TOMATO YELLOW LEAF CURL DISEASE IN TANZANIA: CHARACTERIZATION, ALTERNATIVE HOSTS AND TRANSMISSION

BY

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A DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2003

ABSTRACT

Tomato farms in Arusha, Morogoro, Dodoma, Iringa, Kilimanjaro and Coast regions of Tanzania were surveyed to assess the incidence of the yellow leaf eurl disease, and to collect infected tomato leaf and weed samples for diagnosis and identification. Results indicated that disease incidence and severity were significantly higher ($P < 0.05$) in Dodoma region, but low and least severe in Iringa region. The disease-causing agent was identified to be a geminivirus by electron microscopic examination of the geminate particles in diseased samples. Squash and dot blots of extracts from the samples hybridized to DIG-labelled viral DNA probes under high strigency conditions. Similarly, Southern hybridization of genomic DNA extracted from the samples by phenol-chloroform procedures, revealed viral bands in their open circular, closed circular and single-stranded forms. The putative viral DNA was amplified by polymerase chain reaction using primer pairs designed to yield up to 1.4 kb amplicons. The amplified viral DNA was digested with *Alu*\ and clectrophorcsed in polyacrylamidc gel. Restriction fragment length polymorphisms analysis showed similar banding patterns for all the *Tomato yellow leaf curl Tanzania virus* isolates. Furthermore, the amplified viral DNA was ligated to pBluescript II KS' and transformed into *Escherichia coli* strain JM 83 cells by electroporation or Calcium chloride-mediated transformation. The viral DNA was extracted from colonies containing the insert and sequenced using a Li-Cor DNA Automatic Sequencer. The BLAST programme was used to search for viruses with similar sequences, and phylogenetic relationship was established using the CLUSTAL function of the Vector NTI.5 software. Sequence comparison and phylogeny showed

that *Tomato yellow leaf curl Tanzania virus* was closely related to African tomato leaf curl virus and to *Tomato yellow leaf curl Sardinia virus.* However, the isolates of *Tomato yellow leaf curl Tanzania virus* were biologically and genetically similar. More than 90 % homology in the Rep and/or coat protein was found between *Tomato yellow leaf curl Tanzania virus* and some crop- and weed-infecting geminiviruses. Representative samples of the *Tomato yellow leaf curl Tanzania* v/rw.s-infected tomato plants collected from the regions were maintained in the screenhouse by grafting and through whitefly-mediated inoculations. The biological properties of the virus relating to acquisition and inoculation time, persistence, graft transmission, mechanical transmission and host range were studied. Results obtained indicated that the virus was transmitted persistently by *Bemisia tabaci* Genn., and by grafting, but it was not mechanically transmissible. Minimum acquisition and inoculation times were 30 min for all the virus isolates, except the Kilimanjaro and Iringa isolates, which had minimum acquisition feeding time of 1 h. For the first time, the present study demonstrated that the following weed species are non-cultivated hosts of TYLCTZV: *Commelina erecta, Amaranthus spinosus, Erigeron floribundus, Ageratum conyzoides, Bidens pilosa, Sida acuta, Ipomea batatas, Amaranthus viridis, Portulaca oleracea, Cassia oblusifolia, Euphorbia hirta, Calopogonium mucunoides, Crotalaria retusa, Trianthema portulacastrum, Alternanthera sessilis, Celosia trigyna, Commelina diffusa, Chromolaena odor at a, Kclipta pros (rata, Syne dr el la nodi flora, Cassia occidental is, Spigelia anthelmia, Boerhavia diffusa, Physalis angulata,* and *Acanthospermum hispidium.* Kxperimental studies using screenhouse cultures of the *Tomato yellow leaf*

curl Tanzania virus representative isolates resulted in the infection of five plant species *(Capsicum annuum, Datura stramonium, Lycopersicon esculcntum, Nicoliana glutinosa* and *N. tabacum). Phaseolus vulgaris, Gossypium hirsutum, Solanum melongena, Solanum tuberosum. Glycine max,* and *Arachis hypogea* were not infected. Field and sereenhouse experiments were conducted to determine the resistance of tomato genotypes to *Tomato yellow leaf curl Tanzania virus* by comparing the yield and yield components between inoculated and un-inoculated plants of each genotype. With the exception of the resistant genotype TY172, both sereenhouse and field inoculated plants of all the genotypes showed statistically significant ($P<0.001$) reductions in plant fresh weight, fruit number, fruit weight and relative total yield when compared with the un-inoculated plants. On the overall, tomato genotype TY172 was the most resistant followed by Tengeru 97, Cal-J and Marglobc. It is recommended that the resistance in TY172 be introgressed into the Tengeru 97, Cal-J, Marglobc and Moneymaker, which are commonly cultivated by farmers in Tanzania, to achieve high resistance to the virus. Meanwhile, the cultivation of the tolerant tomato cultivar, Tengeru 97 should be popularised among farmers in Tanzania while the search for sources of resistance among wild *Lycopersicon* relatives continues.

DECLARATION

I, BONIFACE DAVID KASHINA, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work and has neither been submitted nor concurrently being submitted for a degree award in any other University.

Signature.....

Date.....3 November, 2003.....

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ACKNOWLEDGEMENTS

First and foremost, I give thanks to ANSTI-DAAD for awarding me a scholarship to execute this project, and to the staff of the Embassy of the Federal Republic of Germany, Tanzania for being friendly and understanding. I register my appreciation to my employer, the Institute of Agricultural Research/Ahmadu Bello University Zaria, Nigeria for granting me a Study Fellowship. I will ever remain grateful to Prof. R. B. Mabagala and Dr A. A. Mpunami, who tirelessly took great pleasure in supervising, guiding, supporting (materially and logistically) and encouraging me right from inception to the completion of this research project. I deeply appreciate the great deal of co-operation, understanding and friendship of the staff of Department of Crop Science and Production.

I am indebted to Profs. G. Pietersen (Plant Protection Division, South Africa) for providing me with Goat-anti-rabbit conjugate, C. Rey (University of Witwatersrand, South Africa) for primers and R. L. Gilbertson (University of California Davis, USA) for primers. Prof. Dr. H. Jeske (University of Stuttgart, Germany) was magnanimous in accepting to provide laboratory space, equipment and supervision for the molecular aspects of the project. I remember with thanks the love and assistance from Juan Jovel and laboratory staff, University of Stuttgart. I am duely grateful to Drs. S. Winter (DSMZ, Germany) and Jacque Hughes (IITA, Ibadan, Nigeria) for providing me with polyclonal and monoclonal antibodies. I thank Drs. M. Lapidot (Volcani Centre, Israel) and R. Nono-Womdim (formerly with AVRDC-ARP, Arusha) for supplying me with tomato seeds, nitrocellulose/nylon membranes, and for useful discussions. My thanks

also go to Drs K. Gorman (BBSRC, UK) for identifying the whitefly biotype and providing information on rearing of whiteflies, and S. Ekesi (formerly with ICIPE, Nairobi) for all forms of assistance.

I thank the management of the Tanzania Official Seed Certification Agency (TOSCA) for making available to me her screenhouse and laboratory. The co-operation from farmers, Regional and District Officials of the Ministry of Agriculture and Food Security during my field visits is highly appreciated. I remember with thanks the encouragement, support and understanding from friends, colleagues and fellow students, too numerous to mention. Finally, my heart goes to my darling wife, Felicia and daughters Precious and Lois, for their unabridged love, understanding, support and prayers. The generous support, encouragement, love and prayers from members of the Kashina and Pedok families are thankfully acknowledged. Over and above all, I give God the glory for His guidance, protection, strength, mercies and favour granted me in the course of my research. The prayers, love and support of the brethren must not go unmentioned. To them all, I say thank you and God bless you.

DEDICATION

To my late parents Baba David Kashina and Mama Moureen David Kashina, for their parental love and exemplary lives of hardwork, humility, honesty, and patience.

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ABBREVIATIONS AND SYMBOLS

°C	Degrees celsius
g	Gramme(s) or force of gravity
h	Hour(s)
kb	Kilobase(s)
kv	Kilovolts
λ	Lambda
M	Molar
mA	Milliampere
mg	Milligramme(s)
min	Minute(s)
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
µg	Microgramme(s)
µl	Microlitre(s)
µF	*Microfarad(s)*
N	Normality
ng	Nanogramme(s)
nm	Nanometre(s)
%	Percent
pg	Picogramme(s)

S	Second(s)
v	Volt(s)
AAP	Acquisition access period
a. i.	Active ingredient
Alu I	Restriction enzyme from *Arthrobacter luteus*
ANSTI	African Network of Scientific and Technological Institutions
ATPase	Adenosine triphosphatase
AVRDC	Asian Vegetable Research and Development Centre
Bam HI	Restriction enyzme from *Bacillus amyloliquefaciens* H
bp	base pair(s)
cccDNA	Covalent closed circular deoxyribonucleic acid
CDP star	Disodium2-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-yl)-1-phenylphosphate
cm	Centimetre(s)
DAAD	German Academic Exchange Services
DAPI	4, 6-diamidino-2-phenylindole
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
DIG-11-UTP	Digoxigenin-3-O-methylcarbonyl-aminocaproyl-[5-(3-aminoallyl)-uridin-5-triphosphate
DMSO	Dimethyl sulfoxide

DNA	Deoxyribonucleic acid
DNA A	Deoxyribonucleic acid of the A component of bipartite geminiviruses
DNA B	Deoxyribonucleic acid of the B component of bipartite geminiviruses
dNTPs	Deoxyribonucleoside triphospahtes
dsDNA	Double-stranded deoxyribonucleic acid
DTT	Dithiothreitol
dTTP	2'-deoxythymidine 5'-triphosphate
EC	Emulsifiable concentrate
EDTA	Disodium ethylenediamine tetracetate
ELISA	Enzyme-linked immunosorbent assay
GAM-PAL	Goat-anti mouse alkaline phosphatase
HCl	Hydrochloric acid
IAP	Inoculation access period
ICIPE	International Centre for Insect Physiology and Ecology
IPTG	Isopropyl-β-D-thiogalactoside
IR	Intergenic region
ISEM	Immuno-sorbent electron microscopy
LB	Luria Bertani
LiCl	Lithium chloride
Mab	Monoclonal antibody
M. a. s. l	Metres above sea level
$MgCl_2$	Magnesium chloride

mRNA	Messenger ribonucleic acid that serves as a template for protein synthesis
NaAc	Sodium acetate
NaCl	Sodium chloride
Na-EDTA	Sodium-Disodium ethylenediamine tetracetate
NaOH	Sodium Hydroxide
OD	Optical density
opDNA	Open circular deoxyribonucleic acid
ORF	Open reading frame
PAb	Polyclonal antibody
PBS-T	Phosphate bufferred saline-Tween 20
PCR	Polymerase chain reaction
*Pst*I	Restriction endonuclease from *Providencia stuartii*
PVP-40	Polyvinyl pyrollidone-40
REn	Replication enhancer protein
Rep	Replication-associated protein
RF	Replicative form or intermediate
RFLP	Restriction fragment length polymorphisms
rpm	Revolutions per minute
SCRI	Scottish Crops Research Institute
SDS	Sodium dodecyl sulphate
ssDNA	Single-stranded deoxyribonucleic acid
SUA	Sokoine University of Agriculture

TAS-ELISA	Triple antibody sandwich-enzyme-linked immunosorbent assay
TATA box	Invariant nanonucleotide (TAATATTAC) sequence of geminiviruses
Taq	Restriction enzyme extracted from *Thermus aquaticus*
TBE	Tris-borate disodium ethyleneamine tetracetate buffer
TE	Tris- disodium ethylenediamine tetracetate buffer
ToLCTZV	*Tomato leaf curl Tanzania virus*
TrAP	Transcriptional activator protein
tRNA	Transfer ribonucleic acid
tRNAse	Transfer ribonuclease enzyme
TYLCTZV	*Tomato yellow leaf curl Tanzania virus*
UV	Ultraviolet wavelength
X-GAL	5-bromo-4-chloro-3-indolyl-β-D-galactoside
WP	Wettable powder

CHAPTER ONE

1.0 INTRODUCTION

Tomato, *Lycopersicon esculentum* Miller, is one of the most important vegetables cultivated in most regions of the world second only to potatoes, but ranked first in Tanzania (AVRDC, 1993). In Tanzania, there has been progressive increase in hectarage over the years without commensurate increase in tomato fruit yields (Nono-Womdim *et al.*, 1996). This has been attributed to many factors, including pests and diseases. Viral diseases account for much of the economic losses in tomato fruit yield. *Tomato yellow leaf curl disease* (TYLCD) is one of the most widespread and economically important viral diseases worldwide. The disease is caused by *Tomato yellow leaf curl virus* (TYLCV) (Al-Musa, 1982). TYLCV is a major problem of tomato production in many tropical and subtropical regions (Makkouk and Laterrot, 1983). It is a member of the genus *Begomovirus*, one of four genera (*Mastrevirus*, *Curtovirus, Topocuvirus,* and *Begomovirus*), which make up the *Geminiviridae* family. Subdivision into genera is based on genome structure, plant host and insect vector (Fauquet *et al.*, 2000). It has been documented that several different begomoviruses, depending on the geographical areas of cultivation, affect tomatoes grown in tropical and subtropical regions.

The virus has long been known in the Middle East, North, Central and West Africa, Southeast Asia, and it is spreading fast to other regions of the world with favourable weather conditions for the vector, the virus and tomato production. Severe outbreaks of TYLCV have occurred in Southern Europe and America (Nakhla *et al.*, 1994). The virus

has been reported in several African countries (Cohen and Harpaz, 1964; Yassin and Nour, 1965; Cherif and Russo, 1983; D'Hondt and Russo, 1985). In Tanzania the diseases was first detected in Morogoro (Czosnek *et al*., 1990). Of the four viruses so far reported on tomatoes in Tanzania, *Tomato yellow leaf curl Tanzania virus* (TYLCTZV) (Fauquet *et al*., 2000) is the most widespread and economically important (AVRDC, 1993; Nono-Womdim *et al*., 1996), with the highest incidence occurring during the hottest periods of the year, when tomato yield losses can be as high as 100 %.

Typical symptoms of TYLCV infection in tomato include yellowing, leaf curling, stunting, abscission of flowers and fruits, and marked reduction in leaf and fruit sizes (Cohen and Harpaz, 1964; Mansour and Al-Musa, 1992). Quantitative and qualitative reductions in tomato yield due to infection by TYLCV have also been reported (Makkouk, 1978; Al-Musa, 1982). Reduction in the quality of tomato fruits affects market access and increases the costs of grading. Ratul and Brar (1989) observed metabolic changes in infected tomato tissues. However, some tomato varieties have been reported to be symptomless carriers of the virus (Cohen and Nitzany, 1966; Lapidot *et al*., 1997) while other viruses cause symptoms that are similar to TYLCV in tomato (Thomas, 1981; Zitter and Tsai, 1981; Polston *et al*., 1994).

Tomato yellow leaf curl virus is transmitted by grafting and by the aleurodid *Bemisia tabaci* Gennadius, which also vectors other important geminiviruses (Pico *et al*., 1996). In nature, the virus is exclusively transmitted and spread by *B. tabaci* in a persistent

manner (Cohen and Harpaz, 1964). Although the relationship between the virus and its vector has been studied extensively (Cohen and Harpaz, 1964; Mansour and Al-Musa, 1992; Atzmon *et al*., 1998), the interaction between them is still not properly understood (Ghanim *et al*., 1998). There is no record of studies involving vector transmission of TYLCTZV.

The identification of whitefly-transmitted geminiviruses by traditional virological methods has proven difficult. Many of the economically important whitefly-transmitted geminiviruses including TYLCV, are generally, not mechanically transmissible from tomato to tomato, except in very few cases (Abdel-Salam, 1990). Therefore, the use of definitive bioassay hosts for virus identification, evaluation of host range and other biological properties has been very difficult in many cases. Another difficulty, is the production of virus-specific antiserum which has been attributed to the instability of physical and chemical properties of virus particles during purification, poor immunogenicity of virions, and antigenic indistinctiveness of their capsid proteins with available polyclonal and most monoclonal antisera preparations (Roberts *et al*., 1984; Swanson *et al*., 1992). More reliable procedures that have been used for the detection and identification of whitefly-transmitted geminiviruses, and to predict or infer virus relationships at the quasi-species or strain levels, are DNA-DNA hybridization assays (Roberts *et al*., 1984; Swanson *et al*., 1992; Padidam *et al*., 1995a).

The polymerase chain reaction (PCR) and degenerate broad-spectrum oligonucleotide primers have been used for general detection and identification of begomoviruses (Saikia *et al.*, 1988; Rojas *et al.*, 1993; Deng *et al.*, 1994). Tomato leaf samples showing typical TYLCV symptoms collected from different regions in Tanzania could not provide conclusive evidence as to the true identity of the virus by genetic methods (Nono-Womdim *et al.*, 1996). Czosnek *et al.* (1990) identified TYLCV in Morogoro, Tanzania, using nucleic acid probes. Chiang *et al.* (1997) reported the nucleotide sequence of a distinct geminivirus, *Tomato leaf curl Tanzania virus* (ToLCTZV). However, it is generally thought that many viruses/strains may be responsible for the symptoms observed on tomatoes or, that a distinct virus strain is involved. *Tomato yellow leaf curl virus* is the first reported whitefly-transmitted geminivirus that possesses a single genomic component (Navot *et al.*, 1991). However, it is unusually heterogeneous with some isolates having two genomic components (Rochester *et al.*, 1994). A high degree of sequence diversity, greater than normally found among isolates of other viruses, has been reported among genomes of TYLCV strains (Navot *et al.*, 1991; Pico *et al.*, 1996).

Tomato yellow leaf curl virus is reported to have a narrow host range. The virus is endemic on tomato, a seed propagated annual, but it is not seed transmitted. In many areas, TYLCV completes its entire cycle on overlapping tomato crops; however, it is also known to survive on other plants that serve as reservoirs for the virus. Three weed species (*Achyranthes aspera, Euphorbia heterophylla* and *Nicandra physaloides*) have

so far been reported as alternative hosts of the virus in Tanzania (Nono-Womdim *et al.*, 1996). However, preliminary field observations revealed that there is a greater diversity of weed species often associated with tomato farms with the possibility of many serving as alternative hosts of the virus. In order to understand the epidemiology of TYLCV in each infected area, specific studies of the plant flora of the infected regions are necessary to analyze the role that different crops and weeds play as natural hosts of TYLCV.

Because of the increasing and widespread occurrence of TYLCV epidemics and the great economic significance of the disease in tomato production, research efforts have concentrated on the study of the factors involved in the host-virus-vector relationships in order to develop efficient control strategies. However, control methods investigated so far are not very effective in managing the disease. These measures usually involve recommendation for limiting the vector population, which very often, requires the heavy use of pesticide and physical barriers (Cohen and Antignus, 1994; Polston and Anderson, 1997; Hilje *et al.*, 2001). Chemical control of the vector has been difficult and ineffective, since whitefly populations can reach very high numbers, leading to intensive use of pesticides in attempts to eliminate the vector before it transmits the virus (Lapidot and Friedmann, 2002). Moreover, there are concerns that the vector may develop pesticide resistance and the intensive use of pesticides may have deleterious effects on the environment (Palumbo *et al.*, 2001). Physical barriers such as fine-mesh screens have been used in the Mediterranean Basin to protect crops (Cohen and Antignus, 1994). Recently, UV-absorbing plastic sheets and screens have been shown to inhibit

penetration of whiteflies into greenhouses (Antignus *et al*., 1996, 2001b). Furthermore, the filtration of UV light was shown to hinder the whiteflies' dispersal activity, and consequently, reduce virus spread (Antignus *et al*., 2001a). However, adoption of physical barriers adds to production costs and the screens create problems of shading, overheating and poor ventilation.

Therefore, the best way to reduce geminivirus damage is by breeding crops resistant or tolerant to the virus (Cohen and Antignus, 1994; Morales, 2001). The removal of weeds in and around tomato farms could form an important component of integrated pest management package for managing the disease. Only partially resistant F1 hybrids are commercially available (Laterrot, 1993; Pico *et al*., 1996). Progress in breeding is slow primarily because of the complex genetics of the resistance, and the need to set up a reliable protocol for screening for resistance to the virus, which is dependent on the availability of viruliferous whiteflies (Vidavsky *et al*., 1998). For more than two decades, there has been considerable effort to develop tomato cultivars that are resistant to TYLCV. Because all available tomato cultivars are susceptible to the virus, wild *Lycopersicon* species have been screened to determine their level of resistance to the virus (Pilowsky and Cohen, 1974; Pilowsky and Cohen, 1990; Scott *et al*., 1996; Friedmann *et al*, 1998; Hanson *et al*., 2000).

In view of the domestic and industrial value of tomato, and the economic impact of TYLCD on its production, it is imperative to generate the information needed to

effectively manage and control the disease in order to improve the performance and yield of tomato crop in Tanzania. Therefore, the general objective of this study was to characterize the TYLCTZV and study some aspects of its epidemiology.

Specifically, the study was initiated to:

1. Collect, characterize and identify variability among isolates of TYLCTZV.
2. Identify alternative hosts of TYLCTZV.
3. Determine the transmission efficiency of TYLCTZV isolates.
4. Screen tomato varieties for resistance or tolerance to TYLCTZV.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Uses of tomato

Tomatoes are consumed fresh in salads, sauces and as a flavouring ingredient in soups and meat or fish dishes, but can also be made into sweetened candies, dried fruits, and even wine. The fruits, which are rich in vitamins A, B_1, B_2 and C, are eaten raw, baked or stewed while the 24 % non-drying oil contained in seeds is used as salad oil and for the manufacture of margarine and soap (Nath, 1976). However, the most economically important uses of tomatoes are in various processed forms such as purées, juice, sauce, prickles, ketchup, canned whole and diced fruits (Dhamo and Jotwani, 1984). Tomato is also a major trade commodity for both local and international markets (Moustafa *et al.*, 1991). Although tomatoes generally rank low in comparative nutritional value, they outrank all other vegetables in total contribution to human nutrition because so much is consumed in so many different ways.

2.2 History of *Tomato yellow leaf curl disease* (TYLCD)

A disease resembling TYLCD was first reported in 1939 in the Jordan valley, in what later became the state of Israel, and was associated with outbreaks of whiteflies (Avidov, 1946). Nearly 20 years later, farmers started large-scale cultivation of cotton in the Jordan and Bet She'an valleys, which probably supported the establishment of large whitefly populations close to tomato fields. Concomitant with a heavy infestation of whiteflies, a disease of uncertain etiology destroyed the entire tomato crop in the area in

1959 (Cohen and Harpaz, 1964). When diseased plants were sampled, the disease-causing agent was identified to be a virus transmitted by whiteflies. At first, the virus was erroneously identified as 'Tomato yellow top virus', which is transmitted by aphids. Later, it became apparent that a new virus, termed TYLCV, was the causal agent (Cohen *et al.*, 1961; Cohen and Harpaz, 1964). It took another 20 years before the geminate particles were observed by electron microscopy (Russo *et al.*, 1980). Only in 1988 was TYLCV first isolated (Czosnek *et al.*, 1988a), and shortly after it was shown to be a monopartite geminivirus (Navot *et al.*, 1991). Following comparisons of TYLCV isolates from distinct geographical regions, it became apparent that the name TYLCV has been given to a heterogenous complex of begomoviruses (Moriones and Navas-Castillo, 2000).

2.3 Geographical distribution of *Tomato yellow leaf curl disease*

Tomato yellow leaf curl disease has become economically important in many countries of the Middle East, Southern Asia, Eastern, Central and Western Africa, and the Mediterranean Basin (Pico *et al.*, 1996). It has continued to spread throughout the Caribbean Basin (McGlashan *et al.*, 1994), and to southern Europe where severe outbreaks have been reported (Nakhla and Maxwell, 1998). In the early to middle 1990s, TYLCV was identified in the Dominican Republic (Nakhla *et al.*, 1994) and subsequently in Jamaica (McGlashan *et al.*, 1994), India (Grit *et al.*, 1994), and Cuba (Ramos *et al.*, 1996). More recently, TYLCV has been identified in Florida, Georgia, and Louisiana in the United States (Momol *et al.*, 1999; Polston and McGovern, 1999;

Valverde *et al.*, 2001), the Bahamas (Sinisterra *et al.*, 2000), Puerto Rico (Bird *et al.*, 2001), and in Mexico (Ascencio-Ibanez *et al.*, 1999). In Africa, it has been reported from Egypt (Cohen and Harpaz, 1964), Sudan (Yassin and Nour, 1965), Tunisia (Cherif and Russo, 1983), Burkina Faso (Konate *et al.*, 1995), Tanzania (Czosnek *et al.*, 1990), and in Nigeria, Senegal, Cape Verde Islands, Mali, Ivory Coast, Gambia and Mauritania (D'Hondt and Russo, 1985).

2.4 Symptomatology

Typical symptoms of TYLCV in tomato (Plate 1) include yellowing, leaf curling, stunting, abscission of flowers and fruits, and marked reduction in leaf size (Cohen and Harpaz, 1964; Mansour and Al-Musa, 1992). The first symptoms of TYLCV on tomato plants appear 2 - 4 weeks after infection and become fully developed after a period of up to 2 months (Ioannou, 1985a; Credi *et al.*, 1989). However, the type and severity of symptoms vary according to the virus isolate, the host genetic background, the environmental conditions, and the growth stage and physiological condition of the tomato plant at the time of infection (Pico *et al.*, 1996). Temperatures above 25°C enhance development of foliar symptoms (Pico *et al.*, 1996).

Leaves that appear soon after infection have leaflets that are cupped downward and inward in a hook-like shape; leaves appearing later are misshapen and smaller, exhibiting interveinal and marginal chlorosis and upward curling of the leaf margins. Purpling of the lower surface of infected leaflets has been reported (Nitzany, 1975).

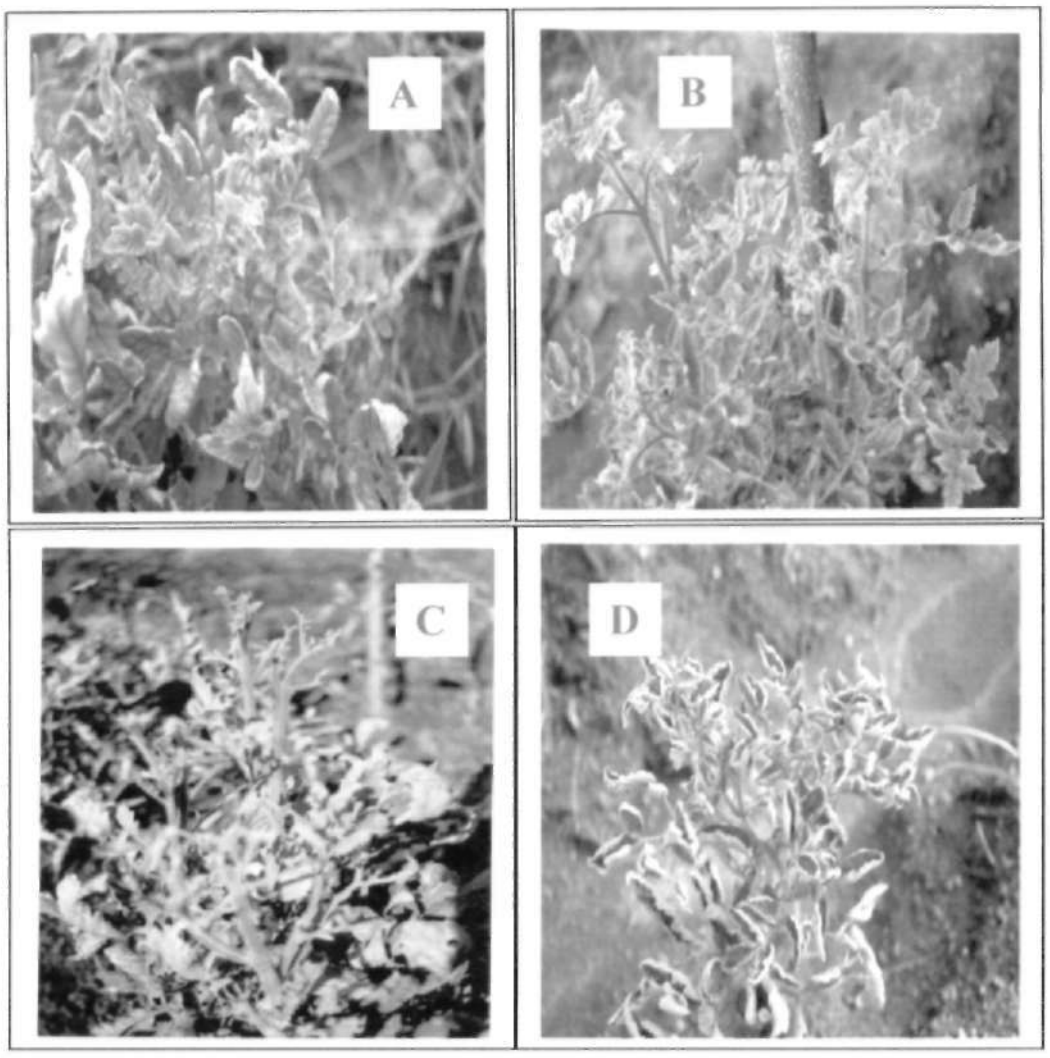


Plate 1: Typical yellowing (A and B), erect appearance (C) and leaf curling (D) symptoms of tomato plant due to infection by *Tomato yellow leaf curl Tanzania virus.*

Tomato plants infected by TYLCV are severely stunted showing an erect growth habit with many small branches and shortened internodes (Mazyad *et al.*, 1979; Credi *et al.*, 1989).

Quantitative and qualitative reductions in tomato yield also occur (Makkouk, 1978; Al-Musa, 1982). Young tomato plants infected at an early stage of plant growth are usually fruitless because of severe flower shedding. Thus, the reduction in yield is higher when plants are infected at earlier than later stages of development. Infected plants produce fewer and smaller fruits that usually drop, but fruits set before infection tend to ripen normally (Nitzany, 1975; Ioannou, 1985a).

Metabolic changes in infected tomato tissues have been observed (Ratul and Brar, 1989). However, some tomato varieties are symptomless carriers of the virus (Cohen and Nitzany, 1966; Kasrawi *et al.*, 1988). Other viruses such as *Beet curly top*, Tomato yellow top, Tomato yellows, *Tomato mottle virus* and Tobacco etch virus cause symptoms similar to TYLCV (Thomas, 1981; Zitter and Tsai, 1981; Polston *et al.*, 1994).

2.5 Economic importance

Tomato yellow leaf curl disease (TYLCD) is a serious problem in areas where it occurs and a potential threat in regions where tomato production is being expanded because of its great economic impact on both tomato fruit yield and quality (Makkouk *et al.*, 1979;

Ioannou 1985b). The TYLCD causes weakening of the whole tomato plant as well as flower and fruit abscission, resulting in serious yield losses, especially when infection occurs before flowering (Pico *et al.*, 1996). There is also a decrease in tomato fruit quality caused by reduction in fruits sizes, which do not often attain the appropriate commercial size.

The incidence of the virus on tomato has been reported to reach 85 – 100 % in many locations (Kegler, 1994). Yield losses in tomato caused by TYLCV may reach 100 % (Ioannou, 1985b; Polston *et al.*, 1994). Makkouk (1978) reported a yield reduction of 63 % when glasshouse tomato plants were inoculated 3 weeks after transplanting. In the Jordan valley, Al-Musa (1982) reported TYLCV incidences of 0 - 13.2 % in spring-grown and 93 – 100 % in autumn-grown tomatoes. Studies by scientists at the AVRDC reported a TYLCV incidence of 70 – 100 % in several farms in Arusha, Coast, Dodoma, and Shinyanga regions in Tanzania (AVRDC, 1994). Recently, Kashina *et al.* (2002b) reported TYLCTZV incidences of 15 - 85 % in a survey of tomato farms from six regions in Tanzania mainland. The incidence, severity, and spread of the disease vary with seasons, and such variations have been correlated with fluctuations in the population of the vector (Ioannou and Iordanou, 1985; Cohen *et al.*, 1988). Under favourable conditions for virus spread, the disease could reach epidemic proportions leading to the abandonment of cultivated fields (Nakhla *et al.*, 1994).

2.6 Biology of the virus

2.6.1 Molecular biology

Geminiviruses are small plant viruses characterised by a 22 x 38 nm geminate particle. Each particle consists of two joined incomplete icosahedra, encapsidating circular single-stranded (ss) DNA genome molecules of about 2700 nucleotides (Goodman, 1977; Francki *et al.*, 1980; Czosnek *et al.*, 2002). The *Tomato yellow leaf curl virus* has a characteristic geminate particle morphology (20 x 30 nm in size) consisting of two incomplete icosahedra (Harrison, 1985; Frischmuth and Stanley, 1993). Although, single particles do appear in most preparations, they are non-infective degraded products (D'Hondt and Russo, 1985).

