DISTRIBUTION AND GENETIC DIVERSITY OF *TOMATO YELLOW LEAF CURL VIRUS,* ASSOCIATED WHITEFLY VECTORS AND THE RESPONSE OF SELECTED TOMATO VARIETIES TO THE VIRUS

AVEDI EDITH KHAMONYA

Bsc.AgEd, (Egerton); Msc. Crop protection (MMUST)

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR DEGREE OF DOCTOR OF PHILOSOPHY IN CROP PROTECTION

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

UNIVERSITY OF NAIROBI

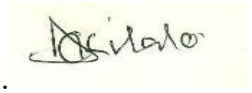
2022

DECLARATION

I declare that this thesis my original work and has not been presented for an award of a degree in any other University

Edith Khamonya Avedi Signature:  Date: 23-11-2022

This thesis has been submitted with our approval as University supervisors

Dr. Dora Kilalo Signature…  Date: 24-11-2022
Department of Plant Science and Crop Protection

University of Nairobi.

Prof. Florence Olubayo Signature:  . Date: 24-11-2022
Department of Plant Science and Crop Protection

University of Nairobi.

Dr. Isaac Macharia Signature……  …. Date : 23-11-2022
Phytosanitary and Biosafety Department

Kenya Plant Health Inspectorate Services.

DECLARATION OF ORIGINALITY

Name of Student: Avedi Edith Khamonya

Registration Number: A80/53432/2018

College of Agriculture and Veterinary Sciences (CAVs)

Faculty/School/Institute Agriculture

Department of Plant Science and Crop Protection

Course Name: PhD in Crop Protection

Title of the work: Distribution and genetic diversity of *Tomato yellow leaf curl virus,* associated whitefly vectors and the response of selected tomato varieties to the virus

DECLARATION

1. I understand what Plagiarism is and I am aware of the University's policy in this regard

2. I declare that this PhD thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

3. I have not sought or used the services of any professional agencies to produce this work

4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work

5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature …  ……. Date: 23- 11-2022

DEDICATION

This thesis is dedicated to my nulfamily. Their cooperation and support made this study possible and valuable. And to my late dad, this is the dream you had for me and I didn’t let you down.

ACKNOWLEDGEMENTS

My sincere gratitude goes to several people who made the completion of this thesis a reality. Firstly, 1 wish to thank my supervisors, Dr. D. Kilalo, Prof. F. Olubayo and Dr. I. Macharia for their valuable time, encouragement and guidance throughout this work.

I particularly wish to thank the Africa Biosciences Challenge Fund (ABCF) from granting me a fellowship that allowed me to undertake part of the research at the Biosciences Eastern and Central Africa-International Livestock Research Institute (BecA- ILRI) hub.

My gratitude goes to the Community Network for African Vector- Borne Plant Viruses (CONNECTED) for the training in Genomic data analysis at SLU-Sweden.

My gratitude goes to my employer Kenya Plant Health Inspectorate Service for supporting the survey and for allowing me to utilize the laboratory for most of the research. I appreciate without mentioning names the support I received from my colleagues in this journey.

Table of Contents

List of tables

List of figures

ABBREVIATIONS AND ACRONYMS

AAP	Acquisition access period
AEZ	Agroecological Zone
ALFP	Amplified length fragment polymorphism
ANOVA	Analysis of Variance
ATLCV	*African tomato leaf curl virus*
BecA	Biosciences Eastern and Central Africa
BLAST	Basic Local Alignment Sequence Tool
CABI	Centre for Agriculture and Bioscience International
CP	Coat protein
CTAB	Cetylmethylammonium bromide
DAS-ELISA	Double Antigen Sandwich Enzyme Linked Immunosorbent Assay
DNA	Deoxyribonucleic acid
DnaSP	DNA Sequence Polymorphism
dNTPS	Dinucleotide triphosphate
EDTA	EthyleneDiamine Tetracetic Acid
ELISA	Enzyme linked immunosorbent assay
EPPO	European plant protection Organization
GARD	genetic algorithm recombination detection
HCD	Horticultural Crop Development
HCL	Hydrochloric acid
IAP	Inoculation access period
ILRI	International Livestock Research Institute
KALRO	Kenya Agricultural Livestock Research Organization
kb	Kilobase
LSD	Least Significant Difference
MEGA	Molecular Evulotionary Genetics Analysis
MMUST	Masinde Muliro University of Science and Technology
mtCO1	Mitochondrial cytochrome oxidase 1
NaCL	Sodium Chloride
NCBI	National Center for Biotechnology Information
NPK	Nitrogen Phosphorus and Potassium
ORF	Open reading frames
PCR	Polymerase chain reactions
QC	Quality Control
RAPD	Random amplified polymorphic DNA
RDP	Recombination Detection Program
REn	Replication enhancer
Rep	Replication
RNA	Ribonucleic acid

SAS	Stastical Analysis System
SBP	single breakpoint scanning
SEM	Standard Error of Means
SRA	Sequence Read Archive
TBE	Tris-borate-EDTA (Ethylene Diamine Tetracetic Acid)
TD	Tagment DNA Buffer
TDE	Tagment DNA Enzyme
TE	Tris- EDTA
TICV	*Tomato infectious chlorosis virus*
TLCUV	*Tomato leaf curl Uganda virus*-Iganga
ToCV	*Tomato chlorosis virus*
ToLCArV	Tomato leaf curl Arusha Virus
ToLCV	*Tomato leaf curl virus*
TrAP	Transcription activator protein
Tris	*Trisaminomethane*
TSP	Triple Super Phosphate
TYCMalV	*Tomato yellow leaf curl Malaga virus*
TYLCAxV	*Tomato yellow curl Axarquia virus*
TYLCCNV	*Tomato yellow leaf curl China virus*
TYLCD	*Tomato yellow leaf curl disease*
TYLCGuV	*Tomato leaf curl Guangdong virus*
TYLCIDV	*Tomato yellow leaf curl Indonesia virus*
TYLCKaV	*Tomato yellow leaf curl Kanchanabari virus*
TYLCMLV	*Tomato yellow leaf curl Mali virus*
TYLCSV	*Tomato yellow leaf curl Sardinia virus*
TYLCTV	*Tomato yellow leaf curl Thailand virus*
TYLCTV	*Tomato yellow leaf curl Tanzania virus*
TYLCV	*Tomato yellow leaf curl virus*
TYLCVNV	*Tomato yellow leaf curl Vietnam virus*
UPGMA	Unweighted pair group method arithmetic mean
V (1,2)	Viral sense polarity

ABSTRACT

Tomato (*Solanum lycopersicum*) is an important fruiting vegetable cultivated worldwide because of it's commercial and high nutritional value. In Kenya, tomato is predominantly cultivated by small scale farmers but its production is constrained biotic constraints such as insect pests and diseases due to bacteria, fungi, nematodes and viruses. Viruses are the third significant constraint to tomato production and this includes viruses in the genus Begomovirus. About 60 begomovirus species affect tomato plants in the tropics and subtropics leading to yield losses of up to 100 %. Begomoviruses are spread by a vector *Bemisia tabaci*. In Kenya, TYLCV-like virus was reported for the first time in tomato crops in 1996. Since then no comprehensive research has been conducted to establish the genetic diversity of the virus, how commonly grown tomato varieties respond to the virus and the diversity of vectors involved in its transmission. This study aimed at contributing to the development of sustainable management strategies of tomato yellow leaf curl disease in tomato crops in Kenya by determining the genetic diversity of *Tomato yellow leaf curl virus* present, that of associated vectors and the response of commonly grown tomato varieties to TYLCV. A field survey was carried out in eight major tomato growing regions in Kenya between September and December 2018 and January to March 2019. A total of 259 fields were surveyed and data collected on tomato leaf curl disease prevalence, incidence, and severity. The presence of the virus was further confirmed using DAS –ELISA and molecular techniques. Estimates of whitefly populations colonizing sampled tomato crops was done through direct counting of adult whiteflies on the underneath of five topmost leaves. Adult whitefly samples were collected using a hand held aspirator, carried in 95% ethanol and analyzed using DNA barcoding technique. Screening of selected tomato varieties to tomato leaf curl disease was done at KALRO Mwea in two seasons; September to December 2018 and May to August 2019. The experiments were laid in randomized complete block design with three replications. Each plot had 25 plants and data was collected on TYLCD disease incidence, severity and population of whiteflies from 15 plants per plot. Data on disease incidence, severity, whitefly populations was analyzed using Analysis of Variance (ANOVA) and Pearson correlations using SAS software (version 9.1). Molecular data was analyzed using phylogenetic relationships, recombination analyses and population genetics. This study established that the disease prevalence, incidences and severity varied between the Counties and among fields within a County. There was a significant difference on TYLCD incidences across the Counties and amongst the sampled varieties ($p \leq 0.05$). Kwale County had the highest disease incidence while Bungoma had the least. The disease incidence was generally lower in hybrids compared to conventional varieties. During screening for the response of selected varieties to the disease, it was observed that all tomato varieties tested were susceptible to the disease and there was no significant difference in disease incidence and severity between the test varieties in both season 1 and 2. Moreover, whitefly populations were not statistically different in both seasons and there was no correlation between whitefly population and TYLCD incidence and severity. However, the whitefly population differed significantly ($p \leq 0.05$) on the test varieties. Serological assays confirmed the presence of *Tomato yellow leaf curl virus* but further analysis using metagenomics revealed that

the begomovirus symptoms observed on tomato crops are caused by *Tomato leaf curl Arusha virus* (ToLCArV). Twelve complete genomes were obtained from the samples with an average coverage of 99.9%. The sequences showed 95.7-100% identity amongst themselves. Analysis of amino acid sequences showed the highest identities in the regions coding for the coat protein gene (98.5–100%) within the isolates, and 97.1–100% identity with the C4 gene of ToLCArV. Phylogenetic algorithms clustered all Kenyan isolates in the same clade with ToLCArV, confirming the isolates to be a variant of the Tanzanian virus. There was no evidence of recombination within the isolates. Estimation of selection pressure within the virus population revealed the occurrence of negative or purifying selection in five out of the six coding regions of the sequences. Though begomoviruses are vectored by *Bemisia tabaci*, all the 163 whitefly samples collected from tomato plants and analysed in this study were *Trialeurodes vaporariorum* species and had no intra population diversity. Demographic analysis indicate population expansion of *T. vaporariorum* observed. It is therefore concluded that begomovirus symptoms found in tomato plants in Kenya are caused by *Tomato leaf curl Arusha virus.* Breeding programs should consider developing cultivars resistant to this virus. However, there is need to evaluate the role of the complex agroecosystems in tomato fields in the transmission of ToLCArV. Further research should be done to determine if the virus is seed transmitted. The information generated in this study will be useful in developing sustainable management strategies of the disease in the country.

CHAPTER ONE

INTRODUCTION

1.1 Background

Tomato (*Solanum lycopersicum*) is one of the most important vegetables cultivated in most parts of the world whose origin is Latin America. The crop is grown in many parts of the world because of its commercial and high nutritional value (Marie *et al*., 2012; Glick *et al*., 2009). Worldwide annual production is around 221 million metric tons valued at 190.4 billion US dollars produced on 6 million hectares (FAOSTAT, 2019). Africa produces about 19 million metric tons, with Egypt taking lead, while Kenya is ranked in the ninth position, with an annual production of 973,304 metric tonnes valued at approximately 170m USD produced on about 30,000 Ha (HCD, 2020). In Kenya, tomato is predominantly cultivated by small scale farmers and the main producing Counties are Kajiado, Taita Taveta, Kirinyaga, Baringo, Bungoma, Nakuru, and Meru (Karuku *et al*., 2017). Growing of tomato is mostly done out door in the open fields, however greenhouses or net houses production have been introduced allowing production all year round (Macharia *et al*., 2015)

Tomato production is constrained by both biotic and abiotic factors. Biotic constraints include arthropod pests and diseases caused by fungi, bacteria, nematodes, viruses (Macharia *et al*., 2015). Although bacteria, fungal and nematodes are believed to cause significant yield losses in tomato production, the effect of viral diseases on production has been overlooked.

Viruses are ranked the third major limitation in tomato production (Macharia *et al*., 2015). The genus *Begomovirus* significantly affects tomato plants all over the world, with about 60 species affecting the crop (Marie *et al*., 2012). They cause severe infections in tomato crops in the tropics and subtropics leading to up to 100 % reduction in yield (Glick *et al*., 2009). The

reduction in yield depends on the crop variety, type of viral strain, environmental conditions, age of plant during infection period, cropping systems and efficiency of vectors (Marie *et al*., 2012)

Tomato yellow leaf curl virus (TYLCV) (family Geminiviridae; genus Begomovirus) is one of the major viruses in this genus that infects tomato, it comprises of several viral species that cause Tomato yellow leaf curl disease (TYLCD) in infected plants. TYLCD significantly impede production of tomatoes worldwide (Hosseinzadeh and Garivani, 2014). Two strains, the mild and the severe have been identified to cause varied disease severity. Studies done in Israel and Spain reported the occurrence of the mild strain, whereas the severe strain has been reported in Southern United States and Carribbean (Basak, 2016). The virus was first reported and described in Israel in the 1960s (Diaz-Pendon *et al*., 2010). It has spread to many parts of the world and about 12 TYLCV-like viruses are now documented (Marie *et al.,* 2012) as indicated in the **table 1.1** below. Natural interspecies recombination between TYLCSV strain and TYLCV strain has occurred in Spain and Italy leading to better ecologically adapted recombinants whose epidemiological consequences has not been established (Diaz-Pendon *et al*., 2010; Monci *et al*., 2002; Navas-Castillo *et al*., 2000). The recombinants are more virulent and are able to attack resistant varieties leading to epiphytotics (Diaz-Pendon *et al.,* 2010).

Table 1. 1 Worldwide occurrence of TYLCV like viruses

TYLCV like virus	Location	References
Tomato yellow leaf curl Axarquia virus (TYLCAxV)	Spain	Abhary *et al.*, 2007.
Tomato yellow leaf curl China virus (TYLCCNV)	China	Yin *et al.*, 2001
Tomato yellow leaf curl Guangdong virus(TYLCGuV)	China	Zi *et al.*, 2007
Tomato yellow leaf curl Indonesia virus (TYLCIDV)	Indonesia	Diaz-Pendon *et al*., 2010
Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV)	Laos,Thailand,Vietnam	Tang *et al*., 2014
Tomato yellow leaf curl Malaga virus(TYLCMalV)	Spain	(Navas-Castillo *et al*., 2000 Monci *et al*.,2002)
Tomato yellow leaf curl Mali virus(TYLCMLV)	Mali, Ethiopia, Cameroon	Lett *et al.*, 2009; Diaz-Pendon *et al*., 2010
Tomato yellow leaf curl Sardinia virus (TYLCSV) (formerly TYLCV-Sardinia)	Tanzania,Italy,Spain Burkina Faso, Uganda	Nono–Womdim, 2005; Glick *et al.*, 2009
Tomato yellow leaf curl virus (TYLCV) (formely TYLCV-Israel)	Europe, America, Asia, Egypt, Sudan, Malawi, Tanzania, Kenya and Zambia	Glick *et al*.,2009; Nono Womdim, 2005
Tomato yellow leaf curl Vietnam virus (TYLCVNV)	Vietnam	Diaz-Pendon *et al*., 2010
Tomato yellow leaf curl Thailand virus (TYLCTV)	Thailand	Tang *et al*., 2014

Source: Own compilation from publications

In Africa the first case of TYLCV was reported in tomato crops in Sudan (Akhtar *et al.*, 2014), since then the virus has spread to Malawi, Uganda, South Africa, Morocco, Zambia, Namibia,

Swaziland, Kenya and Tanzania (Nono–Womdim, 2005). In East Africa *Tomato yellow leaf curl virus* (TYLCV) and *Tomato yellow leaf Sardinia virus* (TYLCSV) are present (**Table 1.2**). In 1994, TYLCV-like symptoms were observed in Tanzania. However serological methods used in identification were not sufficient to allow species identification (Harrison and Robinson, 1999; Chiang *et al*., 1997). Three years later, molecular characterization established the presence of *Tomato leaf curl Tanzania virus* (TLCTV) which caused an epidemic with high disease severity in tomato crops in Makutupora district in Tanzania (Marie *et al*., 2012; Chiang *et al*., 1997). A field survey conducted later indicated the presence of *Tomato yellow leaf curl Sardinia virus* with less severe symptoms (Marie *et al*., 2012; Kashina *et al*., 2002). In Kenya, leaf curl like symptoms were observed in tomato production during a survey conducted in Kibwezi, Kitui, Athi River, Naivasha and Machakos. Through DNA hybridization *Tomato yellow leaf curl virus* was identified as the pathogen responsible for the symptoms observed in the field (Nono-Womdim *et al.*, 2005; Bob *et al*., 2005). Since then no comprehensive research has been conducted to establish the distribution and the existing genetic diversity of the virus. Therefore, there is need to establish the diversity of the causal agents of TYLCD in tomato crops in Kenya. This information will be useful in development of disease management strategies.

Table 1. 2 First reports on TYLCV-like viruses within East Africa

Country	Virus species	Year first reported	Reference
Sudan	TYLCV	1960	Akhtar *et al* (2014)
Tanzania	TYLCSV, TLCTV	2002	Kashina *et al* (2002)
Uganda	TYLCSV	1997	Nono-Womdim (2005)
Kenya	TYLCV	1996	Nono-Womdim (2005)
Ethiopia	TYLCMLV	2005	Czosnek (2007)

Source: Own compilation from publications

Tomato yellow leaf curl virus strains have monopartite genomes and are spread in a persistent, circulative and non-propagative way by whiteflies, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Zehra *et al*., 2017; Al-ani *et al.*, 2011). TYLCV hosts vary in different geographical locations and includes; *Solanaceae* (tomato, peppers, nightshade weeds and ornamental plants), *Malvaceae* (Okra), *Fabaceae* (beans) and weeds species (Marie *et al*., 2012). Symptoms of TYLCD include, yellowing of upper leaves, reduced leaf size, upward curling of leaf margins, stunting and flower abscission. The variation in symptom expression has been shown to indicate different strains of the virus which may emerge from the existing indigenous viruses or may be introduced through plant imports (Marie *et al*., 2012).

Whitefly species, *Bemisia tabaci*, *Aleurodicus dispersus* Russell, *Trialeurodes vaporarium* (West) and *T. abutiloneus* (Haldeman), spread viral infections in plants. However, studies indicate that *Bemisia tabaci* species is responsible for TYLCV transmission. *Bemisia tabaci* exhibits a high genetic variation, where biotypes MEAM 1 and MED (formerly biotype B and Q respectively) have been associated with TYLCV transmission (Weng and Tsai, 2013). The virus circulates and replicates within the vector before transmission occurs. In addition transovarial transmission has been reported where the infected female transmits the virus to its progeny (Weng and Tsai, 2013). Recently, seed transmission of TYLCV in tomato crops was reported (Kil *et al*., 2016) and so far mechanical transmission of the virus has not been documented (Yoon *et al*., 2015). Through transboundary trade and movement of ornamental plants and other hosts, the host range and geographical expansion of this pest beyond their native habitat continues to occur (Lorenzo *et al*., 2016).

Bemisia tabaci is a polyphagous pest and vector with a high fecundity and multiple hosts. These characteristics offer a challenge to the vector and virus management but through the use of

cultural practices, resistant tomato cultivars and use of insecticides in the management of the vector, TYLCV incidences are reduced (Glick *et al*., 2009). Chemical use for vector management is not sustainable since the vectors readily develop resistance. For instance research carried out by McKenzie *et al.* (2009) indicates that the MED biotypes have a great tendency to have resistance to neonicotinoids insecticides and insect growth regulators. Furthermore, the use of chemicals such as buprofezin, acetamiprid, imidacloprid, thiamethoxam, a mixture of fenpropathrin and acephate, and pyriproxyfen (on eggs) have had little effect in reducing the population of MED biotypes (Horowitz *et al*., 2005; Dennehy *et al*., 2005). However, variation in developing resistance to these chemicals among the MEAM (B) and MED (Q) biotypes has not been well studied (McKenzie *et al*., 2009). Moreover, though tomato plants are infested by other whitefly populations alongside *B. tabaci*, the contribution of the others to TYLCV transmission remains unknown.

1.2 Statement of the problem

Tomato production is limited by biotic constraints, one such constraint is Tomato yellow leaf curl disease (TYLCD) which leads to 100% yield loss in infected fields (Zhang *et al*., 2009). This disease is due to TYLCV though other strains of this virus have been reported. Studies indicate that the pathogen is spread by white flies (*Bemisia tabaci* Genn) in a persistent way. The global spread of this virus has been attributed to the worldwide presence of *B. tabaci* and a wide host range for both the vector and the pathogen (Medina *et al*., 2006). In Kenya, TYLCV-like virus was reported for the first time in tomato crops in 1996; where Athi River, Naivasha, Kibwezi and Kitui were identified as hot spots. Since then, no comprehensive research has been done to establish its genetic diversity, how commonly grown tomato varieties respond to the virus and the diversity of vectors involved in its transmission. This leaves a gap in the

information about the extent of its distribution and spread in major tomato growing areas in Kenya. The relationship of the TYLCV strain(s) in Kenya to those reported elsewhere is yet to be evaluated. Although *B. tabaci* has been reported as the sole vector of the virus, it's not well understood if other whitefly populations found in tomato ecosystems are involved in spreading of the virus.

Bemisia tabaci species is complex comprising of about 40 morphologically similar species, variants or biotypes (De Barro *et al*., 2011; Mugerwa *et al*., 2021). These biological types have variations in host range, transmission ability of the virus and resistance to insecticides (Horowitz *et al*., 2005). The spread of TYLCV in tomato plants has been associated with MEAM and MED biotypes. Studies done by Higuchi *et al*. (2007) indicate that selection by pesticides leads to new whitefly biotypes emerging. In Kenya the management of white flies is done mainly by use of pesticides. It is therefore important to understand how this practice has impacted on the diversity of whitefly populations in tomato fields and how different tomato varieties respond to the virus.

1.3 Justification

For proper management of any disease, it is critical to understand the pathogens involved and the mode of transmission. Host plant resistance is a sustainable way of managing plant pathogens. In order to develop resistant varieties, it is important to understand the genetic diversity of the pathogen. New pathogen species having different pathogenic ability and enlarged geographical and/or host range emerge from processes such as mutations, pseudo recombination and genetic recombination (Marie *et al*., 2012). Moreover, though *Bemisia tabaci* has been reported as the only species that transmits TYLCV, it is important to evaluate if any other whitefly populations found within tomato ecosystems are involved in the transmission of this virus. Information generated from this study will assist in coming up with appropriate choices to combat the

disease. Choices that will help in reducing indiscrimate use of synthetic chemicals hence less chances of pesticide resistance among whiteflies on tomatoes. Therefore, this study aims at establishing the distribution of TYLCV, its genetic diversity and the associated whitefly populations found in major tomato growing areas in Kenya and to assess the response of selected tomato varieties to the virus. This knowledge will assist breeders develop resistant cultivars and in developing appropriate management strategies.

1.4 Overall Objective

The overall study aims to contribute to the development of sustainable management strategies of tomato yellow leaf curl disease in tomato crops in Kenya by establishing the genetic diversity of *Tomato yellow leaf curl virus* present and associated vectors.

1.4.1 Specific Objectives of the study

1. To determine the distribution, incidence and severity of Tomato yellow leaf curl disease in major tomato growing areas in Kenya.
2. To establish the genetic diversity of *Tomato yellow leaf curl virus* found in major tomato growing areas in Kenya.
3. To determine the diversity of whitefly populations colonizing tomato plants in Kenya.
4. To evaluate the response of common tomato varieties grown in Kenya to TYLCD

1.4.2 Hypothesis

1. Tomato yellow leaf curl disease does not affect tomato crops found in major growing areas in Kenya.
2. *Tomato yellow leaf curl virus* infecting tomato crops in Kenya is not genetically different.
3. Whitefly species found in colonizing tomato plants are not genetically different.
4. Common tomato varieties grown in Kenya do not vary in their response to TYLCD.

CHAPTER TWO

LITERATURE REVIEW

2.1 Economic importance of tomato

Tomato (*Solanum lycopersicum*) is a fruiting plant that belongs to Solanaceae family and whose origin is Latin America. It is considered a top priority vegetable crop grown worldwide. In Kenya, it is among the major horticultural crops grown by farmers. It is a source of income, the fruits are rich in ascorbic acid and retinol, and also contains lycopene which has antioxidant properties that fights cancer (Sawalha, 2013).

2.1.2 Tomato production in Kenya

Tomato is a significant vegetable grown widely in most parts of Kenya. The major growing Counties include, Kirinyaga, Meru, Nakuru, Bungoma, Taita Taveta, Kajiado and Makueni (Macharia *et al*., 2015), however due to its economic importance most Counties not known to grow this crop have started planting it mainly as an income generating crop. Tomato is a source of income for both small scale, medium and large scale producers. Tomato constitutes about 20% of vegetables produced in Kenya with production of about 973,304 metric tonnes valued at approximately 170m USD (HCD, 2020). The major varieties grown in Kenya include Anna F1, Cal J, Chonto F1, Eden F1, Fortune Maker, Heinz 1350, Kilele F1, M82, Tylka F1, Nema 1400, Onyx, Oxyl Roma VF, Rio Grande among others (Masinde *et al*., 2011). Production is done both in green house and open fields. Though 95% of the production occurs in open fields greenhouse technology allows for production all year round. Tomato production requires low to medium rainfall however supplementary irrigation is important during dry periods (Masinde *et al*., 2011). The desired soils for optimum production are deep, medium-textured sandy loams, fertile, well drained soils with high organic matter (Naika *et al*., 2005). Ideal conditions for the growth of the crop are warm conditions with optimum temperatures of 20 ℃-25 ℃ (Srinivasan, 2010). Fruit

setting and quality are affected by temperatures below 12 °C or above 35 °C, low temperatures delay colour formation and ripening, while high temperatures limit fruit setting, development of lycopene and flavor. Wet conditions lead to many foliar infections on the plant (Masinde *et al*., 2011).

2.1.3 Constraints to tomato production in Kenya

Tomato production in Kenya is limited by a number of factors such as; lack of improved well performing varieties, poor fruit setting due to heavy rains and excessively high temperatures, lack of inputs, sub optimal crop husbandry and due to pests and diseases (Ochilo *et al*., 2018; Macharia *et al*., 2015). Several pests and diseases are known to affect tomatoes in Kenya. Major insect pests include, *Tuta absoluta*, whitefly species (e.g. *Bemisia tabaci*, *Trialeurodes vaporariorum*), African boll worm (*Helicoverpa armigera*), Western flower thrip (*Frankliniella occidentalis*), leaf miner (*Liriomyza sativae*, *L. trifolii* and *L. huidobrensis*), Onion thrips (*Thrips tabaci*), aphids (*Aphis gossypii*) (Macharia *et al*., 2015; Nderitu *et al*., 2010). It has been reported that more than 146 viruses affect tomato plants. These are grouped in about 33 genera of which 15 are of significant importance. In African tropics, 5 genera have been reported to affect tomato and these are; *Tobamovirus*, *Cucumovirus*, *Tospovirus*, *Begomovirus* and *Potyvirus* (Nono-Womdim, 2005).

The family *Geminiviridae* is among the largest group of plant viruses. Geminivirus particles are small geminate, circular, quasi-icosahedra with single stranded DNA genome (Briddon *et al*., 2010; Rojas *et al*., 2005). They are transmitted by insects and are divided into four genera namely, *Mastrevirus*, *Begomoviruses*, *Topocuvirus* and *Curtovirus* based on their biological properties and genome organization (Rojas *et al*., 2005). The most numerous and economically significant are *Begomoviruses*, vectored by whitefly species (*Bemisia tabaci*) to dicotyledonous

plants and cause similar disease symptoms. It is the only genus of family *Geminiviridae* that has viruses infecting tomato in both the New and Old Worlds.

2.2.0 Begomoviruses

Begomoviruses are transmitted by whitefly (*Bemisia tabaci* Gennadius) in a persistent manner leading to yield losses of up to 100% in dicotyledonous plants in tropics and subtropics (Zehra *et al*., 2017; Glick *et al*., 2009). Begomoviruses genomes have a circular single stranded DNA (ssDNA) and are classified as either mono or bipartite (Fauquet *et al*., 2003). The bipartite begomovirus genome possess two ssDNA molecules identified as DNA-A and DNA-B whereas monopartite begomovirus has only DNA-A (Sohrab *et al*., 2016). The molecules (DNA-A and DNA-B) are similar at the Common Region (CR) of 200nt, which is plays a role in replication and transcription (Dhakar *et al*., 2010).

Begomoviruses are grouped into two broad groups, old world (OW) viruses (those found in Africa, Europe and Asia) and new world (NW) viruses (found in the Americas), this classification is based on phylogenetic analyses and genome organization (Prassana *et al*., 2010; Ghosh *et al*., 2012).

Most begomoviruses from the old world are monopartite and have a satellite molecules known as betasatellites and alphasatellite (Briddon and Stanley, 2006; Briddon *et al*., 2003). The genomes of monopartite begomoviruses are ~2.8kb in size and have genes in both directions diverging from a noncoding intergenic region (IR) which has promoter elements plus the *ori* of virion-strand DNA replication (Díaz-Pendón *et al*., 2019). The DNA-A component of begomoviruses contains either five or six ORFs in both directions that encode ~10 kDa proteins. These proteins play roles in virus assembly; viral replication; host gene regulation and silencing suppression; and vector transmission (Silva *et al*., 2014; Yadava, 2010). Viruses are highly evolving

biological entities mainly as a result of recurrent mutations and recombination during replication (Padidam *et al*., 1999). Begomoviruses have a high recombination ability that plays a role in their evolution and emergence of new pathotypes that not only challenge host resistance but also exploit new environments (Lefeuvre and Moriones, 2015; Davino *et al*., 2009; Moriones *et al*., 2007). The number of sequenced and characterized *Begomoviruses* species is increasing and vary in genome components and organization (Fauquet *et al*, 2003). This includes *Tomato yellow leaf curl viruses* (*sensu lato*) (Van Regenmortel *et al*., 1997) reported in many parts in sub Saharan Africa. Examples include; *Tomato leaf curl virus* (ToLCV), *Tomato yellow leaf curl virus*-Israel strain (TYLCV-IL), *African tomato leaf curl virus* (ATLCV), *Tomato leaf curl Uganda virus*-Iganga (TLCUV), *Tomato leaf curl Uganda virus* reported in Sudan, Tanzania, Uganda, Kenya respectively (Chiang *et al*., 1997; Nono-Womdim *et al*., 2005; Shih *et al*., 2006; Kimathi *et al*., 2020). These viruses were identified from samples exhibiting leaf curl symptoms. This is an indication that leaf curling symptoms in tomato plants are caused by several viruses and new begomoviruses continue to emerge due to recombination (Rojas *et al*., 2005).

2.2.1 Structure and genome organization of TYLCV

Tomato yellow leaf curl virus has only one genomic component (Monopartite) i.e. DNA-A which it uses to cause systemic infections (Pratap *et al*., 2011). The DNA-A is single stranded and about 2.5-2.8 kb (Glick *et al*., 2009; Rojas, 2005). This genomic DNA encodes six partly overlapping open reading frames (ORFs) which are arranged in two transcriptional units divided by an intergenic region of about 300 nucleotides (Diaz-Pendon *et al*., 2010). The viral sense strand has one gene (*AV1*) and located on this strand are two of the ORFs i.e. encoding the coat protein (CP) and a movement like protein (V2), the complementary sense strand comprises of three genes (*AC1*, *AC2*, and *AC3*) and four ORFs are located on it i.e. encoding a replication-

associated protein (Rep), a transcription activator protein (TrAP), a replication enhancer protein (REn) and a minute C4 protein entrenched inside the Rep **(Fig 2.1)** (Glick *et al*., 2009; Wartig *et al*., 1997). The CP encloses the genome and conveys it in and out of the nucleus, it's also essential for systemic infection in the plant, vector transmission and determines the specificity of the arthropod vector (Diaz-Pendon *et al*., 2010). Rep and REn are vital for effective viral DNA replication, while the V2 protein of TYLCV functions as a viral suppressor of RNA silencing **(Table 2.1)** (Zrachya *et al*., 2007).

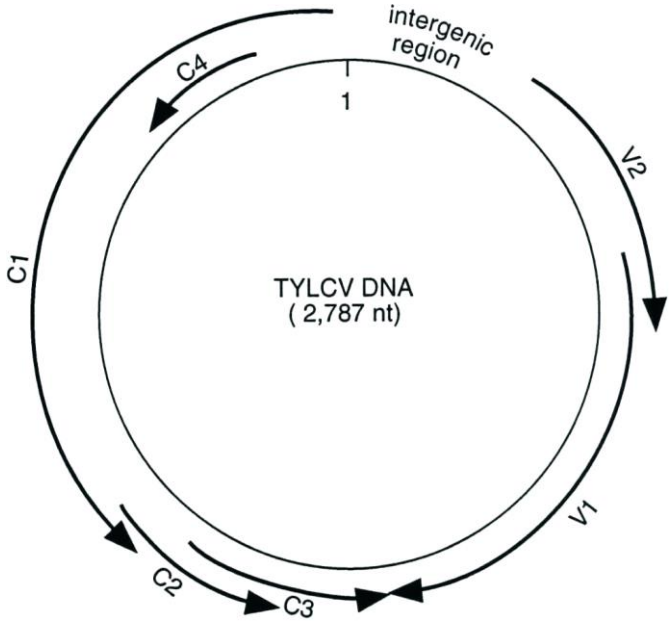


Figure 2. 1 Structure of TYLCV genome (Glick *et al*., 2007)

Table 2. 1 A summary on the functions of the TYLCV genes

Gene	Protein	Function
AV1	**CP**	Assembly of virion capsid and whitefly-mediated transmission
AC1	**Rep**	Replication of viral DNA
AC2	**TrAP**	Subdues RNA silencing and other responses that lead to host defense, Transcriptional activator for the virus-sense genes
AC3	**REn**	Intensifies viral replication

Source: Zrachya *et al*., 2007; Glick *et al*., 2009

2. 2.2 Alternative hosts of TYLCV

Tomato yellow leaf curl virus has many plant hosts i.e. more than 30 species found in 12 families (Polston and Lapidot 2007). The hosts act as reservoirs and they vary among tomato growing regions and include; cultivated vegetables, ornamentals and weeds. Though, tomato (*Solanum lycopersicon*) is the main host of TYLCVs, natural infections also occur in common bean (*Phaseolus vulgaris*), chilli pepper (*Capsicum chinense*) and tobacco (*Nicotiana tabacum*), sweet pepper (*Capsicum annuum*), ornamentals such as petunia and lisianthus (*Eustoma grandiflora*), and weeds i.e. *Solanum nigrum, Cynanchum acutum, Datura stramonium, Chenopodium murale*, *Malva parviflora, Convolvulus* sp, *Eclipta prostrate* and *Cuscuta* sp (Diaz-Pendon *et al*., 2010). In some crops like pepper (*Capsicum sp*) and beans (*Phaseolus vulgaris*) there is variation amongst the genotypes on their susceptibility TYLCV. Lapidot (2002) screened the response of selected bean genotypes to TYLCV and established that only 57% of the tested genotypes were found susceptible to the virus. Similarly though pepper is a symptomless carrier of the virus, not all cultivars are susceptible (Polston *et al*., 2006). Additionally some weeds such as *Malva*

parviflora and undomesticated tomato species e.g. *Lycopersicon chilense, L. hirsutum, L. peruvianum, L. pimpinellifollium* are asymptomatic carriers (Glick *et al*., 2009). Nonetheless, *B. tabaci* effectively acquires and transmits the virus from symptomless carriers and transmits to other hosts (Glick *et al*., 2009).

2.2.3 Transmission of Tomato yellow leaf curl viruses (*Sensu lato*)

*Tomato yellow leaf curl virus*es whose origin is Middle Eastern-Mediterranean region are a serious limitation globally in the production of tomatoes (Moriones and Navas-Castillo, 2000; Hosseinzadeh and Garivani, 2014). According to Navot *et al.* (1992) the presence of viral DNA in a vector is proof of the vector transmission of that particular virus. In this regard *Bemisia tabaci* Genn has been associated with the transmission of TYLCV in a persistent - circulative way (Weng and Tsai, 2013; Ning *et al*., 2015). The high reproductive ability of the vector leads to more generations and thus a higher vector population, this results into faster spread of viral infections.

Bemisia tabaci MEAM and MED biotypes are associated with the spread of TYLCV, though MED biotypes acquires and transmit TYLCV at a higher rate than MEAM biotypes (Ning *et al*., 2015) therefore the type of biotype present within a locality influences the spread of the virus. MEAM biotype is considered invasive and one of the most damaging crop pests found in Africa, Australia, Asia and America leading to significant yield losses (Chu *et al*., 2006; De Barro *et al*., 2011). The vectors acquires TYLCV virus by feeding the on phloem sap of infected plant and transmits to susceptible hosts through feeding (Weng and Tsai, 2013). Horizontal transmission of TYLCV occurs between individuals of the same biotype during copulation (Diaz-Pendon, 2010). *Bemisia tabaci* transmits TYLCV with shorter acquisition access period (AAP) and a brief Inoculation access period (IAP) i.e. $\leq$ 1 hour (Weng and Tsai, 2013). Ghanim *et al.* (2001)

indicated that the latent period (period required for a virus particle to multiply within the vectors body until its ready to be inoculated to a new susceptible host) of TYLCV within the vector is 8 hrs. While the retention period of the virus is lifelong such that a viruliferous *B. tabaci* remains infective for the entire life time and the virus replicates within the vector (persistent-propagative transmission), however the transmission ability of this vector diminishes with age (Weng and Tsai, 2013). And also, the vector's life expectancy and number of eggs laid is reduced by TYLCV by about 20% and 50% respectively (Glick *et al*., 2009). Studies indicate that adult females are more efficient in transmitting the virus than males (Ning *et al*., 2015).

Though *B. tabaci* is the sole vector of TYLCV in tomatoes, the role of other whitefly populations present in tomato ecosystems in transmitting this virus remains unknown. Other whitefly species present in Kenya include *Trialeurodes vaporariorum* and *Aleurodicus dispersus*. Studies on vectors associated with the spread of *Cassava brown streak virus* (CBSV) in Kenya indicated that though *B. tabaci* had been identified as the only vector of CBSV, *Aleurodicus dispersus* was also found to transmit the virus (Mware *et al*., 2009). On the other hand *Trialeurodes vaporariorum* (Westwood, 1856) is responsible for the spread of *Criniviruses* as well as *Torradovirus* (Navas-Castillo *et al*., 2011).

2.2.4 Interaction of TYLCV and *B. tabaci* whitefly species

Tomato yellow leaf curl virus particles are ingested by *B. tabaci* during feeding on infected plant tissues, the particles enters the gut, they are conveyed to the haemolymph then to the salivary gland and later injected back into plant tissues during successive feeding (Ghanim and Medina, 2007). The ability of *B. tabaci* to acquire and transmit TYLCV is determined by the chaperonin GroEL homologue that binds TYLCV particle while preventing its degradation in the haemolymph (Diaz-Pendon, 2010). The viral capsid protein determines all the properties needed

for vector transmission and specificity. Studies done by Caciagli *et al*. (2009) established that amino acids 129 and 134 located on the CP were responsible for transmission of TYLCSV. Plant viruses can directly or indirectly alter the growth and development of their vectors. TYLCV has been reported to adversely affect the fecundity and longevity of MEAM biotypes (Diaz- Pendon, 2010). Though the replication of TYLCV within *B. tabaci* has not been well understood, viral transcripts and TYLCV DNA have been reported to accumulate in the vector after feeding on infected tomato with TYLCV (Diaz- Pendon, 2010). Horizontal transmission between similar biotypes occurs during copulation and this could be due to contamination of heamolymph (Ghanim *et al*., 2007). However studies have indicated possibility of transovarial transmission through at least two generations (Rojas, 2004)

2.2.5 Interaction between tomato and TYLCV

For a disease to occur the virus must spread and replicate within the plant, however, the host plant elicits some responses that minimize infections. The response by plants varies, some exhibit immunity, these are non-host and the virus do not replicate within their protoplast or in initially inoculated cells (Hull, 2009). Infectible plants are host plants in which viruses can infect and replicate in their protoplasts (Hull, 2009). Resistant plants are hypersensitive and limit virus multiplication to initially infected cells by formation of a localized necrotic lesion. Susceptible plants allow systemic movement and replication of viruses while tolerant ones exhibit latent infections (Hull, 2009). Inoculation of TYLCV to host plant cells is done by *B. tabaci*, the virus moves to the nucleus where the genome replicates. TYLCV like other geminiviruses replicates inside the nuclei of mature cells whose DNA is not replicating (Diaz-Pendon *et al*., 2010). The viral proteins significantly impact on the host cell's biological processes such as; differentiation of cells, cell cycle control, replication of DNA, functioning of plasmodesmata and RNA

silencing (Diaz-Pendon *et al*., 2010). Tomato plants inoculated with TYLCV exhibit symptoms after 2-3 weeks but the viral DNA can be detected after one week but the highest viral DNA concentration occurs 4 days before the onset of symptoms (Samarakoon *et al*., 2012).

2.2.6 Mixed infections of TYLCV

Mixed infections of TYLCVs occur and are responsible for the occurrences of epidemics in the world (Diaz-Pendon *et al*., 2010). Recombination which arises due to exchange of genetic material leads to evolution of new recombinant variants with an increased pathogenicity and/or improved environmental adaptation (Froissart *et al*., 2005; Martin *et al*., 2005; Davino *et al*., 2009). Recombination may happen at the level of strain, species, and genus and even inter family (Diaz-Pendon *et al*., 2010). Studies show that in Spain, in the 1990s the species *Tomato yellow leaf curl Sardinia virus* (TYLCSV) was present and stable though with little genetic diversity however, the introduction of isolates of Israel TYLCV strains (mild and severe) led to genetic recombination (Diaz-Pendon *et al*., 2010). Genetic exchange between TYLCSV and TYLCV – Israel isolate resulted to a new recombinant variant identified as *Tomato yellow leaf curl Malaga virus* (TYLCMaV) (Monci *et al*., 2002). Whereas the recombination between TYLCSV and severe strain of TYLCV resulted into *Tomato yellow leaf curl Axarquia virus* (TYLCAxV) (Garcia-Andres *et al*., 2006). Both TYLCAxV and TYCMaV were detected within the populations and with enhanced ecological adaptation than the parental viruses (Diaz-Pendon *et al*., 2010; Monci *et al*., 2002). In Italy both TYLCV and TYLCSV occur either as single or mixed infections, hence resulting in emergence of recombinant variant. Recombinants may be more virulent and cause wide spread epidemics (Davino *et al*., 2009).

2.2.7 Epidemiology of TYLCV in tomatoes

For proper management of TYLCV, it is critical to understand its epidemiology in tomato growing regions. *Tomato yellow leaf curl virus* is likely to be found in most tomato fields in an affected region and more so where the vectors are found. An increase in vector population leads to a rise in TYLCD incidences (Snehi *et al*., 2015). However, there is a variation amongst seasons with relation to whiteflies populations throughout the years (Srinivasan *et al*., 2012). Warm climates favour a reduction in the life cycle of the vector hence more generations occur (Weng and Tsai, 2013). The variation in tomato genotypes ability to support TYLCV and whitefly populations influences epidemics of the virus, resistant cultivars have been reported to be reservoirs for TYLCV and *B.tabaci* (Srinivasan *et al*., 2012). Environmental factors such as wind play a significant role in the spread of vectors, it has been reported that inoculative white flies can spread the virus 7 km from the source (Srinivasan *et al*., 2012). Inoculation in the fields is random and the number of infected plants positively correlates with the time/period taken by the white flies in the fields, however some escapes occur (Ssekweya, 2006). The presence of volunteer tomato plants and alternate hosts (e.g. *Sonchus* sp, *Amaranthus* sp, *Malva* sp, *Solanum melongena*, *Mercuria lisannua*, *Phaseolus vulgaris*) within and near the growing fields provide primary inoculums hence enhanced TYLCV incidences (Sawalha, 2013).

2.2.8 Identification of TYLCV in tomato

Under field conditions identification of TYLCV is done based on symptoms such as; stunting, leaf curling, mottling, chlorosis, reduced leaf size. However, this approach provides unreliable results as they are influenced by pest infestation by sap sucking insects as well as biotic factors (Ssekweya, 2006; Green, 1991) hence need to employ laboratory based methods in proper identification. Such methods include serological tests and molecular techniques.

2.2.8.1 Serological tests for detecting TYLCV in tomato

Enyme Linked Immunosorbent Assay (ELISA) is a serological technique that utilizes antiserum prepared against a given virus. The antiserum contains antibodies generated in blood serum of rabbits inoculated with that particular virus' antigen (Clark and Adams, 1977).

Double-Antigen-Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) is used for rapid identification of viruses such as TYLCV based differences in the coat protein of a virus. It is fairly cheap since the reagents and chemicals required are readily available, and it gives adequate identification of viruses (Clark and Adams, 1977; Ssekweya, 2006). Triple-Antibody-Sandwich (TAS-ELISA) is another form of ELISA. It uses monoclonal antibodies to detect viruses such as TYLCV (Ssekweya, 2006).

2.2.8.2 Molecular techniques for detecting TYLCV infecting tomato

Polymerase Chain Reaction (PCR) is one of the molecular techniques used in identification of viruses. It is based diversity of viral nucleic acid, and is very efficient, and also accurate depending on the type of primers used (specific or general) (Boonham *et al*., 2013). The assays are easy to apply, sensitive and very specific in TYLCV identification, hence they have been widely adopted (Ssekweya, 2006). The application of multiplex PCR has been widely adopted since it is cheaper and faster and employs the use of multiple primer pair targeting a definite section of the gene involved in replication and the intergenic section (Anfoka *et al*., 2008). This is followed by DNA sequence analysis which enables accurate identification of viruses to determine its association with existing viral strains (Samarakoon, 2012). Phylogenetic analysis either through the use of complete sequence or partial nucleotides indicates ancestral relationship among geminiviruses hence useful in virus taxonomy (Samarakoon, 2012).

2.2.8.3 Next generation sequencing of TYLCV isolates from tomato

More recently new advances mostly known as metagenomics have been adopted in the identification of plant diseases (Wu *et al*., 2015). This technique can be used to identify both known and unknown pathogens in a diseased sample (Wu *et al*., 2015). It is also able to determine co-infections in plants (Blawid *et al*., 2017; Akinyemi *et al*., 2016). Through bioinformatics approaches identification of the pathogen sequences is done through comparison with known viruses or virus like motifs (Adams *et al*., 2012).

2.2.9 Management of TYLCV in tomato

Management of TYLCV in both protected and open field protection is challenging and expensive. Several strategies have been developed to manage TYLCV, these include the use of healthy seedlings, chemicals, cultural practices, crop rotation, physical control and use of resistant varieties (Glick *et al*,. 2009). A combination of several strategies is necessary in order to lower the vector population and restrict its movement thus reducing sources of TYLCV inoculum (Polston and Lapidot, 2007).

