter CEQ 8000 Genetic Analysis System set at optimum default long sequencing parameters. The primers used in DNA amplification were also used in pre-sequencing PCR, except in the case of sequencing the intergenic region (IR) for isolates MUBA6, PSA6 and SOROA2, in

which case, a TLCUV specific primer TLCUVseq-rev (5' GATATTCTCATCCATCCATATCTTCCC 3') was used. The sequences generated were checked for overlapping segments and combined using the Bio-Edit molecular sequence analysis programme (Hall, 2007). Complete sequences of the isolates were then compared to each other, in Bio-Edit, and with other *Begomovirus* sequences in the European Molecular Biology Laboratory (EMBL) Genbank using BlastN (Altschul *et al.*, 1997). Identity percentages were then determined. Homology values above the 89% cut point meant that the two viruses were similar while those below meant that they were distinct (Padidam *et al.*, 1995). A Parsimony phylogenetic tree, based on Clustal W Multiple alignment option in BioEdit, was constructed at a bootstrap value of 1000 for isolates MUBA6, PSA6, SOROA2, some leaf curl viruses of crops commonly found in the tomato agro-ecosystem and other tomato leaf curl viruses reported in Africa. Thereafter, the sequences of isolates generated (MUBA6, PSA6 and SOROA2) were submitted to the EMBL Genbank.

Results

The genomic diversity in the Tomato Yellow Leaf Curl Virus Population studied is shown in Figure 1.

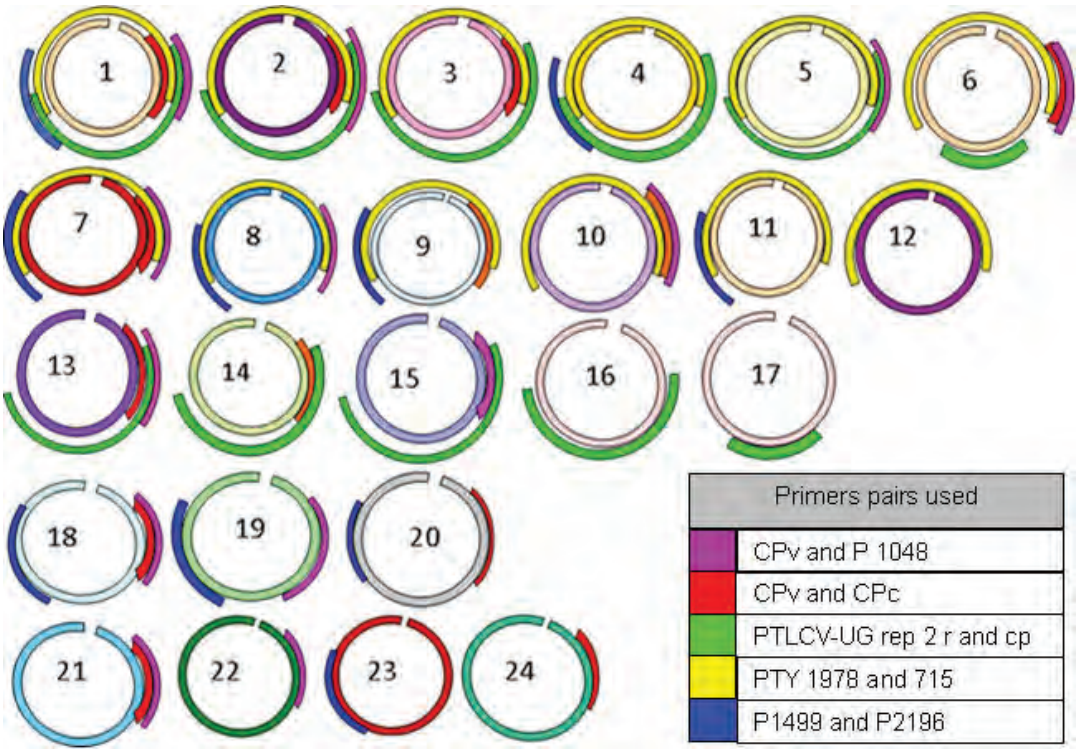

Figure 1: Categories of Isolates Identified

Note: Number of isolates per category ranges from 1-24, with varying colour shades of the inner circle representing DNA. Each row connotes categories of similar overall DNA amplification, from complete coverage of the genome (1-6) to just a quarter of the genome in categories 21-24.

