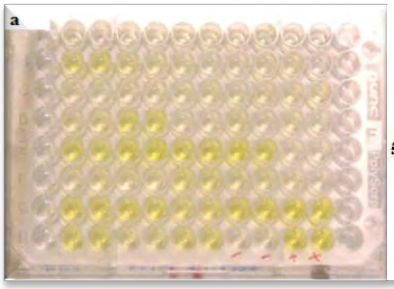
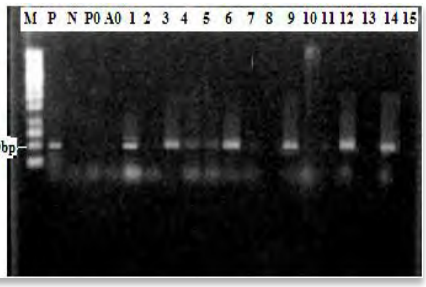
ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTEMENT OF BIOLOGY

SEROLOGICAL AND MOLECULAR BASED ASSAY OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) AND CASSAVA (*MANIHOT ESCULENTA* CRANTZ) VIRUSES IN ETHIOPIA





By: **Eyasu Wada**

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Biology (Applied Genetics)

June, 2010

Addis Ababa, Ethiopia

ACKNOWLEDGEMENTS

First and foremost, I would like to express my genuine appreciation to my advisors, *Dr. Tileye Feyissa,* from Addis Ababa University and *Dr. Adane Abraham*, from Holleta Agricultural Research Center of EIAR. Their excellent guidance, motivation, encouragement and good intellectual suggestion lead not only to the realization of this work but also taught me many more.

I thank *Department of Biology*, Addis Ababa University and *BIO-EARN* both for financial support and *EIAR* for providing laboratory materials and allowing me to conduct laboratory works in Biotechnology Research Laboratory at Holleta Agricultural Research Center. My thanks are not limited to be extended to Holleta Agricultural Research Center for ever cooperation while carrying out laboratory works.

I wish also to extend my deepest thanks to the *Tesfaye Disasa, Gulima Tenkolu, Emebet Admasu, Saba Abdulseged* and other peoples at Holleta Agricultural Research Center for their full collaboration in every aspect. My special thanks as well go to *Tewodros Tesfaye* and *Kero Jemal* for nice time that we had in challenging environment, conducting laboratory works for our M.Sc. thesis. Many thanks to *Tefera Kesho* and *Yohannis Alemayehu* for their progressive deeds to my M.Sc. study. I also owe special thanks to my undergraduate classmates: *Abebe Asale, Washun Wodajew, Taddese Ogato* and *Dawit Beyene* who have been helping me in the way that they passed. I cannot pass without mentioning the helps provided by *Meseret Gano, Mesi!* Your encouragement greatly helped me indeed. I, certainly, must acknowledge my family particularly *my mother* and *my brothers* for all what they did for me.

Above all Glory is to the omnipotent; omniscient *GOD* Who provided me with all to accomplish this thesis work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

A - Absorbance

ap - alkaline phosphatase

bp - base pair

CP - Coat Protein

CR - Common Region

DAS - ELISA - Double Antibody Sandwich Enzyme Linked Immunosorbent Assay

dNTPs - deoxyribonitrotriphosphates

DSMZ - Deutsche Sammlung fur Microorgamismen und Zellkulturen

EIAR - Ethiopian Institute of Agricultural Research

EDTA- Ethylene Diamine Tetra Acetic acid

ELISA - Enzyme Linked Immunosorbent Assay

IC-PCR- Immunocapture PCR

IgG - immunoglobin G

IgG - ap - immunoglobin G alkaline phosphatase conjugate

IR - Intergenic Region

kb - Kilobase

MAb - Monoclonal Antibody

PBS - Phosphate Buffered Saline

PBST – Phosphate Buffered Saline + Tween 20

PCR - Polymerase Chain Reaction

pNPP - p-Nitrophenyl Phosphate

PVP - Polyvinyl Pyrrolidone

Ram - Anti Mouse Rabbit Antibody

Ram-ap - anti mouse rabbit antibody alkaline phosphatase conjugate

ssDNA - Single Stranded DNA

(+) ssRNA - Positive or Sense Single Stranded RNA

TAE - tris acetate EDTA

TAS - ELISA - Triple Antibody Sandwich ELISA

TYLCD - Tomato Yellow Leaf Curl Disease

UAAIE - Upper Awash Agro Industry Interprise

LIST OF VIRUS NAMES AND ACRONYMS

ACMV - *African cassava mosaic virus*

BCTV - *Beet curl top virus*

BGMV - *Bean golden mosaic virus*

CALV - *Cassava American latent virus*

CBSV - *Cassava brown streak virus*

CCMV - *Cassava common mosaic virus*

CGMV - *Cassava green mottle virus*

CIBV - *Cassava Ivorian bacilliform virus*

CMBeV - *Cassava mosaic Begomovirus*

CMV- *Cucumber mosaic virus*

CsXV - *Cassava x virus*

CVMV - *Cassava vein mosaic virus*

EACMV - *East African cassava mosaic virus*

ICMV - *Indian cassava mosaic virus*

MSV- *Maize streak virus*

PVMV- *Pepper veinal mottle virus*

PVY- *Potato virus Y*

TAV - *Tomato asprmy virus*

TMMV - *Tomato mild mottle virus*

TMV - *Tobacco mosaic virus*

SACMV - *South African cassava mosaic virus*

SLCMV - *Sir Lankan cassava mosaic virus*

ToMV - *Tomato mosaic virus*

ToRSV - *Tomato ring spot virus*

TPSTV - *Tomato pseudo curl top virus*

TSWV - *Tomato spotted wilt virus*

TYLCMV - *Tomato yellow leaf curl Mali virus*

TYLCV - *Tomato yellow leaf curl virus*

UgV - *Ugandan cassava mosaic virus*

ABSTRACT

Tomato as well as cassava yield is limited by virus diseases and which has been one of most important constraint for production of both crops. The genus *Begomovirus* belongs to family Geminiviridae and has emerged as more serious constraints to varieties of crops including tomato and cassava worldwide. In addition to it, other major tomato viruses infecting tomato plant in tropical Africa have been included in the genus *Tobamovirus*, *Tospovirus, Potyvirus* and *Cucumovirus*. *African cassava mosaic virus* also causes a devastating cassava yield. To determine the incidence and prevalence of begomoviruses on tomato and cassava in Ethiopia, 570 tomato and 250 cassava leaf samples were collected from 57 tomato and 25 cassava fields respectively from October 21, 2009 to December 21, 2009. Most of the tomato fields were surveyed at East Shewa in Rift Valley and western Ethiopia whereas most cassava fields were surveyed at southern Ethiopia. The tomato samples were tested in DAS-ELISA against seven tomato infecting viruses: *Tomato yellow leaf curl virus*, *Tomato mosaic virus*, *Tomato spotted wilt virus*, *Tomato ring spot virus*-Chickadee strain, *Tobacco mosaic virus, Potato virus Y* and *Cucumber mosaic virus*. Cassava samples were tested in TAS-ELISA against *African cassava mosaic virus*, using universal probe which can detect all African cassava mosaic virus species so far described. Molecular technique based assays were performed based on immunocapture PCR for detection of *Tomato yellow leaf curl virus*, using *Begomovirus* DNA-A specific degenerative primer pair, Begomo_146 and Begomo_672. Immunocapture-PCR and standard PCR techniques were used to test *African cassava mosaic virus*, using three pairs of primers: Begomovirus DNA-A specific, Begomovirus DNA-B specific and *African cassava mosaic virus* DNA-A specific. Tomato samples from Rift Valley areas provided weak positive test to *Tomato yellow leaf curl virus* and clear positive test to *Tomato mosaic virus* in DAS-ELISA. All tomato samples were negative for *Tomato spotted wilt virus*, *Tomato Ring spot virus*-Chickadae strain, *Tobacco mosaic virus, Potato virus Y* and *Cucumber Mosaic virus*. The expected 520 bp PCR product of *Tomato yellow leaf curl virus* was obtained from samples which gave weak positive test for this virus in DAS-ELISA test. TAS-ELISA test as well as molecular technique based assays based on both methods using three pairs of primers all provided negative results for *African cassava mosaic virus*. Research should be broadened to include more samples. *Tomato yellow leaf curl virus* and *Tomato mosaic virus* were detected at 24.6% and 47.37% of the study area respectively. These viruses showed a distinct pattern of distribution in the surveyed areas at incidence level varying from 20% to 70%. Considering the incidence and distribution, appropriate virus management programme should be sought.

Key word: Begomoviruses, Cassava, ELISA, IC-PCR, Tomato

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) has become one of the most widely grown vegetables in the world and is regarded as one of top priority vegetables. Tomato contributes to a healthy, well-balanced diet. It is rich in minerals, essential amino acids, sugars, dietary fibers and is considered to be fairly high in vitamins of high cash value and with potential for better-quality processing. Recently, there has been more emphasis on tomato production as a source of income and food security (Rice *et al*., 1987).

However, there are a number of factors which limit tomato yield. These include: lack of improved well performing varieties, poor fruit setting due to heavy rains and excessively high temperatures and pests and diseases. In eastern and southern Africa, arthropods and fungal as well as bacterial diseases are considered to be the major constraints to tomato production. Viral diseases have been ranked as the third most important constraint among tomato diseases basically because of absence of enough information on them (Varela, 1995). Major tomato infecting viruses in tropical Africa fall into five genera: *Begomovirus*, *Tobamovirus*, *Tospovirus*, *Potyvirus* and *Cucumovirus*. Tomato yellow leaf curl disease (TYLCD) which is caused by *Tomato yellow leaf curl virus* (TYLCV) is one of the most damaging diseases of tomato. Yield losses of 100% are common, particularly when tomatoes are infected early in development (Cohen and Antignus, 1994; Nakhla and Maxwell, 1999).

Different Tomato infecting viruses have been reported from different areas of Ethiopia. Marchoux (1976) observed mosaic symptoms on almost 100% of tomato plants at Melkasa research station and yellowing on most tomato plants in Arbaminch. Agranosky and Anisimoff (1986) reported that *Tobacco mosaic virus* (TMV) was wide spread in areas Sidamo and Wello provinces, reaching the incidence level of 12-15% and 40% respectively and rarely found in areas around Ambo, Guder, Merti, Nazret, Zwai, Awassa, Arbaminch and Upper Awash Valley. Yaynu Hiskias (1998) detected *Tomato mild mottle virus* (TMMV), *Potato virus Y* (PVY) and *Tomato mosaic virus* (ToMV) on infected tomato plants from tomato fields at Melkasa, Guder, Bako, Zwai, Wolliso, Merti and Wondo-Genet. According to his report, the most widespread and predominant viruses occurred frequently in mixed

infections were PVY and TMMV. ToMV was identified in only few samples from tomato plants in the Rift Valley.

In Melka-Werer, about 90% of tomato plants showed leaf curl virus symptoms with reduced size suspected to be caused by TYLCV (Quiot, 1976) and Yaynu Hiskias (1998) observed plant stunting and leaf curling on a few tomato plants at Melkasa but never detected begomoviruses. Shih *et al.* (2006) reported occurrence of *Begomovirus* associated with tomato yellow leaf curl diseases as a first report from a field in Melkasa. After sequencing one of the samples they reported that the Ethiopian virus is a provisional strain of *Tomato yellow leaf curl Mali virus* (TYLCMV) having highest sequence identity (92%) with TYLCMV in BLAST analysis. Apart from this limited report in a single area there is no information on the incidence, prevalence and economic importance of begomoviruses on tomato or other crops in Ethiopia.

Cassava (*Manihot esculenta* Crantz) is ranked among the top ten most significant food crops produced in developing countries and as a major source of carbohydrate for human consumption throughout the tropics. Ability of this species to tolerate drought and degraded soils constitutes a determinant parameter for its high importance (Legg and Fauquet, 2004). Considering the continuous increases in human population and pressure on available land and the decline of soil fertility, it would continue to play a major role for food security in Sub-Saharan Africa (Busogoro *et al*., 2008).

However, cassava is also susceptible to a number of pests and diseases, which are constraint to its production. About 30 diseases have been described worldwide. In Africa, main diseases affecting cassava are cassava bacterial blight, fungal diseases and several viral diseases have been reported (Atiril *et al*., 2004). Among viral diseases, cassava mosaic disease caused by *cassava mosaic Begomovirus* plays an important role and involves considerable losses up to 95%. This disease occurs in almost all cassava main growing areas in Africa, India and Sri-Lanka and induces high levels of yield losses particularly in Africa (Adjata *et al.,* 2008). There was no published information about the cassava infecting viruses in Ethiopia.

Begomoviruses are considered to have caused the earliest known record of plant virus diseases and they have become one of the most important groups of plant viruses in tropical and subtropical areas of the world, causing devastating yield of many crops (Saunders *et al.*, 2003). Agricultural intensification has been proposed as one of the main causes for their dissemination together with increases in population of their white fly vector, *Bemisia tabaci* Gennadius (Xie and Zhou, 2003). Other underlying factor influencing the emergence of begomoviruses have been the evolution of more aggressive virus variants (Seal *et al.,* 2006). Human activities like changes in the cropping system, introduction of new crops, movement of infected cropping materials and introduction of more vulnerable crops have also played an important role in the emergence of serious virus diseases across the world (Anupam and Malathi, 2003).

While most research on plant viruses has concentrated on RNA viruses, the biggest emerging threats to crops worldwide are the single stranded DNA (ssDNA) geminiviruses, especially those of the dicot infecting, whitefly-transmitted begomoviruses (Seal *et al*., 2006). The reason for the emphasis on plant RNA viruses has been the desire to assess the extent and structure of genetic variation created by their use of error prone RNA polymerase, which is necessary to understand their molecular epidemiology and can complicate the creation of resistant crop lines. However, multiple genera of geminiviruses display high levels of within host genetic variation. This suggests ssDNA viruses might exhibit genetic diversity similar to that seen in plant RNA viruses even though they utilize host DNA Polymerases for replication (Duffy and Holmes, 2008).

Begomoviruses as a group have very wide host range, infecting dicotyledonous plants. Common symptoms induced by these viruses include leaf curling, mosaic, vein yellowing or more generalized leaf yellowing, often accompanied by stunting of infected plants (Fauquet *et al.,* 2008). All begomoviruses are transmitted by white fly. They formed the group of viruses formerly known as white fly transmitted geminiviruses (Martinez, 2008).

Compared to mastreviruses and curtoviruses in the family Geminiviridae, begomoviruses have emerged as more serious constraints to varieties of crops. They are responsible for a large amount of economic damage to many important crops such as tomato, cassava, pepper,

bean, squash and cotton worldwide. The frequency with which new begomoviruses appearing shows that these viruses are still evolving and pose a serious threat to sustainable agriculture, particularly in tropics and sub tropics (Anupam and Malathi, 2003; Seal *et al*., 2006).

It is important that viruses occurring in a specific geographical area should be identified prior to developing sustainable and environment friendly disease management programmes (Green and Kim, 1991). However, there is scarcity of information on TYLCV and cassava viruses to take appropriate control/prevention measures in Ethiopia. The main aim of this research was therefore to study the incidence and distribution of TYLCV and *African cassava mosaic virus* (ACMV), which are devastating begomoviruses in causing considerable tomato and cassava yield loss respectively in the world. In addition, other six tomato infecting viruses: ToMV, TMV, PVY, *Tomato ring spot virus* Chickadee strain (ToRSV-Ch), *Tomato spotted wilt virus* (TSWV) and *Cucumber mosaic virus* (CMV), were also tested on tomato samples to determine their relative importance.

2. LITERATURE REVIEW

2.1 Tomato

Tomato (*Lycopersicon esculentum* Mill) belongs to a large family of plants called the Solanaceae which contains many important food crops such as potato, pepper and egg plant (aubergine). Tomato has diploid chromosome number 2n=2x=24. The crop is short lived herb, 0.5-1.5 m high, often densely covered with glandular hairs (Naika *et al*., 2005; Inga *et al*., 2006).

