SCREENING OF FARMER PREFERRED TOMATO LINES (*Solanum lycopersicon* L.) FOR RESISTANCE AGAINST BEGOMOVIRUSES ALONG THE COASTAL BELT OF TANZANIA

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A DISSERTATION SUBMITTED IN PARRTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

MOROGORO, TANZANIA.

2016

EXTENDED ABSTRACT

A study was done to enhance the understanding of the diversity of begomoviruses and to identify tomato varieties resistant to Tomato yellow leaf curl disease (TYLCD) that can be used by farmers in the coastal belt of Tanzania. Twenty four out of 225 diseased leaf samples collected tested positive for DNA-A component for primer pairs VD360/CD1266 and VD360/AC1048. No DNA-B or satellites were detected. Their phylogenetic tree constructed with other closely related sequence from the GeneBank showed four begomovirus clusters each with a distinct species indicating a great diversity of begomoviruses along the coast regions of Tanzania and Zanzibar Islands. The entire cluster IV formed a new species of begomovirus. Four of the begomovirus sequences namely ToLCVTz-Ch-4, ToLCVTz-Ch-7, ToLCVTz-Ch-9 and ToLCVTz-Ch-26 tested positive for DNA-A PCR amplification for primers VD360/CD1266, VC 2305 – F1/VC 442 – R1, C1 – 1700 – F2/C1 – 2422 – R2 and Int 1232 – F3/C1 – 1763 – R3. Their full length genomes were 2760, 2760, 2774 and 2767 base pairs, respectively. ToLCVTz-Ch-9 and ToLCVTz-Ch-26 were found to be variants of a completely new species. Phylogenetic analysis confirmed the viruses to display recombination. Betasatellites were not found to be associated with these begomoviruses. On the other hand, a total of 20 tomato lines were screened for resistance to TYLCD. Fifteen of the lines contained *Ty* genes which confer resistance to TYLCD. In the seventh week after transplanting, tomato lines AVTO1122, AVTO1130, AVTO1141 and AVTO1219 were considered resistant as they had a severity of below 2.4. Tomato Lines AVTO1080, AVTO0301, AVTO0922, AVTO1226, AVTO1229, AVTO1260, AVTO1010, AVTO1132, AVTO1008, AVTO1005, AVTO1143, *Assila F1* and *Mwanga* were considered as moderate resistant with severity scores ranging from 2.4 to 3.4. The susceptible lines were line VI045743 and *Tanya* with severity scores greater than 3.5.

Ty-2 was found to be the strongest gene to show resistance to begomoviruses while *Ty-1* was the weakest gene. The study concludes that there is a great genetic diversity of begomovirus on the coastal areas of Tanzania which has been caused by recombination occurring in the begomovirus populations. Breeding resistant tomato lines is the most reliable and sustainable approach to withstand the begomovirus infections. Therefore more breeding for effective resistant tomato varieties is recommended.

DECLARATION

I, **JACKSON JOSHUA,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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The above declaration confirmed

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ACKNOWLEDGEMENT

My Special thanks go to Professor H. F. J Lyimo and Professor T. J Msogoya, my supervisors at Sokoine University of Agriculture (SUA). They have spent their precious time reading, correcting, and encouraging me at the same time continued being patient with me during the entire process. It is their solid review which has helped the completion of this dissertation.

I would also like to acknowledge and thank the International Institute of Tropical Agriculture (IITA) for allowing me to conduct my research in their laboratories and providing assistance that I requested. Special thanks go to Dr. James Legg who had supervised me at IITA. He always believed in me that I was able to produce such a good work. He personally took his time to teach me how to do bioinformatics to the point that I was able to do it in this study. I'll always be grateful for such training.

Special thanks to Dr. Danny Coyne who had sponsored my MSc. study at Sokoine University of Agriculture. I thank Mr. Rudolf Shirima who helped me to know different techniques of laboratory approach concerning my objectives. My thanks also go to Mr. Mushobozi Baitani and Ms. Digna Sway who gave me a head start at the beginning of my research.

DEDICATION

I dedicate my dissertation to my family, friends and the reader who will benefit from this work.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACMV	*African cassava mosaic virus*
ANOVA	Analysis of variance
AVRDC	Asian Vegetable Research and Development Center
BecA	Biosciences eastern and central Africa
BCTV	*Beet curly top virus*
BGYMV	*Bean golden yellow mosaic virus*
CAN	Calcium ammonium nitrate
CMV	Cucumber mosaic virus
CP	Coat protein
CR	Common region
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic acid
DV	Daily value
GPS	Global Positioning System
ICTV	International Committee on Taxonomy of Viruses
IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management
Kb	Kilobytes
LCA	Last Common Ancestor
NCBI	National Center for Biotechnology Information
NJ	Neighbor joining
NPK	Nitrogen phosphorus
NSP	Nuclear shuttle protein
nt	nucleotides

NW	New World
MSV	*Maize streak virus*
ORF	Open Reading Frames
OW	Old World
PTGS	Post transcriptional gene silencing
RCA	Rolling circle amplification
RCBD	Randomized Complete Block Design
RDP	Recombination Detection Program
REn	Replication enhancer
Rep	Replication associated protein
RNA	Ribonucleic acid
SEM	Standard error of mean
ssDNA	Single-stranded DNA
TGS	Transcriptional gene silencing
TLC	*Tomato leaf curl*
TMV	*Tobacco mosaic virus*
ToLCV	*Tomato leaf curl virus*
ToLCVTz	*Tomato leaf curl virus Tanzania*
ToLCVTz-Ch	*Tomato leaf curl virus Tanzania Chambezi*
ToMV	*Tomato mosaic virus*
ToRMV	*Tomato rugose mosaic virus*
ToSRV	*Tomato severe rugose virus*
TPCTV	*Tomato pseudo-curly top virus*
TrAP	Transcriptional activation protein
TSP	Triple Super Phosphate
TSWV	*Tomato spotted wilt virus*

TYLCD	Tomato yellow leaf curl disease
TYLCV	*Tomato yellow leaf curl virus*
V	Virus
WAT	Weeks after transplant
βC1	Beta C1 protein

CHAPTER ONE

1.0 General Introduction

1.1 Background Information

Tomato (*Solanum lycopersicon* L.) is a fruit, which is consumed as a fresh vegetable or processed products such as tomato paste, sauce, juice, soup or ketchup (Rao and Agarwal, 2000). It is highly nutritious and is a major source of income for small holder farmers. It is known to contain lycopene, a large amount of vitamin C, which amounts to 40% of the daily value (DV), as well as vitamin A, 17 percent DV (Bhowmik *et al*., 2012). Tomato has the highest lycopene content of all known vegetables and fruits, which varies with the tomato variety and increases with fruit ripening (Rao and Agarwal, 2000). Lycopene is accessible in the cell wall of tomato, therefore a cooked or a processed tomato such as ketchup, juice, sauce, paste, or soup have more of lycopene as compared to a fresh tomato fruit (Rao and Agarwal, 2000). Lycopene is a powerful antioxidant which lessen the danger of getting prostate, pancreatic, stomach, colon, rectal, oral, lung and breast cancers (Bhowmik *et al*., 2012; Rao and Agarwal, 2000).

Tomato is a native to the Central America and the modern day Mexico (Bauchet and Causse, 2012), where it was cultivated by the Aztecs. Spanish explores who concurred and colonized Tenochtitlan, spread the crop to all over the world. It is now cosmopolitan and it is cultivated under a broad range of environmental conditions throughout the world (Bauchet and Causse, 2012) and is grown almost in every region of Tanzania both in the coastal and mainland areas. Tomato production is higher than other vegetable yields in Tanzania with an aggregate yearly generation of 129 578 tones, which makes it 51% of the aggregate vegetable production (Minja *et al*., 2011).

The humid coastal areas of Tanzania, being led by the urban centers of Dar es Salaam, Tanga, Bagamoyo, Lindi, Mtwara and Zanzibar have continued to grow exponentially with increase in human population density and general development. A critical problem therefore relates to the sustainable increase in agricultural production in proportional to demand of essential food stuff. There is high demand of tomato to supplement small quantity produced locally in these areas. Much of the current supplies come from the highland regions such as Iringa, Morogoro, Arusha and Kilimanjaro. Disease and pests are the major production constraints that limit tomato production in the coastal regions. Pests such as aphids, whiteflies, locusts and worms, at time can be very difficult to control (Louws *et al*., 2010). Diseases such as fusarium wilt, tomato blight, bacterial wilt, root-knot nematodes and viral diseases can cause substantial yield loss (Louws *et al*., 2010).

Report by Scholthof *et al.* (2011) reveals that of the top 10 most damaging plant viruses, five infect tomato. The five viruses in order of their importance are *Tobacco mosaic virus*, TMV (*Virgavirdae*), *Tomato spotted wilt virus*, TSWV (*Bunyaviridae*), TYLCV (*Geminiviridae*), *Cucumber mosaic virus,* CMV (*Bromoviridae*) and *Potato virus Y,* PVY (*Potyviridae*). All tomato viruses mentioned above can cause severe damage to the crop, which decreases food production, and cause financial losses (Hull, 2009). In the current study, our focus is on the genus Begomovirus of the family *Geminiviridae*, which affects tomato. Begomoviruses are a large group of DNA viruses in the family *Geminiviridae* (Idris *et al*., 2014; Rey *et al*., 2012; Sattar, 2012; Lefeuvre *et al*., 2010; Dellate *et al*., 2005; Kashina *et al*., 2003) with at least 192 species identified globally (Rey *et al*., 2012; Fauquet *et al*., 2008; Brown *et al*., 2011).

The most widely distributed and best studied tomato begomoviruses is *Tomato yellow leaf curl virus* (TYLCV) (Lafeuvre *et al*., 2010). Tomato yellow leaf curl disease (TYLCD) is the most widespread and economically important viral disease of tomato in Tanzania (AVRDC, 1993, 1994; Nono-Womdim *et al*., 1996; Kashina *et al*., 2003). This disease has also been observed in South India where the introduction of TYLCV-resistant tomato lines have produced more than doubled tomato yields, and reduced pesticide application by 50-70% (Colvin, 2005).

1.2 Problem Statement and Justification

Diseases caused by begomoviruses are among the most pervasive diseases that limit tomato production (Mnari-Hattab *et al*., 2014; Rocha *et al*., 2013; Hansen *et al*., 2010). A range of mechanisms are involved in the epidemiology of most of the begomoviruses: recombination between viruses themselves and with satellites (Leke *et al*., 2015), synergism between virus species, emergence of new vector biotypes, genome integration of the virus, host adaptation and long distance dispersal of the whitefly vector. In this group of viruses, recombination appears to have contributed extraordinarily to the genetic diversification of viral populations (Moriones and Navas-Castilo, 2008; García-Andrés *et al*., 2007). In Brazil, recombination analysis indicated that new species are still emerging and therefore the population of begomoviruses on tomato is still evolving (Colariccio *et al*., 2007). This factor has caused the formerly known tomato resistant varieties to be susceptible to new viral strains of begomoviruses.

TYLCV studies in Tanzania have found the disease incidence to be high in Dar es Salaam but low in Iringa and Morogoro (Nono-Womdim *et al*., 1996; Kashina *et al*., 2002a, b). This has contributed to poor tomato production in Dar es Salaam and other areas along the coastal belt of Tanzania. Previous studies in Northern Tanzania (Kashina *et al*., 2002a, 2002b, 2003) have revealed existence of diverse groups of begomovirus. However, no

studies have been carried to evaluate the diversity of begomoviruses in the coastal regions despite high incidence of viral diseases.

Most of cultivated tomato varieties in the coastal belt are susceptible to diseases. Asian Vegetable Research and Development Centre (AVRDC) has released lines that are putatively resistant to begomovirus developed using resistant varieties from Taiwan but the response of these tomato lines against begomovirus diseases in the coastal belt has not been tested. This study aims at revealing the diversity of begomovises in the coastal belt of Tanzania and evaluating the response of AVRDC developed tomato lines to tomato leaf curl disease in the Coastal belt of Tanzania.