Out of the 276 samples, 98 tested positive to five (5) primer pairs. Fourteen isolates had their complete genome amplified. A total of 52 isolates had either the lower half segment or the upper half segment of their genome amplified. Eight isolates were only amplified for the complementary sense (Rep) and virion sense (CP). Five isolates had only the complementary sense amplified while 19 isolates had only the virion sense amplified. A total of 178 isolates were not amplified though they had leaf curl symptoms

recorded in the field. All the isolate amplicons were grouped into 24 categories (Figure 1). Categories 1-6 had their whole genome (2787bp) amplified, 7-12 had the upper part of the genome amplified (1500bp), 13-17 had partial genome (1500bp) amplification only for the lower half, 18-20 had both complementary (697bp) and virion (697bp) segments amplified while 21-24 had either the complementary or the virion segment amplified. Category 7 was the biggest grouping, with 24 isolates that were collected from various places in Mubende (Tall grass savannah). Category 22 followed, with 16 isolates from the western tall savannah agro-climatic zone of Mubende, Fortportal and Hoima (accounting for 87%); and the short grass dry savannah Kyoga plains of Soroti (accounting for 13%). Amplification results indicate that the isolates had *Tomato yellow leaf curl virus*, *Tomato leaf curl Sardinia virus*, or their recombinants, and tomato leaf curl viruses.

The genomic identity of the Ugandan isolates identified is shown in Table 3.

Table 3: Pair wise Identity Percentages for DNA-A of MUBA6, PSA6, SORO2 and Selected Viruses with Complete Genome Sequences in the Genbank

Genbank Accession No. (NCBI Genbank)	Virus Name	Homology (%) (for matches >85%)		
		SORO2 (2828bp)	MUB-A6 (2749bp)	PSA6 (2757bp)
Not yet	SORO 23 (2828bp)	100	77	84
Not yet	MUBA6 (2749bp)	77	100	78
Not yet	PSA6 (2757bp)	84	78	100
AM701768	TLCV-Toliara	86	85	76
DQ 519575	TLCV-Arusha	87	90	83
AM 698119	TYLCV (ZJ8)	81	78	84
AJ 489258	TYLCV-Almeria	83	79	83
DQ 127170	TLCUV-Iganga	82	77	93
AJ 865341	TLCV-Mayotte (Dembeni)	82	79	85
DQ 358913	TYLCV- Mali (Ethiopia)	81	78	82
EF 194760	TLCV- Arusha	86	92	80
AJ 489258	TYLCV-Almeria	80	78	80
AY 594174	TYLCV-Egypt	83	78	84
AJ 519675	TYLCV-Sardinia	80	76	78
AJ 717579	EACMV- Kenya	79	75	82
(AM502328, AJ717528, AJ618956, AM502329 AF259896)	Other EACMV	75-79	73-75	80

Isolates MUBA6 (Category 4), PSA6 (Category 2) and SORO-A2 (Category 1) were sequenced and characterized. Isolate MUBA6 was collected from the tall grass wet savannah while PSA6 and SORO-A2 were collected from the short grass dry Kyoga plains of Pallisa and Soroti respectively. All three isolates were different from each other. Isolate MUBA6 was 92% identical to *Tomato leaf curl Arusha virus* (TLCV-Arusha, AC. No. EF194760). On the other hand, PSA6 and SORO-A2 were, respectively, 93% and 82% homologous with *Tomato leaf curl Uganda virus*-Iganga (TLCUV-Iganga, AC. No. 127170). Isolate MUBA6 DNA-A had an associated DNA genome, which was partially amplified (1050bp). Figure 2 shows that

the alignment results obtained indicated that this DNA has about 700bp segment identical to the DNA-A VI open reading frame while the rest of the sequences generated were different from any known virus in the Genbank.