Tomato was originated in the South America Andes. The cultivated form first taken to Europe by Spanish conquistadors in the 16th century and from there introduced into southern and eastern Asia, Africa and the Middle East and distributed throughout the world (Pursegrove, 1968; Naika *et al*., 2005). In Ethiopia, no local cultivars of tomato have evolved or been developed and hence all varieties grown are introduced (Yayeh Zewdie, 1989). It grows in many parts of the country (Inga *et al*., 2006); on large scale under irrigation at Merti, Upper Awash Agro Industry Enterprise and some irrigated tomato for fresh consumption is found in around Melkasa, Koka, Zwai, Wondo-Genet, Guder, Bako and other areas (Shimeles Aklilu, 2000).

World tomato production in 2001 was about 105 million tons of fresh fruit from an estimated 3.9 million hectare. As it is short duration crop, gives a high yield and economically attractive, the area under cultivation is increasing daily (Naika, 2005). In Ethiopia, it is also among the most important vegetable crops and its production has shown a marked increase since it became the most profitable crop providing a higher income to small scale farmers compared to other vegetable crops (Lemma Dessalegne *et al*., 1992). However, the national average of tomato fruit yield in Ethiopia was often low (125 q/ha) compared even to the neighboring African countries like Kenya (164 q/ha) (FAO, 2004). Current productivity under farmers' condition in Ethiopia is 90 q/ha whereas yield up to 400 q/ha recorded on research plots. Farmers get lower yield mainly due to diseases, pests and sub-optimal fertilization (Tesfaye Balem, 2008).

Tomato served in various raw and processed materials. Fresh tomatoes are key ingredients in all around the world and processed tomatoes are used to make soup, juice and other products.

The tomato fruit contains abundant and well balanced nutrition consists of minerals (calcium, iron, phosphorus), vitamins (vitamin A, vitamin C), protein (essential amino acids), sugars, dietary fiber (pectin), citric acid, etc. (Naika, *et al*., 2005; Ssekyewa, 2006). In addition, the red pigment of the licopene which tomato fruit contains in plenty has attractive interest because the licopene has high antioxidant ability against oxygen radicals that probably cause cancer, aging, arteriosclerosis, etc. Thus, tomato would contribute to our enjoyable diet and good health all over the world (Giovannucci, 1999; Naika *et al*., 2005).

However, tomato plants are susceptible to several fungi, bacteria and viruses. Fungi and bacteria cause fruit, stem or root diseases. A virus infection often leads to dwarfed growth and decreased production. Damage caused by virus disease can result considerable yield losses for a farmer. The most important symptom of viral infections of tomato is the light (white or yellow) color of the leaves, or a mosaic pattern of light and darker shades of green on the leaves. In many cases, viral disease leads to dwarfed growth, rosette or other strange stem formation and leaf deformations like leaf curling. The symptoms of viral infections are often not found everywhere in a cultivated field, as is usually the case with fungal or bacterial diseases. It is always possible to find a number of plants that show no signs of the disease (Naika *et al*., 2005).

2.2 Cassava

Cassava belongs to the Euphorbiaceae family with diploid chromosome number 2n=2x=36 and has about 100 species, among which *Manihot esculenta* is the only one commercially cultivated. Cassava is little branched woody herb that grows up to 5 m. It has large and starchy roots and leaves are 3-5(-7) lobed (Nassar, 1978; Gilbert, 1995).

Cassava and all its wild relatives have their genetic origin in South America. The Americans gave it to the rest of the world after the arrival of early European explorers (Henry and Hershey, 2002). In the 16th century, the Portuguese navigators took cassava from Brazil to Africa (Hillocks, 2002). This crop was introduced to Ethiopia in 19th century and is grown in humid areas and altitude ranging from 480 m to 1,800 m above sea level. In places like Wolayta where it was first introduced, dominantly grown and utilized, it is known by variety of local names. It is known as “Faranja Boyiya” meaning white men yam. Yam was found as

root crop in the area before cassava was introduced. In the same region in some other places it is called "Mita Boyiya" meaning tree yam (Dejene Mekonnen, 2006; Cherinet Abuye *et al*., 2008). It is also known as yefurno duket zaf (Amharic), Batata (Soomaali), Deekikaa (Afaan Oromo) (Gilbert, 1995; Amsalu Nebiyu *et al*., 2000). In southern Ethiopia, it grows in Konso Special district, Amaro special district, Gedeo, Sidama, Wolayta and Gamo-Gofa zone while in southwestern part of Ethiopia it grows in some areas of Illubabor, Jimma, West Wollega and Benshangu-Gumuz zone. It is also found in very few places in Central Ethiopia in Majete and North Shewa. Since the last ten years to date, it has been promoted to many part of the country including the northern Ethiopia where it had not been known before (Amsalu Nebiyu, 2006; Dejene Mekonnene, 2006; Cherinet Abuye *et al*., 2008).

In 2000, world cassava production was estimated to be 172 million tones, Africa accounted for 54%, Asia for 28% and Latin America and the Caribbean for 18% of the total world production (IITA, 2007). In the year 2006, the estimated global cassava production was around 226 million tons (FAO, 2008). In Ethiopia, it has been of immense interest as a food crop since a famine in 1984, but only a limited amount of research has been done on cassava improvement (Amsalu Nebiyu, 2006; Dejene Mekonnene, 2006; Cherinet Abuye *et al*., 2008). Recently, the development of tissue culture protocol for cassava improvement is only at initial stage in Ethiopia (Adane Abraham, 2009).

Cassava is the third most important food crop after cereals and grain legumes. It provides staple food to over 500 million people in tropical countries. The crop is the third most important source of calories in the tropics, after rice and corn. Its leaves are also consumed as vegetables in many regions in Africa and constitute proteins, vitamins and mineral salts (Legg and Fauquet, 2004). It adapts to wide range of uses: food security crop, cash crop, feed crop and raw material for industrial uses such as starch and alcohol production (Wright, 1996).

Root crops such as sweet potato and cassava are usually found to be infected by one or more viruses. This virus infection causes a significant reduction in yield and root quality. Throughout the 20^{th} century, cassava mosaic disease has been a hindrance to cassava production in Africa (Karakacha, 2001).

2.3 General description of plant viruses

Many plant viruses are named after the most conspicuous symptom they cause on the first host in which they have been studied. Thus, a virus causing a mosaic on tobacco is called *Tobacco mosaic virus*, whereas the disease itself is called tobacco mosaic; another virus causing spotted wilt symptoms on tomato is called *Tomato spotted wilt virus* and the disease is called tomato spotted wilt, and so forth. Considering, however, the variability of symptoms caused by the same virus on the same host plant under different environmental conditions, by different strains of a virus on the same host or by the same virus on different hosts, it becomes apparent that this system of nomenclature leaves much to be desired. Viruses are now distinguished as RNA viruses and DNA viruses depending on whether the nucleic acid of the virus is RNA or DNA. Viruses are further subdivided depending on whether they possess one or two strands of RNA or DNA, either filamentous or isometric (George, 2005). The majority (94%) of plant infecting viruses consists of RNA viruses and among them 90% are single stranded RNA viruses. Only 6% of plant viruses are DNA viruses (Dube, 2004). Some viruses have all their genome in one particle (monopartite viruses), but the genome of other (multipartite) viruses is divided among two, three, or rarely four particles (George, 2005).

2.3.1 Viral diseases of plants

Viral disease of plants may be systemic, i.e., spread throughout the host plant or local, i.e., restricted to certain areas. Mostly the symptoms appear on the leaves. Usually many symptoms appear together. The infected plant may be stunted in growth; their leaves may show mosaic symptom and enations may also develop. The followings are the important symptoms of viral plant disease (Dube, 2004).

(i) **Mosaic:** Mixed light green, green and yellow patches appear on the leaves and make a mosaic pattern. This is caused by systemic infection, e.g., tobacco mosaic, tomato mosaic, mosaic of cucurbits, mosaic of potato, mosaic of sugarcane, etc.

(ii) **Yellows:** Uniform chlorosis of the leaves, e.g., Rice chlorosis.

(iii) **Enations:** Hair like out growth called enations appear on the leaves, stems, and etc. Enations frequently accompany mosaic symptoms, e.g., *Dolichos* enation mosaic.

(iv) **Vein clearing, vein banding and vein thickening:** Vein cleaning involves yellowing of the vein and veinlets. The rest of the leaf remains green. In vein banding the opposite occurs.

(v) **Leaf curling or leaf rolling:** It is a very common symptom, e.g., leaf curl of papaya, leaf curl of potato, leaf curl of tomato.

(vi) **Little leaf symptom:** The leaves become small and closely placed to form rosette-like structure, e.g., little leaf of egg plant.

(vii) **Stunting:** General retardation of growth results in stunting of plants, e.g., Bunchy top of banana.

(viii) **Breaking and greening of plants:** Changes the color of petals giving them a variegated appearance. When petals become green due to virus infection it is called virescence.

(ix) **Tumors:** Tumors are outgrowth produced by uncontrolled division and growth of cells.

(x) **Witches broom:** The leaves become reduced in size. The internodes are shortened and the abnormal growth of leaves results in a densely packed structure called witches broom.

2.3.2 Transmission of plant viruses

Viruses being non motile, are carried passively from one place to other. Acknowledge of the mode of their transmission is of great help in controlling the disease caused by them (Dube, 2004).

(i) **Transmission by contact:** Only few viruses are transmitted through this method, the most important being TMV. Plants grow very closely in several crops and frequently rub against each other to cause abrasions.

(ii) **Transmission through seeds and organs of vegetative propagation:** Seeds produced by diseased plants carry virus by their seed coat, endosperm or embryo. Such seeds give rise to infected plants. Organs of vegetative propagation like tubers, bulbs, rhizomes, stem cuttings, etc derived from infected plants give rise to infected plants.

(iii) **Transmission through nematodes and fungi:** The nematodes and soil fungi are important vectors of viruses. These vectors are carried to long distances through irrigation water; and along with them, the viruses they harbor, also reach far off places.

(iv) **Transmission through insects:** No other group of viruses is so much dependent on insects for transmission as are the viruses. Aphids, leafhoppers, beetles and mites transmit large number of viruses.

2.4 Viruses of tomato and cassava

2.4.1 Tomato viruses

About 146 viruses infect tomato worldwide. They are grouped into thirty three genera, of which fifteen genera are of the most economic importance. Twenty seven viruses belong to genus *Potyvirus,* 17 belong to *Begomovirus,* 15 belong to *Nepovirus* and 8 belong to *Tobamovirus* and the remaining 88 viruses distributed among different genera (Nono-Womdim *et al.,* 1996). In tropical Africa, major tomato viruses fall into five genera: *Begomovirus, Tobamovirus, Tospovirus, Potyvirus*, and *Cucumovirus* (Table 1).

Table 1 Some tomato virus taxon name according to Jones *et al*. (1991).

Genome	Virus Species [a]	Genus	Family
ssDNA	TYLCV	*Begomovirus*	Geminiviridae
(+)ssRNA	TMV, ToMV	*Tobamovirus*	Unclassified genus
(+)ss RNA	PVY, PVMV	*Potyvirus*	Potyviridae
(+)ss RNA	CMV, TAV	*Cucumovirus*	Bromoviridae
(-)ssRNA	TSWV	*Tospovirus*	Bunyaviridae
(+)ssRNA	ToRSV	*Nepovirus*	Comoviridae

[a] For virus names see list of virus name and acronyms

TYLCV is included in the genus *Begomovirus* of family Geminiviridae that has viruses infecting tomato in both the New and Old Worlds (Ssekyewa, 2006). TYLCV is not transmitted in seed, soil or from plant to plant by handling. It is only transmitted by whitefly. The whitefly vector has a very wide host range and feeds by sucking plant juices from the underside of leaves of crops such as tomato, cassava, tobacco, cucumber, sweet potato as well as some weeds (Dellate, 2005).

ToMV and TMV are included in the genus *Tobamovirus*. Known common ToMV symptoms include mosaic, systemic chlorosis, leaf abscission, systemic leaf and stem necrosis, uneven ripening and internal browning or brown wall on fruits of certain varieties, but symptoms

vary greatly. It is not easy to correctly identify TMV from ToMV by basing on symptoms because it causes a variety of symptoms caused by ToMV. Both ToMV and TMV transmitted by contact, through seed and from leaf and root debris (Green and Kim, 1991).

TSWV is the known member of genus *Tospovirus* that infects tomato. It is known to cause chlorosis and yellow rings on tomato leaves and fruits. TSWV is transmitted persistently by thrips. Seed transmission also occurs. This virus has wide host range and potentially very damaging (Jones *et al.,* 1991).

Some major viruses in genus *Potyvirus* that infect tomato include PVY and *Pepper veinal mottle virus* (PVMV). Typical symptoms of PVY in tomato include: mosaic, vein chlorosis, mild mottling and necrosis on leaflets, leaf crinkling and drooping. PVY is transmitted by aphids in a non-persistent manner (Jones *et al.,* 1991).

There are two major tomato viruses in the genus *Cucumovirus,* CMV and *Tomato asprmy virus* (TAV). Of the two species, CMV is the most common in tropics. It causes mottling, mosaic, yellow discoloration, vein-clearing, stunting and leaf deformation with extreme shoestring leaf appearance. In severe infections, plants produce no fruits or only very few fruits which are of small size. CMV is transmitted in a non-persistent manner by aphids (Jones *et al.,* 1991). In general, major tomato viruses so far encountered in tropical Africa form two groups based on symptoms are leaf curl-causing viruses, and mosaic/mottling symptom-causing viruses (Nono-Womdim *et al.,* 1996).

ToRSV is a member of *Nepovirus* and causes economically important diseases in vegetables, ornamentals and fruit crops worldwide. It affects 285 plant species in 159 genera of 55 botanical families. It causes local lesions, leaf curling, mosaic, systemic streaking, vein cleaning, vein necrosis and deformed growth. It is naturally transmitted by nematodes. Seed and pollen transmission have been reported in several crops (Samuitiene *et al*., 2003).

2.4.2 Cassava viruses

Cassava is particularly prone to damage by viruses as infection tends to build up in successive cycles of propagation. The viruses include that shown in Table 2. Among these, *cassava mosaic Begomovirus* plays an important role and involves considerable losses up to

95%. This cassava mosaic disease occurs in almost all cassava producing areas in Africa, India and Sri-Lanka and induces high levels of yield losses particularly in Africa (Adjata *et al.,* 2008).

The disease is predominantly a foliar disease and the symptoms range from chlorotic spots, leaf curling, bright mosaic and stunting of severely affected plants. The severity of symptoms depends on environmental factors, type of infection, virus or virus strain and the variety of the cassava plant (Gibson and Otim-Nape, 1997). Although cassava begomoviruses are naturally transmitted by the whitefly, cassava mosaic disease is widely disseminated by the distribution of stem cuttings used for vegetative propagation (Sserubombwe *et al*., 2008).

Table 2 Cassava viruses, disease and countries from where the viruses were reported according to ICTV management report (2006).