The TYLCV genome consists of circular single-stranded DNA (ssDNA) molecules (Harrison, 1985). The number of genomic components (one or two ssDNA molecules) varies according to the virus strain. TYLCV isolates from Israel, Sardinia, Sicily, Egypt, and Spain, have only one circular ssDNA molecule of approximately 2.7 - 2.8 kb, which has sufficient information for virus replication, vector transmission, and systemic infection (Kheyr-Pour *et al.*, 1991; Navot *et al.*, 1991; Nakhla *et al.*, 1992; Noris *et al.*, 1994b; Crespi *et al.*, 1995). The Thailand isolate has a bipartite genome with two circular ssDNA molecules (DNA A and DNA B) of approximately 2.8 kb, separately encapsidated (Rochester *et al.*, 1994). Plants inoculated with only DNA A of a bipartite geminivirus show milder symptoms than those developed on plants inoculated with both components (Rochester *et al.*, 1994).

2.6.2 Genome organization

Sequencing and molecular characterization have provided detailed information on the genomic organization of the different TYLCV isolates. Begomoviruses originating from the New World have bipartite genome organization consisting of two approximately 2.8 kb circular ssDNA genomic molecules (DNA A and DNA B). Begomoviruses from the Old World have either a DNA A-like monopartite genome or a bipartite genome genetically similar to that of the begomoviruses from the New World. The genomes of monopartite viruses encode six open reading frames (ORF) (Fig. 1), two on the virion strand (AV1 and AV2) and four on the complementary strand (AC1 to AC4) (Rochester *et al.*, 1994; Czosnek *et al.*, 2002). AV2 encodes the coat protein (CP) and AV1 may control symptoms and movement. AC1 encodes the Rep protein necessary for replication. AC2 is a transcriptional activator protein (TrAP) involved in the regulation of the ratio between double-stranded (dsDNA) and single-stranded DNA (ssDNA), probably by transactivating the expression of coat protein, which causes a decrease in ssDNA concentration through encapsidation. AC3 is a replication enhancer protein (REn) necessary for efficient viral DNA replication, and AC4 may affect host range, symptom severity and movement because when it is disrupted, the virus is unable to move systemically and infected plants develop no or very mild symptoms (Sunter *et al.*, 1990; Laufs *et al.*, 1995; Czosnek *et al.*, 2002).

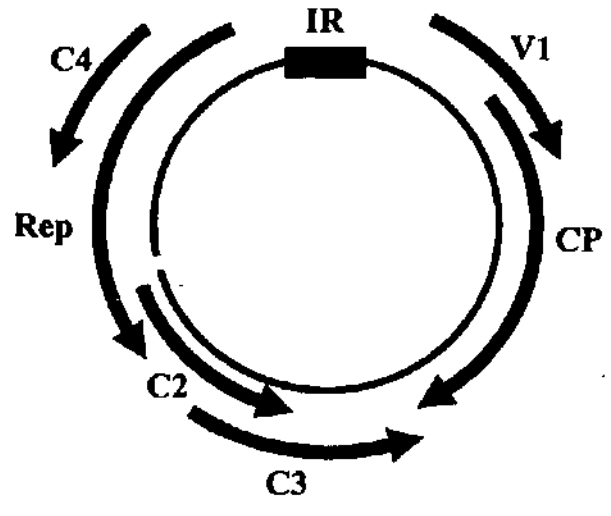


Figure 1: Genome organization of TYLCV: Open reading frames (ORFs) are shown as black arrows; V denotes ORFs on the virion strand, and C denotes ORFs on the complementary strand. IR is intergenic region, CP is the coat protein gene (V2), and Rep is replication-associated protein gene (C1).
Source: Salati *et al.*, (2002).

The organization of TYLCV DNA A in the viral sense resembles that of the monopartite leafhopper-transmitted geminiviruses; it has two ORFs, AV1 and AV2 (Hanley-Bowdoin *et al.*, 1988; Lazarowitz, 1992). In bipartite geminiviruses, the DNA A component lacks the V2 gene. The DNA B component encodes BV1 and BC1 proteins that are essential for cell-to-cell and systemic movement (Gilbertson *et al.*, 1994; Hanley-Bowdoin *et al.*, 1999; Czosnek *et al.*, 2002), and can influence host range (Ingham *et al.*, 1995). Although, not directly involved in interaction with the whitefly vector, DNA B sequences affect the efficiency of virus acquisition by the insect by determining the location of begomoviruses in plant tissues (Liu *et al.*, 1997).

There is a non-coding intergenic region (IR) located between ORFs AC1 and AV1 (Fig. 1), which include potential promoter sequences for bi-directional transcription. Polyadenylation signals are found at the end of ORFs AV2 and AC3, respectively, suggesting that both viral and complementary sense transcripts terminate at this point (Kheyr-Pour *et al.*, 1991; Noris *et al.*, 1994b).

The two genomic components, DNA A and DNA B, of bipartite geminiviruses have a common region (CR) of approximately 200 nucleotides similar to that of the IR. This CR is highly conserved between both genomic components of any single bipartite geminivirus, but it exhibits a great variation among different geminiviruses. A conserved sequence essential for viral replication, has been found in the CR of bipartite and IR of monopartite geminiviruses. It contains the GC-rich inverted repeat that has the potential to form a stem-loop structure, including the nonamer TAATATTAC conserved among all gemininviruses (Rochester *et al.*, 1994; Timmermans *et al.*, 1994).

2.6.3 Virus replication

2.6.3.1 Cytology

The multiplication of TYLCV genome is confined to the cell nucleus, where viral particles accumulate as irregular or crystalline aggregates (Cherif and Russo, 1983; D'Hondt and Russo, 1985). This aggregation induces nucleolus hypertrophy and segregation of nucleolar material into granular and fibrillar regions. Since the fibrillar rings consist of DNA and protein, they can be the sites where viral DNA synthesis and

assembly take place. A repositioning of the chromatin confined to peripheral clumps around the nuclear membrane occurs, and the nucleus is altered showing a nucleoplasm with a low opacity to electrons (D'Hondt and Russo, 1985). These changes precede the accumulation of viral particles and could reflect a shift in the transcription and synthesis machinery of the host plant towards the synthesis of the viral material. Because the cell's synthesizing machinery is required for viral replication, this may limit viral DNA synthesis and assembly to the S phase of the cell cycle when chromosome replication occurs (Timmermans *et al.*, 1994). Examination of the viral DNA in TYLCV-infected plants detected circular dsDNA molecules that can be found in two forms: a supercoiled form (covalently closed circular form, cccDNA) and a relaxed form (open circular form, opDNA) (Czosnek *et al.*, 1989). These dsDNA molecules are considered intermediates for viral replication, named replicative forms (RF). This suggests that TYLCV replication follows a 'rolling circle' mechanism, similar to that used by ssDNA phages, ssDNA plasmids and other geminiviruses (Saunders *et al.*, 1991; Jeske *et al.*, 2001).

2.6.3.2 Rolling circle mechanism

Rolling circle replication is a two-step process in which the leading and lagging-strand DNA synthesis are separate events (Kornberg and Baker, 1992) (Fig. 2).

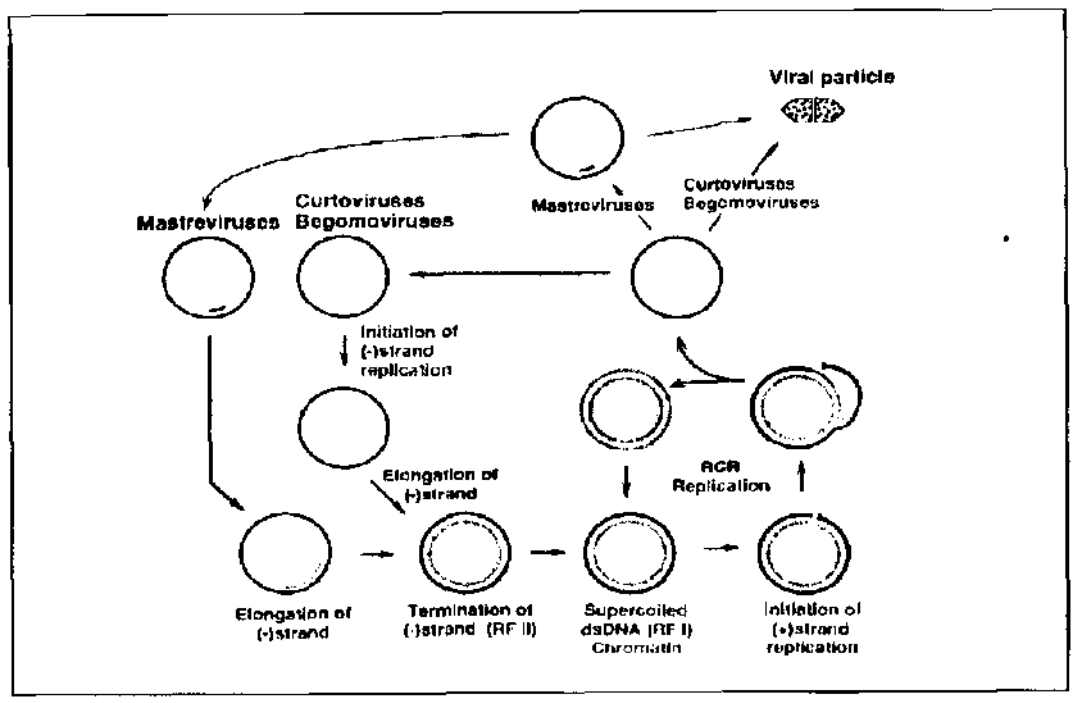


Figure 2: A schematic summary of the geminivirus replicative cycle.

Source: Gutierrez, C. (1999).

First, the virus uses host functions in the cell to synthesise the complementary (-) strand to generate the double-stranded replicative form. An RNA molecule that is generated either through RNA polymerase or DNA primase activity primes the synthesis of the complementary strand (Hanley-Bowdoin *et al.*, 1999). In this respect, monopartite geminiviruses have a small, approximately 80 bp DNA sequence, base-paired to the IR region of the encapsidated ssDNA, which contains a few covalently bound 5' terminal ribonucleotides. This sequence seems to prime the synthesis of the complementary strand in the conversion of ssDNA to dsDNA (RF). Self-priming capacity has not been found in TYLCV (Hanley-Bowdoin *et al.*, 1999). During the second step, the RF serves

as a template for the virion (+) strand synthesis to generate free ssDNA (Lazarowitz, 1992; Hanley-Bowdoin *et al*., 1999; Jeske *et al*., 2001).

2.6.3.3 Functional domains

Geminiviruses use the host polymerases, and only one viral protein [replication protein (Rep)], is essential for replication. The Rep is a multifunctional protein encoded by ORF AC1 that acts as a sequence-specific endonuclease at the replication origin (Laufs *et al*., 1995; Orozco and Hanley-Bowdoin, 1998). In addition to endonuclease activity, rolling circle replication requires helicase and ligase activity. Thus, replication of DNA B depends on the presence of DNA A genic products, whereas DNA A can replicate autonomously (Laufs *et al*., 1995). The study of the organization of the IR of some bipartite and monopartite geminiviruses shows the existence of short iterative sequences located near the TATA box, promoter of the complementary sense transcription. These sequences differ even between closely related geminiviruses, and act as specific binding sites of the AC1 protein (Argüello-Astorga *et al*., 1994). The replication origin located in the intergenic region contains the nonamer TAATATTAC, which is conserved in most geminiviruses and is flanked by two inverted sequences that form the stem-loop structure. The stem-loop is required for replication, but does not contribute to virus-specific recognition for the Rep; the specificity is conferred by the iterative sequences at which AC1 protein binds specifically (Argüello-Astorga *et al*., 1994). The Rep cleaves the viral DNA strand at replication origin between bases 7 and 8 in the conserved nonanucleotide sequence. After cleavage, Rep remains covalently bound to the 5' end of

the cleaved strand. The 3' hydroxyl group primes the synthesis of the viral strand DNA. The Rep also acts as a terminase, releasing a circular ssDNA of the genome size in each replication circle. The releasing process involves a transfer reaction between 5' end bound to the Rep, and the nonamer of the newly synthesized DNA (Laufs *et al.*, 1995). Nicking and joining of the viral DNA at the replication origin are catalyzed by the amino terminal domain of the Rep determining the specificity of the origin of recognition (Heyraud-Nitschke *et al.*, 1995; Jupin *et al.*, 1995). It has also been demonstrated that ATPase activity of the Rep is required for viral DNA replication (Desbiez *et al.*, 1995). Since the Rep cleavage site and the Rep specific binding sites are distantly located on viral strand, two models for the functional organization of the replication origin have been proposed (Laufs *et al.*, 1995; Jupin *et al.*, 1995). The first model requires the helicase activity of the Rep to unwind the dsDNA RF. This activity would start at the TATA box in the direction of the stem-loop structure where Rep cleavage occurs. The second model involves the participation of a host transcription factor that interacts with a TATA-binding protein bound to the complementary strand transcription promoter. This interaction brings the stem-loop structure and the AC1 protein bound to the iterative sequences nearer to allow for DNA cleavage. Besides these functions, Rep acts as a repressor of its own gene, and transactivates the expression of the viral capsid gene promoter (Argüello-Astorga *et al.*, 1994).

2.6.4 Accumulation and spread of the virus in infected tissues.

Hybridization techniques have been used to study the accumulation of TYLCV viral DNA in relation to symptom expression. These methods allow the spread of the virus through the plant to be monitored from the site of inoculation. *Tomato yellow leaf curl virus* accumulates preferentially in tissues containing dividing cells, indicating the need for active DNA synthesis to replicate its own genetic material (Cohen and Antignus, 1994). Viral DNA can be detected at the inoculation site 4 - 5 days after infection, 1 or 2 weeks before the first disease symptoms appear and reaches a peak 12 - 15 days post-inoculation (Rom *et al.*, 1993), spreading to other tissues. In monopartite geminiviruses, the AV1 and AC4 are thought to be involved in cell-to-cell and systemic spread, while in bipartite geminiviruses, BV1 and BC1 proteins are essential for cell-to-cell and systemic movement (Gilbertson *et al.*, 1994; Hanley-Bowdoin *et al.*, 1999; Czosnek *et al.*, 2002) following the photoassimilates pathway (Ber *et al.*, 1990).

The replication and transport of viral particles depend on the development stage of the inoculated tissue. It seems that a susceptiblity gradient exists, which decreases as the leaf ages. Consequently, young tissues are the best targets for insect inoculation. When young leaves are inoculated, replication of viral DNA occurs at the site of inoculation and spreads rapidly. First, it is transported to the roots, then to the shoot apex, and finally to the neighbouring leaves and flowers (Nelson and van Bel, 1998). These tissues accumulate high concentrations of viral DNA, being highest in the shoot apex, and lowest in the roots, stems and expanding leaves. DNA accumulation in the roots seems

to be due to transport of viral DNA and not to viral replication. In non-dividing tissues such as cotyledons and older leaves, viral DNA concentration is practically imperceptible. Inoculation of middle-aged leaves follows the same distribution pattern, but at a slower rate, while inoculation of older tissues is not effective (Czosnek *et al.*, 1988b; Ber *et al.*, 1990).

2.7 Diagnosis

2.7.1 Back indexing

Since mechanical transmission is not effective, reciprocal grafting or vector inoculation on indicator plants is used to detect the presence of TYLCV in infected plants. *Datura stramonium* and susceptible tomato cultivars are the most commonly used test plants. The major disadvantages of the back indexing method are that it is time-consuming and symptom expression is not usually a precise indication of virus identity (Pilowsky and Cohen, 1974; Kasrawi *et al.*, 1988).

2.7.2 Electron microscopy

Electron microscopic examination of thin sections of tissues and extracts prepared from infected plants can assist the rapid identification and characterization of many plant viruses. Visual identification is easy for geminiviruses due to the geminate characteristic particle structure and cytopathological changes they induce. But this method lacks the required specificity, since particle structure and ultrastructural changes are not virus-

specific and cannot be used to distinguish the different whitefly-transmitted geminiviruses (Credi *et al.*, 1989).

2.7.3 Serology

Serological techniques such as immunosorbent electron microscopy (ISEM), dot immunobinding assay (DIBA), enzyme-linked immunosorbent assay (ELISA), and tissue print ELISA work on the principle of amplification of the reaction between viral antigens and their antibodies by utilizing an enzyme and its substrate. ELISA is one of the most widely used serological tests for the detection of plant viruses because of its simplicity, adaptability and sensitivity. The test is different from the classical serological procedures in which immunoprecipitin reactions are used. Immunospecificity is recognized through the action of the associated enzyme label on a suitable substrate rather than by observing the formation of an insoluble antigen-antibody complex.

The basic principle of the ELISA lies in immobilizing the antigen onto a solid surface, or capturing antigen by specific antibodies, and probing with specific immunoglobulins carrying an enzyme label. The enzyme retained in the case of a positive reaction is detected by adding the suitable substrate. The enzyme converts the substrate to a product that can be easily recognized by its colour.

There are the direct and indirect ELISA procedures. In the direct ELISA, antibodies extracted from virus-specific antiserum are used for coating the solid surface to trap the

antigen, and the same antibodies labelled with an enzyme are employed for detection. In this case, the antigen is sandwiched between the two antibodies; thus, it is referred to as the double-antibody sandwich (DAS) ELISA. In indirect ELISA procedure, the antigen is bound to a solid surface, unconjugated antibody (primary antibody) is added, and then detected by the enzyme-labelled secondary antibody. In indirect ELISA, the antigen-specific detecting antibodies or primary antibody are not labelled with enzyme. This ELISA is particularly suitable for virus detection in disease surveys, for testing the presence of virus in seed, and for determining serological relationships, particularly when specific conjugates cannot be prepared. It is more economical, and it is theoretically more sensitive than direct ELISA because of the possibility of attachment of more than one second antibody molecule to primary. However, under optimum conditions, the sensitivity of both ELISA systems is comparable. On the whole, serological techniques, which are based on the antigenic properties of the viral coat protein, are inadequate for geminivirus identification. They lack both the specificity and sensitivity required for diagnosis due to a high degree of homology in the coat protein among related whitefly-transmitted geminiviruses, which is shown by cross-reactions with monoclonal and polyclonal antibodies (Pico *et al.*, 1996). On the contrary, leafhopper-transmitted geminiviruses, which are not so closely related to each other, are efficiently detected by these serological methods (Roberts *et al.*, 1984; Thomas *et al.*, 1986). It is difficult to obtain high-titre antisera against TYLCV as geminivirus particles occur in plants only in moderate concentration (Czosnek *et al.*, 1990). Serological

methods have been found to be effective in detecting TYLCV and other geminiviruses in purified, but not in crude preparations (Noris *et al.*, 1994a).

2.7.4 Hybridization with DNA probes

Molecular hybridization using TYLCV-specific DNA probes is a sensitive technique for quantitative and qualitative detection of low concentrations of TYLCV. The technique is based on the homology between two strands of nucleic acid either in double-stranded DNA or in hybrid molecules that consist of strands of RNA and DNA. The hybridization procedures involve: (i) spotting and immobilizing the target nucleic acid onto a support matrix such as nylon membrane, (ii) blocking of the free binding sites on the membrane with a non-homologous DNA (usually salmon sperm or calf-thymus DNA) or a protein source (bovine serum albumin or non-fat dried milk), (iii) incubating the membrane with the melted probe, which is complementary to the target nucleic acid of interest in annealing conditions, (iv) removing the unhybridized probe from the hybridized material in a series of washing steps, and (v) assaying the presence of the target sequence by detecting the reporter molecule in the hybridized probe sequences.

Several types of DNA probes can be radioactively or non-radioactively labelled for the visualization of results. Because the use of radioactivity has limitations, hybridization techniques have been developed using non-radioactive labelled probes, which can be visualized by a simple immunological reaction or, by using chemilumiscent detection (Crespi *et al.*, 1991). Although, this method of detection takes less time than using DNA

radioactive probes, it has been found to be less sensitive, detecting from 0.45 – 1.3 pg of DNA as opposed to 0.15 pg of the latter (Crespi *et al.*, 1991). A sulphonated DNA probe has also been developed for virus detection by squash blotting, but the yellow-brownish colour of leaves and stems that remain in the membrane can interfere with the final colorimetric detection (Zilberstein *et al.*, 1989). In order to increase detection specificity, cloned DNA probes with deleted coat protein can be used, including the more divergent gene that encodes the AC1 protein and the 200 nucleotide sequence unique for each geminivirus (Navot *et al.*, 1989). Depending on sample treatment, there are several techniques based on DNA hybridization, which have been used for the diagnosis of viruses.

2.7.4.1 Squash blot

The viral DNA of TYLCV can be detected in infected tissues squashed onto nylon or nitrocellulose membrane followed by hybridization with specific probes. This method provides a specific, rapid, and simple means to detect infected tomato tissues and does not require DNA extraction. Squash-blotted samples are very stable, can be taken in the field by unspecialized personnel, and can be maintained at room temperature for several months without losing their hybridization capacity (Navot *et al.*, 1989; Grit *et al.*, 1994).

2.7.4.2 Dot blot

Hybridization of lysates spotted onto a membrane provides a rapid and accurate estimation of viral concentration. This method minimizes the loss of nucleic acid, which

usually occurs in the DNA extraction process. Lysates can be prepared from viruliferous whiteflies or from tomato-infected tissues such as leaves, roots, stem, cotyledons, and shoot apex, enabling analysis of viral distribution throughout the plant. Both squash and dot blot methods are useful for the monitoring of TYLCV epidemics, but cannot discriminate between TYLCV isolates (Czosnek *et al*., 1988b; Crespi *et al*., 1991).

2.7.4.3 Southern blot

This technique allows the detection and quantification of the different forms in which viral DNA (ssDNA or dsDNA) occurs in plants and insect vectors. Furthermore, Southern hybridization can discriminate different TYLCV isolates/strains with a high degree of specificity (Rojas *et al*., 1993). This involves the digestion of the total DNA extracts with restriction enzymes and running the products in agarose gel. The viral DNA is denatured, neutralized and transferred onto nylon or nitrocellulose membrane before hybridization and detection by autoradiography, colorimetry or chemiluminiscence.

2.7.5 Polymerase chain reaction (PCR)

The PCR technique has been used to detect TYLCV in both plants and vectors. It is a rapid, inexpensive and simple means of producing relatively large numbers of copies of DNA molecules from minute quantities of source DNA material, even when the source DNA is of relatively poor quality. Since geminiviruses replicate by a dsDNA intermediate, PCR techniques are adequate for diagnosis in this group (Rojas *et al*.,

1993; Mehta *et al.*, 1994). This method is more sensitive than hybridization techniques and does not require the use of radioactivity (Pico *et al.*, 1996). The use in PCR of degenerate oligonucleotide primers complementary to highly conserved sequences of the whitefly-transmitted geminivirus genomes extends the range of viruses that can be detected. In this sense, the coat protein of the TYLCV isolates from Israel, Italy and Thailand, show three conserved regions that can act as binding sites for PCR primers, which allow the detection and analysis of the heterogeneous TYLCV isolates (Navot *et al.*, 1992). Individual isolates can be distinguished by the restriction fragment length polymorphisms (RFLPs) obtained after digestion of the PCR products with restriction enzymes. This technique can also be used for the identification of viruses in mixed infections (Rojas *et al.*, 1993; Deng *et al.*, 1994).

2.8 Transmission

The whitefly, *Bemisia tabaci* Gennadius, is the only known vector of TYLCV (Cohen and Nitzany, 1966; Nakhla *et al.*, 1978). Consequently, the spread of TYLCV in tomato fields is due entirely to the whitefly vector with females being more efficient transmitters than the males (Cohen and Nitzany, 1966; Nene, 1972; Caciagli *et al.*, 1995; Muniyappa *et al.*, 2000). There is evidence of passage of the virus to the progeny transovarially for at least two generations and through moulting (Cohen and Nitzany, 1966; Ghanim *et al.*, 1998). Although, the greenhouse whitefly, *Trialeurodes vaporariorum*, and the aphid *Myzus persicae* can acquire the virus non-specifically, they are not able to transmit it (Antignus *et al.*, 1994a). *Tomato yellow leaf curl virus* is also

graft-transmissible (Ioannou, 1985b), but attempts to transmit it through seed, soil or mechanical means from tomato to tomato have not been successful (Cohen and Nitzany, 1966; Makkouk *et al.*, 1979; Credi *et al.*, 1989). However, mechanical transmission of TYLCV to tomato has been achieved under artificial conditions from plants of *D. stramonium*, *N. glutinosa*, and *L. pennellii* at a very low transmission rate (Makkouk *et al.*, 1979), and only in few cases, from tomato to tomato (Abdel-Salam, 1990). TYLCTZV is not mechanically transmissible (Kashina *et al.*, 2003a).

2.8.1 Vector biology

2.8.1.1 Life cycle

Bemisia tabaci (Homoptera: Aleyrodidae), commonly called tobacco, cotton, or sweetpotato whitefly, is a cosmopolitan pest of eastern origins, likely from Pakistan or India (Mound, 1983; Fishpool and Burban, 1994). It is a damaging polyphagous outdoor pest infesting more than 600 plant species, especially, in hot and sunny conditions (Pico *et al.*, 1996). Like most whiteflies, *Bemisia tabaci* is arrhenotokous. The females are able to regulate the sex of their progeny by selective egg fertilization (Horowitz and Gerling, 1992). Fertilized females lay both diploid and haploid eggs, which give rise to females and males, respectively; the unfertilised females lay only haploid eggs. The progeny sex ratio is usually 1:1, but can be upsetted by seasonal and environmental changes, and insect age. Young females lay more female-producing eggs than older females (Horowitz and Gerling, 1992). In laboratory experiments, a single B

biotype female was found to have laid between 21 and 324 eggs per plant on egg plant, tomato, sweet potato and garden bean (Wang and Tsai, 1996; Tsai and Wang, 1996).

The eggs hatch into first instar larvae (crawlers), which further develop into second, third and fourth (pupae) instar larvae, and finally into adults (Fig. 3). The rate of development of *B. tabaci* is determined mainly by factors such as temperature, humidity, and plant host quality (Powell and Bellows, 1992). Average life cycle ranges from 17 to 27 days. The range of temperatures for development of *B. tabaci* is wide, being most favourable between 16 and 24 °C. Temperatures below 9 °C and above 40 °C are reported to be lethal to the insect. The relative humidity of 30 - 60 % is optimum for insect development. However, low relative humidity, rainfall, and extreme temperatures can impair oviposition (Zalom *et al*., 1985; Cohen, 1990).

Total developmental times in the field (egg to adult) are highly variable ranging from 14 to 107 days. In the laboratory, egg development occurs in 5.2 to 10.7 days at 21 – 31 °C, but could be faster at higher temperatures (Wang and Tsai, 1996; Tsai and Wang, 1996). Differences in developmental rates of *B. tabaci* of as much as 10 days have been observed on different hosts. On tomato, the egg develops in 6.2 days, crawlers in 2.2 days, second instar larvae in 2.2 days, third instar larvae in 2.2 days, and fourth instar larvae in 5.1 days at 25.5 °C (Tsai and Wang, 1996).

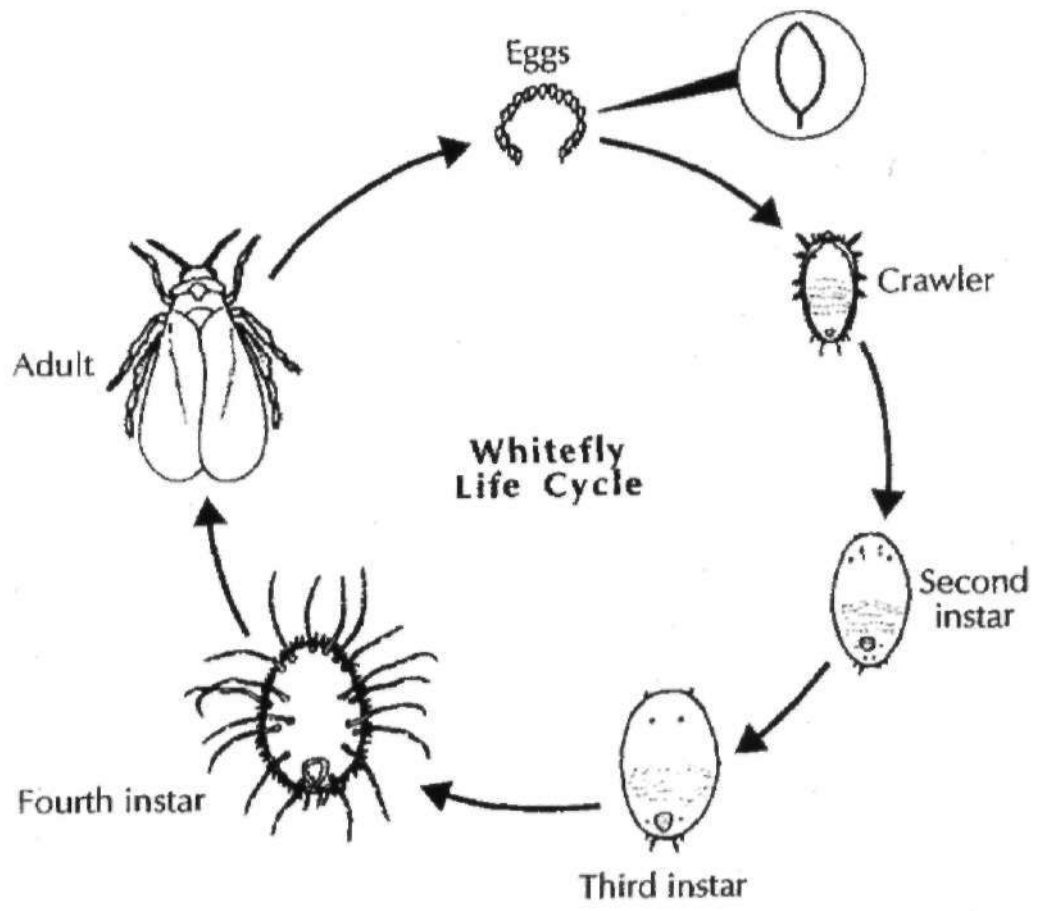


Figure 3: The whitefly life cycle showing stages of development from eggs to adults. Courtesy of Dr. Jack K. Clark, Statewide Integrated Pest Management Project, University of California, USA.

2.8.1.2 Individual behaviour

The adults and young larvae of *B. tabaci* prefer to feed on young leaves, and they seem to have an adaptation for higher concentration of soluble nitrogenous compounds (Pico *et al.*, 1996). This preference is reflected in their distribution on the plant; adults, eggs, and first larval stages are found on the upper part of the plant on young apical leaves,

whereas the pupae and recently emerged adults are only found lower down the older leaves (Mound 1983). The adults and larvae feed mainly on the phloem of the abaxial leaf surface (Plate 2) penetrating the leaf tissues intercellularly using their stylets. This feeding behaviour is effective for the acquisition and subsequent transmission of geminiviruses that characteristically infect phloem-associated tissues (Buntin *et al.*, 1993).

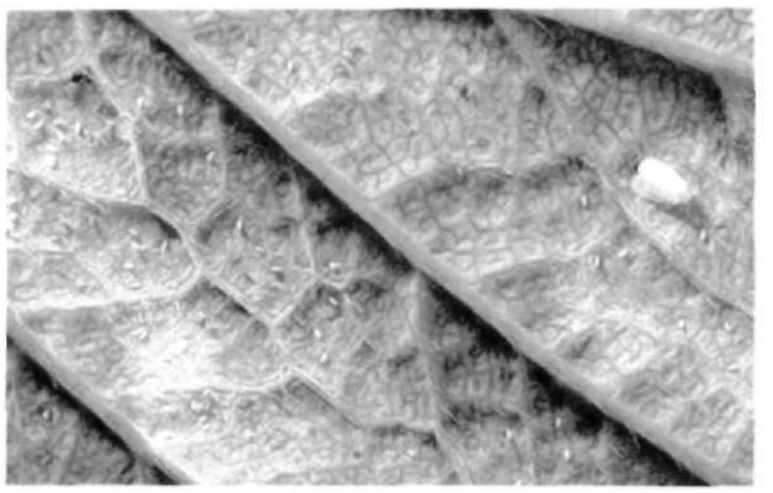


Plate 2: Adult *Bemisia tabaci* at the abaxial surface of a tomato leaf.