2.2. 9.1 Use of resistant varieties to tomato yellow leaf curl disease

Use of resistant varieties is an effective, sustainable and environmentally safe strategy in managing TYLCV (Snehi *et al*., 2015). A resistant variety is one that can reduce virus multiplication hence suppressing disease symptom development (Glick *et al*., 2009). However, efforts to breed for resistant varieties have produced only tolerant varieties, which show delayed symptoms and less accumulation of viral DNA (Glick *et al*., 2009). Introgression of resistant genes obtained from undomesticated tomato types such as (*Solunum chilense, Solanum pimpinellifolium, Solanum peruvianum*, and *Solanum habrochaite*) has resulted into tomato cultivars with variable resistance levels e.g. Tyking and Fiona varieties in Tanzania are resistant

to TYLCV, these varieties show no virus symptoms and on the other hand TY20 exhibits tolerance (Glick *et al*,. 2009; Non-Wondim *et al*., 2005). Breeding programs for cultivated tomato aim at introgressing Ty-1, Ty-2, and Ty-3 genes to confer resistance to TYLCV, however the process is slow due to poor quality of fruits and interspecific barriers that hinder effective transfer of genes between wild and domesticated tomatoes (Polston and Lapidot 2007). Combining host resistance with high quality fruit is still a challenge in the breeding program, additionally some of the resistance collapse in early and severe disease pressure, therefore growers incorporate cultural and chemical control measures (Snehi *et al*., 2015).

2.2.9.2 Cultural practices for managing tomato yellow leaf curl disease

Several cultural practices such use of virus free transplants, field sanitation, use of reflective mulch and sticky traps and crop free periods help reduce TYLCV incidences in tomatoes. Virus free transplants can be produced in a greenhouse located away from production areas, or through use of apical meristem cultures, heat treatment (35-54^{o}C) (Snehi *et al*., 2015). Application of antifeeding insecticide e.g. Pymetrozine on the transplants during their production reduces TYLCV incidences (Polston and Lapidot 2007). Application of a protective dose of a neonicotinoid on young transplants minimizes vector infestation thus offers a two week protection on the seedlings after transplanting in the open field (Polston and Lapidot, 2007)

Weeds and alternate hosts are involved in the epidemiology of TYLCV, therefore maintaining the field free of TYLCV host plants for about 3 months reduces the inoculums and *Bemisia tabaci* incidences (Snehi *et al*., 2015; Gilbertson *et al*., 2007). Additionally, inter-planting tomatoes with trap plants such as squash and cucumber (not hosts of TYLCV), diverts whiteflies from tomato plants thus delaying TYLCV infection by about 2 months (Snehi *et al*., 2015).

2.2.9.3 Chemical control of tomato yellow leaf curl disease

Chemical are efficient in decreasing economic losses due to TYLCV especially in open field production. Whitefly populations have been reduced by different chemical compounds with varied modes of actions (Polston and Lapidot 2007). However, continuous use of pesticides has resulted into resistance in whiteflies hence decreased efficacies; this has also resulted into the outbreak of secondary pests like leaf miners (Polston and Lapidot 2007). Incidences of TYLCV infections in tomato crops are reduced through the use of neonicotinoids. To prevent inoculation of TYLCV in susceptible plants the insecticide should induce death of all whiteflies in 35 - 40 minutes, this is not practical in the field and therefore insecticides only offer partial control (Polston and Lapidot 2007). The use of neonicotinoids as drenches and on transplants for the first two weeks offers protection for 8 weeks, thereafter to minimize on resistance rotation with non-neonicotinoids chemicals like insecticidal soaps, oils and growth regulators and other contact insecticides may be used till harvesting (Snehi *et al*., 2015). Use of insecticides is also ecologically unfriendly (Castle *et al*., 2010).

2.2.9.4 Genetic engineering of varieties to TYLCD

Virus resistant transgenics (pathogen-derived resistance) are developed by introducing a virus capsid protein in a tomato plant (Snehi *et al*., 2015). The incorporation of TYLCV- *CP* gene in tomato plants results in plants which take time to express symptoms, and recover from TYLCD infection and also exhibit resistance upon repetitive inoculations. Experiments on *Nicotania tabacum* and S. *lycopersicum* plants transformed with a 2/5 TYLCV *Rep* gene construct showed TYLCV resistance (Yang *et al*., 2004).

Tomato plants derived from RNA-mediated gene silencing and later artificially inoculated with TYLCV exhibited immunity (Fuentes *et al*., 2006). The use of antisense RNA to Rep protein

gene interferes with the disease caused by TYLCV, however this is type of silencing is specific to TYLCV species or strain (Snehi *et al*., 2015). Similarly, resistance to this pathogen can be achieved through binding with GroEL. Studies done by Edelbaum *et al*. (2009) showed that plants over expressing GroEL gene were tolerant to TYLCV infections. This tolerance occurs through restricted virus movement that occurs due to binding of endosymbiotic bacteria found in the whitefly with the coat protein found in TYLCV (Diaz-Pendo, 2010).

2.3 Whitefly populations in tomato agro ecosystems

Whiteflies are agricultural pests with a worldwide distribution (Lapidot *et al*., 2014). Their agricultural importance is either through direct feeding of both immature and mature stages or secretion of honeydew which is suitable for sooty moldy fungi growth on fruits and leaf surfaces that limits photosynthesis and lowers quality of fruits and fiber (Boykin and De Barro, 2014). Among the most economically important whiteflies are the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood, 1856) and *Bemisia tabaci* Genn. *Trialeurodes vaporariorum* transmits a number of plant viruses of the genera *Crinivirus* and *Torradovirus* (Navas-Castillo *et al*., 2014; Navas-Castillo *et al*., 2011). Of these, the *Criniviruses*, *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV) are of major economic importance in tomato production globally (Wintermantel *et al*., 2009). On the other hand, *Bemisia tabaci* has been reported to spread over 111 economically significant viral infections belonging to several genera such as; *Begomovirus*, *Crinivirus*, *Carlavirus*, and *Ipomovirus* (Weng *et al*., 2013; Liu *et al*., 2012).

2.3.1 *Bemisia tabaci* (Gennadius) species of whitefly

The *Bemisia tabaci* Genn is a sap feeding insect that is widely distributed in the tropics and subtropics (Ahmed *et al*., 2010). Yearly yield losses of between 20 -100% have been associated

with this pest (Pan *et al*., 2012). *Bemisia tabaci* has been reported to spread over 111 economically significant viral infections belonging to several genera such as; *Begomovirus, Crinivirus, Carlavirus* and *Ipomovirus* (Liu *et al*., 2012; Weng *et al*., 2013). Hosts of *B. tabaci* include; *Brassica* spp, tomatoes, cotton, beans, egg plants, squash, cucumber, poinsettia and weeds e.g. *Euphorbia heterophylla* and *Commelina benghalensis* (Weng and Tsai, 2013). *Bemisia tabaci* species has a high genetic variation hence several biotypes/genetic variants have been reported (Weng and Tsai 2013). Identification of the various biotypes is done through consideration of factors like variations in biotic and biotic characteristics, resistance to pesticides, host preference and specificity, virus transmission efficiency, variability in fecundity and also how they are geographically distributed in addition to ability to cause phytotoxicity after feeding (Weng and Tsai 2013; Qiu *et al*., 2009). Several *Bemisia* biotypes are present within agroecosystems, therefore it's important to understand the existing biotypes since this influences pest management choices to be applied in agroecosystems (Mugwerwa *et al*., 2021; Qiu *et al*, 2009).

2.3.2 Life cycle of *B. tabaci*

Bemisia tabaci species has a high fecundity with many generations in a year. The adult is a tiny insect of about 0.88mm length; the life history has three stages (egg, nymph and adult). The eggs are pyriform and are laid in groups underneath the hosts leaves (Weng and Tsai, 2013).

The first instars also referred to as crawlers are mobile with a flat body; the second and third instars lack legs hence immobile. The fourth instar (red eye nymph) has thickened body shapes and turns yellowish, later in this stage they stop feeding and moults into an adult (Weng and Tsai., 2013). An adult female lays about 300 eggs within its lifespan of about 25 to 30 days and

about 8-12 generations occur within a year with more generations occurring in warm climates (Weng and Tsai, 2013).

2.3.3 Identification of *B. tabaci*

Identification of *B. tabaci* can be done using both morphological and molecular methods. However, classification basing on morphological traits is problematic and difficult due to close similarity amongst the genera and species (Shah *et al*., 2013). Morphologically, *Bemisia tabaci* adults have a yellowish body with four membranous wings (Weng and Tsai 2013). The nymphs are difficult to distinguish, however Qui *et al* (2009) were able to morphologically distinguish between MEAM 1 and MED biotypes (formerly biotype B and Q) nymphs basing on the size of the anterior and posterior wax fringes. The study established that MEAM 1 has a larger anterior wax fringe and a smaller posterior wax fringe than the MED biotype.

The use of biochemical, molecular and DNA finger printing techniques have been adopted in identification of morphologically indistinguishable individuals (Shoorcheh *et al*., 2008; Shah *et al*., 2013). RAPD–PCR technology is simple and rapid hence widely used to distinguish haplotypes of *B. tabaci* (Shah *et al*., 2013). Amplified length fragment polymorphism (ALFP) allows the use of large samples in population analyses and produces similar results as RAPD-PCR. However, recently the use of genetic markers like cytochrome oxidase 1 (CO1) sequences of mitochondrial DNA, internal transcribed spacer of the ribosomal DNA and the use of microsatellite markers have contributed significantly to molecular identification of *Bemisia tabaci* haplotypes. These techniques give accurate results though they are difficult to perform and time consuming (Li, 2005; Qui *et al*., 2009). Use of mitochondrial cytochrome oxidase 1 (mt CO1) gene has been widely adopted in studying *B. tabaci* populations since it is able to identify genetic variability amongst *B. tabaci* biotypes (Shah *et al*., 2013). Through phylogenetic analysis

B. tabaci populations have been separated into 12 major groups, namely: Mediterranean/Asia Minor/Africa (includes the B haplotype), Mediterranean (includes the Q biotype), sub-Saharan Africa silver leafing, Indian Ocean, Asia I, Australia, China, Asia II, Italy, New World, sub-Saharan Africa non-silver leafing and Uganda sweet potato (Boykin *et al.*, 2007). According to Boykin and De Barro (2014), it's recommended to use 657 bases of 3' end of the mt COI to analyze the full mitogenome as this allows the comparison of many mt COI sequences already available hence allowing sufficient identity of *B. tabaci*.

CHAPTER THREE

DISTRIBUTION, INCIDENCE AND SEVERITY OF TOMATO YELLOW LEAF CURL DISEASE IN MAJOR TOMATO GROWING AREAS IN KENYA

3.1 Abstract

Tomato (*Solanum lycopersicum* L.) is a fruiting herbaceous plant grown in many parts of the world for commercial and nutritional purposes. In Kenya, tomato is a major source of income for small scale rural farmers and it is produced mostly for domestic markets. The main producing Counties are Kirinyaga, Kajiado, Nakuru, Meru, Bungoma and Taita Taveta. Viruses are a major constraint in tomato production in tropics and sub tropics and induce symptoms like stunting, leaf mosaic, distortion, chlorosis, mottling, and vein clearing which are similar to symptoms caused by abiotic factors. These viruses include begomoviruses such as *Tomato yellow leaf curl virus* that was first reported in Kenya in 1996, however since then no research has been conducted to assess its status in tomato fields, hence the objective of this survey. A field survey was carried out in eight major tomato growing regions in Kenya between September and December 2018 and January to March 2019. A total of 259 fields were surveyed and data collected on tomato leaf curl disease prevalance, incidence, and severity. The presence of the virus was further confirmed using DAS –ELISA. Estimates of whitefly populations colonizing sampled tomato crops was done through direct counting of adult whiteflies on the underneath of five topmost leaves. It was observed that TYLCD was present in all the Counties and AEZs surveyed. The disease prevalence, incidences and severity varied from amongst the Counties, AEZs and between fields. The mean disease prevalence across the Counties ranged from 19.5 to 64%, with Kwale having the highest disease prevalence while Bungoma had the least. Similarly, Kwale had the highest mean disease incidence while Bungoma had the least. Within AEZs, the disease incidence was high in the coastal lowland 3, 4, 5 (38.75 ± 10.02) while LM2 had the least (0.31± 0.16). There was a significant difference ($P \leq 0.05$) in disease incidences amongst the varieties sampled. The disease incidence was generally lower in hybrids compared to non hybrid varieties. Mean disease severity in the varieties was statistically different ($P \leq 0.05$) and ranged from 0.18 to 2.20. Whitefly populations varied across the Counties and among the varieties. There was significant difference ($P \leq 0.05$) in adult whitefly populations both in the Counties and among the tomato varieties. Kwale had the highest whitefly population while Bungoma had the least. The variety, New Fortune maker had the highest whitefly populations, while the variety Star F1 had the least. More than half of the farmers interviewed (58.7%) had observed the disease in their tomato crops but the majority (74%) associated the disease with high temperature. This study confirmed the presence of Tomato yellow leaf curl disease in Kenya using both visual symptoms and ELISA tests

3.2 Introduction

Tomato (*Solanum lycopersicum* L.) is a fruiting herbaceous plant grown in many parts of the world due to its commercial and high nutritional value. In Kenya, tomato is a major source of income for small scale rural farmers and it is produced mostly for domestic markets (Geoffrey *et al*., 2014). The main producing Counties are Kirinyaga, Kajiado, Nakuru, Meru, Bungoma and Taita Taveta. The crop is cultivated both in green houses and open fields, though the latter accounts for 95% of the production. The main varieties grown in Kenya are Moneymaker, Kilele, Oxly, Cal J and Rio grande mainly because they are preferred by the market (Macharia *et al*., 2015; Karuku *et al*., 2017; Ochilo *et al*., 2018). However, though the production of tomato has intensified in Kenya, yields have remained low (Ochilo *et al*., 2018). This is majorly due to biotic constraints that include insect pests such as nematodes, *Tuta absoluta* and diseases caused by bacteria, fungi, viruses (Bob *et al*., 2005; Macharia *et al*., 2015; Peris *et al*., 2018).

Viruses induce symptoms such as stunting, leaf mosaic, distortion, chlorosis, mottling, vein clearing which are similar to symptoms caused by abiotic factors (Syller, 2012; Schreinemachers *et al*., 2015). Among viral diseases, begomoviruses have become the most important viruses of tomato in the tropics and sub tropics. They are transmitted by whitefly (*Bemisia tabaci* Gennadius) in a persistent manner leading to yield losses of up to 100% (Glick *et al*., 2009; Zehra *et al*., 2017). A number of begomoviruses have been described as main constraints of tomato production in Africa. These among others, includes *Tomato yellow leaf curl Mali virus*, *Tomato yellow leaf curl Sudan virus* and *Tomato leaf curl Nigeria virus Tomato leaf curl Uganda virus, Tomato leaf curl Arusha virus* and *Tomato leaf curl Tanzania virus* (Shih *et al*., 2006; Zhou *et al*., 2008; Lafeuvre *et al*., 2010; Kon and Gilbertson, 2012). In Kenya, though *Tomato yellow leaf curl virus* was reported in 1996 in tomato crops as the cause of TYLCD,

recently (Kimathi *et al*., 2020) reported the presence of *Tomato leaf curl Uganda virus* in tomato plants exhibiting begomovirus infection symptoms. As a result of recombination and mutations there is genetic diversification of begomovirus populations leading to emergence of new begomoviruses continue with increased virulence (Moriones and Navas-Castilo, 2008).

Several techniques are used in the identification of begomoviruses, this include serological assays such as Enzyme linked immunosorbent assay (ELISA) and Polymerase chain reactions (PCR). ELISA is widely used as a diagnostic tool to assess the phytosanitary status of plants and for quarantine purposes and has become an essential part of pathogen indexing (Boonham *et al*., 2013). Though previous studies have been carried out on tomato viruses in Kenya, little focus has been given to TYLCD since it was first reported in 1996. Currently, tomato production has intensified in Kenya and efforts such as breeding for TYLCD resistant varieties has been made. This study was carried out to determine the status of TYLCD in tomato production areas in Kenya.

3.3 Materials and Methods

3.3.1 Tomato yellow leaf curl disease (TYLCD) survey and sampling areas

A field survey was conducted in eight major tomato growing Counties in Kenya between September and December 2018 and January to March 2019. The Counties surveyed were; Bungoma, Taita Taveta, Meru, Kwale, Nakuru, Baringo, Kirinyaga and Kajiado (**Figure 3.1**). A combination of purposive and simple random sampling was used to select tomato farms and sampling sites. In each County, tomato farms were sampled by stopping at regular predetermined intervals of 3-8 km along major and feeder roads traversing each sampling area. The number of fields surveyed per County depended on the availability of tomato farms at the time of survey. Thirty plants were examined along an X- shaped transect extending from the four corners of each field. Plants were examined for virus symptoms and the number of plants exhibiting TYLCD symptoms documented. Along the transect 5 symptomatic samples were collected and where no symptoms were observed asymptomatic plants were sampled. Adult whitefly population was determined by direct count method (Sseruwagi *et al*., 2006). This involved direct counting of adult whiteflies on five top most leaves of 10 randomly selected tomato plants along the X transect. Each leaf was held by a petiole and gently inverted so that the adults present on the lower surface could be counted (Mware *et al*., 2009).

A total of 259 tomato fields were assessed for TYLCD symptoms. The number of fields sampled varied across the Counties, based on the intensity of tomato cultivation in the selected regions at the time of the survey. A total of 1275 leaf samples were collected and taken to the laboratory for serological analysis. Moisture on the leaves was removed by blotting with absorbent paper and dehydrated on unhydrous Calcium chloride ($CaCl_2$) contained in a paper bag. The samples were carried to the plant quarantine and biosecurity station laboratory to confirm the presence of

the virus using DAS-ELISA. Coordinates were taken at each sampling site using a global positioning system (GPS) device (Magellan Triton 'Windows CE Core 5.0 X11-15302).

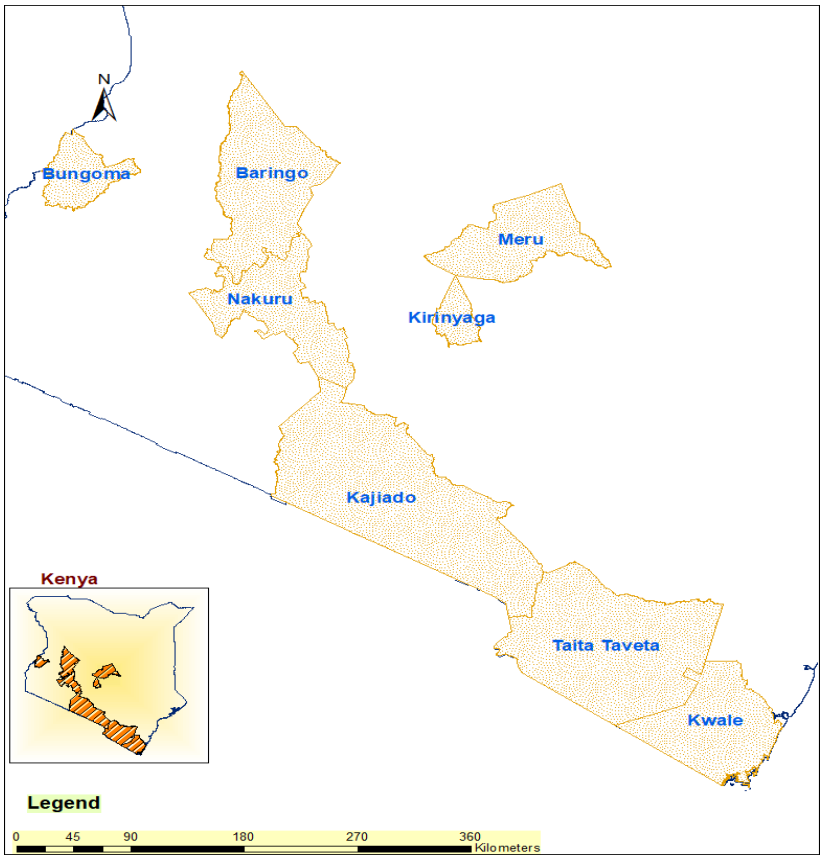


Figure 3. 1 A map of Kenya showing Counties where tomato samples were collected

3.3.2 Data collection and analysis

Data was collected on TYLCD prevalence, incidence, severity, whitefly populations and varieties grown by the farmers. Prevalence was expressed as a percentage of farmer fields in which the disease symptoms was observed. TYLCD viral incidence were assessed by visually examining 30 plants randomly and scored as a percentage. The type of TYLCD symptoms observed were recorded. Disease severity was assessed by randomly selecting ten tomato plants and evaluating the percentage of leaf area infected and scored on a five point scale such that; 1=1-20% (chlorosis), 2=21- 40% (chlorosis, stunting), 3=41-60% (chlorosis, stunting, reduced

leaf sizes), 4= 60-80% (Chlorosis, stunting, reduced leaf size, upward curling of leaves, reduced internodes), 5=81-100% (Chlorosis, stunting, reduced leaf size, upward curling of leaves, reduced internodes, flower abortion, reduced fruit size) (Ssekyewa *et al*., 2006; Mwangi *et al*., 2015). Additional information was captured using a structured questionnaire where information on types of seed planted (whether hybrid or non-hybrid), farmers' knowledge of TYLCD, management choices applied to manage TYLCD, level of education, gender and area under tomato production. Data was collected on percentage of samples that tested positive during laboratory analysis per region. The data was analyzed through descriptive statistics (frequencies, percentages and mean values) for all continuous variables to generate summaries and tables and analysis of variance using SAS version 9.1 at $p \leq 0.05$ significance level (SAS Institute, 2004). Differences between means was determined using Fischer's Protected LSD at P = 5%. The correlation coefficient between disease incidence, severity and whitefly populations was determined.

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

All samples were tested for the presence of TYLCV by Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977). The antisera were purchased from Agdia® USA. Coating of microtiter plates with TYLCV specific antibodies diluted in 1× coating buffer was done by dispensing 200µl per well. The microtiter plate was covered with aluminum foil and placed in a humid box and incubated at 30°C for 4 hours. The plate was later washed three times. Each sample was crushed in 10 ml of extraction buffer and incubated. In each of the wells of the microtiter plate, 200µl of the extracted sap was added. The plate was covered and placed in humid box and incubated in at 4°C overnight. The following day, the plates were taken out and washed. The washing repeated for three times. Thereafter,

secondary enzymes were added to trap the antigen proteins and incubation done for 5 hours at 30°C. Finally the substrate along with para-Nitrophenylphosphate (pNPP) was added to the antibody-antigen coated wells. The plate was incubated in a dark area but at room temperature for 45 minutes for development of yellow color and the optical density values were taken at 405nm in ELISA plate reader. Positive and negative controls were included. The positive controls were purchased from Agdia® (USA) together with the antibodies. All samples were assayed in duplicate and the results inferred to be positive if the absorbance was greater than or equal to twice the average reading of the negative (healthy) controls.

3.4 Results

3.4.1 TYLCD symptoms, prevalence, incidence and severity across Counties and AEZs sampled

A total of 259 farms were surveyed in eight Counties. TYLCD symptoms observed in the fields were mainly stunting, chlorosis, reduced internodes, reduced leaf sizes and upward leaf curling (**Figure 3.2**). TYLCD was present in all the Counties and AEZs surveyed. The disease prevalence, incidences and severity varied amongst Counties, AEZs and between farms. Mean prevalence ranging from 19.5 to 64% were observed in the eight Counties. The disease prevalence was high in Kwale (64%) while Bungoma had the lowest (19.5%). The mean prevalence was 53.06% (**Table 3.1**)

There was variation in disease incidence across the Counties and farms sampled. There was a significant difference on TYLCD incidences across the Counties and AEZs ($P \leq 0.05$). Within AEZs, the disease incidence was high in the Coastal lowlands 3, 4, 5 (38.75 ± 10.02) while LM2 had the least (0.31 ± 0.16). On the other hand, the highest TYLCD severity was recorded in UM1 (2.2 ± 0.35) while LM1 and LM2 had the least at (0.21 ± 0.15) and (0.17 ± 0.08) respectively

(**Table 3.4**). The mean disease incidence in the Counties ranged from 0.3 to 38.8%. Kwale County had the highest mean (38.8%), followed by Baringo (34.2%) while Bungoma had the least (0.3%). The disease incidences in Nakuru, Kirinyaga and Baringo were statistically similar. Meru, Taita Taveta and Kajiado had statistically similar disease incidences. Based on serology results, Kwale County had the highest disease incidence (41.4%) followed by Baringo (33.6%) while Bungoma County had the least (1.7%). Visually assessed incidence levels were consistently lower than that those based on serological assays. TYLCD disease severity was also statistically different ($P \leq 0.001$) across the sampled Counties. TYLCD severity was assessed on a scale of 1-5. Disease severity ranged from 0.18 to 2.20 across the Counties. Bungoma County had the least severity (0.18) while Baringo had the highest TYLCD severity (2.20). Most Counties had scores of <2.00, which indicated very mild infections (**Table 3.1**).

Figure 3. 2 TYLCD symptoms in tomato plants (a) tomato plants exhibiting reduced internodes, chlorosis, reduced leaf size (b) Stunting symptoms; (c) reduced leaf size and upward leaf curling

3.4.2 TYLCD incidences and severity among the most cultivated tomato varieties

During the survey more than 20 tomato varieties were found to be cultivated by farmers. These consisted of Riogrande, Kilele F1, Onyx F1, Elgon, Cal J, Safari, Shanty F1, New Fortune maker, Eden F1, Nyati F1, Asila F1, Money maker, Pesa F1 amongst others. The major varieties cultivated varied between and within the Counties. It was found that 20% of the farmer

repondents across all Counties surveyed preferred Riogrande (**Table 3.1).** However, more than one variety was grown in all the Counties and in some cases several varieties were grown in the same field. Few farmers (3.5%) planted recycled seed, which was obtained by extracting seed from previous crop. Tomato yellow leaf curl disease incidence amongst the varieties sampled was statistically different (P=0.001) and ranged from 0.4 to 59.1%. Recyled seed had the highest disease incidence (59.1%) followed by new fortune maker (36.9%) while onyx F1 had the least (0.4%). Generally, the disease incidence was lower in hybrid varieties compared to non hybrid cultivars (**Table 3.2**). Similarly, there was significance difference between the mean disease severity amongst the varieties (P=0.001). The severity ranged from 0.18 to 3.05. New fortune maker had the highest disease severity (3.05) while Onyx F1 had the least (0.18) (**Table 3.2**).

Table 3. 1 Disease prevalence, incidence and severity across the sampled Counties

County	**No. of fields surveyed**	**Prevalence (%)**	**Mean incidence (%)+SEM (Visual)**	**Mean incidence (%) (ELISA)**	**Mean severity(1-5)+SEM**
Nakuru	55	57.5	22.9^{bc} ±2.50	28.3^{bc} ± 3.70	1.73^{abc} ±0.14
Kirinyaga	60	57.0	23.7^{bc} ±3.20	31.4^{bc} ± 9.30	1.62^{abcd} ±0.15
Meru	19	47.0	18.6^{c} ±3.70	22.6^{c} ± 7.20	1.67^{abcd} ±0.22
Bungoma	41	19.5	0.3^{d} ±0.10	1.7^{e} ± 0.40	0.18^{e} ±0.07
Taita Taveta	39	61.5	16.7^{c} ±3.40	18.5^{c} ±5.80	1.30^{bd} ±0.17
Kajiado	19	58.0	14.8^{c} ±4.40	17.4^{d} ± 3.20	1.37^{bcd} ±0.29
Kwale	11	64.0	38.8^{a} ±10.00	41.4^{a} ± 8.40	1.85^{ab} ±0.38
Baringo	15	60.0	34.2^{ab} ±8.40	33.6^{ab} ± 6.30	2.20^{a} ±0.35
LSD			**11.87**	**13.04**	**0.60**

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

3.4.3 Whitefly populations across the Counties, AEZs and in sampled tomato varieties

Whitefly populations varied across the Counties and amongst the tomato varieties. There was a significance difference (P=0.001) in adult whitefly populations across the Counties, Kwale had the highest number though not statistically different from the population in Kirinyaga and Nakuru. The whitefly population in Kajiado was statistically similar to that of Taita Taveta, Meru and Baringo. Bungoma County had the least number of whitefly population (**Table 3.3**). Based on AEZs, LH5 had high population of whitefly populations with an average of (11.76 ± 1.50) adult whiteflies per plant while LM2 had the least with an average of (0.8 ± 0.35) whiteflies per plant. Whitefly population in UM3, Coastal lowlands 3, 4, 5, UM4, LH4, UM1, LM5 were statistically similar. There was a significant difference (P= 0.001) in adult whitefly populations amongst the tomato varieties sampled (**Figure 3.4**). New fortune maker variety had the highest whitefly population (17.8), followed by recycled seed (16.0) while Onyx had the least (1.24) **(Figure 3.3).** There was a positive correlation between whitefly population and TYLCD incidence in varieties sampled (r =0.70) **(Figure 3.5)**

Figure 3.4 Whitefly populations on tomato leaves of different varieties

3.4.4 Farmers' perceptions on tomato yellow leaf curl disease

Out of the 259 farmers interviewed, 152 (58.7%) farmers recognized tomato yellow leaf curl disease (TYLCD) in the photos provided during the survey and had observed the symptoms in their crops either in the current or in previous crops. Over, 90% of the farmers reported that they did not know the causes on being asked the cause of the disease. They were unable to identify it as a viral infection and the majority (85%) thought it was blight due to the yellowing of leaves. However they pointed out that based on their observations there was reduction in yields on infected plants especially in hot seasons. Most farmers 74.1% believed that the disease symptoms were caused by high temperatures. Other factors attributed to the disease by farmers were dry weather, seed transmitted and insects, only a small number associated the symptoms with whiteflies (**Fig 3.6**).

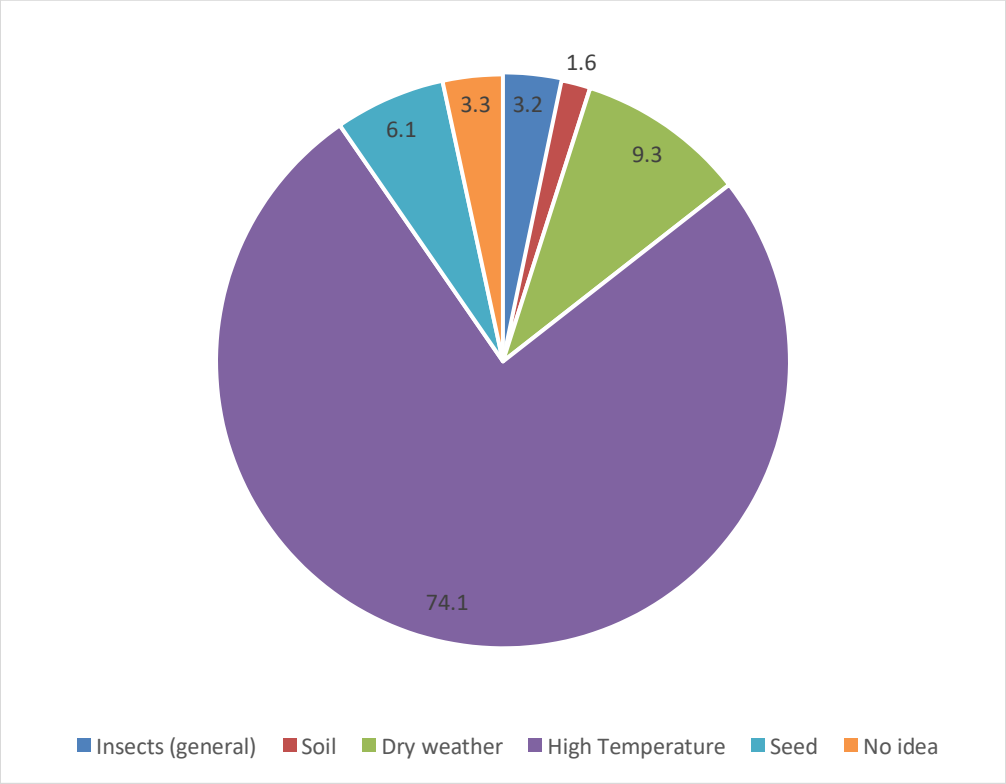


Figure 3. 6 Kenyan farmers' perception on the cause of TYLCD

3.4.5 Type of seed planted

During the farmer interviews the respondents reported the different types of seed they planted. These seed types are classified into hybrids, conventional (non-hybrid) and those that had been recycled from previous harvests. Over half (53.6%) of the farmers planted non hybrid seed while 3.8% and 42.6 % used recycled and hybrid seeds respectively. Overall, majority (96%) of the farmers sampled bought certified seed from local markets and only a minority recycled seed from previous harvests. Riogrande was the most cultivated variety (20.5%) followed by Kilele F1 (8.9%) (**Table 3.2**). The use of non-hybrid seed ranged from 32 to 69% across the Counties with a mean of **53.63** %. Baringo County had the highest percentage number of farmers who planted conventional seed (69%), followed by Kwale (67%) while Bungoma had the least (32%). The use of hybrid seed across the Counties ranged from 22 to 68%. Bungoma had the highest percentage (68%) while Kwale had the least (22%) and the mean average across all the Counties was 42.63% (**Table 3.5**). The percentage of famers who planted recycled seed ranged from 0 to 11%, with an average of 3.75%. Kwale had the highest percentage (11%) while Bungoma and Nakuru had the least.

3.4.6 Land sizes, gender and level of education

Land sizes for tomato production ranged from ≤ 0.125 to more than 1 acre. Majority (76%) of farmers in all the Counties sampled produced tomato on less than a quarter of an acre. Majority farmers interviewed were male (75%). Most farmers (69%) were between 35-50 years old, while most had secondary education (52%).

Table 3. 2 TYLCD incidence and severity observed on the most frequently grown cultivars of tomato in sampled areas.

Variety	No. of farms	Mean TYLCD incidence +SE	Mean TYLCD severity + SE
Recycled	9 (3.5%)	$59.1^{a} \pm 3.40$	$3.04^{ab} \pm 0.11$
New Fortune maker	13 (5.0%)	$36.9^{b} \pm 3.00$	$3.05^{a} \pm 0.15$
Riogrande	53 (20.5%)	$33.7^{bc} \pm 2.60$	$2.08^{acdef} \pm 0.14$
Money maker	14 (5.4%)	$33.1^{bc} \pm 5.90$	$2.15^{acdef} \pm 0.30$
Cal J	15 (5.8%)	$29.4^{bcd} \pm 5.10$	$1.94^{acdef} \pm 0.25$
Tecsim	5 (2%)	$19.7^{bcde} \pm 0.00$	$2.10^{abcde} \pm 0.00$
ATM	11 (4.2%)	$13.8^{bde} \pm 5.30$	$1.70^{acdefg} \pm 0.45$
Nyati F1	7 (2.7%)	$13.7^{bde} \pm 10.50$	$1.47^{cdefgh} \pm 0.22$
Safari	10 (3.9%)	$12.5^{ef} \pm 3.40$	$0.99^{ceghij} \pm 0.20$
DRD	11 (4.2%)	$10.5^{bdefg} \pm 7.30$	$1.50^{acdefgh} \pm 0.30$
Star F1	9 (3.5%)	$9.3^{bcdefg} \pm 0.00$	$2.30^{abc} \pm 0.00$
Big Rock F1	12 (4.6%)	$5.7^{efg} \pm 2.90$	$1.03^{ceghi} \pm 0.22$
Pesa F1	7 (2.7%)	$5.0^{efg} \pm 2.80$	$1.00^{ceghij} \pm 0.31$
Eden F1	12 (4.6%)	$4.1^{efg} \pm 1.60$	$0.84^{ceghijkl} \pm 0.27$
Kilele F1	23 (8.9%)	$3.5^{eg} \pm 0.80$	$0.71^{cehijkl} \pm 0.10$
Shanti F1	8 (3.1%)	$3.4^{efg} \pm 2.10$	$0.54^{ehijklm} \pm 0.14$
Assila F1	13 (5.0)%	$1.6^{eg} \pm 0.60$	$0.53^{ehijklm} \pm 0.19$
Rambo F1	11(4.2%)	$0.7^{eg} \pm 0.70$	$0.37^{ehijklm} \pm 0.37$
Onyx F1	16 (6.2%)	$0.4^{eg} \pm 0.20$	$0.18^{ikm} \pm 0.10$
LSD		**19.27**	**1.11**

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

Table 3. 3 Mean whitefly population across the Counties

County	Mean +SEM
Kwale	10.39^{a} ±1.43
Kirinyaga	10.26^{a}±1.06
Nakuru	9.98^{a} ±0.95
Baringo	9.18^{ab}±2.15
Meru	8.93^{abc}±1.54
Taveta	8.54^{abc}±0.87
Kajiado	4.83^{b} ±1.01
Bungoma	1.11^{d} ±0.35
LSD	**3.71**

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

Table 3. 4 Whitefly population, TYLCD incidence and severity across agro ecological zones

AEZ	Mean whitefly population	Mean TYLCD incidence	Mean TYLCD severity
LH5	11.76^{a} ± 1.50	24.23^{abc} ±3.99	1.89^{ab} ± 0.24
UM3	11.08^{ab} ±2.08	25.01^{abc} ± 4.75	1.82^{abcde} ± 0.24
Coastal	10.39^{ab} ±1.43	38.75^{a} ± 10.02	1.85^{abc} ± 0.38
UM4	9.81^{ab} ±1.14	23.88^{bc} ± 3.133	1.74^{abcde} ± 0.16
LH4	9.79^{ab} ±1.80	26.65^{abc} ± 5.85	1.83^{abcd} ± 0.36
UM1	9.18^{ab} ±2.15	34.22^{ab} ± 8.44	2.2^{a} ± 0.35
LM5	8.54^{ab} ±0.87	16.69^{c} ± 3.41	1.30^{cdef} ± 0.17
LM3	8.32^{b} ±1.10	20.91^{c} ± 3.31	1.5^{bcdef} ± 0.16
LM4	6.07^{abc} ±1.63	6.35^{cd} ± 4.45	0.77^{dgf} ± 0.43
UM2	5.98^{bcd} ±1.98	9.74^{cd} ± 4.52	1.46^{abcdef} ± 0.43
LM1	1.7^{cde} ± 0.76	0.38^{d} ± 0.26	0.21^{g} ±0.15
LM2	0.8^{ce} ± 0.35	0.31^{d} ± 0.16	0.17^{g} ±0.08
LSD	**4.82**	**15.27**	**0.77**

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

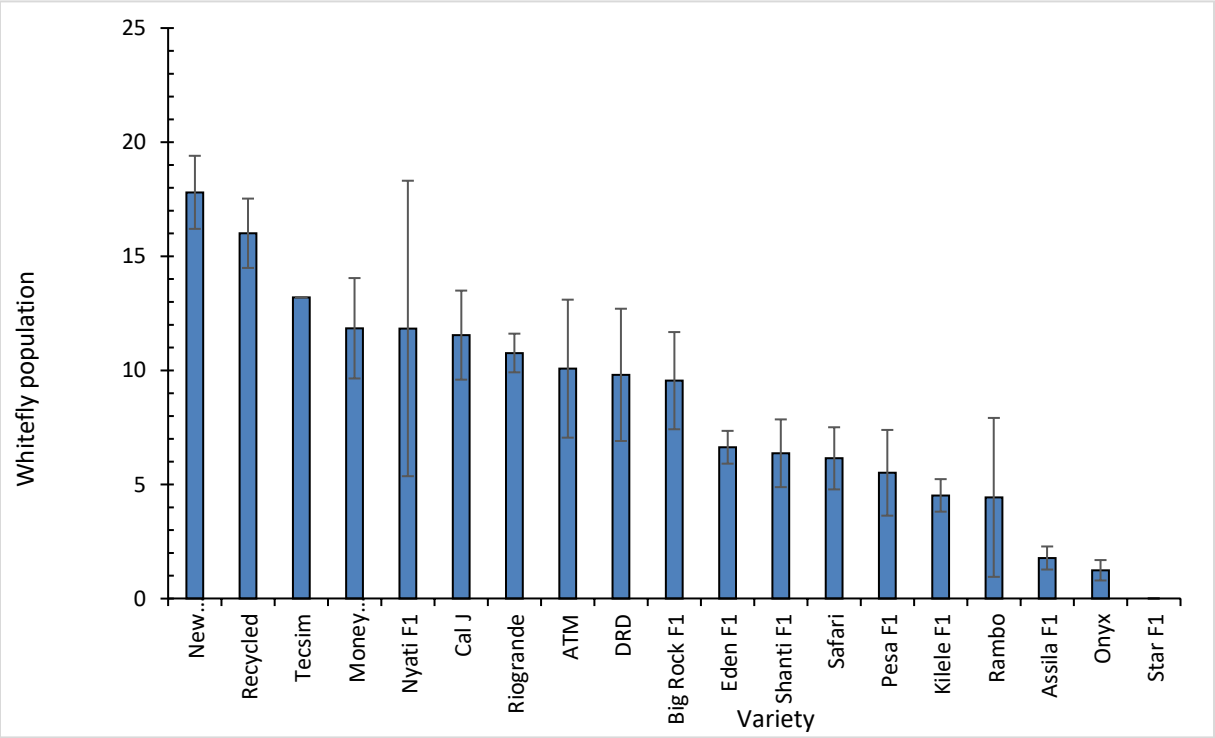


Figure 3. 3 Whitefly populations in tomato varieties sampled during the survey.

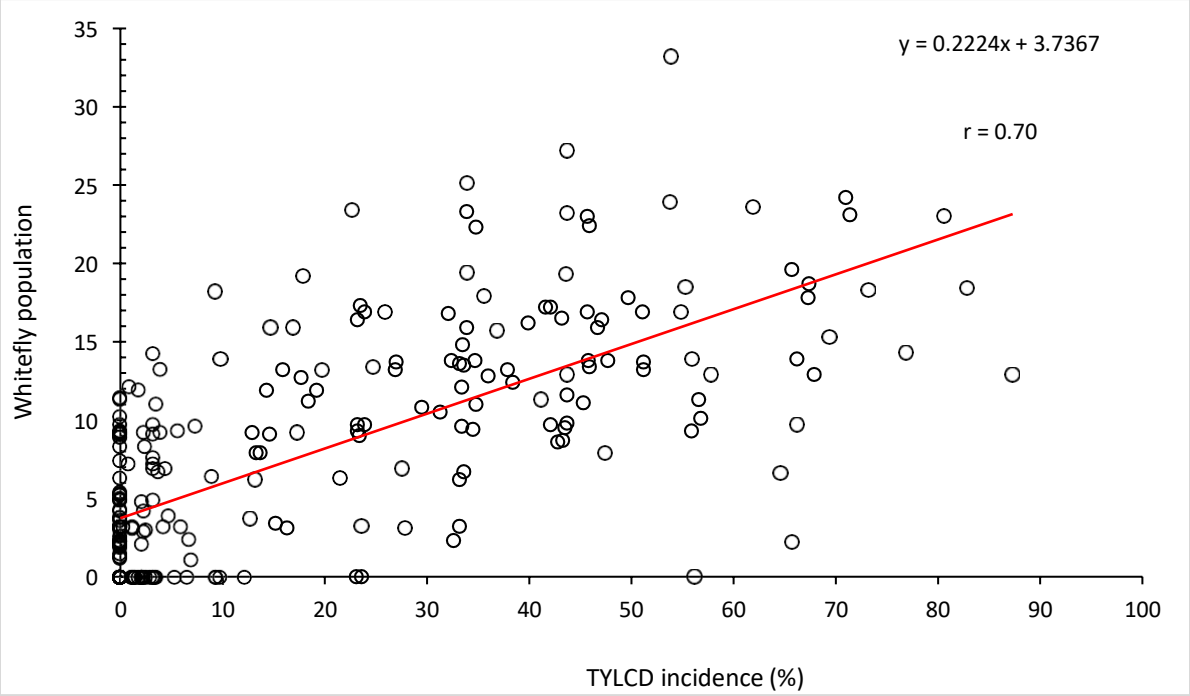


Figure 3. 5 Correlation between whitefly population and TYLCD incidence in sampled tomato varieties

Table 3. 5 Type of seed planted across the surveyed Counties

County	No. of fields surveyed	Prevalence (%)	Mean no. of farmers using hybrid seed (%)	Mean no. of farmers using Conventional seed (%)	Mean no. of farmers using Recycled seed (%)
Nakuru	55	57.5	53	47	0
Kirinyaga	60	57	57	37	6
Meru	19	47	30	69	1
Bungoma	41	19.5	68	32	0
Taita Taveta	39	61.5	43	52	5
Kajiado	19	58	41	56	3
Kwale	11	64	22	67	11
Baringo	15	60	27	69	4
			42.63	**53.63**	**3.75**

3.4.7 Control measures used by farmers in management of TYLCD

Chemical use was the most prevalent pest management practice. It was observed that farmers used multiple pest management methods in managing the disease. The mean across the Counties of farmers who applied insecticides was 76.1%, while 51.9% used fungicides. Application of insecticides was done to target other insect pests such as *Tuta absoluta* alongside whiteflies. Application was done after establishment of insect populations on the crops. The highest number of farmers who applied chemicals to manage TYLCD symptoms was found in Kajiado County with 92% applying insecticides whilst 73% applied fungicides. Bungoma County had the lowest percentage of farmers who applied insecticides (44%) whereas Kwale had the least number of farmers who applied fungicides in the management of TYLCD symptoms. All farmers in the eight Counties practiced crop rotation. Different crops were used as rotational crops across the Counties. Only 0.63% of the farmers didn't apply any control measures. Use of TYLCD resistant varieties was not common, in most Counties except in Kajiado, Kirinyaga and Taita Taveta.

Though, most of the farmers interviewed had several years of growing the crop and considered it as a profitable horticultural crop, they lacked knowledge on the causal agent of the TYLCD and availability of resitant varieties. Rogueing of infected plants was carried out by a few farmers in three Counties i.e Kirinyaga, Taita Taveta and Kajiado at (2%, 1% and 3%) respectively.

3.5 Discussion

During the survey TYLCD symptoms were observed on almost all tomato varieties planted. Viruses induce almost similar symptoms such as stunting, mottling, chlorosis, reduced leaf size. Therefore the use of visual assessment of disease symptoms is not be an accurate means of identifying the causal agent hence need for laboratory diagnosis. This explains why visually assessed incidence levels were consistently lower than that those based on serological assays since some of the symptoms observed may have been associated with other viral diseases other than TYLCD.

The main variety grown in all the Counties was Riogrande, while Kilele F1 was the second most preferred variety, this corroborates previous findings by Macharia *et al*. (2015). This could be attributed to the good agronomic and market qualities like longer storage life of these varieties. However, tomato varieties respond differently to TYLCV infection, with varieties being either susceptible, resistant or tolerant (Yan *et al*., 2018). The high disease incidence in recycled seeds could be attributed to a decline in physical, physiological and health quality of the seeds (Ochichi *et al*., 2018). However, only few farmers were found to use recycled seed and these were mostly resource poor farmers. Through breeding TYLCD resistant varieties have been developed and are available in Kenya, their cost is high hence not affordable by majority of small scale farmers. Nonetheless, some of the varieties bred for TYLCV resistant e.g. Nyati F1, Big rock F1, Assila F1 were found to be infested with TYLCD with diseases severities of 1.47, 1.03, 0.53

respectively. Therefore, there is need to test tomato varieties under TYLCD pressure to assess their levels of resistant/susceptibility. Ochilo *et al*. (2019) observed that varietal characteristics, cost of seed and uses influenced the choice of varieties planted. Most farmers opted for less costly seed and for determinate varieties with good processing qualities. This is contrary to findings in Karnataka in Southern India where 50% of farmers used hybrid tomato seed, mainly due to their high yielding ability and disease resistance (Nagaraju *et al*., 2002).