Cassava virus	**Disease caused by virus**	**Reported from (along)**
Cassava mosaic Begomovirus (CMBeV)	African cassava mosaic	Africa
Cassava brown streak virus (CBSV)	cassava brown streak	Coast of Kenya
Cassava common mosaic virus (CCMV)	cassava common Mosaic	South America
Cassava green mottle virus (CGMV)	cassava green mottle	Solomon islands
Cassava vein mosaic virus (CVMV)	cassava vein mosaic	Brazil
Cassava American latent virus (CALV)	symptomless infections	Brazil and Guyana
Cassava Ivorian bacilliform virus(CIBV)	symptomless infections	Cote d`Ivoire
Cassava x virus (CsXV)	symptomless infection	Columbia
Cassava c virus (an unnamed virus)	symptom disappear after infection	Cote d`Ivoire, Malawi and Cameroon

2.5 Geminiviruses

Geminiviruses are classified within Geminiviridae family which has been divided into four genera that differ with respect to genome organization, host range and vector species (Table 3) (Rybicki, 1994; Harrison and Robinson, 1999; Fauquet *et al*., 2000). They are

characterized by 2.5 kb to 3.0 kb circular ssDNA genomes encapsidated in twinned quasi isometric particles of about 18 x 30 nm. In the last two decades these viruses have emerged as devastating pathogens. Epidemics caused by re-emerging and newly emerging geminiviruses are becoming frequent even in the regions that were earlier free from these viruses (Anupam and Malathi, 2003).

Table 3 Classification of geminiviruses.

No	**Genera**	**Type species**	**Genome organization**	**Host range**	**Vector species**
1	Mastreviruses (formerly subgroup I)	*Maize streak virus* (MSV)	Monopartite	Monocots (with a few exception)	Leafhoppers
2	Curtoviruses (formerly sub group II)	*Beet curly top virus* (BCTV)	Monopartite	Dicot	Leafhoppers
3	Topocuviruses (formerly classified under curtoviruses)	*Tomato pseudo curly top virus* (TPSTV)	Monopartite	Dicot	Treehopper
4	Begomoviruses (formerly sub group III)	*Bean golden mosaic virus* (BGMV)	Either bipartite or monopartite	Dicot	White fly

Source: Harrison and Robinson (1999).

2.5.1 Begomoviruses

The genus *Begomovirus,* coined from *Bean golden mosaic virus*, the type species, contains more than 200 species. The genus is the largest of the Geminiviridae family and more than 80% of known geminiviruses belong to this genus (Fauquet *et al*., 2000).

2.5.1.1 *Structure and Variability of begomoviruses genome*

Begomovirus is the only genus of the family Geminiviridae to be either bipartite with virus genes resident on two different circular ssDNA molecules (DNA- A and DNA- B) each of about 2.5-3.0 kb or a few begomoviruses are monopartite with all genes resident on one

ssDNA (DNA A-like) of about 3.0 kb and are common in the old world. DNA-A contains two genes (*AV1* and *AV2*) in the virus strand and four genes (*AC1, AC2, AC3* and *AC4*) in the complementary strand. The virus (V) and complementary (C) strands of DNA-B contain one gene each; *BV1* and *BC1* respectively (Fig. 1). The DNA-A of the bipartite species is similar in arrangement to the genome of the monopartite begomoviruses (Stenger *et al.*, 1991; Paszkowski *et al*., 1993; Liu *et al.*, 1997; Harrison and Robinson, 1999).

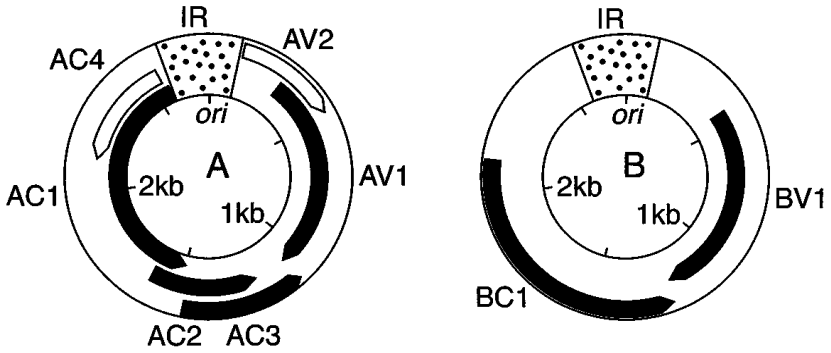


Fig. 1 Genome organization of a typical *Begomovirus.* Black arrows show arrangements of open reading frames (ORFs) occur in all begomoviruses with bipartite genomes where as white arrows show ORFs that occurs only in some. Shared sequences of DNA-A and DNA-B in the intergenic region (IR) are stippled. Approximate number of nucleotides (kb) is indicated on the inner circle, counting from origin of replication (*ori*). **Source:** Harrison and Robinson (1999).

The greatest kind of variation among the genomes of begomoviruses is the lack of a DNA-B component in some, such as the TYLCV from Israel and that from Sardinia (Ssekyewa, 2006), however, not all TYLCV are monopartite. The monopartite forms are reported from old world (Jones *et al*., 1991). There are many others for which no DNA-B has been identified, but the evidence falls short of demonstrating that DNA-B is absent. Despite the lack of the DNA-B-encoded BV1 and BC1 movement proteins, monopartite forms spread efficiently through infected plants (Navot *et al.*, 1991).

Between the initiation codons of *AV2* and *AC1* in DNA-A lies an intergenic region (IR) and in DNA-B there is an equivalent intergenic region between the initiation codons of *BV1* and *BC1* (Fig. 1). In most of the viruses where the nucleotide sequences of both DNA

components have been determined, sequences in the IR in DNA-A and DNA-B are very similar or identical. This is the only region of significant sequence similarity between the two genome parts and for this reason it is often referred to as the common region (CR) (Harrison and Robinson, 1999).

2.5.1.2 *Serological Characteristics of begomoviruses*

Coat protein (CP) forms the shell of the geminate particles that are typical of begomoviruses. It is the basis of serological detection and identification methods. Its properties include binding to and protecting viral ssDNA, self-binding, nuclear targeting and export and mediating systemic movement in plants and transmission by the whitefly vector. It is an outstanding example of a multifunctional viral protein (Harrison *et al*., 2005). The coat proteins of begomoviruses, whether from the new world or old world, have a high degree of homology in their amino acid sequence and hence have common serological determinants (Akad and Czosnek, 2002).

2.5. 2 Begomoviruses infecting tomato and cassava

2.5.2.1 *Tomato begomoviruses*

Tomato appears to be an ideal haven for begomoviruses. Most begomoviruses infecting tomato induce symptoms characteristic of leaf curl disease including severe reduction of leaf size, down ward curling, crinkling of interveinal areas, interveinal and marginal chlorosis, occasional development of enations, shortening of internodes, development of small branches, bright yellow spot on leaves and reduced fruiting. It is, therefore, natural that most of tomato viruses caused by begomoviruses have been collectively described as “leaf curl” or “yellow leaf curl” (Fauquet *et al*., 2003). TYLCV is a generic name that includes 59 virus isolates belonging to 15 different strains associated with TYLCD. The majorities have come to light in the last 21 years and likely many more await identification (Czosnek, 2007).

The first reports on TYLCV infection in tomato were from Israel and other countries in the Middle East in the 1930s and since then the virus has further emerged (Cohen and Antignus, 1994). In Africa, TYLCD was first described in Sudan in 1965, but the causal agent was identified as TYLCV in 1997 (Yassin and Nour, 1965; Czosnek and Laterrot, 1997). In Ethiopia, at Melka-Werer about 90% of tomato plant showed leaf curl virus symptoms with

reduced size suspected to be caused by TYLCV (Quiot, 1976), but only recently occurrence of *Begomovirus* associated with TYLCD was reported for the first time from Melkasa (Shih *et al.,* 2006).

2.5.2.2 *Cassava mosaic begomoviruses*

Cassava mosaic viruses belong to the genus *Begomovirus*. Thus, the cassava mosaic viruses are referred to as CMBeV. They have not been reported in Latin America, the origin of cassava, although their symptoms are similar to those caused by CCMV, genus *Potexvirus* in South America. This suggests that the viruses probably originated in Africa and cassava became infected by CMBeV after its introduction into the continent (Fauquet *et al*., 2000).

When cassava mosaic disease was first described, the causal agent was assumed to be a virus, in the absence of any visible pathogen. After initial uncertainty, the various isolates from Africa and India were regarded as strains of a single virus of the geminivirus group and designated ACMV. Subsequent studies have led to the recognition of several distinct but similar viruses associated with cassava mosaic disease, namely *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *South African cassava mosaic virus* (SACMV) and *Ugandan cassava mosaic virus* (UgV) in Africa; *Sri Lankan cassava mosaic virus* (SLCMV) in sir-Lanka and *Indian cassava mosaic virus* (ICMV) in the Indian subcontinent (Stephen, 1995). Cassava mosaic begomoviruses caused major pandemics of cassava mosaic disease in Africa that led to massive economic losses and destabilization of food security (Legg and Thresh, 2000).

2.6 Methods for detection of plant viruses

Plant viruses cause major losses to several agricultural and horticultural crops around the world. Unlike other plant pathogens, there are no direct methods available to control viruses and consequently the control measures rely on indirect tactics to manage the viral diseases. Hence, methods for detection and identification of viruses play a critical role in virus disease management (Miller and Martin, 1988). Diagnostic techniques for viruses fall into two broad categories. That are (1) biological properties related to the interaction of the virus with its host and/or vector (e.g., symptomatology and transmission tests) and (2) instinct properties of the virus itself (coat protein and nucleic acid) (Naidu and Hughes, 2003).

2.6.1 Methods of detection based on biological properties

Symptomatology: Common virus disease symptoms on plants are used to characterize a disease having viral etiology for rouging of diseased plants in an attempt to control the disease. However, many factors such as virus strain, host plant variety, time of infection, and the environment can influence the symptoms exhibited. Some viruses may induce no apparent symptoms or cause symptomless infection. In addition, different viruses can produce similar symptoms or different strains of a virus cause distinct symptoms in the same host (Matthews, 1980).

Transmission tests: Virus detection and identification techniques originated with mechanical, graft and vector transmission of the viruses to susceptible indicator plants (Jones, 1993). Mechanical transmission by sap inoculation to herbaceous indicator plants can be done with minimal facilities and characteristic symptoms produced by these plants allow both the detection and identification of many viruses (Horvath, 1983). Many of the economically important whitefly transmitted geminiviruses including TYLCV are, however, difficult for mechanically transmission (Dellate, 2005). Cassava begomoviruses are also naturally transmitted by the whitefly (Sserubombwe *et al*, 2008). 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 (Potter *et al*., 2007).

2.6.2 Detection methods based on viral coat protein

Serological or immunological assays have been developed and used successfully for a number of years for the detection of plant viruses. These tests are broadly subdivided into liquid and solid phase tests. In the former, both antigen and antibody react in solution to form a visible precipitate (precipitin or micro precipitin tests, gel diffusion assays) or agglutination of cells (agglutination methods). In the latter, assays are conducted on a solid surface such as on a microtitre plate or nitrocellulose membrane and the antigen-antibody reaction is visualized by means of a suitable detection system such as an enzyme labeled antibody (Naidu and Hughes, 2003).

Although widely used for virus detection, serological methods have certain disadvantages. They are based on the antigenic properties of the virus coat protein and do not take into account the rest of the virus genome (Naidu and Hughes, 2003). The difficulty is poor immunogenicity of virions and antigenic indistinctiveness of begomoviruses capsid proteins with available polyclonal and most monoclonal antisera. The methods have played a major role in identifying TYLCV but need to obtain sufficient purified coat protein for the production of antiserum (Harrison *et al*., 2005).

2.6.3 Detection methods based on virus nucleic acid

Nucleic acid based detection methods, on the other hand, have the advantage that any region of a viral genome can be targeted to develop the diagnostic test. Consequently nucleic acid based diagnostic assays are the methods of choice (Naidu and Hughes, 2003). Viral nucleic acid based techniques like dot-blot hybridization assays and polymerase chain reaction (PCR) using degenerate broad-spectrum oligonucleotide primers have been more reliable procedures for the detection and identification of begomoviruses (Nakhla and Maxwell, 1999; Potter *et al*., 2007).

Viral infections which cannot be detected by serological methods can be easily detected with PCR methods. The technique could be useful in overcoming many of the current difficulties such as low titer of antigens, limited availability of antibodies against geminiviruses associated with serological detection methods because the method is not dependent on the presence of expressed proteins (antigens) but only on the presence of target nucleic acid sequences (Rojas *et al*., 1993; Mehta *et al.,* 1994; Czosnek and Laterrot, 1997). This diagnostic method provides greater flexibility, increased sensitivity and specificity for diagnosis of virus diseases in disease surveys, epidemiological studies, plant quarantine, seed certification and breeding programs (Deng *et al*., 1994; Naidu and Hughes, 2003; Potter *et al*., 2007).

However, conventional PCR protocols require the preparation of total nucleic acids from infected plants for successful amplification of viral DNA templates. Methods of DNA isolation and purification are not only laborious but also may not be universally applicable to different plant species, especially those with high levels of polysaccharides or phenolics and

therefore template preparation can be a limitation on PCR detection (Tigst Demeke and Adams, 1992; LeamKhang *et al.*, 2005).

Immunocapture PCR (IC-PCR), used in this study, is a synthesis of two commonly used diagnostic tools: partly of serological and partly of molecular. This method exploits the high-affinity binding of antibodies to provide a facile method of purification, usually from a complex matrix, supplying the substrate for PCR detection. PCR exponentially amplifies a DNA template in a temperature dependent fashion by the annealing of primers, enzymatic extension of bound primers by a heat stable polymerase, followed by denaturation, annealing and extension in the next steps (Burns, 2004).

The use of clarified plant extracts in detecting viral DNA through PCR amplification provides several advantages. It eliminates the time consuming steps of total nucleic acid isolation and purification, avoids possible inhibitory effects of co-isolated impurities on PCR amplification and allows detection of viruses that occur in low titers (Wetzel *et al.,* 1992; LeamKhang *et al.*, 2005). This PCR based detection method is sensitive enough to detect TYLCV in up to 1:20,000 dilution (1g sample in 20ml extraction buffer) (LeamKhang *et al.*, 2005) and mixed samples up to 25 (1 infected sample to 24 non infected samples) (Mehta *et al.,* 1994). IC-PCR is the most efficient in detecting begomoviruses in different species of plants. This method can be used for detecting and identifying viruses in clarified plant extracts with degenerate primers, without prior isolation of total nucleic acids. The technique takes advantage that it leads to the development of sensitive and rapid single tube assays (Rampersad and Umaharan, 2003).

3. OBJECTIVES

3.1 General objective

- General objective of this research was to assess viruses on tomato and cassava in Ethiopia by serological and molecular based techniques.

3.2 Specific objectives

- To detect tomato viruses through serological test.
- To identify *Tomato yellow leaf curl virus* on tomato using nucleic acid based techniques.
- To test *African cassava mosaic virus* by serological and molecular techniques.
- To determine the incidence and prevalence of tomato and cassava viruses in the study area.

4. MATERIALS AND METHODS

4.1 Study area and collection of samples

4.1.1 Study area

Main tomato and cassava growing areas of Ethiopia were diagnostically surveyed from October 21, 2009 to December 21, 2009. The fields surveyed were varying from small yard to large hectare. East Shewa in the Rift Valley areas and western Ethiopia where tomato grows under irrigation were given more attention for tomato sample collection. The survey was conducted at East Shewa in Rift Valley area at upper Awash Agro Industry Enterprise (UAAIE), Merti, Melkasa, along the road from Mojo to Zwai; Ambo to Guder in western Ethiopia; Wolliso to Jimma in southwestern Ethiopia; Butajira to Wolayta in southern Ethiopia and western and eastern Harerge. In the survey, 57 tomato fields were inspected (Table 4).

Table 4 Locations from which tomato samples were collected.

Region	Zone	Woreda	Field No	Kebele
Oromiya	East Shewa	Dawa-Chefa	1 and 2	UAAIE
			3, 4 and 5	Merti
		Melkasa	6, 7 and 8	MARC
		Mojo	9	Mojo 01
			10	Koka
		Alemgena	11	Ashewa
		Bora	12and 13	Doda
		Adami-Tulu	14 and 15	Ana
			16	Batu (DSAD)
		Golbe-Chale	17 and 18	Alto
		Zwai-Zuriya	19	Zwai 01
			20 and 21	Abne
		Teko-Choronke	22, 23 and 24	Doda
		Meki	25	Giresa
		Wora	26	Enane
			27	Ana-Digida

Table 4 ---continued.

