A Complex of Begomoviruses Affecting Tomato Crops in Nicaragua

Aldo Rojas
Department of Plant Biology and Forest Genetics
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2004

Acta Universitatis Agriculturae Sueciae
Agraria 492

ISSN 1401-6249
ISBN 91-576-6772-1

Tryck: SLU Service/Repro, Uppsala 2004

Abstract

Rojas, A. 2004. A complex of Begomoviruses affecting tomato crops in Nicaragua.
Doctor's dissertation
ISSN 1401-6249, ISBN 91-576-6772-1

Diseases caused by begomoviruses (family *Geminiviridae*, genus *Begomovirus*) constitute a serious constraint to vegetable production in Nicaragua as they are associated with large economical losses. This thesis was done in an effort to identify and characterize the begomoviruses responsible for the tomato diseases and to understand their relationships.

The cropping system used by small-holding farmers comprises essentially five crops: maize and bean as consumption crops, and tomato, pepper and cucurbits as cash crops. These crops are grown in the three different growing seasons all the year around. Except maize, all the other crops are hosts for begomoviruses and whiteflies.

In this study, begomovirus sequences detected with universal and virus specific primers were cloned, sequenced and used for phylogenetic analysis. The plants from which the viruses were detected were tomato, pepper, cucurbits and *Euphorbia heterophylla*. The sequence comparisons revealed high identity with other already described begomovirus species, including Euphorbia mosaic virus (EuMV), *Squash yellow mild mottle virus* (SYMMoV) *Tomato severe leaf curl virus* (ToSLCV), Tomato leaf curl Sinaloa virus (ToLCSinV) and *Pepper golden mosaic virus* (PepGMV). One viral sequence from tomato showed only low identity to previously sequenced begomoviruses (84%) and represents a new tentative species designated as Tomato leaf curl Las Playitas virus (ToLCLPV). The complete nucleotide (nt) sequences of the DNA-A and DNA-B components were determined for ToLCSinV, and the complete nt sequence was determined for the DNA-A component of two isolates of ToSLCV. The genome organization of ToLCSinV and ToSLCV was identical to the bipartite genomes of other begomoviruses described from the Americas. A phylogenetic analysis of DNA-A showed that the indigenous begomoviruses of the New World can be divided into three major clades and an intermediate group, and that ToLCSinV and ToSLCV belong to different clades. Computer-based predictions indicated that recombination with another begomovirus had taken place within *AV1* of ToSLCV dividing this species into two strains. Mixed infection with different strains of the same virus, and mixed infections with up to three begomovirus species were detected in tomato plants. Three begomoviruses were detected in both tomato and pepper in the field.

Detection of predicted recombinant viral isolates is consistent with other findings of this study which indicate that begomoviruses commonly occur as mixed infections in the field, and that intraspecific sequence variability within an infected plant may be as high as between different plants. These conditions provide a high risk for evolution of new virus strains and species via recombination.

Acquisition and transmission of ToLCSinV and ToSLCV by their whitefly vector, *Bemisia tabaci*, required only 10 min on tomato plants. Longer acquisition and inoculation access periods tended to increase the virus transmission rates. Whiteflies transmitted the viruses for seven days without new virus acquisition.

Key words: plant disease, *Lycopersicon esculentum, Capsicum annuum, Cucurbita argyrosperma*, begomovirus evolution.

Author's current address: Aldo Rojas, Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences (SLU), SE-750 07 Uppsala, Sweden. E-mail: Aldo.Rojas@vbsg.slu.se
Author´s home address: Departamento de Proteccion Agricola y Forestal, Universidad Nacional Agraria, km 12 Carretera Norte, Apdo. 453, Managua Nicaragua.

Dedicada a:

Mi madre Adilia Solís, y a todas las madres humildes de mi sufrido pueblo, Nicaragua.

Mis amados hijos Aldo y Fidel, dos poderosas razones para seguir siempre adelante.

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Rojas, A., Kvarnheden, A., and Valkonen, J. P. T. 2000. Geminiviruses infecting tomato crops in Nicaragua. Plant Dis. 84:843-846.

II. Ala-Poikela, M., Svensson, E., Rojas, A., Horko, T., Paulin, L., Valkonen, J. P. T. and Kvarnheden, A. 2004. Genetic diversity and mixed infections of begomoviruses infecting tomato, pepper and cucurbit crops in Nicaragua. (Submitted)

III. Rojas, A., Kvarnheden, A., Marcenaro, D., and Valkonen, J. P. T. 2004. Sequence characterization of Tomato leaf curl Sinaloa virus and *Tomato severe leaf curl virus*: Phylogeny for New World begomoviruses and detection of recombination. Arch. Virol. (accepted pending revision).