TYLCD was found in all agroecological zones and all Counties surveyed. The disease incidence and severity varied amongst the agroecological zones and Counties. The disease levels also varied from one farm to the other even in the same agroecological zone and County. This could be due to factors such as type of tomato variety cultivated, cropping system and pest management options applied. Kwale County had the highest disease prevalence, incidence and whitefly populations. This is mainly due to presence of sweet potato and cassava crops that are hosts of whiteflies. However, there is need for further research to establish the role of these host crops in the TYLCD epidemiology. Most of the farmers in Kwale were small scale farmers who did not consider tomato as a commercial crop in the region.

The presence of tomato yellow leaf curl disease in all the other Counties surveyed could be linked to the intensive horticultural farming in most of the Counties like Kirinyaga, Taita Taveta, Meru, Nakuru, Baringo and Kajiado. Other than tomato, these Counties engage in intensive horticultural production of crops such as Capsicum spp, egg plants, sweet potatoes, beans, cucurbits which are hosts of whiteflies. Egglants, capsicum and beans are alternative hosts of TYLCD (Marie *et al*., 2012). Crop rotation is also practiced using non solanaceous crops. However, due to the small land sizes, farmers attempt to avoid risks by growing several crops, with some of the crops being alternative hosts to TYLCD or its vector. Moreover, the small land

sizes limited long crop rotations (Mwangi *et al.*, 2015). Studies done by Macharia *et al*. (2015) indicate that tomato production in areas surveyed relied on irrigation, this allows for production of the crop throughout the year. This may explain the high prevalence of TYLCD in all Counties surveyed except in Bungoma where tomato production is dependent maily on rain. In studies done by Bob *et al*. (2005), Bungoma had zero prevalence of TYLCD, despite the presence of high incidences of *Bemisia tabaci.* Similarly, Macharia *et al*. (2015) reported no incidence of *Tomato spotted wilt virus* in tomato crops in this County. The low disease prevalence of TYLCD in Bungoma County may be attributed to the fact that tomato crop is a secondary crop after maize and it's grown only during the short rains season. Moreover, Bungoma has less horticultural activities as compared to other tomato growing regions sampled.

The variation in population of whiteflies across the counties and amongst tomato varieties may be attributed to type of tomato varieties planted as well as the complexity of the tomato agroecosystem. Whitefly populations on plants are influenced by antibiosis mechanism where some host plants are preffered over others as further discussed in Chapter 6. Different Counties sampled have different climatic conditions with variations in temperature, the effect of these varitions on whitefly populations on tomato crops should be evaluated. There was a positive correlation between whitefly population and TYLCD incidence and severity. These findings corroborate previous studies by Mansour *et al*. (1992) and Mehta *et al*. (1994) who reported that TYLCD incidence was directly propotional to whitefly populations. Interestingly presence of *Bemisia tabaci* was not detected in whitefly samples collected from tomato plants and analysed in chapter 5. The complex agroecosystem with rich biodiversity in tomato fields should be evaluated to establish its role in TYLCD transmission. This includes evaluating alternative hosts of TYLCD and *B.tabaci*. Transmission of TYLCD from weeds such as, *Physalis floridana*,

Euphorbia heterophylla and *Desmodium* spp has been reported (Nono-Womdim *et al*., 1996). Moreover, crops such as beans, sweet potato and cassava are found within tomato agroecosystems in Kenya and also offer breeding grounds for whiteflies including *B.tabaci.* Therefore, their role in TYLCD transmission should be determined.

The data on farmer perceptions and management options show that most farmers had limited knowledge of TYLCD and as a result no conscious management practices were applied. A small percentage of farmers who had knowledge of the disease were able to correlate it with white flies, while a majority associated the disease to high temperatures. These results are in agreement with previous studies done by Bob *et al*. (2005), when he carried out a survey on TYLCD in Kenya. Similarly Macharia *et al*. (2015) noted that there was scarce knowledge on TSWV and its vectors amongst tomato farmers in Kenya. In Tanzania studies done by Gibson *et al*. (2000) established that there was little knowledge on sweet potato virus disease in sweet potato crops in Lake Victoria region. Similar results were reported by Nagaraju *et al*. (2002) after interviewing tomato farmers in five districts in Karnataka in India on the causes of tomato yellow leaf curl disease.

Lack of this knowledge hinder application of appropriate management options and thus most farmers use chemicals. Mwangi *et al*. (2015) observed that farmers lacked an understanding on application of chemicals and their target pests and as such there was application of wrong chemicals for target pest. In the current study some farmers applied fungicide against TYLCD symptoms, as they thought the disease was blight based on the chlorosis observed. The use of synthetic chemicals is not sustainable in pest management due to development of pest resistance to the active chemical ingredient (Lietti *et al*., 2005; Roditakis *et al*., 2005; Silva *et al*., 2011). The lack of knowledge on TYLCD by most farmers limited timely application of pesticides to

manage the vectors and rouging of infected plants. Scouting and monitoring of vector populations before application of insecticides is important (Schreinemachers *et al.,* 2015) yet very few farmers practiced it. Most farmers in the surveyed areas were literate, thus with proper sensitization they are able to adopt effective integrated management strategies in the management of viruses and their vectors.

CHAPTER FOUR

GENETIC DIVERSITY OF TOMATO YELLOW LEAF CURL VIRUS IDENTIFIED IN MAJOR TOMATO GROWING AREAS IN KENYA

4.1 Abstract

Tomato production is threatened worldwide by the occurrence of begomoviruses which are associated with tomato leaf curl diseases. The genus Begomovirus is the largest and the most economically significant member of *Geminiviridae* family. Begomoviruses are transmitted by *Bemisia tabaci* in a persistent cirulative manner. In Kenya several begomoviruses have been reported in many crops, however little research has been conducted on tomato crops. This study aimed to understand the genetic diversity of viruses associated with tomato leaf curl disease in tomato plants in Kenya. A field survey was carried out in eight major tomato growing regions in Kenya between September and December 2018 and January to March 2019. A total of 259 fields were surveyed and 544 leaves exhibiting leaf curl like symptoms were collected. Samples from each field were pooled prior to genomic DNA extraction. Extraction was done using CTAB (Cetyl trimethylammonium bromide) method. The quality and quantity of the genomic DNA was done using qubit and a nanodrop respectively. Genomic DNA of 48 samples randomly picked from the regions was used in library preparation. DNA libraries were prepared using Nextera DNA library preparation kit at Biosciences Eastern and Central Africa International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya. Illumina sequencing of the DNA libraries was done at the same facility. Quality control of the resultant reads and their assembly into contiguous sequences was done. Twelve complete begomovirus genomes were obtained from our samples with an average coverage of 99.9%. The sequences showed 95.7–99.7% identity among each other and 95.9–98.9% similarities with a *Tomato leaf curl virus Arusha virus* (ToLCArV) isolate from Tanzania. Analysis of amino acid sequences showed the highest identities in the regions coding for the coat protein gene (98.5–100%) within the isolates, and 97.1–100% identity with the C4 gene of ToLCArV. Phylogenetic algorithms clustered all Kenyan isolates in the same clades with ToLCArV. There was no evidence of recombination within the isolates. Estimation of selection pressure within the virus population revealed the occurrence of negative or purifying selection in five out of the six coding regions of the sequences. The begomovirus associated with tomato leaf curl diseases of tomato in Kenya is a variant of ToLCArV, possibly originating from Tanzania and there is low genetic diversity within the virus population.This information is useful in the development of appropriate management strategies for the disease in the country.

4.2 Introduction

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

The genus *Begomovirus* is the largest and the most economically significant member of Geminiviridae family. It includes viruses that are transmitted by the whitefly, *Bemisia tabaci,* (Genn.) biotype B (MEAM 1) and infect only dicotyledonous plants (Zehra *et al*., 2017). Symptoms include yellowing of upper leaves, excessive branching, reduced leaf sizes, stunting and upward curling of leaf margins. 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). The DNA-A component of begomoviruses contains five or six open reading frames (ORFs) that encode ~10 kDa proteins (Yadava *et al*., 2010). The ORFs are; AC1, AC2, AC3, AC4, AV1 and AV2. These proteins play various roles in virus assembly, virus

replication, host gene regulation, silencing suppression and vector transmission (Seal *et al*., 2006). Like most plant viruses, begomoviruses evolve rapidly through recurrent mutations and recombination events, leading to the emergence of novel pathotypes that exploit new environments and challenge host resistance. Natural occurrences of recombinants are known to lead to genetic diversification of viruses with new hosts and properties (Padidam *et al*., 1999; Lefeuvre *et al*., 2015).

Several approaches are available for begomovirus identification, ranging from serological techniques to deep sequencing approaches (Idris *et al*., 2014). Since begomoviruses species and strains cause diseases with similar symptoms in tomato, the use of serological assays has limitations as antibodies are able to cross-react with closely-related viruses or virus strains, thus making strain identification difficult. Recent advances in sequencing technologies have provided better approaches for identification and characterization of plant viruses in Kenya (Wamonje *et al*., 2017; Mutuku *et al*., 2018; Wamaitha *et al*., 2018; Wainaina *et al*., 2019). Metagenomics is the analysis of microbial and virus populations in environmental samples through nucleic acid sequencing methods (Roossinck *et al*., 2015). Motivations for performing plant virus metagenomics include the identification of causal organisms associated with virus diseases in crops, screening for specific viruses when their presence is suspected, detection of asymptomatic or cryptic viruses, and the discovery of novel viruses and other microorganisms (Mutuku *et al*., 2018). In this study we characterized tomato leaf curl viruses from tomato plants in Kenya using both conventional and deep sequencing.

4.3 Materials and methods

4.3.1 Field surveys and Sampling

Field surveys and sampling were carried out between September and December 2018 and January to March 2019 in eight Counties namely, Taita Taveta, Kajiado, Kirinyaga, Meru, Kwale, Bungoma, Nakuru, Baringo that majorly grow tomato in Kenya. Tomato farms were randomly selected based on crop availability, with 30 plants randomly assessed per farm. From each farm, young, trifoliate leaf samples (n=5) were obtained from plants showing symptoms such as chlorosis, reduced leaf size, upward leaf curling, stunting and flower abscission. A total of 544 symptomatic leaf samples were obtained from 259 farms, carried in paper bags with silica gels and stored till further analysis. DNA extraction was done on pooled samples from each farm.

4.3.2 DNA extraction from leaf samples

Extraction of total genomic DNA from the pooled sampled collected from all the surveyed sites was done using 2% Cetylmethylammonium and 0.2 % (v/v) β –mercaptoethanol as well as 50 mg PVP (Poly vinyl pyrrolidine) added prior to use according to the protocol by Pratap *et al.*, (2011). 100mg of leaf samples were homogenized with preheated extraction buffer (0.1M Tris HCL, 0.2 M EDTA, 1.4M NaCl, 25 CTAB, 1% PVP, β –mercaptoethanol). The homogenized sample was transferred into a 1.5ml micro centrifuge tube and incubated at 65ºC for 30 minutes while mixing at 10 min interval. The tube was centrifuged at 10,000 rpm for 5sec, 750µl of supernatant was then transferred into a new tube and an equal volume of chloroform; Isoamyl alcohol (24:1) added, mixing was done by vortexing and later centrifuged at 10,000 rpm for 15 min. The aqueous layer was transferred into a new tube and 300µl of ice cold isopropanol added and mixed by inverting the tube slowly. Overnight incubation at -20 ºC was done, before the

DNA was pelleted at 10,000 rpm for 15 minutes. The supernatant was discarded and the pellets washed with 500 µL of 70% (v/v) ethanol by vortexing followed by centrifuging at 10000 rpm for 5 minutes. The DNA template was dried at room temperature and dissolved in 100 µL of TE (Tris-EDTA) buffer (10mMTris-Hcl, PH 8.0 and 1mM EDTA PH 8.0) and incubated at 37 ºC for 30 minutes and stored at -20 ºC. A nanodrop 2000 (Thermo Fisher Scientific, MA, USA) was used to determine the quality and quantity of the DNA (Pratap *et al.*, 2011).

4.3.3 Library preparation and sequencing

Genomic DNA of 48 samples randomly picked from the regions was used in library preparation, this was based on the available library preparation kit available which was for 48 samples. The genomic DNA were quantified using a Qubit™ fluorometer (Thermo Fisher Scientific, MA, USA) and normalized to 2.5 ng/µl and used for library preparation. Libraries were prepared using Nextera DNA library preparation kit (Illumina, CA, USA) according to the manufacturer's instructions. Briefly, enzymatic fragmentation was carried out on normalized genomic DNA samples (20 µl) via addition of TD buffer (25 µl) and TDE (5 µl). Mixtures were centrifuged (Hettich Centrifugen, D–78532, Germany) at 14,000 rpm at 20 ºC for 1 min and transferred into microtubes. Tagmentation was carried out in a pre-programmed thermocycler at 55 ºC lid and 55 ºC incubation temperature, while holding at 10 ºC. The tagmented DNA was barcoded using indexed adapters then cleaned with AMPure XP magnetic beads (Beckman Coulter, Inc. Indianapolis, IN) to remove shorter DNA fragments and other impurities. Library quality was confirmed with the Agilent Tape Station 2200 System (Agilent Technologies, Santa Clara, CA). All the 48 libraries were quantified using the Qubit™ fluorometer (Thermo Fisher Scientific Inc., Waltham, MA). The indexed DNA libraries of 48 biological samples (**Figure 4.1**) were each normalized to a concentration of 4 nm before being pooled. High-throughput sequencing was

performed on an Illumina MiSeq System using 2 × 251 v2 kit and 12 pM of 1% PhiX v3 spike to create paired-end reads. Sequencing was performed at the facility of the Biosciences Eastern and Central Africa International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya

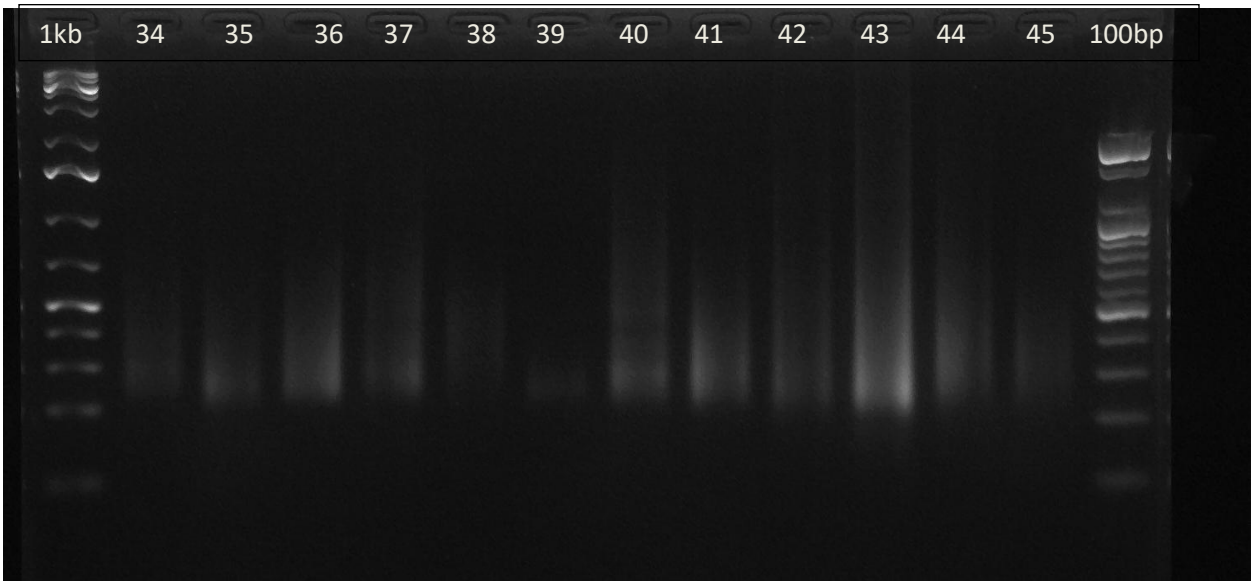


Figure 4. 1 Gel electrophoresis photo of DNA libraries prepared using Nextera DNA kit

4.3.4 Sequence processing and assembly

After sequencing, quality control of fastq paired end reads was performed using FastP v.0.20.0 (Chen *et al*., 2018) to remove adapters, poly-N sequences (≥15%) and filter off low quality reads. High-quality reads were then mapped to the tomato genome (GenBank RefSeq accession number GCA_000188115.3) using Bowtie v.2.3.4.3 (Langmead and Steven 2012) under default parameters. Unmapped reads were assembled into contigs *de novo* using MEGAHIT v.1.1.3 (Li *et al*., 2015) with default settings and those representing ssDNA sequences were verified using Kaiju virus database (Menzel *et al*., 2016). The sequences were then subjected to BLASTN 2.9.0+ (Zhang *et al*., 2000) to determine similarity match and virus identification. Protein prediction of ORFs was determined using ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf).

4.3.5 Sequence validation through Polymerase chain reaction and Sanger sequencing

The assembled begomovirus genomes were validated using a polymerase chain reaction (PCR) step followed by Sanger sequencing of the amplified products. The Illumina assembled virus sequences were aligned together using ClustalW multiple sequence alignment program with default parameters as implemented in BioEdit v.7.2.3 (Hall, 1999). A consensus sequence was obtained and used to design PCR primers ToLCV-Forward (5'-ATTGGCGATTTCCCAGGTATAG-3') and ToLCV-Reverse (5'-ACAATGTGGGCTAGGTCATTAG-3') using the Primer Express v3.0 software (Applied Biosystems, USA). Secondary structures, complementarity and dimer effects of the primers were also checked using the multiple primer analyzer software (Thermo Fisher Scientific, MA, USA). Using PCR, these were tested on the genomic DNA from which the complete begomovirus genomes had been obtained via Illumina sequencing. The PCR product were ethanol-purified and quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA) to determine purity levels. The amplicons were visualized using gel electrophoresis. Sequencing was done at Macrogen Europe (Netherlands). The sequences were manually assembled using BioEdit and consensus sequences verified using BLASTN 2.9.0+ and comparisons made with the complete begomovirus sequences assembled from Illumina reads.

4.3.6 Sequence alignment, distance matrix and evidence of recombination

Complete sequences of monopartite begomoviruses found in tomato were retrieved from GenBank (**Table 4.1**) and aligned with full virus contigs using ClustalW in BioEdit. Deduced amino acids from the ToLCV genomes were compared with GenBank isolates while sequence pairwise identities were performed using SDT v1.2 (Muhire *et al*., 2014) with pairwise gap deletions. A scan for recombination signatures were performed on each protein-coding sequence

data using the single breakpoint scanning (SBP) and genetic algorithm recombination detection (GARD) methods (Kasakovsky *et al*., 2006). These two methods were implemented by the Datamonkey software (Weaver *et al*., 2018). Potential recombination events were further investigated using the default settings of the seven detection algorithms within RDP v 4.13 (Martin *et al*., 2015). Putative recombination events, potential recombinants, and their parental sequences were deemed acceptable only when signals were identified by at least four detection methods, with strong levels of significance ($P \leq 0.05$).

Table 4. 1 List of begomovirus isolates used across all analysis in this study

Virus name	Acronym	GenBank Accession No	Original Host	Year of collection	Isolate and Country of origin	Complete genome size (nt)	Reference
Tomato leaf curl virus	ToLCV	MN894493	*Solanum lycopersicum*	2018	Tom5a:Kenya	2761	This study
Tomato leaf curl virus	ToLCV	MN894494	*Solanum lycopersicum*	2018	Tom5b:Kenya	2765	This study
Tomato leaf curl virus	ToLCV	MN894495	*Solanum lycopersicum*	2018	Tom46:Kenya	2763	This study
Tomato leaf curl virus	ToLCV	MN894496	*Solanum lycopersicum*	2018	Tom13:Kenya	2762	This study
Tomato leaf curl virus	ToLCV	MN894497	*Solanum lycopersicum*	2018	Tom14:Kenya	2760	This study
Tomato leaf curl virus	ToLCV	MN894498	*Solanum lycopersicum*	2018	Tom45:Kenya	2763	This study
Tomato leaf curl virus	ToLCV	MN894499	*Solanum lycopersicum*	2018	Tom39:Kenya	2762	This study
Tomato leaf curl virus	ToLCV	MN894500	*Solanum lycopersicum*	2018	Tom27:Kenya	2762	This study
Tomato leaf curl virus	ToLCV	MN894501	*Solanum lycopersicum*	2018	Tom35:Kenya	2762	This study
Tomato leaf curl virus	ToLCV	MN894502	*Solanum lycopersicum*	2018	Tom28:Kenya	2763	This study
Tomato leaf curl virus	ToLCV	MN894503	*Solanum lycopersicum*	2018	Tom37:Kenya	2762	This study
Tomato leaf curl virus	ToLCV	MN894504	*Solanum lycopersicum*	2018	Tom22:Kenya	2761	This study
Tomato leaf curl virus Arusha virus	ToLCArV	EF194760	*Solanum lycopersicum*	2006	AFTT23:Tanzania	2762	Shih *et al.*, 2006
Tomato leaf curl Cameroon virus	ToLCCMV	FM210278	*Solanum lycopersicum*	2008	TOS2B1F4:Cameroon	2808	Leke *et al.*, 2011

Table 4. 1 Cont'

Virus name	Acronym	GenBank Accession No	Original Host	Year of collection	Isolate and Country of origin	Complete genome size (nt)	Reference
Tomato yellow leaf curl virus	TYLCV-Mld	EF185318	*Solanum lycopersicum*	2006	LBa4:Lebanon	2790	Anfoka *et al.*, 2008
Tomato yellow leaf curl virus	TYLCV	AJ489258	*Capsicum annuum*	2002	Almeria, Spain	2781	Morilla *et al.*, 2005
Tomato yellow leaf curl virus	TYLCV-Mld	AF105975	*Solanum lycopersicum*	1995	pPort2:Portugal	2793	Navas-Castillo *et al.*, 2000
Tomato yellow leaf curl virus	TYLCV-Mld	AF071228	*Solanum lycopersicum*	1997	Sp7297:Spain	2791	Navas-Castillo *et al.*, 2000
Tomato yellow leaf curl virus	TYLCV	AB110218	*Solanum lycopersicum*	1996	Sz:Japan	2791	Kato *et al.*, 1998
Tomato yellow leaf curl virus	TYLCV-Mld	AB116632	*Solanum lycopersicum*	2002	SzY:Japan	2791	Ueda *et al.*, 2004
Tomato yellow leaf curl virus	TYLCV	KF229723	*Solanum lycopersicum*	2012	Tom46:Oman	2791	Khan *et al,* 2014
Tomato yellow leaf curl virus	TYLCV	AJ223505	-	1997	Cuba	2781	Bejarano, 1998
Tomato yellow leaf curl virus	TYLCV	AF024715	*Solanum lycopersicum*	1994	Dominican Republic	2781	Salati *et al.*, 2002
Tomato yellow leaf curl virus	TYLCV	AY134494	*Solanum lycopersicum*	2001	Puerto Rico	2781	Bird *et al.*, 2001

Table 4. 1 Cont'

Virus name	Acronym	GenBank Accession No	Original Host	Year of collection	Isolate and Country of origin	Complete genome size (nt)	Reference
Tomato yellow leaf curl virus	TYLCV	DQ631892	*Solanum lycopersicum*	2005	Culiacan, Mexico	2781	Brown and Idris, 2006
Tomato yellow leaf curl virus	TYLCV	EF060196	*Solanum lycopersicum*	2002	Moroccan	2781	Boukhatem *et al*., 2008
Tomato yellow leaf curl virus	TYLCV	EF523478	*Solanum lycopersicum*	2006	Sinaloa, Mexico	2781	Kon *et al*., 2007
Tomato yellow leaf curl virus	TYLCV	EF539831	*Solanum lycopersicum*	2007	California, USA	2781	Rojas *et al*., 2007
Tomato yellow leaf curl virus	TYLCV	GU325633	*Solanum lycopersicum*	2009	Jeju, South Korea	2781	Kim *et al*., 2011
Tomato yellow leaf curl virus	TYLCV	GU983859	*Solanum lycopersicum*	2009	Beijing3,China	2781	Li *et al*., 2010
Tomato yellow leaf curl virus	TYLCVIL	GU076440	*Solanum lycopersicum*	2006	Ta30:06:Taft, Iran	2781	Lefeuvre *et al*., 2010
Tomato yellow leaf curl virus	TYLCV	HM459851	*Capsicum annuum*	2008	Baja-California, USA	2781	Cardenas-Conejo *et al*., 2010
Tomato yellow leaf curl virus	TYLCV	JX856172	*Nicotiana tabacum*	2012	SDTA, China	2781	Zhu *et al*., 2012
Tomato yellow leaf curl Malaga virus	TYLCMaV	AF271234	*Phaseolus vulgaris*	1999	ES42199: Malaga, Spain	2782	Monci *et al*., 2002

Table 4. 1 Cont'

Virus name	Acronym	GenBank Accession No	Original Host	Year of collection	Isolate and Country of origin	Complete genome size (nt)	Reference
Tomato yellow leaf curl virus	TYLCV-Mld	X76319	*Solanum lycopersicum*	1969	Israel	2790	Antignus and Cohen, 1994
Tomato yellow leaf curl Mali virus	TYLCMLV/ML	AY502934	*Solanum lycopersicum*	2003	Mali	2794	Zhou *et al*., 2008
Tomato yellow leaf curl Anjouan virus	ToLCAnV	AM701758	*Solanum lycopersicum*	2004	Comoros	2781	Lefeuvre *et al*., 2007
Tomato leaf curl Bangalore virus	ToLCBaV/A	Z48182	*Solanum lycopersicum*	1988	Bangalore, India	2749	Hong and Harrison, 1995
Tomato yellow leaf curl virus	ToLCBV	AF188481	*Solanum lycopersicum*	1998	Bangladesh	2761	Shih *et al.*, 1998
Tomato leaf curl Mayotte virus	ToLCKMV	AJ865340	*Solanum lycopersicum*	2001	Kahani:Mayotte	2768	Delatte *et al*., 2005
Tomato leaf curl Diana virus	ToLCDiV	AM701765	*Solanum lycopersicum*	2001	Namakely, Madagascar	2745	Lefeuvre *et al*., 2007
Tomato leaf curl Ghana virus	ToLCGV	EU350585	*Solanum lycopersicum*	2008	FGH53:Ghana	2803	Osei *et al*., 2008

Table 4. 1 Cont'

Virus name	Acronym	GenBank Accession No	Original Host	Year of collection	Isolate and Country of origin	Complete genome size (nt)	Reference
Tomato leaf curl Hanoi virus	ToLCHaV	HQ162270	*Solanum lycopersicum*	2010	HanoiVietnam	2740	Ha et al., 2011
Tomato leaf curl Iran virus	ToLCIRV	AY297924	*Solanum lycopersicum*	2003	Iran	2763	Behjatnia et al., 2004
Tomato leaf curl Java virus	ToLCJaV/A	AB100304	*Solanum lycopersicum*	2002	pToX1:Java, Indonesia	2752	Kon et al., 2006
Tomato leaf curl Kerala virus	ToLCV-K3	EU910141	*Solanum lycopersicum*	2008	K3:Kerala, India	2767	Pandey et al., 2010
Tomato leaf curl Laos virus	TLCV-LA	AF195782	*Solanum lycopersicum*	1998	Laos	2748	Tsai et al., 1999
Tomato leaf curl Madagascar virus-[Morondova]	ToLCMGV-[Mor]	AJ865338	*Solanum lycopersicum*	2001	Morondova, Madagascar	2777	Delatte et al., 2005
Tomato leaf curl Mindanao virus	ToLCMiV	EU487046	*Solanum lycopersicum*	2005	P162:Mindanao, Philippines	2761	Tsai et al., 2011
Tomato leaf curl Moheli virus	ToLCMohV	AM701763	*Solanum lycopersicum*	2005	Fomboni, Comoros	2756	Lefeuvre et al., 2007
Tomato leaf curl Namakely virus	ToLCNaV	AM701764	*Solanum lycopersicum*	2001	Namakely, Madagascar	2769	Lefeuvre et al., 2007
Tomato leaf curl New Delhi virus 2	ToLCNDC2	JQ897969	*Solanum lycopersicum*	2011	IANDS1:India	2735	Chaudhary et al., 2012

Table 4. 1 Cont'

Virus name	Acronym	GenBank Accession No	Original Host	Year of collection	Isolate and Country of origin	Complete genome size (nt)	Reference
Tomato leaf curl Joydebpur virus	ToLCJV	HM991146	*Capsicum annuum*	2010	Varasani:India	2798	Sinha *et al*., 2010
Tomato leaf curl Karnataka virus 2	ToLCKV2	KF551578	*Solanum lycopersicum*	2010	TC289:India	2772	Swarnalatha *et al*., 2013
Tomato leaf curl Karnataka virus 3	ToLCKV3	KF551585	*Solanum lycopersicum*	2009	TC235:India	2757	Swarnalatha *et al*., 2013
Tomato leaf curl Nigeria virus	ToLCNGV	FJ685621	*Solanum lycopersicum*	2006	Nigeria	2784	Kon and Gilbertson, 2012
Tomato leaf curl Palampur virus	ToLCPalV	AM884015	*Solanum lycopersicum*	2006	Palampur-India	2756	Kumar *et al*., 2008
Tomato leaf curl Patna virus	ToLCPatV	EU862323	*Solanum lycopersicum*	2007	Patna-India	2752	Kumari *et al*., 2009
Tomato leaf curl purple vein virus	ToLCPVV	KY196216	*Solanum lycopersicum*	2015	BR-793-15_cloneP793:Brazil	2629	Macedo *et al*., 2016
Tomato leaf curl Rajasthan virus	ToLCRaV	DQ339117	*Solanum lycopersicum*	2002	Rajasthan, India	2758	Sivalingam *et al*., 2005
Tomato leaf curl Seychelles virus	ToLCSCV	AM491778	*Solanum lycopersicum*	2004	Mahe, Seychelles	2742	Lefeuvre *et al*., 2007

Table 4. 1 Cont'

Virus name	Acronym	GenBank Accession No	Original Host	Year of collection	Isolate and Country of origin	Complete genome size (nt)	Reference
Tomato leaf curl Taiwan virus	ToLCTV/A	U88692	*Solanum lycopersicum*	1995	TW1:Taiwan	2739	Chiang *et al.*, 1997
Tomato leaf curl Arusha virus	ToLCTZV	DQ519575	*Solanum lycopersicum*	2006	TZ-Ten-05:Arusha, Tanzania	2766	Shih *et al.*, 2006
Tomato leaf curl Toliara virus	ToLCToV	AM701768	*Solanum lycopersicum*	2001	Miandrivazo, Madagascar	2764	Lefeuvre et al., 2007
Tomato leaf curl Uganda virus - [Iganga]	ToLCUV	DQ127170	*Solanum lycopersicum*	2006	Iganga, Uganda	2747	Shih *et al.*, 2006

4.3.7 Phylogeny, genetic diversity and population genetic analysis

A phylogenetic tree was constructed using the maximum likelihood method based on Jukes-Cantor model in MEGA v.6.06 (Tamura *et al*., 2013). Bootstrap replicate values were set at 1,000 while a strain of Tomato leaf curl purple vein virus (KY196216) was selected as an outgroup. Genetic structure and diversity within ToLCV populations in Kenya were investigated to understand potential evolutionary dynamics that produce variations. Population structure parameters estimated included; average nucleotide diversity (π), haplotype diversity (Hd), number of polymorphic or segregating sites (S), the statistic estimate of population mutation based on the number of segregating sites (θ-W), total number of mutations (Eta), the average number of nucleotide differences between sequences (k) and the statistic estimate of population mutation based on the total number of mutations (θ-Eta). These were estimated using complete genome and protein coding sequences in DnaSP v5.10.01 (Librado *et al*., 2009).

The possible occurrences of selection pressure on individual genes and sites within the ToLCV populations were obtained using the single-likelihood ancestor counting (SLAC) method (Kasakovsky *et al*., 2005) in the HyPhy package (Kasakovsky *et al*., 2020) as implemented on the Datamonkey software (Weaver *et al*., 2018) at http://www.datamonkey.org. The ratio of average number of nucleotide differences between the sequences per nonsynonymous site (dN) to the average number of nucleotide differences between the sequences per synonymous site (dS) were calculated as an indicator of natural selection. These were used to estimate the occurrence of positive and negative selection at typical begomovirus amino acid ORF sites: the movement protein (MP) or V1 protein, coat protein (CP) or V2 protein, replication protein (Rep) or C1 protein, transcription activator protein (TrAP) or C2 protein, Rep enhancer protein (REn) or C3 protein and the C4 protein. Depending on the dN/dS values, the selection pressure was

considered negative or purifying (dN/dS < 1), neutral (dN/dS = 1), or diversifying or positive (dN/dS > 1) for data sets of each coding region. The DNAsp v5.10.01 was used to calculate the Tajima's D, Fu and Li's F* and D*, and Fu's Fs to determine the deviation of ToLCV populations from neutrality assuming a constant population size, with zero recombination and migration (Ramirez-Soriano *et al.*, 2008). A negative Tajima's D statistic indicates superfluous low-frequency polymorphism triggered by background selection, genetic hitchhiking, or population expansions (Alabi *et al*., 2011). Conversely, positive values of Tajima's D statistic suggest minimal levels of low and high frequency polymorphisms, indicating a reduction in population size and/or balancing selection.

4.4 Results

4.4.1 Sequence data, *de novo* assembly

After mapping of sequence reads from leaf samples to the tomato reference genome, unmapped reads were subsequently assembled into contigs. The *de novo* assembly yielded several contigs, with the largest having sizes of >45 kb while N50 values ranged from 135-270 bp (**Table 4.2**). After Kaiju analyses, all assembled virus contigs were subjected to BLASTN 2.9.0+ searches. The results revealed twelve contig matches of lengths >2.7 kb from eleven samples with complete begomovirus genomes within the database (**Table 4.4**) while partial contigs matching other DNA viruses were also present (**Table 4.3**). However, a sample (Tom54) produced the full-length genome of a separate begomovirus, *Chickpea chlorotic dwarf virus* (Avedi *et al*., 2020). Across all the samples, only monopartite begomoviruses with DNA-A-like sequences were recovered. Raw reads from these positive samples have been deposited at the SRA archive (Bioproject number PRJNA646848). The PCR primers designed from the full begomovirus genomes produced the expected 530 bp amplicons from the genomic DNA of infected tomato plants **(Figure 4.2)**. Sanger sequencing of the PCR products revealing 95.6-99.7% identity (**Figure 4.3**) with the complete genomes assembled from the Illumina reads, thus confirming the accuracy of the nucleotides within the assembled virus genomes.

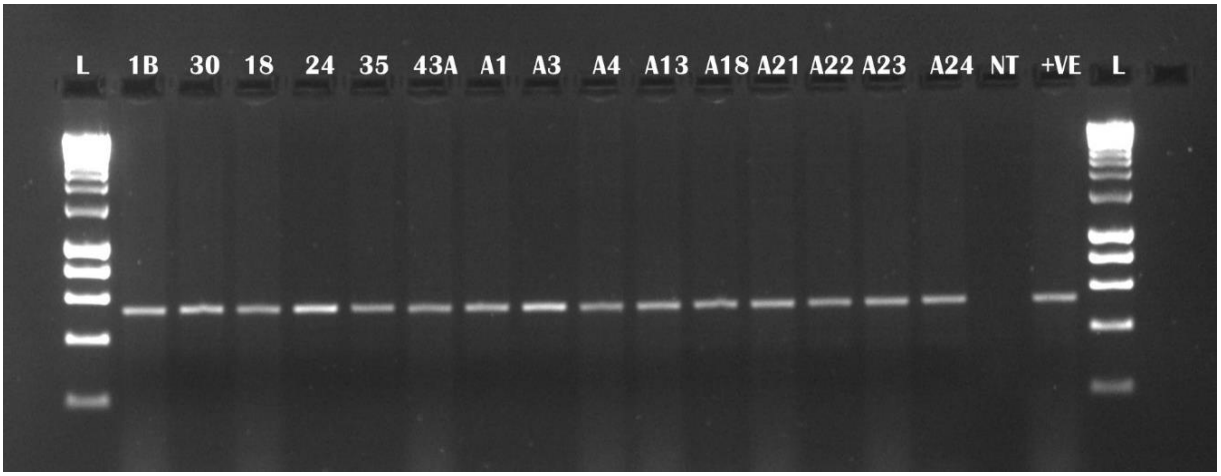


Figure 4. 2 Gel electrophoresis of PCR products, 1kb Hypper ladder (Bioline).

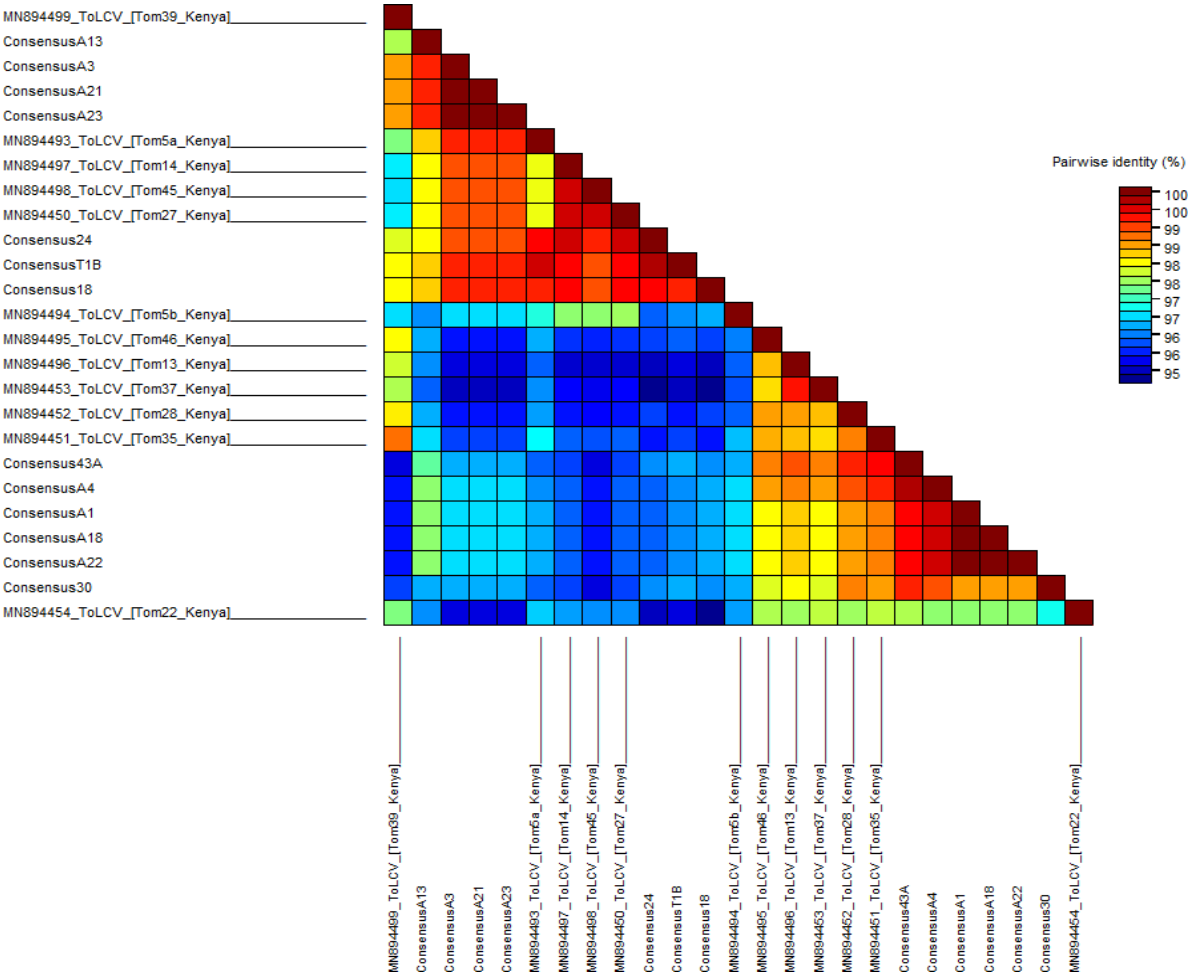


Figure 4. 3 Pairwise comparison of Sanger sequences with complete genomes from Illumina sequencing

Table 4. 1 Summary of *de novo* assembly reads

Sample ID	County	Location	Number of reads before QC	Number of reads after QC	N50 after *de novo* assembly(bp)	Average length after *de novo* assembly(bp)	Maximum contig length (bp)
Tom 2	Kirinyaga	Mwea	740	593	147	148	1023
Tom 3	Kirinyaga	Mwea	319816	314602	157	157	5262
Tom 4	Kirinyaga	Mwea	712833	698797	172	171	8902
Tom 5	Kirinyaga	Mwea	478717	468508	235	231	13612
Tom 6	Meru	Isiolo	926762	910347	182	178	10110
Tom 7	Kirinyaga	Mwea	685854	673575	182	179	7946
Tom 8	Nakuru	Rongai	228919	223686	180	178	7153
Tom 9	Nakuru	Naivasha	234634	230096	183	182	6069
Tom 10	Kirinyaga	Mwea	666739	687749	184	182	6290
Tom 11	Kirinyaga	Mwea	513780	510356	175	175	7911
Tom 12	Nakuru	Naivasha	518378	510356	175	175	6450
Tom 13	Baringo	Pekerra	1294432	1274379	201	214	10139
Tom 14	Baringo	Pekerra	1315827	1298815	201	207	44887
Tom 15	Baringo	Pekerra	681944	672083	159	162	3864
Tom 16	Nakuru	Naivasha	923671	908547	186	182	17400
Tom 17	Kwale	Kwale	534566	527106	162	162	7417
Tom 18	Nakuru	Naivasha	352911	347096	181	181	11029
Tom 20	Baringo	Pekerra	609870	597102	166	165	5871
Tom 21	Nakuru	Naivasha	243975	240633	135	138	947
Tom 22	Baringo	Pekerra	253971	251006	135	138	947
Tom 23	Meru	Meru	989149	975057	163	164	7104
Tom 24	Meru	Meru	786980	774322	171	171	7102
Tom 26	Baringo	Pekerra	728499	719324	164	166	42790
Tom 27	Meru	Isiolo	1058065	1039347	187	190	42301
Tom 28	Baringo	Pekerra	339590	324853	170	173	7369
Tom 33	Makueni	Kibwezi	1093634	1078116	147	149	5805
Tom 34	Kirinyaga	Mwea	619068	610590	152	152	7151
Tom 35	Nakuru	Solai	322666	317651	159	160	5398
Tom 36	Nakuru	Solai	605642	597918	151	152	3604
Tom 37	Baringo	Pekerra	161895	158650	201	206	3608
Tom 39	Makueni	Kibwezi	638580	630445	146	149	7957
Tom 40	Baringo	Kamoskoi	359195	353997	201	208	40180
Tom 41	Machakos	Mavoko	424094	418916	173	173	5839
Tom 42	Nakuru	Rongai	286302	282623	168	167	7249
Tom 43	Kirinyaga	Mwea 6	478583	470428	173	173	7405

Table 4. 2 Cont'

Sample ID	County	Location	Number of reads before QC	Number of reads after QC	N50 after *de novo* assembly(bp)	Average length after *de novo* assembly(bp)	Maximum contig length (bp)
Tom 45	Baringo	Pekerra	547166	540173	208	211	9004
Tom 46	Meru	Isiolo	484669	477311	176	178	6633
Tom 47	Meru	Isiolo	492672	486329	161	162	4876
Tom 48	Meru	Isiolo	656855	649550	270	237	14432
Tom 49	Meru	Isiolo	99706	98214	168	169	5196
Tom 50	Kirinyaga	Mwea	576946	568993	166	167	7263
Tom 51	Nakuru	Rongai	236211	232201	160	162	7294
Tom 52	Baringo	Pekerra	369927	364146	201	212	6670
Tom 53	Meru	Isiolo	590600	581771	179	181	7083
Tom 54	Nakuru	Naivasha	320207	314556	174	174	7225
Tom 55	Nakuru	Solai	387684	382226	165	167	7218
Tom 56	Baringo	Pekerra	219381	214875	224	223	8109

4.4.2 The begomoviruses in Kenyan tomato are a variant of ToLCArV

The full-length genomes of the ToLCArV obtained varied from 2,760 to 2,765 bp (**Table 4.4**). These were subsequently deposited in GenBank database under the accession numbers MN894493 to MN894504. Sequence analyses showed that these genomes encoded the six ORFs (V1, V2, C1, C2, C3 and C4) that are typical of monopartite begomoviruses while the intergenic regions ranged from 245-250 nt. Pairwise alignments of begomoviruses with pairwise deletion of gaps revealed the highest full genome similarity (95.9-98.9%) with an isolate of *Tomato leaf curl Arusha virus* (ToLCArV, GenBank accession EF194760) from Tanzania (**Table 4.5**). This was followed by *Tomato leaf curl Toliara virus* (ToLCToV, GenBank accession AM701768) with 95.9-98.9 % identity and another isolate of *Tomato leaf curl virus Arusha virus* (ToLCArV, GenBank accession DQ519575) at 89.8-90.5% similarity. Furthermore, all isolates exhibited less than 80% pairwise sequence identity to other begomovirus sequences (**Figure 4.4**). Based on the species demarcation criteria of the International Committee for the Taxonomy of Viruses set for

begomoviruses at <91% nucleotide sequence identity (Brown *et al*., 2015), the Kenyan begomoviruses were considered as a variant of ToLCArV. Similar patterns were observed for deduced amino acids as the highest identity was observed with ToLCArV (GenBank accession EF194760) across all the six coding regions (93.3-99.1 for MP, 97.3-98.9% for CP, 95.4-98.6% for Rep, 94.2-97.8% for TrAP, 96.0-98.0% for REn and 97.1-100% for C4 protein). Pairwise comparison across amino acids of other tomato infecting monopartite begomoviruses revealed similar patterns (**Table 4.6**). Further analyses revealed 95.7-99.7% similarity within the twelve Kenyan ToLCArV-like isolates while amino acid residues also revealed high similarities at the MP (94.1-100%), CP (98.5-100%), Rep (94.1-99.4%), TrAP (94.3-100%), REn (95.6-100%) and C4 (95.1-100%) coding regions (**Table 4.7**).