Oromiya	West Shewa	Ambo-Zuriya	28	Ambo 01
			29	AARC
			30	Senkele
		Guder	31, 32 and 33	Taga-Filie
			34	Dogajei
			35	Kilinto
			36 and 37	Birbirsa and Dogama
			38	Guder 01
			39	AUGARC
	Southwest Shewa	Wolliso	40 and 41	Kodu-Kora
SNNPR	Gurage	Abasha	42 and 43	Tawula
Oromiya	Jimma	Kersa	44	Gele
	West Harerge	Tulu	45	Odanagen
			46	Ragesiis
			47	Itu
		Meiso	48	Tokuma
		Haramaya	49	HU
	East Harerge	Kombolcha	50	Jelbe
		Dereteyera	51	Asarge
		Kersa	52	Mada-Oda
SNNPR	Wolayta	Kindo-Koysha	53	Kacho-Na'a
	Hadiya	Semen-Belele	54	Ada-Maka
	Silte	Humbarik	55	Humbaricho
		Silti	56	Agode
	Gurage	Butajira	57	Jole

Southern Ethiopia, where cassava grows abundantly, was given more attention for cassava sample collection respectively. The survey was conducted at 17 cassava fields at Wolayta, 5 cassava fields at Gamo-Gofa, one field at Dawuro Zone, one field at Konta special district in southern Ethiopia, two fields at Hawassa Agricultural Research Center and one field at Jimma Agricultural Research Center (Table 5). In the survey, 25 cassava fields were inspected. The relative locations of the fields where tomato and cassava samples collected are shown in Fig. 2.

Table 5 Locations from which cassava samples were collected.

Region	**Zone**	**Woreda**	**Field No**	**Kebele**
SNNPR	Sidama	Hawassa	1 and 2	HARC
	Wolayta	Damot-Gale	3	Buge
			4	Hagaza-Doge
		Soddo-Zuriya	5	Waja-Kero
		Gununo	6	Shamba
			7	Doge-Shakicho
		Kindo-Koysha	8 and 9	Sorto
			10	Kacho-Na'a
			11and 12	Bele-01
		Damot-Woyde	13 and 14	Mayo-Kote
			15	Tora-Wulsho
			16	Bilbo-Badessa
			17	Badessa 01
	Gamo-Gofa	Arbaminch-Zuriya	18	Gurba
			19	Kola-Shara
			20	Chamo-Deresa
			21and22	Chano-Millae
Oromiya	Jimma	Jimma	23	JARC
SNNPR	-	Konta Special Woreda	24	Bite-Thanga
	Dawuro	Waka	25	Aba-Hargasa

HARC - Hawassa Agricultural Research Center; JARC - Jimma Agricultural Research Center

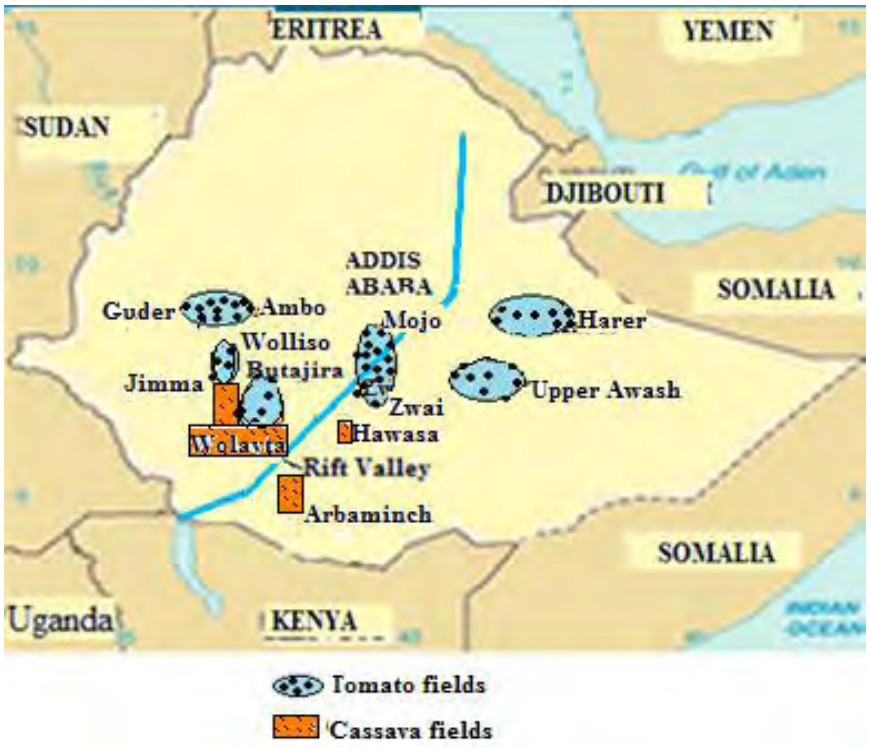


Fig. 2 Map of Ethiopia showing itinerary of surveys for tomato and cassava viruses.

4.1.2 Sample collection

In each field, plants were observed along foliar samples and carefully examined for viral symptoms. Plants showing virus disease-like symptoms were given more attention to pick up sample from individual plant. From each field, 10 samples were collected and total of 570 tomato and 250 cassava samples were collected during the survey periods. The incidence of viral infection, based on field observation, was recorded at each field. Approximately 1 g of leaf (wet weight) was subjected to be dried in approximately 5 g of silica gel simply by placing silica gel in collecting tubes which can be tightly sealed to make the sample leaf to be dried before nucleic acids degraded. The indicator silica gel which "reports" when the silica gel is dehydrated (blue) and hydrated (pink) was used. For some samples collection calcium chloride ($CaCl_2$) was also used by placing approximately 1 g $CaCl_2$ in sample tube with thin layer of cotton above it and placing approximately 1 g sample on the cotton and sealed. To minimize cross contamination of samples ethanol (70%) was used to wash hand.

4.1.3 Labeling and transport

At each field, field number and sample number were given. Region, Zone, Woreda and Kebele were recorded (Table 4 and 5). All the samples were brought to Holleta Agricultural Research Center of Ethiopian Institute of Agricultural Research (EIAR), Biotechnology Research Laboratory.

4.2 Virus detection

In order to confirm virus disease-like symptoms were indeed due to virus infection, intensive serological and molecular techniques based assay were conducted in laboratory.

4.2.1 Serological techniques

Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) and triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) were conducted for serological analysis of tomato and cassava samples respectively in 96 well ELISA plate (Nunc polysorp). For both ELISA tests, generally, volumes for each reaction were kept at 100 µl/well. Between incubations, 3 washing steps each lasting 5 minutes were carried out by soaking of the plates in washing buffer. The compositions of coating, washing and extraction buffer used are listed in Appendix 1. The negative controls were the extraction buffer and leaf samples of health plant used after successive checking in this study. All the antibodies and the positive controls for TYLCV, ToMV, TMV and PVY were from collections of Biotechnology Research, EIAR. The binding of alkaline phosphatase (ap) antibodies were revealed by the use of 1 mg/ml p-nitrophenyl phosphate (pNPP) substrate (sigma Fluka) with an incubation period of 30 minutes to 60 minutes at room temperature or as long as necessary to obtain clear reaction. The result was assessed by visual observation for color development and quantitative measurement was made by determining absorbance at 405_{nm} (A_{405nm}) using an ELISA plate reader model (Human, Germany), an hour after substrate addition. The mean absorbance reading of negative controls was determined and two times to the mean values of negative control was used as the positive/negative thresholds. A sample was regarded as positive when showed clear color development and if its average absorbance readings exceeded two times the mean of the negative control values after 60 minutes of incubation, i.e., when A_{405nm} $x_1+x_2 \div 2$ of infected sample > $A_{450nm}x_1+x_2 \div 2$ of negative control and fairly comparable with positive control reading value.

4.2.1.1 *Double Antibody Sandwich ELISA (DAS-ELISA) test*

DAS-ELISA was conducted basically as described by Clark and Adams (1977) as follows:

Microtitre ELISA plates were coated with a purified immunoglobin G (IgG) diluted in coating buffer (100 µl/well) to TYLCV, ToMV, TSWV, ToRSV-Ch, TMV, PVY and CMV (as recommended) and incubated at 37^0C for 2-4 hours. The antibody adsorbed on the plate becomes the trapping antibody. Aliquots of the test sample, prepared by grinding 20 mg of dried leaf sample in 1 ml extraction buffer, positive control (as available) and negative control were added to the duplicated wells (100 µl/well) in ELISA plate and incubated for overnight at 4^0C. Antigens (viruses) specific to the bound trapping antibody attach them to it, but other proteins remain in solution and are removed by washing. The virus attached to the trapping antibody is detected by adding IgG-ap in conjugate buffer (as recommended) (100 µl/well) and incubating at 37^0C for 4 hours. When aliquots of freshly prepared substrate, 1 mg pNPP, dissolved in 1 ml of substrate buffer was added (100 µl/well) in the final step, color developed as a result of enzyme action for positive samples and positive control after incubation of 30 to 60 minutes at room temperature. The result was confirmed by visual observation and the optical densities of wells were photometrically determined by ELISA plate reader.

4.2.1.3 *Triple antibody sandwich ELISA (TAS-ELISA) test*

To test for ACMV, the ELISA used was TAS-ELISA. It was conducted basically as described by Thomas *et al.* (1986) as follows:

ELISA plate was coated with purified polyclonal a universal probe IgG diluted in coating buffer (100 µl/well) to ACMV (DSMZ AS-0421/2) and incubated at 37^0C for 2-4 hours. Two percent of skim milk in PBST was added (100 µl/well) and incubated at 37^0C for 30 minutes. Aliquots of the test sample (prepared as to DAS ELISA test) was added to the duplicated wells in ELISA plate (100 µl/well) and incubated for overnight at 4^0C. Monoclonal antibody (Mab) which is broad spectrum to ACMV and can react with all ACMV species so far described (DSMZ AS-0421/2) diluted in conjugate buffer (1:1000 recommended) was added (100 µl/well) and incubated at 37^0C for 2-4 hours. An anti mouse rabbit antibody-alkaline phosphatase conjugate (Ram-ap) in conjugate buffer was added (100 µl/well) and incubated at 37^0C for 2 hours. Aliquots of freshly prepared substrate, 1 mg pNPP, dissolved in 1 ml of substrate buffer was added (100 µl/well) and incubated for 30 to 60 minutes or as long as

necessary at room temperature to obtain clear reaction. The result was confirmed as did to DAS-ELISA test.

4.2.2 Testing samples by nucleic acid based technique

A Further confirmatory testing becomes necessary and immunocapture PCR (IC-PCR) was conducted as a second step towards to TYLCV in molecular technique based assay. To that effect, from each field two samples were selected randomly. In addition, mater mix was made in 1.5 ml eppendorf tube by drawing 50 µl aliquots from each of ten samples collected from the same field in case when all sample from a field showed negative ELISA reaction. This was also done to test for ACMV on selected samples. In addition, conventional PCR method was conducted to test for ACMV by purifying the total genomic DNA from selected samples by promega total genomic DNA purification kit.

4.2.2.1 *Template DNA purification*

Immunocapture PCR (IC-PCR): To diagnose begomoviruses of tomato and cassava IC-PCR based technique was used by following the method used by LeamKhang *et al*. (2005) to identify *Tomato leaf curl Thailand virus* (TYLCTV) by PCR without DNA extraction.

A hundred µl of anti TYLCV or anti ACMV antibody diluted in coating buffer was added to PCR tube. Incubated at 37^0C for 3 hours and then the PCR tube was washed 3 times with washing buffer (PBST). Hundred µl aliquot of the test sample (20 mg of dried sample leaf was homogenized in 1 ml extraction buffer) was added to the PCR tube and incubated for overnight at 4^0C. The PCR tube was washed 3 times with PBST and 2 times with deionized distilled water.

Total DNA extraction: Forty mg of dried leaf tissue of selected cassava samples were freeze in liquid nitrogen and ground into fine powder with pestle and mortar. The powder was added into 1.5 ml eppendorf tube and 600 µl of nuclei lysis solution was added. The tube was vortexed for 2 seconds and incubated at 65^0C for 15 minutes. Three µl of RNase solution was added to each cell lysate and mixed with the sample by inverting each tube 4 times. The mixture was incubated at 37^0C for 15 minutes. Two hundred µl of protein precipitation solution was added and vortexed vigorously at high speed to 20 seconds and centrifuged for 3 minutes at 1400 rpm. The supernatant containing the DNA was transferred into a new clean

eppendorf tubes containing 600 µl of room temperature isopropanol. The solution was gently mixed until the tread like strands of DNA become visible and centrifuged at 1400 rpm for 3 minutes. The supernatant was carefully decanted and 600 µl of ethanol (70%) was added and the tube was inverted several times. Then, the ethanol was carefully removed. The tube was inverted in clean absorbent paper and the pellet was air dried for 15 minutes. Hundred µl of DNA rehydration solution was added to rehydrate the DNA by incubating overnight at 4^0C. The total DNA was checked on agarose gel and the viral DNA was checked by taking 2 µl of total DNA using three primer combinations (Table 7) for amplification in thermocycler.

4.2.2.2 *PCR and agarose gel electrophoresis*

PCR primers: For molecular identification of TYLCV and to test for ACMV, a degenerative (general) primer that is genus specific to all begomoviruse*s* was used. Primer pair, Begomo_146 and Begomo_672, derived from sequences that are common to all cassava (and most) begomoviruses, including TYLCV, was used for virus detection purposes. To test for ACMV, primer pair specific to ACMV DNA-A and *Begomovirus* DNA-B specific primers were also used. The primer sequences are shown in Table 6 and the primer combinations are shown in Table 7. The general *Begomovirus* primer combination (Begomo_146 and Begomo_672) with primers designed to anneal in highly conserved regions of begomoviruses, amplify DNA-A fragments of isolates of the following viruses: ACMV, EACMV and UgV as well as ICMV, TYLCV and *Lima bean golden mosaic virus* while the primer combination C154_F and PBL1V2040_R amplify DNA B fragment of these begomoviruses. The primer combination ACMVAL/F_F and ACMV_AROR_R only amplify ACMV DNA-A sequences (Karakacha, 2001).

Table 6 Primer sequences used to test begomoviruses on tomato and cassava.

No	Name of primer	Sequence (5'→3')	$T_m(^0c)$	Strand
1	Begomo_146	TAA TAT TAC C(GT)G (AT) (GT)G (AGC) CC (GC) C	56.6	Sense
	Begomo_672	TGG AC(CT) TT(AG) CA(AT) GG(GCT) CCT TCA CA	61.8	Complimentary
2	ACMVAL/F_F	GCG GAA TCC CTA ACA TTA TC	55.3	Sense
	ACMV_AROR_R	GCT CGT ATG TAT CCT CTA AGG CCT G	64.6	Complimentary
3	C154_F	GGT AAT ATT ATA (ACT) CG GAT GG	51.8	Sense
	PBL1V2040_R	GCC TCT GCA (AG) TG (AG) TC (GT) AT CTT CAT ACA	67.4	Complimentary

T_m - melting temperature.

Table 7 Primer combinations and annealing temperatures used to test begomoviruses [a].

No	Virion sense primer	Complimentary primer	Virus tested	$T_a(^0c)$	Specific to
1	Begomo_146	Begomo_672	TYLCV ACMV	52	Begomovirus DNA-A
2	ACMVAL/F_F	ACMV_ AROR_R	ACMV	52	ACMV DNA-A
3	C154_F	PBL1V2040_R	ACMV	-	Begomovirus DNA-B

T_a - annealing temperature

[a] Number 1 primer combination was used to diagnose both TYLCV and ACMV; number 2 and 3 were used to diagnose ACMV.