5'AGCATTACGATCATATACATCTTAGGGAAAATATGGATGGATGAGAATATCAAGAAGCAG
AACCACACTAATCAGGTCATGTATTTTTTGGTCCGTGATAGAAGGCCCTATGGCACAAGCC
CAATGGATTTTGGGCAGGTATATAACATGTCATAGATAATAGAGCCCAGTACAGCCACGGA
CGATACACCAATCTCCCTACGACACTACCTCGATAGGAACTCAACCGAGAAAGATCCAGCT
GACNGCACAGTTCGAACCGGGACCCTAACTGTACCCACGCGACTCACTATGCAGCAAGCC
ACGGTTCGACCACCACNCCNCTCCGTCCTGACGGTCATAAAAAAAAAAAAAAAAAAAAAA
AACAACCCCCGTTCTTTTTTTTTGTGGCCCGGGGGGTGAGGGGAGGTTAGTAGTGTTTAAT
GTGCAGCCGGGAGGGTGTTGTTTGTAAAGGTAATAGGGAANATAAAATGGTAACAGGATG
GGGGTGTCAGGACAGGTAGAATAGACGCGGGTAATATGGTCAGACAAGTCGTAGGGTGGT
AAGGTGANAACNAGATCAAGGTCGGAGGACGAAGGCNCAGATGGTTACGATCTGCNTCCA
ACNAAGTACGGAGTCGGCGAGGGGTGCGGCATCGATTACTCAGCGTGTCTTTGGGTGACT
CAGTGGTCGTGTGAGTGGCACGCGCGAGATGACTCGCGCGTNCCTCGGGGTCGCGACGG
TGACACGTGCGTCACGTACCGCCATGTTATCNTGAGCGCCGCTCACGACTCTCGTCGCGT
GGCACCGACGTGGTATCTCAGCGCACACCTTTTTTTTACACATGTNGTGGGTAACTATTAAT
AGTAGGTAGTGATTGTCCACTTGTCATTGTACAGTCGGCATTAATGGGCACTGACTATCTG
TGAATCATTCTCGCTCATTTGTAGGTCTTAATGTATGCATTTAAATCACAACTACATAGCTGT
GGTATCTGGAAGCACTCTGAGAATATCCAATATCGCTCTTTCGTTGGGTCTATGACACAATT
TCAGGTCAAATACGCAAACT 3'

Figure 2: Partial Sequence of the MUBA6 DNA-A associated DNA Molecule (1050bp) Reported to Coexist within the Same Ecosystem

Note: These include *Ageratum yellow vein China virus* (AYE564744), *East African cassava mosaic virus*-Kenya (AJ717579), *Cotton leaf curl Bangalore virus* (AY705380), *Papaya leaf curl China virus* (AJ876548), *Sida yellow mosaic virus* (AM 048837), *Tobacco leaf curl Comoros virus* (AM701762), *Tobacco leaf curl virus Comoros virus*-Simboussa (AM701760), *Tomato leaf curl Pakistan virus* (DQ 116884), *Tomato yellow leaf curl virus*-Iran (TYE132711) and *Tomato yellow leaf curl Vietnam virus* (DQ 641697). This was not so for over 400 sequences with which PSA6 DNA-A sequence was aligned.

Phylogenetic results indicated a close relationship between isolate PSA6 and *Tomato leaf curl Uganda virus-Iganga* while isolate SOROA2 is more distant even though it is in the same cluster. Isolate MUBA6 is more closely related to *Tomato leaf curl Arusha virus*. Figure 3 shows that Ugandan tomato leaf curl viruses have a lineage to the tomato leaf curl viruses reported in Madagascar, such as *Tomato leaf curl Mayotte virus* (AC. No. AJ865341) and *Tomato leaf curl Toliara virus* (AC. No. AM701768).