Table 4. 3 Additional DNA viruses obtained from high throughput read sequencing of tomato leaf samples from Kenya

Virus	Reference accession number	Length (bp)	Number of contigs	Contig lengths	Similarity (%)	E value	Sample number
African tomato leaf curl geminivirus	U73498	1523	8	201 to 1642	91.53 to 97.20	≤4e-106	23,37,39,45
Tomato leaf curl Uganda virus	MN381114/5	2769	5	206 to 501	95.04 to 100	≤5e-120	4,7,22,23
Ageratum yellow vein Sri Lanka virus	JN809826	2750	2	201 to 800	86.28 to 90.00	≤4e-65	23
Tomato leaf curl new delhi virus	MG758145	201	1	201	77.30	1e-10	23

Table 4. 4 Summary of virus identification of contigs from tomato samples in Kenya by BLAST and their identity with closest database homologues

Sample	Length of virus contigs (nt)	Virus identified	Accession number	Similarity (%)	Query cover (%)	Identities	E-value
Tom 5a	2761	*Tomato leaf curl Arusha virus*	EF194760	97.72	99	2698/2761	0
Tom 5b	2765	*Tomato leaf curl Arusha virus*	EF194760	97.69	100	2701/2765	0
Tom 46	2763	*Tomato leaf curl Arusha virus*	EF194760	96.16	100	2658/2764	0
Tom 13	2762	*Tomato leaf curl Arusha virus*	EF194760	95.84	100	2648/2763	0
Tom 14	2760	*Tomato leaf curl Arusha virus*	EF194760	98.81	100	2729/2762	0
Tom 45	2763	*Tomato leaf curl Arusha virus*	EF194760	98.84	100	2731/2763	0
Tom 39	2762	*Tomato leaf curl Arusha virus*	EF194760	97.10	100	2683/2763	0
Tom 27	2762	*Tomato leaf curl Arusha virus*	EF194760	98.91	100	2732/2762	0
Tom 35	2762	*Tomato leaf curl Arusha virus*	EF194760	96.45	100	2665/2763	0
Tom 28	2763	*Tomato leaf curl Arusha virus*	EF194760	95.98	100	2653/2764	0
Tom 37	2762	*Tomato leaf curl Arusha virus*	EF194760	95.91	100	2651/2764	0
Tom 22	2761	*Tomato leaf curl Arusha virus*	EF194760	96.60	100	2668/2762	0

Table 4.5 Percentage nucleic acid similarities between full and individual genomic regions of Tomato leaf curl Arusha virus-like isolates from Kenya with DNA-A component of tomato begomoviruses

Begomovirus species [a]	Complete genome	Genomic regions [b]						
		IR	V1	V2	C1	C2	C3	C4
ToLCArV	95.9-98.9	91.8-97.1	95.5-99.4	96.2-98.9	96.3-99.4	95.7-98.3	97.1-98.7	98.4-99.7
ToLCCMV	79.7-80.1	66.5-70.3	79.2-81.7	79.0-80.4	82.2-83.7	74.8-76.7	76.5-77.1	87.1-88.8
ToLCAnV	77.0-77.8	69.3-74.9	78.0-80.5	79.6-80.1	75.4-76.4	73.9-74.8	72.4-73.5	75.8-76.8
ToLCBaV/A	72.9-74.0	68.4-75.4	69.2-72.3	71.5-72.2	75.1-77.0	70.4-71.6	70.0-70.9	75.6-77.0
ToLCBV	74.7-75.5	64.8-67.5	75.6-77.8	74.0-75.1	77.1-78.1	68.7-72.1	70.6-72.6	75.0-76.0
ToLCKMV	79.5-80.3	75.6-80.6	82.6-84.4	82.3-82.9	77.3-78.2	73.9-75.6	74.2-74.9	76.6-77.9
ToLCDiV	75.9-77.2	64.6-67.1	73.6-75.3	74.7-75.9	79.8-81.4	73.9-75.7	74.9-76.0	86.2-86.8
ToLCGV	77.9-78.2	71.0-73.9	80.0-82.3	78.0-78.9	78.3-79.6	74.8-76.5	74.2-75.6	80.4-81.1
ToLCGdV	75.6-76.6	62.3-67.2	72.8-73.9	75.4-76.1	79.9-80.9	71.9-73.4	74.7-75.2	84.9-85.9
ToLCHaiV	73.2-74.0	61.2-65.2	75.8-76.6	71.7-72.5	77.9-78.9	71.5-73.7	70.5-71.5	80.7-81.4
ToLCHaV	74.4-75.1	66.3-74.4	75.8-77.0	73.7-74.0	77.5-78.8	70.1-71.3	70.0-70.8	75.7-77.3
ToLCIRV	73.8-74.6	64.2-72.0	75.3-76.8	71.5-72.3	77.5-78.4	72.0-73.5	70.4-72.0	76.0-77.3
ToLCJaV/A	76.1-76.9	65.5-71.0	73.8-75.0	74.1-75.1	80.9-82.0	71.9-72.7	71.3-72.2	83.9-85.3
ToLCV-K3	74.6-75.6	67.7-71.6	75.3-76.5	73.7-74.5	77.0-80.0	71.1-73.0	71.5-73.5	80.0-81.3
ToLCLV	74.5-75.4	63.5-68.6	69.6-71.8	74.0-75.2	78.1-79.5	71.7-73.4	72.1-73.8	81.4-82.7

Table 4.5 Cont'

Begomovirus species [a]	Complete genome	Genomic regions [b]						
		IR	V1	V2	C1	C2	C3	C4
ToLCMGV/Men	78.6-79.3	70.2-75.5	82.2-83.6	79.8-80.3	78.2-79.5	75.1-77.2	74.0-75.1	80.6-82.0
ToLCMiV	75.1-75.7	61.4-67.7	73.0-74.6	72.4-74.8	79.1-79.1	72.5-73.9	72.3-73.1	80.4-81.7
ToLCMohV	78.0-78.9	70.0-76.7	82.9-84.4	82.1-82.7	74.5-75.5	74.4-76.3	74.9-75.3	68.3-69.2
ToLCNaV	79.8-80.3	75.4-80.0	84.3-87.3	81.9-89.9	77.2-78.7	74.6-76.5	75.3-76.2	79.5-81.1
ToLCNDC2	73.6-74.3	66.7-69.8	71.1-73.6	72.1-73.3	76.9-77.8	73.0-74.2	70.0-72.9	79.5-80.1
ToLCNDC5	71.3-72.2	66.7-72.1	69.8-70.4	71.1-71.8	72.8-73.9	68.2-70.0	66.8-68.5	75.3-76.3
ToLCJV	71.9-73.4	58.3-69.8	68.8-71.0	73.3-74.4	76.1-78.1	66.2-68.6	68.7-70.5	78.6-79.3
ToLCKV2	74.1-74.8	71.9-76.0	67.7-69.3	72.1-73.0	78.1-79.2	72.5-73.5	70.4-72.2	76.6-78.0
ToLCKV3	73.4-74.0	62.9-71.3	73.5-75.8	72.3-73.2	75.7-76.5	70.9-71.5	70.2-72.0	77.3-78.6
ToLCNGV	78.1-78.9	68.4-71.9	78.8-80.7	78.8-79.9	78.8-80.1	75.1-77.0	74.2-78.1	83.0-83.6
ToLCPalV	70.1-70.5	61.6-65.4	67.7-68.3	70.8-71.5	70.9-72.1	68.9-70.6	70.3-72.2	75.0-76.3
ToLCPatV	74.5-75.1	62.1-65.7	72.6-73.2	73.6-74.2	78.7-79.6	71.2-73.1	70.6-71.3	78.5-79.8
ToLCRaV	72.4-72.9	62.8-69.4	75.1-76.2	73.4-74.2	73.0-74.9	69.5-71.6	70.1-71.1	73.1-74.4
ToLCSCV	76.7-77.4	69.7-73.3	83.3-85.2	80.4-80.9	73.5-75.0	72.6-75.4	72.1-73.5	65.7-67.0
ToLCLKV	73.8-74.4	68.3-73.7	68.8-70.0	71.6-72.5	77.7-78.5	71.8-73.7	70.4-72.1	79.2-80.6

Table 4.5 Cont'

Begomovirus species [a]	Complete genome	Genomic regions [b]						
		IR	V1	V2	C1	C2	C3	C4
ToLCTV/A	74.1-74.7	62.7-69.0	70.8-71.7	72.9-73.9	78.3-79.9	71.0-71.7	71.1-72.9	76.7-78.0
ToLCTZV	89.8-90.5	70.8-73.3	85.0-88.3	94.0-95.4	88.7-89.8	95.2-96.7	96.9-98.0	89.4-90.0
ToLCToV	95.9-98.9	75.9-81.7	73.8-75.9	83.4-84.6	91.4-92.5	94.7-96.0	95.1-96.7	92.6-93.6
ToLCUV	78.9-79.3	75.0-76.7	83.5-86.2	82.5-83.3	74.4-75.0	76.5-78.1	74.4-75.6	65.3-66.3

[a] ToLCArV: Tomato leaf curl virus Arusha virus, ToLCCMV: Tomato leaf curl Cameroon virus, ToLCAnV: Tomato leaf curl Anjouan virus, ToLCBaV/A: Tomato leaf curl Bangalore virus, ToLCBV: Tomato leaf curl Bangladesh virus, ToLCKMV: Tomato leaf curl Comoros virus, ToLCDiV: Tomato leaf curl Diana virus, ToLCGV: Tomato leaf curl Ghana virus, ToLCGdV: Tomato leaf curl Guangdong virus, ToLCHaiV: Tomato leaf curl Hainan virus, ToLCHaV: Tomato leaf curl Hanoi virus, ToLCIRV: Tomato leaf curl Iran virus, ToLCJaV/A: Tomato leaf curl Java virus, ToLCV-K3: Tomato leaf curl Kerala virus, TLCV-LA: Tomato leaf curl Laos virus, ToLCMGV: Tomato leaf curl Madagascar virus, ToLCMiV: Tomato leaf curl Mindanao virus, ToLCMohV: Tomato leaf curl Moheli virus, ToLCNaV: Tomato leaf curl Namakely virus, ToLCNDC2:Tomato leaf curl New Delhi virus 2, ToLCNDC5: Tomato leaf curl New Delhi virus 5, ToLCJV: Tomato leaf curl Joydebpur virus, ToLCKV2: Tomato leaf curl Karnataka virus 2, ToLCKV3: Tomato leaf curl Karnataka virus 3, ToLCNGV: Tomato leaf curl Nigeria virus, ToLCPalV: Tomato leaf curl Palampur virus, ToLCPatV: Tomato leaf curl Patna virus, ToLCRaV: Tomato leaf curl Rajasthan virus, ToLCSCV: Tomato leaf curl Seychelles virus, ToLCLKV: Tomato leaf curl Sri Lanka virus, ToLCTV/A: Tomato leaf curl Taiwan virus, ToLCTZV: Tomato leaf curl Arusha virus, ToLCToV: Tomato leaf curl Toliara virus, ToLCUV: Tomato leaf curl Uganda virus.

[b] IR: Intergenic region, V1: Movement protein gene, V2: Coat protein gene, C1: Replication-associated protein gene, C2: Transcriptional activator protein gene, C3: Replication enhancer protein gene, C4: C4 protein gene

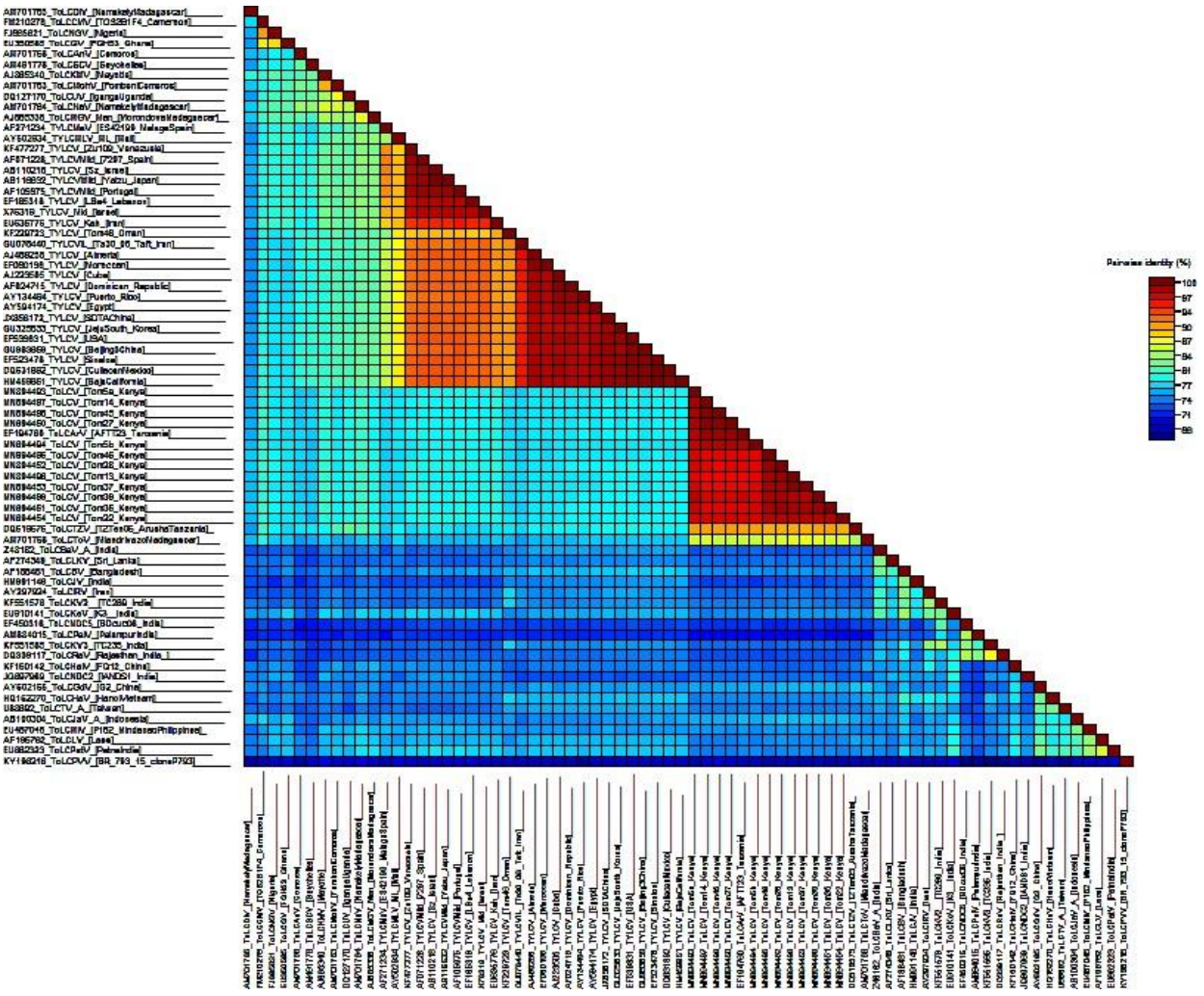


Figure 4. 4 Pairwise identity of Kenyan monopartite tomato begomoviruses with other tomato infecting begomoviruses species

Table 4.6: Percentage amino acid sequence similarities between open reading frames of Tomato leaf curl Arusha virus-like isolates from Kenya with DNA-A component of tomato begomoviruses

Begomovirus species [a]	Genomic regions [b]					
	V1	V2	C1	C2	C3	C4
ToLCArV	93.3-99.1	97.3-98.9	95.4-98.6	94.2-97.8	96.0-98.0	97.1-100.0
ToLCCMV	74.5-76.3	86.6-87.8	81.9-85.4	64.3-68.6	66.2-68.2	69.2-73.1
ToLCAnV	75.0-76.7	88.2-88.9	70.6-72.2	62.1-64.3	65.2-68.1	50.6-53.3
ToLCBaV/A	63.0-64.7	73.9-74.3	75.3-76.9	58.5-60.0	63.0-65.2	52.9-56.5
ToLCBV	67.7-68.6	78.1-79.3	75.7-77.6	57.0-59.3	65.2-68.8	50.0-52.9
ToLCKMV	80.6-82.3	93.1-93.9	76.5-77.9	63.6-65.0	66.0-69.6	54.8-56.7
ToLCDiV	66.3-67.2	81.7-82.9	80.1-81.7	64.7-66.9	71.9-74.8	72.5-73.5
ToLCGV	74.8-76.5	86.2-87.4	78.2-80.1	64.7-68.4	67.5-69.6	56.7-58.6
ToLCGdV	71.4-73.1	79.0-80.5	80.7-81.6	61.0-63.2	62.7-66.7	72.9-76.5
ToLCHaiV	71.2-74.3	75.5-75.9	79.3-80.7	59.2-60.0	57.3-59.4	60.8-61.7
ToLCHaV	72.2-73.9	79.0-80.1	76.4-77.8	56.3-58.5	63.0-64.5	46.1-50.9
ToLCIRV	67.7-68.6	73.5-73.9	73.3-74.4	59.2-61.5	65.2-66.7	48.1-51.0
ToLCJaV/A	67.7-69.5	78.2-79.4	81.8-82.9	56.6-58.8	65.9-66.7	70.6-73.5
ToLCV-K3	67.7-68.6	76.2-77.4	75.1-76.8	62.9-64.4	65.2-66.7	58.6-62.1
ToLCLV	55.0-56.7	81.2-82.4	77.4-78.6	56.6-59.5	62.0-65.2	59.6-62.5
ToLCMGV/Men	82.5-84.2	88.5-90.0	77.7-78.5	67.1-69.3	66.2-68.8	57.6-61.2
ToLCMiV	68.6-69.4	77.8-79.0	77.5-78.9	59.5-61.7	65.2-66.7	62.5-65.4
ToLCMohV	79.8-84.0	91.6-92.4	-	65.4-66.9	66.0-69.6	37.5-38.5
ToLCNaV	81.5-83.2	91.6-92.4	76.8-78.2	66.1-66.9	70.2-73.2	60.0-64.7
ToLCNDC2	61.0-63.5	76.6-77.8	78.0-79.7	61.1-63.3	56.8-58.9	56.7-58.6
ToLCNDC5	61.4-62.2	79.3-80.1	73.8-75.7	50.7-51.5	54.6-57.3	62.1-67.2
ToLCJV	70.8-73.5	78.3-78.7	75.5-77.6	52.0-54.4	55.7-57.2	55.1-56.5

Table 4.6 cont'

Begomovirus species [a]	Genomic regions [b]					
	V1	V2	C1	C2	C3	C4
ToLCKV2	56.7-57.6	77.8-78.5	77.6-78.7	62.2-63.7	64.5-66.7	49.0-51.9
ToLCKV3	63.5-64.4	79.7-80.4	74.3-77.3	52.9-53.7	62.0-63.7	50.0-52.9
ToLCNGV	73.9-75.6	87.4-88.5	78.6-80.7	67.6-69.1	67.5-68.9	62.7-63.7
ToLCPalV	56.8-58.5	76.2-77.4	72.0-73.4	52.9-54.5	60.0-62.3	62.1-67.2
ToLCPatV	70.3-74.5	79.4-80.1	76.6-77.7	59.5-61.7	56.7-60.9	56.3-59.7
ToLCRaV	62.5-66.1	78.9-79.3	72.7-74.4	51.5-52.2	60.0-62.3	62.1-67.2
ToLCSCV	81.7-84.2	90.8-91.2	71.3-73.0	65.6-68.5	63.1-66.7	35.0-39.0
ToLCLKV	63.6-65.4	73.5-73.9	76.1-77.5	59.7-61.8	61.4-63.6	51.7-55.3
ToLCTV/A	67.7-68.6	77.1-77.8	73.9-75.3	55.8-58.1	58.0-63.0	50.9-53.8
ToLCTZV	82.5-84.1	96.2-97.3	88.2-89.8	93.6-95.0	95.3-98.5	78.8-79.8
ToLCToV	68.3-70.9	82.8-84.0	88.6-90.3	93.6-95.0	91.3-94.2	85.3-88.2
ToLCUV	79.8-81.5	93.1-93.5	72.6-74.6	65.0-66.4	67.5-69.6	31.7-33.6

a ToLCArV: Tomato leaf curl virus Arusha virus, ToLCCMV: Tomato leaf curl Cameroon virus, ToLCAnV: Tomato leaf curl Anjouan virus, ToLCBaV/A: Tomato leaf curl Bangalore virus, ToLCBV: Tomato leaf curl Bangladesh virus, ToLCKMV: Tomato leaf curl Comoros virus, ToLCDiV: Tomato leaf curl Diana virus, ToLCGV: Tomato leaf curl Ghana virus, ToLCGdV: Tomato leaf curl Guangdong virus, ToLCHaiV: Tomato leaf curl Hainan virus, ToLCHaV: Tomato leaf curl Hanoi virus, ToLCIRV: Tomato leaf curl Iran virus, ToLCJaV/A: Tomato leaf curl Java virus, ToLCV-K3: Tomato leaf curl Kerala virus, TLCV-LA: Tomato leaf curl Laos virus, ToLCMGV: Tomato leaf curl Madagascar virus, ToLCMiV: Tomato leaf curl Mindanao virus, ToLCMohV: Tomato leaf curl Moheli virus, ToLCNaV: Tomato leaf curl Namakely virus, ToLCNDC2: Tomato leaf curl New Delhi virus 2, ToLCNDC5: Tomato leaf curl New Delhi virus 5, ToLCJV: Tomato leaf curl Joydebpur virus, ToLCKV2: Tomato leaf curl Karnataka virus 2, ToLCKV3: Tomato leaf curl Karnataka virus 3, ToLCNGV: Tomato leaf curl Nigeria virus, ToLCPalV: Tomato leaf curl Palampur virus, ToLCPatV: Tomato leaf curl Patna virus, ToLCRaV: Tomato leaf curl Rajasthan virus, ToLCSCV: Tomato leaf curl Seychelles virus, ToLCLKV: Tomato leaf curl Sri Lanka virus, ToLCTV/A: Tomato leaf curl Taiwan virus, ToLCTZV: Tomato leaf curl Arusha virus, ToLCToV: Tomato leaf curl Toliara virus, ToLCUV: Tomato leaf curl Uganda virus.

b V1: Movement protein gene, V2: Coat protein gene, C1: Replication-associated protein gene, C2: Transcriptional activator protein gene, C3: Replication enhancer protein gene, C4: C4 protein gene

Table 4.7: Percentage pairwise sequence identities among the twelve *Tomato leaf curl virus Arusha virus*-like isolates from Kenya

Segment [a]	Nucleotide (%)	Amino acid (%)
Genome	95.7-99.7	-
V1	95.0-100	94.1-100
V2	95.0-100	98.5-100
C1	95.7-99.6	94.1-99.4
C2	95.0-100	94.3-100
C3	96.8-100	95.6-100
C4	98.7-100	95.1-100

[a] V1: Movement protein gene, V2: Coat protein gene, C1: Replication-associated protein gene, C2: Transcriptional activator protein gene, C3: Replication enhancer protein gene, C4: C4 protein gene

4.4.3 Recombination analyses

Using the automated SBP and GARD tools within Datamonkey, recombination signals were found within the genomic regions of our ToLCArV-like populations. However, further analyses of the isolates using the programs implemented in the RDP4 software did not reveal significant recombination signals within our sequences. Conversely, two isolates Tom5a (MN894493) and Tom39 (MN894499) were identified as potential major and minor parental sequences for the signals detected in ToLCArV (DQ519575) and ToLCDiV (AM701765), respectively (**Table 4.8).**

Table 4.8: Identification of Kenyan *Tomato leaf curl virus Arusha virus*-like isolates as parents of putative recombinant tomato begomoviruses using the RDP4 software

Recombinants [a]	Potential parents [b]		Recombination breakpoints	Average *p* values in detecting algorithms [c]						
	Minor	Major		R	G	B	M	C	S	T
ToLCArV TZTen05-Tanzania (DQ519575)	ToLCUV Iganga-Uganda (DQ127170)	**ToLCArV Tom5a-Kenya (MN894493)**	158-524	2.42E-06	2.04E-04	-	7.13E-09	4.67E-10	-	1.68E-12
ToLCDiV Namakely-Madagascar (AM701765)	**ToLCArV Tom39-Kenya (MN894499)**	ToLCMohV Moheli-Comoros (AM701763)	1091-1583	1.48E-12	4.75E-12	1.32E-10	1.04E-10	1.17E-05	-	4.29E-04

[a] ToLCArV: *Tomato leaf curl virus Arusha virus*, ToLCDiV: *Tomato leaf curl Diana virus.*

[b] ToLCUV: ToLCUV: *Tomato leaf curl Uganda virus*, ToLCMohV: *Tomato leaf curl Moheli virus*.

[c] R: RDP, G: GENCOV, B: BoostScan, M: MaxChi, C: Chimera, S: SiScan, T: 3Seq

4.4.4 Phylogenetic relationships and genetic diversity of Kenyan tomato begomoviruses

A phylogenetic analysis was done using full genome sequences of the 12 ToLCArV isolates from Kenya, together with TYLCV-like sequences and other tomato begomoviruses from GenBank. All the TYLCV-like isolates (n=25) clustered separately from ToLCV-like sequences (n=46) with a clear geographical segregation. African ToLCV-like sequences (n=26) were separated from those of Asian origins (n=20) while isolates from Kenya formed a monophyletic cluster with isolates from Tanzania (ToLCArV, EF194760 and DQ519575) (**Figure 4.5**).

Analyses of haplotype number and haplotype diversity, represented by 'h' and 'Hd', respectively revealed varying values among the 12 Kenyan ToLCArV-like sequences and also among other ToLCV-like sequences from GenBank, based on the six coding regions evaluated **(Table 4.9**). From the total ToLCV-like sequences (n=46), haplotypes number ranged from 43 in the MP region to 46, in the CP, Rep and whole genomes. Similarly, among the Kenyan ToLCArV-like isolates (n=12), 'h' values ranged from 9 (MP gene) to 12 (CP, Rep and complete genomes). Thus, across ToLCV-like sequences from the Genbank and the Kenyan ToLCArV-like sequences, each isolate represented a haplotype at both CP and Rep genes, revealing high genetic variation within the coding regions of each group. This therefore indicates that genetic variation was highest within the CP and Rep coding regions. Interestingly, Hd values were highest for the CP and REn gene and lowest for MP gene, both across ToLCV-like isolates obtained from GenBank and among the 12 Kenyan ToLCV-like sequences obtained in this study (**Table 4.9**). Furthermore, genetic distances for each gene-specific data set were calculated, with highest π values obtained within the REn gene (0.2458) across the ToLCV-like isolates (n=46). The C4 gene and Rep gene recorded the lowest π values i.e. 0.21015 and 0.21165, respectively. Remarkably, the π value of the C4 gene within the 12 Kenyan ToLCArV-like isolates (0.00869)

was more than half the π values of other coding regions, indicating that these coding regions were more variable than the C4 gene (**Table 4.9**). Collectively, these results show high genetic variability among the CP and Rep coding regions across both ToLCV groups, with C4 gene having the least variation across the isolates.

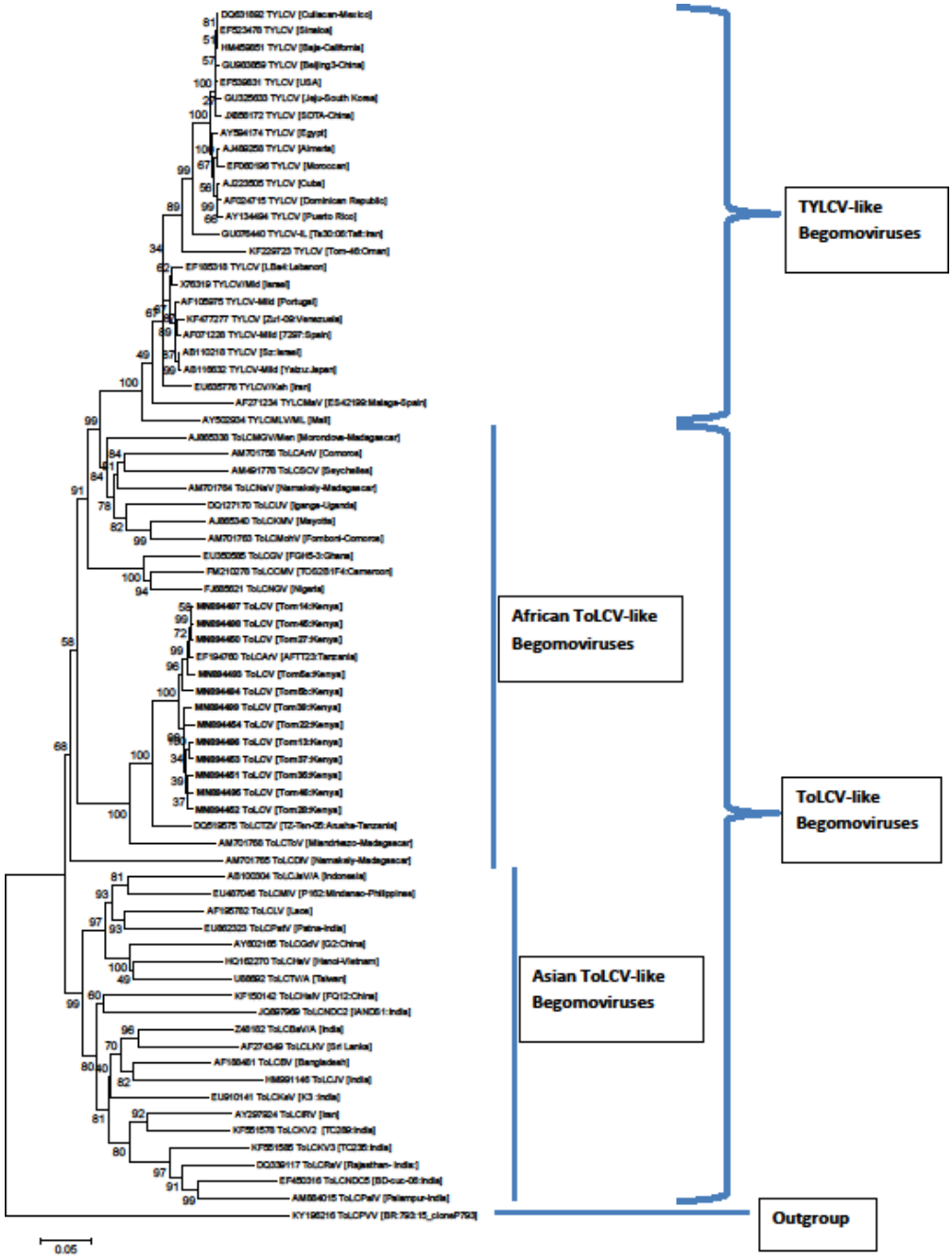


Figure 4. 5 Phylogenetic analyses of tomato leaf curl virus from Kenya (n = 12) with selected worldwide begomoviruses based on alignment of complete DNA-A nucleotide sequences. The tree was generated using the maximum likelihood inference based on the Jukes-Cantor model as implemented in MEGA v.6.06 (Tamura et al., 2013). Percentage bootstrap support values (1,000 iterations) are indicated at the branch nodes. The tree is rooted withTomato leaf curl purple vein virus (accession number KY196216) as an outgroup. The scale bar shows the number of nucleotide substitutions powersite.

Table 4.9: Genetic variability determinants and neutrality tests on *Tomato leaf curl virus Arusha virus*-like populations from Kenya with other worldwide tomato begomoviruses

Population	Gene [a]	N [b]	h [c]	S [d]	Hd [e]	Eta [f]	π [g]	k [h]	θ-W [i]	θ-Eta [j]	Tajima's *D*	Fu and Li's *D*	Fu and Li's *F*
Tomato begomoviruses	Genome	2920	46	1666	1.000	2985	0.22424	569.5749	0.14924	0.2674	-0.5971	-0.2912	-0.4859
(n=46)	V1	372	43	241	0.996	419	0.22538	76.1768	0.16224	0.28206	-0.7389	-0.8229	-0.9463
	V2	1261	46	752	1.000	1334	0.22421	271.971	0.14106	0.25023	-0.3842	-0.0052	-0.1764
	C1	1179	46	692	1.000	1213	0.21165	225.4106	0.14784	0.25915	-0.6771	-0.3609	-0.5737
	C2	431	45	264	0.999	458	0.23777	97.486	0.14651	0.25417	-0.2374	-0.0923	-0.1752
	C3	469	45	303	0.999	550	0.24589	108.1903	0.15669	0.28442	-0.4989	-0.3631	-0.4949
	C4	316	44	210	0.997	360	0.21015	65.1459	0.15414	0.26423	-0.7516	-0.4512	-0.6736
Kenyan ToLCArV-like	Genome	2766	12	211	1.000	224	0.0264	72.955	0.0253	0.0268	-0.0774	-0.4189	-0.3751
isolates (n=12)	V1	360	9	22	0.939	24	0.02298	8.2727	0.02024	0.02208	0.1819	0.1216	0.1564
	V2	1239	12	71	1.000	72	0.0209	25.8788	0.01899	0.01926	0.3958	-0.0631	0.0659
	C1	1116	12	94	1.000	102	0.02744	30.6212	0.02789	0.03027	-0.4355	-0.7775	-0.7841
	C2	422	11	39	0.985	40	0.02761	11.6515	0.0306	0.03139	-0.5481	-1.072	-1.065
	C3	450	11	23	0.985	23	0.01872	8.4242	0.01692	0.01692	0.4703	-0.3193	-0.1286
	C4	312	10	11	0.955	11	0.00869	2.7121	0.01167	0.01167	-1.0628	-1.4718	-1.552

[a] V1: Movement protein gene, V2: Coat protein gene, C1: Replication-associated protein gene, C2: Transcriptional activator protein gene, C3: Replication enhancer protein gene, C4: C4 protein gene
[b] N: Number of nucleotide sites
[c] h: Haplotype number
[d] S: Total number of variable or segregation sites
[e] Hd: Haplotype diversity
[f] Eta: Total number of mutations
[g] π: Nucleotide diversity
[h] k: Average number of nucleotide differences between sequences
[i] θ-W: Waterson's estimate of population mutation rate based on the total number of segregating sites[j] θ-Eta: Waterson's estimate of population mutation rate based on the total number of mutations

4.5 Discussion

Tomato production in Kenya is widespread and has been limited by the impact of the tomato leaf curl disease. *Tomato yellow leaf curl virus* has always been assumed to be the causal because of the typical yellow leaf curl symptoms commonly associated with tomato in Africa. Indeed, a tomato leaf curl-like virus infecting tomato in Kenya has previously been reported (Nono-Womdim, 2001). The paucity of information on viruses of high economic importance is compounded by the fact that only a few studies from Kenya have described the genomic properties of begomoviruses from cassava (Zhou *et al*., 1998), sweet potato (Miano *et al*., 2006) and on a weed (Kyallo *et al*., 2017). Using a metagenomics approach, this study has described the occurrence of monopartite begomoviruses associated with the leaf curl disease of tomato in Kenya. Results show that several begomoviruses are associated with leaf culr disease in Kenya and moreso a genetically distinct ToLCArV-like is present in tomato crops in Kenya. Analyses of the complete genomes and coding regions of these begomoviruses, together with the failure to detect the presence of DNA-B component affirms that these virus populations were members of the Old World monopartite begomovirus species. Findings of this research represent the first comprehensive description of full ToLCArV-like genomes from tomato in Kenya. This information is crucial for understanding the causal agents associated with the tomato leaf curl disease and its properties as a first step towards appropriate robust disease management. The availability of full genome sequences will help to elucidate further the evolutionary behavior of the virus.

All the Kenyan ToLArV-like sequences obtained in this study, shared very high nucleotide and amino acid sequence similarities, indicating low intra-population genetic diversity. Similar conclusions have been reached in other studies on tomato begomoviruses (Ala-Poikella *et al*.,

2005; Sohrab., 2020). Curiously, it was observed that the nucleotide sequences of the 12 ToLCArV-like isolates shared high identities among themselves but shared lower sequence identities with other begomoviruses. This is likely as a result of the genetic bottleneck imposed through the method of begomovirus transmission by whiteflies (Yang *et al*., 2017). This study did not investigate virus occurrence within vectors, and hence there is need for further research on this aspect. Nevertheless, the high genetic similarity within the population in this study could be due to a 'founder effect' arising from ecological and epidemiological factors such as vector or seed-mediated spread possibly from Tanzania. The derived amino acid sequences of the ToLCArV –like isolates of this study show homologous characteristic with other monopartite begomoviruses, indicating possible similar biological behaviors.

Results from sequence similarity indices, together with phylogenetic inferences, suggest that the ToLCArV-isolates associated with tomato leaf curl diseases in Kenya were likely of Tanzanian origin. The homogeneity of nucleotide and amino acids as well as phylogenetic inferences supports a single introduction of the tomato begomovirus into Kenya. There was no evidence of recombination occurring within our ToLCArV population. Intriguingly, five algorithms detected recombination signals ($P \leq 0.05$) from a Tanzanian ToLCArV isolate (GenBank number DQ519575), identifying one of the Kenyan isolates (GenBank number MN894493) as a major parent (Table 4.8). This suggests that, although the properties of the Kenyan isolates are just being characterized, they could be the parents that contributed to the emergence of ToLCArV which was first described by (Shih *et al*., 2006). Thus, it is possible that the Kenyan ToLCArV population could pre-date the Tanzanian isolates which were then only reported earlier.

Since this study reveals clustering of isolates from geographically proximal countries, the dissemination of the ToLCArV-like isolates is likely to have occurred via virus-infected planting

material or spread by cross-border spread of viruliferous whiteflies, leading to genetic similarity among these isolates. Although, this study did not investigate mode of virus transmission, evidence of seed transmission has recently been reported in other closely related begomovirus species from tomato (Kil *et al*., 2016) and other hosts (Sangeetha *et al*., 2018; Kil *et al*., 2018). Thus, further research is required to understand how specific begomovirus species are spread across various borders in East Africa and to determine the epidemiological and ecological implications. Additionally, further studies to investigate the effect of whitefly-mediated transmission on begomovirus diversity in Kenya should be conducted.

Findings of this research show that ToLCArV –like sequences from Kenya have discernible patterns of geographical structuring with other ToLCV-like isolates of African origin. This is in agreement with previous studies that have shown geographical structuring of African Old World begomovirus sub-populations into clear genetically distinct categories (Delatte *et al*., 2005; Prassana *et al*., 2010). This suggests that these viruses perhaps came from a common ancestor that was introduced to the continent and speciation arose as they interacted with various hosts across different geographical locations. In this study, we determined the genetic diversity of ToLCArV-like sequences from Kenyan within tomato fields using coding regions and complete genome sequences. Over the years, tomato begomoviruses in Kenya have received little or no attention in previous studies (Miano and Kuria., 2017). Findings of this research will deepen the knowledge on genetic diversity of tomato begomoviruses, therefore allowing for better diagnostics and appropriate management options. This study indicates that although there is low intra-specific diversity among our isolates, the haplotype number and haplotype diversity analyses revealed varying homogenous levels within the coding regions. Thus, the non-coding

regions could have contributed to the overall low diversity indices, similar to the observations of (Brown *et al*., 2017).

In this study it was observed that varying natural selection pressures appear to be acting on the coding regions of the Kenya ToLCArV-like isolates, indicating independent coevolution of these genes. Analyses of synonymous and nonsynonymous substitutions revealed that, except the C4 gene, all coding regions appear to be under strong negative or purifying selection to conserve its encoded amino acid sequence. This is in line with similar observations for other related tomato begomovirus species from the Old World (Yang *et al*., 2014) and New World (Malgarejo *et al*., 2013). The evolutionary constraints on these coding regions could be intended to preserve their biological functions which include virus replication, accumulation and fidelity to vector transmission. For example, the CP gene has been reported to mediate interactions between begomoviruses and their whitefly vectors (Briddon *et al*., 1990). Any alterations in the CP sequence could subsequently alter their virus-vector interactions or other associated biological functions (Pan *et al*, 2020). This is probably why this phenomenon is more in the CP region with the lowest mean dN/dS values, indicating that it is undergoing a stronger purifying selection. Other studies have also indicated similar patterns within begomoviruses, with the CP gene having the strongest evolutionary constraint (Padidam *et al*., 1995; Duffy and Holmes., 2009; Mondal *et al*., 2019). dN/dS ratios are estimators of evolutionary bottlenecks imposed on a coding region at intra-specific levels. Due to the natural selection functions largely on these regions, synonymous and nonsynonymous mutations are usually under varying selective pressures and are fixed at different rates within begomovirus genomes (Briddon *et al*., 2010; Gibbs *et al*., 2010). Thus, comparison of synonymous and nonsynonymous substitution rates can reveal the direction and strength of natural selection acting on virus proteins. Importantly, it was

observed that the C4 gene within the Kenyan isolates was selectively neutral as its estimated dN/dS ratio (1.1491) suggesting that neither purifying nor diversifying selection was ongoing. This neutral selection could be as a result of its divergent but crucial role in modulating disease severity, determination of host range, virus movement and suppression of host silencing mechanisms (Jupin *et al*., 1994; Luna *et al*., 2012).

The information obtained in this study will assist in the design and implementation of quarantine plans to manage virus-host dynamics. Sequence information and genetic diversity data obtained in this study are also important for the development of rapid and robust detection tools towards the production of virus-free tomato seedlings for farmers. This will ultimately improve tomato production across the country for better food security.

CHAPTER FIVE

GENETIC DIVERSITY OF WHITEFLY POPULATIONS INFESTING TOMATO CROPS IN KENYA

5.1 Abstract

Whiteflies are agricultural pests with a worldwide distribution. They cause damage to plants through direct feeding, secretion of honey dew and transmission of viruses. Overall, whiteflies contribute to yield loss of about 50% in infested crops. Among the most economically important whiteflies are the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood, 1856) and *Bemisia tabaci* Genn. Management of whiteflies in agroecosystems majorly involve use of pesticides leading to emergence of new biotypes that exhibit pesticide resistance and also leads to reduction of natural enemies. This study was conducted to determine the genetic diversity of whitefly populations colonizing tomato plants in Kenya. A field survey was carried out in seven major tomato growing regions in Kenya between September and December 2018 and January to March 2019. A total of 259 farms were sampled and in each farm thirty plants were randomly examined for the presence of whiteflies. Adult whiteflies were collected using a hand held aspirator into a vial. The whiteflies were preserved in 95% ethanol and stored at 4 ºC before molecular analysis. DNA extraction was done using Chelex procedure and Sanger sequencing done at Macrogen Europe. Sequences were analyzed using Bioedit software. One hundred and sixty three (163) sequences were deposited in the Gene bank under accession number MZ191168 to MZ191330. BLASTn similarities of all sequences with those in the database revealed 98.93-99.75% identities with *T. vaporariorum.* A phylogenetic tree was constructed using Maximum likelihood method and Kamura 2 parameter model. Population genetic analysis and neutrality tests were performed using DnaSp software and median joining network evaluated using NETWORK software. These sequences with other *T. vaporariorum* sequences obtained from the gene bank formed one monophyletic clade indicating lack of intraspecies diversity. One hundred and fourteen (114) haplotypes were identified, demographic analysis of the whitefly populations revealed negative Tajima's D, Fu and Li tests indicating population expansions. Median joining networks indicated that dominant haplotypes exists amongst the populations in all the Counties. This study concluded that, tomato plants in Kenya are colonized by *T.vaporariorum* and there was no genetically diversity amongst the existing populations. *T. vaporariorum* present in tomato crops in Kenya is similar to those reported in other parts of the world. This information is useful in developing management strategies that will enhance tomato production in Kenya.

5.2 Introduction

Whiteflies are agricultural pests with a worldwide distribution (Lapidot *et al*., 2014). Their agricultural importance is through direct feeding of both immature and mature stages, transmission of pathogenic viruses, secretion of honeydew which is suitable for growth of sooty moldy fungi on fruits and leaf surfaces, thus limiting photosynthesis and lowers quality of fruits and fiber (Boykin and De Barro, 2014). Overall, whiteflies contribute to yield loss of about 50% in infested crops (Khamis *et al*., 2021). Among the most economically important whiteflies are the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood, 1856) and *Bemisia tabaci* Genn. In the genus *Trialeurodes, Trialeurodes abutilonea, Trialeurodes vaporariorum*, and *Trialeurodes ricini* transmit viruses (Fiallo-Olivé *et al*., 2020). *Trialeurodes vaporariorum* affects numerous ornamental crops and vegetables and transmits a number of plant viruses belonging to the genera *Crinivirus* and *Torradovirus*, both genera in the family Closteroviridae (Navas-Castillo *et al*., 2011; Navas-Castillo *et al*., 2014). Of these, the *Criniviruses*, *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV) are of major economic importance in tomato production globally (Wintermantel *et al*., 2009). In Africa TICV has been reported in Morocco, South Africa and Sudan, while ToCV has been reported in many African countries including Kenya (Kimathi *et al*., 2020).

Trialeurodes vaporariorum is considered a single species based on sequences of mtCO1 and internal transcribed spacer (ITS) genes. Phylogenetic analysis of mtCO1 sequences also reveals one monophyletic clade (Roopa *et al*., 2012; Kipantaidaki *et al*., 2015).

In the genus *Bemisia, B. tabaci* (Genn.) is a species complex that has been implicated to spread about 111 virus species belonging to several genera such as; *Begomovirus* (90%), *Crinivirus* (6%), *Closterovirus, Carlavirus,* as well as *Ipomovirus* (Liu *et al*., 2012; Weng *et al*., 2013;

Fahmy and Abou-Ali 2015). Significant yield losses are associated with *B. tabaci* transmitted viruses. Management of whiteflies in agroecosystems majorly involve use of pesticides leading to reduction of natural enemies and emergence of new biotypes that exhibit pesticide resistance (Fahmy and Abou-Ali 2015).

Phylogenetic relationships of *B.tabaci* isolates reported globally has been resolved into approximately 40 cryptic putative species. In Africa, *B. tabaci* species Mediterranean (MED), (East Africa 1 (EA1), Middle East-Asia Minor (MEAM1, MEAM2-Africa), Morocco, Indian Ocean (IO), New World (NW1)-Sudan and sub-Saharan Africa (SSA) species SSA1-SSA13 have been reported on host plants such as cassava, tomato, beans, eggplant, sweet potato, cotton, or several uncultivated plants (Frohlich *et al*., 1999; Berry *et al*., 2004; Sseruwagi *et al*., 2006; Boykin *et al*., 2012; Esterhuizen *et al*., 2013; Mugerwa *et al*., 2012, 2018). These whitefly species exhibit differences in their geographical location, host plants and ability to transmit viruses (Ssweruwagi *et al*., 2006; Tocko-Marabena *et al*., 2017).

Whitefly species identification can be done using morphological or molecular techniques. However, morphological identification is not sufficient to provide information on existing biotypes in an agroecosystem. Molecular techniques such as DNA barcoding of mtCO1 gene have been used to identify genetic variability in whiteflies (Shah *et al*., 2013; Roopa *et al*., 2012; Wainaina *et al*., 2018; Mugerwa *et al*., 2018). This technique utilizes a short standardized DNA sequence in insects i.e. a 658bp fragment of the mitochondrial cytochrome oxidase (CO1) gene and it is useful in discriminating closely and distantly related taxa (Herbert *et al.*, 2003; Herbert *et al.*, 2004; Smith *et al.*,2008). The method has been used globally in studying genetic diversity in insect and virus vectors (Gariepy *et al.*, 2007). This is mainly because mitochondrial gene such as CO1 lack introns and has less exposure to recombination since most of the nucleotide

substitutions occur at neutral sites, moreover it is maternally inherited with reliable inter specific variations, as compared to other markers (Saccone *et al.,* 1999; Savolainen *et al.*, 2005).

Recently, there have been concerns on the use of mtCO1 in identification of cryptic species of *B.tabaci*, studies indicate that we have errors in classifications that arise from chimeric PCR products or nuclear mitochondrial DNA (NUMTs) and also species status does not correlate reliably with differences in the mtCO1 barcode region (Tay *et al*., 2017; Vyskočilová *et al*., 2018; de Moya *et al*., 2019; Kunz *et al*., 2019; Mugerwa *et al*., 2021). And as such, techniques like SNP-genotyping using NextRAD sequencing have been recommended to be more reliable in distinguishing *Bemisia tabaci* genotypes. In spite of these concerns scientists have continued to use us this method to identify whitefly species colonizing crops and weeds (Mugerwa *et al*., 2021). Identification of a species is achieved through comparison of the sequences to a reference database through similarity methods such as BLAST (Lee *et al.*, 2010). This study investigates the diversity of whitefly populations colonizing tomato plants by evaluating the evolutionary relationship based on sequence variation of the mitochondrial cytochrome oxidase-I (CO-I) gene, pairwise nucleotide identities, population genetics and haplotype analyses.