1.3 Objectives

1.3.1 Overall objective

To enhance the understanding of the diversity of begomoviruses on coastal belt of Tanzania and screen tomato lines for resistance to TYLCD.

1.3.2 Specific objectives

i. To determine the diversity of begomoviruses along the coastal belt of Tanzania
ii. To evaluate experimental lines and varieties for resistance to Tomato leaf curl disease in the coastal belt of Tanzania

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CHAPTER TWO

2.0 Diversity of Begomoviruses Affecting Tomato (*Solanum lycopersicon* L.) on Coastal Belt of Tanzania

2.1 Abstract

The purpose of the current study was to determine the diversity of begomoviruses along the coastal belt of Tanzania. Twenty four viral samples tested positive for DNA-A PCR amplication for primer pairs VD360/CD1266 and VD360/AC1048. No DNA-B or satellites were detected. Their phylogenetic tree with other closely related sequence from the GeneBank showed four begomoviruses clusters each with distinct species. This shows a great diversity and emergence of new species of begomoviruses on coast regions of Tanzania and Zanzibar Island. Four of the begomovirus sequences namely ToLCVTz-Ch-4, ToLCVTz-Ch-7, ToLCVTz-Ch-9 and ToLCVTz-Ch-26 tested positive for DNA-A PCR amplification for primers VC 2305 – F1/VC 442 – R1, C1 – 1700 – F2/C1 – 2422 – R2 and Int 1232 – F3/C1 – 1763 – R3, respectively. These primers together with VD360/CD1266 made a complete circular genome of ssDNA begomvirus. Their full length genomes were 2760, 2760, 2774 and 2767 base pairs, respectively. Begomoviruses ToLCVTz-Ch-9 and ToLCVTz-Ch-26emerged to be variants of a completely new species based on analysis of pairwise comparison. Recombination analysis proved all the four begomoviruses were recombinants possessing a better adaptation to their hosts than their predecessor. The findings from this study provide evidence on the evolution into new species of Begomovirus in the Coast belt of Tanzania. This evolution has implication on tomato breeding programs for resistance against Begomoviruses.

***Keywords*:** Begomovirus, Tomato, Tanzania, Zanzibar, Recombination, Phylogenetic analysis

2.2 Introduction

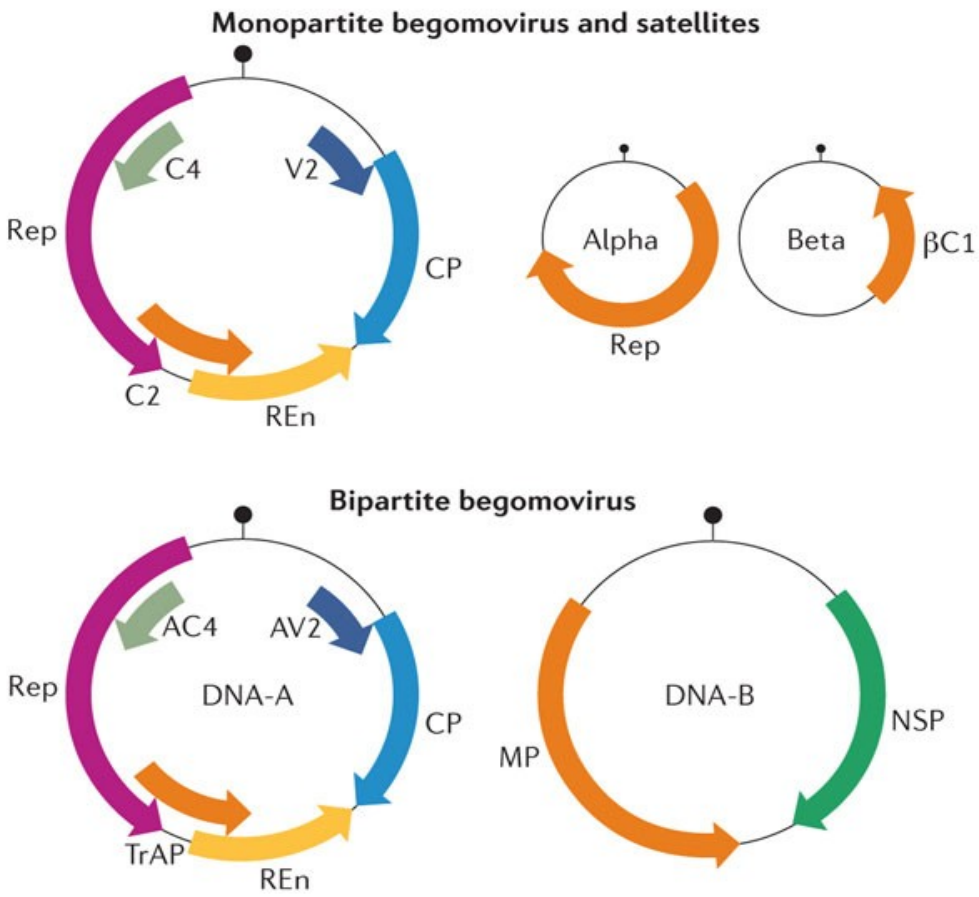
The *Geminiviridae* is the second largest plant virus family. Geminiviruses are structurally characterized by twinned (geminate) quasi-icosahedral capsids and genetically by having one or two small circular, single stranded DNA molecules (Rocha *et al.*, 2013; Fauquet *et al*., 2008; Delatte *et al*., 2005). They infect a broad range of plants including both monocots and dicots (Sattar, 2012). Members of *Geminiviridae* are divided into four genera based on the type of insect vector, number and organization of genome components, diversity of hosts and phylogeny (Rocha *et al.*, 2013; Rey *et al.*, 2012; Sattar, 2012; Colariccio *et al*., 2007): Curtoviruses (type member: *Beet curly top virus* BCTV; transmission by leaf hopper; characterized by circular single stranded DNA, ssDNA; monopartite); Mastreviruses (type member: *Maize streak virus* MSV, transmission by leaf hopper; ssDNA; monopartite); Topocuviruses (type member: *Tomato pseudo-curly top virus* TPCTV; transmission by leaf hopper; ssDNA; monopartite); and Begomoviruses (type member: *Bean golden yellow mosaic virus* BGYMV; transmission by whiteflies; can be monopartite or bipartite).

Begomovirus is the largest and the most economically important genus of the *Geminiviridae* family. It includes viruses that are exclusively transmitted by the whitefly, *Bemisia tabaci*, (Genn.) biotype b and infect only dicotyledonous plants (Huang *et al*., 2013). Begomoviruses have either bipartite or monopartite genomic components, which comprise single-stranded circular DNA. The two components are known as DNA-A and DNA-B and are each approximately 2.8kb in size (Melgarejo *et al*., 2013; Briddon *et al*., 2010; Fauquet *et al*., 2008).

Monopartite *Begomovirus* consists of only DNA-A (example *Tomato leaf curl virus* (*ToLCV*), while bipartite begomovirus consists of two components, which are DNA-A

and DNA-B (example *East African cassava mosaic Cameroon virus*, EACMCV) (Leke *et al*., 2015). These molecules (DNA-A and DNA-B) are similar only in the Common Region (CR) of 200nt, which is important in replication and transcription (Dhakar *et al*., 2010). The DNA-A is responsible for viral proteins associated with replication, control of gene expression, suppression of host defense responses, insect transmission and encapsidation whereas the DNA-B harbors proteins associated with intra- and intercellular movement (Silva *et al*., 2014; Briddon *et al*., 2010). Despite the immense variability of viral parts, both bipartite and monopartite begomoviruses bring about comparative symptoms and significant yield losses (Maruthi *et al*., 2003).

Based on phylogenetic studies and genome arrangement, bipartite begomoviruses have been divided broadly into two groups: the Old World (OW) viruses (Eastern hemisphere: Europe, Africa, Asia) and the New World (NW) viruses (Western hemisphere: the Americas) (Ghosh *et al*., 2012; Prassana *et al*., 2010). The majority of the begomoviruses are bipartite nature (Marwal, 2013). Monopartite begomoviruses originated from the Old World (OW) (Melgarejo *et al*., 2013) and are frequently associated with betasatellites or alphasatellites (Fig. 1) (Hanley-Bowdoin *et al*., 2013; Melgarejo *et al*., 2013).



Source: Hanley-Bowdoin *et al*. (2013)

Figure 1: Genome components of begomoviruses.

DNA-A has 6 Open Reading Frames (ORF) which are identified in most viruses in this genus. These ORF are *AC1, AC2, AC3, AC4, AV1* and *AV2*. *AC1* (*AL1*) encodes for Replication initiation protein (Rep) while *AC2* (*AL2*) produces transcription activator protein (TrAP). *AC3* (*AL3*) encodes for replication enhancer (REn) proteins whereas *AC4* is responsible for determining symptoms expression. On the other hand, *AV1* (*AR1*) produces Coat Protein (CP) while *AV2* is the movement protein ("precoat" ORF) (Marwal *et al*., 2012).

DNA-B has two ORF which are identified in all bipartite viruses in this genus. *BV1* (*BR1*) encodes Nuclear shuttle protein (NSP) whereas *BC1* (*BL1*) instructs for movement protein for cell to cell transfer. *AV1, AV2* and *BV1* are the plus (+) virion sense strand whereas, *AC1, AC2, AC3, AC4* and *BC1* represent for negative (-) complementary sense strand (Marwal *et al*., 2012).

A satellite is a subviral agent composed of nucleic acid that depends on the co-infection of a host cell with helper or master virus for its replication. Thus, satellites may be viruses or satellite nucleic acids. Satellite viruses encode a structural protein, which encapsidates its own nucleic acid, while satellite nucleic acids rely on the helper virus structural protein for encapsidation and do not necessarily encode additional nonstructural proteins. Satellites usually lack nucleotide sequence identity to the helper virus (Leke *et al*., 2015).

Betasatellites (formerly DNA-β) are satellite molecules associated with monopartite begomoviruses and approximately half the size of the helper virus genome (~1360 nucleotides in length) (Huang *et al*., 2013). Betasatellites have a sequence that is unrelated to their helper begomoviruses (Briddon *et al*., 2010). In spite of this, they depend entirely upon their helper begomoviruses for their replication, movement in plants, and transmission between plants, apparently by trans-encapsidation in the helper virus coat protein (CP) (Huang *et al*., 2013). The only gene product encoded by betasatellite, *βC1*, plays an important role in the function of betasatellite. *βC1* is a symptom determinant, a suppressor of both *transcriptional* (TGS) and *post-transcriptional gene silencing* (PTGS), and can repress plant defense (Huang *et al*., 2013).

Alphasatellite (formerly DNA-1) are ssDNA components that associate with the majority of monopartite begomovirus-betasatellite complexes (Briddon *et al*., 2010). These components are not considered true satellite molecules because they are capable of self-replication (Sattar, 2012), and by definition, satellites require a helper virus for replication. However, alphasatellites do require helper begomoviruses for movement in plants as well as insect transmission (Xie *et al*., 2010). The molecules are approximately half the size of the genomes of their helper begomoviruses (Sattar, 2012; Xie *et al*., 2010). The alphasatellites are originated from the nanoviruses (Briddon *et al*., 2010). The function of alphasatellites is not clear but it is evident that alphasatellites functionally interact with geminivirus/betasatellite complexes resulting in symptom alteration and a reduction in the levels of viral DNA and betasatellites (Xie *et al*., 2010).

Several techniques which can be used for the detection of begomoviruses are serology, electron microscopy, indicator plant assays and Polymerase chain reaction (PCR). PCR is widely used as it allows the detection of very small amounts of the disease agent in the infected plant and vectors using specific primers (Rojas *et al*., 1996). PCR amplifies the viral nucleic acid and is extremely useful for it bypasses problems associated with serology (Ghosh *et al*., 2012). PCR is also more sensitive than hybridization procedures and does not oblige the utilization of radioactivity (Pico *et al*., 1996). Sequencing of PCR results is usually done commercially by companies such as Biosciences eastern and central Africa (BecA), which is in the Nairobi, Kenya.