PCR reaction: PCR reaction contained a mixture of 10 µl of 5xTaq polymerase buffer (PCR buffer), 5 µl $MgCl_2$ (25 mM), 2.5 µl dNTPs (10 mM), 1 µl of each primer (100 pmol), 0.25 µl (5 units) of Taq polymerase (promega) and 30.25 µl PCR water (nuclease free water) in a total volume of 50 µl. Master mix for PCR reaction was made on ice and divided into PCR tubes that contained immunocaptured viral DNA. In addition, similar PCR reaction mixture was prepared to test for ACMV by adding 2 µl of the total extracted DNA on 48 µl of the above PCR reaction mix, PCR water was made to 28.25 µl per reaction in this case. The reaction mix was subjected to amplification by thermocycler (TECHNE TC-3000, USA).

PCR programme: After an initial denaturation step of 3 min at 95^0C, 35 amplification cycles followed with 1 min denaturation at 95^0C, 1.5 min primer annealing at temperature specified for respective primer combinations (Table 7) and 1 min strand extension at 72^0C. PCR amplification was terminated by a final extension period of 10 min at 72^0C and hold at 10^0C. The expected length of the DNA amplified by the primer combination 1 and 3 of Table 7 was 520 bp and primer combination 2 was 975 bp (Karakacha, 2001).

Agarose gel electrophoresis: one percent (w/v) agarose powder in 1x TAE buffer was melted in micro wave for 2 minutes. The gel comb was placed at the top of the gel box. Melted agarose was gently poured into the box until the TEA buffer was approximately halfway up the teeth of the comb and left to solidify. Two µl of 6x loading dye was placed on piece of parafilm, separately each drop from one another. PCR product (10 µl) was gently added on each of the loading dye and was mixed together by drawing the liquid back into pipette tip and then expelling into the parafilm. Finally, 12 µl of liquid was drawn and placed in one well of the agarose gel. This was repeated for remaining samples, 1 kb ladder, negative controls and/or positive control (as available) and allowed to run for 45 minutes to 1 hour at 100 V. Gels were then stained with 1 µg/ml ethidium bromide (100 µl of 10 mg/ml ethidium bromide in 1000 ml of d H_2O) for 20 minutes with gentle shaking. The result was visualized with UV light and photographed using Sony digital photo camera connected with computer. The expected size of unknown DNA (TYLCV) was determined by comparing with the DNA molecule of known sizes (1 kb DNA size marker, promega).

4.3 Data analysis

The virus disease-like symptoms observed during the survey periods were related to area distribution. The incidence was estimated based on virus disease-like symptoms recorded at a field and number of samples assayed. The recorded disease incidence level was estimated as percentage infection whereby ≤20% = low incidence; 21-49%= moderate incidence and ≥50%= high incidence, the range used by Nono-Womdim *et al*. (1996). Based on the serological and IC- PCR positive results, the distribution of viruses tested positive was evaluated as to Adjata *et al.* (2008).

5. RESULTS

5.1 Virus diseases-like symptoms and incidence at tomato fields

Virus disease-like symptoms observed in the tomato field were leaf curling, bright mosaic, leaf yellowing with curling, and stunted growth (Fig. 3a-d). These symptoms were observed particularly in fields surveyed at UAAIE, Merti, Melkasa, and along the road Mojo to Zwai in East Shewa. All these areas were located in the Rift Valley. Virus disease-like symptoms were also observed in few fields surveyed along the road from Wolliso to Jimma. Tomato plants inspected at fields along the road from Ambo to Guder, Butajira to Wolayta and Harer were free from any virus disease-like symptoms (Fig. 3e).

Fig. 3a-d Tomato plants showing virus disease-like symptom: leaf curling (a); bright mosaic (b) leaf yellowing and curling (c); stunted growth (d). Samples were from Rift Valley areas. **Source:** photo Eyasu Wada, December 2009.

Fig. 3e Tomato plant showing no virus disease-like symptom; sample was from Guder

Source: Photo Eyasu Wada, December 2009.

Based on data recorded at tomato fields, estimate of virus disease-like incidence ranges from 90-95% at UAAIE, 20-75% at Merti, 20-30% at Melkasa, 0 (no symptom observed) to 45% at fields along the road from Mojo to Zwai and 2-3% at a few fields surveyed along the road from Wolliso to Jimma. Virus disease-like symptom was observed at 47.37% of the tomato fields inspected during the survey periods. Of 47.37%, at 18.5% of the fields high incidence was recorded (≥ 50%); at 25.9% of the fields, moderate incidence was recorded (21-49%) and at 55.6% of the fields, low incidence was recorded (≤ 20%) (Appendix 2). No virus disease-like symptoms were observed at the remaining 30 (52.6%) tomato fields inspected during the survey periods.

5.2 Serological test results

5.2.1 DAS-ELISA test result of TYLCV

A total of 570 tomato samples tested against TYLCV, 12.1% of the samples (Table 8) gave weak positive ELISA reaction. In some plate wells color development seems somehow half intensity between positive and negative controls (Fig. 4a). Furthermore, samples averaged ELISA reader values were not greater than two times to the negative control and not coincide with positive control. However, samples from the rest of fields gave no ELISA reaction, still the positive control tested positively (Fig. 4b), showing that the samples were negative for TYLCV.

Of 12.1% TYLCV infected samples, 17.4% were from Melkasa, 20.3% were from UAAIE, 24.6% were from Merti and 37.7% were from fields along the road from Mojo to Zwai.

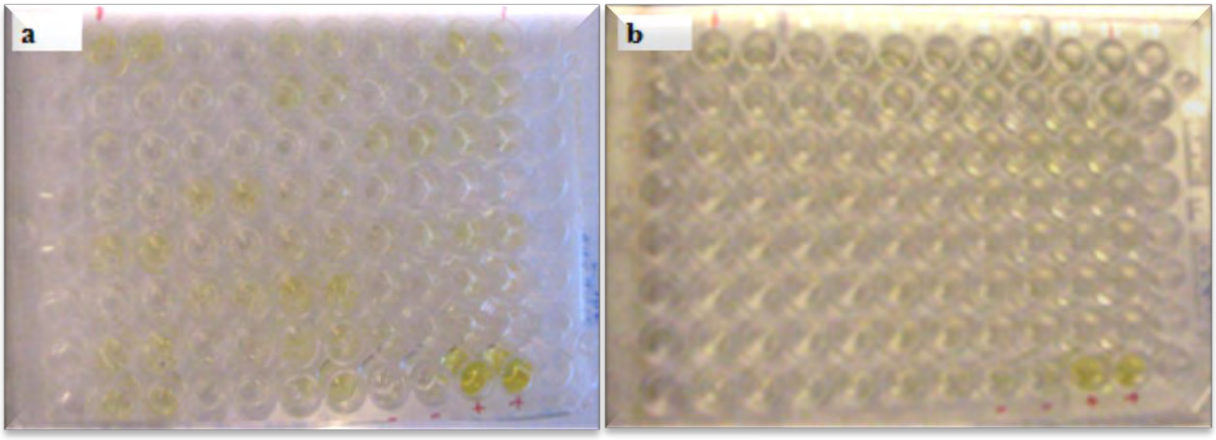


Fig. 4 DAS-ELISA test result of TYLCV, ssDNA virus: samples gave weak positive reaction (a) and samples gave negative reaction (b); Yellow colors show positive reactions; - Negative control; + positive control. Samples were added in duplicated well from left to right and in left and right edge wells no sample was added.

5.2.2 DAS-ELISA test results of tomato RNA viruses

Total of 570 samples tested against ToMV, 17.9% of samples gave positive ELISA reaction to ToMV showing clear color development (Fig. 5a) and these samples ELISA reader averaged values were greater than two times to negative control and fairly comparable with averaged ELISA reader value of positive control.

Of 17.9% ToMV infected samples, 5.9% were from Merti, 6.9% were from Melkasa and 87.2% were from fields along the road from Mojo to Zwai. All the samples tested against other RNA viruses: TSWV, ToRSV-Ch, PVY, TMV and CMV were provided negative reaction to DAS-ELISA test (Fig. 5b-f). ELISA reader averaged value of the duplicated wells of these viruses was similar and not differs from the negative control, i.e., all the samples were negative for these viruses.

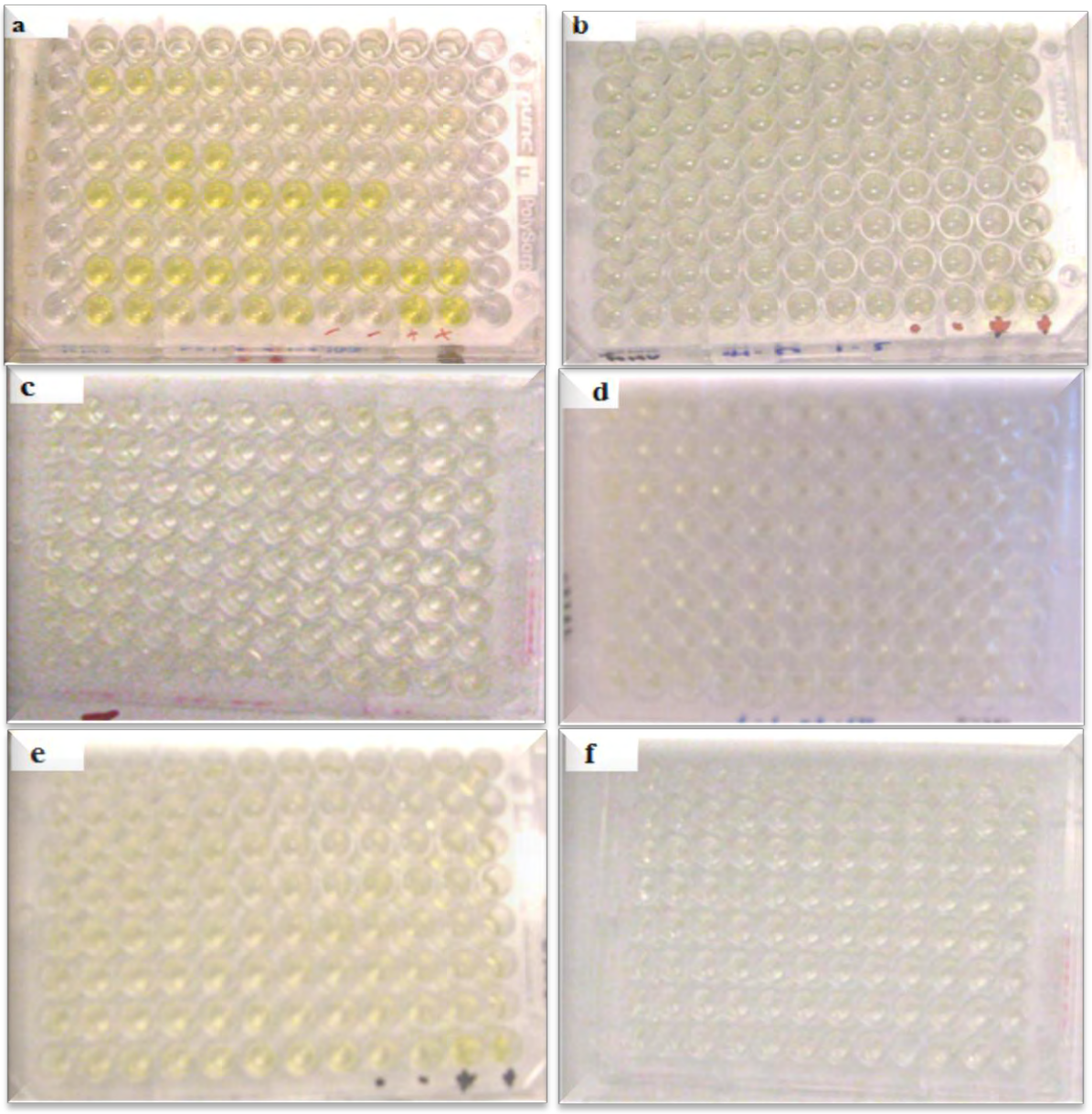


Fig. 5 DAS-ELISA test results of tomato RNA viruses: ToMV (a), PVY (b), ToRSV-Ch (c), TSWV (d), TMV (e) and CMV (f); Yellow colors show positive reactions; - Negative control; + positive control. Samples were added in duplicated wells from left to right. In left and right edge wells no sample was added in plate (a).

5.2.3 Mixed infection of TYLCV and ToMV

At least one sample was infected by both viruses at fields where both viruses were detected, i.e., 6.2% of samples from both viruses detected field showed mixed infection, 3.3% of samples from Melkasa, 6% of samples from fields along the road from Mojo to Zwai and 6.7%

of samples from Merti showed mixed infection (Fig. 6). Half of samples collected from 27 virus disease-like symptoms observed fields were infected by TYLCV and/or ToMV. All 40 samples from four virus disease-like symptom observed fields at Wolliso and Abasha/Gurage provided negative test result for all viruses. In contrary, 10 samples from four non symptomatic fields (among 40 samples), in Rift Valley (DSAD, Zwai, Doda and Enane), provided positive test for ToMV. Number and percentage of samples infected by TYLCV and/or ToMV at the respective fields is shown in Table 8. TYLCV occurred at high incidence level (70%) in UAAIE and 56.7% in Merti, moderate incidence level (40%) in Melkasa, low incidence level (12.6%) in fields surveyed along the road from Mojo to Zwai and not detected in samples from the rest fields. ToMV was distributed at fields surveyed along the road from Mojo to Zwai and Melkasa at moderate incidence level, reaching 41.6% and 23.3% respectively. At Merti it occurred at low incidence level (20%) and not detected in tomato samples from the other fields. Seventy percent of samples from UAAIE, 66.7% of samples from Merti, 60% of samples from Melkasa and 47.4% of samples from fields along the road from Mojo to Zwai were virus infected (Fig. 6).

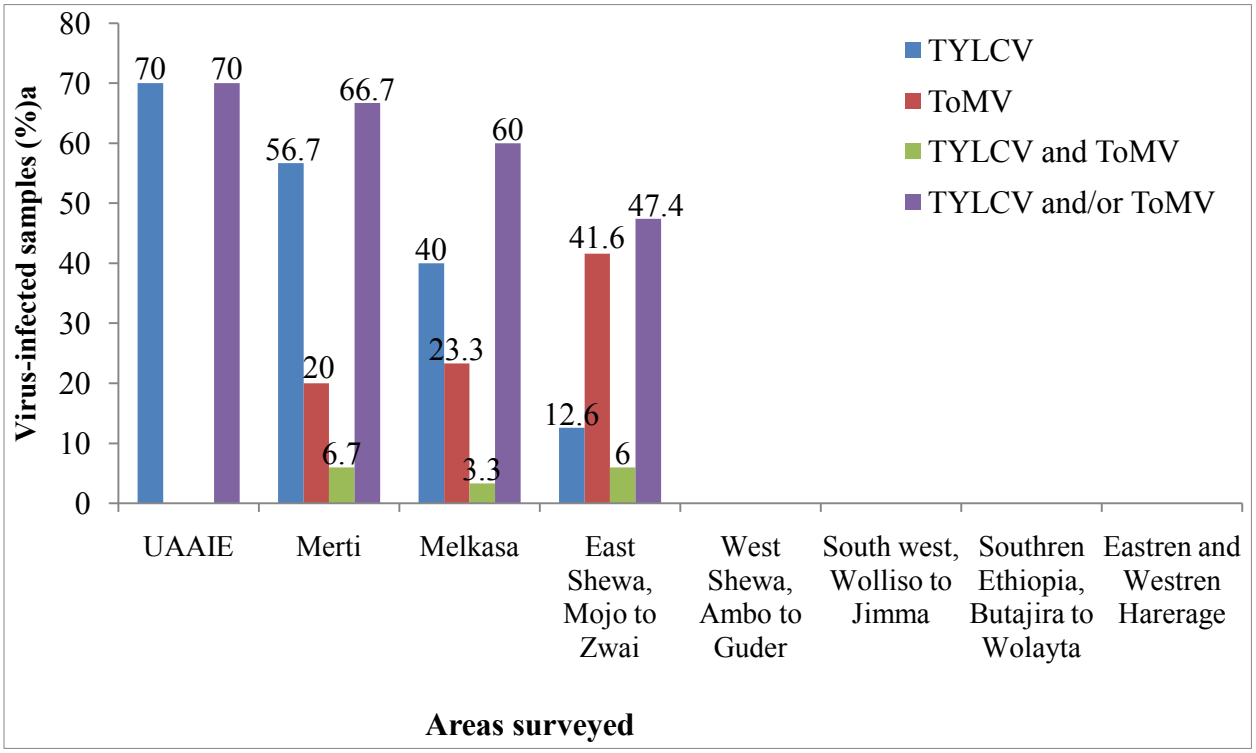


Fig. 6 The percentages of virus-infected samples at respective areas. [a] Individual % of TYLCV and ToMV do not sum up to 100% infected samples because of cases of mixed infections. UAAIE - Upper Awash Agro Industry Enterprise.