5.3 Materials and methods

5.3.1 Collection of whitefly populations from tomato fields

A field survey was carried out between July 2018 and March 2020 to determine the whitefly species associated with tomato crops in different tomato growing regions in Kenya. The study was conducted in major tomato growing regions in Kenya in seven Counties, Bungoma, Baringo, Taita Taveta, Nakuru, Meru, Kirinyaga and Kajiado (**Table 5.1**). These geographical regions have different agroecological zones and climatic conditions. Adult whitefly samples were collected from tomato crops at different growth stages. Within each tomato field 30 plants were randomly selected on an X-shaped transect and the underneath of top five leaves checked for presence of adult whitefly populations. Five to ten adult whiteflies were collected using a hand held mouth aspirator (JohnW. Hock Company, Gainesville, FL, USA), and aspirated directly into a falcon tube and 95% ethanol added to preserve the whiteflies. Information on the name of the County, sub-County and field number and the corresponding geographical coordinates using Global Positioning System (GPS) were recorded for each field. The number and distribution of collection sites varied depending on the number of tomato fields that were found in each region. The collected whiteflies were transported to Kenya Plant Health Inspectorate Service, Plant Quarantine and Biosecurity Laboratory at Muguga and stored at 4 ºC pending molecular analysis.

5.3.2 DNA extraction from whitefly adults

Genomic DNA was extracted from individual insect collected from all the sites using Chelex modified (White *et al*., 2009). Five adult whiteflies from each sample were used in genomic DNA extraction. Briefly, a single insect previously preserved in 95% ethanol was removed using a 200 µl pipette tip (Gilson, Middleton, WI, USA) and dried out on a clean tissue paper for 20

seconds. The single insect was then crushed using a sterile micropestle in 1.5 mL Eppendorf tube containing lysis buffer in 120 µl TE solution (10 mM Tris–HCl and 1 mM EDTA, pH 8.0) (FisherBiotech, Bridgewater, NJ, USA) containing 20% Chelex (BIO-RAD, Watford, UK) and 300 µg proteinase K (Jena Bioscience, Dortmund, Germany). The mixture was then vortexed and spun down on a microcentrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). This was followed by incubation at 60 °C for 15 min then protein denaturation at 96 °C for 10 min using Grant SUB Aqua 18 machine (Grant Instruments, Cambridge, England). The lysis mixture was centrifuged at 13,000 rpm for 10 min in a microcentrifuge and the lysate collected and stored at -20 °C for downstream use. Determination of the DNA quantity and quality was done using NanodropONE (Thermo Scientific, Wilmington, DE, USA).

5.3.3 PCR amplification of the extracted whitefly DNA

DNA extracted from 163 individual whiteflies from all the sampled locations was used for PCR amplification. PCR was undertaken using universal primers that amplify the mitochondrial cytochrome oxidase -1 gene. The primers used were, forward LCO 1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and reverse was HCO 2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') as described by Macharia *et al*. (2015). The PCR reaction was carried out in 25 µl volume containing 2.5 µl DNA template, 12.5 µl OneTaq 2 × MM with standard buffer (New England biolabs), 0.5 µl (10 pMol) of both forward and reverse primer. The PCR reaction was carried out on an Eppendorf master cycler machine pro (Eppendorf, Hamburg Germany) under the following conditions; initial denaturation at 94°C for 5 min, 35 cycles of 30s at 94°C denaturation, 30s at 53°C annealing, 1 min at 72°C extension and a final extension of 72°C for 10 min. The expected amplicon size ranged between 700-800bp (Boykin and De Barro 2014).

5.3.4 Gel electrophoresis and Purification of PCR products

The PCR amplicons were separated on 2% agarose gels pre stained with Gel red (Biotium, CA, USA) and run in 0.5 ×Tris Boric EDTA (TBE) electrophoresis buffer for 60 minutes at 100 Volts and gel bands visualized in a C280 gel documentation system (Azure Biosytems, Dublin, CA, USA). Estimation of expected amplicon band sizes was done by comparison to a direct Load PCR 1kb-bp ladder (Bioline). PCR products were purified using Gene JET PCR purification kit. (Thermo Scientific cat K0702; Thermoscientific, Vilnius, Lithuania) according to manufacturer's instructions. Quantification of the purified PCR products was performed using Nano drop One (Thermo Scientific,Wilmington, DE, USA). Purified PCR products were submitted for bi-directional at Sanger sequencing unit at Macrogen (Macrogen Europe, B.V, Amsterdam, The Netherlands). All the 163 whitefly samples were successfully sequenced and used for the downstream analysis.

5.3.4 Sequence Alignment

A total of n=163 forward and reverse sequences of the 3' end of mtCOI were obtained after sequencing. Raw chromatograms were inspected manually across all the bases, using Bioedit v.7.2.3 software and ambiguities corrected. Consensus sequences were generated for each whitefly, and used for subsequent analysis. The resulting consensus sequences (696 bp) were compared and identified using sequences in the gene bank database using Basic local alignment (BLAST) program (Altschul *et al.*, 1990). The sequences were subsequently submitted to GenBank for accession number curation while other sequences homologous to the mtCOI genes were retrieved from the database.

5.3.5 Phylogeny and Network analyses

To assess phylogenetic relationship amongst the isolates, we utilized twenty eight mtCO1 consensus sequences randomly selected across the sampled areas with other isolated reported in other parts of the world. Sequences of *Bemisia tabaci* (Accession number KY523874; KY523884) from Bungoma and Kilifi respectively, were used outgroups. Multiple sequence alignment was done using Clustal W program in MEGA 7.0 software with default parameters. The final dataset composed of 36 sequences was trimmed to 656 bp and used in contruction of a phylogenetic tree, using Maximum Likelihood method and Kimura 2-parameter model in MEGA 7.0 software (Tamura *et al*., 2013). Bootstrap values were set at 1,000 iterations.

5.3.6 Haplotype Network analyses

Haplotypes networks based on mtCOI sequences of mtCO1 of *T. vaporarorium* sequences ofbtained per County was calculated using the median-joining method in NETWORK v.5.0 (Bandelt *et al*., 1999) under default parameters.

5.3.7 Pairwise nucleotide identities, population genetics and haplotype analyses

All the sequences were aligned using ClustalW multiple sequence alignment program with default parameters as implemented in BioEdit v.7.2.3 program (Hall, 1999). The aligned file outputs were used for the sequence pairwise identities performed using SDT v1.2 (Muhire *et al.,* 2014) with pairwise gap deletions. The structure, diversity and dynamics within the *T. vaporariorum* populations across the various sampled Counties in Kenya were investigated to identify possible evolutionary interplay that produces genetic variations. The parameters estimated include number of polymorphic sites (S), haplotype diversity (Hd), average nucleotide diversity (π), average number of nucleotide differences between sequences (k),total number of mutations (Eta), the statistic estimate of population mutation based on the number of segregating

sites (θ-W) and the statistic estimate of population mutation based on the total number of mutations (θ-Eta). Additionally, haplotype differentiations and frequencies were also calculated within each *T. vaporariorum* population. All population genetics and haplotype analyses were estimated in DnaSP v5.10.01 (Librado and Rozas, 2009).

5.3.8 Neutrality and demographic history

Statistical tests originally designed to assess the selective neutrality of mutations have been implemented to test for demographic expansion in recent years (Ramos-Onsins, S.E., and Rozas, J. 2002). These tests are intended to distinguish between neutrally evolving sequences under mutation drift equilibrium, and sequences evolving under non-neutral processes including directional or balancing selection, and demographic expansion or contraction. In order to test for past population expansion, we used three statistical tests commonly used to analyze demographic events. Tajima's D statistical test (Ramírez-Soriano *et al*., 2008) was used to evaluate the nucleotide polymorphism occurring within the isolates while Fu and Li's Fs (Fu and Li 1993) were used to compare the number of derived singleton mutations and mean pairwise differences between sequences and Fu and Li's D which compares the number of derived singleton mutations and the total number of derived nucleotide variants, analyses were estimated in DnaSP v5.10.01 (Librado and Rozas, 2009).

5. 4 Results

5.4.1 Sequence properties and pairwise similarities of partial mtCOI sequences

Amplification of the CO1 gene resulted in fragment of ~ 696bp (**Fig. 5.1**) for each of the individual adult whitefly. From the forward and reverse sequences we obtained partial mtCOI sequences of 163 individual whiteflies and were assigned GenBank accession numbers MZ191168 to MZ191330 (**Table 5.1**). The BLASTn similarities of all sequences with those in

the database revealed 98.93-99.75% identities with *T. vaporariorum* and produced statistical E-values of 0.0 and alignment scores of ≥200. The highest query coverage of the partial CO1 genomes ranged from 97 to 100%. For the *T. vaporariorum* populations obtained from Meru, identities ranged from 98.71 to 100% (**Figure 5.2**) while samples from Kajiado were 98.99 – 99.86 % similar (**Figure 5.3**). **Figure 5.4** revealed 99.14 - 100% similarities among the whitefly samples from Bungoma while isolates were 97.69 – 100% identical among those from Baringo County (**Figure 5.5**). Identities from Nakuru, Taveta and Kirinyaga Counties ranged from 98.56 - 100, 98.85 - 100 and 96.83 - 100% respectively (**Figure 5.6-5.8**). All these reveal very low genetic diversity within the *T. vaporariorum* populations sampled across the seven countries in Kenya.

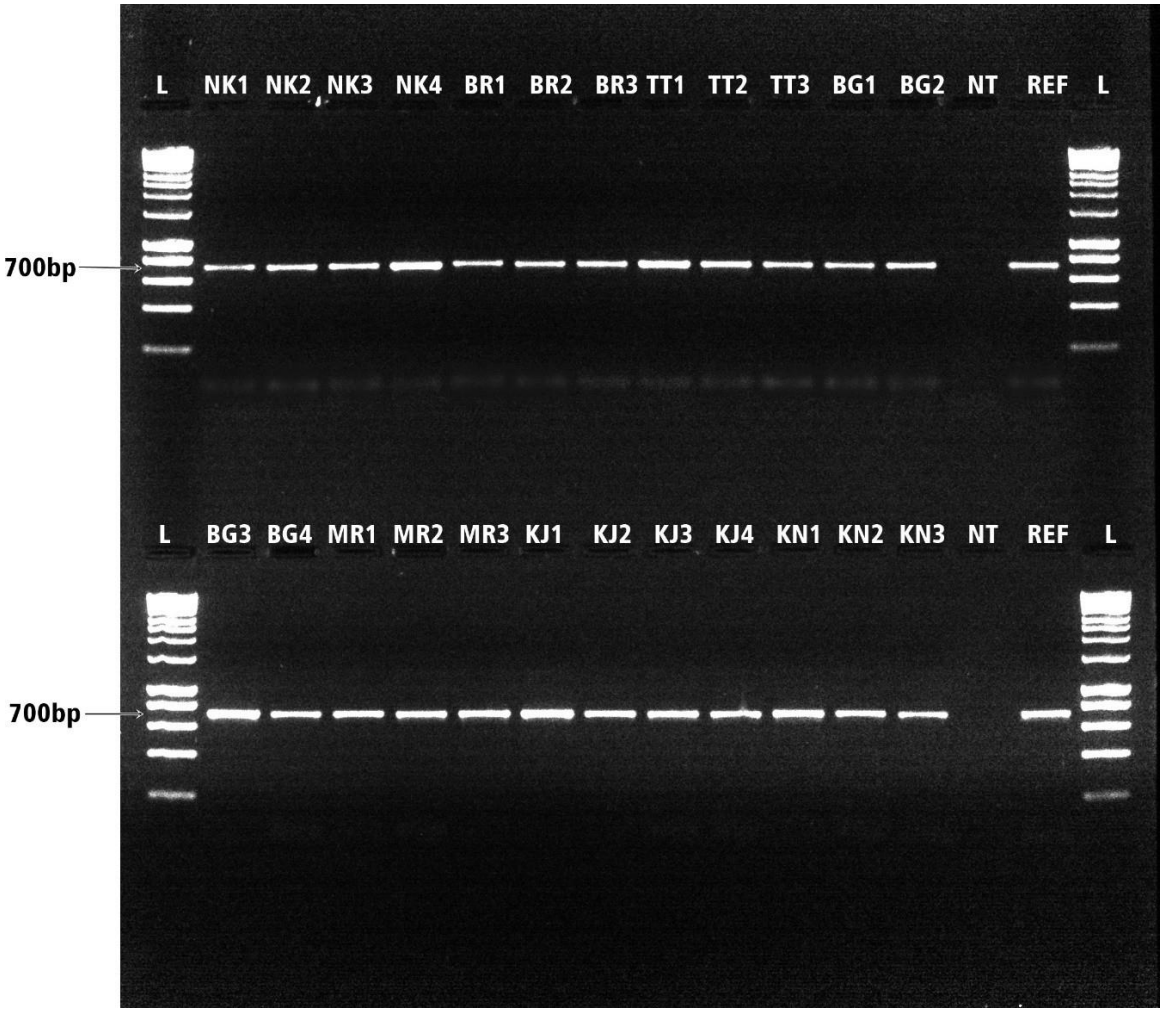


Figure 5. 1 Polymerase chain reaction amplified product (~696bp) of *T. vaporariarum*

NK- Whitefly samples from Nakuru; TT- Whitefly samples from Taita Taveta, MR-Whitefly samples from Meru; KN-Whitefly samples from Kirinyaga; BR-Whitefly samples from Baringo; BG-Whitefly samples from Bungoma; NT- Non template; REF- Positive control

Table 5.1: Summary of Trialeurodes vaporariorum collected from tomato crops in Kenya

Sample Number	Sample ID	County	Area	Latitude	Longitude	Altitude	Identification	Accession Number
1	Bu-Si-BG01A_Kenya	Bungoma	Sirisia	0.780611	34.52744	1572.9 4.78	T. Vaporariorum	MZ191168
2	Bu-Si-BG01B_Kenya	Bungoma	Sirisia	0.780611	34.52744	1572.9 4.78	T. Vaporariorum	MZ191169
3	Bu-Si-BG01C_Kenya	Bungoma	Sirisia	0.780611	34.52744	1572.9 4.78	T. Vaporariorum	MZ191170
4	Bu-Si-BG01D_Kenya	Bungoma	Sirisia	0.780611	34.52744	1572.9 4.78	T. Vaporariorum	MZ191171
5	Bu-Sa-BG06A_Kenya	Bungoma	Saboti	0.900223	34.84998	1821.3 4.85	T. Vaporariorum	MZ191172
6	Bu-Sa-BG06B_Kenya	Bungoma	Saboti	0.900223	34.84998	1821.3 4.85	T. Vaporariorum	MZ191173
7	Bu-Sa-BG06C_Kenya	Bungoma	Saboti	0.900223	34.84998	1821.3 4.85	T. Vaporariorum	MZ191174
8	Bu-Sa-BG06D_Kenya	Bungoma	Saboti	0.900223	34.84998	1821.3 4.85	T. Vaporariorum	MZ191175
9	Bu-Sa-BG09A_Kenya	Bungoma	Saboti	0.945303	34.8113	2004.3 4.6	T. Vaporariorum	MZ191176
10	Na-Su-W54A_Kenya	Nakuru	Subukia	0.047077	36.21748	1971.5 4.5	T. Vaporariorum	MZ191177
11	Na-Su-W54B_Kenya	Nakuru	Subukia	0.047077	36.21748	1971.5 4.5	T. Vaporariorum	MZ191178
12	Ba-Mo-W68A_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191179
13	Ba-Mo-W68B_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191180
14	Ba-Mo-W68C_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191181
15	Ba-Mo-W68D_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191182
16	Ba-Mo-W68E_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191183
17	Na-So-WF15A_Kenya	Nakuru	Solai	0.274265	37.55384	1360.6 4.9	T. Vaporariorum	MZ191184
18	Na-So-WF47A_Kenya	Nakuru	Solai	-0.10892	36.145	1973.8 2.9	T. Vaporariorum	MZ191185
19	Na-So-WF49B_Kenya	Nakuru	Solai	-0.10892	36.145	1973.8 2.9	T. Vaporariorum	MZ191186
20	Na-So-WF49C_Kenya	Nakuru	Solai	-0.10892	36.145	1973.8 2.9	T. Vaporariorum	MZ191187
21	Na-So-WF49D_Kenya	Nakuru	Solai	-0.10892	36.145	1973.8 2.9	T. Vaporariorum	MZ191188
22	Na-Vi-WF50B_Kenya	Nakuru	Visoi	-0.12075	35.92693	1758.4 4.9	T. Vaporariorum	MZ191189
23	Na-Vi-WF50C_Kenya	Nakuru	Visoi	-0.12075	35.92693	1758.4 4.9	T. Vaporariorum	MZ191190

Table 5.1 conti'

Sample Number	Sample ID	County	Area	Latitude	Longitude	Altitude	Identification	Accession Number
24	Na-Vi-WF50D_Kenya	Nakuru	Visoi	-0.12075	35.92693	1758.4 4.9	T. Vaporariorum	MZ191191
25	Na-Vi-WF50E_Kenya	Nakuru	Visoi	-0.12075	35.92693	1758.4 4.9	T. Vaporariorum	MZ191192
26	Na-Vi-WF50F_Kenya	Nakuru	Visoi	-0.12075	35.92693	1758.4 4.9	T. Vaporariorum	MZ191193
27	Na-So-WF51A_Kenya	Nakuru	Solai	-0.0757	36.11947	1776.5 4.6	T. Vaporariorum	MZ191194
28	Na-So-WF51B_Kenya	Nakuru	Solai	-0.0757	36.11947	1776.5 4.6	T. Vaporariorum	MZ191195
29	Na-So-WF51C_Kenya	Nakuru	Solai	-0.0757	36.11947	1776.5 4.6	T. Vaporariorum	MZ191196
30	Na-So-WF52A_Kenya	Nakuru	Solai	-0.09261	36.10942	1776.3 4.8	T. Vaporariorum	MZ191197
31	Na-So-WF52B_Kenya	Nakuru	Solai	-0.09261	36.10942	1776.3 4.8	T. Vaporariorum	MZ191198
32	Na-So-WF52C_Kenya	Nakuru	Solai	-0.09261	36.10942	1776.3 4.8	T. Vaporariorum	MZ191199
33	Na-So-WF52D_Kenya	Nakuru	Solai	-0.09261	36.10942	1776.3 4.8	T. Vaporariorum	MZ191200
34	Na-So-WF52E_Kenya	Nakuru	Solai	-0.09261	36.10942	1776.3 4.8	T. Vaporariorum	MZ191201
35	Na-We-WF54A_Kenya	Nakuru	Wei	0.047077	36.21748	1971.5 4.5	T. Vaporariorum	MZ191202
36	Na-We-WF54E_Kenya	Nakuru	Wei	0.047077	36.21748	1971.5 4.5	T. Vaporariorum	MZ191203
37	Ba-Ki-WF56C_Kenya	Baringo	Kipsogon	-0.07219	35.92367	1679.5 2.5	T. Vaporariorum	MZ191204
38	Ba-Ki-WF56D_Kenya	Baringo	Kipsogon	-0.07219	35.92367	1679.5 2.5	T. Vaporariorum	MZ191205
39	Ba-Ki-WF58A_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191206
40	Ba-Ki-WF58B_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191207
41	Ba-Ki-WF58C_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191208
42	Ba-Ki-WF58D_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191209
43	Ba-Ki-WF58F_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191210
44	Ba-Ki-WF59A_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191211
45	Ba-Ki-WF59C_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191212

Table 5.1 Conti'

Sample Number	Sample ID	County	Area	Latitude	Longitude	Altitude	Identification	Accession Number
46	Ba-Ki-WF59D_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191213
47	Ba-Ki-WF59E_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191214
48	Ba-Ki-WF59F_Kenya	Baringo	Kipsogon	-0.05654	35.93843	1625.5 4.9	T. Vaporariorum	MZ191215
49	Ba-Mo-WF60A_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191216
50	Ba-Mo-WF60C_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191217
51	Ba-Mo-WF60D_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191218
52	Ba-Mo-WF60E_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191219
53	Ba-Mo-WF60F_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191220
54	Ba-Mo-WF62A_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191221
55	Ba-Mo-WF62B_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191222
56	Ba-Mo-WF62C_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191223
57	Ba-Mo-WF62D_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191224
58	Ba-Mo-WF62E_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191225
59	Ba-Mo-WF62F_Kenya	Baringo	Mogotio	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191226
62	Na-Gi-WF64A_Kenya	Nakuru	Gicheha	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191227
63	Na-Gi-WF64B_Kenya	Nakuru	Gicheha	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191228
64	Na-Gi-WF64C_Kenya	Nakuru	Gicheha	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191229
65	Na-Gi-WF64D_Kenya	Nakuru	Gicheha	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191230
66	Na-Gi-WF64E_Kenya	Nakuru	Gicheha	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191231
67	Na-Gi-WF64F_Kenya	Nakuru	Gicheha	0.005857	35.97355	1553.9 3.0	T. Vaporariorum	MZ191232
68	Na-Gi-WF66A_Kenya	Nakuru	Gicheha	-0.19814	5.874368	1875.3 4.3	T. Vaporariorum	MZ191233
69	Na-Gi-WF66B_Kenya	Nakuru	Gicheha	-0.19814	5.874368	1875.3 4.3	T. Vaporariorum	MZ191234

Table 5.1 Conti'

Sample Number	Sample ID	County	Area	Latitude	Longitude	Altitude	Identification	Accession Number
70	Na-Gi-WF66C_Kenya	Nakuru	Gicheha	-0.19814	5.874368	1875.3 4.3	T. Vaporariorum	MZ191235
71	Na-Gi-WF66D_Kenya	Nakuru	Gicheha	-0.19814	5.874368	1875.3 4.3	T. Vaporariorum	MZ191236
72	Na-Gi-WF66E_Kenya	Nakuru	Gicheha	-0.19814	5.874368	1875.3 4.3	T. Vaporariorum	MZ191237
73	Na-Gi-WF66F_Kenya	Nakuru	Gicheha	-0.19814	5.874368	1875.3 4.3	T. Vaporariorum	MZ191238
74	Me-Bu-WF1A_Kenya	Meru	Buuri	-0.09153	37.6854	1374.2 4.8	T. Vaporariorum	MZ191239
75	Me-Bu-WF1B_Kenya	Meru	Buuri	-0.09153	37.6854	1374.2 4.8	T. Vaporariorum	MZ191240
76	Me-Bu-WF1C_Kenya	Meru	Buuri	-0.09153	37.6854	1374.2 4.8	T. Vaporariorum	MZ191241
77	Me-Bu-WF1D_Kenya	Meru	Buuri	-0.09153	37.6854	1374.2 4.8	T. Vaporariorum	MZ191242
78	Me-Ny-WF2B_Kenya	Kirinyaga	Nyangata	-0.5903	37.35516	1290.8 3.4	T. Vaporariorum	MZ191243
79	Me-Ny-WF2C_Kenya	Kirinyaga	Nyangata	-0.5903	37.35516	1290.8 3.4	T. Vaporariorum	MZ191244
80	Me-Mw-WF3A_Kenya	Kirinyaga	Mwea	-0.5903	37.35516	1290.8 3.4	T. Vaporariorum	MZ191245
81	Me-Mw-WF3B_Kenya	Kirinyaga	Mwea	-0.5903	37.35516	1290.8 3.4	T. Vaporariorum	MZ191246
82	Me-Mw-WF3C_Kenya	Kirinyaga	Mwea	-0.5903	37.35516	1290.8 3.4	T. Vaporariorum	MZ191247
83	Me-Mw-WF3G_Kenya	Kirinyaga	Mwea	-0.5903	37.35516	1290.8 3.4	T. Vaporariorum	MZ191248
84	Me-Mw-WF3H_Kenya	Kirinyaga	Mwea	-0.5903	37.35516	1290.8 3.4	T. Vaporariorum	MZ191249
85	Me-Mw-WF4B_Kenya	Kirinyaga	Mwea	-0.61157	37.34238	1217.2 4.6	T. Vaporariorum	MZ191250
86	Me-Mw-WF4H_Kenya	Kirinyaga	Mwea	-0.61157	37.34238	1217.2 4.6	T. Vaporariorum	MZ191251
87	Me-Km-WF5D_Kenya	Kirinyaga	Kimbimbi	-0.6247	37.37162	1203.6 4.3	T. Vaporariorum	MZ191252
88	Me-Km-WF5H_Kenya	Kirinyaga	Kimbimbi	-0.6247	37.37162	1203.6 4.3	T. Vaporariorum	MZ191253
89	Me-Mw-WF6B_Kenya	Kirinyaga	Mwea	-0.63653	37.37067	1184.3 3.6	T. Vaporariorum	MZ191254
90	Me-Mw-WF6C_Kenya	Kirinyaga	Mwea	-0.63653	37.37067	1184.3 3.6	T. Vaporariorum	MZ191255
91	Me-Mw-WF7A_Kenya	Kirinyaga	Mwea	-0.63868	37.3952	1153.7 4.8	T. Vaporariorum	MZ191256

Table 5.1 Conti'

Sample Number	Sample ID	County	Area	Latitude	Longitude	Altitude	Identification	Accession Number
92	Me-Mw-WF7B_Kenya	Kirinyaga	Mwea	-0.63868	37.3952	1153.7 4.8	T. Vaporariorum	MZ191257
93	Me-Mw-WF7C_Kenya	Kirinyaga	Mwea	-0.63868	37.3952	1153.7 4.8	T. Vaporariorum	MZ191258
94	Me-Mw-WF7D_Kenya	Kirinyaga	Mwea	-0.63868	37.3952	1153.7 4.8	T. Vaporariorum	MZ191259
95	Me-Mw-WF8A_Kenya	Kirinyaga	Mwea	-0.64277	37.39909	1149.4 3.0	T. Vaporariorum	MZ191260
96	Me-Mw-WF8C_Kenya	Kirinyaga	Mwea	-0.64277	37.39909	1149.4 3.0	T. Vaporariorum	MZ191261
97	Me-Mw-WF8D_Kenya	Kirinyaga	Mwea	-0.64277	37.39909	1149.4 3.0	T. Vaporariorum	MZ191262
98	Me-Mw-WF9A_Kenya	Kirinyaga	Mwea	-0.64189	37.41017	1138.4 4.2	T. Vaporariorum	MZ191263
99	Me-Mw-WF9B_Kenya	Kirinyaga	Mwea	-0.64189	37.41017	1138.4 4.2	T. Vaporariorum	MZ191264
100	Me-Mw-WF9C_Kenya	Kirinyaga	Mwea	-0.64189	37.41017	1138.4 4.2	T. Vaporariorum	MZ191265
101	Me-Mw-WF9D_Kenya	Kirinyaga	Mwea	-0.64189	37.41017	1138.4 4.2	T. Vaporariorum	MZ191266
102	Me-Mw-WF9E_Kenya	Kirinyaga	Mwea	-0.64189	37.41017	1138.4 4.2	T. Vaporariorum	MZ191267
103	Me-Mw-WF9F_Kenya	Kirinyaga	Mwea	-0.64189	37.41017	1138.4 4.2	T. Vaporariorum	MZ191268
104	Me-Mw-Mu10A_Kenya	Kirinyaga	Mwea	-0.61744	37.44548	1184.8 3.9	T. Vaporariorum	MZ191269
105	Me-Mw-EA11A_Kenya	Kirinyaga	Mwea	-0.69501	37.39139	1129.2 3.4	T. Vaporariorum	MZ191270
106	Me-Mw-EA11B_Kenya	Kirinyaga	Mwea	-0.69501	37.39139	1129.2 3.4	T. Vaporariorum	MZ191271
107	Me-Mw-EA11C_Kenya	Kirinyaga	Mwea	-0.69501	37.39139	1129.2 3.4	T. Vaporariorum	MZ191272
108	Me-Mw-EA11D_Kenya	Kirinyaga	Mwea	-0.69501	37.39139	1129.2 3.4	T. Vaporariorum	MZ191273
109	Me-Mw-EA11E_Kenya	Kirinyaga	Mwea	-0.69501	37.39139	1129.2 3.4	T. Vaporariorum	MZ191274
110	Me-Mw-KU12A_Kenya	Kirinyaga	Kiumbu	-0.72505	37.45103	1107.3 3.7	T. Vaporariorum	MZ191275
111	Me-Mw-KU12B_Kenya	Kirinyaga	Kiumbu	-0.72505	37.45103	1107.3 3.7	T. Vaporariorum	MZ191276
112	Me-Mw-KU12D_Kenya	Kirinyaga	Kiumbu	-0.72505	37.45103	1107.3 3.7	T. Vaporariorum	MZ191277
113	Me-Mw-KU12E_Kenya	Kirinyaga	Kiumbu	-0.72505	37.45103	1107.3 3.7	T. Vaporariorum	MZ191278

Table 5.1 Conti'

Sample Number	Sample ID	County	Area	Latitude	Longitude	Altitude	Identification	Accession Number
114	Me-Mw-KU12F_Kenya	Kirinyaga	Kiumbu	-0.72505	37.45103	1107.3 3.7	T. Vaporariorum	MZ191279
115	Me-Me-WF13A_Kenya	Meru	Meru	0.215822	37.52612	1625.6 4.7	T. Vaporariorum	MZ191280
116	Me-Me-WF13D_Kenya	Meru	Meru	0.215822	37.52612	1625.6 4.7	T. Vaporariorum	MZ191281
117	Me-Me-WF13E_Kenya	Meru	Meru	0.215822	37.52612	1625.6 4.7	T. Vaporariorum	MZ191282
118	Me-Me-WF14A_Kenya	Meru	Meru	0.21572	37.52608	1617.8 4.8	T. Vaporariorum	MZ191283
119	Me-Me-WF14E_Kenya	Meru	Meru	0.21572	37.52608	1617.8 4.8	T. Vaporariorum	MZ191284
120	Me-Me-WF15A_Kenya	Meru	Meru	0.261963	37.52346	1444.9 5.0	T. Vaporariorum	MZ191285
121	Me-Me-WF15B_Kenya	Meru	Meru	0.261963	37.52346	1444.9 5.0	T. Vaporariorum	MZ191286
122	Me-Me-WF15F_Kenya	Meru	Meru	0.261963	37.52346	1444.9 5.0	T. Vaporariorum	MZ191287
123	Me-Ke-WF16B_Kenya	Kirinyaga	Kerugoya	-0.5451	37.31082	1355.1 2.9	T. Vaporariorum	MZ191288
124	Me-Ki-WF17C_Kenya	Kirinyaga	Kianjogu	-0.55161	37.30437	1365.1 5.0	T. Vaporariorum	MZ191289
125	Ka-Ro-WF7A_Kenya	Kajiado	Rombo	-3.06002	37.72437	1124.3 3.6	T. Vaporariorum	MZ191290
126	Ka-Ro-WF7B_Kenya	Kajiado	Rombo	-3.06002	37.72437	1124.3 3.6	T. Vaporariorum	MZ191291
127	Ka-Ro-WF7C_Kenya	Kajiado	Rombo	-3.06002	37.72437	1124.3 3.6	T. Vaporariorum	MZ191292
128	Ka-Ro-WF7D_Kenya	Kajiado	Rombo	-3.06002	37.72437	1124.3 3.6	T. Vaporariorum	MZ191293
129	Ka-Ro-WF7E_Kenya	Kajiado	Rombo	-3.06002	37.72437	1124.3 3.6	T. Vaporariorum	MZ191294
130	Ka-Ro-WF7F_Kenya	Kajiado	Rombo	-3.06002	37.72437	1124.3 3.6	T. Vaporariorum	MZ191295
131	Ka-Nj-WNA_Kenya	Taita Taveta	Njukini	-3.19576	37.71333	1000.9 4.5	T. Vaporariorum	MZ191296
132	Ka-Nj-WNB_Kenya	Taita Taveta	Njukini	-3.19576	37.71333	1000.9 4.5	T. Vaporariorum	MZ191297
133	Ka-Nj-WND_Kenya	Taita Taveta	Njukini	-3.19576	37.71333	1000.9 4.5	T. Vaporariorum	MZ191298

Table 5.1 Conti'

Sample Number	Sample ID	County	Area	Latitude	Longitude	Altitude	Identification	Accession Number
134	Ka-Nj-WNF_Kenya	Taita Taveta	Njukini	-3.19576	37.71333	1000.9 4.5	T. Vaporariorum	MZ191299
135	Ka-Nj-WNG_Kenya	Taita Taveta	Njukini	-3.19576	37.71333	1000.9 4.5	T. Vaporariorum	MZ191300
136	Ka-Ro-WF6A_Kenya	Kajiado	Rombo	-3.03541	37.68649	1214.7 3.5	T. Vaporariorum	MZ191301
137	Ka-Ro-WF6B_Kenya	Kajiado	Rombo	-3.03541	37.68649	1214.7 3.5	T. Vaporariorum	MZ191302
138	Ka-Ro-WF6C_Kenya	Kajiado	Rombo	-3.03541	37.68649	1214.7 3.5	T. Vaporariorum	MZ191303
139	Ka-Ro-WF6D_Kenya	Kajiado	Rombo	-3.03541	37.68649	1214.7 3.5	T. Vaporariorum	MZ191304
140	Ka-Ro-WF6E_Kenya	Kajiado	Rombo	-3.03541	37.68649	1214.7 3.5	T. Vaporariorum	MZ191305
141	Ka-Ro-WF6F_Kenya	Kajiado	Rombo	-3.03541	37.68649	1214.7 3.5	T. Vaporariorum	MZ191306
142	Ka-Ro-WF6G_Kenya	Kajiado	Rombo	-3.03541	37.68649	1214.7 3.5	T. Vaporariorum	MZ191307
143	Ka-El-WFEA_Kenya	Kajiado	Elerai	-3.16353	37.73556	997.4 4.8	T. Vaporariorum	MZ191308
144	Ka-El-WFEB_Kenya	Kajiado	Elerai	-3.16353	37.73556	997.4 4.8	T. Vaporariorum	MZ191309
145	Ka-El-WFEC_Kenya	Kajiado	Elerai	-3.16353	37.73556	997.4 4.8	T. Vaporariorum	MZ191310
146	Ka-El-WFED_Kenya	Kajiado	Elerai	-3.16353	37.73556	997.4 4.8	T. Vaporariorum	MZ191311
147	Ka-El-WFEE_Kenya	Kajiado	Elerai	-3.16353	37.73556	997.4 4.8	T. Vaporariorum	MZ191312
148	Ka-El-WFEF_Kenya	Kajiado	Elerai	-3.16353	37.73556	997.4 4.8	T. Vaporariorum	MZ191313
149	Ka-Ta-WFTA_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191314
150	Ka-Ta-WFTB_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191315
151	Ka-Ta-WFTC_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191316

Table 5.1 Conti'

Sample Number	Sample ID	County	Area	Latitude	Longitude	Altitude	Identification	Accession Number
152	Ka-Ta-WFTD_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191317
153	Ka-Ta-WFTE_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191318
154	Ka-Ta-WFTF_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191319
155	Ka-Ta-WFTG_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191320
156	Ka-Ta-WFTH_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191321
157	Ka-Ta-WFTI_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191322
158	Ka-Ta-WFTJ_Kenya	Taita Taveta	Taveta	-3.46718	37.7416	700.6 4.2	T. Vaporariorum	MZ191323
159	Ka-Ta-WF31A_Kenya	Taita Taveta	Taveta	-3.39573	37.71284	750.2 3.1	T. Vaporariorum	MZ191324
160	Ka-Ta-WF31B_Kenya	Taita Taveta	Taveta	-3.39573	37.71284	750.2 3.1	T. Vaporariorum	MZ191325
161	Ka-Ta-WF31C_Kenya	Taita Taveta	Taveta	-3.39573	37.71284	750.2 3.1	T. Vaporariorum	MZ191326
162	Ka-Ta-WF31D_Kenya	Taita Taveta	Taveta	-3.39573	37.71284	750.2 3.1	T. Vaporariorum	MZ191327
163	Ka-Ta-WF31E_Kenya	Taita Taveta	Taveta	-3.39573	37.71284	750.2 3.1	T. Vaporariorum	MZ191328
164	Ka-Ta-WF31F_Kenya	Taita Taveta	Taveta	-3.39573	37.71284	750.2 3.1	T. Vaporariorum	MZ191329
165	Ka-Ta-WF31G_Kenya	Taita Taveta	Taveta	-3.39573	37.71284	750.2 3.1	T. Vaporariorum	MZ191330

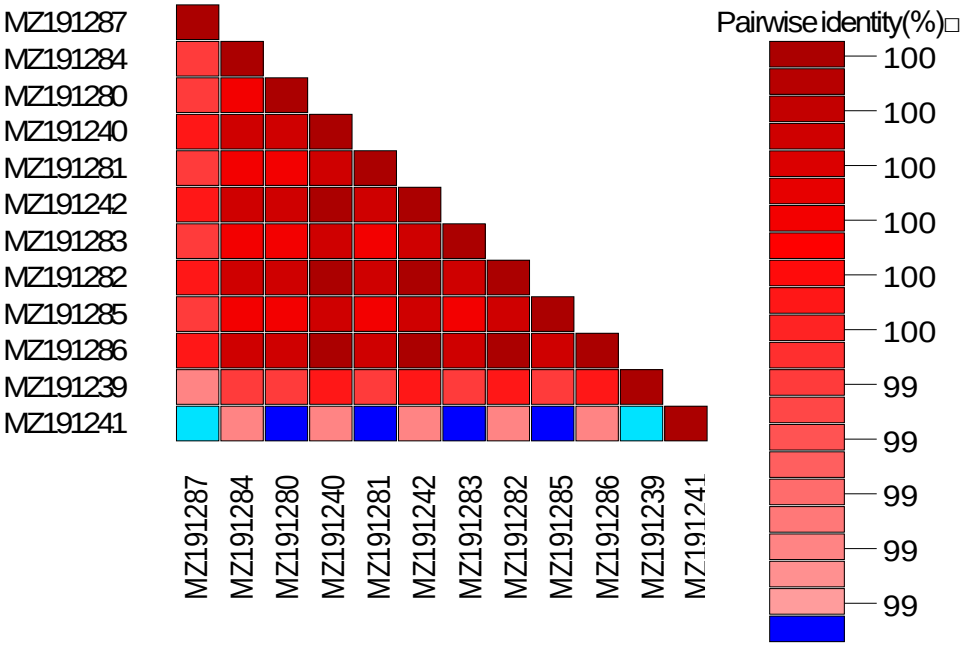


Figure 5. 2 Pairwise identity of partial mitochondrial cytochrome oxidase sub-unit 1 gene obtained from whiteflies (*Trialeurodes vaporariorum*) populations (n=12) colonizing tomato plants in Meru County, Kenya

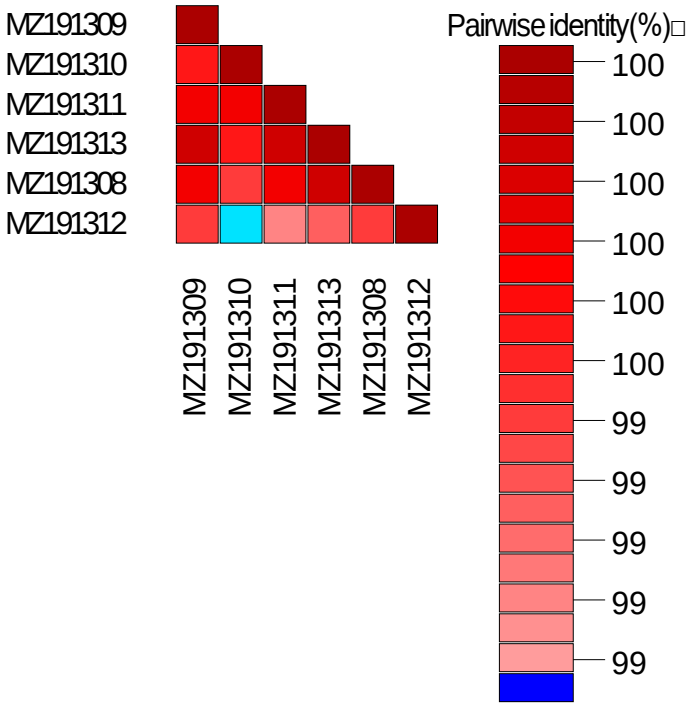


Figure 5. 3 Pairwise identity of partial mitochondrial cytochrome oxidase sub-unit 1 gene obtained from whiteflies (*Trialeurodes vaporariorum*) populations (n=6) colonizing tomato plants in Kajiado County, Kenya

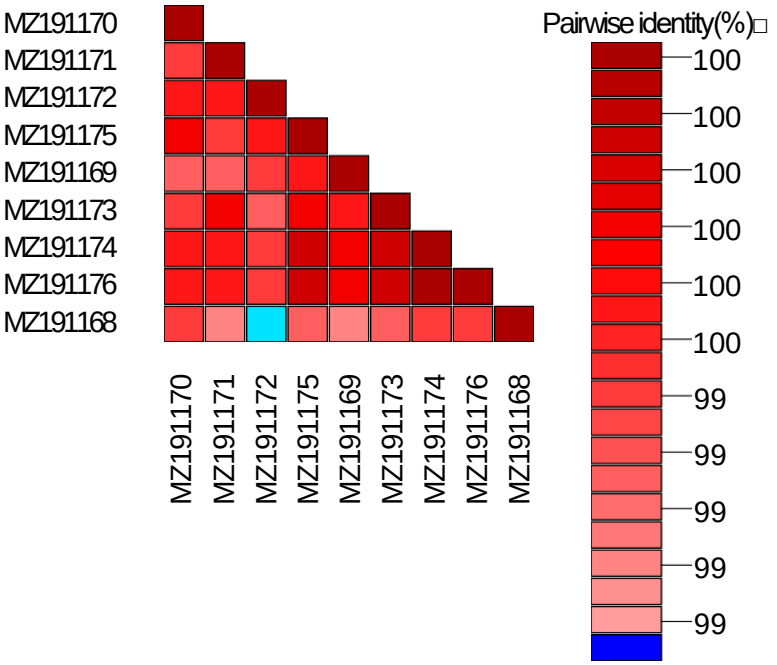


Figure 5. 4 Pairwise identity of partial mitochondrial cytochrome oxidase sub-unit 1 gene obtained from whiteflies (*Trialeurodes vaporariorum*) populations (n=9) colonizing tomato plants in Bungoma County, Kenya

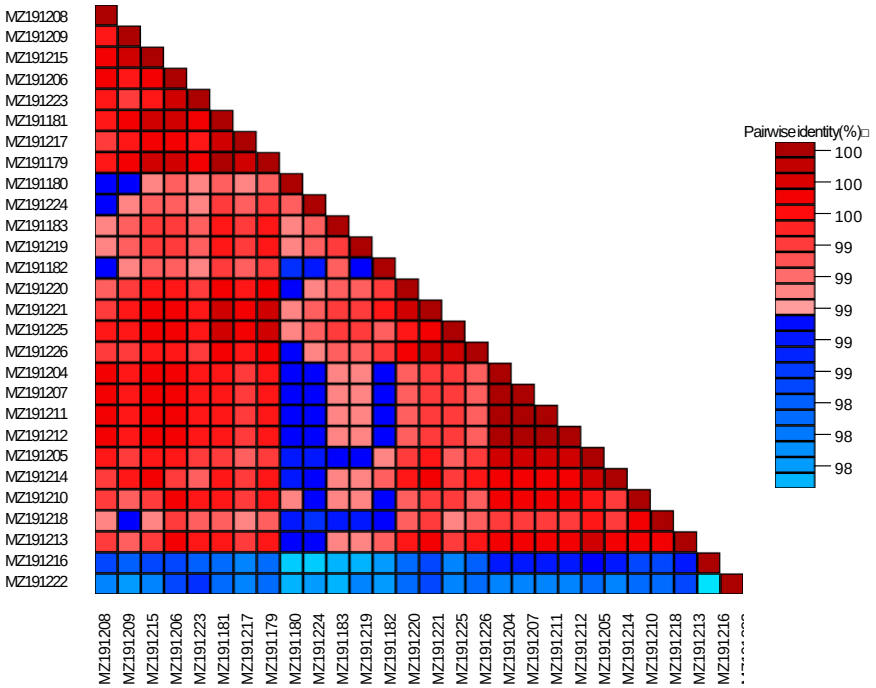


Figure 5. 5 Pairwise identity of partial mitochondrial cytochrome oxidase sub-unit 1 gene obtained from whiteflies (*Trialeurodes vaporariorum*) populations (n=28) colonizing tomato plants in Baringo County, Kenya

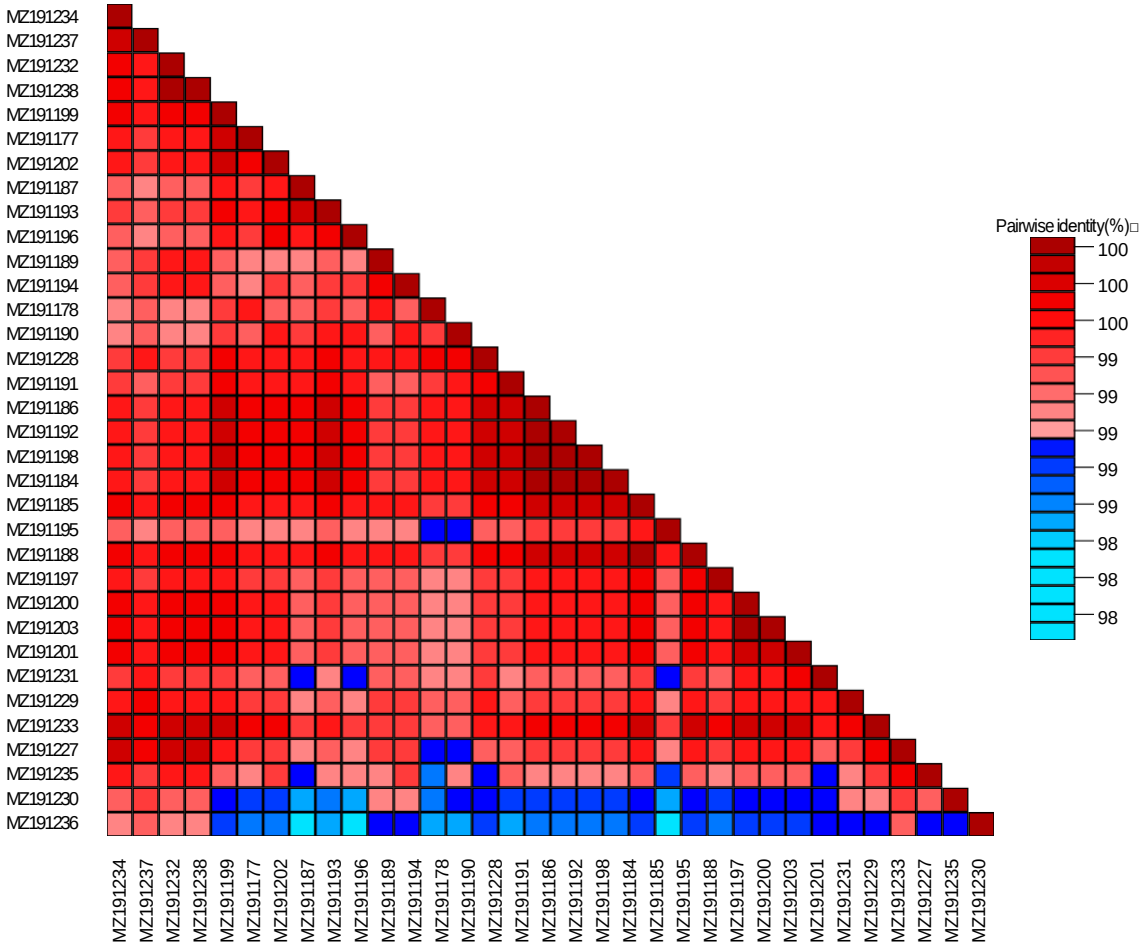


Figure 5. 6 Pairwise identity of partial mitochondrial cytochrome oxidase sub-unit 1 gene obtained from whiteflies (*Trialeurodes vaporariorum*) populations (n=34) colonizing tomato plants in Nakuru County, Kenya