Begomoviruses causing disease in tomato are globally important, although the effects are greatest in tropical regions where conditions are most suitable for the whitefly vector, *Bemisia tabaci* (Lapidot *et al*., 2014). Several cases of begomoviruses have been reported as main constraints of tomato production in Africa. These among others, includes *Tomato*

yellow leaf curl Sudan virus, *Tomato yellow leaf curl Mali virus* and *Tomato leaf curl Nigeria virus* (Kon and Gilbertson, 2012; Lafeuvre *et al*., 2010; Zhou *et al*., 2008). Begomovirus namely *Tomato leaf curl Uganda virus* has been reported (Shih *et al*., 2006). *Tomato leaf curl Arusha virus* and *Tomato leaf curl Tanzania virus* have been revealed in Arusha and Mukutopora regions, respectively in Tanzania (Shih *et al*., 2006).

Recombination appears to have contributed extraordinarily to the genetic diversification of begomovirus populations (Moriones and Navas-Castilo, 2008; Colariccio *et al*., 2007; García-Andrés *et al*., 2007). Recombination involving DNA-A components are viewed as a noteworthy wellspring of molecular variation for begomoviruses and may bring about an increase of virulence (Silva *et al*., 2014). This means that the population of begomoviruses on tomato is still evolving, with new species that are more virulent emerging. This factor has caused the formerly known tomato resistant varieties to be susceptible as they succumbed by evolving new viral strains of begomoviruses.

Begomovirus studies in Tanzania have found high disease incidence in coastal areas around the major city of Dar es Salaam but low in the inland and higher altitude areas of Iringa and Morogoro (Nono-Womdim *et al*., 1996; Kashina *et al*., 2002a, b). This has contributed to poor tomato production in Dar es Salaam and other areas along the coastal belt of Tanzania. Yield losses due to begomoviruses can reach up to 100% (Glick *et al*., 2009; Pico *et al*., 1996). Fruits developing at the time of infection remain on the plant but very few fruit will continue to grow once infection has occurred. An understanding of the diversity of begomovirus is needed for formulation of breeding strategies for the control begomovirus diseases (Prassana *et al*., 2010). Previous studies in Northern Tanzania (Kashina *et al*., 2002a, b, 2003) have revealed existence of diverse groups of begomoviruses. No studies have been carried to evaluate the diversity of begomoviruses

in the coastal regions despite the high incidence of the viral diseases. Therefore, the objective of this study was to characterize the begomoviruses that are available in the coastal belt of Tanzania.

2.3 Material and Methods

2.3.1 Field sampling

A total of 105 tomato leaf samples were collected from 23 sites in coastal areas of Tanzania namely Dar es Salaam, Pwani, Tanga, Lindi and Mtwara regions as well as the island of Zanzibar (Table 1). These sites are located at altitudes of less than 500m above the sea level. In addition, 120 samples were collected from an experimental field site at Chambezi Research Station, Bagamoyo District (Pwani Region). Co-ordinates of sampling sites were recorded with a Garmin E-Trex Global Positioning System (GPS) unit and shown below in Table 1.

Young leaves of tomato showing leaf curling symptoms were collected by placing a small portion of symptomatic leaf tissue in a microfuge tube and cutting it using its cover to avoid cross-contamination between samples. The tubes were then labeled, placed in a polythene bag and stored in a coolbox for transport back to the laboratory. Once in the laboratory, samples were stored in a - 45°C freezer prior to nucleic acid extraction.

Table 1: Description of locations where diseased tomato leaf samples were collected

Number of sample	Region	District	Sites[1]	Latitude(°S)	Longitude (°E)
9	**Tanga**	Korogwe	Bungu	5.03643	38.39089
		Pangani	Pangani	5.406	38.99579
		Mkinga	Daluni	4.78653	38.76838
169	**Pwani**	Kibaha	Misugusugu	6.47547	38.82219
		Bagamoyo	Msata	6.21741	38.22505
			Magomeni	6.45297	38.88989
			Chambezi	6.51667	38.91667
		Mkuranga	Miekela	7.24438	39.17788
			Hoyoyo	7.08634	39.19092
			Kiparang'anda	7.1833	39.16046
			Kisse	7.15761	39.16046
21	**Dar es salaam**	Kinondoni	Mpiji	6.72279	39.0602
			Ununio	6.63201	39.18028
			Kawe	6.59321	39.1079
		Temeke	Vijibweni	7.01851	39.11355
13	**Zanzibar**	Mjini magharibi	Dimani	6.26737	39.26588
		Kaskani	Donge Chanjani	5.92973	39.25633
		Kusini Unguja	Kati/Bungi	6.24068	39.33111
		Unguja Kaskazini	Mahonda	6.00843	39.24015
6	**Lindi**	Kilwa	Kilwa Masoko	8.92323	39.5202
		Lindi rural	Mtwalonga	10.21907	39.49698
7	**Mtwara**	Mtwara rural	Kitere	10.352	39.7726
			Lipwidi	10.38036	39.84428

[1]Sites where samples were collected.

2.3.2 DNA extraction and PCR amplification

A modified CTAB (Cetyl Trimethyl Ammonium Bromide) method was used to extract the DNA (Lodhi *et al*., 1994 and Xu *et al*., 2010). Seven pairs of primers were used for PCR amplification of DNA-A, DNA-B and betasatellites. Three primer pairs were developed in the current study using CLC Mainworkbench 6.9.2 whilst the remaining sets were developed by other authors as indicated in Table 2. The first four primer sets produced overlapping PCR products which were used to develop a full length genome sequences.

PCR reactions for primer pairs 4, 5, 6 and 7 (Table 2) were carried out in 20 ml volumes with the following programme: a cycle of 5 min at 94˚C, then 30 cycles at 95˚C for 1 min, 55˚C for 1 min, 72˚C for 1 min, and a final cycle at 72˚C for 5 min. The protocol for primer pairs 1, 2 and 3 was the same except that an annealing temperature of 50˚C was used.

Table 2: PCR primers used in the current study

Index	Primer names	Primer sequences	Expected size (nt)
	DNA-A (detection)		
1	VC2305-F1	5'-RTAGGAMTTGACRTCDGA-3'	894
	VC442-R1	5'-CTTKCKRTACATGGGCCT-3'	
2	C1-1700-F2	5'-TGGATTGCARAGGAAGATWG-3'	722
	C1-2422-R2	5'-CATGCYCTCATCCARTTC-3'	
3	Int1232-F3	5'-AAKAAAMGACCAGTCTGA-3'	531
	C1-1763-R3	5'-TGGTAYAACGTCATTGAT-3'	
4	VD 360	5'-AGRCTGAACTTCGACAGC-3'	906
	CD1266 (Delatte *et al*., 2005)	5'-TCTCAACTTCARGGTCTG-3'	
5	AV494	5'-GCCYATRTAYAGRAAGCCMAG-3'	552
	AC1048 (Wyatt and Brown, 1996)	5'-GGRTTDGARGCATGHGTACAT-3'	
	DNA-B (detection)		
6	PBL1v2040	5'-GCCTCTGCAGCARTGRTCKATCTTCATACA-3'	600
	PCRc1 (Rojas *et al*., 1993)	5'-CTAGCTGCAGCATATTTACRARWATGCCA-3'	
	DNA-β (detection)		
7	Beta 01	5'-GGTACCACTACGCTACGCAGCAGCC-3'	600–700 and 1350
	Beta 02 (Briddon *et al*., 2002)	5'-GGTACCTACCCTCCCAGGGGTACAC-3'	

2.3.3 Sequence analysis

Sequencing of PCR products was done commercially by Macrogen Inc., in Maryland, USA. Sequences were trimmed using Ridom Trace Edit 1.1.0 (Rothgänger *et al*., 2006) before forward and reverse sequences were assembled into contigs using CLC Main Workbench 6.9.2. Full DNA-A sequences of related viruses used in phylogenetic analyses

were obtained from the NCBI public sequence database (Table 3). Multiple sequence alignment was performed using the alignment method of ClustalW (Larkin *et al*., 2007) within MEGA6 (Tamura *et al*., 2013). Final sequence editing was carried out by cross-referencing the MEGA6 alignment results with the original sequence data from the chromatogram files received from Macrogen.

Table 3: GenBank accession numbers of complete begomovirus DNA A-like used in the current study

Begomovirus name	Accession no.
Tomato leaf curl Arusha virus	DQ519575
Tomato leaf curl Arusha virus	EF194760
Tomato leaf curl Tanzania virus Makutopora	U73498
Tomato leaf curl Comoros virus, isolate Comoros: Bambas: 2004.	AM701759
Tomato leaf curl Uganda virus - [Iganga] segment A	DQ127170
Tomato yellow leaf curl virus-Israel [Japan:Haruno:2005] DNA	AB192966
East African cassava mosaic virus-Tanzania segment	Z83256
Tomato leaf curl Namakely virus, isolate Madagascar:Namakely:2001	AM701764
Tomato leaf curl Ghana virus	NC_010313
Tomato leaf curl Seychelles virus	AM491778
Tomato leaf curl Sudan virus	HE819244

Source: www.ncbi.com

2.3.4 Short Length Sequences

Partial genome sequences were obtained for 24 of the sampled isolates. The full alignment of short sequences together with references from GenBank comprised of 35 sequences that were 567 bases long and one sequence (ToLCVTz-Zanzibar_Dimani_23-AC1048) that was 531 bases in length. Following the alignment, a phylogenetic tree was constructed and evolutionary relationships were inferred using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993) in MEGA6 with 1000 bootstraps.

2.3.5 Full length genomes

Whole genome sequences were assembled as contigs for the four overlapping consensus sequences obtained from PCR amplicons derived from primer pairs 1-4 (Table 2). This was done for four virus isolates that represented contrasting genotypes from within the sample set. Full length genome sequences were analyzed using the same methods applied to the short sequences, involving the alignment and phylogenetic analysis. Pairwise comparison analysis was computed using 14 sequences.

The detection of recombinant sequences together with the identification of likely parental sequences and localization of possible recombination breakpoints were carried out using Recombination Detection Program, RDP 3.44 at default settings (Martin *et al*., 2015). The analysis was conducted at default setting of the program with Bonferroni corrected P-value cut-off of 0.05.

2.4 Results

2.4.1 Short length sequences

PCR amplification was positive for DNA-A (Fig. 2) which yielded a total of 24 products, of which three were from Tanga, three from Zanzibar and the remaining 18 were from the tomato screening trial in Chambezi. No DNA-B or betasatellites specific PCR yielded amplification products for any of the examined tomato leaf samples.

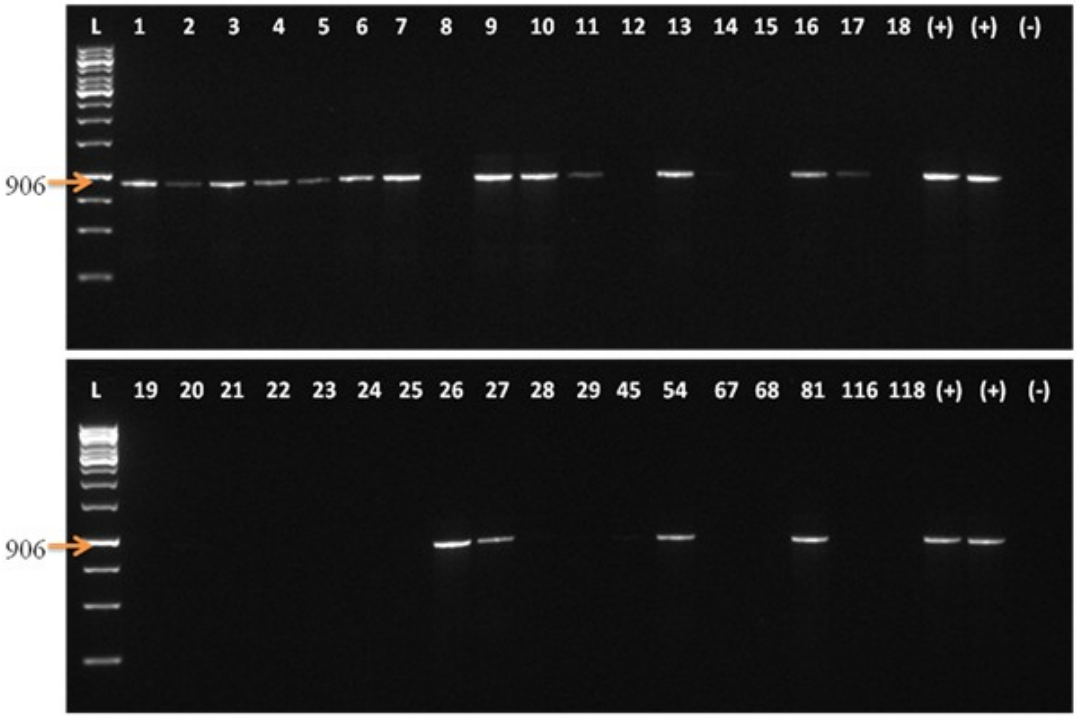


Figure 2: Detection of PCR products for primer VD360/CD1266. L is ladder ruler and numbers 1 to 118 are the samples tested. 906 base pair is the expected length of the PCR product for primer VD360/CD1266.