IV. Rojas, A., Marcenaro, D., Rayo, M., Salinas, C., Zeledón, K., Kvarnheden, A., and Valkonen, J. P. T. 2004. Transmissión of Nicaraguan begomovirus isolates by whiteflies on tomato. (Manuscript)

Abbreviations

aa	Amino acid
AAP	Acquisition access period
bp	Base pair (s)
CNIA	Centro Nacional de Investigaciones Agropecuarias
CP	Coat protein
DNA	Deoxyribonucleic acid
EMV	Euphorbia mosaic virus
IAP	Inoculation access period
ICTV	International Committee on Taxonomy of Viruses
kb	Kilo bases (nucleotide)
nt	Nucleotide
ORF	Open reading frame
PCR	Polymerase chain reaction
PepGMV	*Pepper golden mosaic virus*
SYMMoV	*Squash yellow mild mottle virus*
ToLCLPV	Tomato leaf curl Las Playitas virus
ToLCSinV	Tomato leaf curl Sinaloa virus
ToSLCV	*Tomato severe leaf curl virus*
UNA	Universidad Nacional Agraria

Introduction

In many developing countries, the majority of the population still produces most of their own food and depend on small-scale farming for their incomes and livelihoods. Crops can be affected by diseases showing a wide range of symptoms. The causal agents of these diseases are biotic or abiotic. Among the biotic disease agents, viruses can attack all types of plants. Plant virus diseases can in extreme cases reduce yields to zero leading to catastrophic effects on people. The yield reduction depends on many factors like crop variety, virus disease, crop system and vector efficiency in the case of vector-transmitted viruses. Some virus diseases have caused catastrophic losses in agriculture, such as hoja blanca on rice, citrus tristeza, and geminiviruses in many crops (Agrios, 1997).

The earliest known written record of a virus disease was made in a Japanese poem referring to *Eupatorium lindleyanum*, a plant very susceptible to a virus disease, which causes yellowing symptoms (Hull, 2002). These symptoms have recently been shown to be caused by a geminivirus-satellite complex: *Eupatorium yellow-vein virus* (EpYVV) and a DNA β-satellite component (Saunders *et al*., 2003).

The study of plant diseases caused by viruses can be historically separated into three phases. A descriptive phase in 1883-1951, is called "Classical Discovery Period". The second phase evidenced development of new techniques and further descriptions of virus properties in 1952-1983 ("Early Molecular Era"). In the current, third phase (Recent Period) more techniques are available for studies on virus genome, gene functions, and plant transformation for resistance to virus diseases (Zaitlin and Palukaitis, 2000).

Many definitions of viruses have been proposed through time, but the definition by Hull (2002) could be considered as the most complete until now: "A virus is a set of one or more nucleic acid template molecules, normally encased in a protective coat or coats of protein or lipoprotein, that is able to organize its own replication only within suitable host cells. It can usually be horizontally transmitted between hosts. Within such cells, virus replication is (1) dependent on the host's protein-synthesizing machinery, (2) organized from pools of the required materials rather than by binary fission, (3) located at sites that are not separated from the host cell contents by a lipoprotein bilayer membrane, and (4) continually giving rise to variants through various kind of change in the viral nucleic acid".

Virus nomenclature and classification have long been a troublesome area of virology. The ideal goal is to establish groups that reveal the evolutionary and phylogenetic relationships between viruses. The development of this goal has been strongly supported in the "Recent period" of plant virology (Zaitlin and Palukaitis, 2000). Actually, 977 plant viruses have been named and listed in the ICTV seventh report, of which 701 are true species and 276 are tentative species (Van

Regenmortel *et al*., 2000). Now there are 70 genera, 14 families and three orders of plant viruses recognized (Hull, 2002).

Plant viruses can attack a wide range of plant species, both cultivated or wild, and they cause from very low to total crop losses. One of the plant families heavily attacked by viruses is the Solanaceae which includes several widely cultivated plants, such as tomato, potato, tobacco, pepper, eggplant, and petunia. Tomato (*Lycopersicon esculentum*), has its center of origin in the mountainous region of the Andes in South America. The actual name "tomato" was derived from the Nahuatl language of Mexico, where the indigenous tomato was domesticated and cultivated by early civilizations. Tomato plants are herbaceous perennials but have been used as an annual crop and their fruits can be used fresh or processed. They are good sources of vitamins A and C (Jones *et al*., 1997). Peppers are members of the genus *Capsicum* and originated in the tropical Americas. This genus includes 25 species, but only five of them have been domesticated and among those *C. annuum* is the species that is economically most important and widely cultivated worldwide. Mexico and Mesoamerica are the centres of genetic diversity of this species. The other four species are *C. baccatum, C. pubescens, C. chinense, and C. frutescens.* Like tomato, pepper fruits are consumed as a fresh vegetable or processed as a spice providing essential vitamins and minerals for humans (Pernezny *et al*., 2003). Cucurbitaceae family contains several species used as human food. Five species (*Cucurbita argyrosperma*, *C. ficifolia, C. moschata, C. maxima,* and *C. pepo*) have been domesticated in the New World and from very ancient times they have contributed essential food products to the diet of rural and urban communities on the American continent and in many other parts of the world. With the exception of *C. maxima*, whose centre of origin is in South America, it is assumed that the other four cultivated species were domesticated in Mesoamerica, although this has not been confirmed in all cases (Lira, 1991).