2.8.1.3 Population dynamics

Winged *B. tabaci* adults moving in short active distances and long passive distances by wind, spread *Tomato yellow leaf curl virus*. During new invasions, a greater density of the insect population can be found at the sides of fields and close to greenhouse doors and windows (Cohen, 1990). Since whiteflies have a broad host range, they can

complete several generations (about 11 – 15) per year under favourable conditions (Powell and Bellows, 1992). In tropical regions, *B. tabaci* does not diapause; it is a mobile pest moving from one host to another throughout the year. In these regions, host plant and biological equilibrium with natural enemies are the main factors affecting insect populations, whereas in temperate regions where low temperature is the principal factor controlling populations, the insects overwinter as nymphs (Horowitz and Gerling, 1992). In Mediterranean countries, populations are highest in late summer or early autumn and are drastically reduced in winter. The adults overwinter on leaves of perennial plant species (Cohen, 1990; Horowitz and Gerling, 1992).

2.8.2 Vector biotypes

Bemisia tabaci was first described on tabacco in Greece (Gennadius, 1889). It has been traditionally considered an outdoor pest, mainly in tropical and sub-tropical regions (Mound, 1983). Outbreaks of this pest have been reported in many regions of the world, with most spectacular increases in population due partly to the development of resistance to pesticides and to changes in agronomic practices (Pico *et al.*, 1996). The increasing importance of protected crops has encouraged the presence of *B. tabaci* in less favourable climates and it is fast displacing the greenhouse whitefly, *T. vaporariorum* (Pico *et al.*, 1996). *B. tabaci* is an insect species complex that has geographically distinct phenotypic and genotypic variants, which occur as a series of biotypes that have different geographical distributions and differ in their ability to transmit the viral disease (Bird and Maramorosch, 1978; Brown *et al.*, 1995; Frohlich *et*

al., 1999). Several biotypes (up to S) have now been described (Brown *et al.*, 1995), supporting the idea of a species complex, rather than of a number of distinct species, such as *B. argentifolii*. Adaptation exists between the different whitefly-transmitted geminiviruses and specific vector biotypes (Harrison *et al.*, 1994). Each vector population is linked to a restricted number of crops in each area, and a change to new crops demands nutritional adaptation that implies high insect mortality (Markham and Bedford, 1993). Therefore, the mobility of geminiviruses from one host to another depends on the mobility of their vector, which is determined by vector-host compatibility (Brown, 1994).

The appearance of B biotype of greater fecundity, strong pesticide resistance and a broad host range, compared with other biotypes of *B. tabaci*, has increased the importance of geminivirus infections and broadened the range of infected crops. Many recent, new geminivirus outbreaks are probably associated with known geminiviruses infecting alternative crops. This new biotype, also called poinsettia strain or silverleaf whitefly, induces phytotoxic responses in squash and tomato (Paris *et al.*, 1993). Some scientists consider the B biotype as a new species, *B. argentifolli*. It can be distinguished from other populations of *B. tabaci* with biological and genetic differences by the sterase banding pattern (Perring *et al.*, 1993; Bellows *et al.*, 1994). A recent study by Rosell *et al.* (1997) which used Scanning Electron Microscopy to examine the morphological characters documented by Bellows *et al.* (1994) for identifying the B biotype showed that most Old World populations of *B. tabaci* were morphologically indistinguishable

from the B biotype. The biotype B has become established in Southern Europe (Italy, France, Spain, Portugal, and Greece), in Northern European greenhouses, the Caribbean Basin, South and Central America, United States and Canada (Bedford *et al.*, 1994).

2.8.3 Virus-vector relationships

2.8.3.1 Mode of transmission

The existing virus-vector interaction had been described as "periodic acquisition" (Cohen and Harpaz, 1964) since the insect is not able to re-acquire the virus until the end of the previous infective period. TYLCV is transmitted in a persistent and circulative manner. The nymphs can acquire the virus as effectively as the adults and transmit it in the adult stage (Pico *et al.*, 1996). Females transmit the virus six times more efficiently than the males (Cohen and Nitzany, 1966; Caciagli *et al.*, 1995).

The infection pathway involves the insect acquiring the virus while feeding on phloem sap by inserting its stylets into plant tissue and locating the vascular tissue (Pollard, 1955). The stylet bundle is composed of three stylets: the maxillary stylet, which contains the food canal (through which phloem is acquired) and the lateral salivary canal (through which saliva is injected into the plant), and two mandibulary stylets (Rosell *et al.*, 1995). Viral particles ingested through the *B. tabaci* stylets enter the oesophagus and the digestive tract, penetrate the gut membranes into the body cavity to enter the haemolymph, accumulate and reach the salivary glands, and finally enter the salivary duct from where they are egested with the saliva during insect feeding (Markham *et al.*,

1994; Ghanim *et al.*, 2001). This circulation of the virus inside the insect accounts for the latent transmission period that ends once the virus reaches the salivary glands and the insect becomes viruliferous (Markham *et al.*, 1994; Caciagli *et al.*, 1995).

The coat protein is the only begomoviral gene product that directly interacts with whitefly factors during the circulative transmission of the virus (Rybicki, 1994; Padidam *et al.*, 1995a; Czosnek *et al.*, 2002). Its essence for vector recognition has been demonstrated in experiments involving the replacement of geminivirus coat protein (Briddon *et al.*, 1990). Consequently, mutations in the coat protein can impair virus recognition or acquisition by the vector (Accoto *et al.*, 1994). This is a possible explanation for the genome homologies of the viral capsid and antigenic relationships found among whitefly-transmitted geminiviruses, unlike the leafhopper-transmitted geminiviruses (Howarth and Vandemark, 1989). Different vector biotypes differ in their specificity and in the frequency of transmission of the different geminiviruses. The selection of variants in the coat protein for an effective transmission by the local vector biotype explains the similar epitope profiles of the whitefly-transmitted geminiviruses within a particular region (Harrison *et al.*, 1994; McGrath and Harrison, 1995). Transmission specificity seems to be determined by the virus passage from the haemocoel into the salivary glands (Markham *et al.*, 1994). However, the movement of the virions through the insect gut wall seems to be non-specific and related to some epitopes on coat protein (Antignus *et al.*, 1994a).

Evidence concerning virus replication in the vector is contradictory. On one hand, the increase in virus concentration in viruliferous whiteflies that have stopped feeding on infected plants, suggests virus replication in the vector (Mehta *et al.*, 1994). On the other hand, the analysis of the viral DNA content of infected whiteflies has shown that the DNA replicative form does not occur, indicating that TYLCV does not multiply in the insect vector (Czosnek *et al.*, 1989; Antignus *et al.*, 1994a). In any case, the acquisition of the TYLCV by a whitefly is not a passive process because the concentration of the virus in the vector is at least 1000-fold greater than that found in the plant (Navot *et al.*, 1989). However, there seems to be a limit to the rate at which the virus is transferred to the salivary glands, which is slower than the rate of accumulation (Caciagli *et al.*, 1995). The amount of viral particles that an insect can accumulate is limited, which suggests the existence of a mechanism, probably based on the saturation of geminivirus receptors or on the cyclical production of antiviral factors by viruliferous whiteflies, that regulates the number of acquired viral particles (Cohen and Marco, 1970; Zeidan and Czosnek, 1991; Cohen and Antignus, 1994).

2.8.3.2 Transmission conditions

The rate of TYLCV transmission increases with vector population (Cohen *et al.*, 1988). Although, a single viruliferous insect per plant can transmit the virus to more than 50 % of test plants, five to 20 whiteflies per plant are required to achieve 100% transmission of the virus (Cohen and Nitzany, 1966; Brown and Nelson, 1988; Mehta *et al.*, 1994). These researchers also reported minimum access, inoculation and latent periods of 30 -

60, 30 - 60 min and 20 h, respectively, and maximum access, inoculation, and latent periods of 24 - 48, 24 - 48 and 48 h, respectively. The transmission rate increases with longer acquisition access period. Viral DNA can be found in the insect vector 1 h after the start of acquisition. Its concentration increases with time, and after an acquisition period of 48 h, the viral DNA is retained for more than three weeks. However, the TYLCV coat protein is only retained for 10 days after acquisition (Antignus *et al.*, 1994a). This might be related to the vector infectivity, since the vector remains infective for an average period of 10 - 12 days after acquisition (Ioannou, 1985b; Mansour and Al-Musa, 1992).

Virus-vector relationships differ slightly among several TYLCV isolates from different geographical origins. These differences are not considered significant since they can be due to variability in experimental conditions, although they could also reflect the adaptation of the different TYLCV isolates to transmission by different whitefly populations (Cohen and Nitzany, 1966; Mansour and Al-Musa, 1992; Caciagli *et al.*, 1995).

2. 8. 4 Host range of the virus

Whitefly-transmitted geminiviruses generally have a narrow host range among dicotyledonous plants (Harrison, 1985): TYLCV is not an exception. In spite of the polyphagous nature of its vector the virus infects only a few species of several families. Plants from six botanical families have been found to be hosts of TYLCV:

Asclepiadaceae, Compositae, Leguminosae, Malvaceae, Solanaceae, and Umbelliferae (Cohen and Antignus, 1994). Some plants develop clear disease symptoms, whereas others are susceptible symptomless carriers of the virus. TYLCV mainly affects members of the Solanaceae family, particularly tobacco and mainly tomato (Cohen and Nitzany, 1966; Nitzany, 1975; Ioannou, 1985a)

The host range varies with the virus isolate. For example, the Jordan, Sardinia and Yemen isolates of TYLCV do not infect *Phaseolus vulgaris* unlike the Israeli isolate. On the other hand, these isolates infect some tobacco cultivars not affected by the Cyprus isolate (Mansour and Al-Musa, 1992). The detection of plants that serve as viral or vector reservoirs is most important in regions where tomato is not grown throughout the year. In order to understand the epidemiological cycle of TYLCV in each infected area, specific studies of the flora of the infected regions must be done to analyze the role that different crops and weeds play as natural hosts of TYLCV and its vector. In many areas, TYLCV completes its entire life cycle on overlapping tomato crops. Hosts of TYLCV reported from different geographical areas are listed in Table 1.

2.9 Taxonomy

2.9.1 Classification based on viral genome and biological characteristics

Geminiviruses are plant viruses that belong to the family *Geminiviridae*, and have circular ssDNA genomes packaged within geminate particles. They are classified into four genera based on genome organization, vectors and hosts (Lazarowitz, 1992;

Table 1: Natural reservoirs of *Tomato yellow leaf curl virus* from different geographical regions

Host	Area	Reference
Lycopersicon esculentum, Nicotiana tabacum, Phaseolus vulgaris, Lens esculenta, Datura stramonium, Malva nicaensis, M. parviflora, Cynancum acutum	Israel, Jordan	Cohen and Nitzany (1966), Cohen *et al.* (1988), Mansour and Al-Musa (1992)
Lycopersicon esculentum, N. tabacum, D. stramonium, wild Lycopersicon spp.	Cyprus	Ioannou (1987)
Acanthospermum hispidium, Ageratum conyzoides, Bidens biternata, Centratherum anthelminticum, Conyza stricta, Galinsoga parviflora, Sonchus brachyotis, Synedrella nodiflora, Zinnia elegans, Euphorbia geniculata, Althae rosea, Oxalis acetosella, Capsicum annuum, D. stramonium, Lycopersicon esculentum, L. glandulosum, L. hirsuta, L. peruvianum, Nicotiana benthamiana, N. glutinosa, N. tabacum, Physalis minima, Solanum nigrum	Southern India	Saikia and Muniyappa (1989)
Achyranthes aspera, Euphorbia heterophylla, Nicandra physaloides, Commelina erecta, Amaranthus spinosus, Erigeron floribundus, Ageratum conyzoides, Bidens pilosa, Sida acuta, Ipomea batatas, Amaranthus viridis, Portulaca oleracea, Cassia obtusifolia, Euphorbia hirta, Calopogonium mucunoides, Crotalaria retusa, Trianthema portulacastrum, Alternanthera sessilis, Celosia trigyna, Commelina diffusa, Chromolaena odorata, Eclipta prostrata, Synedrella nodiflora, Cassia occidentalis, Spigelia anthelmia, Boerhavia diffusa, Physalis angulata, and *Acanthospermum hispidium*	Tanzania	Nono-Womdim *et al.* (1996) Kashina *et al.* (2002b)

Padidam *et al.*, 1995a; van Regenmortel *et al.*, 2000). Those that have a monopartite genome and are transmitted by leafhoppers to monocotyledonous plants are members of the genus *Mastrevirus*, of which *Maize streak virus* is the type species. The genus *Curtovirus* is comprised of viruses that have a monopartite genome and are transmitted by leafhoppers to dicotyledonous plants; *Beet curly top virus* is the type species. The genus *Topocuvirus* has only one member (also the type species): *Tomato pseudo-curly top virus*, which has a monopartite genome and is transmitted by treehoppers to dicotyledonous plants. The fourth genus, *Begomovirus* include viruses that are transmitted by whiteflies to dicotyledonous plants; *Bean golden mosaic virus* is the type species. Most of the "Old World" and all "New World" begomoviruses have bipartite genomes (A and B components). However, some "Old World" begomoviruses including *Tomato yellow leaf curl virus*, *Cotton leaf curl virus*, and *Tomato leaf curl virus* have monopartite genomes and no B components have been found (Timmermans *et al.*, 1994; Fauquet *et al.*, 2000). In this genus, the identity of some tomato-infecting geminiviruses, which cause yellowing or curling symptoms, remains unclear. In some cases, the differences found in symptomatology, mode of transmission, host range, and molecular biology clearly indicate different causal agents; in other cases, the identity of the virus is still confused.

2.9.2 Classification based on serology

Serological studies based on epitope profiles of geminivirus coat protein indicate that the geminiviruses transmitted by different species of leafhoppers (*Mastrevirus* and *Curtovirus*) show less cross-reactivity with monoclonal or polyclonal antibodies than the geminiviruses within the genus *Begomovirus*, which are transmitted by a single species of whitefly, *Bemisia tabaci*. This has been demonstrated by comparison of the coat protein sequence, which indicates a greater sequence divergence among leafhopper- than among whitefly-transmitted geminiviruses (Roberts *et al.*, 1984; Thomas *et al.*, 1986). In the latter group, coat protein is the most conserved sequence of the genome. These differences lead to a division of the geminivirus group into two main clusters clearly correlated with viral-vector specificity, suggesting that the evolution of coat protein may have been directed to accommodate transmission by one or another type of insect vector.

The analysis based on the comparison of the Rep protein sequence leads to the division of begomoviruses into two main groups correlated with viral-host specificity. Therefore, the divergence among viruses affecting monocotyledonous or affecting dicotyledonous host plants may have been due to the Rep protein evolution directed to infect different hosts (Roberts *et al.*, 1984; Howarth and Vandermark, 1989).

2.9.3 Classification based on geographical location

In addition to biological characteristics, geographical isolation may have played an important role in geminivirus evolution, affecting sequence diversity. In this sense,

phylogenetic trees have been constructed based on genome comparisons, dividing *Begomoviruses* into two groups according to geographical origin of the virus, from the Old or the New World. *Tomato yellow leaf curl virus* is classified among the Old World geminiviruses together with *Tomato leaf curl virus* (ToLCV), *African cassava mosaic virus* (ACMV), *Indian cassava mosaic virus* (ICMV), and *Mungbean yellow mosaic virus* (MYMV). However, unlike many other begomoviruses, TYLCV has a monopartite genome. This made it necessary to propose a new taxonomic structure for the family *Geminiviridae* (Maxwell *et al.*, 1994; Padidam *et al.*, 1995a).

2.9.4 Classification based on evolution

In an evolutionary context, the greater diversity found among the leafhopper-transmitted geminiviruses, suggests that monopartite geminiviruses may be ancestral to their bipartite counterparts. The transition would have then involved three steps (Kikuno *et al.*, 1984) (Plate 3). First, leafhopper-transmitted geminiviruses with a single genomic component affecting monocotyledonous hosts would be the simplest and ancestral forms. Second, this group would have acquired the capacity for infecting dicotyledonous hosts. Finally, bipartite geminiviruses with two genomic components would have evolved by genomic duplication and later, divergence, with partitioning of replication and movement functions in both genomic components. Most of the TYLCV isolates have single genomic components. Even the second component found in the Thailand isolate is not essential for infection. This places TYLCV in an intermediate evolutionary step (Kikuno *et al.*, 1984; Lazarowitz *et al.*, 1992).

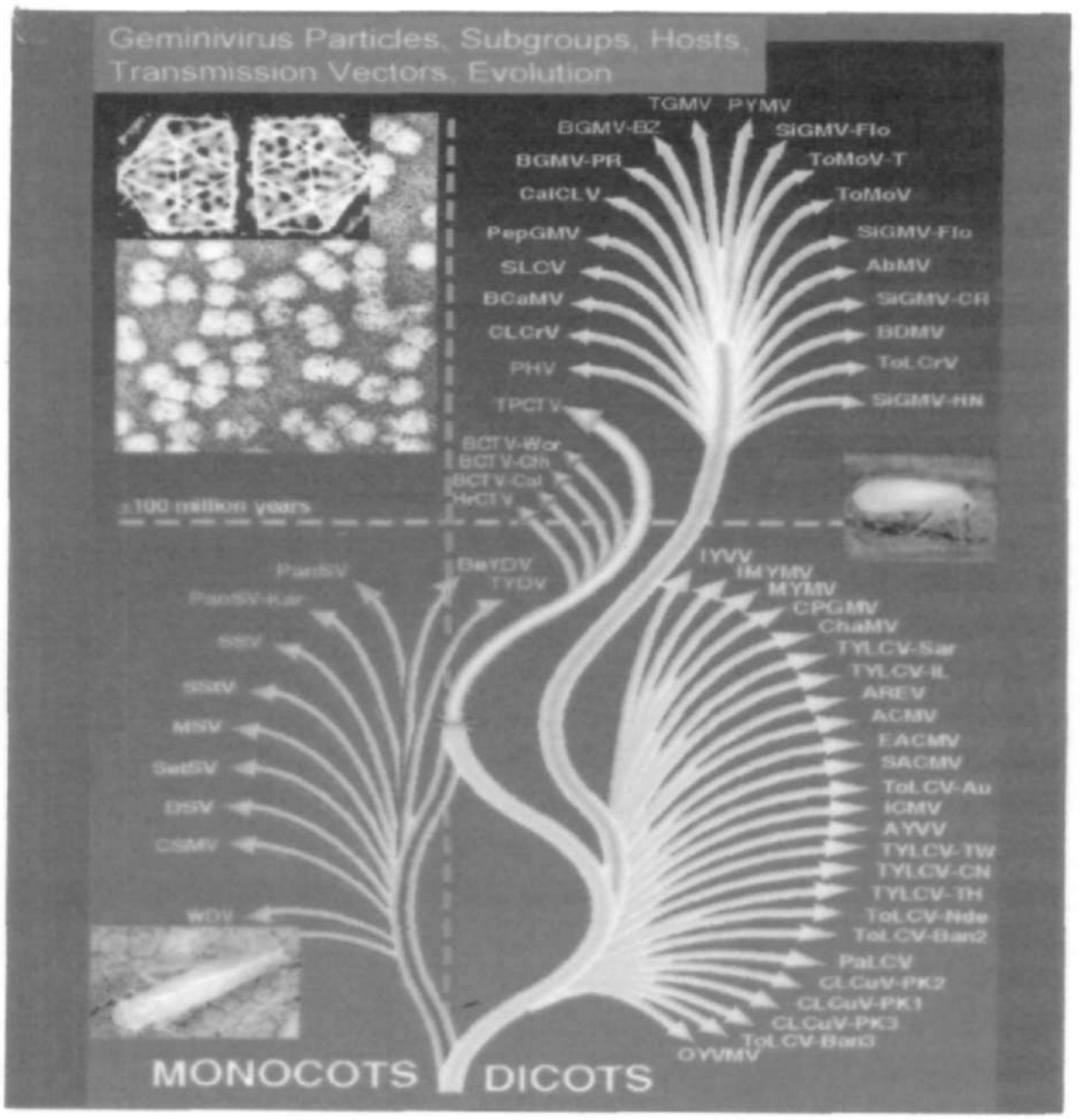


Plate 3: Geminivirus evolution depicting virus names, their vectors and hosts. Courtesy of Dr S. C. Zhang, University of Stuttgart, Germany.

2.10 Pathotypes

The bipartite geminivirus group is the most distant, including *Tomato yellow leaf curl Thailand virus* (TYLCTHV), which seems closest to ToLCV. Among TYLCV isolates, it maintains the highest similarity to the isolate from Israel, in spite of both isolates showing great sequence divergence (25%) (Rochester *et al.*, 1994). The DNA B of TYLCTHV does not show any particular homology with DNA B of the geminiviruses studied so far, unlike the New World geminiviruses that exhibit a greater homology in their DNA B sequences (Rochester *et al.*, 1994). TYLCTHV is serologically related to *Tobacco leaf curl Japan virus* (TbLCJV), which has been found to induce TYLCV-like symptoms in tomatoes in Japan (Chiemsombat *et al.*, 1991).

Studies carried out using restriction fragment length polymorphisms (RFLP) revealed different patterns for Tomato yellow leaf curl Sardinia virus-Sicily (TYLCSV-Si) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) on one hand, and African and Asian isolates on the other. *Tomato yellow leaf curl Nigeria virus* (TYLCNV), *Tomato leaf curl Senegal virus* (ToLCSV), and *Tomato leaf curl Bangalore virus* (ToLCBV) gave different patterns from one another and from those of European viruses. Similar results were obtained when the coat protein epitope profiles were compared (Muniyappa *et al.*, 1991b; Macintosh *et al.*, 1992).

Tomato yellow leaf curl Yemen virus (TYLCYV), shares the highest sequence homology with Tomato yellow leaf curl Sardinia virus-Spain [1] (TYLCSV-ES[1]), but the study

of its biology brings it closer to a TYLCV isolate from Jordan (Bedford *et al.*, 1994). Finally, American isolates from Mexico, Dominican Republic, Jamaica and North America are closely related to those of Cyprus and Israel, pointing to the Near East as the origin of the initial infection inoculum (Grit *et al.*, 1994; McGlashan *et al.*, 1994; Nakhla *et al.*, 1994).

The Tomato yellow leaf curl virus-[Egypt] (TYLCV-[EG]) is generally believed to be the most prevalent in Tanzania. However, based on nucleic acid hybridization tests of tomato samples, only 3 % of 420 samples from Tanzania and Uganda hybridized to TYLCV-[EG]-specific probe; 4 % reacted with the broadspectrum TYLCV-[EG] probe and none reacted with the Indian, Taiwan or the broadspectrum Thailand probe (AVRDC, 1994). The probe of TYLCV-[EG] hybridized strongly with samples from Arusha, Coast and Morogoro regions. Chiang *et al.* (1997) reported a new virus, *Tomato leaf curl Tanzania virus*, (ToLCTZV) (Fauquet *et al.*, 2000) from Tanzania that is different from the Old World geminiviruses. Recent molecular studies (Kashina *et al.*, 2002a) revealed that the virus causing yellow leaf curl symptoms on tomatoes in Tanzania is most similar to the African tomato leaf curl virus, but also shares similarity with TYLCSV in the replication-associated protein (AC1).

2.11 Disease control strategies

Current TYLCV control practices are mainly focused on the prevention of infection and genetic resistance. However, for any strategy to be effective, it must recognize the

relative importance of three key elements crucial for infection to occur, namely: the virus, vector and host plant, and their interactions (Pico *et al.*, 1996; Kashina *et al.*, 2003b). Measures that have been adopted to check disease development and spread are discussed below.

2.11.1 Reduction of potential sources of inoculum

2.11.1.1 Use of virus-free planting materials

The early appearance of the disease with subsequent high yield loss is aggravated if infected seedlings are transplanted. These young infected plants normally act as primary sources for secondary spread of the virus to healthy plants. The protection of tomato seedlings in nurseries using chemical or physical means against the vector reduces yield losses on the field (Ioannou, 1985b, 1987).

2.11.1.2 Elimination of virus reservoirs

This strategy emphasises the removal and destruction of tomato plants in which the virus has overwintered, weeds and other solanaceous crops that are hosts of the virus. However, the knowledge of both the wild and domesticated hosts of the virus is essential for a purposeful and result-oriented implementation of this strategy. The age-old practice of farm sanitation, which involves the clearing of weeds, is a core component of this strategy. It has been possible to limit the migration of viruliferous whiteflies using this strategy (Ioannou, 1987; Cohen *et al.*, 1988).

2.11.2 Control of virus transmission

The underlying principle of this practice is to reduce the spread of the virus by keeping the vector population in check, since the two are positively correlated (Ioannou and Iordanou, 1985; Cohen *et al.*, 1988).

2.11.2.1 Chemical control

As with other persistent viruses, the spread of TYLCV can be partially controlled by the use of insecticides against the vector (Cohen *et al.*, 1974; Berlinger and Dahan, 1989). Complete control is difficult to achieve because of the low sensitivity of *B. tabaci* to insecticides and its ability to develop resistance against them. Al-Musa (1986) observed other difficulties such as the simultaneous presence of different developmental stages of the whitefly vector on tomato plants and migration of vector populations among neighbouring fields. Some level of success in reducing the vector population can be achieved if insecticide application is targeted to coincide with the early stages of insect development. However, this failed to reduce the incidence of TYLCV as the number of insects required for field epidemics is often very low, and the transmission efficiency is very high (Al-Musa, 1986). Sometimes, a small increase in tomato yield is achieved, but it does not usually justify the intensive treatment programmes economically or ecologically. For a more effective chemical control, Kisha (1981) and Sharaf (1986) recommended a combination of the chemical approach with other methods. Plant extracts are well known as control agents (Hesler and Plapp, 1986), especially, against stored-product pests (Hill and Schoonhoven, 1981), foliar pests such as mites and scale

insects (Lawson and Weires, 1991), aphids and whiteflies (Fenigstein *et al.*, 2001). Vegetable oils, like other spray oils have the desirable properties of less risk of arthropod resistance and cross-resistance to other pesticides (Hesler and Plapp, 1986). They are also environmentally friendly, and pose little danger to the health of the user or consumer of agricultural products (Cornish *et al.*, 1993).

2.11.2.2 Biological control

This is an alternative to chemical control; it involves the use of natural enemies of the vector, which are usually grouped according to their mode of action as predators or parasites. This strategy has been successfully used in the Mediterranean regions with *Encarsa formosa, E. lutea* and *Eretmocerus mundus* to control the vector population and virus spread. However, to achieve better control, knowledge of the delicate interactions between *B. tabaci* and its natural enemies is necessary since indiscriminate use of control agents can offset this delicate balance to bring about undesirable consequences (Henneberry and Bellows, 1995).

2.11.2.3 Physical methods

These involve the use of fine-meshed screens to prevent insect to plant contact in seedbeds, low-tunnel and greenhouse tomato crops. The effectiveness of the screen depends on their mesh. Fine mesh nets are associated with overheating, excessive shading and poor ventilation. Consequently, coarse meshes are sometimes recommended, but this means less protection (Ioannou, 1987). In Italy and Israel, up to

90 % reductions in infection incidence was achieved when two types of bioscreens (antivirus and antiaphid) were used to cover doors and windows of greenhouses (Nucifora, 1992). Growing of tomato crops under plastic and muslin tunnels, which allow ventilation, has proven effective for TYLCV control (Al-Musa, 1986; Ioannou, 1987). Yellow and green sticky traps are also effective when complemented with chemical sprays (Al-Musa, 1986).

2.11.2.4 Planting date

The manipulation of tomato sowing date to avoid periods of peak vector populations, which often occur after periods of high temperature and low relative humidity, can be effective in avoiding the disease. This has been adopted with recorded successes in greenhouse and low-tunnel tomato cultures in Jordan and Cyprus (Makkouk and Laterrot, 1983; Ioannou and Iordanou, 1985).

2.11.2.5 Mulching

The practice of mulching transplanted tomato seedlings using fresh and/or dried straw is commonplace among tomato growers in Tanzania (Kashina *et al*., 2003b). This has the advantage of delayed spread of TYLCTZV disease for at least two weeks by discouraging vector landing on the crops, if used on seedbeds. Other mulching materials that have been used elsewhere to achieve the same results of either discouraging vector landing and feeding or killing the vector by reflective heating, are yellow polyethylene

materials and ultraviolet-absorbing films (Makkouk and Laterrot, 1983; Antignus *et al.*, 1995).

2.11.2.6 Intercropping

Tomato growers in Tanzania intercrop tomatoes with other crops such as, cucumbers, peppers and eggplant (Kashina *et al.*, 2003b). This strategy is effective in diverting the whiteflies from the tomato crop to other more preferred hosts, especially if planted earlier than tomatoes, thus delaying and reducing tomato infection by TYLCV, and the eventual fruit yield losses that would have been otherwise incurred (Al-Musa, 1982, 1986; El-Serwiy *et al*, 1987).

2.11.3 Breeding for resistance

The most promising approach for the control of TYLCV infection is the use of tolerant or resistant tomato varieties. However, breeding for viral disease resistance is often limited by the lack of reliable and efficient inoculation and screening procedures, which prevent an adequate selection of sources of resistance, lack (until recently) of consistency in viral nomenclature, and relative frequent recombination between species of geminiviruses (Padidam *et al.*, 1999; Fauquet *et al.*, 2000; Lapidot and Friedmann, 2002). Researchers of the Asian Vegetable Research Development Centre (AVRDC), Arusha, Tanzania, working in close collaboration with national scientists continue to search for tomato cultivars that are resistant/tolerant to the TYLCTZV. While none of

the tomato cultivars currently being cultivated in Tanzania is immune to the virus, Tengeru 97, a tolerant cultivar, possesses an appreciable level of resistance to the virus.

2.11.3.1 Inoculation procedures

The most commonly used inoculation method in tomato breeding programmes is the whitefly-mediated inoculation of tomato plants in insect-proof cages with viruliferous whiteflies previously allowed to feed on TYLCV-infected plants (Pico *et al.*, 1996). Less frequently, transmission by grafting of infected cuttings onto healthy plants, have also been used. However, the main limitation of these methods is how to ensure a 100 % success in transmission. This often leads to contradictory results, attributing different resistance levels to the same genetic source (Ioannou, 1985a; Pilowsky and Cohen, 1990).

Mechanical transmission of TYLCV to tomato has only been reported in artificial conditions from plants of *Datura stramonium*, *Nicotiana glutinosa*, and *Lycopersicon pennellii* (Makkouk *et al.*, 1979), and in few cases, from tomato to tomato (Abdel-Salam, 1990). Consequently, this type of transmission is not used in inoculation trials.

More effective inoculation is achieved when molecular procedures are used. Inoculation techniques have been developed using agroinoculation, which involves the infection of the plant with a strain of *Agrobacterium tumefaciens*, transformed using dimeric copies of an infectious TYLCV-DNA clone (Kheyr-Pour *et al.*, 1994). The viral genome is

capable of replicating to start an infection process. Infection using *Agrobacterium* can be performed mechanically or by vector transmission since *B. tabaci* is capable of acquiring the transformed bacteria (Zeidan and Czosnek, 1991). The technique can be applied on the whole plant or leaf discs, thereby accelerating the selection process because the study of viral accumulation, being positively correlated with symptom severity, permits discrimination between susceptible genotypes, which support viral replication and spread, and resistant genotypes that do not support viral replication (Rom *et al.*, 1993; Kheyr-Pour *et al.*, 1994). The main disadvantage of this method lies in its inability to identify those tolerant genotypes that support viral replication and its cell-to-cell movement, but prevent long distance movement as well as reduce and delay infection development. Natural resistance has been overcome in certain accessions after inoculation using this technique (Pico *et al.*, 1996). Consequently, its use in breeding programmes has been questioned (Czosnek *et al.*, 1993; Rom *et al.*, 1993; Kheyr-Pour *et al.*, 1994).

Since liposomes protect viral DNA against plant nucleases, liposome-mediated cell transformation provides another inoculation tool to study TYLCV replication in tomato plant cells (Rosenberg *et al.*, 1990).