A phylogenetic tree showing the evolutionary relationship of 24 begomovirus sequences based on prime pairs VD360/CD1266 and VD360/AC1048 as compared to the close related viruses retrieved from the GeneBank is presented in Figure 3. Four begomovirus clusters were formed with four types of species emerging in the clusters. Two new species namely ToLCVTz-Ch-9 and ToLCVTz-Ch-26 were detected in cluster 4. This shows that there is a great diversity of begomoviruses on the coast areas of Tanzania.

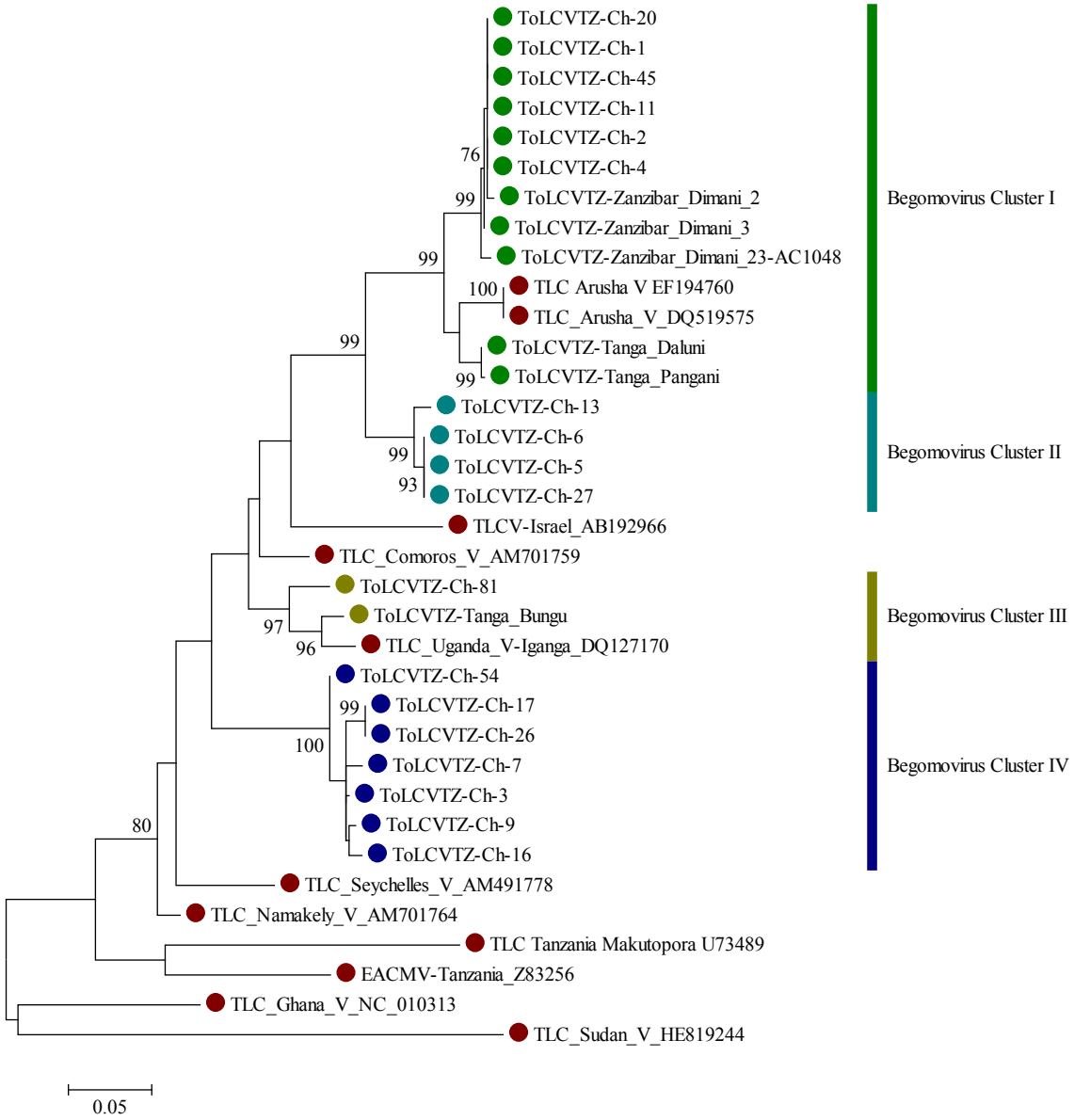


Figure 3: Molecular Phylogenetic analysis for short sequences by Maximum Likelihood method. Light green, deep yellow, deep green, purple and blue represent the 4 distinct begomovirus clusters. Red color represents retrieved sequences from the GeneBank.

2.4.2 Full length genome

Of the 24 short length sequences, four of them namely ToLCVTz-Ch-4, ToLCVTz-Ch-7, ToLCVTz-Ch-9 and ToLCVTz-Ch-26 tested positive for DNA-A PCR amplification for primers VC 2305 – F1/VC 442 – R1, C1 – 1700 – F2/C1 – 2422 – R2 and Int1232 – F3/C1 – 1763 – R3, respectively. These primers together with VD360/CD1266 made a complete circular genome of begomovirus. The full length genomes of begomoviruses ToLCVTz-Ch-4, ToLCVTz-Ch-7, ToLCVTz-Ch-9 and ToLCVTz-Ch-26 were 2760, 2760, 2774 and 2767 base pairs, respectively. Their phylogenetic tree together with other full length closely related begomoviruses from the GeneBank is shown in Figure 4.

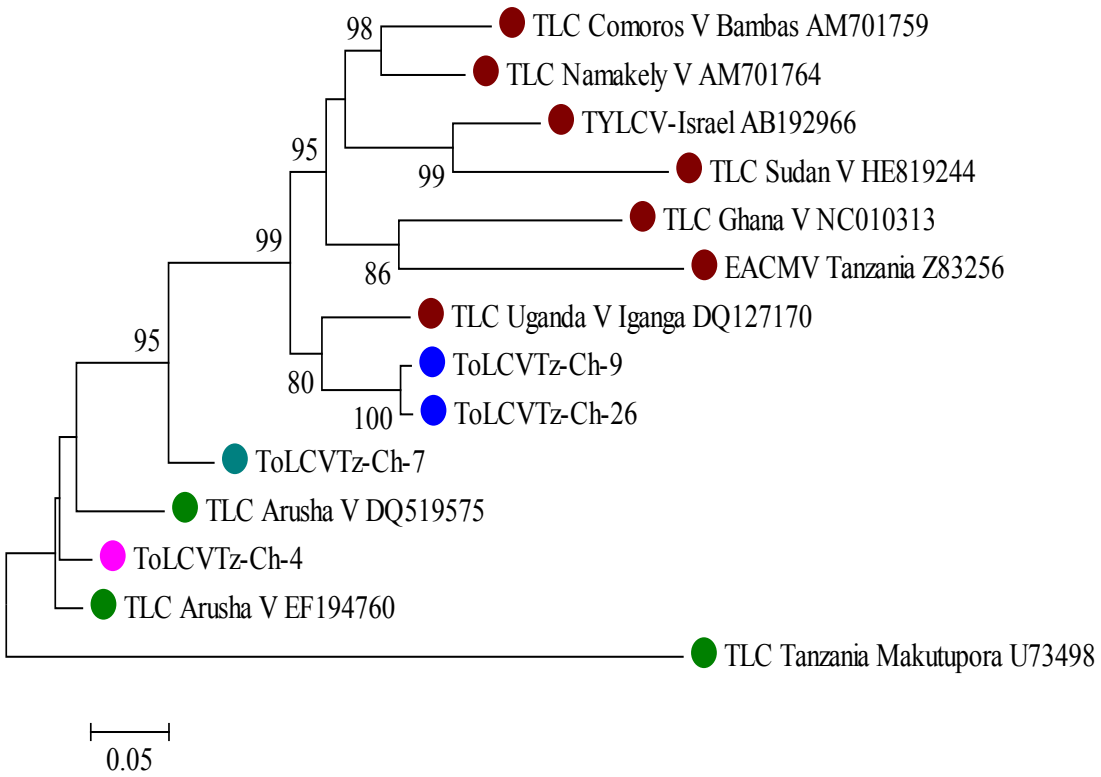


Figure 4: Molecular phylogenetic analysis for full length sequences by Maximum Likelihood method.

2.4.3 Pairwise Sequence Comparison (PASC)

The pairwise sequence comparison for four full length begomovirus genome is shown in Table 4. ToLCVTz-Ch-4 and ToLCVTz-Ch-7 had nucleotide identity of 94.75%, which makes the two variants of the same species. They are the strains of TLC_Arusha_V_EF194760, which has a nucleotide identity of 93.12% with ToLCVTz-Ch-4, and 89.29% with ToLCVTz-Ch-7.

ToLCVTz-Ch-9 and ToLCVTz-Ch-26 are also variants of the same species as they are similar by 97.73%. The two form one completely new species as they both share less than 89% similarity to their closely related begomovirus available on the GeneBank. ToLCVTz-Ch-9 share 83.39% similarity with TLC_Namakely_V_AM701764 while the later share 82.82% similarity with ToLCVTz-Ch-26.

Table 4: The pairwise sequence comparison for four begomovirus full length genomes

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
TLC-Arusha-V-DQ519575	1														
TLC-Arusha-V-EF194760	2	**89.15**													
ToLCTZV-Ch-9	3	**82.27**	79.96												
TLC-Tanzania-Makutoora-V-U73498	4	33.59	35.73	31.87											
TLC-Comoros-V-Bambas-AM71759	5	79.95	76.47	**80.39**	31.65										
TLC-Namakely-V-AM701764	6	79.18	78.24	**82.35**	32.35	**86.44**									
TLC-Uganda-V-Iganga-DQ127170	7	78.84	76.50	**82.72**	32.82	**81.72**	**81.94**								
ToLCTZV-Ch-4	8	**86.13**	93.01	**83.87**	35.72	77.67	79.34	77.69							
ToLCTZV-Ch-26	9	**81.76**	79.69	97.73	31.84	**80.50**	**82.23**	**82.03**	**83.78**						
ToLCTZV-Ch-7	10	**83.36**	**89.18**	**88.29**	33.98	78.43	**80.49**	78.62	94.75	**88.03**					
TLC-Ghana-V-NC010313	11	75.12	75.34	78.11	30.30	78.57	79.28	77.32	75.91	77.51	76.23				
TYLCV-Israel-AB192966	12	73.24	73.19	75.30	34.13	76.77	76.60	75.52	74.20	75.10	74.49	72.41			
EACMV-Tanzania-Z83256	13	71.82	69.68	72.81	28.60	74.05	73.50	72.11	70.39	72.81	71.64	70.77	68.81		
TLC-Sudan-V-HE819244	14	74.01	73.66	76.61	31.14	77.56	79.45	75.93	74.16	76.51	74.38	76.56	77.00	69.20	

Note: Percentages of identity above 90% are underlined; percentages of identity between 80 and 90% are in bold.

2.4.4 Recombination Analysis

The results of the recombination analysis that used the same data set alignment as in the phylogenetic tree (Fig. 4) are shown in the schematic sequence display on Figure 5 as generated by RDP.