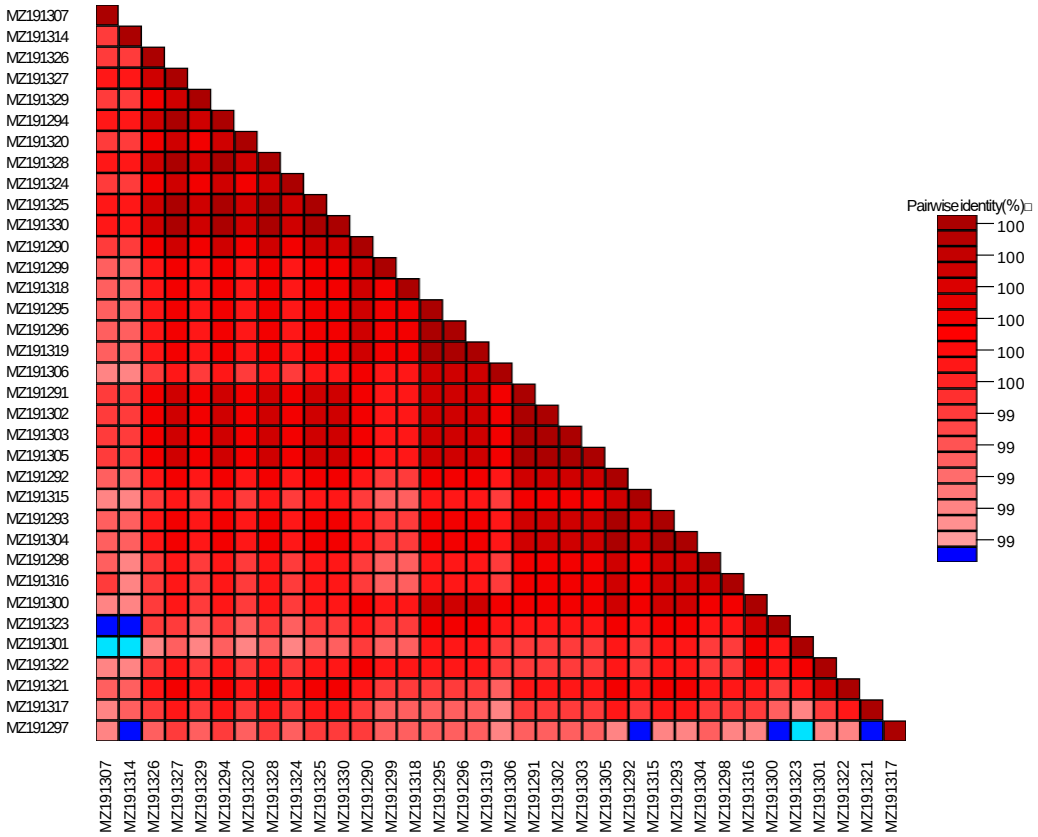


Figure 5. 7 Pairwise identity of partial mitochondrial cytochrome oxidase sub-unit 1 gene obtained from whiteflies (*Trialeurodes vaporariorum*) populations (n=35) colonizing tomato plants in Taita Taveta County, Kenya

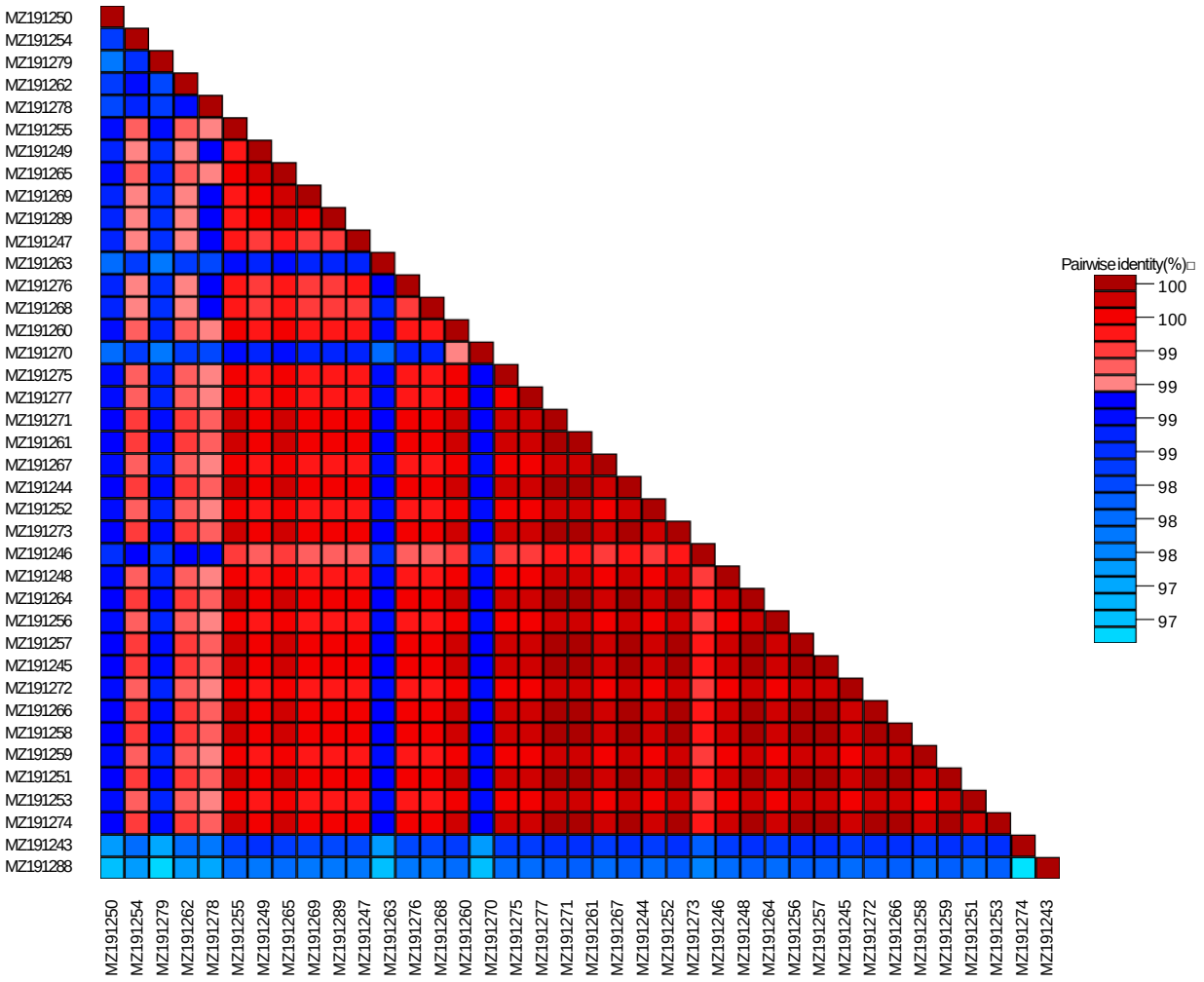


Figure 5. 8 Pairwise identity of partial mitochondrial cytochrome oxidase sub-unit 1 gene obtained from whiteflies (*Trialeurodes vaporariorum*) populations (n=39) colonizing tomato plants in Kirinyaga County, Kenya

5.4.2 Phylogenetic analyses

Phylogenetic analyses of the *T. vaporariorum* populations from Kenya and those from other parts of the world reveal a monophyletic clade with nucleotide identity of 95-99%. *Bemisia tabaci* isolates were used to root the phylogenetic tree (**Figure 5.9).**

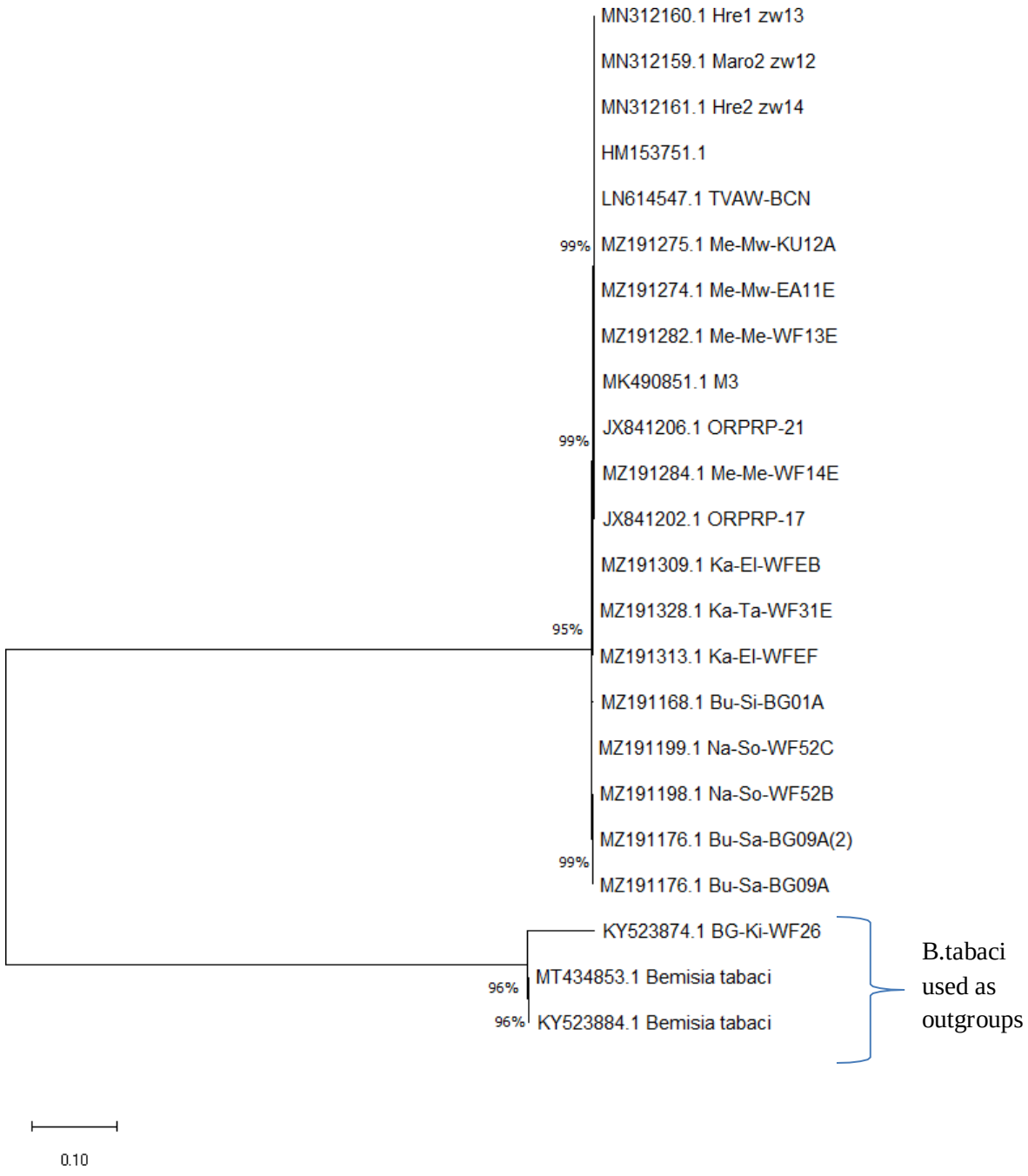


Figure 5. 9 Maximum Likelihood method showing evolutionary relationships of some whiteflies (*Trialeurodes vaporariorum*) colonizing tomato plants in Kenya with other worldwide isolates based on the partial cytochrome oxidase subunit 1 gene. The tree was inferred using the Tamura Nei in model MEGA v 7.0. Numbers at tree nodes represent bootstrap percentages based on 1,000 replicates. The scale bar shows the number of nucleotide substitutions per site.

5.4.3 Genetic variability and neutrality tests

The genetic variability determinants and neutrality tests on the partial CO1 sequences from *T. vaporariorum* populations in Kenya are shown in **Table 5.2**. Analyses of haplotype number and haplotype diversity, represented by 'h' and 'Hd', respectively revealed varying values among the insect species obtained in this study. A total of 114 haplotypes were identified from the *T. vaporariorum* populations (n=163) in Kenya. Haplotype diversity (0.981), nucleotide diversity (0.00651), and a negative Tajima's D (-2.6775) suggest that the population is undergoing purifying selection and is undergoing rapid expansion. Kirinyaga had the highest number of haplotypes followed by Nakuru County. Isolates from Kajiado and Bungoma had high haplotypes vis-à-vis *T. vaporariorum* populations. The lowest diversity occurred in Kirinyaga County with Hd value of 0.926 **(Table 5.2).** Furthermore, genetic distances were calculated, with highest π value obtained from Baringo County (0.00714) while Taveta had the least π value at 0.00419 (**Table 5.2**).

A negative Tajima's D value was reported in all the samples across the Counties, with significant values observed within populations from Baringo ($p < 0.05$), Meru ($p < 0.01$) and Kirinyaga ($p < 0.001$). A negative Tajima's D value indicates an excess of rare nucleotide site variants compared to the expectation under a neutral model of evolution. Similary, a negative value for Fu and Li's F value was observed across all the populations in all the Counties, with significant difference in all the Counties except Kajiado and Bungoma. A negative value indicates an excess of singletons indicative of population expansion. On the other hand, except for the *T. vaporariorum* populations from Bungoma, that had a positive Fu and Li's D value, the other Counties recorded negative values. The negative value indicates excess number of

singletons while the positive Fu and Li's D value observed in Bungoma shows lack of singleton mutations (**Table 5.3)**.

Table 5.2: Genetic variability determinants of *Trialeurodes vaporariorum* populations colonizing tomato plants in Kenya based on the partial mitochondrial cytochrome oxidase sub-unit 1 gene

Populations	N	h	S	Hd	Eta	π	θ-W	k	θ-Eta
Kenyan population (n=163)	702	114	125	0.981	159	0.00651	0.04011	4.5266	27.8763
Meru (n=12)	702	9	17	0.909	18	0.00429	0.00856	2.9848	5.9605
Kajiado (n=6)	696	6	7	1.000	8	0.00450	0.00503	3.1333	3.5036
Bungoma (n=9)	696	8	9	0.972	10	0.00484	0.00529	3.3611	3.6794
Baringo (n=28)	696	24	33	0.981	37	0.00714	0.01368	4.9603	9.5080
Nakuru (n=34)	696	28	23	0.984	28	0.00574	0.00985	3.9875	6.8480
Taveta (n=35)	696	24	21	0.963	25	0.00419	0.00872	2.9160	6.0706
Kirinyaga (n=39)	696	29	79	0.926	84	0.00674	0.02859	4.6815	19.8680

N = Number of nucleotide sites, h = Number of haplotypes, S = Number of variable sites, Hd = Haplotype diversity, Eta = Number of mutations, π = Nucleotide diversity, θ-W = Waterson's estimate of population mutation rate based on the total number of segregating sites, k = number of nucleotide differences between sequences, θ-Eta = Waterson's estimate of population mutation rate based on the total number of mutations

Table 5.3: Test of neutrality tests on the partial mitochondrial cytochrome oxidase sub-unit 1 (CO1) gene sequences from *Trialeurodes vaporariorum* populations colonizing tomato plants in Kenya

Population	Tajima's D	Fu and Li's D	Fu and Li's F
Kenyan population (n=163)	-2.6775	-7.8306	-6.5027
Meru County (n=12)	-2.1760**	-2.7167**	-2.9311**
Kajiado County (n=6)	-0.6231	-0.3024	-0.3989
Bungoma County (n=9)	-0.4027	0.0496	-0.0661
Baringo County (n=28)	-1.7751*	-2.5214*	-2.6858*
Nakuru County (n=34)	-1.4684	-1.9568	-2.1204*
Taveta County (n=35)	-1.7996	-3.2204*	-3.2482*
Kirinyanga County (n=39)	-2.7935***	-5.6218**	-5.5017**

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at P<0.001

5.4.3 Haplotype Network

The haplotype network diagrams using the *T. vaporariorum* populations confirmed the occurrence of multiple haplotypes within the sequences from the various Counties. For example, only one haplotype was dominant and radial network formed mainly in Meru (Figure 5.10), Baringo (Figure 5.13) and Kirinyaga (Figure 5.16). For all the network figures, each circle represents a haplotype, and its size is proportional to the frequency of individual occurrence. Median vectors are marked with short lines with mutated positions numbered in red.

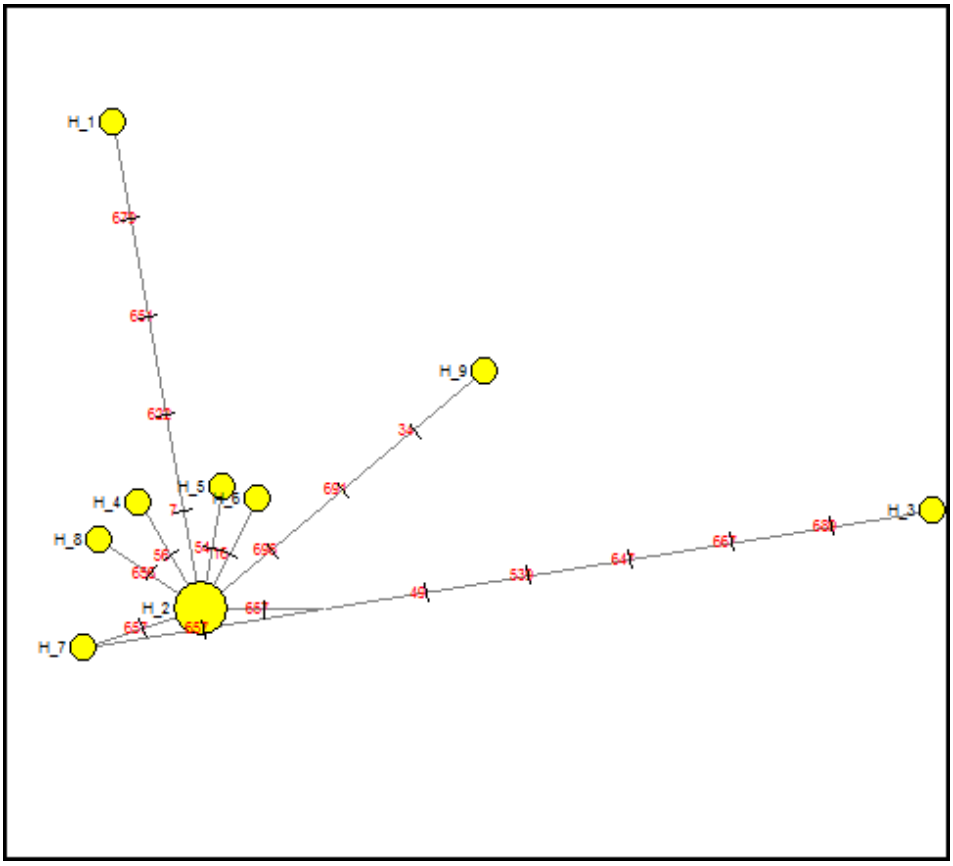


Figure 5. 10 Median-joining network of *Trialeurodes vaporariorum* populations from Meru County based on the single genes of COI haplotypes. 9 Haploypes were revelead and H_2 is the most dominant.

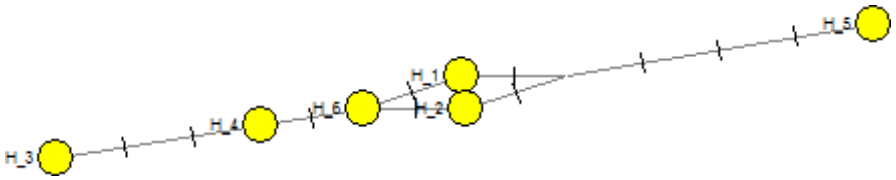


Figure 5. 11 Median-joining network of *Trialeurodes vaporariorum* populations from Kajiado County based on the single genes of COI haplotypes. 6 haplotypes revealed, with H_2 and H-1 being dominant.

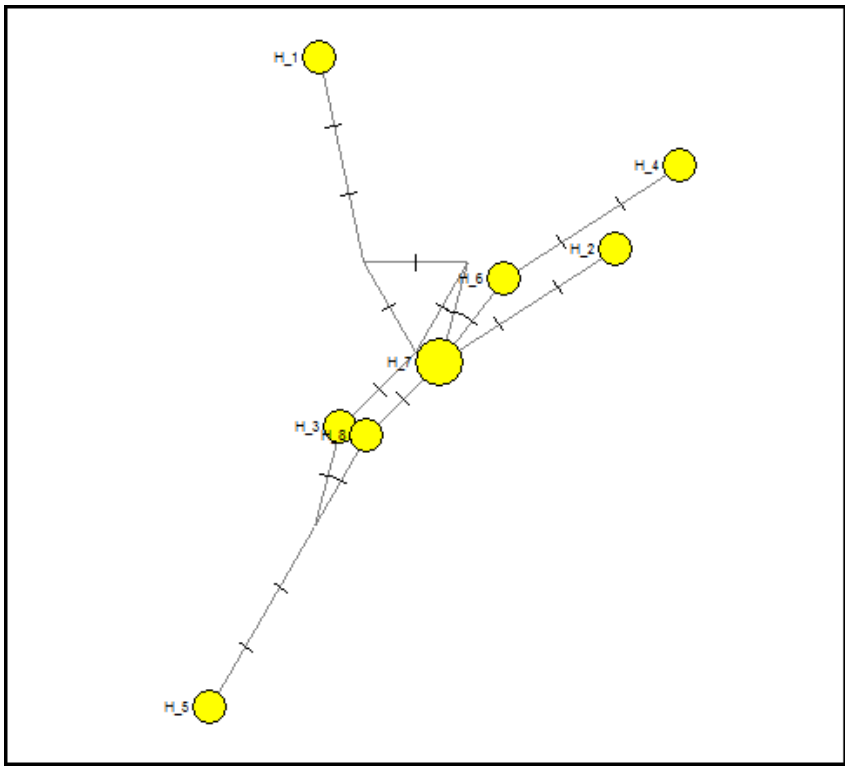


Figure 5. 12 Median-joining network of *Trialeurodes vaporariorum* populations from Bungoma County based on the single genes of COI haplotypes. 8 haplotypes identified, with H_7 being dominant.

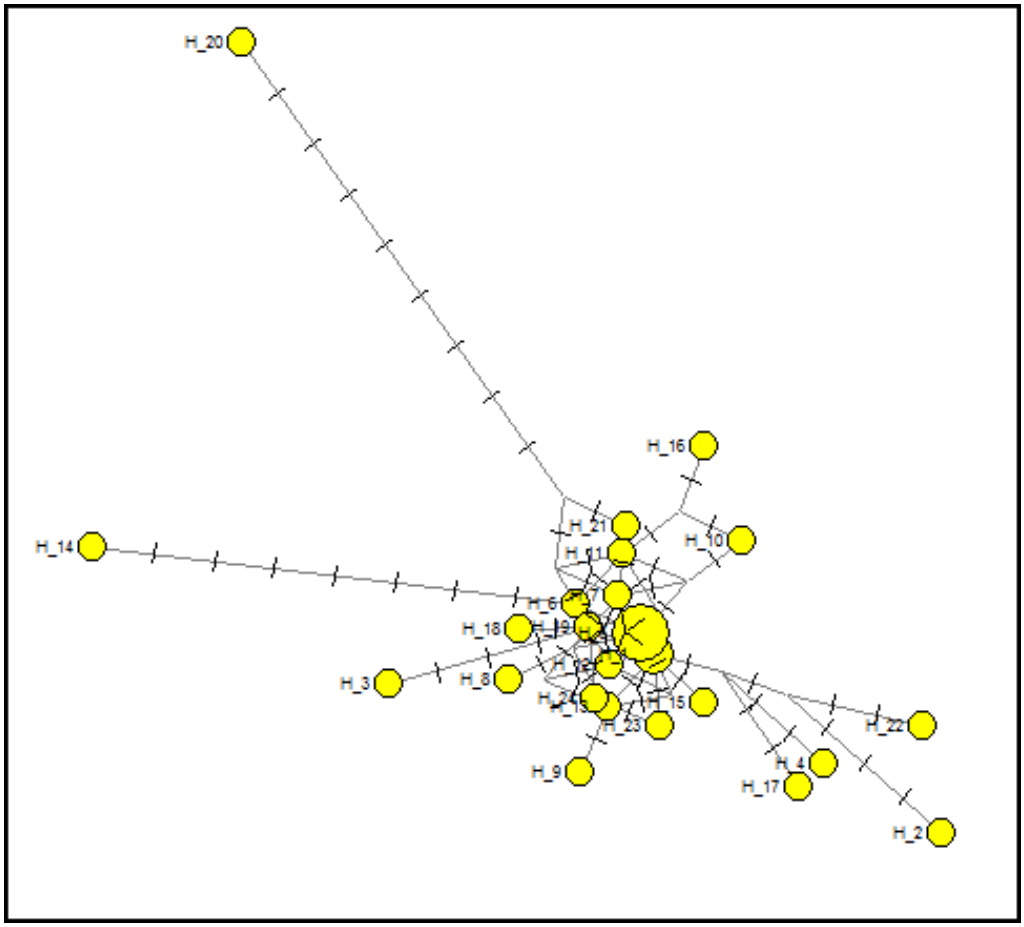


Figure 5. 13 Median-joining network of *Trialeurodes vaporariorum* populations from Baringo County based on the single genes of COI haplotypes. 23 haplotypes identified with H_4 being dominant.

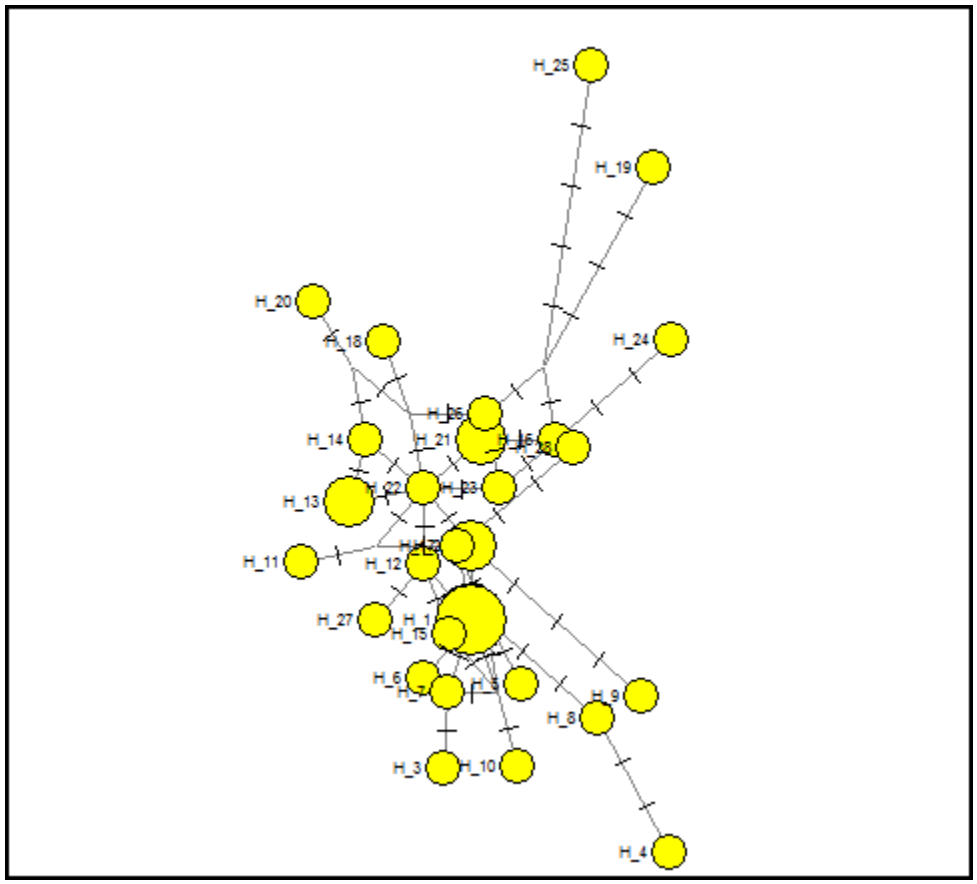


Figure 5. 14 Median-joining network of *Trialeurodes vaporariorum* populations from Nakuru County based on the single genes of COI haplotypes. 28 haplotypes revealed.

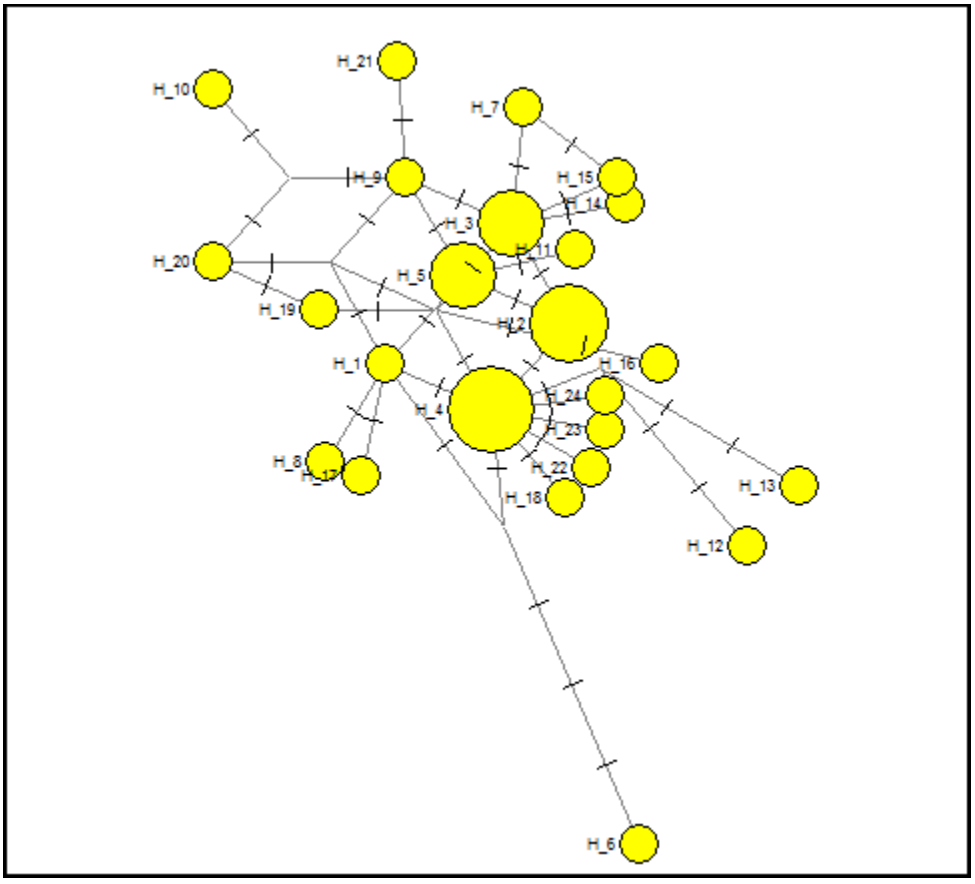


Figure 5. 15 Median-joining network of *Trialeurodes vaporariorum* populations from Taveta County based on the single genes of COI haplotypes. 24 haplotypes identified

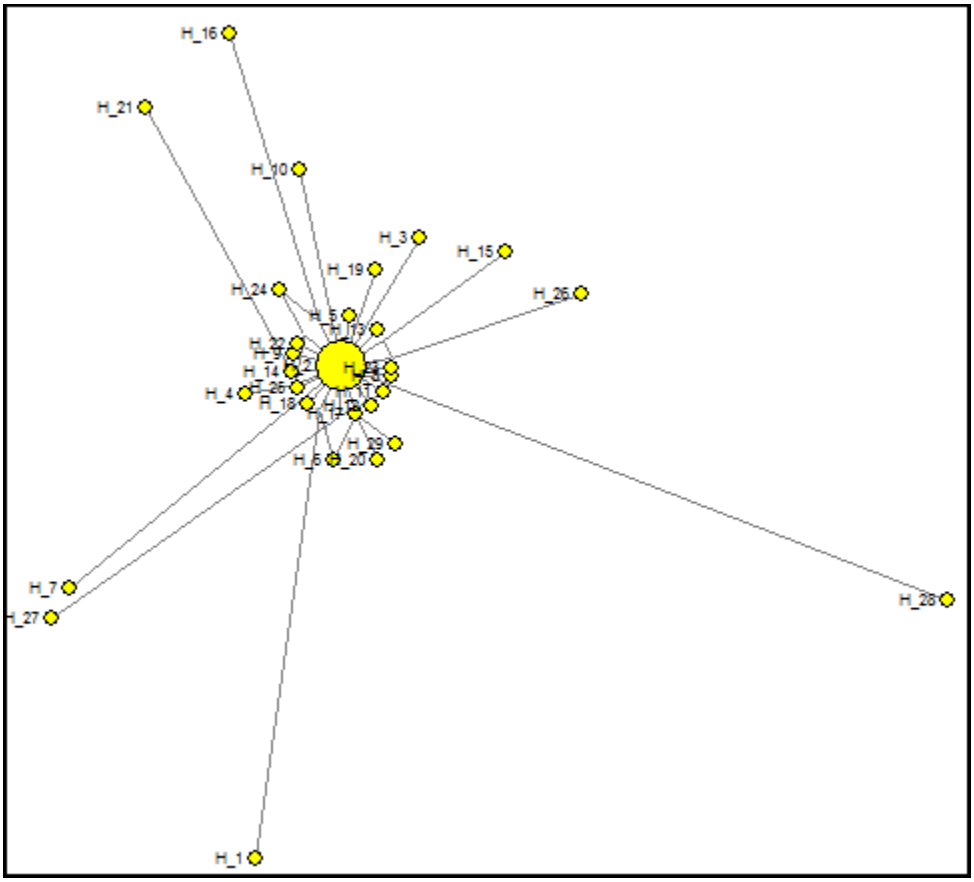


Figure 5. 16 Median-joining network of *Trialeurodes vaporariorum* populations from Kirinyaga County based on the single genes of COI haplotypes. 29 haplotypes revealed, with H_18 being dominant.

5.5. Discussion

This study presents the first extensive sampling, molecular identification and population genetics analysis of whitefly populations colonizing tomato crops in Kenya. Whiteflies are important agricultural pests and understanding their genetic diversity in agroecosystems is important as information on emergence of new species, biotypes, cryptic species and haplotypes is generated (Roopa *et al.*, 2012). In Africa, most studies on whiteflies have focused on *Bemisia tabaci* mainly on cassava crops (Khamis *et al*., 2021). However, in the recent past many researchers have focused on *Trialeurodes vaporariorum* because of its ability to develop resistance to insecticides such as neonicotinoids (Gorman *et al*., 2007; Karatolos *et al*., 2011). Using the mtCOI gene as a molecular marker, this study expected that several whitefly species would be found colonizing tomato crops, this included *Bemisia tabaci* and *Trialeurodes vaporariorum.* Previous researchers have reported the presence of *B.tabaci* on tomato crops in Kenya (Jones and Markham 2005). In Sub-Saharan Africa, *B.tabaci* has been reported on tomato crops (Romba *et al*., 2018; Mugerwa *et al*., 2021). On the contrary, this reseach only found *T. vaporariorum* on tomato crops. These findings are similar to recent reports by Khamis *et al*. (2021). However, the reason behind the contrasting findings are not currently apparent, hence need for future study.

In an experimental research to understand host preference for *T. vaporariorum* and *B.tabaci* whiteflies in tomato and pepper crops in Uruguay, Lorenzo *et al*. (2016) observed that the former whitefly species preffered pepper while latter species was exclusively found on tomato. The study was conducted using non conditioned test, where the whitefly species had no previous access or experience with the two crops. The research further established that *B.tabaci* whitefly species was able to develop easily on tomato crop, but it was rare to find it on tomato crop under

field conditions. This could be attributed to antibiosis where some plants are preffered hosts to whitefly species. Antibiosis based resistance affects adult survival and oviposition rate (Lorenzo *et al*., 2016). Previous studies on plant resistance of *B.tabaci* whitefly species have found that this whitefly is affected by plant surface characteristics, such as glandular trichomes, leave colour, shape and hairiness (Oriani and Vendramim 2010; Lorenzo *et al*., 2016). *Bemisia tabaci* species exibits a low oviposition rate on tomato plants, this is mainly due to hairness of tomato leaves, occurrence of glandular trichomes type IV, while the production of acylsucrose discourage the insect from making contact and settling on tomato (Rodrıguez-Lo´pez *et al*., 2011). In other studies, it was observed that non cassava colonizing *B. tabaci*, were unable to reproduce and colonize cassava plants, partially since they are unable to effectively feed on cassava plants (Milenovic *et al*., 2019). This confirms that whitefly species have a preference for laying eggs on host plants that provide conducive feeding and/or offspring development conditions (Courtney and Kibota 1990). Therefore, in order to develop a better understanding of host preference and antibiosis mechanisms for whitefly populations found in tomato agroecosystems in Kenya, further research needs to be done. The research should monitor the relative abundance of the two whitefly species in tomato crops, the interrelationships between whitefly populations on crops and wild hosts and an evaluation of any apparent and interference competition. This will answer a recent hypotheses by Khamis *et al*. (2021) which suggests displacement of *Bemisia tabaci* whitefly by *T. vaporariorum* whitefly, out-competition, eradication or low populations of existing *B. tabaci* whitefly biotypes in tomato agroecosystems in Kenya. Moreover, transmission studies should be conducted to elucidate the possible role of *T. vaporarioum* whitefly species in transmission of leaf curl viruses in tomato plants.

Phylogenetic analysis of sequences from this study with those reported elsewhere showed a single monophyletic clade indicating close relationship. Similary, pairwise sequence analysis indicated minimum divergence within the group. This results are similar to previous findings on *T. vaporariorum* species from other parts of the world (Roopa *et al*., 2012; Cavalieri *et al*., 2014; Prijovic´ *et al*., 2014). Recent studies on genetic diversity and phylogeographical structuring of *T. vaporariorum* based on mtCOI gene indicate that a single large group is present, though several populations exists (Gao *et al*., 2014; Wainaina *et al*., 2018). Similarly, Kapantaidaki *et al*. (2015) while analyzing mtCOI sequences from 18 countries across the world using phylogeny observed that the sequences clustered in only two clades and with very low diversity. Barboza *et al*. (2018) also reported low genetic variations in *T. vaporariorum* species sampled from tomato and pepper crops in Costa rica. Therefore, based on the mtCO1 sequence data available, the findings of this study confirms what other researchers have reported that there is low levels of genetic variation in *T. vaporariorum* from different parts of the world.

Analysis of sequence divergence of the mtCO1 gene in our samples revealed the presence of 114 haplotypes with 125 variable sites. Our findings reported many haplotypes than previously reported in Kenya on bean crops (Wainaina *et al*., 2018). The high number of haplotypes could be due the criteria for selection used in this study, where many whitefly sequences were selected from several Counties. Overall, it is evident that there is an expansion in the number of *T. vaporariorum* haplotypes reported globally, the increase is due to transboundary trade in ornamental plants that are pathways for the pest (Wainaina *et al*., 2018). Kenya is a key importer of cuttings of ornamental plants mainly from Europe. These cuttings are a pathway for introducing eggs or other developmental stages of *T. vaporariorum* into the country. The cuttings are propagated in greenhouses, which have a conducive microclimate that supports

multiplication, establishment and subsequent spread of the pest. Therefore, there is need to enhance phytosanitary measures to mitigate against introduction of *T. vaporariorum* whitefly species into Kenya via transboundary trade.

To further understand population genetics of *T. vaporariorum*, we evaluated haplotype and nucleotide diversity. There was low haplotype and nucleotide diversity indicating a very small genetic difference between the haplotypes, hence further confirming presence of one genetic group. However, a negative Tajima's D, Fu and Li's D and Fu and Li's F values observed across all the populations in all Counties gives an indication of possible expansions in the population (Alabi *et al*., 2011). The significant values in Tajima's D, Fu and Li's D and Fu and Li's F observed in Meru, Baringo and Kirinyaga signifies more demographic expansions. Hence, there is need for understand aspects on host plants, insecticide resistance and its influence on gene flow of *T. vaporariorum* species as well as dispersal patterns.

Bacterial endosymbionts present in *T. vaporariorum* haplotypes may cause genetic sweeps within the mitochondria and hence lead to diversification of *T. vaporariorum* (Kapantaidaki *et al*., 2015). Therefore there is need for studies to infer the existing bacterial endosymbionts in *T. vaporariorum* found in Kenya. Further surveys should be conducted to identify whitefly species in both cultivated and non-cultivated hosts within tomato agroecosystems as this could have inferences on the dynamics of viruses found in tomato crops in Kenya.

CHAPTER SIX

SCREENING OF SELECTED TOMATO VARIETIES RESPONSE TO TYLCD UNDER SPONTENOUS FIELD INOCULATION

6.1 Abstract

Tomato yellow leaf curl disease (TYLCD) caused by begomoviruses is a major constraint to tomato worldwide. Infected susceptible plants exhibit symptoms such as upward curling of leaves, severe dwarfing and flower and fruit abortion. TYLCD symptoms on tomato depends on the, tomato variety, inoculation date, and virulence of the virus strain causing the infection. Resistant varieties to TYLCD are developed by introgression of resistant genes from wild relatives of tomato into farmer preferred tomato varieties. The objective of this study was to determine the response of selected tomato varieities grown in Kenya to TYLCD under spontenous field inoculation. A total of 20 varieties were assessed in field experiments for two seasons; September to December 2018 and May to August 2019. The experiments were laid in randomized complete block design with three replications at KALRO Mwea. Each plot had 25 plants and data was collected on TYLCD disease incidence, severity and population of whiteflies from 15 plants per plot. There was no significance difference ($P>0.05$) in disease incidence and severity between the test varieties in both season season 1 and 2. The plants exhibited symptoms such as chlorosis and upward leaf curling. Whitefly populations were not statistically different in both seasons, and there was no correlation between whitefly population and TYLCD incidence and severity, however the whitefly population on the test varieties was significantly different. This study established that varieties considered to be resistant to TYLCD were found susceptible. It is recommended for tomato breeding programs in Kenya to breed for genotypes that are resistant to the existing leaf curl viruses in Kenya. The breeding should adopt use of molecular techniques such as gene pyramiding in order to develop materials with durable resistance that will enhance productivity of tomato in Kenya.

6.1 Introduction

Tomato yellow leaf curl disease caused by begomoviruses is a major limitation to tomato globally. Infected susceptible plants exhibit symptoms such as upward curling of leaves, severe dwarfing and flower and fruit abortion, with up to 100% yield losses (Abhary *et al*., 2007). TYLCD symptoms on tomato depends on the, tomato variety, date of inoculation and the virulence of the virus strain causing the infection (Lapidot, 2007). Nonetheless, some tomato varieties are known to be symptomless carriers of the virus (Kashina *et al*., 2003). Begomoviruses are transmitted by *Bemisia tabaci* (Genn) in a persistent circulative manner, though seed transmission has been reported. Management of TYLCD includes use of insecticides to manage the vector, cultural practices and use of resistant/tolerant varieties. Resistant varieties to TYLCD are developed by introgression of resistant genes from wild relatives of tomato into farmer preferred tomato varieties (Lapidot, 2007). The resistant genes include *Ty-1*, *Ty-3*, *Ty-2, Ty-5, ty-5* obtained wild relatives of tomato with resistant genes such as *Solanum arcanum*, *S. chilense*, *S. corneliomulleri, S. cheesmaniae, S. galapagense, S. pennellii, S. chmielewskii, S. habrochaites, S. neorickii*, *S. peruvianum*, and *S. pimpinellifolium*, *Solanum pimpinellifolium*, *peruvianum* and *Solanum habrochaites* (Vidavski *et al*., 2008; De la Peña *et al*., 2010; Pereira-Carvalho *et al*., 2010; Tomás *et al*., 2011; Verlaan *et al*., 2013). In this study we evaluated the response of farmer preferred varieties to tomato yellow leaf curl disease under field experiment. Some of the cultivars are marketed as TYLCD resistant varieties while others are known to be susceptible.

6.2 Materials and methods

6.2.1 Experimental site

The experiment was conducted at Kenya agricultural and livestock research organization (KALRO) Mwea in Kirinyaga County,located at an elevation of 1159 m above sea level and on latitude 0 37'S and longitude 037 20'E. The average rainfall was about 850 mm with a range of 500 - 1250 mm divided into long rains (March – June with an average of 450 mm) and short rains (Mid-October to December with an average of 350 mm). The rainfall was characterized by uneven distribution in total amounts, time and space. The temperature ranged from 15.6 ºC to 28.6 ºC with a mean of about 22 ºC. The experiment was carried out during the short rain season between September 2018 and January 2019 and during the long rains between March and June 2019. The soil was characterized by a mixture of loamy and sandy soils. The study site was selected purposively because it a hot spot for TYLCD.

6.2.2 Experimental design

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Twenty commonly planted tomato varieties were tested for their resistance to yellow leaf curl disease (Table 6.1). Tomato cultivar Asilla F1 was the resistant control while Money maker was used as a susceptible control. Twenty five (25) plants of each variety were planted on five rows at a spacing of 90×45 (cm). A spreader crop of TYLCD susceptible variety (Money maker) was planted one month earlier before transplanting, this also acted as inoculation source for whitefly.

6.2.3 Crop establishment and management

6.2.3.1 Nursery

The seedlings were propagated on trays with holes size of about 4.5 cm diameters and 4 cm deep filled with coco peat media and watered. This was done in an insect proof screen-house

where the trays were placed on wire-meshed tables at the Plant quarantine and biosecurity station. Sowing was done on 4th September 2018 and 6th May 2019 for short and long rain seasons respectively. The germination of the seeds varied amongst the varieties and ranged between four to seven days from the date of sowing. Watering of the seedlings was done twice a day. Weekly application of ridomyl (Metalaxyl-M+Mancozeb) fungicide was done at a rate of 2 g/l. No application of insecticide was done as this would kill the whitefly vector of the tomato leaf curl virus.

Table 6. 1 Tomato cultivars used in the study

Variety	Company [1]	Disease resistance[2]	Growth type	Open field or green house
Assila FI (resistant control)	**Monsanto**	**bdfgm**	**Determinate**	**Open field**
Bingwa F1	EAseed	gf	Indeterminate	Open/green house
Cal J	East African	gi	Determinate	Open field
DRD 8551	**Monsanto**	**abdfgm**	**Determinate**	**Open field**
Eden F1	Monsanto	dfghjim	determinate	Open field
Geo 12 F1	**Johnseed**	**abdfgm**		**Open/greenhouse**
Julia f1	Hygrotech	dfghjim	Determinate	Open field
Libra F1	**Hygrotech**	**kb**	**Determinate**	**Open field**
M82	Simlaw		indeterminate	Open field
Money maker (susceptible control)	Simlaw	fg	Determinate	Open field
Nuri F1	**East African**	**abdgi**	**Determinate**	**Greenhouse**
Pamela F1	**Hygrotech**	**abdfgm**	**Indeterminate**	**Green house**
Pesa F1	**Hy-Genebiotech**	**bi**	**Determinate**	**Green house**
Rambo F1	Royal seed	fgm	Determinate	Open field
Riogrande F1	simlaw	fg	Determinate	Open field
Roma VF	Starke ayres	fgm	Determinate	Open field
Sandokan FI	Royal	fgkm	Determinate	Open field
Super Rio	Simlaw	lh	Determinate	Open field
TM 20	Gromost	bji	Determinate	Open field
Oxyl	Royal	d	Determinate	Open field

[1] Seed company from where the seed will be purchased

[2] Disease resistance: TSWV[a], **TYLCV[b]**, CMV[c], ToMV[d], TMV[e], Fusarium wilt[f], Verticilium wilt[g],Grey leaf spot[h], Fusarium crown rot[i], Alternaria stem canker[j], Bacterial wilt[k], Bacterial speck[i], Nematodes[m]

Varieties in bolded are marketed as TYLCD resistant varieties

6.2.3.2 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. Each plot measured $15m^2$ with five rows and one replication had twenty plots. Seedlings at 4 - 5 leaf growth stage were transplanted in the field on 5^{th} October 2018 and 6^{th} June 2019 for short and long rain seasons respectively.