Table 8 Tomato samples infected by TYLCV and/ToMV and the respective fields*

Area surveyed	Particular site where samples were collected	F	N	No of samples infected with			
				TYLCV	ToMV	TYLCV and ToMV	TYLCV and/or ToMV
UAAIE	Unit B and Unit C	2	20	14(70)	(-)	(-)	14(70)[a]
Awash	Merti	3	30	17(56.7)	6 (20)	2(6.7)	20(66.7)
Melkasa	MARC	3	30	12(40)	7(23.3)	1(3.3)	18(60)
East Shewa, Mojo to Zwai Rift Valley	Mojo (01 and Koka)	2	20	4(20)	9(45)	2(10)	11(55)
	Alemgena (Ashewa)	1	10	5(50)	7(70)	3(30)	9(90)
	Bora (Doda)	2	20	9(45)	11(55)	5(25)	15(75)
	Adama-Tulu (Ana, Batu-DSAD)	3	30	(-)	11(36.7)	(-)	6(20)
	Golbe-Chale (Alto)	2	20	(-)	10(50)	(-)	10(50)
	Wora (Enane, Digida)	2	20	(-)	6(30)	(-)	6(30)
	Meki (Gerisa)	1	10	(-)	6(60)	(-)	6(60)
	Techo-Choronqe (Doda)	3	30	(-)	17(56.7)	(-)	15(50)
	Zwia (Zwai 01, Abne)	3	30	8(26.7)	12(40)	2(6.7)	15(50)
West Shewa, Ambo to Guder	Ambo, Toke-Kutaye, Guder	12	120	(-)	(-)	(-)	(-)
Southwest Ethiopia, Wolliso to Jimma	Wolliso, Abasha, Kersa	5	50	(-)	(-)	(-)	(-)
Southern Ethiopia, Buta-Jira to Wolayta	Buta-Jira, Silti, Humbark, Semene-Belele, Kindo-Koysha	5	50	(-)	(-)	(-)	(-)
Western and Eastern Harerage	Tulu, Meiso, Kombolcha, Dereteyera, Kersa	8	80	(-)	(-)	(-)	(-)
Total		57	570	69 (12.1)	102 (17.9)	15 (2.6)	145 (25.4)

* Figures in parentheses are the percentage of virus infected samples. F - number of fields; N - number of sample collected; UAAIE - Upper Awash Agro Industry Enterprise; MARC - Melkasa Agricultural Research Center; DSAD - Dadicho Sengele Agricultural Development; (-) samples gave negative result to DAS-ELISA test. [a] Individual % of TYLCV and ToMV do not sum up to 100% infected samples because of cases of mixed infections.

5.3 Molecular technique based assay results of TYLCV

IC-PCR, using genus, *Begomovirus,* specific degenerative primers Begom_146 and Begomo_672, for identification of TYLCV was successful. The specific PCR product was obtained in positive control and samples positive for IC-PCR test, but not with the negative control. The TYLCV positive sample which was added into PCR tube with no antibody to capture the virus and only antibody with no plant extract was added were subjected to the PCR reaction, both did not provide a band. Samples from some fields also yielded faint bands were also observed. The specific bands are at the position corresponding to the expected 520 bp (Fig. 7).

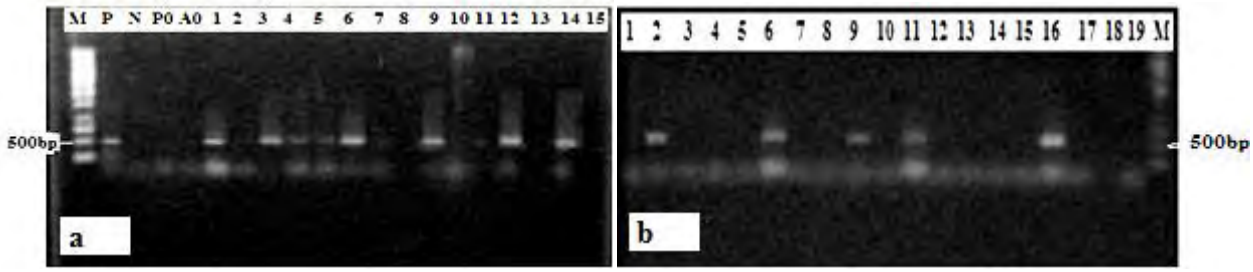


Fig. 7 Agarose gel electrophoresis showing IC-PCR products of TYLCV, using the primer combinations Begomo_146 and Begomo_672. (a) M -1kb size marker; P - positive control; N -negative control; P0 - only antibody added (no plant material); A0 - no antibody added (only a positive sample); number 1 to 15 indicate samples tested, (b) 1-19 samples tested.

5.4 Prevalence of tomato viruses

DAS-ELISA test and IC- PCR test revealed the occurrence of TYLCV in 24.6% of the total fields surveyed. All these fields are in Rift Valley. Samples from the fields in other areas provided negative result with both modern technique based assays. ToMV was wide spread virus among the tomato viruses tested by DAS-ELISA being prevalent at 47.37% of the study area. Samples from all fields were negative for other tomato viruses: TSWV, ToRSV-Ch, TMV, PVY and CMV (Table 8).

5.5 Virus diseases-like symptoms at cassava fields

Unlike tomato fields, in almost all cassava fields no typical cassava mosaic disease symptom was observed during the survey period (Fig. 8a) except in few cassava fields where bright mosaic revealing cassava plants were observed at the surveyed areas (Fig. 8b) and that were collected as sample to test ACMV. It was, therefore, not possible to estimate the incidence and incidence level of cassava mosaic virus disease based on symptom, without clear symptom observation.

Fig. 8 Cassava plants from surveyed areas exhibits: no virus disease-like symptom from Arbaminch Zuriya (a); virus disease-like symptom from Wolayta (Kindo-Koysha) (b).

Source: Photo Eyasu Wada

5.6 serological and molecular test results of cassava samples

TAS-ELISA test result of ACMV suggested that all 250 cassava samples tested were not infected by ACMV. No nucleic acid based amplified fragment of ACMV DNA was detected from the samples tested by purifying DNA by IC-PCR method. This was done by using primer pairs Begomo_146 and Begomo_672 which were designed from sequence common to all cassava mosaic viruses and most begomoviruses. Although the primer pair successfully amplified TYLCV from positive tomato samples, no band was obtained with the tomato samples which were negative to TYLCV and with all cassava samples tested for ACMV (Fig. 9).

This result was obtained by applying similar method to both tomato and cassava plants at similar condition from IC step to visualizing result with UV light and photographing except that for each virus its specific unlabeled antibodies was used at IC step to capture the virus. In addition to this method, selected cassava samples tested by conventional PCR method were also provided no band. So that all cassava samples from all fields were negative for ACMV.

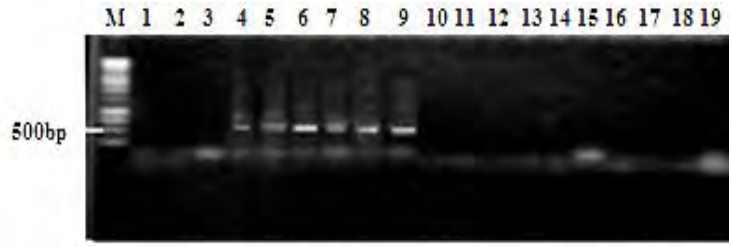


Fig. 9 Agarose gel electrophoresis for TYLCV and ACMV, using the primer combinations Begomo_146 and Begomo_672. M-1kb size marker; number 1-3 tomato samples negative for TYLCV; 4-9 tomato samples positive for TYLCV; 10-19 cassava samples tested for ACMV.

6. DISCUSSION

6.1 Virus disease survey results

Leaf curling, leaf yellowing, bright mosaic and stunted growth were the predominantly observed symptoms at tomato fields. Tomato plants revealed virus disease-like symptoms reach 90-95 % at UAAIE, 20-75% at Merti, up to 45% at fields along the road Mojo to Zwai and 20-30% at Melkasa. No virus disease symptom was observed at tomato fields surveyed at West of Ethiopia. This supports previous report that the greater number of tomato plants showing virus disease-like symptoms were found at Zwai when compared with west of Ethiopia, where a few tomato plants showed virus disease-like symptoms (Yaynu Hiskias, 1998). Common symptoms on plants are used to study a disease having a viral etiology in attempt to control disease. However, many factors can influence the symptom exhibition (Matthews, 1980). Usually it is necessary that visual inspection for symptom is done in conjugate tests to ensure accurate diagnosis for virus infection (Block, 1982). Thus, serological and molecular based assays were conducted as further test to detect particular virus.

6.2 Serological test results

All the samples selected among those with weakly positive serological reaction gave samples were provided specific bands for TYLCV with IC-PCR test. This suggests that all the samples that gave weak positive reaction by DAS-ELISA test could be taken as positive test for TYLCV. The weak positive reaction of DAS-ELISA test against TYLCV may be due to the strain variance of the TYLCV occurring in study areas from the antibodies are produced for or the low antigenisity properties of the coat proteins of the begomoviruses in general (Harrison *et al*., 1991).

ToMV detected from 41.6% of samples from along the road from Mojo to Zwai, 23.3% of samples from Melkasa, 20% of samples from Merti in Rift Valley areas and absent in sample from western Ethiopia. This is similar to the report of Yaynu Hiskias (1998) that ToMV was not detected at fields along the road Ambo to Guder in western Ethiopia, but unlike this report the incidence and distribution was increased at fields along the Rift Valley areas and the highest incidence was recorded at farmers' tomato fields along the road from Mojo to

Zwai. This may be for the fact that it is transmitted mechanically by contact and infected seeds, i.e., the infected seeds can move freely from one place to another for sowing across these areas may have contributed to the present wider distribution of this virus compared to the previous reports.

CMV infects tomato worldwide causing significance yield losses up to 50% (Jones *et al*., 1991) and it had been previously reported from tomato plants in Ethiopia (Marchoux, 1976). However, there have been some doubts with this virus on tomato in Ethiopia (Yaynu Hiskias, 1998; Shih *et al.,* 2006). DAS-ELISA test conducted in this study indicated the absence of CMV in 570 tomato samples from 57 fields from different parts of Ethiopia. This may suggest that CMV is not important virus infecting tomato at study areas in Ethiopia. PVY was earlier reported from tomato fields at Melkasa, Guder, Zwai, Wolliso and Merti in a low incidence level (Yaynu Hiskias, 1998), all of these areas were surveyed during this study, however, no sample provided positive test for PVY in DAS-ELISA test. Shih *et al.* (2006) also reported that PVY was not detected in samples from Melkasa by DAS-ELISA test and which was similar to this study result that all samples from Melkasa were negative for PVY. TMV was reported as it was widely spread in areas Sidamo and Wello provinces (Agranosky and Anisimoff, 1986), these areas were not surveyed in this study. According to their report it was rarely found in areas around Ambo, Guder, Merti, Zwai and Upper Awash Valley. In accordance with their report all samples gave negative serological result this study indicating that this virus has no importance to these areas. TSWV and ToRSV-Ch was the first to be checked on tomato plant samples in Ethiopia and all of the samples were negative for these viruses in DAS-ELISA test. More and repeated diagnosis is necessary to confirm the absence of these viruses in Ethiopia.

6.3 Molecular test results

All the positive results obtained by IC-PCR test were those samples that gave weak positive reaction to DAS-ELISA test. This suggests that all the samples that gave weak positive reaction by DAS-ELISA test could be taken as positive test for TYLCV. The expected band was obtained in samples tested TYLCV by IC-PCR from 24.6% of fields were at the position corresponding to the expected 520bp. No specific band was obtained from samples that showed negative reaction to DAS-ELISA test.

On testing for TYLCV by IC-PCR, some samples only produce weak bands. Such results could be attributed to low concentration of the virus in the PCR tubes due to low concentration of the virus on the test plant. Most previous virus identification works in Ethiopian laboratories relies only on symptomatology and serological tests. This work is among the first attempt to use sensitive PCR based techniques for plant virus identification and paved the way for the use of with other crop virus combination locally.

6.4 Prevalence of viruses

In the present survey, TYLCV is an important tomato virus to be presented at 24.6% of the surveyed areas. The first definitive report of a virus associated with TYLCV in Ethiopia was reported by Shih *et al*. (2006) from Melkasa area. The result of this study shows that the virus is much more distributed particularly in Rift Valley areas in East Shewa but not at all areas inspected during the study time. This virus was also detected from Melkasa which supports the previous report of Shih *et al. (*2006). Since first described in Israel, TYLCV has caused tomato yield loss up to 100% (Nakhla and Maxwell, 1999). ToMV was identified as a wide spread virus among the tomato viruses tested by DAS-ELISA being prevalent at 27 fields that covers 47.37% of the study areas. All the samples infected by either or both virus were collected from Rift Valley areas may be due to the permanent presence of susceptible reservoir hosts, vector (white fly) for TYLCV or infected tomato seeds, root or leaf debris since which can reserve ToMV.

Although the collection of samples was carried out systematically, i.e., samples representing different virus disease-like symptoms were collected, no virus was detected in half of the tomato samples from virus disease-like symptom showing fields while some tomato samples from non symptomatic fields were infected with ToMV. This may indicate that neither the presence nor the absence of virus disease-like symptom necessary proves the presence or absence of a certain virus infection. The absence of virus on tested symptomatic plants may be due to the occurrence of other viruses or the virus disease-like symptom may not be due to virus infection. According to Matthews (1980), plants can also exhibit virus disease-like symptoms as response to unfavorable environmental conditions such as weather conditions, soil mineral nutrient content imbalances, infection by non viral pathogens, damage caused by insects or air pollution.

6.5 ACMV test results

At cassava fields no clear virus disease symptom was observed during the surveyed periods. Visual inspections are relatively easy when symptoms are clear and characteristic of specific diseases. The ACMV causes a devastating disease on cassava in other African countries. A disease incidence of up to 100% was observed in many fields in Kenya (Karakacha, 2001). However, all 250 cassava samples from 25 cassava fields gave negative result in TAS-ELISA test for ACMV in this study. A single diagnosis method or assay may provide adequate information on viruses, but a combination of methods generally needed for unequivocal diagnosis (Naidu and Hughes, 2003). To better conclude as the cassava samples are free from the virus infection further testing based on IC-PCR and conventional PCR technique were conducted for ACMV and both provided no specific band (negative result). Several possibilities can be put towards this result although experimental evidence to that effect is lacking. It may be speculated that there is no ACMV introduction to the study areas. Since cassava plant is only recently introduced in to Ethiopia in 19th century even though the plant is originated in South America over several years ago, the introduced varieties might be the ACMV resistant The Agro-ecology of cassava cultivated areas of Ethiopia from where samples were collected might not be suitable for the virus.