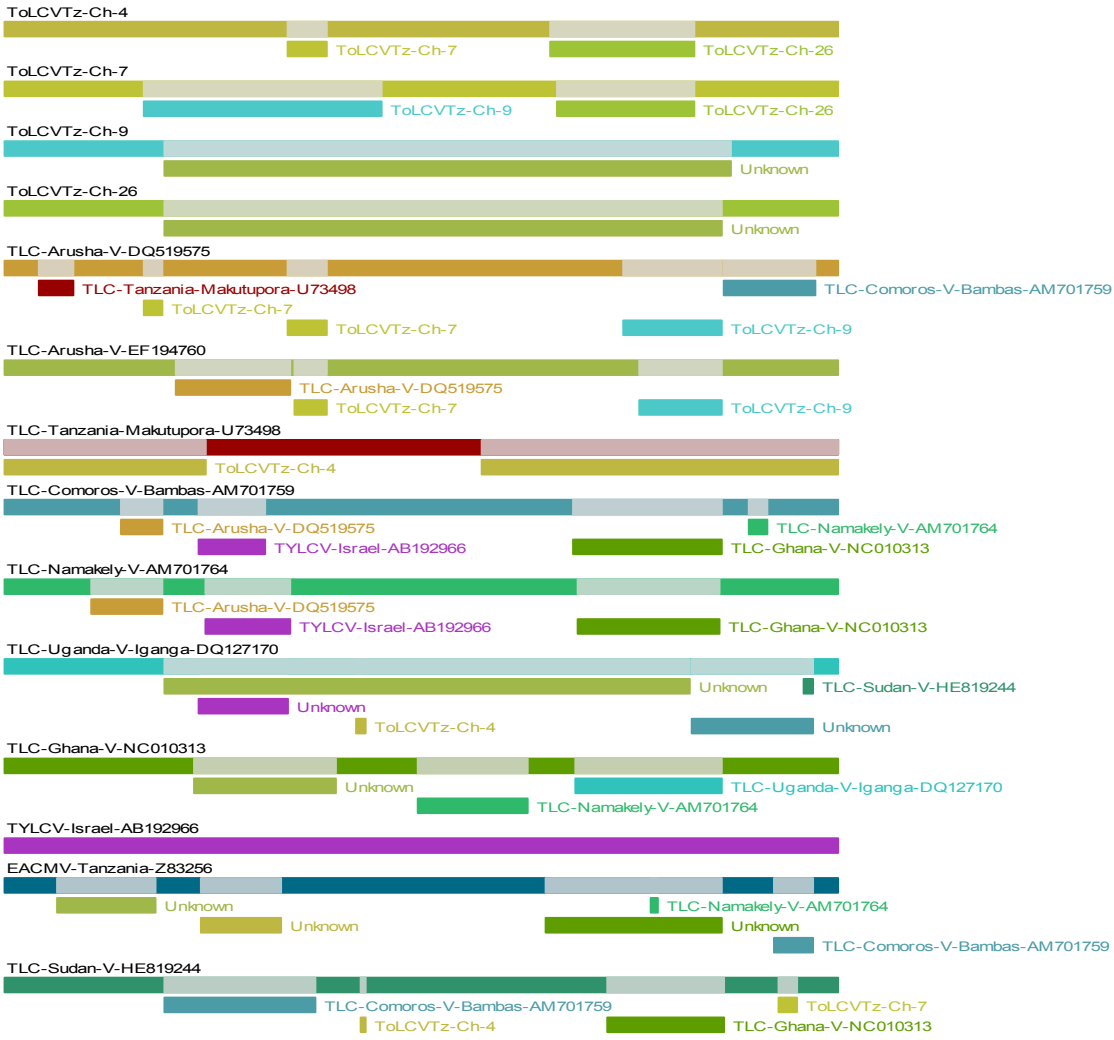


Figure 5: Schematic sequence diagram representing the RDP recombination map of the recombinant fragments for ToLCVTz-Ch-4, ToLCVTz-Ch-7, ToLCVTz-Ch-9 and ToLCVTz-Ch-26. Each colour pattern represents a sequence specific of a virus and recombination events are observed under each virus.

The schematic sequence display is used to manually trace the recombinant positions for observation of the analysed breakpoints and the possible parental sequences, represented graphically, in the RDP plot. From Fig. 5, ToLCVTz-Ch-4 has 2 recombination breakpoint positions; ToLCVTz-Ch-7 also has 2 recombination breakpoint positions while ToLCVTz-Ch-9 and ToLCVTz-Ch-26 each has only one recombination breakpoint position.

The first recombination event in ToLCVTz-Ch-4 (Fig. 6) breakpoint begins from 929th (position 951 in alignment) position and ending breakpoint ends at 1068th (position 1091 in alignment). The approximate p-value for this region was 1.861 x 10^{-01}. The major parent was basically unknown (with slightest probability that it could be TLC Namakely VAM701764) and minor parent was ToLCVTz-Ch-7. According to Delatte *et al*. (2005), for each recognized event the minor parent is apparently the contributor of the sequence within the indicated segment while the major parent is the apparent contributor of the rest of the sequence. These parental sequences are not the actual parents but those sequences most similar to the actual parents in the analysed data set. The region probability (MC Uncorrected) was 1.431 E-03 and region probability (MC Corrected) was 0.521.

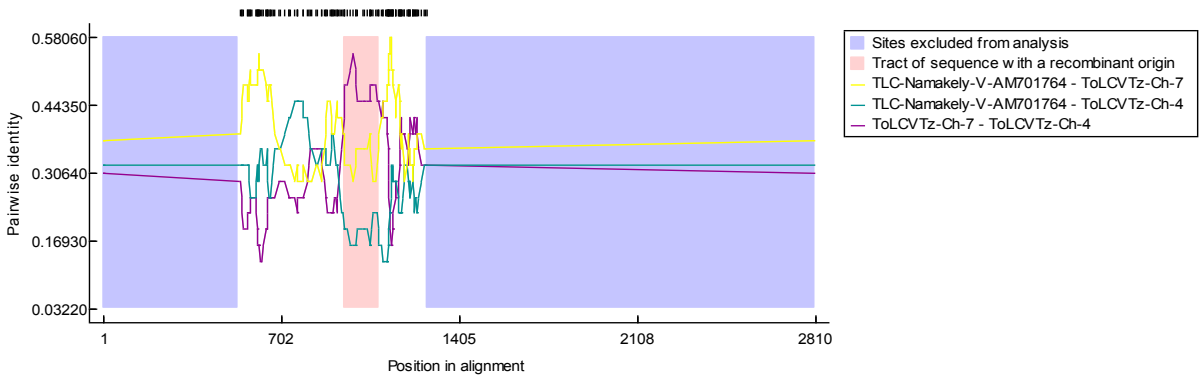


Figure 6: First recombination event of ToLCVTz-Ch-4

The second recombination event of ToLCVTz-Ch-4 (Fig. 7), breakpoint begins from 1812th (position 1836 in alignment) position and ending breakpoint ends at 2305th (position 2329 in alignment). The approximate p-value for this region was 1.089 x 10^{-36}. The major parent was identified as TLC Arusha V EF194760, and the minor parent was found to be ToLCVTz-Ch-26. The region probability (MC Uncorrected) was 2.974 E-44 and region probability (MC Corrected) was 1.082 E-41.

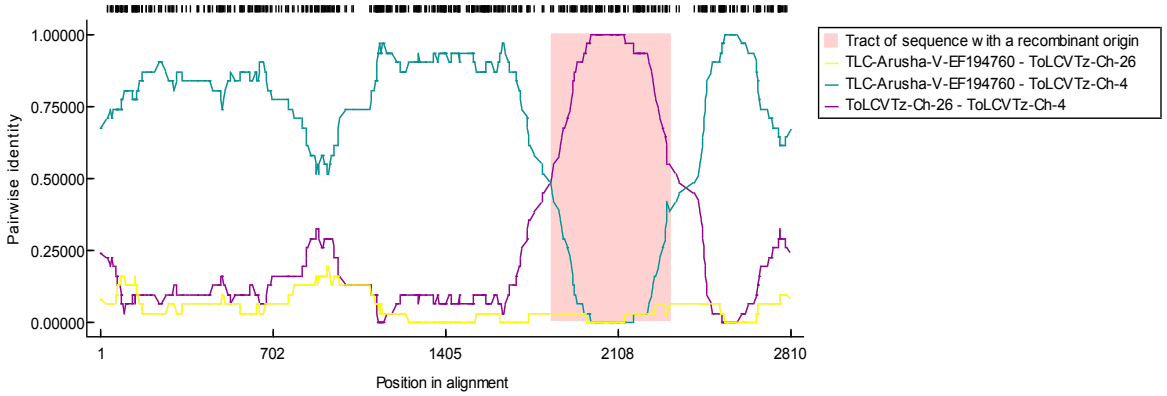


Figure 7: **Second recombination event of ToLCVTz-Ch-4**

As for ToLCVTz-Ch-7, the first recombination event (Fig. 8), breakpoint begins from 448th (position 470 in alignment) position and ending breakpoint ends at 1255th (position 1278 in alignment). The approximate p-value for this region was 3.892 x 10^{-54}. The major parent was identified as ToLCVTz-Ch-4 and the minor parent was found to be ToLCVTz-Ch-9. The region probability (MC Uncorrected) was 1.082 E-41 and region probability (MC Corrected) was 3.892 E-54.

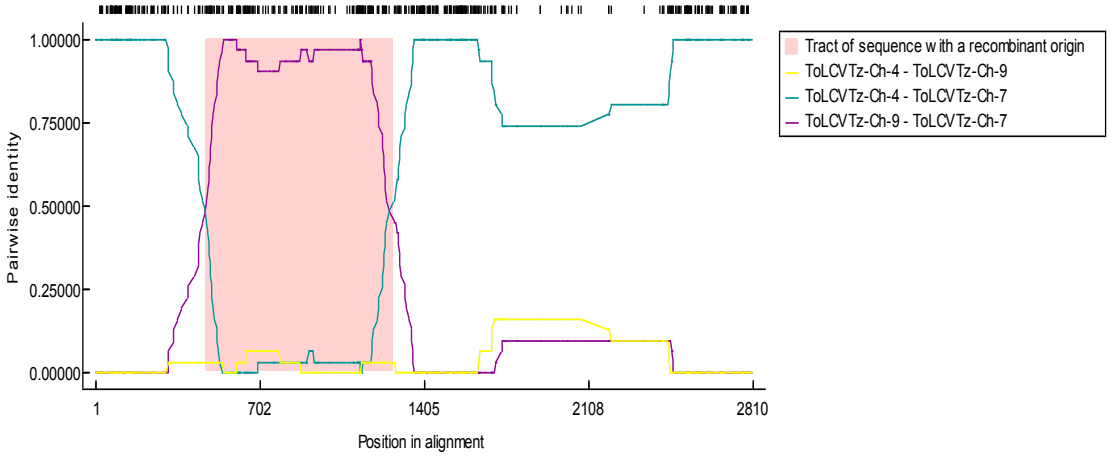


Figure 8: First recombination event of ToLCVTz-Ch-7

In the second recombination event (Fig. 9), breakpoint begins from 1831th (position 1855 in alignment) position and ending breakpoint ends at 2305th (position 2329 in alignment). The approximate p-value for this region was 1.089 x 10^{-36}. The major parent was identified as TLC Arusha V EF194760 and minor parent was ToLCVTz-Ch-26. The region probability (MC Uncorrected) was 3.009 E-34 and region probability (MC Corrected) was 1.095 E-31.