6.2.3.3 Fertilizer application

Before transplanting Triple Super Phosphate (TSP) with 46% P2O5 was applied at the rate of 150 Kg/ha. 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. Staking was done for indeterminate varieties. Furrow irrigation was provided daily except on rainy days. Application of a fungicide (Ridomyl) was done weekly while no insecticides was applied.

6.2.3.4 Data collection

Data on disease incidence, severity, whitefly populations was collected bi-weekly starting with the first week after transplanting up to the seventh week. Data was collected on fifteen plants per plot i.e three plants per plot on five rows. Disease incidence was expressed as the percentage of diseased plants in the sample of plants and genotypes were classified as highly resistant (0-20%), moderately resistant (21-40%), susceptible (41-60%), and highly susceptible (61-100%) to infection with TYLCD. Scoring for severity was done basing on a leaf curl virus disease rating scale of 1 - 5 where; 1=1-20% (chlorosis), 2=21- 40% (chlorosis, stunting), 3=41-60% (chlorosis, stunting, reduced leaf sizes), 4= 60-80% (Chlorosis, stunting, reduced leaf size, upward curling of leaves, reduced internodes), 5=81-100% (Chlorosis, stunting, reduced leaf

size, upward curling of leaves, reduced internodes, flower abortion, reduced fruit size) (Ssekyewa *et al*., 2006; Mwangi *et al*., 2015)

6.2.3.5 Data analysis

Disease severity and incidence as well as whitefly populations data were analyzed using Analysis of Variance (ANOVA) and Pearson correlations using SAS software (version 9.1) (SAS Institute, 2004). Means generated were used in Excel to develop related graphs. Standard error values (SE) were used as basis to determine variability within treatment while F-distribution values were used to determine significance of differences between treatments (Nono Womdim., 2005)

6.3 Results

6.3.1 Disease incidence

There was no significance difference ($P>0.05$) in disease incidence between the test varieties in both season season 1 and 2. However, variety F350 had the highest disease incidence in both seasons while DRD 8551 and Pesa F1 recorded the lowest incidences in season 1 and 2 respectively. The disease incidences in all the test varieties were above 50% and did not vary between the two seasons, hence rated as susceptible.

6.3.2 Disease severity

There was no significance difference ($P>0.05$) in disease severity between the test varieties in both season season 1 and 2. In both seasons, the severity scores were above 2, indicating mild infections. In season one varieties money maker, Pamela F1 and TM 20 had a score of 2.5 while the rest had a score of ≤ 2.4. In season two Sandokan F1, Pamela F1 and F350 had a score of 2.5 while the rest had scores of less than ≤ 2.4.

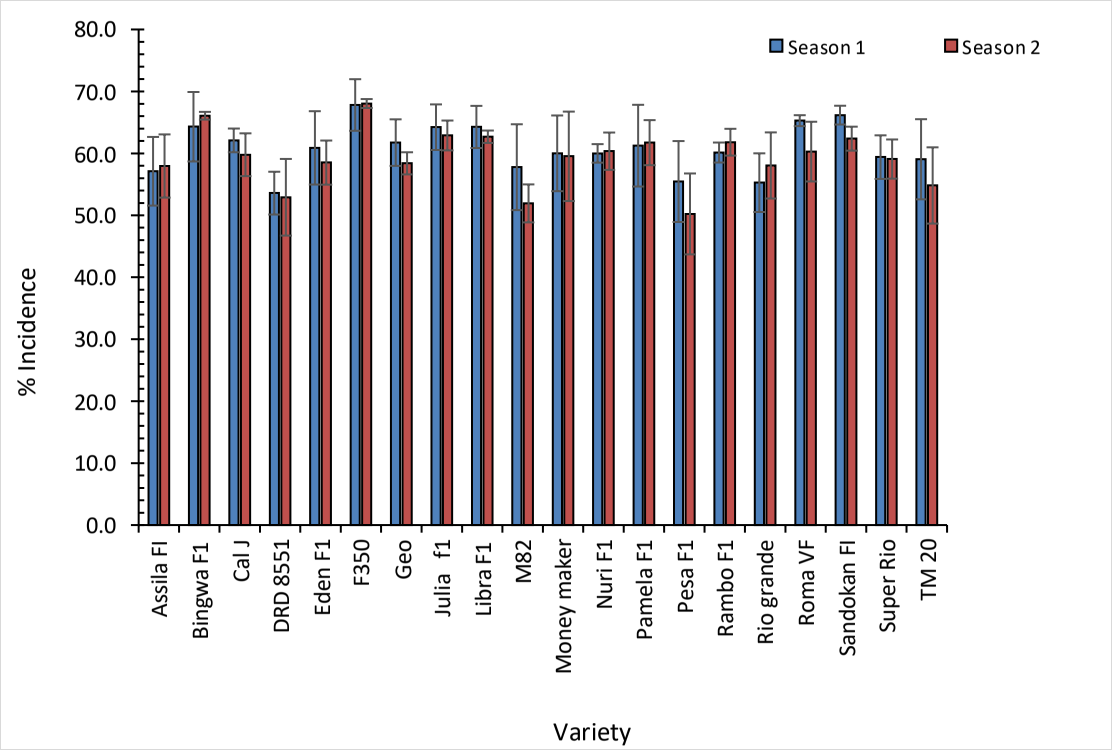


Figure 6. 1 TYLCD incidences amongst different tomato varieties

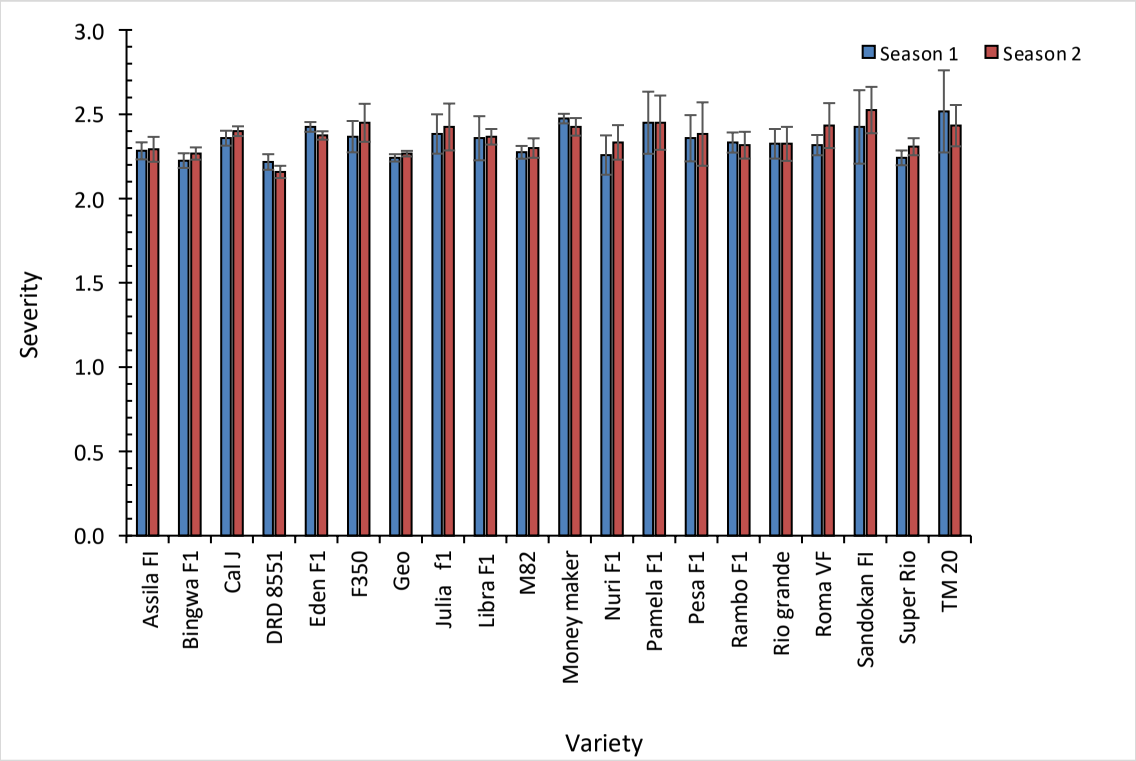


Figure 6. 2 TYLCD severity amongst different tomato varieties

6.3.3 Whitefly populations

There was significant difference (P=0.01) in whitefly populations amongst the test varieties. Riogrande, Julia F1and Eden F1 had the highest whitefly populations while Rambo F1 had the least population of whiteflies. However, there was no significance difference (P>0.05) in the whitefly population between the two seasons and the replications. There was no correlation between whitefly populations and disease incidence and severity (**Fig 6.4**)

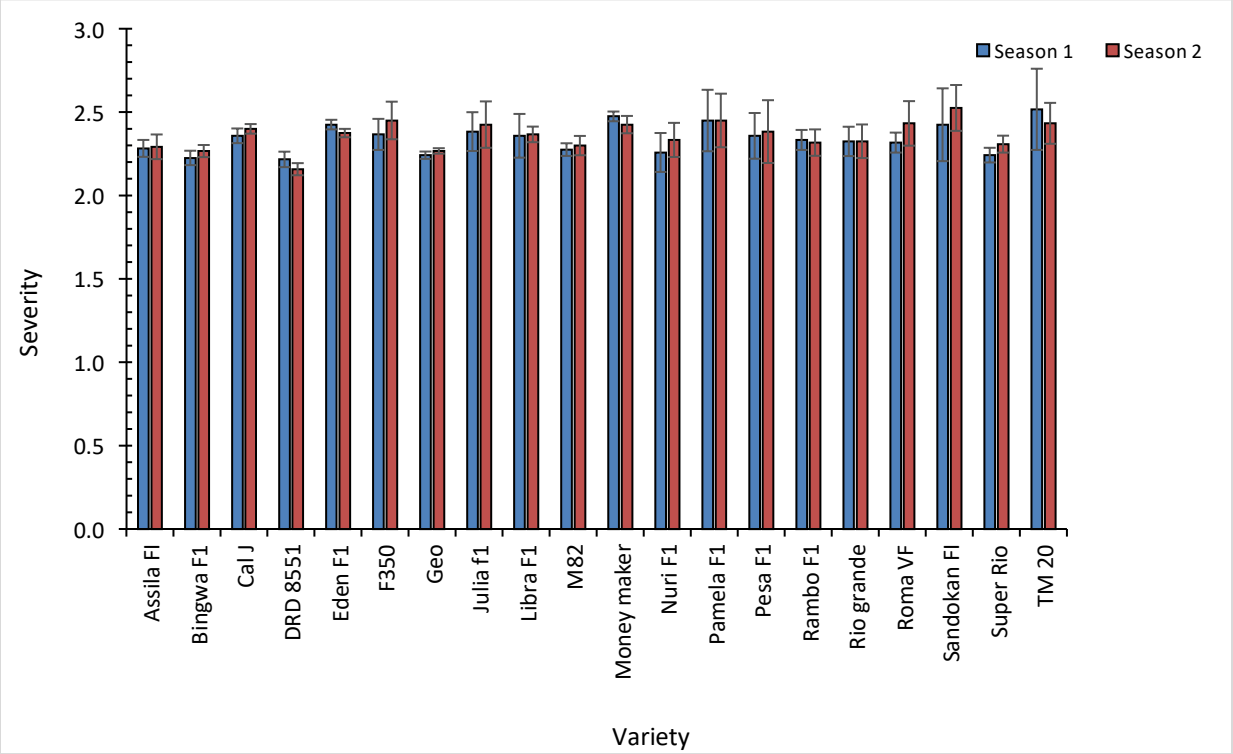


Figure 6. 3 Mean disease severity across the test varieties in season one and two

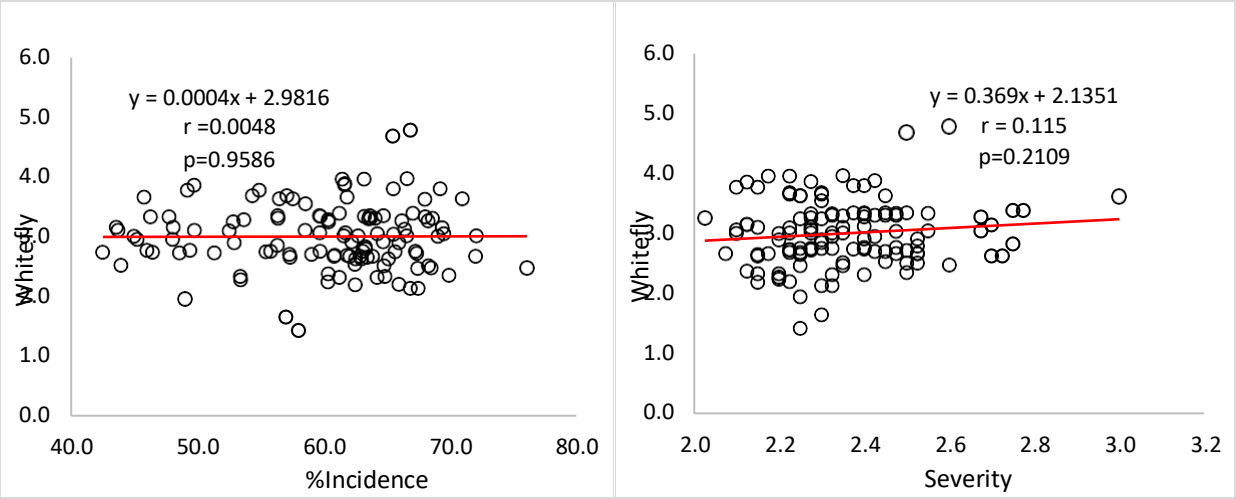


Figure 6. 4 Correlation between whitefly population and TYLCD incidence and severity

6.5 Discusion

In this study, tomato cultivars assessed were infected with Tomato yellow leaf curl disease in both seasons. We considered resistance as absence of symptoms (Yan *et al*., 2018) and therefore all the tomato varieties tested were considered susceptible to TYLCD based on visual observation of symptoms such as leaf chlorosis and upward or inward leaf curling. The disease incidences between the cultivars were not significantly different, this is despite some of them being marketed as TYLCD resistant. Similarly, the disease severity did not vary stastistically amongst the test varieties.

Breeding for TYLCD resistance in tomato plants is based mainly on three genes, Ty-1, Ty-2, and Ty-3, other genes include Ty-4, Ty-5 and Ty-6 (Yan *et al*., 2018). These genes are sourced from wild relatives of tomato such as *S. pimpinellifolium*, *S. habrochaites*, *S. peruvianum*, and *S. chilense* and introgressed in cultivated tomato. Resistance is conferred in commercial breeding materials using either a single resistance gene or joint response of different genes. Some of the genes are not strong enough to confer resistance and therefore to achieve high levels of resistance pyramiding of genes through use of molecular biotechnology is adopted. For instance under high disease pressure, Ty-1-mediated resistance easily breaks down, therefore pyramiding

of Ty-1/Ty-2 and Ty-3 provides higher resistance (Elbaz *et al*., 2016; Mejia *et al.*, 2010; Yan *et al*., 2018). Nonetheless, this resistance may be overcome as a result of changes in TYLCV strains that arise due to factors such as mutations, recombination, inclusions of satellite and invasion of alien whitefly (Hosseinzadeh *et al*., 2014; Yan *et al*., 2018). Studies show that some TYLCV strains are able to overcome the resistance conferred by some of the genes for instance TYLCSV and TYLCV-mild are able to overcome the Ty-2 gene (Barbieri *et al*., 2010; Tomás *et al*., 2011). Yan *et al*. (2018) suggested the adoption of strain specific resistance breeding programs this would be more beneficial in tomato breeding. Previous studies have suggested that TYLCV resistance genes may contribute to emergence of virulent virus species with higher multiplication rates and superior fitness on resistant genotypes (Van den Bosch *et al*.,2006).

As indicated in previous chapters, TYLCD is caused by different strains of TYLCV, ToLCV or TYLCV-like viruses. In chapter 4 it is elucidated that the TYLCD symptoms observed on tomato crops in Kenya are caused by *Tomato leaf curl Arusha virus* and previously Kimathi *et al*. (2020) reported the presence of *Tomato leaf curl Uganda virus* in plants exhibiting similar symptoms. This confirms that *Tomato leaf curl viruses* and not *Tomato yellow leaf curl virus* (*es*) are responsible for the symptoms observed in tomato fields, this is contrary to the earlier perceived knowledge that the symptoms were due to the latter virus and as such breeding programs focused on developing resistant varieties to TYLCV. Though no laboratory confirmatory tests were carried out on samples from this experiment it's assumed that since symptoms are similar to those observed in plants sampled in Chapter 4, the symptoms are probably caused by the same causal agent. Nevertheless, mixed viral infections exhibiting similar symptoms are common in fields some of which are transmitted by whitefly populations (Díaz-Pendón *et al*., 2010). Therefore it's possible that the varieties were infected with mixed viral species. This

notwithstanding its important for breeding programs in Kenya to develop strain-specific resistant accessions considering the leaf curl viruses that are endemic in the country. This will be beneficial to farmers and will enhance tomato productivity in the country.

In the current research, there was no correlation between whitefly populations with either disease incidence or severity. This was contrary to findings in Tanzania by Nono Womdim *et al*. (1996) who established that there was a positive correlation between TYLC disease incidence and whitefly populations. Data of Lapidot (2007) reported that low levels of viruliferous whiteflies in the field affect the disease incidence of whitefly transmitted viruses. However, in the current study the disease incidence was high regardless of the number of whiteflies. The low number of whitefly populations could be attributed to the manner in which their population was determined in this case. Estimation of whitefly populations in this study was done at micro-level, where individual whitefly populations were counted on randomly selected plant leaves. Though this approach is used in estimating population dynamics of whiteflies, it has some limitations since some fly away immediately there is any disturbances in their environment. Therefore the use of relative measures or estimates using sticky traps, vacuum collector, sunmica plate, passive fan trap are recommended as opposed to direct counting (NonoWomdim *et al*., 2005). The data collected from this study did not focus on diversity of whitefly populations found within the experimental site, however in Chapter 5 we reported that no *Bemisia tabaci* Genn species was collected from tomato agroecosystems during the survey. This is in agreement with findings of Khamis *et al*. (2021). This could explain why the whitefly population did not influence the disease incidence and severity. Tomato leaf curl disease is spread by *Bemisia tabaci* Genn, though seed transimission has also been reported (Kil *et al*., 2016) and there is no evidence of

mechanical transmission (Yoon *et al*., 2015). Hence, there is need to determine the possibility of seed transmission of leaf curl viruses in tomato seeds in Kenya

It was observed that there was significant difference in whitefly populations on the varieties tested. This could be attributed to preference, physical barriers, such as waxy or thick cuticles or the presence of specialized trichomes that inhibit whiteflies from settling and feeding on some leaves (Bellotti and Arias, 2001; Rakha *et al*., 2017). Additionally, tomato glandular trichomes release secondary metabolites that influence the whiteflies' feeding behavior and preference for or avoidance of specific plants (Rodríguez-López *et al*., 2011). This phenomenon contributes to antibiosis based resistance to whiteflies amongst the tomato cultivars. Previous studies have demonstrated that plants exibiting natural resistance against the transmission vector may not be resistant to its transmittable viral species (Yan *et al*., 2018). This may explain why the variation in whitefly populations did not correlate with TYLCV –like symptoms observed.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Discussion

From our results we conclude that tomato leaf curl disease is present in tomato crops in major tomato growing regions of Kenya. The disease is distributed in all tomato growing regions though with varied incidence and severity. The disease is caused by several begomoviruses namely, *Tomato leaf curl Arusha virus*, *African tomato leaf curl geminivirus*, *Tomato leaf curl Uganda virus*, *Ageratum yellow vein Sri Lanka virus* and *Tomato leaf curl New Delhi virus*. Tomato leaf curl viruses are amongst major viruses whose incidence and spread are influenced by presence of the whitefly vector (*Bemisia tabaci*). This study observed that high disease prevalence and incidences was observed in Counties with high horticultural production of crops such as Capsicum spp, egg plants, sweet potatoes, beans, cucurbits among others. Some of these crops are alternative hosts of *Bemisia tabaci* and begomoviruses. Previous studies have established that weeds such as *Physalis floridana*, *Euphorbia heterophylla* and *Desmodium* spp are alternative hosts of tomato leaf curl viruses. Therefore further studies should be conducted to understand the role of crops and weeds found in tomato agro ecosystems in the epidemiology of tomato leaf curl disease.

Tomato varieties sampled during the survey were found to be infected by tomato leaf curl disease though with varied incidences and severities. Similar observations were made during screening of selected varieties under spontaneous field inoculation. In this study only twenty tomato varieties were screened to assess their response to tomato leaf curl disease. It is therefore important to screen all tomato cultivars present in Kenya to assess their response to existing leaf

curl viruses. Through virus strain specific resistance should be developed in breeding programs. Breeding for leaf curl disease resistance in tomato plants is based mainly on three genes, Ty-1, Ty-2, and Ty-3, other genes include Ty-4, Ty-5 and Ty-6 (Yan *et al*., 2018). These genes are sourced from wild relatives of tomato such as *S. pimpinellifolium*, *S. habrochaites*, *S. peruvianum*, and *S. chilense* and introgressed in cultivated tomato. Breeding programs in Kenya should develop strain-specific resistant accessions considering the leaf curl viruses that are endemic in the country. Since several leaf curl viral strains are present durable resistance could be achieved by pyramiding several genes using molecular biotechnology tools. Such varieties will be beneficial to farmers as they will enhance tomato productivity in the country.

Findings of this study established that there was variation in whitefly populations across AEZs, Counties and on tomato plants. This could be due to factors such as type of tomato variety cultivated, cropping system and pest management options applied by different farmers across the surveyed areas. Different AEZs have variations in weather conditions and this could have an influence on whitefly populations. High whitefly populations have been observed during the dry season and decreasing with the onset of rain (Ssekyewa, 2006). Therefore future research should focus on understanding the role of weather in different agro ecological zones on white population dynamics.

Variation in whitefly populations on tomato varieties could have been due to antibiosis, where some cultivars were preferred over others. Antibiosis based resistance affects adult survival and oviposition rate of whiteflies (Lorenzo *et al*., 2016). Tomato characteristics such as hairness of leaves, occurrence of glandular trichomes type IV, influence oviposition rate, while the production of acylsucrose discourage the insect from making contact and settling on tomato (Rodrıguez-Lo´pez *et al*., 2011). In this study only *Trialeurodes vaporariorum* populations were

observed on tomato crops in all the regions sampled. Previous studies on plant resistance of *B. tabaci* whitefly species found that this whitefly species is affected by plant surface characteristics, such as glandular trichomes, leaf colour, shape and hairiness (Oriani and Vendramim 2010; Lorenzo *et al*., 2016). The research further established that *B.tabaci* whitefly species was able to develop easily on tomato crop, but it was rare to find it on tomato crop under field conditions. This may explain why *B. tabaci* was not detected in this study since all whitefly samples were collected from tomato plants. Thus there is need for establishing the diversity of whitefly populations from alternative crops and weeds found within the complex agroecosystem in which tomato production in done in Kenya. This will explain the presence of leaf curl diseases in tomato crops.

Several methods, among others, use of serology and molecular techniques are used routinely to identify begomovirus in tomato crops in Kenya. The accuracy, reliability and robustness of these methods varies. In this study, metagenomics was adopted to understand the diversity of begomoviruses affecting tomato crops in Kenya. This technology has the ability to detect viruses either as single agents or as components in mixed infections and can reveal the presence of novel or unpredicted agents (Roossinck *et al*., 2015). The study established that tomato leaf curl disease in Kenya is caused by *Tomato leaf curl virus Arusha virus* (ToLCArV) and other viruses listed in table 4.4, *Chickpea chlorotic dwarf virus* a novel virus in Kenya was detected in one sample. This shows the need to use recent approaches in identification of causal agents of plant diseases. Hence, more research should focus on understanding diversity of viruses infecting tomato crops in Kenya, and evaluation of resistance of various tomato cultivars to tomato diseases. Information obtained from these studies will be useful to tomato breeders and will improve tomato productivity in the country. An understanding on viruses present in the country

will greatly inform the idea of designing various molecular techniques which can be used routinely in detecting tomato viruses.

Results from sequence similarity indices, together with phylogenetic inferences, suggest that the ToLCArV-isolates associated with tomato leaf curl diseases in Kenya were likely of Tanzanian origin. Other recent studies have reported plant viruses that are likely to be introductions into the country (Wamaitha *et al*., 2018; Mutuku *et al*., 2018). Taken together with our discovery of a ToLCArV –isolate, there is need for increased vigilance to prevent accidental importation of alien viruses that may threaten African food security. Nonetheless, more research should focus on understanding diversity of viruses infecting tomato crops in Kenya and evaluation of resistance of various tomato cultivars to tomato diseases. Information obtained from these studies will be useful to tomato breeders and will improve tomato productivity in the country. An understanding on viruses present in the country will greatly inform the idea of designing various molecular techniques which can be used routinely in detecting tomato viruses.

7.2 Conclusions

Results from this study present a good starting point for understanding begomoviruses found tomato plants in Kenya. We have established tomato leaf curl disease is present in tomato production fields in Kenya and exhibit symptoms such as reduced leaf nodes, upward leaf curling, chlorosis and stunting. The disease is caused by *Tomato leaf curl Arusha virus*, *African tomato leaf curl geminivirus, Tomato leaf curl Uganda virus, Ageratum yellow vein Sri Lanka virus* and *Tomato leaf curl New Delhi virus* not *Tomato yellow leaf curl viruses* as earlier perceived. *Tomato leaf curl Arusha virus* present in Kenya is a variant of ToLCArV with origins from Tanzania. Tomato varieties assessed in this study are infected by tomato leaf curl disease though at different severities. Farmers should be encouraged to adopt the use of hybrid varieties,

though this should be augmented with practices such as rouging of infected plants, management of insect vectors and field sanitation, this will reduce losses associated with viral infections. Breeders should adopt breeding programs that target viruses present in the country. The breeding program should aim at introgressing into varieties specific Ty-genes that will confer durable resistance to tomato leaf curl viruses present in tomato fields in Kenya. This can be achieved through use of recent advances in molecular breeding that enables gene pyramiding. Screening of developed genotypes should be done under controlled environment or agroinoculation as opposed to spontaneous field inoculation, this will limit risks of disease escapes in the genotypes.

T. vaporariorum evaluated in this study is similar to those reported elsewhere in the world. *T. vaporariorum* species is associated with transmission of viruses causing damaging diseases in vegetables and ornamental crops. There is therefore need to conduct a comprehensive assessment of the host range, geographical distribution genetic diversity, population dynamics, of *T. vaporariorum* species in Kenya. Additionally, though *Bemisia tabaci* was not detected in tomato crops sampled, there is need establish the role of the complex agroecosystem found in tomato fields in transmission of tomato leaf curl disease. Information generated from the diversity of whitefly populations found in tomato plants will be useful in application of pest management practices and will help in indiscriminate use of pesticides. This will ultimately improve tomato production across the country for better food security.

7.3 Recommendations

Having documented the distribution of tomato leaf curl disease in tomato crops in Kenya, the diversity the causative virus and of whitefly populations found in tomato crops and response of selected cultivars to the disease we recommend that;

- Breeding programs to focus on developing cultivars resistant to *Tomato leaf curl Arusha virus* and other leaf curl viruses present in Kenya.
- The host range of tomato leaf curl viruses in Kenya to be determined, this should include both cultivated and wild plant species
- There is need to determine the possibility of seed transmission of *Tomato leaf curl Arusha virus* in tomato plants in Kenya
- It is important to study the biodiversity and molecular relationship between whiteflies observed on tomato and those found on other plants within the agro-ecosystem.
- More research should be done on other begomoviruses that were detected from metagenomic analysis conducted in this study.
- There is need to sensitize farmers on appropriate management options of both the virus and *Trialeurodes vaporariorum*

REFERENCES

Abhary, M., Patil, B. L., and Fauquet, C. M. (2007). Molecular biodiversity, taxonomy, and nomenclature of tomato yellow leaf curl-like viruses. In: Czosnek H (ed.), Tomato yellow leaf curl virus disease. Dordrecht: Springer 85-118.

Adams, I. P., Miano, D.W., Kinyua, Z. M., Wangai, A., and Kimani, E. (2013). Use of next-generation sequencing for the identification and characterization of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* causing maize lethal necrosis in Kenya. *Plant Pathology* 62:741-49

Ahmed, M. Z., Ren, S.X., Mandour, N., Maruthi, M.N., Naveed, M., and Qiu, B. L. (2010). Phylogenetic analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations from cotton plants in Pakistan, China, and Egypt. *Journal of Pest Science* 83: 135-41.

Ajlan, A.M., Ghanem, G.A.M., and Abdulsalam, K.S. (2007). *Tomato yellow leaf curl virus* (TYLCV) in Saudi –Arabia: Identification, partial characterization and virus –vector relationship. *Arab Journal of Biotechnology* 10: 179-192.

Akhtar, S., Khan, A.J., Singh, A.K., Briddon, R.W. (2014). Identification of a disease complex involving a novel monopartite begomovirus with beta-and alpha satellites associated with okra leaf curl disease in Oman. Archives of Virology 159:1199–205.

Akinyemi, I. A., Wang, F., Zhou, B., Qi, S., and Wu, Q. (2016). Ecogenomic survey of plant viruses infecting Tobacco by Next generation sequencing. Virol. J. 13, 1–12.

Alabi, O.J., Al Rwahnih, M., Karthikeyan, G., Poojari, S., Fuchs, M., Rowhani, A., and Naidu, R.A. (2011). Grapevine leafroll-associated virus 1 occurs as genetically diverse populations. *Phytopathology*101:1446-56.

Al-ani, R.A., Adhab, M.A., Hamad, S.A.H., and Diwan, S.N.H. (2011). *Tomato yellow leaf curl virus* (TYLCV), identification, virus vector relationship, strains characterization and a suggestion for its control with plant extracts in Iraq. *African Journal of Agricultural Research* 6: 5149-5155

Ala-Poikela, M., Svensson, E., Rojas, A., Horko, T., Paulin, L., Valkonen, J.P.T., and Kvarnheden, A. (2005). Genetic diversity and mixed infections of begomoviruses infecting tomato, pepper and cucurbit crops in Nicaragua. *Plant Pathology* 54:448-9.

Anfoka, G., Abhary, M., Ahmad, F.H., Hussein, A.F., Rezk, A., Akad, F., Abou-Jawdah,Y., Lapidot,M., Vidavski, F., Nakhla, M.K., Sobh, H., Atamian, H., Cohen, L., Sobol, I., Mazyad, H., Maxwel, D.P., and Czosnek, H. (2008). Survey of Tomato yellow leaf curl disease associated viruses in Eastern Mediterranean basin. *Journal of Plant Pathology* 90:313-322

Avedi, E.K., Kilalo, D., Olubayo, F., Macharia, I., Adediji, A.O., Ateka, E.M., Machuka, E.M., and Mutuku, J.M. (2020). Complete genome sequence of a new Chickpea chlorotic dwarf virus strain from Tomato in Kenya, obtained from Illumina sequencing. *Microbiology Resource Announcement* 9:e01344-19.

AVRDC (2013). Research Proposal: Multi-Location Evaluation of Tomato Lines Carrying Different Combinations of Ty Genes For Resistance Against Begomovirus Infection. Asian Vegetable Research and Development Centre, Taiwan. 19pp.

Bandelt, H.J., Forster, P., and Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. Molecular Biology 16: 37–48.

Barbieri, M., Acciarri, N., Sabatini, E., Sardo, L., Accotto, G. P., and Pecchioni, N. (2010). Introgression of resistance to two Mediterranean virus species causing tomato yellow leaf curl into a valuable traditional tomato variety. *Journal of Plant Pathology* 92: 485–493.

Barboza, N.M., Esker, P., Inoue-Nagata, A.K., and Moriones, E. (2019). Genetic diversity and geographic distribution of *Bemisia tabaci* and Trialeurodes vaporariorum in Costa Rica. *Annals of Applied Biology* 1–14. https://doi.org/10.1111/aab.12490

Basak, J. (2016). *Tomato Yellow Leaf Curl Virus*: A Serious Threat to Tomato Plants World Wide. *Journal of Plant Pathology and Microbiology* 7: 346.

Bellotti, A.C., and Arias, B. (2001). Host plant resistance to whiteflies with emphasis on cassava as a case study. *Crop Protection* 20: 813–823.

Berry, S.D., Fondong, V.N., Rey, C., Rogan, D., Fauquet, C.M., and Brown, J.K. (2004). Molecular evidence for five distinct *Bemisia tabaci* (Homoptera: Aleyrodidae) geographic haplotypes associated with cassava plants in sub-Saharan Africa. *Annals of the Entomological Society of America* 97: 852–859.

Blawid, R., Silva, J. M. F., and Nagata, T. (2017). Discovering and sequencing new plant viral genomes by next-generation sequencing: description of a practical pipeline. *Annals of Applied Biology* 170: 301–314.

Bob, A.M., Odhiambo, B., Kibata, G., and Ong'aro, J. (2005). White flies as pests and vectors of viruses in vegetables and legume mixed cropping systems in Eastern and Southern Africa. In; Anderson, P.K., Morales, J.F. (eds) Whitefly and Whitefly-borne Viruses in the Tropics: Building a knowledge base for global action. *CIAT publications* 129-143

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., and Mumford, R. (2013). Methods in virus diagnostics: from ELISA to Next Generation Sequencing, *Virus Research* 186:20-31

Boykin, L.M., and De Barro, P.J. (2014). A practical guide to identifying members of the *Bemisia tabaci* species complex and other morphologically identical species. *Frontiers in Ecological Evolution* 2: 1-5

Briddon, R.W., Bull, S.E., Amin, I., Mansoor, S., Bedford, I.D., Rishi, N., Siwatch, S.S., Zafar, M.Y., Abdel-Salam, A.M. and Markham, P.G. (2004). Diversity of DNA 1; a satellite-like molecule associated with monopartite begomovirus-DNA- β complexes. *Virology* 324: 462-474

Briddon, R.W., Patil, B.L., Bagewadi, B., Nawaz-ul Rehman, M.S., and Fauquet, C.M. (2010). Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses. *BMC Evolution Biology* 10:97

Briddon, R.W., Pinner, M.S., Stanley, J., and Markham, P.G. (1990). Geminivirus coat protein gene replacement alters insect specificity. *Virology* 177:85-94.

Brown, J.K., Fauquet, C.M., Briddon, R.W., Zerbini, M., Moriones, E. and Navas-Castillo, J. (2012). Family Geminiviridae. In: A. M. Q. King, M. J. Adams, E. B. Carstens and E. J. Lefkowitz (Ed), Virus Taxonomy: Classification and Nomenclature of Viruses - Ninth Report of the International Committee on Taxonomy of Viruses. In: *Elsevier Academic Press*, USA, pp. 351-373.

Brown, J.K., Ur-Rehman, M.Z., Avelar, S., Chingandu, N., Hameed, U., Haider, S., and Ilyas, M. (2017). Molecular diagnostic development for begomovirus-betasatellite complexes undergoing diversification: A case study. *Virus Research* 241:29-41.

Caciagli, P., Medina, P.V., Marian, D., Vecchiati, M., Masenga, V., Mason, G., Falcioni, T., and Noris, E. (2009). Virion stability is important for the circulative transmission of *Tomato yellow leaf curl Sardinia virus* by *Bemisia tabaci*, but virion access to salivary glands does not guarantee transmissibility. *Journal of Virology* 83: 5784–5795.

Castle, S.J., Palumbo, J.C., Prabhaker, N., Horowitz, R., and Delholm, I. (2010). Ecological determinants of *Bemisia tabaci* resistance to insecticides. In: Stansly, P. A., Naranjo, S. E. (eds) Bemisia: Biomics and Management of a Global Pest. Dordrecht, Heidelberg, London, New York, The Netherlands, Germany, UK, USA: *Springer* 423-465.

Chen, S., Zhou,Y., Chen., and Gu, J. (2018). Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34: i884–i890.

Chiang, B.T., Nakhla, M.K., and Maxwell, D.P. (1997). A new geminivirus associated with a leaf curl disease of tomato in Tanzania. *Plant Disease* 81: 111

Chu, D., Wan, H.F., Zhang, Y. J., and Brown, J.K. (2010). Change in the biotype composition of *Bemisia tabaci* in Shandong province of China from 2005 to 2008. *Environmental entomology* 39: 1028-1036

Chu, D., Zhang, Y.J., Brown, J.K., Cong, B., Xu, B., Wu, Q. J., and Zhu, G. R. (2006). The introduction of exotic Q biotype of *Bemisia tabaci* from the Mediterranean region into China on ornamental crops. *Florida Entomologist* 89**:** 1-10

Clark, M.F., and Adams, A. N. (1977). Characteristics of the microplate method of enzyme - linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34: 475-483

Cohen, J., Gera, A., Ecker, R., Ben-Joseph, R., Perlsman, M., Gokkes, M., Lachman, O., and Antignus, Y. (1995). Lisianthus leaf curl a new disease of lisianthus caused by *Tomato yellow leaf curl virus*. *Plant Disease* 79:416-420

Cohen, S., and Harpaz, I. (1964). Periodic, rather than continual, acquisition of a new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci*). *Entomologica Experimentalis Applicata* **7**:155–166

Courtney, S.P., and Kibota, T.T. (1990). Mother doesn't know best: selection of hosts by ovipositing insects. In: Bernays EA (ed) Insect–plant interactions, vol 2. *CRC Press*, Boca Raton, pp 161–188

Czosnek, H. (2007). Interactions of Tomato yellow leaf curl virus with its insect vector. In: Czosnek, H (ed.), The Tomato Yellow Leaf Curl Virus Disease: Management, Molecular Biology, Breeding for Resistance. Dordrecht, The Netherlands: *Springer*, 157-170.

Davino, S., Napoli, C., Dellacroce, C., Miozzi, L., Noris, E., Davino, M., and Accotto, G.P. (2009). Two new natural begomovirus recombinants associated with the tomato yellow leaf curl disease co-exist with parental viruses in tomato epidemics in Italy. *Virus Research* 143: 15–23.

De Barro, P.J., Liu, S.S., Boykin, L.M., and Dinsdale, A.B. (2011). *Bemisia tabaci*: A statement of species status. *Annual Review Entomology* 56: 1-9

de Jong, M.A., Wahlberg, N., van Eijk, M., Brakefield, P.M., and Zwaan, B.J. (2011). Mitochondrial DNA Signature for Range-Wide Populations of Bicyclus anynana suggests a Rapid Expansion from Recent Refugia. *PLoS ONE* 6: e21385.

De la Peña, R., Kadirvel, P., Venkatesan, S., Kenyon, L., and Hughes, J. (2010). Integrated approaches to manage Tomato Yellow Leaf Curl Viruses. In Hou, C.T., Shaw, J.-F. (eds) Biocatalysis and Biomolecular Engineering. Hoboken New Jersey, John Wiley & Sons 105–132.

de Moya, R.S., Brown, J.K., Sweet, A.D., Walden K.K., Paredes-Montero, R.J.,Waterhouse, M.R., and Johnson, P. K. (2019). Nuclear orthologs derived from whole genome sequencing indicate cryptic diversity in the *Bemisia tabaci* (Insecta: Aleyrodidae) complex of whiteflies. *Diversity* 11:151

Delatte, H., Martin, D.P., Naze, F., Goldbach, R., Reynaud, B., Peterschmitt, M., and Lett, J.M. (2005). South West Indian Ocean islands tomato begomovirus populations represent a new major monopartite begomovirus group. *Journal of General Virology* 86:1533-42.

Dennehy, T. J., Degain, B. A., Harpold, V. S., Brown, J. K., Morin, S., Fabrick, J. A., Byrne, F. J., and Nichols, R. L. (2005). New challenges to management of whitefly resistance to insecticides in Arizona. The University of Arizona-Cooperative Extension Report, Tucson, AZ.

Dhakar, K., Gupta, V. K., Rathore, M. S. and Gaur, R. K. (2010). Virus resistance and gene silencing in plants infected with begomovirus. *Journal of Applied Sciences* 10: 1787 – 1791.

Diaz-Pendon, J.A., Carmen, M.C, Moriones, E. Eduardo, R.B, Czonek, H., and Navas-Castillo, J. (2010). Tomato yellow leaf curl viruses: ménage ‘a trois between the virus complex, the plant and the whitefly vector. *Plant Molecular Biology* 1: 19-21.

Díaz-Pendón, J.A., Sánchez-Campos, S., Fortes, I.M., and Moriones, E. (2019). Tomato Yellow Leaf Curl Sardinia Virus, a Begomovirus Species Evolving by Mutation and Recombination: A Challenge for Virus Control. *Viruses* 11: 45

Duffy, S., and Holmes, E.C. (2009). Validation of high rates of nucleotide substitution in geminiviruses: phylogenetic evidence from East African cassava mosaic viruses. *Journal of General Virology* 90:1539-47.

Edelbaum, D., Gorovits, R., Sasaki, S., Ikegami, M. and Czosnek, H. (2009). Expressing a whitefly GroEL protein in *Nicotiana benthamiana* plants confers tolerance to *Tomato yellow leaf curl virus* and *Cucumber mosaic virus,* but not to *Grapevine virus* A or *Tobacco mosaic virus*. *Archive of Virology* 154: 399–407.

Elbaz, M., Hanson, P., Fgaier, S., and Laarif, A. (2016). Evaluation of tomato entries with different combinations of resistance genes to Tomato yellow leaf curl disease in Tunisia. *Plant Breeding*. 135:525–530.

Esterhuizen, L.L., Mabasa, K.G., Van Heerden, S.W., Czosnek, H., Brown, J.K., Van Heerden, H., and Rey, M.E. (2013). Genetic identification of members of the *Bemisia tabaci* cryptic species complex from South Africa reveals native and introduced haplotypes. *Journal of Applied Entomology* 137: 122–135.

European and Mediterranean Plant Protection Organization (2005). *Diagnostic Bulletin* 35: 271–273

Excoffier, L., Laval, G., and Schneider., S. (2005). Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolution Bioinformatics Online* 1:47–50

Fahmy, F.I., and Abou-Ali, M.R. (2015). Studying genetic diversity of whitefly *B. tabaci* Egyptian isolates in relation to some worldwide isolates. *Journal of Genetic Engineering and Biotechnology* 13, 87–92

FAOSTAT (2019). Global tomato production in 2019. FAO Rome.

Fauquet, C.M., Briddon, R.W., Brown, J.K, Moriones, E., Stanley, J., Zerbini, M., and Zhou, X. (2008). Geminivirus strain demarcation and nomenclature. *Archives of Virology* 153:783-821

Fiallo-Olivé, E., Hernández-Zepeda, C., Trejo-Saavedra, D., Carrillo-Tripp, J., Rivera-Bustamante, R, F., and Martínez-Zubiaur, Y. (2012). Complete genome and pathogenicity of *Tomato yellow leaf distortion virus*, a bipartite begomovirus infecting tomato in Cuba. *European Journal of Plant Pathology* 134:13–21

Fiallo-Olivé, E., Pan, L., Liu, S., and Navas-Castillo, J. (2020). Transmission of Begomoviruses and other whitefly-borne viruses: Dependence on the vector species. *Phytopathology* 110:10-17

Frohlich, D. R., Torres-Jerez, I., Bedford, I. D., Markham, P. G., and Brown, J. K. (1999). A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Journal of Molecular Ecology* 8: 1683 -1691.

Froissart, R., Roze, D., Uzest, M., Galibert, L., Blanc, S. and Michalakis, Y. (2005). Recombination every day: abundant recombination in a virus during a single multi-cellular host infection. *PLoS Biology* 3: 389–395.

Fu, Y.X., and Li, W.H. (1993). Statistical tests of neutrality of mutations. *Genetics* 133: 693–709

Fuentes, A., Ramos, P.L., Fiallo, E., Callard, D., Sánchez, Y., Peral, R., Rodríguez, R., and Pujol M. (2006). Intron-hairpin RNA derived from replication associated protein C1 gene confers immunity to *Tomato yellow leaf curl virus* infection in transgenic tomato plants. *Transgenic Research* 15:291-304.

Gao, R.R., Zhang, W.-P., Wu, H.-T., Zhang, R.-M., Zhou, H.-X., Pan, H.-P., Zhang, Y-J., Brown, J.K. and Chu, D. (2014) Population structure of the greenhouse whitefly, Trialeurodes vaporariorum (Westwood), an invasive species from the Americas, 60 years after invading China. *International Journal of Molecular Sciences* 15: 13388–13400.

García-Andrés, S., Monci, F., Navas-Castillo, J. and Moriones, E. (2006). Begomovirus genetic diversity in the native plant reservoir *Solanum nigrum*: evidence for the presence of a new virus species of recombinant nature. *Virology* 350: 433–442.

Gariepy, T.D., Kuhlmann, U., Gillott, C., and Erlandson, M. (2007). Parasitoids, predators and pcr: The use of diagnostic molecular markers in biological control of arthropods. *Journal of Appllied Entomol*ogy 131: 225- 240

Ghanim, M., and Medina, V. (2007). Localization of *Tomato yellow leaf curl virus* in its whitefly vector *Bemisia tabaci*. In: Czosnek, H. (ed) *Tomato Yellow Leaf Curl Virus* Disease: Management, Molecular Biology, Breeding for Resistance. Dordrecht: *Springer* 171–183.

Ghanim, M., Morin, S., and Czosnek, H. (2001). Rate of *Tomato yellow leaf curl virus* translocation in the circulative transmission pathway of its vector, the whitefly *Bemisia tabaci*. *Phytopathology* 91: 188-196.

Ghanim, M., Sobol, I., Ghanim, M., and Czosnek, H. (2007). Horizontal transmission of begomoviruses between *Bemisia tabaci* biotypes. *Arthropod Plant Interaction* 1: 195–204

Ghosh, R., Palit, P., Paul, S., Ghosh, S. K., and Roy, A. (2012). Detection of Corchorus golden mosaic virus Associated with Yellow Mosaic Disease of jute (Corchorus capsularis). *Indian Journal of Virology* 23: 70 – 74.

Gibbs, A.J., Fargette, D., García-Arenal, F., and Gibbs, M.J. (2010). Time–the emerging dimension of plant virus studies. *Journal of General Virology* 91:13-22.

Gibson, W. R., Jeremiah, C. S., Aritua, V., Msabaha, P.R., Mpembe, I., and Ndunguru, J. (2000). Sweet potato virus disease in Sub-Saharan Africa: evidence that neglect of seedlings in

the traditional farming system hinders the development of superior resistant landraces. *Journal of Phytopathology* 148:441-447

Gilbertson, R.L., Rojas, M.R., Kon, T., and Jaquez, J. (2007). Introduction of Tomato yellow leaf curl virus into the Dominican Republic: the development of a successful integrated pest management strategy. In: Czosnek, H. (ed) The Tomato Yellow Leaf Curl Virus Disease: Management, Molecular Biology, Breeding for Resistance by Dordrecht, The Netherlands: *Springer* 283-307.

Glick, E., Levy, Y., and Gafni, Y. (2009). The viral etiology of Tomato Yellow Leaf Curl Disease A review. *Plant Protection Science* 45: 81-97

Gorman, K., Devine, G., Bennison, J., Coussons, P., Punchard, N., and Denholm, I. (2007). Report of resistance to the neonicotinoid insecticide imidacloprid in Trialeurodes vaporariorum (Hemiptera: Aleyrodidae). *Pest Management Science* 63: 555–558.