2.11.3.2 Exploitation of natural resistance

Attempts made earlier to find resistance within *Lycopersicon esculentum* by testing tomato lines and cultivars in affected regions yielded only moderately susceptible or

slightly tolerant genotypes, with none of them showing satisfactory level of resistance or tolerance (Nitzany, 1975; Makkouk *et al.*, 1979; Ioannou, 1985b). Consequently, breeding programmes for resistance to TYLCV are mainly based on introgressing resistance or tolerance found in wild tomato relatives, which have been proven to be important reservoirs of resistance to many tomato pathogens. Breeding for TYLCV resistance in tomato was initiated in Israel using accession line LA 121 of the wild species *L. pimpinellifolium* as the source of resistance (Pilowsky and Cohen, 1974). The tolerance found in this species did not result in the release of any useful tomato variety, hence, a new breeding programme was initiated in 1977 based on the tolerance found in the accession PI-126935 of *L. peruvianum* (Pilowsky *et al.*, 1989). This resulted in the development of the hybrid TY-20, which was first used commercially in 1988. The hybrid TY-20 exhibits delay in symptom development and viral DNA accumulation, thus producing good yield even if infected (Pilowsky *et al.*, 1989; Pilowsky and Cohen, 1990). In addition to TY-20, two other series of hybrids have been derived from the tolerance found in *L. peruvianum* (Pilowsky and Cohen, 1995). Tolerance to TYLCV has also been found in different accessions of other wild species such as *L. cheesmanii* ssp. Minor and *L. hirsutum* (Mazyad *et al.*, 1982; Ioannou, 1985b; Moustafa and Nakhla, 1990). In some of the *L. peruvianum* and *L. hirsutum* accessions, TYLCV tolerance operates indirectly, preventing vector feeding by means of physical barriers, such as leaf hairs, or by secretion and presence in the sap of anti-feedants (Muniyappa *et al.*, 1991a, Channarayappa *et al.*, 1992).

The great variability found among TYLCV isolates has resulted in discrepancies in the response of the same resistance source in different affected areas. This led to the initiation in 1985, of an EEC-supported multi-site breeding programme to identify resistant sources by testing them under natural conditions in different affected areas of Cyprus, Egypt, Israel, Lebanon, Mali, Senegal, and Sudan. As a result, composite populations with tolerance derived from one or more wild relatives were obtained for use in local breeding programmes, and tolerant tomato cultivars with reduced sensitivity to TYLCV have been selected (Laterrot, 1993).

Most recent studies point to the wild species *L. chilense* as the best source of resistance for the improvement of resistance to TYLCV and other geminiviruses in tomato (Scott and Schuster, 1991; Lapidot *et al.*, 1997). The main problem with using this species is the crossability of barriers with the cultivated tomato that prevent the production of interspecific hybrids. However, these barriers can be overcome by using pollen mixture technique, genetic bridges, and embryo culture (Laterrot, 1983; Ayuso *et al.*, 1987). Several seed companies offer hybrids tolerant to TYLCV. However, their tolerance decreases with increase in disease incidence (Nucifora, 1992; Laterrot, 1993).

2.11.3.3 Genetic engineering

Three approaches are used to obtain protection against TYLCV infection by genetic engineering. These approaches include coat protein-mediated resistance by inoculation with mild TYLCV clone or by plant transformation with viral coat protein gene; use of

antisense or catalytic (ribozyme) RNA, and plant transformation with partial or defective viral sequences (Beachy *et al.*, 1990; Kunik *et al.*, 1994). Cross-protection using coat protein has been used against viral infection (Beachy *et al.*, 1990). In the case of TYLCV, transgenic plants have been produced expressing the coat protein gene, showing high positive correlation between disease remission and presence of the protein coat. The protection mechanism is probably based on the competition for membrane transporters, which import the coat protein into the cell nucleus. The over-expression of coat protein starts a competition for the limited number of membrane carriers, thereby decreasing the number of virions, which can enter the nucleus, and delaying symptom development (Kunik *et al.*, 1994). Based on the same mechanism, infections with mild mutants of TYLCV can efficiently cross-protect susceptible tomato varieties from the severe wild-type infection (Antignus and Cohen, 1994; Antignus *et al.*, 1994b). Cross-protection against TYLCV has also been achieved by inoculation with defective mutants in AC4 gene. This method is reported to offer protection even against TYLCV isolates with up to 25 % of sequence diversity, and mutants can be spread easily in the field by vector transmission (De Kouchkovsky *et al.*, 1993).

Antisense RNA and transformation with defective viral sequences have been used to control other geminivirus diseases (Bejarano *et al.*, 1994). Transgenic defective AC1 gene plants, with certain level of resistance to TYLCV have been obtained by inhibiting viral replication through the expression of mRNA AC1 antisense gene (Brunetti *et al.*, 1994; Noris *et al.*, 1994c).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey

Surveys were conducted in 2000/01 to determine the incidence of TYLCTZV in six regions of Tanzania mainland, and to collect isolates for biological and molecular characterization. The regions, which were selected based on tomato production history and TYLCTZV incidence (Nono-Womdim *et al.*, 1996), included Morogoro and Iringa (low incidence), Kilimanjaro (moderate incidence), and Coast, Arusha and Dodoma (high incidence).

Symptomatic samples were collected from three randomly selected farms of 2 - 3 month old tomato plants within each region and were either analyzed fresh or dried over calcium chloride before assay. Within each farm, the incidence and severity of TYLCTZV were assessed in five, 4 x 4 m^2 quadrants (one from each corner and at the centre of the farm). An average of 28 tomato plants were assessed along a diagonal within each quadrant. Farmers provided information on tomato variety, crop age, farming practices and cropping history.

3.2 Characterization of variability among isolates of TYLCTZV

3.2.1 Triple Antibody Sandwich-Enzyme-Linked Immunosorbent Assay Tests (TAS-ELISA)

Detection of TYLCTZV was done using commercial polyclonal and monoclonal antisera (Adgen Diagnostic Systems, Scotland). The TAS-ELISA was adopted as described by Pico *et al.* (1999). Polyclonal antibody (PAb) was diluted in carbonate buffer, pH 9.6 at 1:1000 µl (v/v) to coat for viruses and the coated plates were incubated at 37 °C for 4 h. After 4-h incubation and washing with phosphate buffered saline-Tween 20 (PBS-T), pH 7.2, 100 µl of each test sample ground in extraction buffer, pH 8.5 (1:10 w/v) was added and incubated overnight at 4 °C. Monoclonal antibodies, SCRI 17, SCRI 20, SCRI 23 and SCRI 33 were diluted in conjugate buffer at 1:1000 µl (v/v) and used as probe antibodies. The plates were incubated for 2 h at 37 °C, washed and goat anti-mouse alkaline phosphatase (GAM-PAL) conjugate diluted in conjugate buffer (1:3000 v/v) was added. The plates were incubated at 37 °C for 1 h, washed to remove unbound conjugate and the substrate (p-Nitrophenyl phosphate) 1:1 (mg/ml) dissolved in Diethanolamine buffer (pH 9.8), was added to the test wells. After incubation for 1 h, the plates were assessed visually and by the spectrophotometer (405 nm) reader. ELISA readings were rated as described by McGrath and Harrison, (1995) to compare reaction strengths. Samples with absorbance values twice those of the healthy control were rated to be positive.

3.2.2 Electron microscopy

Purified extracts (Muniyappa *et al.*, 1991b) of TYLCTZV DNA were embedded in carbon-coated copper grids and negatively stained with 2 % aqueous uranyl acetate (Dykstra, 1993). Caesium chloride-ethidium bromide purified DNA was spread using the on-drop technique (Coggins, 1987). Glutaraldehyde was added to a final concentration of 0.2 % and the sample was fixed for 10 min at 37 °C. With a supplement of ethidium bromide (100 µg/ml), the solution was pipetted onto paraffin as a drop of 50 µl. The drops were kept in the humid chamber for 2 h to spread the complex. The complexes were attached to a carbon-coated 300 mesh copper grid, washed for 30 s in water, stained for 30 s with uranyl acetate (1 µl of a 50 mM stock solution in 50 mM HCl was added to 999 µl of 90 % ethanol just before use), washed with 90 % ethanol and air-dried. Viral particles were examined with a ZEISS EM10 transmission electron microscope at an acceleration voltage of 60 kV.

3.2.3 Polymerase chain reaction (PCR)

3.2.3.1 Extraction of DNA by phenol-chloroform procedures

The DNA was extracted from tomato leaves infected with TYLCTZV using the phenol-chloroform method described by Sambrook *et al.* (1989). Leaf samples placed in 2 ml-microcentrifuge tubes were dipped in liquid nitrogen and ground to powder using a sterile glass rod. One ml of extraction buffer (1M Tris, 1M NaCl, 100 mM Na-EDTA, 1M DTT, 10 % SDS) and phenol-chloroform (9:1 v/v) mixed in equal volumes was

added to each tube. After shaking for 15 min and centrifuging for 5 min at 15 300 g, the aqueous phase was transferred to new microcentrifuge tubes, and the DNA extracted with an equal volume of phenol: chloroform: iso-amyl alcohol (25:24:1). The mixture was centrifuged at 15 300 g for 5 min and the aqueous phase was transferred into a new sterile tube for DNA precipitation overnight at –20 °C after adding a tenth volume of 3 M sodium acetate, pH 4.8 and 750 µl of absolute ethanol. The DNA was washed with 500 µl 70 % ethanol for 5 min after centrifugation (20 200 g for 30 min at 4 °C). The pellet was vacuum-dried for 10 min and re-suspended in 50 µl of TE. DNA amounts were quantified using 4, 6-diamidino-2-phenylindole (DAPI) (Tanious *et al.*, 1992) with the fluorescence normalized against CsCl gradient-purified total DNA of known concentration.

3.2.3.2 Caesium chloride-ethidium bromide gradients

The supercoiled viral DNA was purified from solutions of DNA in TE containing 0.8 mg/ml of ethidium bromide and adjusted to Caesium chloride (CsCl) density of 1.55 gml^{-1} (Sambrook *et al.*, 1989). Gradients were generated by centrifugation (Vti 65.1 rotor, Beckman; 50 000 rpm) for 16 h at 20 °C. Fractions were recovered from the bottom of the quick-seal tubes and the Ethidium bromide was removed by extraction with water-saturated 1-butanol. Carrier tRNA (50 µg/ml), 3 volumes of TE and 2 volumes of ethanol were added to precipitate the DNA. After centrifugation at 20 200 g for 30 min at 4 °C and washing with 70 % ethanol, the DNA was dissolved in TE.

3.2.3.3 The PCR procedure

The putative viral DNA isolated from the samples (section 3.2.3.1) was amplified by PCR. The PCR reaction medium contained 1 µl of sample DNA (7 ng/µl), 0.4 µl of dNTP mix (0.25 µM each), 4 µl of Q-Reagent, 0.4 µl of each primer (30 pmol/µl), 2.0 µl of 10x PCR buffer, 0.25 µl of *Taq* polymerase (1 U) and sterile water to a total volume of 20 µl. The primer pairs used were either PAC1v1978/PAV1c715 or OTYA2/OTYA6 (Table 2). DNA was denatured in one cycle of 95 °C for 5 min, and amplified for 30 cycles (Thermobaid TouchDown Cycler) of 95 °C, 1 min for melting, 55 °C, 1 min for annealing and 72 °C, 2 min for DNA extension. This was followed by a final cycle of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 10 min for melting, annealing and DNA extension, respectively. The reaction products were analyzed by electrophoresis in 1 % agarose gels in Tris-borate-EDTA buffer, pH 8.0.

For restriction fragment length polymorphisms (RFLP) analysis, 5 µl of amplified viral DNA was digested with *Alu* I restriction endonuclease for 2 h at 37 °C. The restriction enzyme was inactivated at 65 °C for 10 min and the digested DNA electrophoresed in 8 % polyacrylamide gel in 1X TBE at 45 v, 200 mA. The gel was stained in ethidium bromide (0.5 µg/ml) for 20 min, washed with water and photographed under UV light.

Table 2: Primer sequences, percentage GC content and sizes.

Primer	Sequence	% GC	Size (mers)
PAC1v1978*	5'-GCATCTGCAGCCCCACATYGTCTTYCCNGT-3'	57.1	21
PAV1c715*	5'-GATTTCTGCAGTTDATRTTYTCRTCCATCCA-3'	28.6	21
OTYA2	5'-GTGAATCCCCAGTTCCTTCCT-3'	52.4	21
OTYA6	5'-CTACATGAGAATGGGGAACC-3'	50.0	20

*The underlined is a *Pst*I restriction site engineered into the primer sequences; primer size excludes the restriction site and degenerate positions (D, N, R, Y).

3.2.4 Hybridization

3.2.4.1 DNA probes

(i) Elution of probe DNA from clone

One microlitre of clone pTYA58 (1 ng/µl) was digested with 1 µl of *Bam* HI (1 U) in 16 µl of sterile water and 2 µl of 10X buffer #3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9) for 2 h at 37 °C to release the viral DNA. After digestion, the enzyme was inactivated at 80 °C for 20 min, and the digested product was electrophoresed (3 µl) in 1 % agarose gel. The *Bam* HI-digested fragment of viral DNA was cut from the gel under long wavelength (360 nm) UV light. The gel piece containing the linear viral DNA was placed in a microcentrifuge tube and dissolved in a buffered solution (capture buffer) containing acetate and chaotrope (1:1 v/w), at 60 °C for 5 - 10 min on a Thermomixer (Eppendorf). The solution was transferred to a GFX

column of gel band purification kit (Amersham Pharmacia) for 2 min to fix the DNA on the negatively charged glass fibre matrix. After centrifugation at 12 000 g for 30 s, the matrix-bound DNA was washed in 500 µl of an ethanolic buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, 80 % ethanol) for 2 min to remove salts and other contaminants, and to dry the matrix prior to elution. The purified DNA was eluted in 50 µl of low ionic strength buffer (10 mM Tris-HCl pH 8.0; 1mM EDTA) at 20 200 g for 2 min, after 5 min dissolution of the DNA.

(ii) Concentration of eluted DNA

The eluted DNA was further concentrated using Microcon 100 tubes, which can only allow the passage of nucleotides of about 300 bp while, retaining larger fragments. After centrifugation at 500 g for 12 min, 25 µl of double distilled sterile water was added to dissolve the DNA. The concentrated DNA was obtained after 2 min of centrifugation at 12 000 g with the Microcon 100 tube inverted into a 1.5 ml microcentrifuge tube.

(iii) Labelling of DNA probe

The concentrated viral DNA was labelled with DIG-11-dUTP using a DIG-High Prime labelling kit (Bochringer, Mannheim) according to manufacturer's instructions. Twenty microlitres of the viral DNA (7 ng/µl) were added to a sterile 1.5 ml microcentrifuge tube. The DNA was denatured at 95 °C for 10 min and chilled quickly in an ice/ethanol bath. Four microlitres of DIG-High Prime mix (1 U/µl Klenow enzyme, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.65 mM dTTP, 0.35 mM DIG-11-dUTP) was added to the

denatured DNA and incubated at 37 °C overnight. The enzyme activity was stopped by heating for 10 min at 65 °C, and the following were added: 2.5 µl of 4 M LiCl, 0.5 µl herring sperm DNA and 75 µl of pre-chilled (-20 °C) ethanol. After thorough mixing and incubation for 2 h at –20 °C, the mixture was centrifuged at 12000 g for 15 min at 4 °C. The pellet was washed in 50 µl of 70 % ethanol at room temperature for 5 min, centrifuged as above, vacuum-dried and dissolved in 50 µl of TE buffer.

3.2.4.2 Squash blot

Leaf samples of tomato and weed plants showing symptoms of TYLCTZV were collected (see sections 3.1 and 3.3) and squash blotted on pre-marked nylon membranes (Hybond N^+, Amersham Pharmacia) following procedures described by Zilberstein *et al.* (1989). Sap from longitudinal sections of either stem portions or rolled leaves were blotted on the membranes after making fresh razor cuts to expose the tissues. The membranes were air-dried and hybridized as described in 3.2.4.4.

3.2.4.3 Dot blot

Tomato and weed leaf samples were macerated and incubated in 0.5 N NaOH for 30 min at 50 °C (Czosnek *et al.*, 1988a; Zilberstein *et al.*, 1989). After neutralization in 1 N HCl and centrifugation at 20200 g for 5 min, 5 µl of the supernatant were dot-blotted on marked squares on nylon membrane (Hybond N^+, Amersham Pharmacia). The membranes were air-dried to fix the viral DNA and hybridized as described under 3.2.4.4.

3.2.4.4 Southern hybridization

Genomic DNA was extracted from tomato leaf samples as described earlier (3.2.3.1), and electrophoresed (5 µl) for 2 h in 1 % agarose gel in 0.5X Tris-borate-EDTA (TBE) buffer at 120 v, 200 mA. After electrophoresis, the gel was washed for 30 min in transfer solution (0.6 M NaCl; 0.4 M NaOH) to denature the DNA. The denatured DNA was transferred onto nylon membranes (Hybond N^+) in transfer solution overnight (Southern, 1975). The blots were neutralized for 15 min in neutralization solution (1.5 M NaCl; 0.5 M Tris, HCl (ca. 35 ml, 32 %), pH 7.5) incompletely dried afterwards between two pieces of Whatmann paper and the DNA was fixed in a UV cross-linker (700 heat units for 1 min) (Amersham Pharmacia). Membranes of the squash and dot blots were treated similarly. The membranes were rolled into hybridization tubes and pre-hybridized for 3 h at 65 °C in 20 ml of pre-hybridization solution {0.2 g Glycine, 3.2 ml 20X SSPE, 0.2 ml sonicated herring sperm DNA (10 mg/ml), 1 ml 100X Denhardt, 10 ml de-ionized formamide, 0.6 ml 10% SDS, 3.2 ml sterile water} using a hybridization oven (Biometra AT line, Germany). For hybridization, the pre-hybridization solution was refreshed with the inclusion of 0.1 g/ml of dextran sulphate and 0.1 µl/ml of the Dig-labelled probe, exclusion of glycine. Hybridization was allowed overnight at 65 °C. Post-hybridization washes included one brief washing in 2X SSPE, four 15-min high stringency washes in 0.2X SSPE containing 0.3% SDS at 65 °C, 5 min-washing in wash buffer {0.3% Tween 20 prepared in buffer 1(0.1 M maleic acid, 0.15 M NaCl, pH 7.5)} at room temperature and 30-min wash in buffer 2 {blocking reagent diluted in buffer 1(1:10 v/v)}. The membrane was incubated for 30 min in antibody-conjugate solution (anti-Dig alkaline

phosphatase in buffer 2, 1:10 000 v/v), and washed twice (15 min each) in washing buffer and once (5 min) in buffer 3 (100 mM NaCl; 50 mM $MgCl_2$, pH 9.5). After further incubation in substrate solution (CDP Star diluted 1: 100 in buffer 3, w/v) (Tropix) for 15 min, the membrane was incompletely dried between two pieces of Whatmann paper. The semi-dry membrane was placed in-between pieces of transparent plastic sheets and sealed (Electric Petra Vacuplus, Germany). The sealed membrane was incubated for 15 min at 37 °C and exposed for 25 min to X-ray film (Fujifilm, Tokyo). The film was developed (Curix 60, Agfa, Germany) to view the viral signals. The relative intensity of hybridization signals was rated as described by Salati *et al.* (2002): 0 = no signal, 1 = weak signal, 2 = moderate signal, 3 = strong signal, and 4 = very strong signal.

3.2.5 Cloning of the amplified DNA

3.2.5.1 Ligation of viral DNA to plasmid vector

The amplified viral DNA was isolated from the PCR product by phenol-chloroform procedures described in section 3.5.4.1. After the isolation and ethanol precipitation of the PCR product, the viral DNA was washed in 70 % ethanol and digested (10 µl) with *Pst*I (1µl) in a solution containing 2 µl of buffer #2 (50mM NaCl, 10mM Tris-HCl, 10mM $MgCl_2$, 1mM DTT, pH 7.9) and 7 µl of sterile water at 37 °C for 2 h. The enzyme was inactivated at 80 °C for 20 min and the product was run in 1 % agarose gel. Viral DNA was eluted from the gel using a GFX column. The eluted viral DNA was ligated overnight at 15 °C to *Pst*I-digested plasmid vector, pBluescript II KS^+

(Stratagene) in a ligation mixture composed of 3 µl viral DNA, 1 µl vector DNA, 1 µl T4 DNA ligase, 4 µl 5X ligase buffer (50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 25 µg/ml bovine serum albumin) and sterile water to a total volume of 20 µl.

3.2.5.2 Preparation of competent cells

(i) Frozen competent *Eschirichia coli* strain JM 83 cells

A sterile toothpick was dipped into *E. coli* JM 83 (Promega) cells and dropped into a conical flask containing 100 ml of Luria Bertani (LB) medium without antibiotics. This was incubated overnight with shaking at 37 °C for bacterial cells to grow. To prepare electro-competent cells, 250 ml of LB medium (incubated overnight at 37 °C) was inoculated with 1 ml of the overnight JM 83 culture in an Ehrlenmeyer's flask. This was incubated with shaking at 37 °C for 1.5 - 2 h to attain an OD value of 0.5 – 0.6 at a wavelength of 600 nm. The flask was immediately transferred to an ice bath and the content was shaken three times at an interval of 5 min. Two hundred millilitres of the culture was centrifuged for 20 min at 5000 g and 2 °C (Sorvall RC 56). After centrifugation, the tubes containing the cells were immediately placed back on ice. The supernatant was carefully discarded and the pellet was dissolved in 150 ml of ice-cold autoclaved double distilled water and centrifuged as above, but for 10 min. The supernatant was discarded and the pellet was dissolved in 45 ml of cold 10 % glycerine and centrifuged for 10 min as before. The pellet was dissolved quickly in 2 ml of cold 10 % glycerine, and 50 µl was pipetted quickly into 1.5 ml microcentrifuge tubes, which

had been chilled overnight at –80 °C. The tubes were transferred quickly into a paper box containing liquid nitrogen, and stored at –80 °C.

(ii) Fresh competent *Eschirichia coli* strain JM 83 cells prepared using $CaCl_2$

One hundred millilitres of antibiotic-free LB medium was inoculated with 1 ml of an overnight culture of JM 83 bacterial cells, and incubated with shaking at 37 °C for 2 - 3 h. Fifty millilitres of this culture was centrifuged at 3000 g and 4 °C for 5 min, and the pellet was dissolved in 5 ml of ice-cold 0.1 M $CaCl_2$. The suspension was kept at 4 °C for transformation after 2 days.

3.2.5.3 Transformation

(i) Electrotransformation

The ligated plasmid was transformed into electro-competent *E. coli* strain JM 83 cells. Electro-competent cells were transformed by high voltage electroporation as described in the users' manual (Life Technologies, Catalogue Series 1612). Two and a half microlitres of ligated DNA was pipetted into sterile, ice-cold curvette, and mixed thoroughly by gentle pipetting with 100 µl of frozen JM 83 cells that had been melted over ice. The outside of the curvette was wipe-dried with kimwipes to prevent short-circuiting. The ligated DNA was electrically transformed into the JM 83 cells at a voltage of 2.5 kV and capacitance of 25 µF (Gene Pulser II, Biorad Laboratories, USA). Under a sterile chamber, 1 ml of SOC medium (see Appendix 1) was pipetted into the transformed DNA-cell mixture. After thorough mixing, 1 ml of the mixture was pipetted

into a sterile 1.5 ml microcentrifuge tube and incubated with shaking at 37 °C for 1 h. As a control, 2.5 µl of supercoiled DNA was transformed. After the incubation, the mixture was centrifuged at 20 200 g for 30 s. The supernatant was poured off leaving just enough to mix the pellet. The vortex-mixed pellet was used to plate the ampicillin-treated LB agar medium in petri dishes to which, 10 µl of isopropyl-β-D-thiogalactoside (IPTG) and 40 µl of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-GAL) had been added to produce a blue-white background for insert selection. The plated dishes were incubated overnight at 37 ° C. Single bacterial colonies with recombinant plasmids (white) were selected from the blue-white background for sub-culturing and further characterization following procedures described by Sambrook *et al.* (1989).

(ii) $CaCl_2$-mediated transformation

Three hundred microlitres of chemically prepared competent JM 83 cells were transferred into 1.5 ml microcentrifuge tubes containing 10 µl of ligated DNA. The mixture was incubated on ice for 1 h, transferred to a water bath set at 42 °C for 2 min and placed back on ice for 10 min. One millilitre of SOC medium was added and the mixture was incubated with shaking for 1 h at 37 °C. After centrifugation at room temperature for 30 s at 20 200 g, the supernatant was discarded and pellet was plated on IPTG- and X-GAL-treated LB agar. The plates were incubated overnight at 37 °C. Single positive (white) colonies were picked for sub-culturing.

3.2.5.4 Purification of plasmid DNA

Colonies containing the inserts were subcultured on ampicillin-treated LB agar media and purified as described by Sambrook *et al.* (1989). A colony from the sub-culture containing the insert was picked with a sterile toothpick, dropped into a conical flask containing ampicillin–treated LB liquid medium and incubated at 37 °C with shaking overnight at 250 rpm (New Brunswick Scientific Company, U.S.A). One hundred and fifty microlitres of the suspension were transferred to a sterile 2 ml microcentrifuge tube and centrifuged for 5 min at 20 800 g. The supernatant was discarded and the whole media suspension removed after centrifugation for 30 s. The pellet was dissolved in 100 µl of MIX-I (20 % Glucose, 100 mM Na-EDTA (pH7.5), 1 M Tris-HCl (pH 7.5), 2-10 mg Lysozyme, sterile water) and incubated on ice for 5 min. The suspension was mixed by 5 - 6 quick inversions of the tube after adding 200 µl of MIX II (2 M NaOH, 10 % SDS, sterile water), and incubated on ice for 5 min. One hundred and fifty microlitres of 3 M Sodium acetate (NaAC), pH 4.8 was added and vortexed gently with the tubes in an inverted position. After a 5-min incubation on ice, the mixture was centrifuged at room temperature for 5 min at 20 200 g. The supernatant was transferred into sterile microcentrifuge tube and 200 µl of phenol-chloroform (1:1) was added and shaken for 5 min. After centrifugation as described earlier, the supernatant was transferred into new sterile microcentrifuge tubes and precipitated with one tenth volume of 3 M NaAc and two volumes of absolute ethanol for 1 h on ice. The supernatant was discarded after centrifugation for 30 min at 20 200 g and the pellet was washed with 500 µl of 70 % ethanol for 5 min at room temperature. After a 10-min centrifugation at 20 200 g, the

pellet was vacuum-dried for 10 min and re-suspended in 30 µl of sterile water. Two microlitres of tRNAse were added.

3.2.6 DNA sequencing and analysis

Viral DNA purified from the positive clones was sequenced using the Li-Cor DNA Automatic Sequencer (MWG Biotech, Germany). Two microlilitres each of nucleotide A, C, G, and T were pipetted into two sets of four PCR tubes containing 6 µl of a template mixture {1 µl DMSO, 1 µl of either forward (5'-AAG GTT TTC CCA GTC ACG ACG TT-3') or reverse (5'-GAG CGG ATA ACA ATT TCA CAC AGG-3') primer, 7 µl of purified DNA and 16 µl of sterile water}. The contents of the tubes were spinned briefly for 30 s and run for 31 cycles using a Thermobaid TouchDown Cycler. Cycling conditions for the set containing the forward primer were, 1 cycle of 92 °C, 2 min for denaturation, 58 °C, 15 s for annealing, 70 °C, 30 s for synthesis, and 30 cycles of 92 °C, 15 s, 58 °C, 15 s, and 70 °C, 30 s for denaturation, annealing and synthesis, respectively. For the set of tubes containing the reverse primer, cycling conditions were one cycle of 92 °C, 2 min for denaturation, 60 °C, 15 s for annealing, 70 °C, 30 s for synthesis, and 30 cycles of 92 °C, 15 s, 60 °C, 15 s, and 70 °C, 30 s for denaturation, annealing and synthesis, respectively. After the amplification, 6 µl of acrylamide loading buffer were added and spinned for 30 s. The DNA was again denatured for 3 min at 95 °C, and loaded (1 µl) in the wells of the acrylamide gel assembled in the Sequencer. Sequencing was done overnight. The sequences generated were assembled using the programme manual of the semi-automatic sequencing roboter (Li-Cor 4000L; MWG).

The BLAST programme (*http://www.nbci.nlm.nih.gov/BLAST*) was used to search the Genebank database for viruses with sequences similar to TYLCTZV.

3.2.7 Phylogeny

The CLUSTAL function of the Vector NTI.5 software was used to establish the phylogenetic relationships of TYLCTZV with other geminiviruses. The geminiviruses used for phylogenetic analysis are shown in Table 3.

3.3 Alternative hosts of TYLCTZV

The plant species that were used for the determination of the host range of TYLCTZV, included plant species that had been previously used for the identification of whitefly-transmitted diseases of tomatoes (Cohen and Nitzany, 1966; Makkouk, 1978; Al-Musa, 1982; Ioannou, 1985b). Seeds of the test plants were sown in 30 cm-diametre plastic pots, thinned to five plants per pot for host range and back-indexing inoculations, respectively. Inoculation was done using adult whiteflies. The whiteflies were allowed to acquire the virus for 48 h on tomato plants infected with each TYLCTZV isolate. Thereafter, 15 viruliferous whiteflies were transferred to each test plant for inoculation feeding periods of 24 h. For back-index transmission from inoculated test plants, the tomato cv. Moneymaker was used as an indicator host. Symptom assessment was done 2 weeks after inoculation.

Table 3: List of geminiviruses aligned for comparison of phylogenetic relationship with *Tomato yellow leaf curl Tanzania virus*.

Virus name	Acronym	Accession number
Abutilon mosaic virus	AbMV	X15983
African cassava mosaic virus-[Ivory Coast]	ACMV-[IC]	AF259894
African cassava mosaic virus-[Kenya]	ACMV-[KE]	J02057
African cassava mosaic virus-[Nigeria]	ACMV-[NG]	X17095
Bean dwarf mosaic virus	BDMV	M88180
Bean golden mosaic virus	BGMV	M88686
Bean golden yellow mosaic virus-[Dominican Republic]	BGYMV-[DO]	L01635
Bean golden yellow mosaic virus-[Guatemala]	BGYMV-[GT]	M91604
Beet curly top virus-California	BCTV-Cal	M24597
Indian cassava mosaic virus	ICMV	Z24758
Cotton leaf curl Multan virus-Faisalabad1	CLCuMV-Fai1	X98995
Squash leaf curl virus	SLCV	M38182
Potato yellow mosaic virus-[Tomato]	PYMV-[Tom]	AF026553
Tomato leaf curl Laos virus	ToLCLV	AF195482
Tomato mottle virus-[Florida]	ToMoV-[FL]	L14460
Tomato golden mosaic virus-Common	TGMV-Com	M73794
Tomato leaf curl New Delhi virus-[Mild]	ToLCNdV-[Mld]	U15016
Tomato leaf curl virus	ToLCV	S53251
Tomato yellow leaf curl Sardinia virus	TYLCSV	X61153
Tomato yellow leaf curl virus	TYLCV	X15656

At least, four leaf samples from weed plants showing TYLCV-related symptoms (Cohen and Harpaz, 1964; Mansour and Al-Musa, 1992) were randomly collected within and outside the tomato farms during the tomato cropping and off seasons from each of the sites described in section 3.1. The samples were either squash-, dot-blotted or both on nylon membranes, and hybridized as described in 3.2.4.4. Weed species were identified as described by Ivens (1967), Akobundu and Agyakwa (1987) and Johnson (1999).

3.4 Transmission efficiency of TYLCTZV isolates

3.4.1 Establishment and maintenance of virus culture

The virus cultures were established and maintained in the screenhouse at Sokoine University of Agriculture (SUA), Tanzania. Young tomato shoots with typical yellow leaf curl symptoms suggestive of TYLCTZV infection, were collected from tomato farms in six regions of Tanzania (Dodoma, Arusha, Kilimanjaro, Iringa, Coast and Morogoro) (section 3.1), and grafted onto tomato seedlings, cv. Moneymaker. Subsequently, the virus cultures were maintained through whitefly-mediated inoculations. The regions were selected based on differences in TYLCTZV incidence reported by Nono-Womdim *et al.* (1996).

3.4.2 Establishment and maintenance of *Bemisia tabaci* colony

To establish the *B. tabaci* colony, the insects were reared in insect-proof cages following procedures described by the International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, modified from Rom *et al.* (1993). About 50 adult whiteflies

were collected from field-grown tomato plants in Morogoro using a battery-operated aspirator. These were transferred into vials and chilled in the refrigerator (4 °C) for 5 - 10 min. The chilled whiteflies were transferred to the leaves of young tomato seedlings maintained in insect-proof cages in the screenhouse, and confined in 5 cm-diametre clip cages on the underside of the upper leaves (Plate 4). After 2 - 3 h of confinement to allow for oviposition, the clip cages were removed and the eggs were allowed to develop on the plants. The plants were tested regularly by TAS-ELISA to ensure that they were virus-free. The whitefly vector was identified as biotype B by esterase banding patterns in polyacrylamide gel electrophoresis.