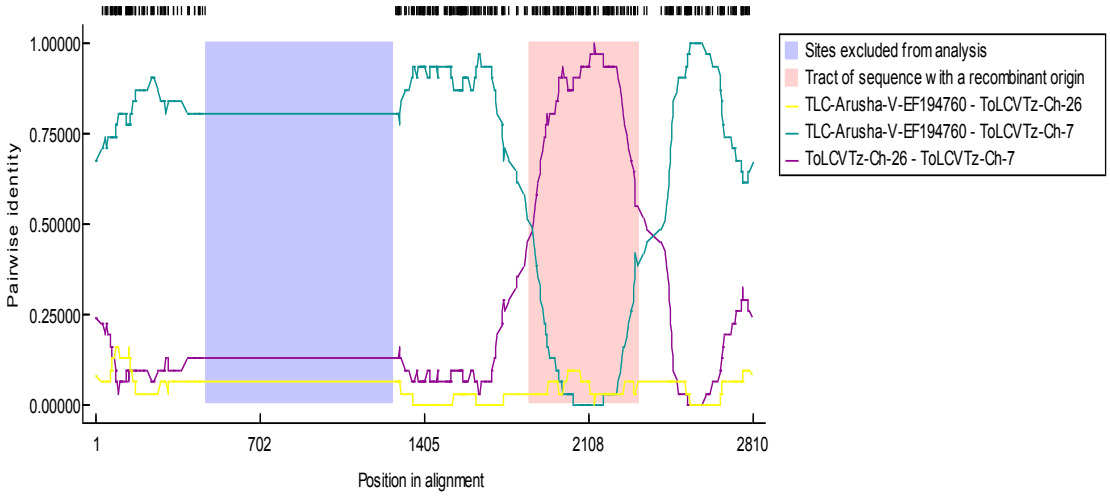


Figure 9: Second recombination event in ToLCVTz-Ch-7

The recombination event of ToLCVTz-Ch-9 (Fig. 10), breakpoint begins from 519th (position 536 in alignment) position and ending breakpoint ends at 2428th (position 2447 in alignment). The approximate p-value for this region was 1.089 x 10^{-36}. The major parent was identified as TLC Arusha V DQ519575 and the minor parent was unknown. The region probability (MC Uncorrected) was 1.029 E-17 and region probability (MC Corrected) was 3.747 E-15.

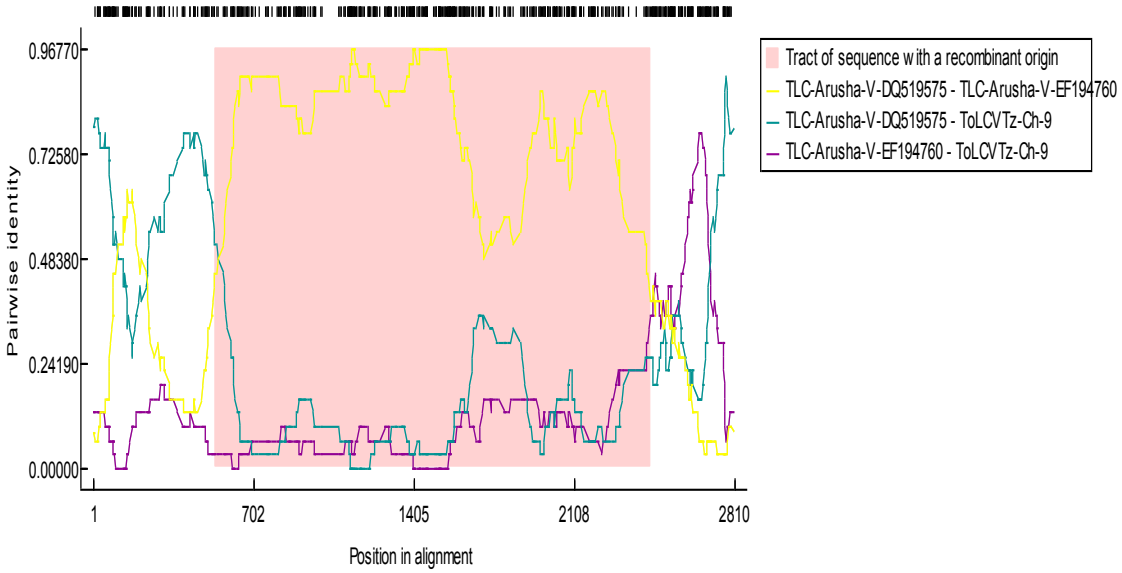


Figure 10: Recombination breaking positions of ToLCVTz-Ch-9

The recombination event of ToLCVTz-Ch-26 (Fig. 11), breakpoint begins from 514th (position 536 in alignment) position and ending breakpoint ends at 2392th (position 2416 in alignment). The approximate p-value for this region was 2.827 x 10^{-13}. The major parent was identified as TLC Arusha V DQ519575 and the minor parent was unknown. The region probability (MC Uncorrected) was 2.018 E-19 and region probability (MC Corrected) was 7.349 E-17.

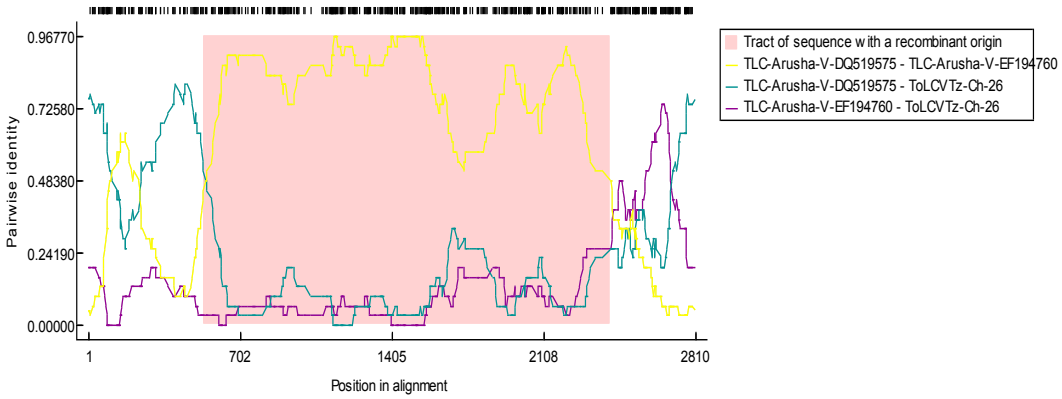


Figure 11: Recombination breaking positions of ToLCVTz-Ch-26

2.5 Discussion

In the current study, DNA-A was detected from the symptomatic leaf curling and stunting samples of tomato while DNA-B genomic component was not detected. The presence of DNA-B genomic component suggests that begomoviruses that are present on the coastal belt of Tanzania are monopartite. This confirms reports that monopartite begomoviruses still dominate the Old World which is the Eastern hemisphere (Africa, Asia and Europe) where it originates (Melgarejo *et al*., 2013). Bipartite begomoviruses are more dominant in the New World which is the Western hemisphere: the Americas (Melgarejo *et al*., 2013).

The partial genome sequences of the DNA-A isolates from the coast belt of Tanzania when compared to the related sequences from GeneBank revealed a great diversity of up to four distinct species of begomoviruses with cluster iv indicating new viral species. The full genome sequence of ToLCVTz-Ch-4, ToLCVTz-Ch-7, ToLCVTz-Ch-9 and ToLCVTz-Ch-26 revealed the four viruses to be strains of begomoviruses according to the ICTV guidance concerning family *Geminiviradae* guidelines (Fauquet *et al*., 2003).

Recombination analysis confirmed all four full length genomes of begomoviruses to be highly recombinants. Recombination may change the biology of a virus by making it better adapted to their host, and possibly by acquiring some new hosts (Marwal *et al*., 2014). The occurrence of new viral hosts may lead emergence of new diseases (Marwal *et al*., 2014). The ability of begomoviruses to quickly form new genotypes by recombination leads to their high diversity. These results therefore conclude that there is a high evolution and diversity of tomato infecting begomoviruses on the coast regions of Tanzania. This results into complications with breeding for resistant and tolerant cultivars against begomoviruses (Leke *et al*., 2015).

Besides breeding for resistant varieties, begomoviruses are controlled by killing the vector whitefly (*Bemisia tabaci*). The most common used insecticides are Karate and Cydium Super (Avicor *et al*., 2014). Other combination of integrated pest management such as removing weeds and use of virus-free transplants can also be practiced.

2.6 Conclusion and Recommendations

The current study has revealed four strains of begomovirus, which are ToLCVTz-Ch-4, ToLCVTz-Ch-7, ToLCVTz-Ch-9 and ToLCVTz-Ch-26. These begomoviruses have shown to display high degree of genetic recombination along the coastal areas of mainland Tanzania and Zanzibar Island. The strains of begomovirus must be taken into consideration when developing varieties that are resistant to the viruses. Another strategy to control begomoviruses is the control of their vector *B. tabaci* by using a combination of Integrated Pest Management (IPM) methods such as removing weeds and use of virus-free transplants.

Further studies on diversity of begomovirus are required especially on other crops and weeds. Moreover, Mtwara and Lindi regions should be revisited and sampled again as they were sampled during the off season of tomato production. As whiteflies are the spreaders of the virus, there is a need to investigate how recombination might occur inside the insect.

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CHAPTER THREE

3.0 Screening Tomato Lines (*Solanum lycopersicon* L.) for Resistance to Tomato Yellow Leaf Curl Disease

3.1 Abstract

Diseases caused by begomoviruses are a major hindrance to tomato production in Tanzania. The objective of the study was to evaluate experimental lines and commercial varieties for resistance to Tomato yellow leaf curl disease (TYLCD) in the coastal belt of Tanzania. A total of 20 tomato lines, 16 of which are from the Asian Vegetable Research and Development Centre (AVRDC), were screened for their resistance against TYLCD. Fifteen of the AVRDC tomato lines were introgressed with *Ty* genes that confer a resistance to TYLCD. Tomato lines AVTO1122, AVTO1130, AVTO1141 and AVTO1219 with severity scores of below 2.4 in the 7th week after transplanting were considered resistant. Tomato lines AVTO1080, AVTO0301, AVTO0922, AVTO1226, AVTO1229, AVTO1260, AVTO1010, AVTO1132, AVTO1008, AVTO1005, AVTO1143, *Assila F1* and *Mwanga* with severity scores ranging from 2.4 to 3.4 were regarded as moderate resistant in the 7th week. Conversely tomato lines VI045743 and *Tanya* were considered as susceptible with severity scores greater than 3.5. *Ty-2* was found to be the strongest gene to show resistance to TYLCD while *Ty-1* was the weakest gene. Since *Assila Fi* and *Mwanga* seeds are already available in the markets for farmers to purchase, and the fact they have proven to be moderate resistant in the study, they should be encouraged to continue to be used by farmers in the coast regions of Tanzania. As for the resistant and moderate resistant AVRDC tomato lines, multi-location trials should be perfumed and the best lines be commercialized for the farmers in the region to purchase.

Keywords: Resistance, Severity, Incidence, Tomato yellow Leaf curl disease, new tomato lines, Tomato.

3.1 Introduction

Tomato yellow leaf curl disease (TYLCD) is caused by begomoviruses. *Begomovirus* is the largest genus of the family *Geminiviridae* with all its hosts being dicotyledonous. It has monopartite or bipartite component genomes. Monopartite species have a single genomic DNA of approximately 2.9 Kb while bipartite species have a size of approximately 2.6 Kb for each of its two DNA components, known to as DNA A and DNA B (Melgarejo *et al*., 2013; Briddon *et al*., 2010; Fauquet *et al*., 2008).

Whitefly (*Bemasia tabaci)* (Genn), biotype b is the vector responsible for transmission of begomoviruses to tomato. The vector belongs to the family *Aleyrodidae* in the order *Homoptera* (Glick *et al*., 2009). It is widely distributed around the world in tropical and warm temperate areas of Africa, India, America (North, Central and South), Europe and China. The vector can acquire the virus after feeding on infected plants, and can transmit the virus to tomato plants after about 8 hours of incubation within the insect (Liu *et al*., 2013). The whitefly retains the virus from 11 up to 12 days and does not transmit it to its progeny (Ioannou, 1985; Mansour and Al-Musa, 1992). Symptoms develop on young plants after 10 to 14 days.