Green, S. K. (1991). Guidelines for diagnostic work in plant Virology. AVRDC Technical Bulletin. No.15. 63 pp

Hall, T.A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-8.

Harrison, B.D., and Robinson, D.J. (1999). Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). *Annual Review of Phytopathology* 37: 369–398

Hebert, P.D., Cywinska, A., Ball, S.L., and deWaard, J.R. (2003). Biological identifications through DNA barcodes. *Proceedings of Biological Science 270*, 313-321.

Hebert, P.D., Penton, E.H., Burns, J.M., Janzen, D.H., and Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly astraptes fulgerator. *Proceedings of the National Academic of Science* USA 101: 14812-14817

Higuchi, S., Maeda, M., Gyoutoku, Y., Ueda, S., and Komaki, K. (2007). Distribution and seasonal fluctuations in the population of different biotypes of *Bemisia tabaci* (Gennadius) in Kumamoto Prefecture. *Kyushu Plant Protection Research* 53: 59-65.

Hodges, G.S. and Evans, G.A. (2005). An identification guide to the whiteflies (Hemiptera: Aleyrodidae) of the Southeastern United States. *Florida Entomologist* 88: 518-534.

Horowitz, A.R., Kontsedalov, S., Khasdan, V., and shaaya, I. (2005). Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and Pyriproxyfen resistance. *Archive of Insect Biochemical Physiology* 58: 216-25

Horticultural Crops Development Authority, (2020). Horticulture data 2019-2020 Validation Report, Kenya.

Hosseinzadeh, M. R., Shams-Bakhsh, M., Osaloo, S. K., and Brown, J. K. (2014). Phylogenetic relationships, recombination analysis, and genetic variability among diverse variants of tomato yellow leaf curl virus in Iran and the Arabian Peninsula: further support for a TYLCV center of diversity. *Archives of Virology* 159: 485–497.

Hosseinzadeh, M., and Garivani, H. (2014). Emerging two distinct groups of the tomato yellow leaf curl virus-severe strain (TLYCV-IL) variant in Iran. *Trakia Journal of Science* 2: 149-161.

Hull, R. (2009). Comparative Plant Virology. 2nd edition. Academic Press, New York

Idris, A., Al-Saleh, M., Piatek, M.J., Al-Shahwan, I., Ali, S., and Brown, J.K. (2014). Viral metagenomics: analysis of begomoviruses by Illumina high-throughput sequencing. *Viruses* 6:1219–36.

Idriss, M., Abdallah, N., Aref, N., Haridy, G., M., and Madkour, M. (1997). Biotypes of the castor bean whitefly Trialeurodes ricini (Misra)(Hom, Aleyrodidae) in Egypt.

Biochemical Characterizaion and efficiency of geminivirus transmission. *Journal of Applied Entomology* 121: 501-509

Jones, A. L., and Markham, R. H. (2005). Whitefly and whitefly-borne viruses in the tropics: building a knowledge base for global action. CIAT 341, 129–140.

Jupin, I., De Kouchkovsky, F., Jouanneau, F., and Gronenborn, B. (1994). Movement of tomato yellow leaf curl geminivirus (TYLCV): Involvement of the protein encoded by ORF C4. *Virology* 204:82-90.

Kapantaidaki, D., Ovcarenko, I., Fytrou, N., Knott, K. E., Bourtzis, K., and Tsagkarakou, A. (2015). Low levels of mitochondrial DNA and symbiont diversity in the worldwide agricultural pest, the greenhouse whitefly Trialeurodes vaporariorum (Hemiptera: Aleyrodidae). *Journal of Heredity* 106: 80–92.

Karatolos, N., Pauchet, Y., Wilkinson, P., Chauhan, R., Denholm, I., Gorman, K., and Williamson, M. S. (2011). Pyrosequencing the transcriptome of the greenhouse whitefly, Trialeurodes vaporariorum reveals multiple transcripts and detoxifying enzymes. *BMC Genomics* 12: 56.

Karuku. G.N., Kimenju, J.W., and Verplancke, H. (2017). Farmers' perspectives on factors limiting tomato production and yields in Kabete, Kiambu County, Kenya. *East African Agricultural and Forestry Journal* 82: 70–89

Kashina, B.D., Mabagala, R.B., and Mpunami, A. A. (2002). Molecular characterization of isolates of Tomato yellow leaf curl from Tanzania. *Archive of Phytopathology* 35: 255–267.

Khamis, M.F., Ombura, L.O.F., Ajene, J.I., Akutse, S.K., Subramanian, S., Mohamed, A. S., Dubois, T., Tanga, M. C., and Ekesi, S. (2021). Mitogenomic analysis of diversity of key whitefy pests in Kenya and its implication to their sustainable management. *Scientific reports* 11:6348

Kil, E.J., Park, J., Choi, E.Y., Byun, H.S., Lee, K.Y., An, C.G., Lee, J.H., Lee, G.S., Choi, H.S., Kim, C.S., et al. (2018). Seed transmission of Tomato yellow leaf curl virus in sweet pepper (Capsicum annuum). *European Journal of Plant Pathology* 150:759-64.

Kil, E-J., Lee,Y-J., Byun, H-S., Park,J., Seo,H., Kim, C-S., Shim, J-K., Lee,K-Y., Choi,H-S., and Lee,S. (2016). *Tomato yellow leaf curl virus* (TYLCV-IL): a seed-transmissible geminivirus in tomatoes. *Scientific Report.* **6**: 19013

Kimathi, R. H., Wilisiani, F,. Mashiko, T., Neriya, Y., Miinda, A. E., Nishigawa, H., and Natsuaki, T. (2020). First report of *Tomato chlorosis virus* infecting tomato in Kenya. *Scientific African* 7:e00286

Kimathi, R. H., Wilisiani, F., Mashiko, T., Neriya, Y., Miinda, A. E., Nishigawa, H., and Natsuaki, T. (2020). Molecular characterization of Tomato leaf curl Uganda virus infecting tomato plants in Kenya. *Journal of General Plant Pathology*. https://doi.org/10.1007/s10327-020-00950-2

Kon, T. and Gilbertson, R. L. (2012). Two genetically related begomoviruses causing tomato leaf curl disease in Togo and Nigeria differ in virulence and host range but do not require a betasatellite for induction of disease symptoms. *Archives of virology* 157: 107 – 120.

Kosakovsky Pond, S. L., and Frost, S. D.W. (2005). Datamonkey: Rapid detection of selective pressure on individual sites of codon alignments. *Bioinformatics* 21:2531-3.

Kosakovsky Pond, S. L., Poon, A. F.Y., Velazquez, R., Weaver, S., Hepler, N.L., Murrell, B., Shank, S. D,, Magalis, B. R., Bouvier, D., Nekrutenko, A., et al. (2020). HyPhy 2.5—A customizable platform for evolutionary hypothesis testing using phylogenies. *Molecular Biology and Evolution* 37:295-9

Kosakovsky Pond, S. L., Posada, D., Gravenor, M. B., Woelk, C. H., and Frost, S. D. W. (2006). Automated phylogenetic detection of recombination using a genetic algorithm. *Molecular Biology and Evolution* 23:1891-1901.

Kunz, D., Tay, W.T., Elfekih, S., Gordon, H.J.K., and De Barro, J. P. (2019). Take out the rubbish– Removing NUMTs and pseudogenes from the *Bemisia tabaci* cryptic species mtCOI database. bioRxiv

Kyallo, M., Sseruwagi, P., Skilton, R.A., Ochwo-Ssemakula, M., Wasswa, P., and Ndunguru, J. (2017). Deinbollia mosaic virus: A novel begomovirus infecting the sapindaceous weed Deinbollia borbonica in Kenya and Tanzania. *Archives of Virology* 162:1393-6.

Langmead, B., and Steven, S. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9:357-9.

Lapidot, M. (2002). Screening common bean (*Phaseolus vulgaris*) for resistance to *Tomato yellow leaf curl virus. Plant disease* 86: 429-432

Lapidot, M., Legg, J. P., Wintermantel, W. M., Polston, J. E. (ed.) (2014). Management of Whitefly- Transmitted Viruses in Open-Field Production Systems. Control of Plant Virus Diseases (1st ed.). Elsevier Inc.

Lee, W., Kim, H., Lim, J., Choi, H., Kim, Y., Kim, Y-S., Ji, J., Foottit, R., and Lee, S. (2010). Barcording aphids (Hemiptera; Aphididae) of the Korean Peninsula: Updating the global data base. *Molecular ecology resources* doi: 10.1111/j.1755-0998.2010.02877.x

Lefeuvre, P., and Moriones, E. (2015). Recombination as a motor of host switches and virus emergence: Geminiviruses as case studies. *Current Opinion in Virology* 10: 14–19

Lefeuvre, P., Martin, D. P., Harkins, G., Lemey, P., Gray, A. J. A., Meredith, S., Lakay, F., Monjane, A., Lett, J. M., Varsani, A. and Heydarnejad, J. (2010). The spread of tomato

yellow leaf curl virus from the Middle East to the world. *PLoS Pathogens* 6(10): e1001164

Lett, J.M., Lefeuvre, P., Couston, L., Houreau, M., Thiery, M., Reynaud, B., Martin, D.P., and Varsani, A. (2009).Complete genomic sequence of *Tomato yellow leaf curl Mali virus* infecting tomato and pepper from the North Province of Cameroon. *Springer* 154: 535-540

Li, D., Liu, C.M., Luo, R., Sadakane, K., and Lam, T.W. (2015). MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* 31:1674-6.

Li, Z., Hu, D., Song, Y., and Shen, Z. (2005). Molecular differentiation of the B biotype from other biotypes of *Bemisia tabaci* (Hemiptera: Aleyroididae) based on internally transcribed spacer 1 sequences. *European Journal of Entomology* 102: 293-297.

Librado, P., and Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452.

Lietti, M. M.M,, Botto, E., and Alzogaray, R.A. (2005). Insecticide resistance in Argentine populations of Tutaabsoluta (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* 34:113-119.

Liu, S.S., Colvin, J.J., and De Barro, P. J. (2012). Species concept as applied to the whitefly *Bemisia tabaci* systematics: How many species are there? *Journal of Integrated Agriculture* 11: 176-186

Lorenzo, M.E., Grille, G., Basso, C., and Banato, O. (2016) Host preferences and biotic potential of Trialeurodes vaporariorum and *Bemisia tabaci* (Hemiptera: Aleyrodidae) in tomato and pepper. *Arthropod-Plant Interactions* 10:293–301.

Luna, A. P., Morilla, G., Voinnet, O., and Bejarano, E.R. (2012). Functional analysis of gene-silencing suppressors from tomato yellow leaf curl disease viruses. *Molecular Plant Microbe Interaction* 25:1294-306.

Macharia, I., Backhouse, D., Ateka, E. M., Wu, S-B., Harvey, J., Njahira, M., and Skilton, R.A. (2015). Distribution and genetic diversity of *Tomato spotted wilt virus* following an incursion into Kenya. *Annals of Applied Biology* 1-9.

Macharia, I., Backhouse, D., Skilton, R., Ateka, E., Wu, S-B., Njahira, M., Maina, S., and Harvey J. (2015). Diversity of Thrips species and vectors of *Tomato spotted wilt virus* in tomato production systems in Kenya. *Journal of Economic Entomology* 1-9.

Mansour, A., and Al-Musa, A. (1992). Tomato yellow leaf curl virus: host range and virus vector relationships. Plant Pathology 41: 122-125.

Marie, E. C. R., Ndunguru, J., Berrie, L.C., Paximadis, M., Berry, S., Cossa, N., Valter N. N., Kenneth G. M., Abraham 1, N., Rybicki, E, P., Martin, D., Pietersen, G., and Esterhuizen, L.L. (2012). Diversity of Dicotyledonous-Infecting Geminiviruses and Their Associated DNA Molecules in Southern Africa, Including the South-West Indian Ocean Islands. *Viruses* 4: 1753-1791

Martin, D.P., Murrell, B., Golden, M., Khoosal, A., and Muhire, B. (2015). RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evolution* 1: vev003.

Martin, D.P., Van der Walt, E., Posada, D., and Rybicki, E.P. (2005). The evolutionary value of recombination is constrained by genome modularity. *PLoS Genetics* 1: 475–479.

Masinde, A.O.A., Kwambai, K.T., and Wambani, N.H. (2007). Evaluation of tomato (Lycopersicon esculentum L.) variety tolerance to foliar diseases at Kenya Agricultural

Research Centre-Kitale in North west Kenya. *African Journal of Plant Science* 5: 676-681

McKenzie, C.L., Hodgers, G., Osborne, S.L., Byrne, J.F., and Shatters, R.G. (2009). Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotypes in Florida investigating the Q invasion. *Journal of Economic Entomology* 102: 670-676.

Medina, V., Pinner, M.S., Bedford, I.D., Achon, M. A., Gemeno, C., and Markham, P.C. (2006). Immunolocalization of *Tomato yellow leaf curl Sardinia virus* in natural host plants and its vector *Bemisia tabaci*. *Journal of Plant Pathology* 88: 299-308

Mehta P., Wyman, J. A., Nakhla, M. K., and Maxwell, D. P. (1994). Transmission of Tomato yellow leaf curl virus by *Bemisia tabaci* (Homoptera: Aleyrodidae). *Economic Entomology* 87: 1291-1297

Mejia, L., Teni, R.E., Garcia, B.E., Fulladolsa, A.C., and Mendez, L. (2010). Preliminary observations on the effectiveness of five introgressisons for resistance to begomoviruses in tomatoes. *TGC Report* 60: 41–53

Melgarejo, T.A., Kon, T., Rojas, M.R., Paz-Carrasco, L., Zerbini, F.M., and Gilbertson, R.L. (2013). Characterization of a new world monopartite begomovirus causing leaf curl disease of tomato in Ecuador and Peru reveals a new direction in geminivirus evolution. *Journal of Virology* 87:5397-413.

Menzel, P., Ng, K.L., and Krogh, A. (2016). Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nature Communication* 7:11257.

Miano, D.W., and Kuria, P.K. (2017). Status of begomovirus research and management in Kenya. In: Saxena S, Tiwari AK, editors. Begomoviruses: Occurrence and Management in Asia and Africa. Singapore: *Springer Nature* 285-95.

Miano, D.W., LaBonte, D.R., Clark, C.A., Valverde, R.A., and Hoy, M.W. (2006). First report of a begomovirus infecting sweet potato in Kenya. *Plant Disease* 90:832.

Milenovic, M., Wosula, E.N., Rapisarda, C., and Legg, J.P. (2019). Impact of host plant species and whitefly species on feeding behavior of *Bemisia tabaci*. *Frontiers in Plant Science* 10:1 doi:10.3389/fpls.2019.00001

Misaka, B.C., Wosula, E.N., Marchelo-d'Ragga. P.W. et al (2019) Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Alyerodidae) colonizing sweet potato and cassava in South Sudan. *Insects* 11:58. https ://doi.org/10.3390/insects110 10058

Monci, F., Sanchez-Campos, S., Navas-Castillo, J., and Moriones, E. (2002). A natural recombinant between the Geminiviruses *Tomato yellow leaf curl Sardinia virus* and *Tomato yellow leaf curl virus* exhibit a novel pathogenic phenotype and is becoming prevalent in Spanish populations. *Virology* 303: 317-326.

Mondal, D., Mandal, S., Shil, S., Sahana, N., Pandit, G.K., and Choudhury, A. (2019). Genome wide molecular evolution analysis of begomoviruses reveals unique diversification pattern in coat protein gene of Old World and New World viruses. *Virus Disease* 30:74-83.

Moriones, E., and Navas-Castillo, J. (2008). *Tomato yellow leaf curl virus*, an emerging virus complex causing epidemics worldwide. *Virus Research* 71: 123–134.

Moriones, E., García-Andrés, S., and Navas-Castillo, J. (2007). Recombination in the TYLCV Complex: A Mechanism to Increase Genetic Diversity. Implications for Plant Resistance Development. In Czosnek, H (eds), Tomato Yellow Leaf Curl Virus Disease Management, Molecular Biology, Breeding for Resistance. Springer: Berlin/Heidelberg, Germany. 119–138.

Mugerwa, H., Rey, M.E., Alicai, T., Ateka, E., Atuncha, H., Ndunguru, J., and Sseruwagi, P. (2012). Genetic diversity and geographic distribution of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) genotypes associated with cassava in East Africa. *Ecology and Evolution* 2: 2749–2762.

Mugerwa, H., Seal, S., Wang, H.L., Patel, M.V., Kabaalu, R., Omongo, C.A., Alicai, T., Tairo, F., Ndunguru, J., Sseruwagi, P., et al. (2018). African ancestry of New World, *Bemisia tabaci*-whitefly species. *Scientific Report* 8: 1–11.

Mugerwa, H., Sseruwagi, P., Colvin, J., and Seal, S. (2021). Is high whitefly abundance on cassava in sub-Saharan Africa driven by biological traits of a specific, cryptic *Bemisia tabaci* species? *Insects* 12: 260

Muhire, B.M., Varsani, A., and Martin, D.P. (2014). SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS One* 9:e108277.

Mutuku, J.M., Wamonje, F.O., Mukeshimana, G., Njuguna, J.N., Wamalwa, M., Choi, S.K., Tungadi, T., Djikeng, A., Kelly, K., Entfellner, J-B. D., et al. (2018). Metagenomic Analysis of Plant Virus Occurrence in Common Bean (Phaseolus vulgaris) in Central Kenya. *Frontiers of Microbiology* 9:2939.

Mwangi, M.W., Kimenju, J.W., Narla, R.D., Kariuki, G.M., and Muiru. (2015). Tomato management practices and diseases occurrence in Mwea West Sub County. *Journal of Natural Science Research* 5: 119-120

Mware, B.O., Ateka, E.M., Songa, J.M., Narla, R.D., Olubayo, F., and Amata, R. (2009). Transmission and distribution of cassava brown streak virus disease in cassava growing areas of Kenya. *Journal of Applied Bioscience* 16:864-870.

Nagaraju, N., Venkatesh, H.M., Warburton, H., Muniyappa, V., Chancellor, T. C. B., and Colvin, J. (2002). Farmers' perceptions and practices for managing tomato leaf curl virus disease in southern India. *International Journal of Pest Management* 48:333-338

Naika, S., Jeude, J.v.L., Goffau, M.d., Hilmi, M., and Dam, B.v. (2005). Cultivation of tomato: production, processing and marketing Wagennigen, Netherlands: Agromisa Foundation and CTA

Navas-Castillo, J., Fiallo-Olive, E., and Sanchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual Review of Phytopathology* 49: 15.1–15.30.

Navas-Castillo, J., Lopez, J. J., and Aranda, M. A. (2014). Whitefly-transmitted RNA viruses that affect intensive vegetable production, 165: 155–171.

Navas-Castillo, J., Sanchez-Campos, S., Noris E., Louro, D., Accotto, G.P., and Moriones, E. (2000). Natural recombination between *Tomato yellow leaf curl virus*-Is and *Tomato leaf curl virus*. *Journal of General Virology* 81: 2797-2801.

Navot, N., Zeidan, M., Pichersky, E., Zamir, D., and Czosnek, H. (1992). Use of thepolymerase chain reaction to amplify Tomato yellow leaf curl virus DNA infected plants and viruliferous whiteflies. *Phytopathology* 82: 1199 - 1202.

Nderitu, J., Mwangi, F., Nyamasyo, G., and Kasina, M. (2010). Utilization of synthetic and botanical insecticides to manage Thrips (Tysan: Thrip) on snap beans (Fabaceae) in Kenya. *International Journal of Sustainable Crop Production* 5: 1-4

Ndunguru, J., Sseruwagi, P., Tairo, F., Stomeo, F., Maina, S., Djinkeng, A., Kehoe, M., and Boykin, L. (2015). Analyses of Twelve New Whole Genome Sequences of Cassava Brown Streak Viruses and Ugandan Cassava Brown Streak Viruses from East Africa: Diversity, Supercomputing and Evidence for Further Speciation. *PloS One* 10: 1-18

Ning, W., Shi, X., Liu, B., Pan, H., Wei, W., Zeng, Y., Sun, X., Xie, W., Wang, S., Wu, Q., Cheng, J., Peng, Z., and Zhang, Y. (2015). Transmission of *Tomato yellow leave curl virus* by *Bemisia tabaci* as affected by whitefly sex and biotype. *Scientific report*

Nono-Womdim R. (2001). An overview of major virus diseases of vegetable crops in Africa and some aspects of their control. In: Hughes JA, Odu BO, editors. Plant Virology in sub-Saharan Africa. Ibadan: IITA Publications p. 213-2.

Nono-Womdim, R., Swai, I., Chadha, M.M.L., and Green, S. (2005). Tomato yellow leaf curl virus and tomato leaf curl viruses in Eastern and Southern Africa. In; Anderson, P.K., Morales, J,F (eds),Whitefly and Whitefly-borne Viruses in the Tropics: Building a knowledge base for global action. *CIAT publications* 157-162

Ochichi, P. B., Muthomi, J.W., and Wagacha, J. (2018). Contribution of seed quality to thr occurrence of fungal and bacterial diseases of farmer produced common bean in varied agro ecological zones of Western Kenya. *American Journal of Agricultural Science* 5: 19-27

Ochilo, N.W., Nyamasyo, N.G., Kilalo, D., Otieno, W., Otipa, W., Chege, F., Karanja, T., and Lingeera, K. E. (2018). Characteristics and production constraints of smallholder tomato production in Kenya. *Scientific African*

Oo, M.L., Yabe, M., and Khai, H.V. (2012). Farmers' perception, knowledge and pesticide usage practices: a case study of tomato production in Inlay Lake, Myanmar. J. Faculty of Agriculture Kyushu University. 57: 327-331.

Oriani, M.A., and Vendramim, J.D (2010). Influence of trichomes on attractiveness and ovipositional preference of *Bemisia tabaci* (Genn.) B biotype (Hemiptera: Aleyrodidae) on tomato genotypes. *Neotropical Entomology* 39:1002–1007

Padidam, M., Beachy, R.N., Fauquet, C.M. (1995). Classification and identification of geminiviruses using sequence comparisons. *Journal of general Virology* 76: 249-263.

Padidam, M., Sawyer, S., and Fauquet, C.M. (1999). Possible emergence of new geminiviruses by frequent recombination. *Virology* 265: 218–225

Pan, H., Chu, D., Yan, W., Su, Q., Liu., B. (2012). Rapid Spread of *Tomato yellow leaf curl virus* in China is aided differentially by two invasive whiteflies. *PLoS One* 7: 348-317.

Pan, L.L., Chi, Y., Liu, C., Fan, Y.Y., Liu, S.S. (2020). Mutations in the coat protein of a begomovirus result in altered transmission by different species of whitefly vectors. *Virology Evolution Journal* 6: veaa014.

PARM (2017). Crop pests and disease management in Uganda: status and investment needs (by M. Kansiime, J. Mulema, D. Karanja, D. Romney and R. Day). Rome: PARM/IFAD. Available at:http://p4arm.org/document/crop-pests-and-d…investment -needs/). Date accessed: 2021–06–08

Pereira-Carvalho, R. C., Boiteux, L. S., Fonseca, M. E. N., Díaz-Pendón, J. A., Moriones, E., Fernández-Muñoz, R., et al. (2010). Multiple resistance to Meloidogyne spp. and bipartite and monopartite Begomovirus spp. in wild Solanum (Lycopersicon) accessions. *Plant Disease* 94: 179–185. doi: 10.1094/PDIS-94-2-0179

Peris, W.N., W. P., Muturi, J.J., Mark, O., Esther, A., and Johnson, M. (2018). Tomato leaf miner (Tuta absoluta) (Meyrick 1917) (Lepidoptera: Gelechiidae) prevalence and farmer management practices in Kirinyaga County, Kenya. *Journal of Entomology and Nematology* 10:43-49

Polston, J.E., and Capobianco, H. (2013). Transmitting plant viruses using whiteflies. *Journal of Visualized Experiments* 81. e4332

Polston, J.E., and Lapidot, M. (2007). Management of *Tomato yellow leaf curl virus*: US and Israel perspectives. In; Czosnek, H (eds), Tomato yellow leaf curl disease. *Springer* 251-262.

Polston, J.E., Cohen, L., Sherwood, T.A., Joseph, R.B., and Lapidot, M. (2006). Capsicum species symptomless hosts and reservoirs of *Tomato yellow leaf curl virus. Phytopathology* 96: 447-452

Prasanna, H. C., Sinha, D. P., Verma, A., Singh, M., Singh, B., Rai, M. and Martin, D. P. (2010). The population genomics of begomoviruses: Global scale population structure and gene flow. *Virology Journal* 7: 1 – 12.

Pratap, D., Kashikar, A.R., and Mukherjee, S.K. (2011). Molecular characterization and infectivity of a *Tomato leaf curl New Delhi virus* variant associated with newly emerging yellow mosaic disease of eggplant in India. *Virology Journal* 8: 305

Prijović, M., Skaljac, M., Drobnjaković, T., Zanić, K., Perić, P., Marčić, D., and Puizina, J. (2014) Genetic variation of the greenhouse whitefly, Trialeurodes vaporariorum (Hemiptera: Aleyrodidae), among populations from Serbia and neighbouring countries, as inferred from COI sequence variability. *Bulletin of Entomological Research* 1: 1–10.

Qiu, B., Chen, Y., Peng, W., Li, X., Ahmed, M. Z., Mathur, V., Du, Y., and Ren, S. 2009. Identification of three major *Bemisia tabaci* biotypes in the China based on morphological and DNA polymorphism. *Progress in Natural Science* 19: 713-718

Rakha, M., Hanson, P., and Ramasamy, S. (2017). Identification of resistance to *Bemisia tabaci* Genn. in closely related wild relatives of cultivated tomato based on trichome type analysis and choice and no-choice assays. *Genetic Resources and Crop Evolution* 64: 247–260.

Ramírez-Soriano, A., Ramos-Onsins, S.E., Rozas, J., Calafell, F., and Navarro, A. (2008). Statistical power analysis of neutrality tests under demographic expansions, contractions and bottlenecks with recombination. *Genetics* 179:555-7.

Ramos-Onsins, S.E., and Rozas, J. (2002). Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* 19: 2092–2100.

Roditakis, E., Roditakis, N. E., and Tsagkarakou, A. (2005). Insecticide resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) populations from Crete. *Pest Management Science* 61: 577–582

Rodrıguez-Lo´pez, M. J., Garzo, E., Bonani, J.P., Fereres, A., Ferna´ndez-Mun˜oz, R., and Moriones, E. (2011). Whitefly resistance traits derivedfrom the wild tomato Solanum pimpinellifolium affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of Tomato yellow leaf curl virus. *Phytopathology* 101:1191–1201

Rojas, A. (2004). A complex of begomoviruses affecting tomato crops in Nicaragua. PhD thesis. Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Science, Uppsala, Sweden.

Rojas, M.R., Gilbertson, R.L., Russell, D.R., and Maxwell, D.P. (1993). Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Disease* 77:340–347

Rojas, M.R., Hagen, C., Lucas, W.J., and Gilbertson, R.L. (2005). Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. *Annual Review of Phytopathology* 43:361–394.

Romba, R., Gnankine, O., Drabo, S.F., Tiendrebeogo, F., Henri, H., Mouton, L., and Vavre, F. (2018). Abundance of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and its

parasitoids on vegetables and cassava plants in Burkina Faso (West Africa). *Ecology Evolution* 8: 6091–6103

Roopa, H.K., Kumar, N.K.K., Asokan, R., Rebijith, K.B., Mahmood, R. and Verghese, A. (2012). Phylogenetic analysis of Trialeurodes Spp. (Hemiptera: Aleyrodidae) from India based on differences in mitochondrial and nuclear DNA. *Florida Entomologist* 95: 1086–1094.

Roossinck, M. J., Martin, D. P., and Roumagnac, P. (2015). Plant virus metagenomics: advances in virus discovery. *Phytopathology* 105: 716–727

Saccone, C., de Giorgi, C., Gissi, C., Pesole, G., and Reyes, A. (1999). Evolutionary genomics in metazoa: The mitochondrial DNA as a model system. *Genetics* 238:195-209.

Samarakoon, S.A.M.C., Balasuriya, A., Rajapaksha, R.G.A.S., and Wickramarachchi, W.A.R.T. (2012). Molecular detection and partial characterization of Tomato yellow leaf curl virus in Sri Lanka. *Pakistan Journal of Biological Sciences* 15: 863-870

Sangeetha, B., Malathi, V.G., Alice, D., Suganthy, M., and Renukadevi., P. (2018). A transmissible strain of Tomato leaf curl New Delhi virus infecting chayote in India. *Virus Research* 258:81-91.

SAS (2001).Version 8 software. SAS Institute Inc., Cary NC. USA.

Sattar, N. M. (2012). Diversity and Interactions of Begomoviruses and Their Associated DNA-Satellites PhD thesis. Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Science, Uppsala, Sweden.

Savolainen, V., Cowan R. S., Vogler, A. P., Roderick, G. K., and Lane, R. (2005). Towards writing the encyclopaedia of life: an introduction to DNA barcoding. Phil. Trans. Roy. Soc. London, B. *Biological Sciences* 360: 1805- 1811.

Sawalha, H. (2013). Epidemiology of Tomato yellow leaf curl virus in the Northern region of the west bank Palestine. *The open Agriculture Journal* 7: 80-85

Schreinemachers, P., Balasubramaniam, S., Boopathi, N.M., Ha, C.V., Kenyon, L., Praneetvatakul, S., Srijinda, A., Le, N.T., Srinivasan, R., and Wu, M-H. (2015). Farmers perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. *Crop Protection* 75: 115-123

Seal, S.E., vanden Bosch, F., and Jeger, M. J. (2006). Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. *Critical Reviews in Plant Science* 25:23-46.

Shah, S.H.J., Malik, A.H., and Quazi, J. (2013). Identification of new genetic variants of *Bemisia tabaci* from Pakistan. *International Journal of Entomological Research* 1:16-24

Shih, S. L., Green, S. K., Tsai, W. S. and Ssekyewa, C. (2006). Molecular characterization of a begomovirus associated with tomato leaf curl disease in Uganda. *Plant Disease* 90: 246 – 246.

Shih, S.L., Tsai, W.S., Green, S.K., and Lee, L.M. (2006). Molecular characterization of a distinct begomovirus associated with tomato leaf curl disease in Arusha of Tanzania. *Plant Disease* 90:1550.

Shoorcheh, H.R., Kazemi, B., Manzari, S., Brown, J.K., and Sarafrazi, A. (2008). Genetic variation and mtCOI phylogeny for *Bemisia tabaci* (Hemiptera, Aleyrodidae) indicate that the 'B' biotype predominates in Iran. *Journal of Pest Science* 81: 199-206

Silva, G.A., Picanco, M.C., Bacci, L., Crespo, A.L., Rosado, J.F., and Guedes, R.N.C. (2011). Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, Tuta absoluta. *Pest Management Science* 67:913-920.

Smith, M.A., Rodriguez, J.J., Whitfield, J.B., Deans, A.R., Janzen, D.H., Hallwachs, W, and Hebert, P.D.N. (2008). Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections**.** *Proceedings of National Academy of Science USA* 105:12359-12364.

Snehi, S.K., Raj, S.K., Prasad, V., and Singh, V. (2015). Recent research findings related to management strategies of begomoviruses. *Journal of Plant Pathology Microbe* 6: 273.

Sohrab, S.S. (2020). Genetic diversity of begomoviruses infecting tomato plant in Saudi Arabia. Saudi Journal of Biological Science 27:222-8.

Srinivasan, R., Riley, D., Diffie, S., Sparks, A., and Adkins, S. (2012). Whitefly population dynamics and evolution of whitefly transmitted Tomato yellow leaf curl virus resistant tomato genotypes as whitefly and TYLCV reservoirs. *Journal of Economic Entomology* 105: 1447-56

Ssekyewa, C. (2006). Incidence, Distribution and Characteristics of Major Tomato Leaf Curl and Mosaic Virus Diseases in Uganda. PhD-thesis. Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium.

Sseruwagi, P., Maruthi, M.N., Colvin, J., Re, M.E.C., Brown, J.K., and Legg, J.P. (2006). Colonization of non-cassava plant species by cassava whiteflies (*Bemisia tabaci*) in Uganda. *Entomologia experimentalis et applicata* 119:145-153.

Syller, J. (2012). Facilitative and antagonistic interactions between plant viruses in mixed infections. *Molecular Plant Pathology* 13: 204e216

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA 6 Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725-9.

Tang, Y.F., He, Z.F., Du, Z.G., and Lu, H.L (2014). First report of *Tomato yellow leaf curl Kachanaburi virus* in Laos. *The American Phytopathological Society* 98: 428

Tay, W., Elfekih, S., Court, L., Gordon, K.H.J., Delatte, H., and De Barro, P.J. (2017). The trouble with MEAM2: implications of pseudogenes on species delimitation in the globally invasive *Bemisia tabaci* (Hemiptera: Aleyrodidae) cryptic species complex. *Genome Biology and Evolution* 9:2732–2738

Tocko-Marabena, B.K., Silla, S., Simiand, C., Zinga, I., Legg, J., Reynaud, B., and Delatte, H. (2017). Genetic diversity of *Bemisia tabaci* species colonizing cassava in Central African Republic characterized by analysis of cytochrome c oxidase subunit I. *PLoS ONE* 12,e0182749.

Tomás, D. M., Cañizares, M. C., Abad, J., Fernández-Muñoz, R., and Moriones, E. (2011). Resistance to Tomato yellow leaf curl virus accumulation in the tomato wild relative Solanum habrochaites associated with the C4 viral protein. *Molecular and Plant Microbe Interaction* 24, 849–861.

Van den Bosch, F., Akudibilah, G., Seal, S., and Jeger, M. (2006). Host resistance and the evolutionary response of plant viruses. *Journal of Applied Ecology* 43: 506–616.

Verlaan, M. G., Hutton, S. F., Ibrahem, R. M., Kormelink, R., Visser, R. G., Scott, J. W., Edwards, J. D. and Bai, Y. (2013). The tomato yellow leaf curl virus resistance genes *Ty-1* and *Ty-3* are allelic and code for DFDGD-class RNA-dependent RNA polymerases. *PLoS Genetics* 9: e1003399.

Vidavski, F., Czosnek, H., Gazit, S., Levy, D., and Lapidot, M. (2008). Pyramiding of genes conferring resistance to Tomato yellow leaf curl virus from different wild tomato species. *Plant Breeding* 127: 625–631.

Vyskočilová, S., Tay, W.T., van Brunschot, S., Seal, S., and Colvin, J. (2018). An integrative approach to discovering cryptic species within the *Bemisia tabaci* whitefly species complex. *Scientific Report* 8:10886

Wainaina, J. M., Ateka, E., Makori, T., Kehoe, M.A., and Boykin, L.M. (2019). A metagenomic study of DNA viruses from samples of local varieties of common bean in Kenya. *Peer J* 7:e6465

Wamaitha, M.J., Nigam, D., Maina, S., Stomeo, F., Wangai, A., Njuguna, J.N., Holton, T., Wanjala, B.W., Wamalwa, M., Lucas, T., et al. (2018). Metagenomic analysis of viruses associated with maize lethal necrosis in Kenya. *Virology Journal* 15:90.

Wamonje, F.O., Michuki, G.N., Braidwood, L.A., Njuguna, J.N., Mutuku, J.M., Djikeng, A., Harvey, J.J.W., and Carr, J.P. (2017). Viral metagenomics of aphids present in bean and maize plots on mixed-use farms in Kenya reveals the presence of three dicistroviruses including a novel Big Sioux River virus-like dicistrovirus. *Virology Journal* 14:188.

Wang, X., Tu, W. C., Huang, E. J., Chen, Y. H., Chen, J. H., and Yeh, W. B. (2017). Identification of disease-transmitting mosquitoes: Development of species-specific probes for DNA Chip Assay using mitochondrial COI and ND2 genes and ribosomal internal transcribed spacer 2. *Journal of medical entomology* 54: 396-402.

Wartig, L., Kheyr-Pour, A., Noris, E., De Kouchkovsky, F., Jouanneau, F., Gronenborn, B., and Jupin, I. (1997). Genetic analysis of the monopartite tomato yellow leaf curl geminivirus: roles of V1, V2, and C2 ORFs in viral pathogenesis. *Virology* 228: 132–140.

Weaver, S., Shank, S. D., Spielman, S. J., Li, M., Muse, S. V., and Kosakovsky Pond, S. L. (2008). Datamonkey 2.0: A modern web application for characterizing selective and other evolutionary processes. *Molecular Biology and Evolution* 35:773-7.

Weng, S.H., and Tsai, C.W. (2013). Insect transmission of *Tomato yellow leaf curl virus*. Proceedings of the 2013 International Symposium on Insect vectors and Insect – Borne Diseases.

White, J.A., Kelly, S.E., Perlman, S.J., and Hunter, M.S. (2009). Cytoplasmic incompatibility in the parasitic wasp Encarsia inaron: Disentangling the roles of Cardinium and Wolbachia symbionts. *Heredity* 102: 483–489.

Wintermantel, W. M., Hladky, L. L., Gulati-Sakhuja, A., Li, R., Liu, H. Y., and Tzanetakis, I. E. (2009). The complete nucleotide sequence and genome organization of tomato infectious chlorosis virus: A distinct crinivirus most closely related to lettuce infectious yellows virus. *Archives of Virology* 154: 1335–1341.

Wu, Q., Ding, S., Zhang, Y., and Zhu, S. (2015). Identification of viruses and viroids by next generation sequencing and homology dependent and homology independent alogarithms. *The Annual Review of Phytopathology* 53: 425-44

Xu, C., Sun, X., Taylor, A., Jiao, C., Xu, Y., Cai, X., Wang, X., Ge, C., Pan, G., Wang, Q., Fei, Z., and Wang, Q. (2017). Diversity, distribution, and evolution of tomato viruses in China uncovered by small RNA sequencing. *Journal of Virology* 91:e00173-17.

Yadava, P., Suyal, G., and Mukherjee, S.K. (2010). Begomovirus DNA replication and pathogenicity. *Current Science* 98:360-8.

Yan, Z., Pérez-de-Castro, A., Díez, M.J., Hutton, S.F., Visser, R.G.F., Wolters, A-M.A., Bai, Y., and Li, J. (2018). Resistance to Tomato Yellow Leaf Curl Virus in Tomato Germplasm. *Frontier in Plant Science* 9:1198. doi: 10.3389/fpls.2018.01198

Yang, X., Wang, B., Luan, J., Xie, Y., Liu, S., and Zhou, X. (2017). Molecular variation of Tomato yellow leaf curl virus in the insect vector *Bemisia tabaci*. *Scientific Reports* 7:16427.

Yang, X.L, Zhou, M.N., Qian, Y.J., Xie, Y., and Zhou, X.P. (2014). Molecular variability and evolution of a natural population of tomato yellow leaf curl virus in Shanghai, China. *Journal of Zhejiang University Science* B 15:133-42.

Yang, Y., Sherwood, T.A., Patte, C.P., Hiebert, E., and Polston, J.E. (2004). Use of *Tomato yellow leaf curlvirus* (TYLCV) Rep gene sequences to engineer TYLCV resistance in tomato. *Phytopathology* 94:490-496.

Yin, Q., Yang, H., Gong, Q., Wang, H., Liu, Y., Hong, Y., and Tien, P. (2001). Tomato yellow leaf curl virus: monopartite genome organization and agroinfection of plants. *Virus Research* 81:69-76

Yoon, J., Kim, S., Choi, G., and Choi, S. (2015). A simple and reliable molecular detection method for *Tomato yellow leaf curl virus* in *Solanum lycopersicum* without DNA extraction. *Research in Plant Diseases* 21: 180-185

Zehra, S. B., Ahmad, A., Sharma, A., Sofi, S., Lateef, A., Bashir, Z., and Rathore, J. P. (2017). Chilli Leaf Curl Virus an Emerging Threat to Chilli in India. *International Journal of Pure Applied Biosciences*, 5: 404-41

Zhang, H., Gong, H., and Zhou, X. (2009). Molecular Characterization and Pathogenicity of *Tomato yellow leaf curl virus* in China. *Virus Genes* 39: 249-255

Zhang, L. P., Zhang, Y. J., Zhang, W. J., Wu, Q. J. B., Xu, Y., and Chu, D. (2005). Analysis of genetic diversity among different geographical populations and determination of biotypes of *Bemisia tabaci* in China. *Journal of Applied Entomology* 129: 121-128.

Zhang, Z., Schwartz, S., Wagner, L., and Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7:203-14.

Zhou, X., Robinson, D.J., and Harrison, B.D. (1998). Types of variation in DNA-A among isolates of East African cassava mosaic virus from Kenya, Malawi and Tanzania. *Journal of General Virology* 79:2835-40.

Zhou, Y. C., Noussourou, M., Kon, T., Rojas, M. R., Jiang, H., Chen, L. F., Gamby, K., Foster, R. and Gilbertson, R. L. (2008). Evidence of local evolution of tomato infecting begomovirus species in West Africa: characterization of tomato leaf curl Mali virus and tomato yellow leaf crumple virus from Mali. *Archives of Virology* 153: 693 – 706

Zi, H.F., Hao, Y., Ming-Jie, M., and Sui-Tao, W. (2007). Tomato yellow leaf curl diseases in Guangdong caused by *Tomato leaf curl Taiwan virus*. *Chinese Journal of Agricultural Biotechnology* 4: 127-131.

Zrachya, A., Glick, E., Levy, Y., Arazi, T., Citovsky, V., and Gafni, Y. (2007). Suppressor of RNA silencing encoded by *Tomato yellow leaf curl virus*-Israel. *Virology* 358: 159–165.

APPENDICES

Appendix 1: DAS ELISA Buffers

1. Coating Buffer (pH 9.6)

1.59g sodium carbonate (Na_2CO_3)

2.93g sodium bicarbonate ($NaHCO_3$)

0.20g sodium azide (NaN_3)

Dissolve in 900ml of distilled water and adjust the pH to 9.6 using HCl to make up 1l

2. Phosphate Buffered Saline (PBS pH 7.4)

8.0g sodium chloride (NaCl)

0.2g monobasic potassium phosphate (KH_2PO_4)

1.15g dibasic sodium phosphate (Na_2HPO_4)

0.2g potassium chloride (KCl)

0.2g sodium azide (NaN_3)

Dissolve in 900ml of distilled water and adjust the pH to 7.4 using NaOH or HCl to make up 1l

3. PBS-T

PBS + 0.5ml Tween-20 per 1l

4. Extraction Buffer pH 8.5

0.05M Tris containing 0.06Msodium sulphite

5. Conjugate Buffer

PBST +0.2% PVP + 0.2% Egg albumin (e.g. Sigma A-5253)

6. Substrate Buffer pH 9.8

97ml diethalomine

600ml distilled water

0.2g sodium azide (NaN_3)

Adjust pH to 9.8 using HCl to make up 1l

All the above buffers were stored at 4°C and warmed to room temperature before use.

Appendix 2: Analysis of Variance Tables of the data analysed

a) ANOVA table for TYLCD % incidence across Counties surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
County	7	24346.6	3478.1	8.21	<.001***
Residual	251	106293.1	423.5		
Total	258	130639.7			

b) ANOVA table for TYLCD severity across Counties surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
County	7	82.161	11.737	10.82	<.001***
Residual	251	272.403	1.085		
Total	258	354.565			

c) ANOVA table for Whitefly population in tomato farms across Counties surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
County	7	2776.7	396.67	9.62	<.001***
Residual	251	10353.08	41.25		
Total	258	13129.78			

d) ANOVA table for TYLCD percentage incidences in tomato cultivars found in farmers' fields across the Counties surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	18	78415.3	4356.4	20.02	<.001***
Residual	240	52224.4	217.6		
Total	258	130639.7			

e) ANOVA table for TYLCD severity in tomato cultivars found in farmers' fields across the Counties surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	18	181.6826	10.0935	14.01	<.001***
Residual	240	172.882	0.7203		
Total	258	354.5646			

f) ANOVA table for whitefly populations in tomato cultivars found in farmers' fields across the Counties surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Variety	18	4779.67	265.54	7.63	<.001	***
Residual	240	8350.11	34.79			
Total	258	13129.78				

g) ANOVA table for TYLCD percentage incidence across AEZs surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	11	26204.4	2382.2	5.63	<.001***
Residual	247	104435.3	422.8		
Total	258	130639.7			

h) ANOVA table for TYLCD severity across AEZs surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	11	88.653	8.059	7.49	<.001
Residual	247	265.912	1.077		
Total	258	354.565			

i) ANOVA table for whitefly population across AEZs surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
AEZ	11	2710.73	246.43	5.84	<.001	***
Residual	247	10419.05	42.18			
Total	258	13129.78				

j) ANOVA table for whitefly populations in field experiment to screen the response of selected cultivars to TYLCD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Rep	2	0.8905	0.4453	1.56		
Season	1	0.2358	0.2358	0.82	0.367	Ns
Variety	19	11.6794	0.6147	2.15	0.010	*
Season.Variety	19	0.4644	0.0244	0.09	1.000	Ns
Residual	78	22.3135	0.2861			
Total	119	35.5836				

k) ANOVA table for TYLCD severity in field experiment to screen the response of selected cultivars to TYLCD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Rep	2	0.13176	0.06588	2.08		
Season	1	0.012	0.012	0.38	0.540	ns
Variety	19	0.75696	0.03984	1.26	0.238	ns
Season.Variety	19	0.08321	0.00438	0.14	1.000	ns
Residual	78	2.47449	0.03172			
Total	119	3.45842				

l) ANOVA table for TYLCD incidence in field experiment to screen the response of selected cultivars to TYLCD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Rep	2	43.95	21.98	0.38		
Season	1	60.35	60.35	1.04	0.311	ns
Variety	19	1770.28	93.17	1.61	0.075	ns
Season.Variety	19	181.02	9.53	0.16	1.000	ns
Residual	78	4518.25	57.93			
Total	119	6573.85				

Appendix 3: Questionnaire used in the survey

Date	Interview start time	Field No.
GPS Coordinates		Administrative location
Latitude		County
Longitude		Sub-County or Constituency
Altitude (m)		Location or Ward
Nearest town or shopping centre		Sub-Location or Village
Farmers/growers Name		Male or female:

Age of respondent (a) 20- 35 (b) 35-55 (c) Above 55		
Level of education a) No school b) Primary c) Secondary d) Tertiary e) University		
Cell Phone number (or other contact)		
Varieties of tomato grown (list names)	Area normally planted (acres)	Source(s) of planting material
1.		
2.		
3.		
Symptoms observed: (a) marginal leaf yellowing (b) stunting (c) reduced leaf size (d) reduced internodes (e) upward or downward leaf cupping (f) flower and/or fruit drop		

Plant no.	Severity disease score	Describe symptoms	Sample collected	Variety

State disease incidence.....

Does the interviewee do crop rotation		Yes	No
If Yes, give length of rotation period (No. of crop seasons)		State crops used in rotation	
Interviewer shows tomato plant with TYLCD symptoms, and asks them what causes the disease If there are no virus-diseased plants present, show them a picture.			

(a) Can the producer recognize the disease? Yes_____(01), No_____(02) (b) What do you call the disease ..... (c) What causes/ spreads it.....
Is there a disease problem in your farm? Yes_____(01), No_____(02) Don't know (03) Do these problems appear every year? Yes_____(01), No_____(02) List the methods you use to control the disease problem (a) (b) (c) (d) (e)
Other notes (e.g. photos of TYLCD on tomato crops)
State the number of samples taken
Other comments/observations