7. CONCLUSIONS

- TYLCV is one of tomato infecting virus in the study areas in Ethiopia. Serological and molecular techniques based assay showed 24.6% relative occurrence of the virus in the surveyed areas in the country. These assay results indicate that the virus incidence was high (≥50%) at tomato fields in UAAIE, Merti and Alemgena (Ashewa) where tomato grows in large scale under irrigation.
- The study result revealed that ToMV is widely spread being distributed at 47.4% of the tomato fields surveyed. This virus incidence was high (≥ 50%) from farmers' tomato field along the road from Mojo to Zwai.
- This study result suggests that TYLCV and ToMV display distinct pattern of spread within the study areas and this is the first to present the incidence and distribution of TYLCV although the virus has been already reported from Ethiopia.
- It is important to notice that ToMV has been distributed widely than the previous information suggests.
- Tomato samples from all fields were negative for TSWV, ToRSV-Ch, TMV, PVY and CMV.
- The field survey study followed by TAS-ELISA, IC-PCR test and standard PCR test suggested that ACMV was not present in all the samples tested.
- The result has provided updated information on the incidence and distribution of tomato viruses and about ACMV on cassava in Ethiopia.

8. RECOMMENDATIONS

- TYLCV is a very aggressive virus that spreads rapidly throughout the world and it has become a major problem for tomato growers in recent years. To better understand the disease situation in Ethiopia, further study on its epidemiology including the strain variability, biodiversity relationship with whitefly vectors and host range should be initiated. It is also important to start study on management methods such as host resistance, screening insecticides to control the white fly vector and cultural practices including planting date adjustments.
- Considering the distribution of ToMV appropriate virus disease management progmamme should be sought, i.e., since ToMV is seed-born and contact transmitted, the use of virus free seeds by using treated seeds and appropriate hygiene in seedbed preparation to avoid contact transmission can be practiced by farmers.
- It is also recommended to apply tissue culture practices to have virus free planting materials.
- Such information can be utilized in the development of virus disease management packages.
- It is interesting to notice that absence of ACMV in all the samples tested and the necessity to be ready to set strong ACMV prevention measures since the virus can be disseminated with vegetative propagating materials. Hence, it is important to test cassava planting materials coming into the country for research or production purpose by strengthening quarantine. Research should be broadened to include more samples for begomoviruses as well as other viruses like *Cassava brown streak virus* which is known to be major important in other cassava main producing countries.
- This study is not exhaustive in terms of area coverage. Hence, having this information, it is important to conduct a survey of tomato and/or cassava viruses to cover areas that were not surveyed during this study time.

9. REFERENCES

Adane Abraham (2009). Agricultural biotechnology development and research in Ethiopia. *Afr. J. Biotechnol*. **8(25):**7196-7204.

Adjata, K.D., Muller, E., Aziadekey, M., Gumedzoe, Y.M.D. and Peterschmitt, M. (2008). Incidence of cassava viral diseases and first identification of *East African cassava mosaic virus* and *Indian cassava mosaic virus* by PCR in cassava fields in Togo. *Amer. J. plant physiol.* **3:**73-80.

Agranovsky, A.A. and Anisimoff, B.V. (1986). Virus diseases of tomato in Ethiopia. *Acta Hort*. **190:**115-119.

Akad, F. and Czosnek, H. (2002). Virus detection: *Tomato yellow leaf curl virus* (TYLCV) and other begomoviruses. Technical sheet No. 36. The Hebrew University, Israel, 5 pp.

Amsalu Nebiyu (2006). Genetic variations in cassava at Jimma, Southwest Ethiopia. *Trop. Sci.* **46(3)**:171-175.

Amsalu Nebiyu, Weyessa Garedew, Assefa Tofa, Wubishet Abebe, Asfaw Kifle and Edossa Etisa (2000). Cassava. **In**: *Root and Tuber Crops, the Untapped Resources*, pp. 304-309, (Gebremdhin Woldegiorgis, Endale Gebre and Berga Lemaga, eds), EIAR, Ethiopia.

Anupam, V. and Malathi, V.G. (2003). Emerging geminiviruses problems: A serious threat to crop production. *Ann. Appl. Biol.* **142:**145-146.

Atiri, G.I., Ogbe, F.O., Dixon, A.G.O., Winter, S. and Ariyo, O. (2004). Status of cassava mosaic virus diseases and cassava begomoviruses in Sub-Saharan Africa. *J. Sust. Agri*. **24:**5-35.

Bock, K.R. (1982). The identification and partial characterization of plant viruses in the tropics. *Trop. Pest Man.* **28:**399-411.

Burns, R. (2004). *Methods in Molecular Biology: Immunochemical Protocols*, 3rd ed., Humana press Inc., Totowa, New Jersey, pp. 317-326.

Busogoro, J.P., Masquellier, L., Kummert, J., Dutrecq, O., Lepoivre, P. and Jijakli, M.H. (2008). Application of a simplified molecular protocol to reveal mixed infections with begomoviruses in cassava. *J. Phytopathol.* **156:**452-457.

Cherinet Abuye, Yemane Berhane and Tessema Ersumo (2008). The role of changing diet and altitude on goitre prevalence in five regional states in Ethiopia. *East Afri. J. Public Heath* **5(3):**163-168.

Clark, M.F. and Adams, A.N. (1977). Characteristics of the micro plate method of enzyme linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol*. **34:**475-483.

Cohen, S. and Antignus, Y. (1994). *Tomato yellow leaf curl virus*: a white fly born geminivirus of tomatoes. *Adv. Dis. Vect. Res.* **10:**259-288.

Czosnek, H. and Laterrot, H. (1997). A worldwide survey of tomato yellow leaf curl viruses. *Archi. Virol.* **142:**1391-1406.

Czosnek, H. (2007). *Tomato Yellow Leaf Curl Disease: Management, Molecular Biology, Breeding for Resistance.* Published by Springer, Dordrecht, The Netherlands, pp. 67-78.

Dejene Mekonnen (2006). The potentials and prospects of cassava as food security crop in Ethiopia. **In:** *Cassava Improvement to Enhance Livelihoods in Sub-Saharan Africa and Northeastern Brazil*. *First International Meeting on Cassava Breeding, Biotechnology and Ecology*, pp. 102-108, (Ortiz, R. and Nassar, N.M.A., eds). Brasilia, Brazil.

Delatte, H. (2005). Study of the pathosystem *Begomovirus/Bemisia tabaci*/ tomato on the South West Islands of the Indian Ocean. Ph.D Thesis. Wageningen University, Wageningen, The Netherlands, 160 pp.

Deng, D., McGrath, P.F., Robinson, D.J. and Harrison, B.D. (1994). Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. *Ann. Appl. Biol.* **125:**327-336.

Dube, H.C. (2004). *A Text Book of Fungi, Bacteria and Viruses*, 2nd ed., Sanjoy Printers, Delhi, pp. 228-236.

Duffy, S. and Holmes, E.C. (2008). Phylogenetic evidence for rapid rates of molecular evolution in the single stranded DNA *Begomovirus*. *J.Virol*. **82(2):**957-965.

FAO (2008). Cassava as alternative food source. Food and Agricultural Organization of UN. (http://www.bt.com.bn/life/2008/07/27/cassava_as_alternative_food_source) (Accessed on June 1, 2010).

FAO (2004). *Production Year Book*. Vol. 90, World Agriculture Information Center Database, Rome, Italy.

Fauquet, C.M., Besaro, D.M., Briddon, R.W., Brown, J.K., Harrison, B.D., Rybicki, E.P., Stenger, D.C. and Stanley, J. (2003). Revision for taxonomic criteria for species demarcation in the family Geminiviridae and an updated list of *Begomovirus* species. *Arch. Virol.* **148:**405-421.

Fauquet, C.M., Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M. and Zhou, X. (2008). Geminiviruses strain demarcation and nomenclature. *Archi. Virol.* **153:**83-821.

Fauquet, C.M., Maxwell, D.P., Gronenborn, B. and Stanley, J. (2000). Revised proposal for naming geminiviruses. *Arch. Virol*. **145:**1743-1761.

George, N.A. (2005). *Plant Pathology*, 5th ed., Elsevier Academic Press, London, UK, pp. 724-822.

Gibson, R.W. and Otim-Nape, G.W. (1997). Factors determining recovery and reversion in mosaic-affected African cassava mosaic virus resistant cassava. *Ann. Appl. Biol.* **131:**259-271.

Gilbert, M.G. (1995). Euphorbiaceae. **In**: *Flora of Ethiopia and Eritrea,* vol.2, No 2, pp. 265-380, (Edwards, S. and Hedberg, I., eds). The National Herbarium of Addis Ababa, Addis Ababa, Ethiopia.

Giovannucci, E. (1999). Tomatoes, tomato based products, lycopene and cancer: Review of the epidemiologic literature. *J. Natl. Cancer Inst.* **91(4):**317-331.

Green, S.K. and Kim, J.S. (1991). Characterization and control of viruses infecting peppers: Literature review. *AVDRC*, *Tech. Bull.* No.18, 60 pp.

Harrison, B.D. and Robinson, D.J. (1999). Natural, genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). *Ann. Rev. Phytopathol*. **37:**369-98.

Harrison, B.D., Swanson, M.M. and Fargette, D. (2005). *Begomovirus* coat protein: Serology, variation and functions. *Nucleic acids res*. **25:**2325-2330.

Harrison, B.D., Liu, Y.L., Khalid, S., Hameed, S., Otim-Nape G.W. and Robinson, D.J. (1991). Detection and relationships of cotton leaf curl virus and allied whitefly transmitted geminiviruses occurring in Pakistan. *Ann. Apl. Biol.* **130:** 61-75.

Henry, G. and Hershey, C. (2002). Cassava in South America and Caribbean. **In:** *Cassava: Biology, Production and Utilization,* pp. 17-40, (Hillocks, R.J. Thresh, J.M. and Bellotti, A.C., eds). CABI Publishing Series, London, UK.

Hillocks, R.J. (2002). Cassava in Africa. **In:** *Cassava: Biology, Production and Utilization,* pp. 41-54, (Hillocks, R.J. Thresh, J.M. and Bellotti, A.C., eds). CABI Publishing series, London, UK.

Horvath, J. (1983). Host plants in diagnosis. **In:** *Diagnosis of Plant Virus Diseases*, pp.15-48, (Matthews, R.E.F., ed). CRC Press, Florida, USA.

ICTV Management report (2006). International Committee on Taxonomy of Viruses. (http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/) (Accessed on January 5, 2010).

IITA (2007). Research for Development. International Institute of Tropical Agriculture. (http://www.iita.org/cms/details/cassava_project_details.aspx?zoneid=63&articl (Accessed on January 5, 2010).

Inga, H., Ensermu Kelbessa, Sue, E., Sebsebe Demisiew and Eva, P. (2006). *Flora of Ethiopia and Eretria: Gentianaceae to Cyclocheilaceae*, Vol.5, Addis Ababa, Ethiopia, 690 pp.

Jones, A.T. (1993). Experimental transmission of viruses in diagnosis. **In:** *Diagnosis of Plant Virus Diseases*, pp. 49-72, (Matthews, R.E.F., ed). CRC Press, Florida, USA.

Jones, J.B., Jones, J.P., Stall, R.E. and Zitter, T.A. (1991). *Compendium of Tomato Diseases*. American Phytopathological Society, USA, pp. 31-41.

Karakacha, H.W. (2001). Serological and molecular characterization of begomoviruses infecting cassava (*Manihot esculenta* Crantz) in Africa. Ph.D dissertation, University of Hanover, Hanover, Germany, 187 pp.

LeamKhang, S., Riangwong, L. and Chatchawankanphanch, O. (2005). Detection of TYLCTV by PCR without DNA extraction. *Mol. Biotechnol.* **31:**567-578.

Legg, J.P. and Fauquet, C.M. (2004). Cassava mosaic geminiviruses in Africa. *Plant Mol. Biol*. **56:**585-599.

Legg, J.P., and Thresh, M. J. (2000). Cassava mosaic virus disease in East Africa: a dynamic disease in a changing environment. *Virus Res*. **71:**135-149.

Lemma Dessalegne, Yayeh Zewdie and Herath, E. (1992). Agronomic studies in tomato. Horticulture research and development in Ethiopia: Proceedings of the Second National Horticultural Workshops of Ethiopia. Addis Ababa, Ethiopia, 163 pp.

Liu, S., Bedford, I.D., Briddon, R.W. and Markham, P.G. (1997). Efficient whitefly transmission of African cassava mosaic geminivirus requires sequences from both genomic components. *J. Gen. Virol.* **78**:1791-1794.

Marchoux, G. (1976). Virology problems in Ethiopia with special reference to Solanaceae and the central south eastern provinces. Account of a Mission to Awassa Experimental Station. 17 January to 3 February 1976, 35 pp.

Martinez, Y. (2008). Emergence of begomoviruse*s* in Cuba. *Revol. Protec. Veg.* **23:**11-15.

Matthews, R.E.F. (1980). Host plant response to virus infection. **In:** *Comprehensive Virology: Virus Host Interaction, Viral Invasion, Persistence and Diagnosis*, pp. 297-359, (Fraenkel, H., Conral, H. and Wanner, R.R., eds). Prenium press, NewYork, USA.

Mehta, P., Wyman, J.A., Nakhla, M.K. and Maxwell, D.P. (1994). Polymerase chain reaction detection of viruliferous *Bemisia tabaci* (Homoptera: Aleyrodidea) with two tomato infecting geminiviruses. *J. Econ. Entomol*. **87(5):**1287-1290.

Miller, S.A. and Martin, R.R. (1988). Molecular diagnosis of plant disease. *Ann. Rev. Pythophytol.* **26:** 409-432.

Naidu, R.A. and Hughes, J.A. (2003). Methods for detection of plant virus diseases. **In:** *Plant Virology in Sub Saharan Africa,* pp. 233-253, (Hughes, J.d. A. and Odu, B.O. eds). IITA, Ibadan, Nigeria.

Nakhla, M.K. and Maxwell, D.P. (1999). Epidemiology and management of tomato yellow leaf curl disease. **In:** *Plant Virus Disease Control,* pp. 565-583, (Hadidi, A., Khetarpal, R.K. and koganzawa, H. eds). The American phytopathological society, St. Paul, USA.

Naika, S., Jeude, J.L., Goffau, M., Hilmi, M. and Dam, B. (2005). *Cultivation of Tomato: Production, Processing and Marketing.* Agromisa Foundation and CTA, Wageningen, The Netherlands, pp. 6-92.

Nassar, N.M. A. (1978). Genetic resources of cassava: chromosome behavior in some *Manihot* species. *Indian J. Gen. Plant Bre*. **38**:135-137.

Navot, N., Pichersky, E., Zeidan, M., Zamir, D. and Czosnek, H. (1991). TYLCV: a whitefly-transmitted geminivirus with a single genomic component. *Virology* **185:**151-161.

Nono-Womdim, R., Swai, I.S., Green, S.K., Laterrot, H., Marchoux, G. and Opena, R.T. (1996). Tomato viruses in Tanzania: Identification, distribution and disease incidence. *J.S.Afri.Soc. Hort. Sci.***38:**41-44.

Paszkowski, U., Zhang, S., Potrykus, I. and Paszkowski, J. (1993). Replication of the DNA-A component of ACMV in a heterologous system. *J.Gen.Virol*. **74:**2725-2729.

Potter, J.L., Nakhla, M.K., Rojas, M., Gilbertson, R.L. and Maxwell, D.P. (2007). *Protocols for Detection of Begomoviruses*. University of Wisconisin, Madison, pp.1-25.

Purseglove, J.W. (1968). *Tropical Crops: Dicotyledons*. Longmans, London, pp. 323-332.

Quiot, B. (1976). Virus diseases of market garden crops. Account of a Mission to Ethiopia. Awassa Experimental Station. July 1976, 29pp.