Besides transmitting plant viruses, the vectors also damage crops directly through their feeding habits. They suck plant sap which results in the death of seedlings and reduction of harvests in older plants. As the vectors feed on leaves, they excrete honeydew which enhances of the growth sooty molds. The Sooty molds interfere with transpiration and photosynthesis, and degrade fruit quality (Gruenhagen *et al*., 1993). The vectors have adapted well to survive against insecticides as they increasingly became resistant to chemicals (Horowitz and Ishaaya, 2014) and also due to their feeding habits on the lower sides of leaves, which make them hard to be caught off by the insecticide sprays.

Other hosts of the viruses include solanaceous crops such as potato, pepper and tobacco. Weeds are also known to harbor viruses with the most notable ones being *Achyranthes aspera*, *Euphorbia heterophylla* and *Nicandra physaloides* (Nono-Womdim *et al*., 1996; Kashina *et al*., 2003).

Symptoms of TYLCD on tomato depend on the date of inoculation, tomato variety and the severity of the virus strain responsible for the infection (Lapidot, 2007). Such symptoms are plant dwarfing, reduced leaflet sizes that curl upwards, leaf crumpling, and leaves with prominent yellowing along margins regions (Mansour and Al-Musa, 1992). Flowers wither and plants set a very few fruits after infection. Plants infected before flowering stage produce extremely low yields. There are tomato varieties which have been found to be symptomless carriers of the virus (Kashina *et al*., 2003).

One of the ways of overcoming the begomovirus devastation to crops is to control whitefly using pesticides to kill the insects. However, pesticides control of whiteflies is now becoming obsolete as they continue to become more resistant mainly due to improper use of these chemicals and increasing costs of pesticides (Horowitzi *et al*., 2005). Thus the most effective way to reduce begomovirus damage and spread is by introgression of resistant genes from the wild relatives of tomato into farmers' preferred tomato varieties (Lapidot, 2007). Such wild relatives include *Solanum pimpinellifolium*, *Solanum peruvianum*, *Solanum chilense* and *Solanum habrochaites*. Among the important resistant genes include *Ty-1* and *Ty-3*, which have been introgressed from *S. chilense* (Verlaan *et al*., 2013).

In the current study, 16 tomato lines from AVRDC- African Program with the *Ty* resistant alleles were tested in the field for their resistance level to TYLC disease. Other 4

commercial varieties which are familiar to farmers were also included to test their level of resistance to begomoviruses. These were *Carl J, Tanya, Mwanga* and *Assila F1*. *Carl J* was the susceptible control while *Assila F1* was the control for resistance. A spontaneous field-exposure infection of whiteflies was used for the inoculation of begomoviruses (Lapidot, 2007). The purpose of this study was to identify the best lines that can withstand TYLCD and produce high yield of better quality at a coastal belt of Tanzania.

3.2 Materials and Methods

3.2.1 Description of experiment site

The experiment was conducted at Chambezi in Bagamoyo district in Pwani region, Tanzania which is located at an elevation of 39 m above the sea. Its GPS coordinates are shown on Table 1. The average minimum and maximum temperatures were 27 and 34˚C, respectively. The experiment was carried out at the end of the long rain season between May and September 2014. The soil was characterized by a mixture of loamy and sandy soils.

3.2.2 Experimental design

The experimental layout was a Randomized Complete Block Design (RCBD) with three replications. Sixteen AVRDC tomato lines with different combinations of *Ty* genes were tested for their resistance to yellow leaf curl disease (Table 5). Tomato cultivar *Asilla F1* was resistant control while *Cal-J, Mwanga* and *Tanya* were used as susceptible controls. A replicate consisted of 12 tomato plants planted at a spacing of 0.6 m x 0.6 m. Spreader plants were used as inoculation source for whitefly.

Table 5: List of tomato lines and local cultivars used in the study

Code	Genotype	Source	Putative response to TYLCVD
L1	AVTO1080	AVRDC	Unknown
L2	VI045743	AVRDC	*Ty-1* Resistant
L3	AVTO0301	AVRDC	*Ty-2* Resistant
L4	AVTO0922	AVRDC	*Ty-1, Ty-2* Resistant
L5	AVTO1226	AVRDC	*Ty-3* Resistant
L6	AVTO1229	AVRDC	*Ty-5* Resistant
L7	AVTO1122	AVRDC	*Ty-2, Ty-5* Resistant
L8	AVTO1130	AVRDC	*Ty-2* Resistant
L9	AVTO1141	AVRDC	*Ty-2* Resistant
L10	AVTO1260	AVRDC	*Ty-2, Ty-5* Resistant
L11	AVTO1010	AVRDC	*Ty-2, Ty-3* Resistant
L12	AVTO1132	AVRDC	*Ty-2, Ty-3* Resistant
L13	AVTO1008	AVRDC	*Ty-2, Ty-3* Resistant
L14	AVTO1219	AVRDC	*Ty-2, Ty-3* Resistant
L15	AVTO1005	AVRDC	*Ty-2, Ty-3* Resistant
L16	AVTO1143	AVRDC	*Ty-2, Ty-3, ty-5* Resistant
L17	*Assila F1*	Seminis	Resistant
L18	*Mwanga*	Kibo Seeds Co. Ltd	Unknown
L19	*Tanya*	Mkulima Seeds	Unknown
L20	*Cal J*	Pop Vriend Seeds	Susceptible

Ty-1, Ty-2, Ty-3, Ty-5, ty-5 are genes encoding resistance to TYLCD. The allele *ty-5* is derived from tomato variety '*TyKing*'

3.2.3 Crop establishment and management

3.2.3.1 Nursery

Seedling trays with holes size of about 4 cm deep and 4.5 cm diameters were filled with peat moss media, watered and then seeds were sown on 11 April, 2014. This was done in a screen-house where the trays were placed on wire-meshed tables. Seeds germinated from four to seven days from the date of sowing depending upon the type of the line. The seedlings were watered twice a day. Fungicide Metalaxyl-M+ Mancozeb (Ridomyl)

at a rate of 2 g/l was applied at an interval of one week. No insecticide was applied as this would kill the whitefly vector of the tomato leaf curl virus.

3.2.3.2 Field preparation and transplanting

The land was ploughed and harrowed by a tractor, and leveling was done by hand hoes. Raised beds were prepared to facilitate drainage of excess water. A bed had a dimension of 17 m x 9 m = 153 m² and this constituted a replicate. Good seedlings at 4 - 5 leaf growth stage were transplanted in the field on 10 May, 2014.

3.2.3.3 Fertilizer application

Triple Super Phosphate (TSP) with 46% P_2O_5 was applied at the rate of 150 Kg/ha before transplanting. Urea (46% N) and Calcium Ammonium Nitrate (21% N) were both applied at the rate of 200 Kg/ha in the 3rd and 5th week from the date of transplanting, respectively. Top dressing with Nitrogen Phosphorus and Potassium (NPK) at 200 Kg/ha was applied at flowering.

Indeterminate tomato plants were supported by staking. Weeding was done using a hand hoe and irrigation was done using a watering can in the morning except during the rainy days. Application of a dual purpose curative and protective fungicide Linkomyl (Metalaxyl 80 g/kg + Mancozeb 640 g/kg) at the rate of g/l of water was done weekly. No insecticide was used as this could kill the whitefly that are the vectors for leaf curl virus.

3.2.4 Data collection

Disease incidence and severity

Disease incidence is the percentage of diseased plants in the sample or population of the plants. Disease severity is the percentage of relevant host tissues or organ covered by

symptom or damaged by disease. Disease severity tells about the extent of the damage caused by the disease. Scoring for incidence and severity of TYLCD on the plants was done at 2 weeks intervals starting with the first week after transplanting up to the seventh week. A leaf curl virus disease rating scale of 1 - 6 from AVRDC (Fig. 12**)** was used for scoring as follows:

1 = no symptoms,

2 = slight yellowing, mosaic or leaf curling symptoms on youngest (top) leaves

3 = mild yellowing, mosaic or leaf curling symptoms on youngest (top)leaves

4 = moderate yellowing and leaf curling symptoms on the youngest (top)leaves

6 = severe yellowing, leaf curling, blistering, leaf deformation, small leaves and stunting (internode-shorting)

A scale rating of 1.0 – ≤2.4 was regarded as resistant; >2.4 – ≤3.4 was tolerant and >3.5 was considered susceptible lines/cultivars.

Source: AVRDC (2013).

Figure 12: TYLCV symptom severity scale.

3.2.5 Data analysis

Disease severity and incidence data were analyzed using One Way Analysis of Variance (ANOVA), using Shapiro-Wilk normality statistic.

3.3 Results

3.3.1 Disease severity

There were no significant differences in TYLC disease severity in all tomato lines during the first and third week from the date of transplanting (P> 0.005) (Table 6). All the lines in the 1st and 3rd had a score of less than 2.4 and therefore were regarded as resistant. Disease severity significantly increased in the fifth and seventh weeks after the date of transplanting. The tomato lines VI045743 and *Tanya* were the most susceptible cultivars with the disease severity values of 3.3 in 5th week, which increased to 4.06 and 3.71 in 7th week, respectively. By considering week seven after transplanting, AVTO1122, AVTO1130 and AVTO1219 were identified to be resistant to TYLCVD. Tomato lines AVTO1080, AVTO0301, AVTO0922, AVTO1226, AVTO1229, AVTO1141, AVTO1260, AVTO1010, AVTO1132, AVTO1008, AVTO1005, AVTO1143, *Assila F1* and *Mwanga* were considered moderate resistant.

Table 6: TYLCD severity on different tomato genotypes evaluated at Chambezi in Bagamoyo, Pwani region

Codes	Genotypes	1 WAT	3 WAT	5 WAT	7 WAT
		Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
L1	AVTO1080	2.0^{b}±0.0	2.5^{b}±0.2	3.1^{a}±0.3	2.8^{b}±0.4
L2	VI045743	2.0^{b}±0.0	2.8^{b}±0.3	3.3^{a}±0.1	4.1^{a}±0.4
L3	AVTO0301	2.0^{b}±0.0	2.9^{b}±0.4	3.0^{b}±0.2	3.0^{b}±0.2
L4	AVTO0922	2.0^{b}±0.0	2.3^{b}±0.3	2.4^{b}±0.2	2.6^{b}±0.1
L5	AVTO1226	2.0^{b}±0.0	2.4^{b}±0.1	2.2^{b}±0.2	2.7^{b}±0.2
L6	AVTO1229	2.0^{b}±0.0	2.5^{b}±0.1	2.5^{b}±0.2	2.6^{b}±0.1
L7	AVTO1122	2.0^{b}±0.0	2.6^{b}±0.3	2.6^{b}±0.2	2.3^{b}±0.2
L8	AVTO1130	--	2.0^{b}±0.0	2.4^{b}±0.2	2.3^{b}±0.2
L9	AVTO1141	2.0^{b}±0.0	2.2^{b}±0.2	2.1^{b}±0.1	2.4^{b}±0.2
L10	AVTO1260	2.0^{b}±0.0	2.3^{b}±0.2	2.1^{b}±0.1	2.7^{b}±0.1
L11	AVTO1010	--	2.0^{b}±0.0	2.3^{b}±0.2	2.8^{b}±0.3
L12	AVTO1132	2.0^{b}±0.0	2.6^{b}±0.2	2.4^{b}±0.2	2.7^{b}±0.3
L13	AVTO1008	2.0^{b}±0.0	2.7^{b}±0.2	2.3^{b}±0.3	2.6^{b}±0.2
L14	AVTO1219	2.0^{b}±0.0	2.3^{b}±0.3	2.2^{b}±0.2	2.1^{b}±0.1
L15	AVTO1005	2.0^{b}±0.0	2.7^{b}±0.2	2.5^{b}±0.4	2.7^{b}±0.5
L16	AVTO1143	2.0^{b}±0.0	2.3^{b}±0.2	2.3^{b}±0.2	2.5^{b}±0.1
L17	*Assila F1*	2.2^{b}±0.2	2.4^{b}±0.2	2.4^{b}±0.3	2.6^{b}±0.2
L18	*Mwanga*	2.3^{b}±0.3	2.1^{b}±0.0	2.7^{b}±0.1	2.8^{b}±0.1
L19	*Tanya*	2.0^{b}±0.0	2.5^{b}±0.2	3.3^{a}±0.3	3.7^{a}±0.5
L20	*Cal J*	2.0^{b}±0.0	2.8^{b}±0.1	2.6^{b}±0.3	3.2^{a}±0.3
***P* value (5%)**		0.745	0.061	0.003	0.002

Disease severity was assessed on a scale 1-6 whereby 1.0 – ≤2.4 was regarded as resistant; >2.4 – ≤3.4 was tolerant and >3.5 was considered susceptible lines/cultivars. Values followed by same letter within the column are not significantly ($P< 0.05$) different. WAT - Weeks after transplanting. SEM - Standard Error of Means.