3.4.3 Graft transmission

Each of the six viral isolates was wedge-grafted onto four-week old tomato seedlings cv., Moneymaker (stock). Five seedlings were grafted per viral isolate, and repeated twice. A longitudinal cut was made way down the stock using a sterile razor blade. The stem portion of the scion infected with the isolate was shaped into a wedge and inserted into the position of the cut made in the stock in such a way that there was close contact between the exposed tissues of both the scion and stock. Using a budding tape, the union was held in place making sure that no spaces existed between the two. The grafted seedlings were maintained in the screenhouse (25 ± 5 °C, 72.4 % relative humidity) for 2 months to assess graft success, viral incidence and symptom severity for each isolate.

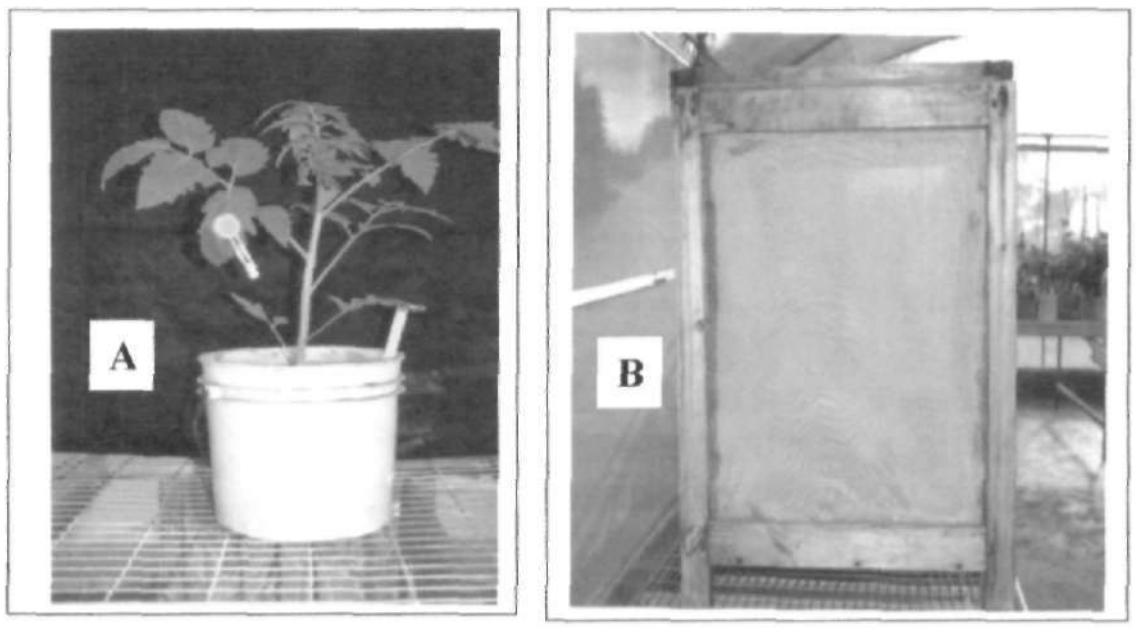


Plate 4: Whitefly transmission of *Tomato yellow leaf curl Tanzania virus*. A: Clip cages on the leaf of tomato plant; B: Insect-proof cage.

3.4.4 Mechanical transmission

Mechanical transmission tests were conducted as described by Ioannou (1985b). Infected leaf tissues from the top most leaves were macerated with a pestle in a mortar containing 0.01M phosphate buffer, pH 7.4 (1:10 w/v), 1.0 % polyvinyl pyrrolidone (PVP-40). The sap obtained was inoculated by rubbing the leaves of test plants pre-dusted with carborundum powder (500 mesh, 50 µm). Five plants each of tomato cv. Moneymaker, *Datura stramonium* L. and *Nicotiana tabacum* L. were rub-inoculated in each of the three replications. Non-inoculated plants were kept as controls. The plants were maintained in the screenhouse for observation and assessment of disease incidence and symptom severity.

3.4.5 Virus-vector relationships

3.4.5.1 Acquisition access period (AAP)

Groups of adult (not more than one week old) non-viruliferous *B.tabaci* were allowed acquisition access feeding periods of 10, 30 min, or 1, 2, 4, 8, 16, 24 or 48 h before being transferred to indicator plants for a 48-h IAP. The whiteflies were killed with Karate 5EC (a.i 50 gl^{-1} λ-cyhalothrin) after inoculation feeding. Fifteen whiteflies were transferred to each plant and 15 plants were used per trial.

3.4.5.2 Inoculation access period (IAP)

To determine the inoculation access feeding time, adult whiteflies were given AAP feeding of 24 h on tomato plants infected with each TYLCTZV isolate, and transferred

to indicator plants for an IAP of 10, 30 min, or 1, 2, 4, 8, 16, 24 or 48 h. The same number of plants and whiteflies per plant was used as in AAP tests. The whiteflies were killed with Karate 5EC (a.i 50 gl^{-1} λ-cyhalothrin) at the expiration of each IAP.

3.4.5.3 Persistence of TYLCTZV in the vector

Three groups of 25 adult non-viruliferous *B. tabaci* were caged for 48 h on tomato plants infected with the TYLCTZV isolates. Thereafter, the viruliferous whiteflies were moved in daily serial transfers to healthy tomato plants. The indicator plants were sprayed with Karate 5EC (a.i 50 gl^{-1} λ-cyhalothrin) after each transfer and maintained in the screenhouse for TYLCTZV symptom development.

3.4.6 Vector transmission

The ability to transmit each of the six virus isolates was tested with whiteflies of the same age from the same colony. Adult *B.tabaci* from the non-viruliferous colony were allowed an acquisition feeding period (AAP) of 48 h on the tomato plants infected with each isolate before being transferred in groups of 15 (using a battery-operated aspirator) to healthy tomato seedlings cv. Moneymaker at the two-leaf growth stage. Five seedlings of the TYLCTZV-susceptible tomato cv. Moneymaker were used as assay hosts per test per isolate, and replicated three times. Graft-inoculated tomato plants with prominent yellow leaf curl symptoms were used as sources of inoculum. After inoculation access feeding period (IAP) of 24 h, the seedlings were sprayed with Karate 5EC (a.i 50 gl^{-1} λ-cyhalothrin) to kill the whiteflies. The plants were monitored under

screenhouse conditions (25 ± 5 °C, 72.4 % relative humidity) and symptoms of virus infection were recorded at weekly intervals for 4 weeks. The triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) (Pico *et al.*, 1999) procedure was used to check for the presence of the virus in test plants.

3.5 Evaluation of tomato genotypes for resistance to TYLCTZV

3.5.1 Screenhouse experiments

Seeds of each tomato genotype were sown in 3 (30 cm-diametre) plastic pots containing sterilized soil. The seedlings were inoculated with TYLCTZV isolate from Morogoro using *B. tabaci*. Whiteflies from the virus-free colony were allowed an acquisition access feeding of 48 h on TYLCTZV-infected tomato plants, and then transferred to the test plants of each tomato genotype for an inoculation feeding time of 72 h. The TYLCTZV isolate used for inoculation was maintained in the screenhouse on tomato cv., Moneymaker by whitefly-mediated inoculations.

Tomato plants at the first leaf stage were mass inoculated using an estimated viruliferous whitefly population twice (30 whiteflies/plant) that used by Cohen and Nitzany (1966), which could be expected to achieve 100% infection (Lapidot *et al.*, 1997). Fifteen tomato genotypes consisting of four tomato cultivars and 11 breeding lines were evaluated for their reaction to TYLCTZV. Nine breeding lines (CLN1–CLN7, H24 and TLB) derived from *Lycopersicon hirsutum* and two cultivars (Tengeru 97 and Moneymaker) were obtained from the Asian Vegetable Research and Development

Centre-African Regional Programme, Arusha, Tanzania, while the Volcani Centre, Israel supplied two lines (TY 172 and Marmande). Two cultivars (Cal-J and Marglobe) were purchased from commercial stores. TY 172 and Tengeru 97 were used as resistant and tolerant control, while Marmande and Moneymaker were included to serve as susceptible checks. The level of resistance of the tomato lines TY172 and Marmande to TYLCV had been reported (Lapidot *et al.*, 1997; Friedmann *et al.*, 1998).

After inoculation, the seedlings were sprayed with Karate 5EC (a.i 50 gl^{-1} λ-cylohalothrin) to kill the whiteflies. Non-inoculated seedlings of each tomato line were used as controls. The experiment was repeated twice in completely randomised design. Fertilizer was applied in two split doses of NPK 20-10-10 (30 kg N, 30 kg P and 30 kg K ha^{-1}) five weeks after sowing and two months thereafter. Disease severity was evaluated weekly according to the following scale described by Friedmann *et al.* (1998): 0 = no visible symptoms, inoculated plants show same growth and development as non-inoculated plants; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, and pronounced cupping and curling; plants cease to grow. Tomato fruits were picked in two harvests. During the first harvest, only the ripe mature fruits were picked, while both mature and immature fruits were picked at the second harvest two weeks later. Data were collected and calculated to determine the

plant fresh weight (without roots) taken immediately after the second harvest, number of fruits per plant, fruit weight per plant, and total yield per plant.

3.5.2 Field experiments

Field experiments to evaluate the tomato genotypes were conducted for two seasons (November 2000 to May 2001, and December 2001 to June 2002) at the Horticulture Farm, Sokoine University of Agriculture Tanzania (06° 19'S and 37° 40'E, elevation of 518 metres above sea level). Inoculation of tomato seedlings was done as described in the screenhouse experiments. The same tomato genotypes (Cal-J, CLN1 CLN2 CLN3 CLN4, CLN5, CLN6, CLN7, H24, TLB, Tengeru 97, Moneymaker, Marglobe, Marmande and TY 172) used for the screenhouse evaluation tests were used for the field experiments. The seedlings were all maintained in the screen house until transplanting.

Tomato seedlings were transplanted four weeks after inoculation in paired inoculated and non-inoculated ridges at an intra-row spacing of 60 cm replicated three times. Each plot consisted of six 1.5 m-long ridges constructed 60 cm apart and separated from each other by a gap of 50 cm. The replicates were spaced 1.5 m apart. One week before transplanting, the tomato plants were sprayed with Ridomil MZ 68 WP (a.i Metalaxyl-M 40 g/kg + Mancozeb 640 g/kg)) and Karate 5EC (a.i 50 g/l λ-cylohalothrin) for protection against fungal/bacterial diseases and insect vectors. Cultural management practices were adopted as recommended by Hanson *et al.* (2000). The transplanted seedlings were irrigated by canal irrigation water. Fertilizer was applied manually at the

same rate as in the screenhouse experiments one week after transplanting and two months later. The tomato plants were kept vector-free throughout the duration of the experiment by spraying weekly with Karate 5EC (a.i 50g/l λ-cylohalothrin) to prevent secondary spread of the virus. Harvesting and recording of data was done as described in the screenhouse experiments from the four inner ridges of each plot.

3.6 Data analyses

Data from the screenhouse and field experiments were analysed using the one-way analysis of variance test (SAS Institute, Cary, NC). Disease severity data were transformed by square root transformation before the analysis. Means were separated and compared between inoculated and non-inoculated treatments using the T test. After combined analysis, the inoculated and non-inoculated plants of respective tomato genotypes were ranked using the Duncan's Multiple Range Tests. Data on vector and graft transmission tests were similarly analyzed using the PROC ANOVA procedures, and Duncan's Multiple Range Tests was used to compare the means.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Incidence and severity of TYLCTZV

Tomato farms in 6 major tomato-growing regions in Tanzania (Table 4) were surveyed for the presence of TYLCTZV symptoms. The incidence of disease varied between and within the regions (Table 4). The highest TYLCTZV incidence, which was significantly different ($P<0.05$) from other regions, was recorded in Dodoma, and lowest incidence was recorded in Iringa. The severity of TYLCTZV was highest in tomato farms in Dodoma, followed by farms surveyed in the Coast and Arusha regions. However, TYLCTZV was less severe in tomato farms located in Kilimanjaro and Iringa regions (Table 4).

During the survey, TYLCTZV symptoms were recognized without problems. However, 89 % of the farmers could neither recognize the disease by symptoms, especially, at early stages of infection nor identify the whitefly vectors, which is crucial for the initiation of control measures. Hence, no attempt was made by farmers to control the TYLCTZV vectors. This contributed, among other factors, to the high incidence of the disease observed in most of the farms visited (e.g Ikowa) compared with low incidences in a few that applied insecticides (e.g. Kidamali and Kwarc). High incidence of the TYLCTZV disease was often associated with high vector population. Weather conditions were variable during the time of the survey.

Table 4: Incidence and severity of *Tomato yellow leaf curl Tanzania virus* from tomato farms surveyed in six regions of Tanzania.

Site	Incidence (%)*	Severity§
Dodoma		
Makutopora	79.83^{a}	3.53^{a}
Veyula	48.77^{bc}	2.30^{abc}
Ikowa	84.57^{a}	3.50^{ab}
Morogoro		
SUA^{+}	43.27^{cde}	2.0^{cdef}
Mlali	51.47^{bc}	2.30^{cde}
Kilosa	47.63^{bc}	2.03^{cdef}
Iringa		
Ilula	36.07^{cde}	1.50^{def}
Mgama	28.83^{def}	1.37^{ef}
Kidamali	15.27^{f}	1.37^{ef}
Arusha		
Movoiro	65.43^{b}	2.43^{abcd}
Madiira	52.13^{bc}	2.57^{abc}
Manyire	55.33^{bc}	2.13^{cdef}
Kilimanjaro		
Bomangombe^{1}	39.70^{cde}	2.0^{cdef}
Bomangombe^{2}	45.20^{cde}	2.23^{cde}
Kware	27.43^{ef}	1.3^{f}
Coast		
Kibaha	53.07^{bc}	2.53^{abc}
Chanika^{1}	47.27^{bcd}	2.40^{bcd}
Chanika^{2}	51.70^{bc}	2.63^{abc}
CV (%)	15.63	14.08
LSD (0.05)	11.56	1.98

Means are averages of five replicates. Means in the same column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Tests

* Incidence and severity data were arcsine- and square root-transformed before analysis.

+ Sokoine University of Agriculture (Horticulture Farm)

1, 2 indicate that both farms were selected from the same named location.

§ Severity was rated on a scale of 0 (no symptoms) – 4 (severe symptoms) according to Friedmann *et al.* (1998).

For example, Iringa was generally colder (average temperature 18.8 °C) than Dodoma (28.6 °C); consequently, vector population and disease incidence were, respectively, low and high. The disease incidence was also high in farms in which the susceptible tomato cultivars Moneymaker, Roma VF and Marglobe, were planted. The reverse was the case in farms planted with Tengeru 97, a TYLCTZV-tolerant cultivar.

In some farms, especially, in Iringa and Kilimanjaro, tomato was intercropped with, or planted near other crops such as maize, cassava, pepper. This could mean low disease incidence if tomato was intercropped with crops more preferred by the vector and vice versa. Similarly, crops such as maize could impede the free movement of the vectors by acting as physical barriers, thereby causing a reduction in TYLCTZV disease incidence on tomatoes intercropped with such crops. In tomato farms, which received more than one insecticide treatments, especially, at the early stages of seedling growth of tomato, the TYLCTZV disease incidence was comparatively lower than in non-treated farms within the same location.

In previous studies (Nono-Womdim *et al*., 1996), TYLCTZV was detected in 11 regions in Tanzania and rated as high in 4 regions (Arusha, Coast, Dodoma and Shiyanga), moderate in 2 (Kilimanjaro and Mwanza) and low in 5 regions (Iringa, Morogoro, Rukwa, Tabora and Tanga). The present study placed the surveyed regions in the same TYLCTZV incidence categories. This implies that TYLCTZV is endemic in these regions; hence, integrated strategies, which may involve the planting of resistant/tolerant

tomato varieties, rotating tomato with non-host crops of the virus, removal of alternative and volunteer hosts of the virus and application of insecticides, are required to manage the disease.

4.2 Characterization of isolates of TYLCTZV

4.2.1 Enzyme-linked immunosorbent assay (ELISA)

The six TYLCTZV isolates reacted with strong signals to the polyclonal probe (PAb) for geminiviruses. The reaction of all the isolates was rated the same (4) (Table 5). When the isolates were further tested with monoclonal antibodies (MAbs) SCRI 17, SCRI 20, SCRI 23 and SCRI 33, the Dodoma and Arusha isolates were rated highest in optical density reading with MAb SCRI 20 and 23. The remaining isolates produced lower OD values (Table 5). All the isolates rated low (2) when tested with SCRI 33.

In the TAS-ELISA tests, all the TYLCTZV isolates recorded high reaction values (4) with the polyclonal antibody. However, it was the Arusha and Dodoma isolates of TYLCTZV that recorded the highest values when tested with SCRI 20 and 23, indicating a closer relationship to the European strains of TYLCV. The monoclonal antibodies SCRI 20 and 23 have been used for the detection of the European strains of TYLCV, while SCRI 33 detects ACMV (McGrath and Harrison, 1995).

Table 5: Results of triple antibody sandwich-enzyme linked immunosorbent assay tests showing absorbance values of *Tomato yellow leaf curl Tanzania virus* isolates.

	OD reading at A_{405nm}[§]				
Virus isolate	PAb*	SCRI 17	SCRI 20	SCRI 23	SCRI 33
Arusha	4	2	4	4	2
Kilimanjaro	4	2	3	2	2
Dodoma	4	3	4	4	2
Morogoro	4	2	3	2	2
Iringa	4	2	2	3	2
Coast	4	2	3	2	2
Infected control	4	4	4	4	4
Healthy control	0	0	0	0	0

[§]A_{405nm} 4 = > 1.8; 3 = 1.21 - 1.8; 2 = 0.61 - 1.2; 1 = 0.3 - 0.6; 0 = < 0.3

*PAb=Polyclonal probe for detecting geminiviruses.

According to documented reports (Pico *et al.*, 1996), a high degree of homology in coat protein exists among related whitefly-transmitted geminiviruses. This explains why the TYLCTZV isolates reacted strongly to the geminivirus polyclonal probe. However, monoclonal antibodies are specifically produced against single coat protein epitopes. Thus, the differences in reaction to the monoclonal antibodies of TYLCV indicate that variability exists between the coat protein epitopes of TYLCV and TYLCTZV on one hand, and among the TYLCTZV isolates on the other. Only the two TYLCTZV isolates from Arusha and Dodoma share a high sequence homology in coat protein with the European and related TYLCV isolates. The other TYLCTZV isolates are only distantly related to TYLCV. Furthermore, based on the differences in reaction, the serological relationships between the TYLCTZV isolates can be determined. For example: The reaction with either SCRI 20 or SCRI 23 show that the isolates from Arusha and Dodoma share a high degree of homology, and could belong to one serotype. The other isolates from Morogoro, Coast and Kilimanjaro could form another serotype, while the isolate from Iringa is a different serotype. On the other hand, reaction with SCRI 17 groups the isolates in two serotypes, the Dodoma isolate alone, and another that groups the other five isolates together.

All the TYLCTZV isolates reacted weakly with SCRI 33, which detects ACMV, indicating that they are all distantly related to the African cassava mosaic virus. Testing the reaction of the TYLCTZV isolates to additional geminivirus monoclonal antibodies,

including those specifically produced to TYLCTZV would yield profiles that would be useful in for the identification of TYLCV strains based on coat protein sequences.

Differences in coat protein epitopes have implications for whitefly transmission of begomoviruses. It has been reported that the begomoviral coat protein is the only gene product that interacts directly with whitefly vectors during the circulative transmission of the virus (Rybicki, 1994; Padidam *et al*., 1995b; Czosnek *et al*., 2002). Additional evidence shows that the coat protein plays a role in vector recognition and acquisition (Briddon *et al*., 1990; Accoto *et al*., 1994). Furthermore, the specificity and frequency of virus transmission by the whitefly vector has been associated with the selection of variants in the coat protein for effective transmission by the vector (Harrison *et al*., 1994; McGrath and Harrison, 1995). All these imply that variation in the virus coat protein epitopes will influence the efficiency of transmission by the whitefly vector. This would explain the observed differences in the transmission efficiency of the TYLCTZV isolates by the vector, *B. tabaci* (Table 13), and the variation in disease incidence and severity at different locations (Table 4). The European TYLCV isolates have been reported to be identical in sequence with the Eastern Mediterranean TYLCV isolates. However, this sequence identity was found to decrease with increasing geographical distance (Noris *et al*., 1994a; Crespi *et al*., 1995).

4.2.2 Electron microscopy

Typical twin-like particles associated with geminiviruses were observed in tomato samples with typical symptoms of TYLCTZV, which were collected from the six regions (Plate 5). Particles from purified virus preparations were observed to be isometric and pentagonal in shape with single and paired gemini particles. Individual components of these geminate pairs were morphologically pentagonal in shape and 20 x 30 nm in size.

The electron micrograph revealed that a geminivirus was responsible for disease symptoms observed on tomatoes in Tanzania. TYLCV has a characteristic geminate particle morphology consisting of incomplete icosahedra (Harrison, 1985; Frischmuth and Stanley, 1993). Single viral particles, which are non-infective degraded products, have been observed in most infected samples (D'Hondt and Russo, 1985). The particle structure and cytopathological changes observed in infected tissues have been used to confirm the causal agent of a disease to be a geminivirus (Russo *et al.*, 1980; Cherif and Russo, 1983; D'Hondt and Russo, 1985).

4.2.3 Hybridization

4.2.3.1 Dot-blot hybridization

Plates 6A and 6B show the results of the dot-blot hybridization assay of extracts from tomato leaf samples collected from the six regions covered in this study.

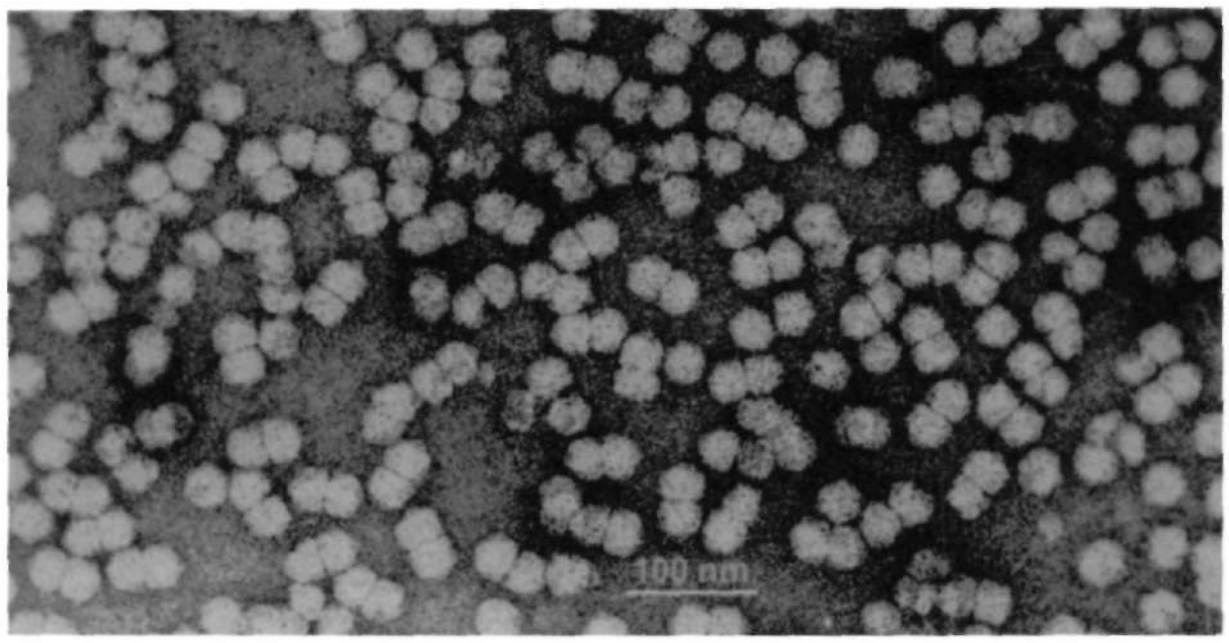


Plate 5: Electron micrograph showing the geminate particles of *Tomato yellow leaf curl Tanzania virus*.

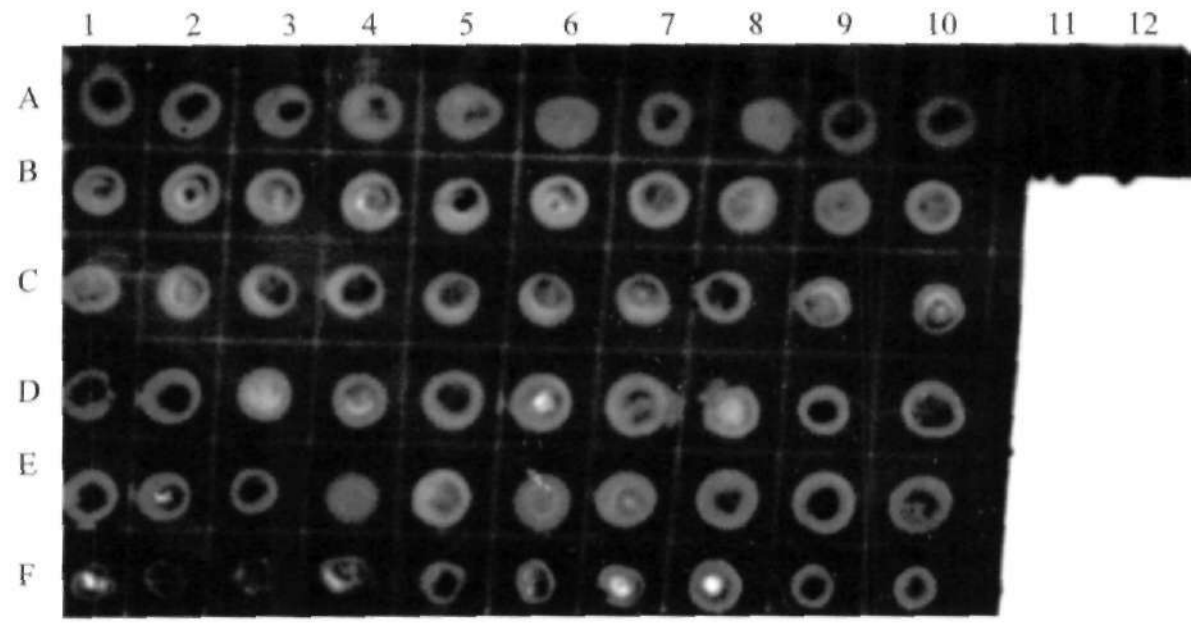


Plate 6A: Dot blot analysis showing reaction signals of *Tomato yellow leaf curl Tanzania virus* samples from different regions of Tanzania when tested with a DIG-labelled probe of TYLCSV. Numbers indicate infected tomato leaf samples collected from farms in Arusha (A), Kilimanjaro (B), Dodoma (C), Morogoro (D), Iringa (E) and Coast (F) regions. A11 = Samples from tomato leaves infected with TYLCTZV; A12 = Samples from tomato leaves infected with TYLCSV

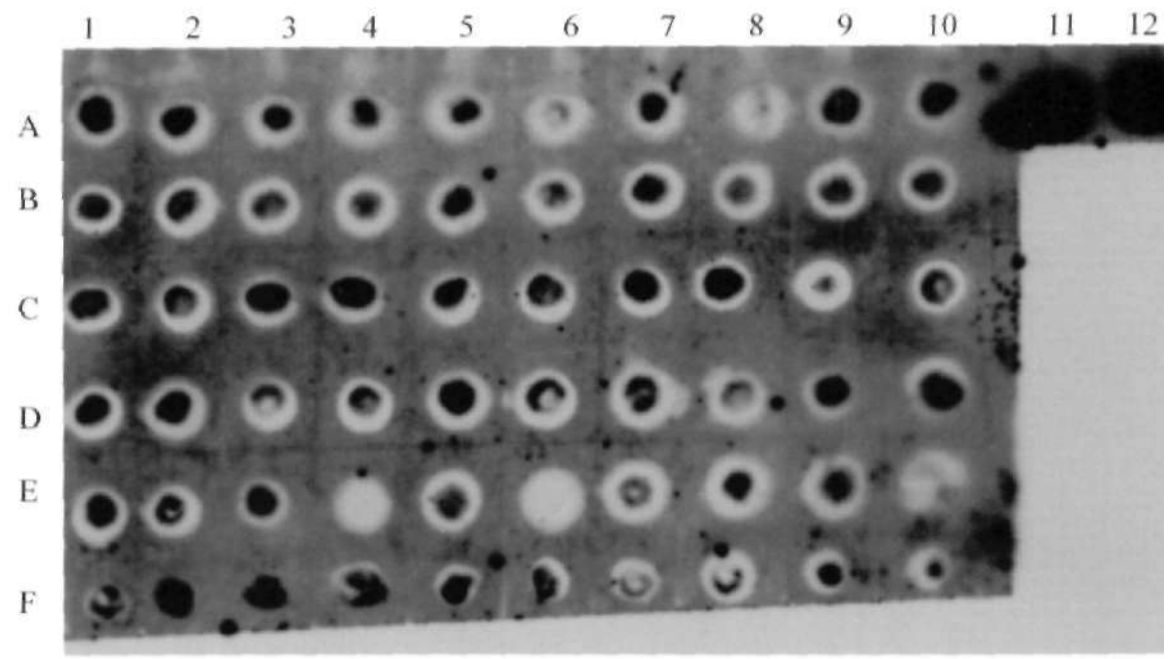


Plate 6B: Dot blot analysis showing reaction signals of *Tomato yellow leaf curl Tanzania virus* samples from different regions of Tanzania when tested with a DIG-labelled probe of TYLCTZV. Numbers indicate infected tomato leaf samples collected from farms in Arusha (A), Kilimanjaro (B), Dodoma (C), Morogoro (D), Iringa (E) and Coast (F) regions. A11 = Samples from tomato leaves infected with TYLCTZV; A12 = Samples from tomato leaves infected with TYLCSV

These figures indicate reaction signals of samples from Arusha (A1-A10), Kilimanjaro (B1-B10), Dodoma (C1-C10), Morogoro (D1-D10), Iringa (E1-E10) and Coast (F1-F10). With the exception of a few, samples from all the regions hybridized to the DIG-labelled viral DNA probes. The dot blotted samples hybridized to the riboprobes synthesized for the detection of TYLCTZV and TYLCSV. Riboprobes have been used for the survey and detection of geminiviruses (Roberts, *et al.*, 1984; Czosnek *et al.*, 1990; Swanson, *et al.*, 1992; Faria, *et al.*, 1994; Padidam, *et al.*, 1995b).

Although the reaction signals were not of the same strength, the results confirmed the presence of TYLCTZV on tomatoes in all the regions surveyed. It further demonstrated the usefulness and reliability of riboprobes for quick detection of field infection and diagnosis of TYLCTZV. Failure to detect the TYLCTZV DNA in some of the suspect samples indicates that symptoms alone are not reliable indicators of the presence of TYLCTZV.

It has been reported that TYLCV field-infected plants exhibit variation in symptoms (Czosnek *et al.*, 1990). This is more important for the farmers because the majority of them could not recognize the disease by symptoms. Therefore, they did not take any management measures for TYLCTZV. The ability to detect the virus with both probes (TYLCTZV and TYLCSV) indicated a possible degree of sequence homology with both virus strains. Chiang *et al.* (1997) reported that the virus causing leaf curl symptoms in Tanzania is different from previously characterized Old World geminiviruses. However,

recent molecular studies revealed a closer relationship of the TYLCTZV from the surveyed regions with ToLCTZV and TYLCSV (Kashina *et al.*, 2002a).

Previous work has indicated that hybridization procedures overcome the limitations encountered with serological reactions, because they have a higher degree of sensitivity, they are easier to apply, and have the ability to differentiate strains of viruses (Zilberstein *et al.*, 1989; Crespi *et al.*, 1991; Abou Jawdah *et al.*, 1995). Similar sensitivity has been found with squash and dot blot hybridization assays (Czosnek *et al.*, 1988b; Navas-Castillo *et al.*, 1998). Nakhla *et al.* (1994) detected TYLCV in samples of *Euphorbia* sp. using a DNA probe specific to begomoviruses from the Western Hemisphere. Riboprobes have been widely used for the field detection of diseases, and are especially recommended for early detection of TYLCV infection.

4.2.3.2 Southern blot hybridization

The probe was able to detect TYLCTZV genomic circular single-stranded DNA (CSS), as well as the circular double-stranded TYLCTZV replicative form in its supercoiled (covalently closed circular, CCC) and relaxed (open circular, OC) forms. A higher molecular weight TYLCTZV-related DNA, which was probably a CCC polymer was also detected (Plate 7).