Rampersad, S.N. and Umaharan, P. (2003). Detection of begomoviruses in clarified plant extracts: A comparison of standard, direct-binding and immunocapture polymerase chain reaction techniques. *Phytopathology* **93:**1153-1157.

Rice, R.P., Rice, L.W. and Tindall, H.D. (1987). *Fruit and Vegetable Production in Africa.* Macmillan Publishers, UK, pp. 371-399.

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

Rybicki, E.P. (1994). A phylogenetic and evolutionary justification for three genera of geminiviridae. Literature review. *Arch. Virol.* **139:**49-77.

Samuitiene, M., Zittikaite, I., Lavalinskiene, M. and Valiunas, D. (2003). Identification of tomato ring spot nepovirus by RT-PCR. *Biologija* **4:**1392-1401.

Saunders, K., Bedford, I.D., Yahara, T. and Stanley, J. (2003). The earliest recorded plant virus disease. *Nature* **422:**831-839.

Seal, S.E., VandenBosch, F. and Jeger, M.J. (2006). Factors influencing *Begomovirus* evolution and their increasing global significance: Implications for sustainable control. *Plant Sci.* **25:**23-46.

Shih, S.L., Green, S.K., Tsai, W.S., Lee, L.M. and Wang, J.T. (2006). First report of begomoviruses associated with tomato yellow leaf curl disease in Ethiopia. *Plant dis.* **90(7):**974-979.

Shimeles Aklilu (2000). Research achievement on variety development and seed production of vegetable crops in Ethiopia. Nazret Research Center, Nazret, Ethiopia, 112 pp.

Ssekyewa, C. (2006). Incidence, distribution and characteristics of major tomato leaf curl and mosaic virus diseases in Uganda. Ph.D Thesis. Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium, 233 pp.

Sserubombwe, W.S., Briddon, R.W., Baguma, Y.K., Ssemakula, G.N., Bull, S.E., Bua, A., Alicai, T., Omongo, C., Otim-Nape, G.W. and Stanley, J. (2008). Diversity of begomoviruses associated with mosaic disease of cultivated cassava (*Manihot esculenta* Crantz) and its wild relative in Uganda. *J. gen. virol.* **89:**1759-1769.

Stenger, D.C., Revington, G.N., Stevenson, M.C. and Bisaro, D.M. (1991). Replicational release of geminivirus genomes from tandemly repeated copies: Evidence for rolling-circle replication of a plant viral DNA. *Genetics* **88**:8029-8033.

Stephen, K.O. (1995). Cassava: new crop factsheet. Tropical Research and Education Center, University of Florida, Florida, USA, 10 pp.

Tesfaye Balem (2008). Response of tomato cultivars differing in growth habit to nitrogen and phosphorus fertilizers and spacing on vertisol in Ethiopia. *Acta Agri.* **91(1):**103-119.

Thomas, J.E., Massalski, P.R., Harrison, B.D. (1986). Production of monoclonal antibody to *African cassava mosaic virus* and differences in their reactivity in other white fly transmitted geminiviruses. *J. Gen. Virol.* **67:**2739-2748.

Tigst Demeke and Adams, R.P. (1992). The effects of plant polysaccharide and buffer additives on PCR. *BioTechniques* **12:**332-334.

Wetzel, J., Candresse, T., Macquaire, G., Ravelonandro, M. and Dunez, J. (1992). A highly sensitive immunocapture polymerase chain reaction method for plum pox potyvirus detection. *J. Virol. Metho.* **39:**27-37.

Wright, R.M. (1996). *Jamaica's Energy: Old Prospective, New Resource.* Litho press, Kingston, Jamaica, pp. 123-134.

Xie, Y. and Zhou, X.P. (2003). Molecular characterizations of squash leaf curl Yunnan virus, a new *Begomovirus* and evidence for recombination. *Arch. Virol.* **148:**2047-2054.

Yassin, A.M. and Nour, M.A. (1965). Tomato leaf curl disease in the Sudan and its relation to tobacco leaf curl disease. *Ann. App. Biol.* **56:**207-217.

Yayeh Zewdie (1989). Hot pepper and tomato production in Ethiopia. **In:** *Tomato and Pepper Production in Tropics*, pp. 442-451, (Green, S.K., Griggs, T.D. and McLean, B.D. eds). AVRDC, Taiwan.

Yaynu Hiskias (1998). Characterization of *Potyvirus* isolates from hot pepper and tomato in Ethiopia. Doctorial dissertation submitted for fulfillment for Doctorial Degree in Faculty of Agricultural Science. George-August-University, Gottingen, Germany, 116 pp.

Appendix 1

ELISA equipments, Antibodies and buffers

Equipments: ELISA plate, polyethene bag, ELISA plate reader, Micro pipettes of different volume, Bottles of different types, tissue paper, permanent marker and incubators.

Antibodies: anti tomato viruses' unlabelled immunoglobin G (IgG) for TYLCV, ToMV, TMV, TSWV, ToRSV-Ch, PVY and CMV; ap conjugate of all these antibodies (IgG-ap); anti ACMV polyclonal unlabelled antibody (IgG), anti ACMV monoclonal antibody (ACMV Mab), ap conjugated anti mouse rabbit antibody (ACMV Ram-ap) and Substrate.

Composition of buffers:

Phosphate buffered saline (PBS), 10x PBS stock:

80.0 g sodium chloride (NaCl)

2.0 g monobasic potassium phosphate (KH_2PO_4)

11.5 g dibasic sodium phosphate (Na_2HPO_4)

2.0 g potassium chloride (KCl) dissolved in 1litre deionized distilled water. pH was adjusted to 7.4 per liter after converting the buffer to 1x.

Washing buffer-PBST (pH 7.4 per liter):

100 ml 10x PBS

500 µl Tween 20 make up to 1 liter with distilled water.

Extraction buffer (pH 7.4):

1. PBST + 2% PVP (serva PVP-15 polyvinyl pyrrolidone)

2. 0.05M tris base containing 0.06M sodium sulfide pH adjusted 7.4 with HCl and/or NaOH was used to test for ACMV

Coating buffer (pH 9.6):

1.59g sodium carbonate ($NaCO_3$) and 2.93g sodium bicarbonate ($NaHCO_3$) were dissolved in 900ml deionized distilled water, pH adjusted to 9.6 with HCL and make up to 1l).

Blocking solutions (for triple antibody sandwich ELISA):

2% skim milk in PBST

Conjugate buffer, pH 7.4 (per liter):

PBST + 2% PVP +0.2% Bovine Serum albumin make up to 1litre with deionized distilled water.

Substrate buffer (pH 9.8)**:**

97ml diethanolamine + 600ml distilled H_2O. pH was adjusted to 9.8 with HCl and made up to 1 litter with distilled H_2O or dissolving 1mg tris base tablet in 1ml deionized, distilled water.

- The buffers were used within 15 days of preparation because there was no preserving reagent, sodium azide (NaN_3) to be used.
- All the reagents used to make solutions were SIGMA products (SIGMA, USA).

Appendix 2

Tomato and cassava virus ELISA data sheet

Experment_____Samples_____Date_____

				Virus							
F	**Sym**	**Inc (%)**	**N**	**TYLCV**	**ToMV**	**ToRSV-Ch**	**TSWV**	**TMV**	**PVY**	**CMV**	**ACMV**
1	Yes	95	1	+*+							
			2								
			3	+							
			4	+*+							
			5	+							
			6	+							
			7								
			8	+							
			9	+							
			10	+							
2	Yes	90	1								
			2	+							
			3	+							
			4	+*+							
			5	+							
			6								
			7	+*+							
			8	+							
			9								
			10								
3	Yes	50	1	+							
			2								
			3								
			4	+ *+							
			5	+							
			6								
			7								
			8	+*+							
			9	+							
			10								
4	Yes	20	1								
			2	+*+							
			3	+							
			4	+							
			5	+							
			6								
			7	+*+							
			8								
			9	+							
			10	+							

F-field number; sym-symptomatic; Inc- incidence; N-sample number; + showed weak positive r/n; * selected for IC-PCR; *$^{+}$provided specific band; ++ positive reaction; □- has no response to ELISA test. For names of viruses see list of virus name and acronyms. The respective fields of the field number given to are at section 4.1.1.

Appendix 2---cont'd

Tomato and cassava virus ELISA data sheet

Experment_____ Samples_____Date_____

				Virus							
F	**Sym**	**Inc (%)**	**N**	**TYLCV**	**ToMV**	**ToRSV-Ch**	**TSWV**	**TMV**	**PVY**	**CMV**	**ACMV**
5	Yes	75	1	+*+	++						
			2	+*+	++						
			3	+	++						
			4		++						
			5	+							
			6								
			7		++						
			8	+							
			9		++						
			10								
6	Yes	30	1	+*+							
			2								
			3	+							
			4		++						
			5	+							
			6	+							
			7								
			8	+*+							
			9								
			10		++						
7	Yes	25	1	*							
			2	+							
			3	+							
			4		++						
			5								
			6								
			7								
			8	+*+							
			9	+	++						
			10	+							
8	Yes	20	1		++						
			2								
			3								
			4	+							
			5	+*+							
			6		++						
			7								
			8								
			9	*							
			10		++						

F-field number; sym- symptomatic; Inc- incidence; N-sample number; + showed weak positive r/n; * selected for IC-PCR; *$^{+}$provided specific band; ++ positive reaction; □- has no response to ELISA test. For names of viruses see list of virus name and acronyms. The respective fields of the field number given to are at section 4.1.1.

Appendix 2---cont'd

Tomato and cassavs virus ELISA data sheet

Experment_____ Samples_____Date_____

				Virus							
F	**Sym**	**Inc(%)**	**N**	**TYLCV**	**ToMV**	**ToRSV-Ch**	**TSWV**	**TMV**	**PVY**	**CMV**	**ACMV**
9	Yes	Trace	1		+						
			2								
			3		++						
			4		++						
			5								
			6								
			7								
			8								
			9								
			10								
10	Yes	30	1	*	++						
			2	+	++						
			3	+*+							
			4	+	++						
			5		++						
			6								
			7		++						
			8	+							
			9		++						
			10								
11	Yes	20	1		++						
			2	+*+							
			3	+	++						
			4		++						
			5	+							
			6		++						
			7	+	++						
			8	+	++						
			9	*	++						
			10								
12	Yes	40	1	+*+	++						
			2		++						
			3								
			4								
			5	+							
			6								
			7		++						
			8	*	++						
			9	+							
			10								

F-field number; sym- symptomatic; Inc- incidence; N-sample number; + showed weak positive r/n; * selected for IC-PCR; *$^{+}$provided specific band; ++ positive reaction; □- has no response to ELISA test. For names of viruses see list of virus name and acronyms. The respective fields of the field number given to are at section 4.1.1.

Appendix 2---cont'd

Tomato and cassava virus ELISA data sheet

Experment_____ Samples_____Date_____

				Virus							
F	**Sym**	**Inc (%)**	**N**	**TYLCV**	**ToMV**	**ToRSV-Ch**	**TSWV**	**TMV**	**PVY**	**CMV**	**ACMV**
13	Yes	45	1		++						
			2		++						
			3	+	++						
			4	+	++						
			5		++						
			6	+*+							
			7	+*+	++						
			8								
			9	+							
			10	+	++						
14	Yes	5	1								
			2		++						
			3								
			4								
			5		++						
			6								
			7								
			8								
			9								
			10								
15	Yes	5	1								
			2		++						
			3		++						
			4								
			5		++						
			6								
			7		++						
			8		++						
			9								
			10								
16	No		1		++						
			2								
			3		++						
			4								
			5								
			6		++						
			7		++						
			8								
			9								
			10								

F-field number; sym- symptomatic; Inc- incidence; N-sample number; + showed weak positive r/n; * selected for IC-PCR; *$^{+}$provided specific band; ++ positive reaction; □- has no response to ELISA test. For names of viruses see list of virus name and acronyms. The respective fields of the field number given to are at section 4.1.1.

Appendix 2---cont'd

Tomato and cassavs virus ELISA data sheet

Experment_____ Samples_____Date_____

				Virus							
F	**Sym**	**Inc (%)**	**N**	**TYLCV**	**ToMV**	**ToRSV-Ch**	**TSWV**	**TMV**	**PVY**	**CMV**	**ACMV**
17	Yes	30	1		++						
			2								
			3		++						
			4		++						
			5		++						
			6		++						
			7								
			8		++						
			9								
			10		++						
18	Yes	15	1		++						
			2								
			3								
			4								
			5		++						
			6								
			7		++						
			8								
			9								
			10								
19	Yes	5	1		++						
			2	+*+							
			3		++						
			4	+							
			5	+							
			6		++						
			7	+	++						
			8	*							
			9	+	++						
			10								
20	No		1								
			2								
			3								
			4								
			5								
			6								
			7								
			8		++						
			9		++						
			10								

F-field number; sym- symptomatic; Inc- incidence; N-sample number; + showed weak positive r/n; * selected for IC-PCR; *$^{+}$provided specific band; ++ positive reaction; □- has no response to ELISA test. For names of viruses see list of virus name and acronyms. The respective fields of the field number given to are at section 4.1.1.

Appendix 2---cont'd

Tomato and cassavs virus ELISA data sheet

Experment_____ Samples_____Date_____

				Virus							
F	**Sym**	**Inc (%)**	**N**	**TYLCV**	**ToMV**	**ToRSV-Ch**	**TSWV**	**TMV**	**PVY**	**CMV**	**ACMV**
21	Yes	20	1		++						
			2	+							
			3		++						
			4		++						
			5	*	++						
			6								
			7								
			8								
			9	+							
			10	+*+	++						
22	Yes	50	1		++						
			2		++						
			3		++						
			4		++						
			5		++						
			6		++						
			7		++						
			8								
			9		++						
			10		++						
23	Yes	40	1								
			2		++						
			3		++						
			4		++						
			5								
			6		++						
			7		++						
			8								
			9								
			10		++						
24	No		1								
			2								
			3								
			4		++						
			5		++						
			6								
			7								
			8								
			9								
			10								

F-field number; sym- symptomatic; Inc- incidence; N-sample number; + showed weak positive r/n; * selected for IC-PCR; *$^{+}$provided specific band; ++ positive reaction; □- has no response to ELISA test. For names of viruses see list of virus name and acronyms. The respective fields of the field number given to are at section 4.1.1.

Appendix 2---cont'd

Tomato and cassavs virus ELISA data sheet

Experment_____ Samples_____Date_____

				Virus							
F	**Sym**	**Inc (%)**	**N**	**TYLCV**	**ToMV**	**ToRSV-Ch**	**TSWV**	**TMV**	**PVY**	**CMV**	**ACMV**
25	Yes	5	1		++						
			2								
			3								
			4								
			5		++						
			6		++						
			7								
			8		++						
			9		++						
			10		++						
26	No		1								
			2		++						
			3								
			4								
			5								
			6		++						
			7								
			8								
			9								
			10								
27	Yes	5	1								
			2		++						
			3		++						
			4								
			5		++						
			6		++						
			7								
			8								
			9								
			10								
28	No		1								
			2								
			3								
			4								
			5								
			6								
			7								
			8								
			9								
			10								

F-field number; sym- symptomatic; Inc- incidence; N-sample number; + showed weak positive r/n; * selected for IC-PCR; *$^{+}$provided specific band; ++ positive reaction; □- has no response to ELISA test. For names of viruses see list of virus name and acronyms. The respective fields of the field number given to are at section 4.1.1

Declaration

I hereby declare to have independently prepared this work with no other than the indicated sources and support. This thesis is my original work and its composition has never been submitted elsewhere. All sources of materials used for the thesis have been duly acknowledged.

Name: **Eyasu Wada Wachamo**

Signature_____

Date_____

This thesis has been presented under my supervision

Name: **Dr. Tileye Feyissa**

Signature _____

Date _____