3.3.2 Disease incidence

There was no significant ($P<0.005$) difference in disease incidence among the genotypes during 1st and 7th weeks after transplanting (Table 7). Significant differences were however observed at 3rd week and 5th week after the date of transplanting. Starting from 3rd disease incidence increased, and by 7th week the majority of tomato lines had the highest incidence of over 85%, except AVTO1122 (80.6%) and AVTO1008 (83.3%) which recorded lower incidence.

Table 7: Disease incidence (%) of tomato yellow leaf curl virus on different tomato new lines

Tomato line Codes	Genotypes	1 WAT (%) Mean± SEM	3 WAT (%) Mean± SEM	5 WAT (%) Mean± SEM	7 WAT (%) Mean± SEM
L1	AVTO1080	8.3[b]±4.8	97.2[a]±2.8	100.0[a]±0.0	100.0[a]±0.0
L2	VI045743	8.6[b]±0.3	93.3[a]±6.7	100.0[a]±0.0	100.0[a]±0.0
L3	AVTO0301	17.2[b]±8.1	87.9[a]±12.1	87.9[a]±12.1	100.0[a]±0.0
L4	AVTO0922	6.1[b]±3.0	61.1[a]±19.4	87.5[a]±7.2	100.0[a]±0.0
L5	AVTO1226	11.9[b]±3.2	76.7[a]±12	62.4[a]±20.6	100.0[a]±0.0
L6	AVTO1229	22.2[b]±9.1	62.5[a]±7.2	81.1[a]±11.6	90.0[a]±10.0
L7	AVTO1122	17.7[b]±0.5	60.6[a]±14.3	79.2[a]±15.0	80.6[a]±19.4
L8	AVTO1130	0.0±0.0	31.3[b]±2.0	64.4[a]±5.9	90.2[a]±0.8
L9	AVTO1141	11.4[b]±7.3	41.9[a]±11.0	65.6[a]±8.7	88.0[a]±7.2
L10	AVTO1260	16.7[b]±12.7	38.4[b]±3.6	49.7[a]±16.3	88.4[a]±5.8
L11	AVTO1010	0.0±0.0	47.2[a]±8.6	61.0[a]±4.7	94.2[a]±2.9
L12	AVTO1132	11.1[b]±2.8	68.2[a]±12	77.8[a]±12.1	91.7[a]±4.8
L13	AVTO1008	13.9[b]±10.0	71.7[a]±10.9	82.4[a]±12.5	83.3[a]±16.7
L14	AVTO1219	19.4[b]±19.4	66.7[a]±13.2	74.4[a]±10.6	90.9[a]±9.1
L15	AVTO1005	6.1[b]±6.1	80.3[a]±15.4	88.9[a]±11.1	97.2[a]±2.8
L16	AVTO1143	26.0[b]±13.1	95.8[a]±4.2	96.3[a]±3.7	96.3[a]±3.7
L17	*Assila F1*	25.8[b]±12.4	78.5[a]±12	93.9[a]±6.1	100.0[a]±0.0
L18	*Mwanga*	18.9[b]±10.6	86.8[a]±8.3	100.0[a]±0.0	100.0[a]±0.0
L19	*Tanya*	5.8[b]±2.9	87.3[a]±3.6	100.0[a]±0.0	100.0[a]±0.0
L20	*Cal J*	14.9[b]±3.3	66.4[a]±12.9	93.6[a]±3.2	97.0[a]±3.0
***P* value (5%)**			<0.001	0.011	0.731

Values followed by same letter within the column are not significantly (P< 0.05) different. WAT - Weeks after transplanting. SEM - Standard Error of Means.

3.4 Discussion

In this study genotypes AVTO1122, AVTO1130, AVTO1141 and AVTO1219 had disease severity of less than 2.4 and are therefore considered as resistant to TYLCD. All these four genotypes contain *Ty-2* gene, and in particular AVTO1122 has an addition of *Ty-5* gene while AVTO1219 has an addition of *Ty-3* gene. Tomato genotypes AVTO1080, AVTO0301, AVTO0922, AVTO1226, AVTO1229, AVTO1260, AVTO1010, AVTO1132, AVTO1008, AVTO1005, AVTO1143, *Assila F1* and *Mwanga*, with disease severity between 2.4 and 3.4 are regarded as moderate resistant to TYLCD. *Ty-2* gene is again found in all these genotypes except for AVTO1226 which has *Ty-3* and

AVTO1229 which has *Ty-5* as the only genes. *Ty-2* is therefore the strongest gene in resistance to begomoviruses. VI045743 and *Tanya* had disease severity greater than 3.5 and therefore are regarded as susceptible to TYLC disease. *Ty-1* is the only gene present in VI045743. Although VI045743 was bred as resistant, its *Ty-1* resistant gene might have been compromised by the high pressure of different begomovirus species available on the trial location. Report by Butterbach *et al*. (2014) reveals that resistance conferred by the gene *Ty-1* has been compromised when plants become infected with multiple RNA viruses. Another report by Camara *et al*. (2013) reveals that 12 varieties of 41 tested in a trial carried out in Senegal proved to be resistant to TYLCD, but when the same varieties were tested simultaneously in Benin, Burkina Faso, Ghana, Mali, and Togo, the varieties performed differently in the different countries.

On disease incidence, in the first week after transplanting only a few plants were infected but their number increased as time went up to the seventh week perhaps due to increase of vector population. This caused late and unsynchronized infection in the field, which caused the differences in symptom manifestations by the plants (Pico *et al*., 1998: Vidavsky *et al*., 1998). Plants that were infected in the first week might have severe symptoms by the seventh week after transplanting as compared to the plants that were infected in the late weeks which could mistakenly be considered as genetic resistant genotypes. This could not have occurred if a controlled screenhouse inoculation was applied, where all plants could easily be inoculated.

3.5 Conclusion and Recommendation

Four tomato lines AVTO1122, AVTO1130, AVTO1141 and AVTO1219 are identified to be resistant to high diversity of begomovirus on the coastal belt of Tanzania. Tomato lines AVTO1080, AVTO0301, AVTO0922, AVTO1226, AVTO1229, AVTO1260,

AVTO1010, AVTO1132, AVTO1008, AVTO1005, AVTO1143, *Assila F1* and *Mwanga*, are considered moderate resistant on the coastal belt of Tanzania. It was VI045743 and *Tanya* which were susceptible to TYLCD. *Ty-2* gene proved to provide strongest resistance. *Ty-1* is the weakest in genetic resistance against TYLCD.

Assila F1 and *Mwanga* seeds are encouraged to continue to be used on the coastal belt of Tanzania as they have proven to be moderate resistant to the recombinant begomoviruses. Multi-location trials of the resistant and moderate resistant AVRDC tomato lines should be carried out by also considering other quality aspects such as yield performance, and the proved resistant lines should be made available for commercial purchases to the farmers of the coastal belt of Tanzania.

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CHAPTER FOUR

4.1 General Discussion and Conclusion

The overall objective of this study was to provide better understanding of diversity of begomovirus and to identify tomato varieties which are resistant to TYLCD in the coastal belt of Tanzania. There specific objectives were to determine the diversity of begomoviruses along the coastal belt of Tanzania, and the second was to evaluate experimental tomato lines and commercial varieties for resistance to Tomato leaf curl disease in the coastal belt of Tanzania.

In the first objective, the study has revealed a great diversity of begomoviruses on the coastal regions of Tanzania. This diversity is shown by the presence of four clusters of begomoviruses that form four unique species, one of which has been identified as new species. This new species is represented by two variants of full begomovirus genome namely ToLCVTz-Ch-9 and ToLCVTz-Ch-26 with size of 2774 and 2767 nucleotide base pairs, respectively. The study also revealed other two full genomes strains relating to *Tomato leaf curl Arusha virus*, which were ToLCVTz-Ch-4 and ToLCVTz-Ch-7, each with 2760 nucleotide base pairs. These new variants and strains have shown to be highly recombinant and therefore they can be lethal to tomato production.

In the second objective, sixteen tomato lines from AVRDC and other four commercial varieties were tested for their response to TYLCD at Chambezi. Of the fifteen of the tomato lines from AVRDC introgressed with *Ty* genes for resistance to TYLCD, only AVTO1122, AVTO1130, AVTO1141 and AVTO1219 were proved to be resistant in the trial. All these four genotypes contained *Ty-2* gene, and in particular AVTO1122 had an addition of *Ty-5* gene while AVTO1219 had an addition of *Ty-3* gene. Tomato lines

AVTO1080, AVTO0301, AVTO0922, AVTO1226, AVTO1229, AVTO1260, AVTO1010, AVTO1132, AVTO1008, AVTO1005, AVTO1143, *Assila F1* and *Mwanga*, were considered moderate resistant. Again it was *Ty-2* gene which proved to be the strong resistant gene in the majority of these tomato lines. VI045743 and *Tanya* were found to be susceptible to TYLCD. *Ty-1* gene which was contained in VI045743 proved to be the weakest in genetic resistance against TYLCD and should not be used in breeding programs.

Tomato samples taken from Chambezi, have shown to have all the four species of begomoviruses, and are coded in the study as ToLCVTz-Ch, meaning *Tomato leaf curl virus Tanzania Chambezi*. All the full genome species and strains of begomoviruses in this study were sampled from the trial at Chambezi. As they have proved to be highly recombinant, it means the tested tomato lines where all exposed to a mixture of viruses with high lethality. This further explains why only four of tomato lines were resistant and the majorities were tolerant, even though they are introgressed with *Ty* resistant genes. *Assila F1* which is known to be resistant to TYLCD is also regarded as tolerant in the study. This is because this variety was exposed to the new recombinant begomoviruses which are more lethal than their parent viruses.

The study has revealed that even for just a small field of tomato, there can be a great diversity of begomoviruses ranging from different groups of species to different groups of strains and variants. Therefore, if a single tomato line has the ability to withstand a lethal pressure of one or three species of begomoviruses, it can still be attacked by just another available species. That species can be a new one or a strain of an already existing species that has developed due to recombination.

4.2 Recommendation

The study recommends that breeding programs should be initiated to develop resistant tomato lines with the strongest resistant genes that have the ability to tackle a wide range of begomovirus species. They can start by using *Ty-2* gene, and a combination of (*Ty-2, Ty-3*) and (*Ty-2, Ty-5*), which have proved strong genetic resistance to four different species of begomoviruses. *Ty-1* gene which has shown weak resistance to TYLCD is not suitable to be introgressed in tomato lines that are intended to be grown in the coastal areas of Tanzania.

The seeds of the resistant and moderate resistant tomato lines from AVRDC should be further tested on multi-locations trials in Tanznaia and the best performers should be released and made available for farmers to purchase. *Assila F1* and *Mwanga* have proved to be moderate resistant to TYLCD, therefore are encouraged to be used by these farmers, as their seeds are already commercially available in the market. This will increase tomato production in the coast, which will eventually tackle its high demand in the coastal cities, and lowering purchasing costs, as their availability will be near.

Begomoviruses are a production constraint to tomato as demonstrated by this study. However this is not the only crop that is seriously affected by begomoviruses, and not only the *Solanaceae* family. Begomoviruses are a problem to both horticultural and field crops particularly tomato, okra, cassava and cotton. The exploration of the diversity of begomoviruses should not only be limited to the rest of important crops and weeds alike. Their recombination can be studied and hence used by breeders to modify the genes, for getting resistant tomato lines and other crops with better adaptability and yield.

As for the control of begomoviruses, a combination of IPM approach should be used to combat the problem. This may include removing weeds, removing whiteflies and use of virus-free transplants.