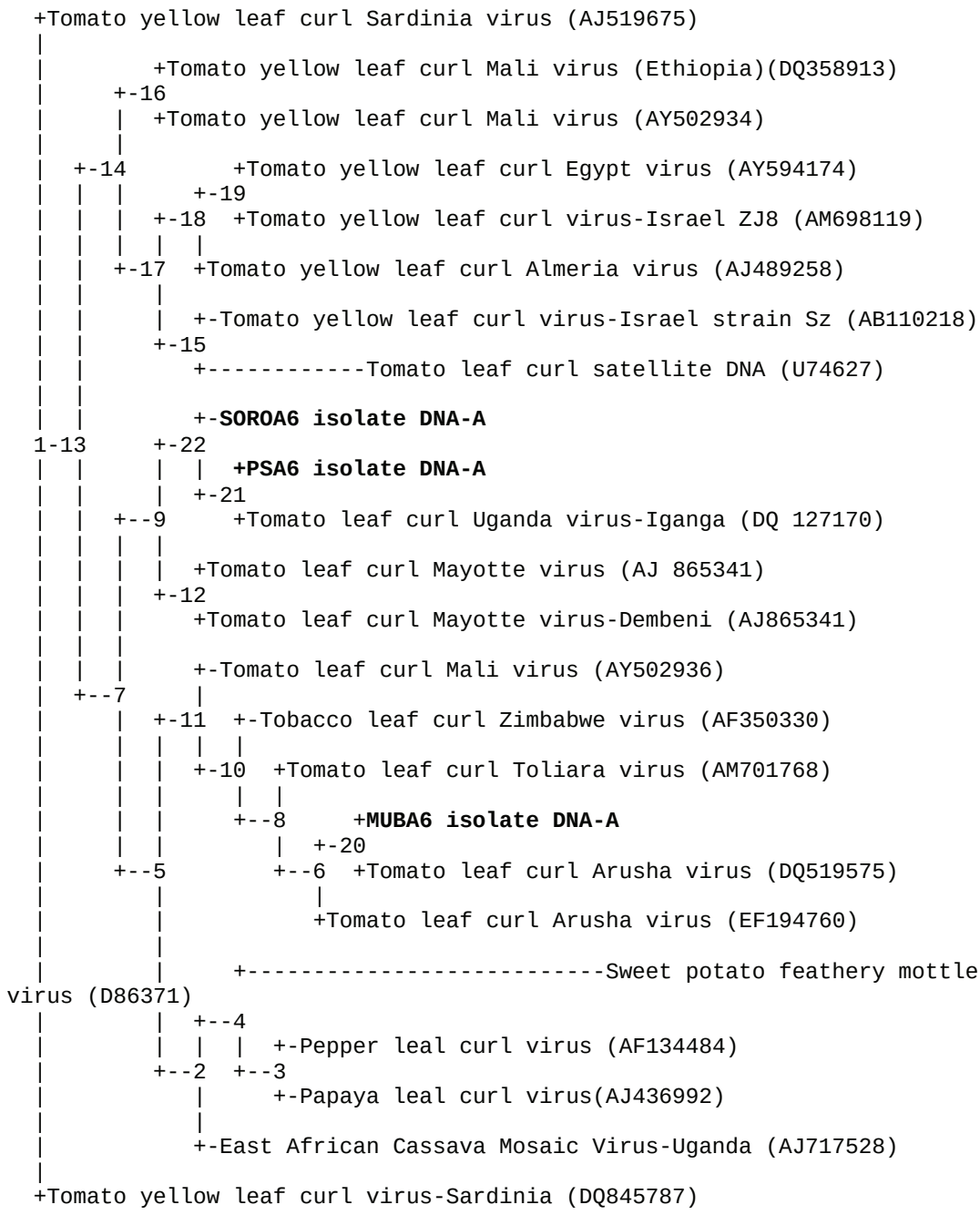

Figure 3: Phylogenetic relationship between the Isolates identified, other Tomato Viruses in Africa and Leaf Curl Viruses Infecting Crops in the Tomato Agro-ecosystem

Note: Ugandan isolates are underlined.

It was noted that isolate SOROA2 had a conserved segment that is also found in strains of *East African cassava mosaic Uganda virus* (EACMV-Uganda) i.e. 100% for 5'ACCGGATGGCCGCGCCC GAAAAAG 3' 24bp segment of EACMV-Uganda 2Mild AF126804, isolate K223 AJ717530, and clone 24-ug 177-10 (AM 502326); 90% for 5'AGAGATTATGTCGAAGCGACCCGGAGATATAA TCATTTCAA 3'

41bp segment of the EACMV DNA-A Coat protein gene (AC. No. AF230374); and 92% identity for the 5'ACCGGATG GCCGCGCCCGAAAAAGAAATAGTGGACCCC 3' 1-42bp segment of EACMV-Uganda (K67) AJ717526. Isolate PSA6 also shares the later conserved segment with EACMV strains (AJ717540, AJ717543, AJ717550, AJ717539 and AJ717536). On the other hand, isolate PSA6 had the same (100%) sequence for the 2734-2757bp (AAAAGCGGCCATCCGTATA ATATT) segment of the Intergenic Region (IR) with sequences of viruses infecting crops and weeds.