The dsDNA molecules are intermediates for viral replication, and their presence is a further confirmation of the incidence and replication of a virus. These intermediates are

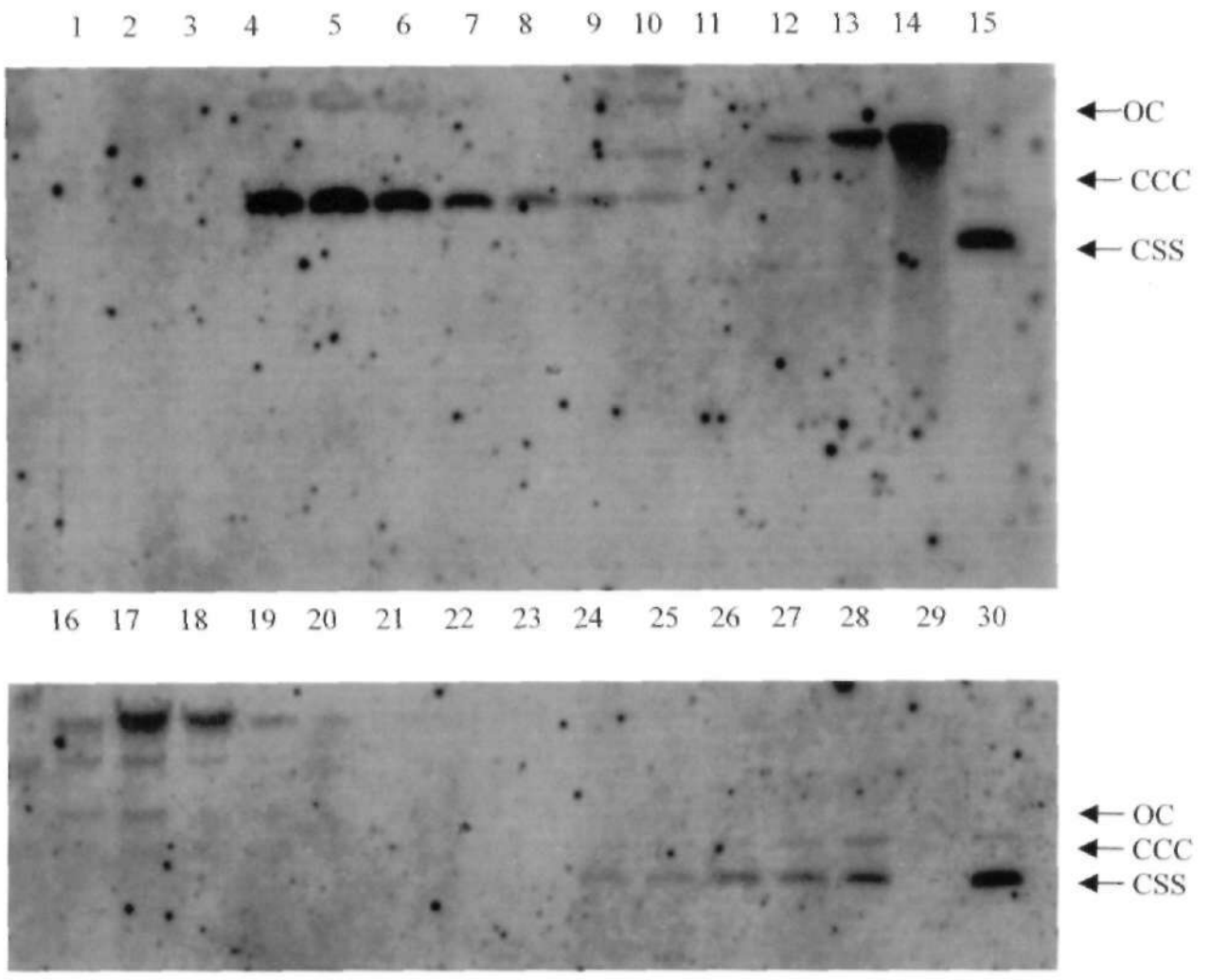


Plate 7: Southern hybridization analysis of Tomato yellow leaf curl Tanzania virus samples using the TYLCTZV DIG-labelled probe. OC: Replicative form of viral DNA in its relaxed (open circular) form; CCC: Circular double-stranded DNA replicative form in its supercoiled (covalently closed circular) form; CSS: TYLCTZV genomic circular single-stranded DNA. Numbers 2 – 14 and 16 – 28 represent DNA samples extracted from tomato leaves. 1 and 29 are negative controls (non-infected *Lycopersicon esculentum*); 15 and 30 represent postive controls (1 pg of TYLCTZV DNA).

products of the first step of the rolling circle replication process reported for geminiviruses (Saunders *et al.*, 1991; Kornberg and Baker, 1992; Jeske *et al.*, 2001). Czosnek *et al.* (1989) detected circular dsDNA molecules in their CCC and OC forms in TYLCV-infected plants.

4.2.4 Molecular analysis

Using the primer pair OTYA 2 and OTYA 6 or PAC1v1978 and PAVc715, the predicted 1.2 - 1.4 kb PCR product was amplified from the TYLCTZV isolates (Plate 8). This fragment was expected to include the replication-associated gene (Rep), pre-coat and coat protein (CP) genes, and the intergenic region. The Vector NTI.5 software was used to analyse virtual restriction fragment patterns generated by a panel of restriction endonuleases. *Alu*I restriction endonuclease was chosen for restriction fragment length polymorphisms (RFLP) analysis based on its predicted ability to generate differential RFLP patterns from three TYLCV sequences in the GenBank database [TYLCSV (accession no. X61153), TYLCV (accession no. X15656) and Tomato yellow leaf curl Thailand virus-[1] (TYLCTHV-[1] (accession no. X63015)]. The RFLP patterns generated from the Tanzanian isolates are shown in Plate 9.

The viral DNA was amplified from the TYLCTZV samples. These primers had been used to amplify viral DNA from other geminiviruses (Rojas *et al.*, 1993). The digestion of the PCR product by *Alu*I endonuclease and analysis by RFLP revealed consistent bands that did not arise from chance variation during the PCR stage. The patterns did not

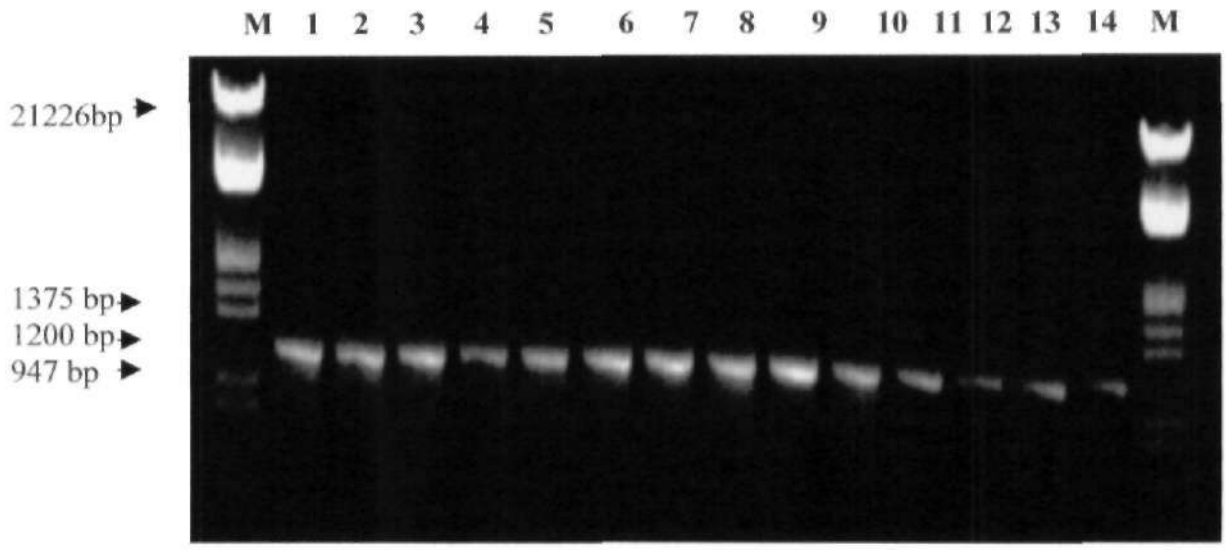


Plate 8: Viral DNA fragment amplified by polymerase chain reaction technique from isolates of *Tomato yellow leaf curl Tanzania virus*. M=Marker (*Hind* III-digested λ-DNA), 1, 2 =Dodoma, 3, 4 =Arusha, 5, 6 =Morogoro, 7, 8 =Coast, 9, 10 = Kilimanjaro, 11, 12 =Iringa, 13 =TYLCSV, 14 = TYLCTZV

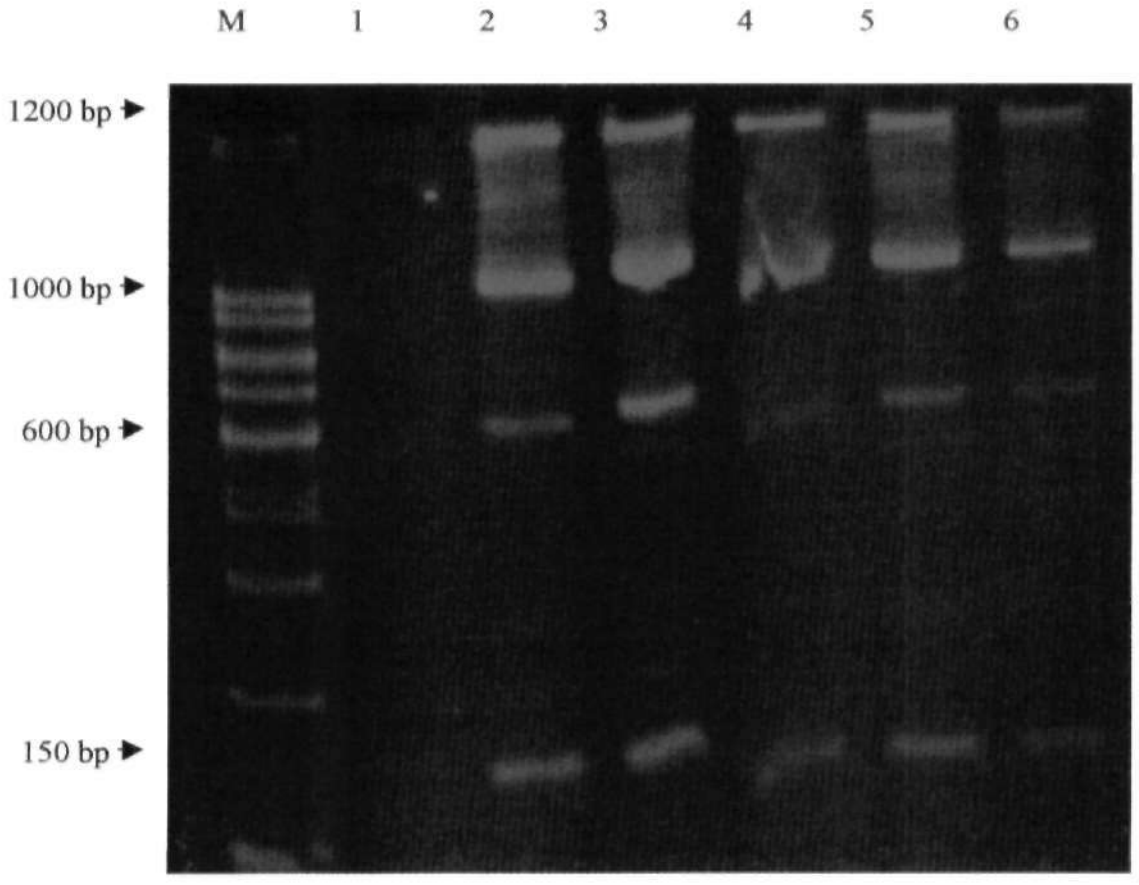


Plate 9: Restriction fragment length polymorphisms analysis of viral DNA from isolates of *Tomato yellow leaf curl Tanzania virus*. M = 1 kb ladder; 1 = healthy tomato leaf tissue; 2 = Dodoma; 3 = Arusha; 4 = Morogoro; 5 = Coast; 6 = Iringa.

appear to be generated from partial digestion by *Alu*I either. Furthermore, the changes in the *Alu*I sites predicted by the sequences, correlated with the RFLP patterns that were observed. Consistency in RFLP patterns suggests that the isolates are similar. These bands were consistently produced. Sequence data did not show significant variation among the TYLCTZV isolates in nucleotides (Figs. 4 and 5).

However, when the CP and C1 nucleotide sequences of the clones were compared with those of other geminiviruses, TYLCTZV was most similar to the African tomato leaf curl virus (95 and 97 % identity in C1 and CP, respectively), which shares the same sequence with *Tomato leaf curl Tanzania virus* (ToLCTZV; Accession number U73498) (Table 6). There was more than 90 % homology in C1 with Tobacco leaf curl virus-Zimbabwe, *Tobacco leaf rugose virus*, *Wissadula golden mosaic virus*, *Tomato yellow leaf curl virus*, and *Tomato curly stunt virus*. Isolates of the same geminivirus usually share an identity of more than 90 % (Martin and Bristow, 1988). The frequent occurrence of mixed infections with the possibility of recombination among geminiviruses in the field could be responsible for the high degree of homology between TYLCTZV and the viruses mentioned above.

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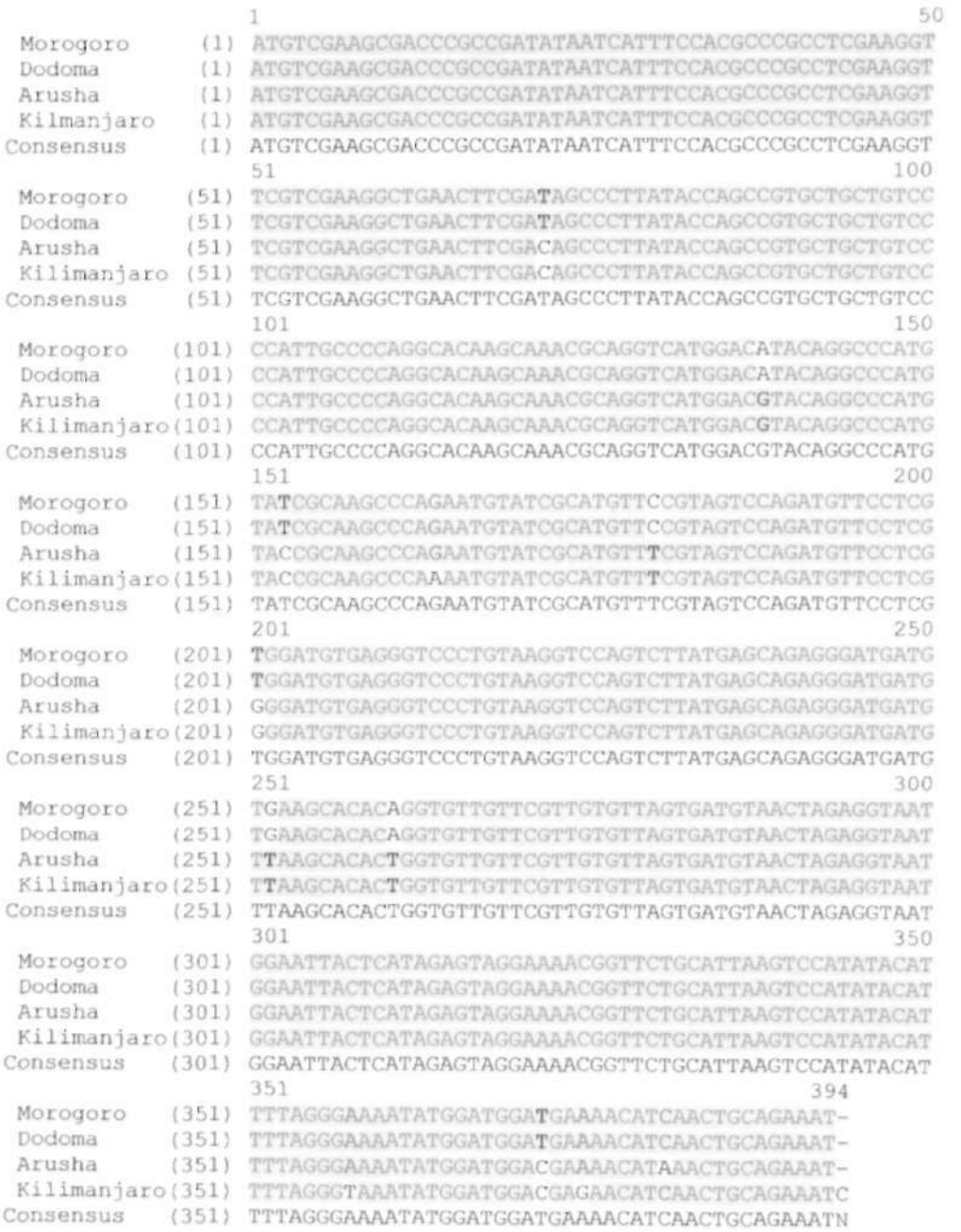
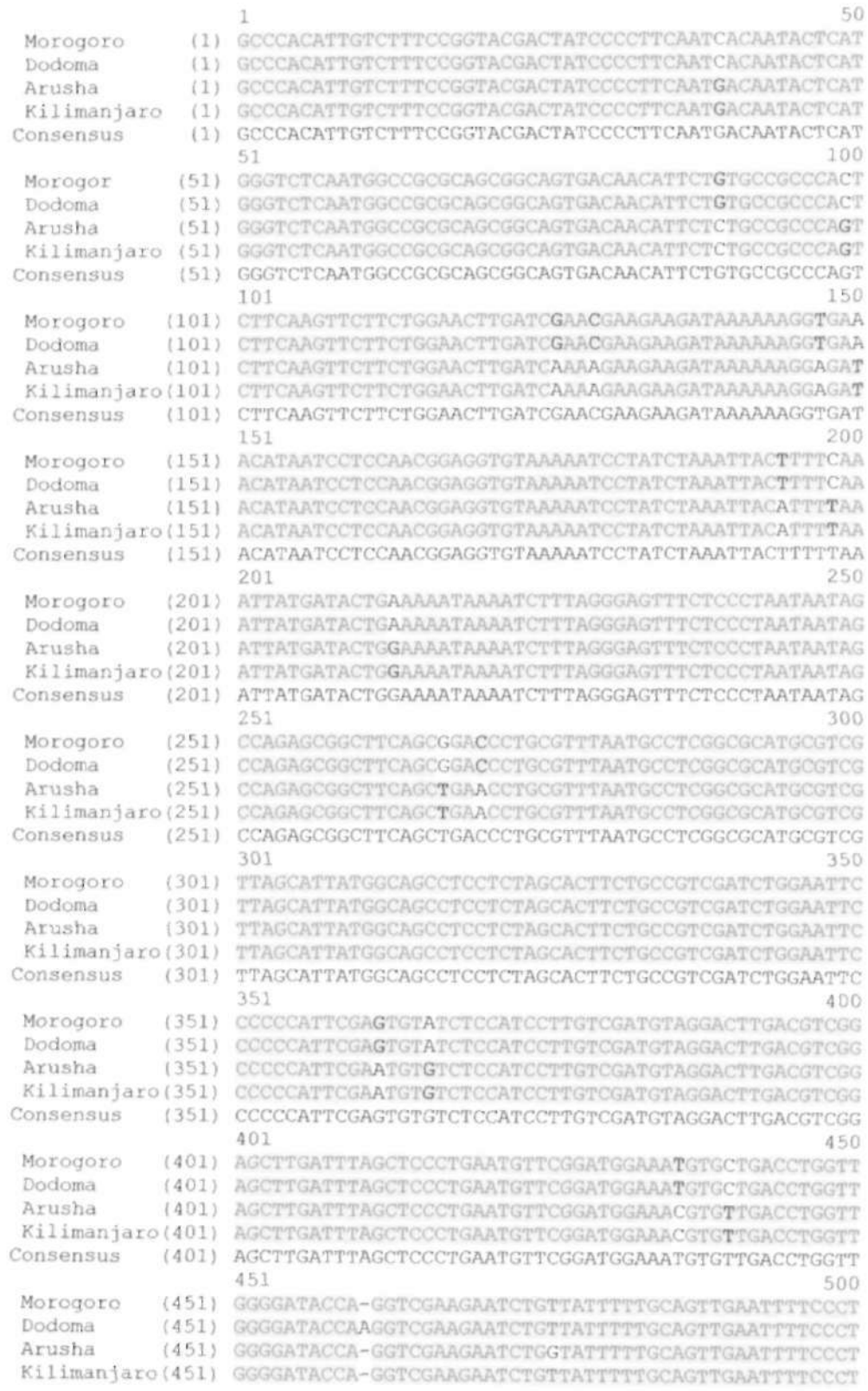


Figure 4: Nucleotide sequence alignments of coat protein (CP) for cloned viral DNA fragments amplified from tomato samples collected from farms in Morogoro, Dodoma, Arusha and Kilimanjaro. Alignments showed a sequence identity of 96.7% among the sequences.



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```
Consensus    (451) GGGGATACCANGGTCGAAGAATCTGTTATTTTTGCAGTTGAATTTTCCCT
                   501                                             550
 Morogoro    (500) CGAACTGAATAAGCACGTGGAGATGAGGCTCCCCATCTTCGTGTAGTTCT
 Dodoma      (501) CGAACTGAATAAGCACGTGGAGATGAGGCTCCCCATCTTCGTGTAGTTCT
 Arusha      (500) CGAACTGAATAAGCACGTGGAGATGAGGTTCCCCATCTTCGTGTAGTTCT
 Kilimanjaro(500) CGAACTGAATAAGCACGTGGAGATGAGGTTCCCCATCTTCGTGTAGTTCT
Consensus    (501) CGAACTGAATAAGCACGTGGAGATGAGGTTCCCCATCTTCGTGTAGTTCT
                   551                                             600
 Morogoro    (550) CTGCAAATTTT-GATGAATTTTTTATTTGGTGGAGTATCAAGTATTTAAT
 Dodoma      (551) CTGCAAATTTTTGATGAATTTTTTATTTGTTGGAGTATCA-GTATTTAAT
 Arusha      (550) CTGCAGATTTT-GATGAATTTTTTATTTGGTGGTGTTTCA-ATATTTAAT
 Kilimanjaro(550) CTGCAGATTTT-GATGAATTTTTTATTTGTTGGTGTTTCA-ATATTTAAT
Consensus    (551) CTGCAGATTTTNGATGAATTTTTTATTTGTTGGTGTTTCANGTATTTAAT
                   601                                             650
 Morogoro    (599) AATT-GAGA-TAGTGCTTCTTCTTTATTTAAAGAGCATTTGGGGATAAA
 Dodoma      (600) AATTTGAGAATAGTGCTTCTTCTTTATTTAGA-GAGCATTTGGGGATAAG
 Arusha      (598) AATC-GAGA-TAGTGCTTCTTCTTTATTTAGA-GAGCATTTTGG-ATAA-
 Kilimanjaro(598) AATT-GAGA-TAGTGCTTCTTCTTTATTTAGA-GAGCATTTTGG-ATAA-
Consensus    (601) AATTNGAGANTAGTGCTTCTTCTTTATTTAGANGAGCATTTTGGGATAAN
                   651                                             700
 Morogoro    (647) GTGAGGGAAATAATTTTTGGGAATTT-ATTTTGGAAACN-CTTAAG-ANG
 Dodoma      (649) GTGAGGAAAATAATTTTTGGGAATTTTATTTTGGAAACGGCTTAAGGAGG
 Arusha      (643) GTGAGG-AAATAATTTTTGG-AATTT--ATTTGGAAAC--CTTANG-ANG
 Kilimanjaro(643) GTGAGG-AAATAATTTTTGG-AATTT--ATTTGGAAACG-CTTANG-AGG
Consensus    (651) GTGAGGNAAATAATTTTTGGGAATTTNATTTTGGAAACGNCTTANGNANG
                   701
 Morogoro    (694) AGGCAT
 Dodoma      (699) AGGCAT
 Arusha      (686) AGGCAT
 Kilimanjaro(687) AGGCAT
Consensus    (701) AGGCAT
```

Figure 5: Nucleotide sequence alignments of replication-associated protein (Rep) for the cloned viral DNA fragments amplified from tomato samples collected from farms in Morogoro, Dodoma, Arusha and Kilimanjaro. Alignments showed a sequence identity of 94.9 % among the sequences.

Table 6: Comparison of nucleotide sequences of coat (CP) and replication-associated (Rep) protein genes of *Tomato yellow leaf curl Tanzania virus* with corresponding sequences of other geminiviruses.

Name of virus	Identity in nucleotides (%)	
	Rep protein	Coat protein
African tomato leaf curl virus (U73498.1)	95	97
African cassava mosaic virus (AF011785.1)	-	82
Bean dwarf mosaic virus (M88179.1)	87	-
Beet curly top virus- Logan (AF379637.1)	89	-
Cassava geminivirus-Swaziland (AF329236.1)	-	82
Cotton leaf curl virus (AJ228573.1)	89	-
Dicliptera yellow mottle virus (AF139168.1)	89	-
East african cassava mosaic virus-Malawi[M11] (AJ006459.1)	85	84
Jatropha mosaic virus (AF324410.1)	90	-
Macroptilium yellow mosaic virus (AJ344452.1)	89	-
Potato yellow mosaic virus (AF039031.1)	88	-
Sida golden mosaic Florida virus-[A11] (U77964.1)	89	-
Tomato leaf curl Sinaloa virus (AF131213.1)	89	-
South African cassava mosaic virus (AF155806.1)	-	83
Soybean crinkle virus (AB020977.2)	89	-
Tomato yellow leaf curl virus (AJ010790.1)	91	89
Tomato pseudo curly top (X84735.1)	89	-
Tomato curly stunt virus (AF261885.2)	93	85
Tomato rugose mosaic virus (AF291705.1)	86	-
Tomato mottle virus-[Florida-B1] (M90495.1)	89	-
Tomato leaf curl virus (AF327436.1)	90	-
Tobacco leaf curl virus-Zimbabwe (AF350330.1)	93	87
Tobacco leaf rugose virus (AJ488768.1)	92	-
Wissadula golden mosaic virus-[Jamaica1] (U69281.1)	91	-

An alignment of approximately 1200 nucleotides was used to construct a dendogram of 21 geminiviruses. The TYLCTZV was similar to TYLCSV, and both were closely related to TYLCV (Fig. 6). Phylogenetic analysis is an important tool that has been used to establish ancestral relationships among geminiviruses, and it is critical for virus taxonomy. Clustering of geminiviruses is sometimes dependent upon the part of the genome used for analysis. This has been reported for bipartite geminiviruses: *Cabbage leaf curl virus* (Hiebert *et al.*, 1995), *Beet curly top virus* (Stanley *et al.*, 1986; Padidam *et al.*, 1995a) and *Pepper huasteco yellow vein virus* (Torres-Pacheco *et al.*, 1993). Although, complete viral genomes or sequences are very often used for this purpose, partial nucleotide sequences are also useful for geminivirus species identification where complete sequences are not available.

Padidam *et al.* (1995a), suggested the use of 5′end of the coat protein gene to show relationship among geminiviruses. Rojas (1992) used partial nucleotide sequence of the 5′end of the Rep gene and the common region to generate cladograms to show phylogenetic relationships among geminiviruses. Faria *et al.* (1994) showed the same clustering using the complete gene sequences. Using 450 nucleotides of the Rep gene and common region, Roye *et al.* (1997) established phylogenetic relationships between the Western Hemisphere and several weed-infecting geminiviruses from Jamaica.

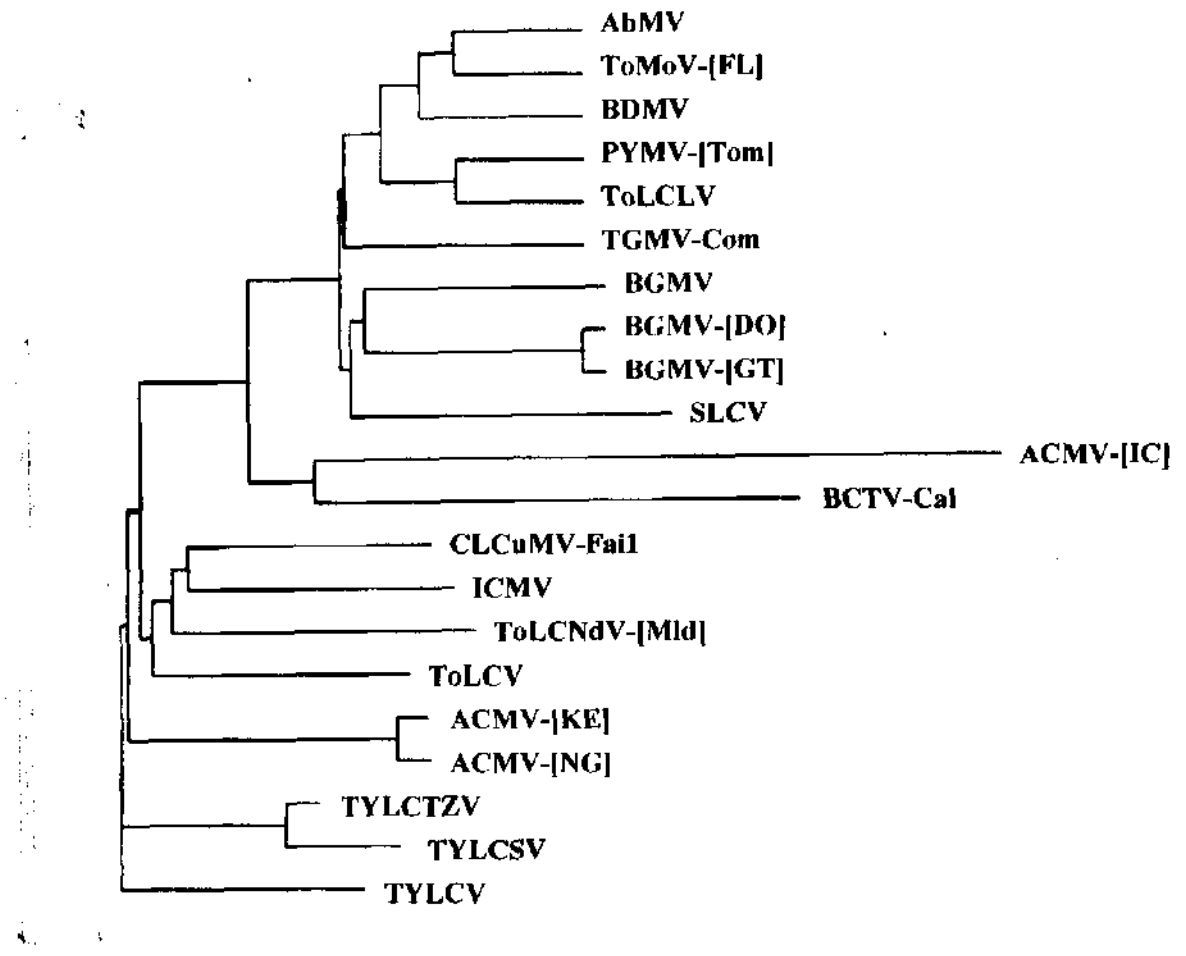


Figure 6: Phylogenetic relationship of *Tomato yellow leaf curl Tanzania virus* compared with other geminiviruses using the replication-associated protein sequences.

The results of this study have shown that TYLCTZV is closely related to the Mediterranean monopartite geminiviruses. Phylogenetic analysis showed that TYLCTZV is similar to TYLCSV. This was supported further by results from host range studies in which TYLCTZV did not infect *Phaseolus vulgaris*. Brunt *et al.* (1996) reported that *P. vulgaris* is not infected by TYLCSV. Hitherto, the Mediterranean strains of TYLCV have been considered to be prevalent in Tanzania. The RFLP analysis did not reveal variation among the TYLCTZV isolates. Sequence data of the viral clones from the isolates did not differ significantly either (96.7 and 94.9 % identity in CP and C1, respectively); hence, they are considered biologically and genetically similar. The isolates were also most similar (97 %) in coat protein to the *Tomato leaf curl Tanzania virus* (ToLCTZV).

4.3 Host range.

The results of host range studies are summarized in Table 7. All the TYLCV isolates used in this study did not infect *Gossypium hirsutum, Solanum melongena, Phaseolus vulgaris Glycine max, Arachis hypogea and Solanum tuberosum*. However, the virus was infected *Datura stramonium, Nicotiana glutinosa, N. tabacum, Capsicum annuum* and *L. esculentum*. *N. tabacum* was symptomeless, but infected. The infected plant species produced the same symptom types (Table 7) when inoculated with each of the TYLCTZV isolates. A test plant was considered to be a host of the virus if characteristic TYLCTZV symptoms were observed on the tomato cultivar Moneymaker (which was used as an indicator plant) after inoculation of the test plants and back indexing with the

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Table 7: Reaction of plant species when inoculated with isolates of *Tomato yellow leaf curl Tanzania virus.*

	Symptoms[a]/host status[b]					
Test plant	Arusha*	Kilimanjaro	Dodoma	Morogoro	Iringa	Coast
Gossypium hirsutum	ns/-	ns/-	ns/-	ns/-	ns/-	ns/-
Capsicum annuum	m,mo,vc/+	m,mo,vc/+	m,mo,vc/+	m,mo,vc/+	m,mo,vc/+	m,mo,vc/+
Datura stramonium	s,mo,lc/+	s,mo,lc/+	s,mo,lc/+	s,mo,lc/+	s,mo,lc/+	s,mo,lc/+
Nicotiana glutinosa	m,lc/+	m,lc/+	m,lc/+	m,lc/+	m,lc/+	m,lc/+
Nicotiana tabacum	ns/+	ns/+	ns/+	ns/+	ns/+	ns/+
Solanum melongena	ns/-	ns/-	ns/-	ns/-	ns/-	ns/-
Solanum tuberosum	ns/-	ns/-	ns/-	ns/-	ns/-	ns/-
Phaseolus vulgaris	ns/-	ns/-	ns/-	ns/-	ns/-	ns/-
Glycine max	ns/-	ns/-	ns/-	ns/-	ns/-	ns/-
Arachis hypogea	ns/-	ns/-	ns/-	ns/-	ns/-	ns/-
Lycopersicon esculentum	s,lc,y/+	s,lc,y/+	s,lc,y/+	s,lc,y/+	s,lc,y/+	s,lc,y/+

* *Tomato yellow leaf curl Tanzania virus* isolates

[a]ns = no symptoms, m = mild symptoms, s = severe symptoms, lc = leaf curling, mo = mottle or mosaic, vc = vein clearing, y = yellowing

[b]+ = host, - = non-host

B. tabaci. Similarly, a test plant was considered a non-host if typical TYLCTZV symptoms failed to develop on Moneymaker indicator plants by back indexing with *B. tabaci*.

The host range of a virus has been reported to vary with virus isolate. The reaction of the various species of plants tested to TYLCTZV isolates was similar to results reported earlier (Yassin and Nour, 1965; Makkouk, 1978; Mazyad *et al*., 1979; Cherif and Russo, 1983; Ioannou, 1985b;). However, unlike TYLCV (Cohen and Nitzany, 1966), the isolates from Tanzania did not infect *Phaseolus vulgaris* (Table 10), which has been reported as a non-host of Sardinian, Jordanian and Yemen isolates of TYLCV (Mansour and Al-Musa, 1992; Brunt *et al*., 1996).

Reaction of the weed species tested for TYLCTZV infection by both squash and dot blot assay is summarized in Plates 10 and 11. A total of 38 samples were tested with the nucleic acid probe for the presence of TYLCTZV. Of the 17 weed species dot blotted on nylon membranes, 12 hybridized to the nucleic acid probes (Plate 10). Dot blots of extracts from *Euphorbia heterophylla, Ipomea batatas, Amaranthus viridis*, and *Cassia obtusifolia* hybridized with very strong signals (4), while, *Amaranthus spinosus, Ageratum conyzoides,* and *Sida acuta* produced strong hybridization signals (3). Faint hybridization signals (2) were produced when extracts from *Commelina erecta, Erigeron floribundus, Bidens pilosa, Portulaca oleracea*, and *Euphorbia hirta* were tested. The remaining nine weed samples did not hybridize to the probe (Plate 10).

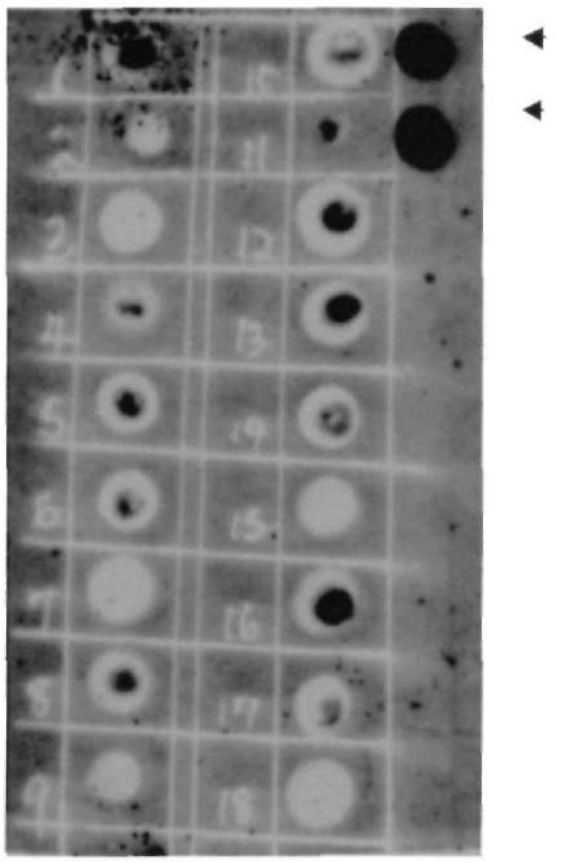


Plate 10: Dot blot analysis of extracts from weed species tested with DIG-labelled DNA probe. 1 = *Euphorbia heterophylla*; 2 = *Neonotonia whightii*; 3 = *Phaseolus vulgaris*; 4 = *Commelina erecta*; 5 = *Amaranthus spinosus*; 6 = *Erigeron floribundus*; 7 = *Ipomea eriopcarpa*; 8 = *Ageratum conyzoides*; 9 = *Synedrella nodiflora*; 10 = *Bidens pilosa*; 11 = *Sida acuta*; 12 = *Ipomea batatas*; 13 = *Amaranthus viridis*; 14 = *Portulaca oleracea*; 15 = *Tridax procumbens*; 16 = *Cassia obtusifolia*; 17 = *Euphorbia hirta*; 18 = Healthy *Lycopersicon esculentum*; Arrows indicate positive controls of TYLCSV (upper arrow) and TYLCTZV (lower arrow).

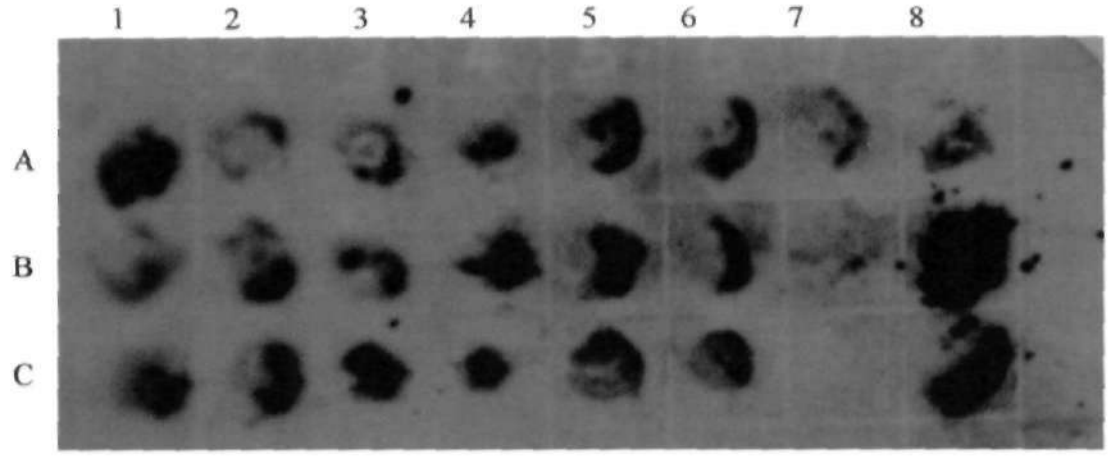


Plate 11: Squash blot analysis of extracts from weed species tested with DIG-labelled DNA probe. A1 = *Ageratum conyzoides*; A2 = *Cassia obtusifolia*; A3 = *Calopogonium mucunoides*; A4 = *Crotalaria retusa*; A5 = *Trianthema portulacastrum*; A6 = *Alternanthera sessilis*; A7 = *Amaranthus viridis*; A8 = *Celosia trigyna*; B1 = *Commelina diffusa*; B2 = *Bidens pilosa*; B3 = *Chromolaena odorata*; B4 = *Eclipta prostrata*; B5 = *Erigeron floribundus*; B6 = *Synedrella nodiflora*; B7 = *Euphorbia hirta*; B8 = Infected *Lycopersicon esculentum* (TYLCSV); C1 = *Cassia occidentalis*; C2 = *Spigelia anthelmia*; C3 = *Boerhavia diffusa*; C4 = *Physalis angulata*; C5 = *Sida acuta*; C6 = *Acanthospermum hispidium*; C7 = Healthy *Lycopersicon esculentum*; C8 = Infected *Lycopersicon esculentum* (TYLCTZV).

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The results of the squash blot analysis showed that all the weed samples gave positive signals when hybridized to the probe (Plate 11). Only blots from *Cassia obtusifolia, Commelina diffusa,* and *Euphorbia hirta* produced faint hybridization signals; the rest produced signals ranging from strong to very strong (Plate 11).

The inability of the virus to infect *P. vulgaris* while infecting other hosts shows that the virus is related to the Sardinia strain of TYLCV. *Capsicum annuum, D. stramonium* and *N. glutinosa* were identified as hosts of the virus in addition to the previously known host, *Lycopersicon esculentum. Nicotiana tabacum* was latently infected.

Hitherto, three weed species (*Achyranthes aspera, Euphorbia heterophylla* and *Nicandra physaloides*) have been reported as weed hosts of the virus in Tanzania (Nono-Womdim *et al.*, 1996). The differences in intensity of hybridization signals were probably, a reflection of the concentration of the virus present in the samples. With the exception of *E. heterophylla*, this study has revealed, for the first time, the presence of other non-cultivated hosts of the virus in Tanzania. These weed species included *Euphorbia heterophylla, Commelina erecta, Amaranthus spinosus, Erigeron floribundus, Ageratum conyzoides, Bidens pilosa, Sida acuta, Ipomea batatas, Amaranthus viridis, Portulaca oleracea, Cassia obtusifolia, Euphorbia hirta, Calopogonium mucunoides, Crotalaria retusa, Trianthema portulacastrum, Alternanthera sessilis, Celosia trigyna, Commelina diffusa, Chromolaena odorata, Eclipta prostrata, Synedrella nodiflora, Cassia occidentalis, Spigelia anthelmia,*

Boerhavia diffusa, Physalis angulata, and *Acanthospermum hispidium* (Plates 10 and 11). These twenty six plant species are potentially important TYLCTZV reservoirs because of one or more of the following reasons: 1) they occurred in abundant numbers within the tomato farms, 2) they were associated with whitefly vectors, and 3) many were found naturally infected with TYLCTZV. Some of the species are perennial in growth habit; others are herbaceous annuals or annual-perennial. These results indicate that, TYLCTZV survives in weeds within and outside tomato farms, which are potential sources of virus inoculum for primary and secondary spread of the disease. TYLCTZV-like symptoms observed in some of the weeds are shown in Plate 12. This is more precarious in areas where tomatoes are grown all-year round because of the possibility of the virus and the vectors becoming more adaptable to yet undetected hosts, some of which could be latently infected. This, by implication, complicates the strategies to manage and control the disease.

4.4 Transmission of TYLCTZV isolates

4.4.1 Graft transmission

The TYLCTZV was readily graft-transmitted to the test cultivar, Moneymaker (Plate 13). Initial symptoms developed within 2 - 4 weeks and the full syndrome within 1 - 2 months. Graft success of 80 % was achieved with the Dodoma, Coast and Iringa isolates compared with Kilimanjaro (73.33 %), Arusha (66.67 %), and Morogoro (66.67 %).

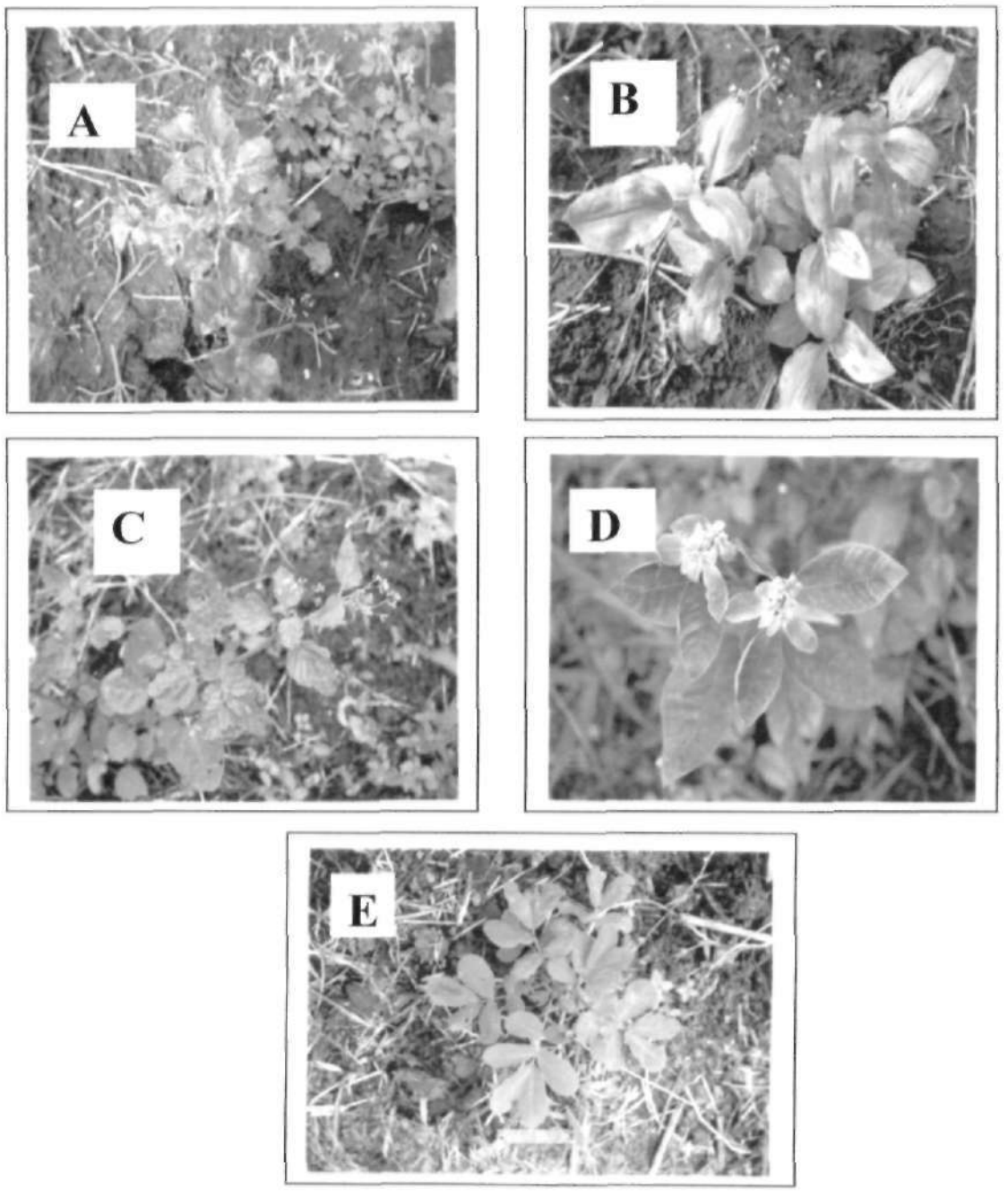


Plate 12: Weed species with *Tomato yellow leaf curl Tanzania virus*-like symptoms. A = *Ageratum conyzoides*, B = *Commelina erecta*, C = *Boerhavia diffusa*, D = *Euphorbia heterophylla*, E = *Cassia obtusifolia*.

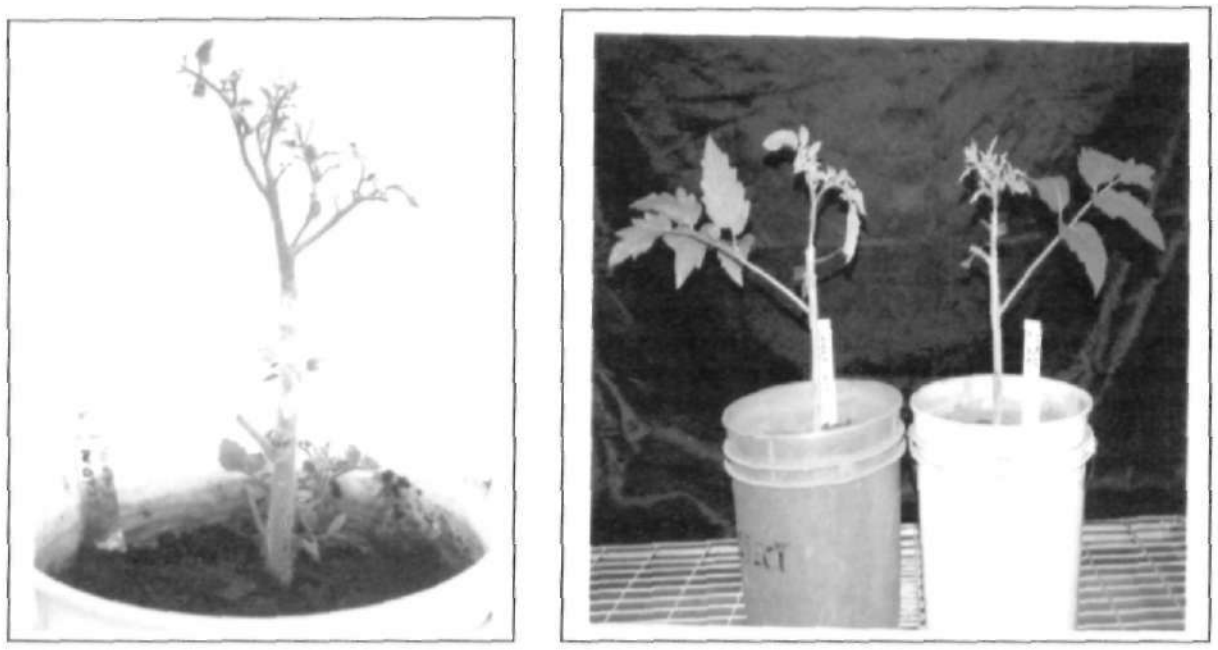


Plate 13: Tomato cultivar Moneymaker, graft-infected with *Tomato yellow leaf curl Tanzania virus*.

However, all the test plants succesfully grafted with the Dodoma and Morogoro isolates, were infected (100 %) with TYLCTZV 8 weeks after grafting compared with the Coast (91.67 %), Arusha (90 %), Kilimanjaro (81.81 %) and the Iringa (75 %) isolates. Similarly, TYLCTZV symptoms were most severe on plants graft-infected with the Dodoma (3.54) and Morogoro (3.12) isolates and least severe (1.68) on plants graft-inoculated with the Iringa isolate (Table 8).

Graft transmission of TYLCV had been reported (Ioannou, 1985b). The differences in disease incidence and symptom severity that were observed among the viral isolates were probably due to differences in the concentration of the virus in the scions at the time of grafting, physiological conditions of both scion and stock, and intial recognition activities in the infection build-up process between scion and stock.

4.4.2 Mechanical transmission

Characteristic TYLCTZV symptoms did not develop on all the inoculated test plants. Addition of PVP-40 in the inoculation buffer to improve transmission did not result in mechanical transmission of TYLCTZV. To confirm these results, the test plants were back-indexed using *B.tabaci*, yet no TYLCTZV symptoms developed. Similarly, none of the plants tested reacted positively with both the polyclonal and monoclonal antibody probes (Table 9).

Table 8: Comparative transmission efficiency of *Tomato yellow leaf curl Tanzania virus* isolates by grafting.

	Transmission			
Virus isolate	Rate[a]	Graft success (%)[b]	Incidence (%)[c]	Severity[d]
Arusha	9/10	66.67^{c}	90.0^{b}	2.56^{c}
Kilimanjaro	9/11	73.33^{b}	81.81^{c}	1.89^{d}
Dodoma	12/12	80.0^{a}	100.0^{a}	3.54^{a}
Morogoro	10/10	66.67^{c}	100.0^{a}	3.12^{ab}
Iringa	9/12	80.0^{a}	75.0^{d}	1.68^{d}
Coast	11/12	80.0^{a}	91.67^{b}	2.98^{bc}
CV (%)	2.02	2.24	4.16	
SE	1.21	2.01	0.07	

Means followed by the same letter within a column are not significantly different ($P \leq 0.05$) by Duncan's Multiple Range Tests

[a] Number of plants infected /number of plants successfully grafted

[b] (Number of plants successfully grafted/total number of plants grafted) x 100

[c] (Number of plants infected /number of plants successfully grafted) x 100

[d] Severity assessed based on scale of 0 (no symptoms)- 4 (very severe symptoms) (Friedmann *et al.*, 1998).

Table 9: Mechanical inoculation of plant species with *Tomato yellow leaf curl Tanzania* virus isolates.

Virus isolate	Number of plants tested/ Number with symptoms			OD at A $_{405nm}$*		
	Lycopersicon esculentum	*Datura stramonium*	*Nicotiana tabacum*	*Lycopersicon esculentum*	*Datura stramonium*	*Nicotiana tabacum*
Arusha	15/0	15/0	15/0	0	0	0
Kilimanjaro	15/0	15/0	15/0	0	0	0
Dodoma	15/0	15/0	15/0	0	1	0
Morogoro	15/0	15/0	15/0	0	0	0
Iringa	15/0	15/0	15/0	0	0	0
Coast	15/0	15/0	15/0	0	0	0

*Reaction of leaf extracts with polyclonal antibody measured at A_{405nm} 4 = > 1.8; 3 = 1.21 - 1.8; 2 = 0.61 - 1.2; 1 = 0.3 - 0.6; 0 = < 0.3

Results of transmission tests by mechanical inoculation, shows that TYLCTZV is not mechanically transmissible (Kashina *et al*., 2003a). These results corroborate previous reports that the yellow leaf curling symptoms caused by TYLCV isolates were not induced mechanically from tomato to tomato (Cohen and Nitzany, 1966; Ioannou, 1985b; Brown and Nelson, 1988; Credi *et al*., 1989). However, under artificial conditions, mechanical transmission of TYLCV to tomato from plants of *D. stramonium*, *N. glutinosa* and *L. pennellii*, has been achieved at low rate, and only in few cases from tomato to tomato (Makkouk *et al*., 1979; Abdel-Salam, 1990). Cytopathological changes associated with TYLCV infections have been observed in the nuclei of phloem parenchyma, companion cells and differentiating sieve tubes (Cherif and Russo, 1983; D'Hondt and Russo, 1985). This phloem localization is thought to be responsible for the difficulty in mechanical transmission, which distinguishes TYLCV from other bipartite geminiviruses capable of invading the mesophyll tissues (Wege *et al*., 1994). The TYLCTZV exhibited this difficulty.

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4.4.3 Virus-vector relationships

The minimum AAP for transmission of the virus isolates was 30 min except, for the Kilimanjaro and Iringa isolates, which were transmitted after acquiring the virus for 1 h (Table 10). After exposing the insects to 48 h feeding on source plants, they were able to transmit the virus from the six isolates to all the test plants. Similarly, the virus isolates were transmitted after 30 min following a 24-h AAP (Table 11). Thereafter, transmission increased with increase in AAP and IAP. Transmission of the Dodoma and

Table 10: Effect of acquisition access time on the transmission of *Tomato yellow leaf curl Tanzania virus* isolates.

Virus isolate	Rate of transmission								
	Acquisition Access Time (AAP)								
	10 min	30 min	1 h	2 h	4 h	8 h	16 h	24 h	48 h
Arusha	0/15	1/15	3/15	6/15	6/15	9/15	12/15	12/15	15/15
Kilimanjaro	0/15	0/15	2/15	6/15	6/15	11/15	12/15	12/15	15/15
Dodoma	0/15	2/15	4/15	6/15	7/15	12/15	13/15	15/15	15/15
Morogoro	0/15	2/15	3/15	4/15	7/15	11/15	13/15	15/15	15/15
Iringa	0/15	0/15	2/15	5/15	6/15	8/15	11/15	11/15	15/15
Coast	0/15	1/15	3/15	5/15	6/15	9/15	12/15	12/15	15/15

Table 11: Effect of inoculation access period on the transmission of *Tomato yellow leaf curl Tanzania virus* isolates.

Virus isolate	Inoculation Access Time (IAP)								
	10 min	30 min	1 h	2 h	4 h	8 h	16 h	24 h	48 h
Arusha	0/15	2/15	4/15	7/15	9/15	11/15	13/15	15/15	15/15
Kilimanjaro	0/15	2/15	3/15	5/15	8/15	9/15	12/15	15/15	15/15
Dodoma	0/15	2/15	5/15	8/15	10/15	13/15	15/15	15/15	15/15
Morogoro	0/15	2/15	4/15	7/15	9/15	12/15	15/15	15/15	15/15
Iringa	0/15	1/15	3/15	6/15	8/15	9/15	11/15	15/15	15/15
Coast	0/15	1/15	2/15	7/15	9/15	9/15	13/15	15/15	15/15

Morogoro isolates to all the test plants was achieved after an AAP and IAP of 24 and 16 h, respectively. The other virus isolates were transmitted to all test plants when the insects were allowed an IAP of 24 h (Table 11).

The minimum AAP was 30 min for each of the TYLCTZV isolates used, except for the Kilimanjaro and Iringa isolates, which had a minimum AAP of 1 h. However, all the TYLCTZV isolates tested had a minimum IAP of 30 min. A minimum AAP of 1 h was reported for *Tomato leaf crumple virus* (TolCrV), a whitefly-transmitted virus (Brown and Nelson, 1988). Mansour and Al-Musa (1992), reported a minimum AAP of 1 h for TYLCV. The increase in the number of infected plants with increasing AAP, suggests that a possible dose response relationship existed between the vectors and TYLCTZV isolates. It could also mean more females were involved in transmitting the virus than males.

After a 24-h AAP, the whiteflies retained the ability to transmit the virus for up to 11 days for the Dodoma and Morogoro isolates. There was a gradual decline in the number of plants showing symptoms after the 4^{th} day of transfer except for the Morogoro and Dodoma isolates whose decline started after the 5^{th} day (Table 12).

The ability of the whiteflies to retain the virus for 10 -11 days after acquisition, was an indication of a persistent type of relationship between the virus and the host. Similar observations have been reported elsewhere (Cohen and Nitzany, 1966; Rubinstein and

Table 12: Persistence of *Tomato yellow leaf curl Tanzania virus* isolates in *Bemisia tabaci*.

Virus isolate	Number of test plants infected out of five													
	1*	2	3	4	5	6	7	8	9	10	11	12	13	14
Arusha	3	5	5	5	4	4	2	2	1	1	0	0	0	0
Kilimanjaro	4	5	5	5	3	3	2	1	1	0	0	0	0	0
Dodoma	5	5	5	5	5	4	3	2	1	1	1	0	0	0
Morogoro	5	5	5	5	5	4	3	2	1	1	1	0	0	0
Iringa	4	5	5	5	4	2	2	1	1	0	0	0	0	0
Coast	5	5	5	5	4	3	2	1	1	0	0	0	0	0

*Indicates days

Czosnek, 1997). *Tomato yellow leaf curl virus* is transmitted in a persistent and circulative manner by the whitefly vectors. Antignus *et al.* (1994a) reported that the CP is only retained in the vector for 10 days after an AAP of 48 h. This means that the CP influences vector infectivity since viruliferous vectors have been reported to remain infective for 10 – 12 days (Ioannou, 1985b; Mansour and Al-Musa, 1992).

4.4.4 Vector transmission

Whitefly transmission of the TYLCTZV to healthy tomato plants was achieved for all the viral isolates collected from the six regions. The highest virus incidence was recorded for the Dodoma isolate followed by the Morogoro isolate. The two isolates, followed by the isolate from the Coast region, were the most severe, (severity scores of 3.62, 3.21 and 3.12 respectively), while the Arusha and Iringa isolates were mild (Table 13).

Results of the current investigation indicated that there were significant differences in the transmission of TYLCTZV isolates from the different locations by the whitefly vectors, and those that were readily transmitted were the most severe. The overall situation, including the results of the virus-vector relationship studies is assessed in deducing a possible explanation for the observed differences. Although the minimum AAP and IAP for all isolates (except 2 for AAP) was 30 min, there was preferential acquisition and transmission of the different viral isolates by the whitefly vectors with increasing time.

Table 13: Transmission efficiency of *Tomato yellow leaf curl Tanzania virus* isolates by *Bemisia tabaci*.

Virus isolate	Transmission		
	Rate*	Incidence** (%)	Severity***
Arusha	12/15	80.0^{cd}	2.71^{ab}
Kilimanjaro	11/15	73.33^{d}	2.0^{b}
Dodoma	15/15	100.0^{a}	3.62^{a}
Morogoro	14/15	93.33^{ab}	3.21^{a}
Iringa	12/15	80.0^{cd}	1.92^{b}
Coast	13/15	86.67^{bc}	3.12^{a}
CV (%)	2.85	8.08	
SE	0.07	0.02	

Means followed by the same letter within a column are not significantly different ($P \leq 0.05$) by Duncan's Multiple Range Tests

*Number of plants infected /number of plants tested

**(Number of plants infected /number of plants tested) x 100.

***Severity assessed on a scale of 0 - 4 (Friedmann *et al.*, 1998).

The isolates from Dodoma and Morogoro were readily acquired and transmitted as the AAP increased (Table 10). They were also more readily transmitted with increasing IAP compared to other isolates (Table 11). Similarly, the two were more persistent in the vector than the other isolates (Table 12). Therefore, it can be deduced that a complementary mechanism seems to exist whereby isolates that are more readily acquired and persists longer in the vector are more efficiently transmitted than the others, and hence, show higher incidence and severity.

4.5 Evaluation of tomato genotypes for resistance to TYLCTZV

4.5.1 Screenhouse experiments

Tomato genotypes were evaluated in the screenhouse to determine their levels of resistance to TYLCTZV (Plate 14). Infected tomato plants of the different genotypes differed significantly in the severity of symptoms assessed. Tomato plants of the susceptible genotypes Moneymaker and Marmande were most severely affected by the virus and were the first to show symptoms, which commenced with chlorosis and yellowing, but gradually advanced in severity to the cupping of the leaf lamina and stunting of the plants (Table 14). The tolerant cv. Tengeru 97, exhibited only mild symptoms, while plants of the resistant control, TY172 were symptomless. Other genotypes varied in symptom expression from mild to severe symptoms. There were significant differences in the effect of TYLCTZV on yield components when the inoculated genotypes were compared with their corresponding un-infected controls. However, there were no significant differences in all the paramcters assessed between