Discussion

The results suggest that there are many begomovirus species causing Tomato Leaf Curl Virus Disease in Uganda. Earlier, a *new* begomovirus (isolated from samples taken from the major tomato growing equatorial, montane and savannah agro-climatic zones of Uganda) was reported (Ssekyewa, 2006; Shih *et al*., 2006). The leaf curl virus found in the equatorial/tall savannah agro-climatic zone seems to differ from the one isolated from the short grass/dry land zone. Some isolates had their virus DNA-A amplified by more than one primer pair while others had part of their genome amplified by one pair. This variation in reaction to different primers targeting specific open reading frames is indicative of differences in nucleotide composition among the isolates. It is also a sign of recombination (Padidam *et al*., 1999). Padidam *et al*. (1999) found 420 recombination sites in the geminivirus genome by comparing sequences of identified isolates and identifying homologous fragments within their respective genomes. Among 12 viruses isolated from Africa, 13 recombinations were identified. These were spread out to different parts of the genome. They, therefore, reported that recombination is very frequent among species, within and across genera. Idris and Brown (2004), while working with tomato begomoviruses in Sudan, were able to detect interspecific recombination. This high rate of recombination was said to be responsible for the evolution of geminiviruses and it could be true for the begomoviruses in Uganda.

Furthermore, intensive cultivation, varying cropping systems and ecological differences contribute to the evolution and diversity of begomoviruses (Seal *et al*., 2006). It is these conditions that might be playing a role in the evolution and diversity of tomato begomoviruses in Uganda. Isolates PSA6 and SOROA2 were collected from the short grass dry savannah while MUBA6 was collected from the tall grass wet savannah. Whereas the three isolates are distinct from each other, SOROA2 is a new species, related to TLCUV-Iganga by 82% identity, and has no homology percentage equal to or above 89% with any begomoviruses whose complete sequences exists in the Genbank (Padidam *et al*., 1995). Therefore, it is tentatively named *Tomato leaf curl Uganda virus-Soroti* (TLCUV-Soroti). PSA6, which is 93% identical to TLCUV-Iganga DNA-A, is considered to be a strain and, therefore, referred to as *Tomato leaf curl Uganda virus*-Iganga (Pallisa). Likewise, MUBA6, with 92% identity, is a strain of TLCV-Arusha, and is, therefore, referred to as *Tomato leaf curl Arusha virus*-Mubende (Uganda). The DNA-A associated molecule in MUBA6 could be a satellite DNA-B. Briddon *et al*. (2004) reported the existence of satellite molecules associated with tomato leaf curl viruses' DNA-A and designated them as DNA-B. This is the first time to report a satellite DNA-A associated with tomato leaf curl viruses in Uganda. According to Bull *et al*. (2004), the DNA-B satellite is responsible for high virulence. The high (50%) incidence of the tomato leaf curl disease and diversity of responses in PCR for isolates from Mubende hints on this fact. Even more diversity is expected among the tomato begomovirus population in Uganda, given the fact that, during this research, 24 different categories of PCR amplicons were generated and that only three of them could be characterized during the study. Thus, further investigation is recommended.

It was noted that isolated tomato leaf curl viruses have conserved DNA-A sequence segments in common with other begomoviruses of crops (*Manihot* spp, *Malva* spp.) and weeds (*Agerantum* spp., *Sida* spp.) that co-exist with tomato in the agro-ecosystem. This could mean co-evolution and recombination events. Seal *et al*. (2006) reported that prevailing hosts and vector populations lead to geminivirus diversity.

According to Padidam *et al.* (1999), diversity in viruses is generated by mutations, recombination, re-assortment and de novo gene acquisition. For example, Zhou *et al.* (1997) established the existence of recombination in the EACMV-Uganda. Furthermore, these begomoviruses are transmitted by the biotype-B whitefly, which is hosted by different species that may have mixed infections and hence, an enabling environment for recombination to take place (Xie *et al.*, 2003). This situation was observed in Uganda, which is why recombination cannot be ruled out.

Evolutionally, the origins of tomato leaf curl begomoviruses in Uganda seem to start in Madagascar, as close relationship between Ugandan and Madagascar isolates was observed. It is not clear how the two virus populations co-evolved; nevertheless, tomato leaf curl viruses are now known to be widespread, especially in the highly cultivated Lake Victoria basin and wet tall grass savannah of Western Uganda, with a 50% incidence. These are places where old exotic varieties are mainly grown while in the North and North-east regions, which have low tomato leaf curl viruses, the incidence of tomato leaf curl viruses is still low (10%). This and the issues of recombination raised above are subjects for future investigation.

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