Plate 14: An overview of the screenhouse evaluation tests of tomato genotypes for resistance to *Tomato yellow leaf curl Tanzania virus*.

Table 14: Severity of *Tomato yellow leaf curl Tanzania virus* disease in infected tomato genotypes evaluated in the screenhouse.

Genotype§	Mean severity score
Moneymaker	3.7^{a}
Marmande	3.7^{a}
CLN4	3.0^{4}
CLN1	2.8^{bc}
CLN6	2.8^{bc}
TLB	2.8^{bc}
CLN3	2.8^{bc}
Marglobe	2.8^{bc}
Cal-J	2.7^{bcd}
CLN7	2.7^{bcd}
CLN2	2.6^{bcd}
CLN5	2.6^{bcd}
H24	2.4^{cd}
Tengeru 97	2.3^{d}
TY172	0.0^{e}
SE	0.04
CV (%)	12.01
R^2	0.74

Means followed by the same letter(s) are not statistically significant at $P \leq 0.05$ according to Duncan's Multiple Range Tests.

§See Appendix 3 for full nomenclature and sources of tomato genotypes

the inoculated and un-inoculated plants of the resistant genotype (TY172) (Table 15). The infected susceptible plants of genotypes Marmande and Moneymaker did not develop normally: the infected plants did not produce fruits compared to 3.5 and 4.6 kg produced by un-inoculated plant (Table 15). When the relative total yield per plant of inoculated versus un-inoculated plants of each respective entry was compared, line TY172 and cultivar Tengeru 97 performed much better than the other genotypes tested. The inoculated plants produced 75 and 50 %, respectively, of the total yield of their respective un-inoculated control plants (Table 15). In contrast, the infected cultivars Cal-J and Marglobe produced 41 % when compared to the un-inoculated control plants. The other genotypes produced between 5 and 34 % of the yield of their respective un-inoculated control plants. When the performance of the inoculated plants of the genotypes was ranked to assess the effect of the virus on yield, plants of genotype TY172 were least affected, producing a significantly higher yield compared with the other test genotypes. Next, were plants of the tolerant cultivar Tengeru 97, Cal-J and Marglobe (Table 16). The yield of the susceptible genotypes was most severely affected.

One of the most pronounced effects of TYLCTZV on tomato plants is a severe stunting of growth (Antignus and Cohen, 1994). In this study, infected plants of genotype TY172, again exhibited the best resistance level, as measured by relative plant fresh weight, with a statistically insignificant reduction in fresh weight compared to the un-inoculated control plants (Table 15).

Table 15: Comparison of yield and yield components between inoculated and un-inoculated plants of tomato genotypes evaluated in the screenhouse.

Genotype[§]	Fresh weight/plant (kg)	Fruit number/plant	Weight/fruit (g)	Average total yield (kg)
Cal-J				
Un-inoculated	**1.86**	40.7	137.1	**5.6**
Inoculated	0.18	25.7	86.5	2.3
P<	0.0001	0.0055	0.0005	0.0011
CLN1				
Un-inoculated	**1.43**	36.3	122.4	**4.6**
Inoculated	0.10	19.3	65.2	1.3
P<	0.0001	0.0002	0.0001	0.0001
CLN2				
Un-inoculated	1.48	37.7	126.9	**4.8**
Inoculated	0.14	18.7	62.9	1.2
P<	0.0001	0.0001	0.0001	0.0003
CLN3				
Un-inoculated	1.74	30.4	102.6	**3.2**
Inoculated	**0.1**	17.7	59.5	1.1
P <	0.0001	0.0001	0.0001	0.0001
CLN4				
Un-inoculated	0.98	45.7	153.9	**7.2**
Inoculated	**0.18**	16.7	56.2	**1.0**
P<	0.0001	0.0001	0.0001	0.0001
CLN5				
Un-inoculated	1.40	43.2	145.7	**6.3**
Inoculated	0.09	22.3	75.26	1.7
P <	0.0001	0.0001	0.0001	0.0001
CLN6				
Un-inoculated	**1.52**	**40.4**	136.3	**5.6**
Inoculated	0.09	15.3	51.7	0.8
P <	0.0001	0.0001	0.0001	0.0001
CLN7				
Un-inoculated	**1.62**	40.3	135.9	**5.5**
Inoculated	**0.08**	**16.7**	56.2	1.0
P <	0.0001	0.0001	0.0001	0.0001
H24				
Un-inoculated	**1.56**	50.5	170.4	**8.7**
Inoculated	**0.15**	**18.0**	60.7	1.1
P <	0.0001	0.0001	0.0001	0.0001
Marglobe				
Un-inoculated	**1.88**	39.7	133.7	5.3
Inoculated	0.21	25.7	86.5	2.2
P <	0.0001	0.0067	0.0001	0.0001

Moneymaker				
Un-inoculated	1.80	32.0	107.8	3.5
Inoculated	0.19	0.0	0.0	0.0
P <	0.0001	0.0001	0.0001	0.0001
Marmande				
Un-inoculated	1.84	36.0	122.1	4.6
Inoculated	0.15	0.0	0.0	0.0
P <	0.0001	0.0001	0.0001	0.0001
Tengeru 97				
Un-inoculated	1.84	37.4	126.2	4.8
Inoculated	0.75	26.7	89.87	2.4
P <	0.0001	0.0127	0.0001	0.0001
TLB				
Un-inoculated	0.98	44.3	149.4	6.8
Inoculated	0.23	17.7	59.5	1.1
P <	0.0001	0.0001	0.0001	0.0001
TY 172				
Un-inoculated	1.55	39.3	132.6	5.2
Inoculated	1.53	34.0	114.6	3.9
P >	0.5471	0.8669	0.8901	0.7494

[§]See Appendix 3 for full nomenclature and sources of tomato genotypes

Table 16: Effect of *Tomato yellow leaf curl Tanzania virus* on yield and yield components of tomato genotypes evaluated in the screenhouse.

	Plant fresh weight (kg)		Fruit number per plant		Mean fruit weight (g)		Yield per plant (kg)	
Genotype[§]	U	I	U	I	U	I	U	I
Cal-J	1.86^{a}	0.18^{f}	40.7^{cde}	25.7^{b}	137.05^{cde}	86.50^{b}	5.6^{cde}	2.3^{b}
Marglobe	1.86^{a}	0.21^{d}	39.7^{de}	25.7^{b}	133.68^{de}	86.50^{b}	5.3^{de}	2.2^{b}
Tengeru	1.84^{b}	0.75^{b}	37.4^{e}	26.7^{b}	126.19^{e}	89.87^{b}	4.8^{e}	2.4^{b}
Marmande	1.84^{b}	0.15^{g}	36.0^{e}	0.0^{g}	122.07^{e}	0.0^{g}	4.6^{ef}	0.0^{f}
Moneymaker	1.80^{c}	0.19^{e}	32.0^{f}	0.0^{g}	107.84^{f}	0.0^{g}	3.5^{fg}	0.0^{f}
CLN3	1.74^{d}	0.10^{j}	30.4^{f}	17.7^{def}	102.6^{f}	59.54^{def}	3.2^{g}	1.1^{de}
CLN7	1.62^{e}	0.08^{k}	40.3^{cde}	16.7^{ef}	135.92^{cde}	56.17^{ef}	5.5^{de}	1.0^{de}
H24	1.56^{f}	0.15^{g}	50.5^{a}	18.0^{de}	170.37^{a}	60.66^{de}	8.7^{a}	1.1^{de}
TY172	1.55^{f}	1.53^{a}	39.3^{de}	34.0^{a}	132.55^{de}	114.58^{a}	5.2^{de}	3.9^{a}
CLN6	1.52^{g}	0.09^{j}	40.4^{cde}	15.3^{f}	136.3^{cde}	51.67^{f}	5.6^{cde}	0.8^{e}
CLN2	1.48^{h}	0.14^{h}	37.7^{e}	18.7^{de}	126.94^{e}	62.91^{de}	4.8^{e}	1.2^{d}
CLN1	1.43^{i}	0.10^{j}	36.3^{e}	19.3^{d}	122.44^{e}	65.15^{d}	4.6^{ef}	1.3^{d}
CLN5	1.40^{j}	0.09^{j}	43.2^{bcd}	22.3^{c}	145.66^{bcd}	75.26^{c}	6.3^{bcd}	1.7^{c}
CLN4	0.98^{k}	0.18^{f}	45.7^{b}	16.7^{ef}	153.9^{b}	56.17^{ef}	7.2^{b}	1.0^{de}
TLB	0.98^{k}	0.23^{c}	44.3^{bc}	17.7^{def}	149.4^{bc}	59.54^{def}	6.8^{bc}	1.1^{de}
CV (%)	1.34	3.09	11.11	12.51	11.11	12.51	21.97	24.80
R^2	0.99	0.99	0.59	0.94	0.59	0.94	0.59	0.90

Means followed by the same letter(s) are not statistically significant at $P \leq 0.05$ according to Duncan's Multiple Range Tests.

[§]See Appendix 3 for full nomenclature and sources of tomato genotypes

*U = Un-inoculated, I = Inoculated

All the other genotypes including the tolerant cultivar Tengeru 97, suffered significant plant fresh weight losses compared to the corresponding un-inoculated control plants. Similarly, when the other yield components were analysed to address the effect of viral infection on yield, there was significant reduction in fruit number and fruit weight per infected plant for all the test genotypes, except the resistant genotype TY172, which had insignificantly reduced fruit number and fruit weight compared with un-inoculated control plants. However, the un-inoculated plants of genotype 1124 produced the highest number (50.5) of fruits followed by CLN4 (45.7).

4.5.2 Field experiments

Plate 15 shows an overview of the field evaluation experiment. The trend of results of the field experiments was similar to that obtained in the screenhouse experiments when disease severity was assessed among inoculated tomato plants (Table 17). Disease severity was not significantly affected by the season. However, there were significant ($P<0.05$) variations in disease severity between genotypes. Disease symptoms were most severe on infected plants of genotype Marmande and Moneymaker. This was significantly different ($P < 0.05$) from the severity recorded on infected plants of other genotypes. No disease symptoms were observed on plants of genotype TY172 (Table 17).

In 2000/01, there were highly significant differences in all the parameters assessed between inoculated and un-inoculated plants of the tomato genotypes tested.

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Plate 15: An overview of the field experiment to evaluate the reponses of tomato genotypes to *Tomato yellow leaf curl Tanzania virus* at the Horticulture farm, Sokoine University of Agriculture, Morogoro.

Table 17: Severity of *Tomato yellow leaf curl Tanzania virus* disease in infected tomato genotypes evaluated in the field at Sokoine University of Agriculture.

Genotype§	Mean severity score
Marmande	3.74^{a}
Moneymaker	3.71^{a}
CLN6	3.0^{b}
Marglobe	2.84^{bc}
CLN7	2.84^{bc}
CLN1	2.78^{cd}
CLN2	2.68^{cde}
CLN4	2.68^{cde}
CLN5	2.68^{cde}
CLN3	2.68^{cde}
Cal-J	2.59^{def}
TLB	2.53^{ef}
H24	2.47^{f}
Tengeru 97	2.28^{g}
TY172	0.0^{h}
SE	0.04
CV (%)	11.97
R^2	0.74

Means followed by the same letter(s) are not statistically significant at $P \leq 0.05$ according to Duncan's Multiple Range Test.

§See Appendix 3 for full nomenclature and sources of tomato genotypes

Only infected plants of the resistant genotype TY172 did not suffer significant reduction in all parameters when the infected plants were compared with their respective un-inoculated control plants (Table 18). The tomato-growing season neither significantly affected the yield parameters nor the treatment. However, the values were generally lower than those obtained in the screenhouse experiment. None of the plants of the resistant control TY172 exhibited symptoms. The infected plants of the resistant tomato genotype TY172 had the highest mean plant fresh weight, fruit weight and relative total fruit yield, which were not significantly different from the respective values of the un-inoculated plant (Table 18). The infected plants of the susceptible genotypes Moneymaker and Marmande produced no fruits leading to total (100 %) yield loss. The infected plants of the tolerant cultivar, Tengeru 97 had its yield reduced by 83 %. Other tomato genotypes recorded yield losses above 95 %.

Results of the 2001/02 experiments followed similar trends (Table 19). When the infected plants were ranked, the resistant genotype TY172 and tolerant cultivar, Tengeru 97, consistently produced the highest plant fresh weight, fruit number, fruit weight and fruit yield, followed by either genotype TLB (plant weight) or Cal-J (fruit number, fruit weight and fruit yield) (Table 20). Tomato genotype CLN6 produced the least plant fresh weight, while infected plants of the susceptible genotypes, Marmande and Moneymaker, consistently produced the least number of fruits, fruit weight and fruit yield.

Table 18: Effect of *Tomato yellow leaf curl Tanzania virus* on yield and yield components of tomato genotypes evaluated in field experiments at Sokoine University of Agriculture in 2000/01.

Genotype[§]	Mean plant fresh weight (kg)	Mean fruit number per plant	Mean fruit weight (g)	Average total yield (kg)
Cal-J				
Un-inoculated	1.79	52	185.64	9.1
Inoculated	0.19	15.2	54.15	0.9
P<	0.0001	0.0001	0.0001	0.0001
CLN1				
Un-inoculated	1.37	22.3	79.73	2.1
Inoculated	0.11	8.7	30.94	0.3
P<	0.0001	0.0001	0.0001	0.0001
CLN2				
Un-inoculated	1.51	31	110.67	3.2
Inoculated	0.15	7.2	25.59	0.2
P<	0.0001	0.0001	0.0001	0.0009
CLN3				
Un-inoculated	1.61	34.7	123.76	4.1
Inoculated	0.11	11.2	39.87	0.5
P<	0.0001	0.0001	0.0001	0.0001
CLN4				
Un-inoculated	0.98	37.2	132.69	5.1
Inoculated	0.17	13.2	47	0.6
P<	0.0001	0.0001	0.0001	0.0001
CLN5				
Un-inoculated	1.32	40.5	144.59	6.0
Inoculated	0.10	10	35.7	0.4
P<	0.0001	0.0001	0.0001	0.0004
CLN6				
Un-inoculated	1.40	39.5	141.02	5.4
Inoculated	0.07	8.2	29.16	0.3
P<	0.0001	0.0001	0.0001	0.0001
CLN7				
Un-inoculated	1.40	43.5	155.3	6.3
Inoculated	0.07	9.3	33.32	0.3
P<	0.0001	0.0001	0.0001	0.0001
H24				
Un-inoculated	1.45	42.7	152.32	6.7
Inoculated	0.16	14	49.98	0.7
P<	0.0001	0.0001	0.0001	0.0001

Marglobe				
Un-inoculated	1.80	45	160.65	6.5
Inoculated	0.21	10.7	38.08	0.4
P<	0.0001	0.0001	0.0001	0.0001
Moneymaker				
Un-inoculated	1.76	49.7	177.31	8.9
Inoculated	0.12	0.0	0.0	0.0
P<	0.0001	0.0001	0.0001	0.0001
Marmande				
Un-inoculated	1.73	52.7	188.02	9.8
Inoculated	0.14	0.0	0.0	0.0
P<	0.0001	0.0001	0.0001	0.0001
Tengeru 97				
Un-inoculated	1.83	51.5	183.86	10.3
Inoculated	0.87	39.8	142.21	5.7
P<	0.0001	0.0001	0.0001	0.0001
TLB				
Un-inoculated	0.96	43.3	154.7	6.7
Inoculated	0.22	15.8	56.53	0.9
P<	0.0001	0.0001	0.0001	0.0001
TY 172				
Un-inoculated	1.88	54.7	195.16	11.6
Inoculated	1.55	53.7	191.59	10.3
P>	0.8501	0.3539	0.2032	0.6211

[§]See Appendix 3 for full nomenclature and sources of tomato genotypes

Table 19: Effect of *Tomato yellow leaf curl Tanzania virus* on yield and yield components of tomato genotypes evaluated in field experiments at Sokoine University of Agriculture in 2001/02.

Genotype[§]	Fresh weight/plant (kg)	Fruit number/plant	Weight/fruit (g)	Average total yield (kg)
Cal-J				
Un-inoculated	1.67	48.5	173.2	8.4
Inoculated	0.20	16.5	58.9	1.1
P<	0.0001	0.0001	0.0001	0.0001
CLN1				
Un-inoculated	1.51	24.8	88.7	2.3
Inoculated	0.15	10.1	36.3	0.4
P<	0.0001	0.0001	0.0001	0.0001
CLN2				
Un-inoculated	1.52	28.5	101.8	2.9
Inoculated	0.11	6	21.4	0.2
P<	0.0001	0.0001	0.0001	0.0005
CLN3				
Un-inoculated	1.65	32.5	116.0	3.8
Inoculated	0.11	8	28.6	0.3
P<	0.0001	0.0001	0.0001	0.0001
CLN4				
Un-inoculated	0.97	37.7	134.47	5.1
Inoculated	0.19	13.7	48.8	0.7
P<	0.0001	0.0001	0.0001	0.0003
CLN5				
Un-inoculated	1.37	41.2	147.0	6.1
Inoculated	0.10	8	28.6	0.3
P<	0.0001	0.0001	0.0001	0.0001
CLN6				
Un-inoculated	1.43	38.3	136.9	5.3
Inoculated	0.07	9	32.1	0.3
P<	0.0001	0.0001	0.0001	0.0005
CLN7				
Un-inoculated	1.60	40.2	143.4	5.8
Inoculated	0.10	11.3	40.5	0.5
P<	0.0001	0.0001	0.0001	0.0001
H24				
Un-inoculated	1.47	43.3	154.7	6.8
Inoculated	0.16	14.8	53.0	0.8

P<	0.0001	0.0001	0.0001	0.0001
Marglobe				
Un-inoculated	1.75	40	142.8	5.7
Inoculated	0.14	12.5	44.6	0.6
P<	0.0001	0.0001	0.0001	0.0001
Moneymaker				
Un-inoculated	1.70	50.2	179.1	9.0
Inoculated	0.13	0.0	0.0	0.0
P<	0.0001	0.0001	0.0001	0.0001
Marmande				
Un-inoculated	1.79	51.7	184.5	9.6
Inoculated	0.14	0.0	0.0	0.0
P<	0.0001	0.0001	0.0001	0.0001
Tengeru 97				
Un-inoculated	1.84	55	196.4	10.9
Inoculated	0.82	40	142.8	5.7
P<	0.0001	0.003	0.0001	0.0001
TLB				
Un-inoculated	0.97	42.7	152.3	6.6
Inoculated	0.22	12.8	45.8	0.6
P<	0.0001	0.0001	0.0001	0.0001
TY 172				
Un-inoculated	1.83	58.8	210.0	12.4
Inoculated	1.62	54.8	195.8	10.8
P>	0.9803	0.3520	0.8675	0.3747

[§]See Appendix 3 for full nomenclature and sources of tomato genotypes

Table 20: Effect of *Tomato yellow leaf curl Tanzania virus* on yield and yield components of tomato genotypes evaluated in field experiments at Sokoine Univerisity of Agriculture.

	Plant fresh weight (kg)		Fruit number per plant		Mean fruit weight (g)		Yield per plant (kg)	
Genotype[§]	U*	I	U	I	U	I	U	I
TY172	1.85^{a}	1.58^{a}	56.8^{a}	54.3^{a}	202.6^{a}	193.7^{a}	11.6^{a}	10.6^{a}
Tengeru	1.84^{a}	0.84^{b}	53.3^{b}	39.9^{b}	190.1^{b}	142.5^{b}	10.3^{b}	5.7^{b}
Marglobe	1.77^{b}	0.18^{e}	42.5^{de}	11.6^{e}	151.7^{de}	41.4^{e}	6.5^{de}	0.5^{ef}
Marmande	1.75^{bc}	0.14^{g}	52.2^{b}	0.0^{i}	186.2^{b}	0.0^{i}	9.8^{b}	0.0^{h}
Cal-J	1.73^{c}	0.20^{d}	50.3^{c}	15.8^{c}	179.4^{c}	56.5^{c}	9.1^{c}	1.0^{c}
Moneymaker	1.73^{c}	0.13^{h}	49.9^{c}	0.0^{i}	178.2^{c}	0.0^{e}	8.9^{c}	0.0^{h}
CLN3	1.63^{d}	0.11^{i}	33.6^{g}	9.6fg	119.9^{g}	34.2^{fg}	4.1^{h}	0.4^{fg}
CLN2	1.52^{e}	0.13^{gh}	29.8^{h}	6.9^{h}	106.2^{h}	23.5^{h}	3.2^{i}	0.2^{gh}
CLN7	1.50^{e}	0.08^{j}	41.8^{de}	10.3^{f}	149.3^{de}	36.9^{f}	6.3^{de}	0.4^{fg}
H24	1.46^{f}	0.16^{f}	43^{d}	14.4^{d}	153.5^{d}	51.5^{d}	6.7^{d}	0.8^{cd}
CLN1	1.44^{fg}	0.13^{gh}	23.6^{i}	9.4^{fg}	84.2^{i}	33.6^{fg}	2.1^{j}	0.3^{fg}
CLN6	1.42^{g}	0.07^{j}	38.9^{f}	8.6^{g}	138.9^{f}	30.6^{g}	5.4^{fg}	0.3^{fg}
CLN5	1.34^{h}	0.10^{i}	40.8^{e}	9^{fg}	145.8^{e}	32.1^{fg}	6.0^{ef}	0.3^{fg}
CLN4	0.98^{i}	0.18^{e}	37.4^{f}	13.4^{d}	133.6^{f}	47.9^{d}	5.1^{g}	0.7^{de}
TLB	0.96^{i}	0.22^{c}	43^{d}	14.3^{d}	153.5^{d}	51.2^{d}	6.7^{d}	0.8^{cd}
CV (%)	4.40	9.21	8.78	18.38	8.78	18.38	17.83	32.03
R^2	0.95	0.99	0.86	0.97	0.86	0.97	0.84	0.97

Means followed by the same letter(s) are not statistically significant at $P \leq 0.05$ according to Duncan's Multiple Range Test.

[§]See Appendix 3 for full nomenclature and sources of tomato genotypes

*U = Non-inoculated, I = Inoculated

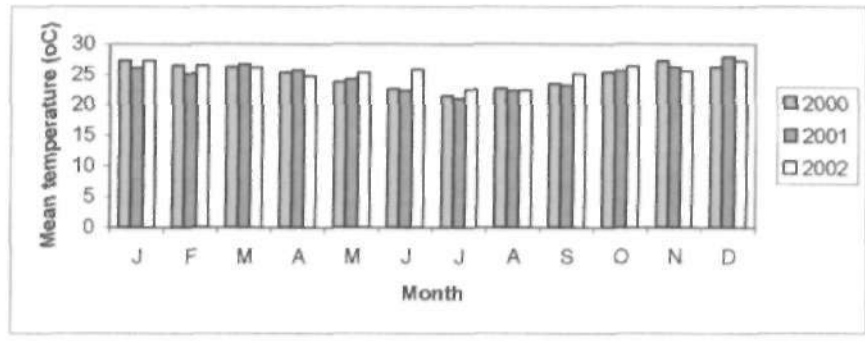
Similarly, results of the yield parameters of un-inoculated plants of tomato genotypes showed that the TY172 and Tengeru 97, consistently produced the highest plant fresh weight, fruit number, fruit weight and fruit yield, followed by Marglobe (plant fresh weight) or Marmande (fruit number, fruit weight and fruit yield). Tomato genotype TLB produced the least plant fresh weight, while CLN1 consistently produced the least fruit number, fruit weight and fruit yield (Table 20). The parameters assessed were significantly positively correlated to one another (Table 21).

Infection by TYLCTZV causes severe yield losses in the tomato crop. Undoubtedly, the best way to reduce TYLCTZV-induced damage is by breeding tomatoes resistant to the virus. The resistance level of tomato genotypes alongside resistant and susceptible controls, was evaluated in the screenhouse and in the field by comparing the yield performance of inoculated versus un-inoculated plants. The various genotypes were evaluated for the extent of yield loss per plant caused by the viral infection compared to un-inoculated control plants, as well as for reduction in fruit number and plant fresh weight. The experiments were conducted during periods of favourable environmental conditions for high symptom severity (average maximum temperature values were 27.4 and 30.5° C, and relative humidity values were 77.7 and 76.1% for the 2001 and 2002 experiments, respectively) (Fig. 7). The inoculum pressure was high and plants were inoculated at an early stage of development. Indeed, the infected susceptible genotypes Moneymaker and Marmande did not produce fruits, and no yield was recorded in the screenhouse and field tests.

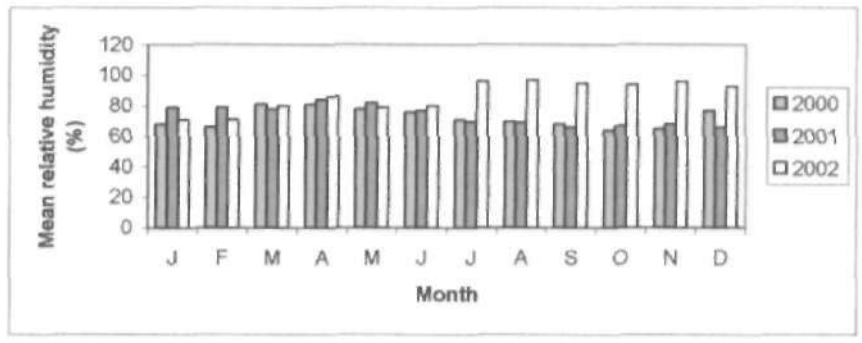
Table 21: Partial correlation coeffiecients of yield and yield components of tomato genotypes evaluated in field experiments at Sokoine University of Agriculture.

	Plant fresh weight	Number of fruits per plant	Mean fruit weight	Average yield per plant
Plant fresh weight	1.0**	0.13**	0.13**	0.12**
Number of fruits per plant	0.13**	1.0**	1.0**	0.91**
Mean fruit weight	0.13**	1.0**	1.0**	0.91**
Average yield per plant	0.12**	0.91**	0.91**	1.0**

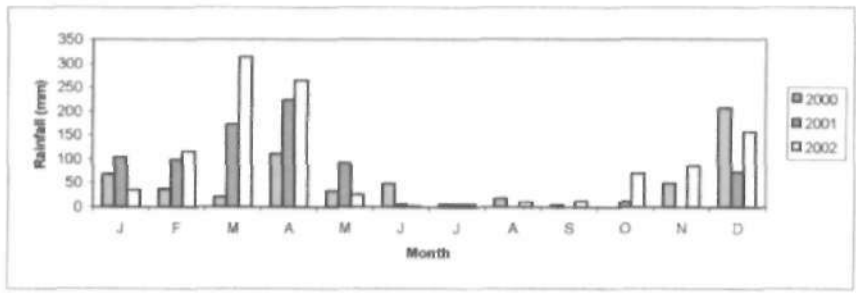
** Statistically significant at $P < 0.0001$



A



B



C

Figure 7: Weather data showing the mean temperature (A), relative humidity (B) and rainfall conditions (C) at the Sokoine University of Agriculture where the tomato genotypes were evaluated in the field.

However, the infected resistant genotype TY172 was able to grow and set fruits. Consequently, the plants resisted the adverse effects of the virus even under the extreme inoculation conditions. The infected plants of the other genotypes performed poorly, in some cases even lower than the susceptible genotypes, Moneymaker and Marmande. Since the differences between the inoculated and non-inoculated plants of the resistant genotype were not significant, it means that plants of genotype TY172 were able to grow to full size and develop full-sized fruits compared with the significant reductions in all the parameters recorded for the rest of the genotypes.

The current results were similar to the findings of Lapidot *et al.* (1997) and Friedmann *et al.* (1998). In most cases, relative yield loss was due to a lower number of fruits and also to a reduction in fruit weight. Most of the genotypes were obtained from AVRDC breeding programme in Taiwan, which uses resistance from the *L. hirsutum* for breeding. Two genes acting epistatically control this resistance (Hanson *et al.*, 2000). Therefore, the relatively poor performance of these tomato genotypes was not unexpected as the screening was done against TYLCTZV, a new virus strain and under a different set of environmental conditions. Moreover, the tomato genotypes were mainly segregating populations of F1 hybrids. Scott *et al.* (1996) recorded a high number of susceptible tomato progenies in segregating populations derived from *L. chilense*. The tolerance to TYLCV of tomato hybrids offered by several commercial seed companies was reported to decrease with increase in disease incidence (Laterrot, 1993). Therefore, there is a need to incorporate the resistance observed in the exotic resistant tomato

genotype TY172 to cultivars such as Tengeru 97, Moneymaker and Cal-J, which are cultivated by farmers in Tanzania. This is because it resisted the adverse effects of TYLCTZV even under severe disease conditions. This will make the tomato cultivars more adaptable to prevailing local conditions in Tanzania, and result in less tomato yield losses due to increased resistance to TYCTZV.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The following conclusions are drawn from results of the current investigations:

1. *Tomato yellow leaf curl Tanzania virus* disease is prevalent in the six regions (Dodoma, Arusha, Kilimanjaro, Iringa, Morogoro and Coast) surveyed. The dot and squash blot techniques are useful for the detection of early and latent infections so that management strategies can be initiated and implemented.
2. The virus causing the disease is a geminivirus transmitted by *B. tabaci* in a persistent manner and by grafting, but not mechanically transmissible.
3. The TYLCTZV isolates from the six regions were genetically similar and shared the highest similarity (95% in the Rep and 97 % in the coat protein genes) with the African tomato leaf curl virus. More than 90 % homology in Rep protein was also shared with some crop- (*Tomato curly stunt virus*, Tobacco leaf curl-Zimbabawe, Tobacco leaf rugose mosaic virus, TYLCV) and weed-infecting (*Jathropha mosaic virus*, *Wissadula golden mosaic virus*) geminiviruses.
4. The virus was transmitted to *Datura stramonium, Nicotiana glutinosa, N. tabacum, Capsicum annuum* and *L. esculentum*, but not *Phaseolus vulgaris*. Twenty-six weed species growing within and around tomato farms were identified as new hosts of TYLCTZV.
5. The tomato genotype TY172 was the most resistant to TYLCTZV, followed by Tengeru 97, Cal-J and Marglobe.

5.2 Recommendations

The following recommendations are deduced from the results of the current research work:

1. Further research work is required to characterize the geminiviruses associated with crops and weeds that grow in and around tomato farms in Tanzania since the virus shares similarity with some crop- and weed-infecting geminiviruses, and recombination is a frequently occurring field phenomenon. Comprehensive information that will be generated from such studies can be used to breed for resistance to TYLCTZV.
2. The precise role of weeds in disease epidemics and as hosts of the whitefly vector over seasons and/or during host-free periods should be investigated further. In the interim, the identified susceptible weeds within and outside tomato farms should be removed to eliminate or reduce possible sources of TYLCTZV inoculum, especially during the off-season tomato cultivation.
3. The resistance genes in tomato genotype TY172 should be introgressed into susceptible tomato cultivars that are locally cultivated (Tengeru 97, Cal-J, Marglobe and Moneymaker) by farmers in Tanzania to reduce yield losses caused by TYLCTZV. Meanwhile, farmers should be encouraged to grow the tolerant cultivar, Tengeru 97 while the search for resistance to TYLCTZV among wild *Lycopersicon* species continues.

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7.0 APPENDICES

Appendix 1: Solutions and buffers

DNA extraction buffer

1 M Tris----------24.23 g

1 M NaCl----------11.69 g

100mM Na-EDTA----------7.45 g

10 % SDS----------20 g

1 M Dithiothretol (DTT)----------1.54 g

Double distilled water----------200 ml

Homogenization buffer (200 ml)

1 M Tris----------20 ml

100 mM Na-EDTA----------2 ml

1 M NaCl----------20 ml

10 % SDS----------12 ml

1 M DTT----------1 part

Phenol-Chloroform (9:1)----------Equal volume

LB medium

Peptone casein----------10 g

Yeast for bacteria----------5 g

NaCl----------10 g

Distilled water----------1 litre

pH----------7.0

Autoclave for 20 min at 120 °C. Cool to 50 °C and add ampicillin to a concentration of 0.1 µg/µl. Pour the plates and allow agar to solidify. Keep at 4 °C.

LB Agar

Add 15 g of agar for bacteria to the composition for LB medium.

100 mM ATP

ATP----------60 mg

Double distilled water----------80 µl

Adjust pH with 0.1 N NaOH to 7.0. Top to 1 ml and filter sterilize.

10X Ligation buffer

1 M Tris-HCl, pH 7.5----------660 µl

1 M $MgCl_2$----------50 µl

1 M DTT----------50 µl

100 mM ATP----------100 µl

Sterile double distilled water----------140 µl

SOB Medium per litre

Bacto-tryptone----------20 g

Yeast extract----------5 g

NaCl----------0.58 g

KCl----------0.186 g

Mix, adjust pH to 7.0 with NaOH and autoclave

2 M Mg^{++} stock

$MgCl_2.6H_2O$----------20.33 g

$MgSO_4.7H_2O$----------24.65 g

Add distilled water to 100 ml. Autoclave or filter sterilize.

2 M Glucose

Glucose----------36.04 g

Add distilled water to 100 ml. Filter sterilize.

SOC Medium

Add 1 ml of 2 M Mg^{++} stock and 1 ml of 2 M Glucose to 98 ml of SOB medium

1 M Tris-HCl buffer

Trizma base----------121.1 g

Distilled water----------800 ml

Adjust pH with HCl. Bring to 1 litre. Autoclave

Appendix 2: IUPAC ambiguity code

A	Adenine
C	Cytosine
G	Guanine
T	Thymine
a	Questionable A
c	Questionable C
g	Questionable G
t	Questionable T
R	A or G
Y	C or T (U)
M	C or A
K	G or T (U)
W	A or T (U)
S	C or G
B	C or G or T (U)
D	G or A or T (U)
H	A or C or T (U)
V	A or C or G
N	Either A, C, G, or T (U)

Appendix 3: Full nomenclature of tomato cultivars and lines.

Code	Full name	Source
Cal-J	Cal-J VF	Holland
CLN1	CLN 2026B	AVRDC
CLN2	CLN 2114 BC_1F_1-2-42-4-1-9-22-0	AVRDC
CLN3	CLN 2116 BC_1F_1-180-31-10-25-16-1-0	AVRDC
CLN4	CLN 2114 BC_1F_1-2-29-20-16-5-12-0	AVRDC
CLN5	CLN 2116 BC_1F_1-180-31-10-25-16-3-0	AVRDC
CLN6	CLN 2116 BC_1F_1-180-10-25-8-12-0	AVRDC
CLN7	CLN 2116 BC_1F_1-180-31-10-25-16-2-0	AVRDC
H24	H24	AVRDC
TLB	TLB 182	AVRDC
Tengeru 97	Tengeru 97	AVRDC
Moneymaker	Moneymaker	AVRDC
Marglobe	Marglobe	Purchased locally
Marmande	Marmande	Volcani Centre, Israel
TY 172	TY 172	Volcani Centre, Israel

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