Family *Geminiviridae*

The family *Geminiviridae* is one the largest groups of plant viruses. The morphology of geminivirus particles is unique and they are characterized by geminate shape and the small size ≈ 30 x 20 nm. They have a circular single-stranded DNA genome which replicates in the host cell nucleus. The transmission of these viruses by the insect vectors is in a persistent manner. They have the propensity to infect phloem cells (Arguello-Astorga *et al*., 1994; Sunter *et al*., 1994; Harrison and Robinson, 1999; Varma and Malathi, 2003). Geminiviruses infect a wide range of weeds and cultivated plants, including both monocots such as maize and wheat, and dicots such as cassava and tomato. The infections can affect plants in many ways. One of the physiological processes seriously affected is photosynthesis with decreasing yields of starch as a result. Geminiviruses also disrupt flower and fruit formation in crops such as tomato, pepper, and cotton (Moffat, 1999). Since the late 1980s, the horticultural-producing areas of Southern USA, such as Arizona and Florida, the Caribbean, Mexico, Central America, Venezuela and Brazil have been heavily attacked by whitefly-borne geminiviruses, with devastating economic consequences for their respective agro-industries. The

whitefly-transmitted geminiviruses have thus become a major group of pathogens of vegetables in the subtropics and tropics of the Western Hemisphere (Polston and Anderson, 1997).

The genome organization and biological properties of geminiviruses allow them to be divided into four genera. Those that have a monopartite genome and are transmitted by leafhoppers in monocotyledonous and dicotyledonous plants are members of the genus *Mastrevirus*, of which *Maize streak virus* (MSV) is the type species. The genus *Curtovirus* comprises viruses that have a monopartite genome and are transmitted by leafhoppers in dicotyledonous plants; *Beet curly top virus* (BCTV) is the type species. The genus *Topocuvirus* has only one member (the type species), *Tomato pseudo-curly top virus* (ToPCTV) which has a monopartite genome and is transmitted by treehoppers in dicotyledonous plants. The fourth genus, *Begomovirus*, includes viruses that are transmitted by whiteflies to dicotyledonous plants; *Bean golden mosaic virus* (BGMV) is the type species. Begomoviruses have bipartite genomes (A and B components), with some exceptions [e.g., *Tomato yellow leaf curl virus* (TYLCV), *Cotton leaf curl virus* (CLCuV), *Tomato leaf curl virus* (ToLCV)] for which no B components has been found (Fauquet *et al*., 2003).

Genus *Begomovirus*

Begomoviruses have emerged as constraints to the cultivation of a variety of crops in various parts of the world. Some of the diseases caused by begomoviruses that are appearing show that these viruses are still evolving and pose a serious threat to sustainable agriculture, particularly in the tropics and sub-tropics. Another concern is the emergence of diseases that are caused by a complex of begomovirus and satellite DNA molecules (Saunders *et al*., 2001; Varma and Malathi, 2003; Bull *et al*., 2004; Stanley, 2004).

Some crops appear to be a paradise for begomoviruses. So far, 45 recognised and 30 tentative species of begomoviruses have been found to naturally infect tomato, pepper and cucurbits in the New and Old World. Some of the viruses have a large number of distinct strains (Jones, 2003). According to Polston and Anderson (1997), 17 begomoviruses were infecting tomato in the Western Hemisphere in the middle of 1990s. Tomato, pepper and cucurbits are now known to be infected by at least 39 begomoviruses species, with 22 of them confirmed and 17 considered as tentative species (Table 1) (Fauquet *et al*., 2003). Begomoviruses have been considered as the most numerous and widespread group of whitefly-transmitted viruses causing severe epidemics in Central America and the Caribbean basin. These epidemics seem to be in connection with some factors like the appearance of efficient vectors, evolution of new variants of the viruses, changing cropping systems, and introduction of susceptible plant varieties (Brown, 1997; Morales and Anderson, 2001; Zhou *et al*., 2001; Ramos *et al*., 2003; Ribeiro *et al*., 2003; Varma and Malathi, 2003).

Table 1. Species and tentative species of begomoviruses infecting tomato, pepper and cucurbit in the New and Old World.

New World	**Old World**
Species	
Chino del tomate virus (CdTV)	*Chilli leaf curl virus* (ChiLCuV)
Cucurbit leaf curl virus (CuLCuV)	*Pepper leaf curl Bangladesh virus* (PepLCBV)
Melon chlorotic leaf curl virus (MCLCuV)	*Pepper leaf curl virus* (PepLCV)
Pepper golden mosaic virus (PepGMV)	*Squash leaf curl China virus* (SLCCNV)
Pepper huasteco yellow vein virus (PHYVV)	*Squash leaf curl Yunnan virus* (SLCCYV)
Potato yellow mosaic Panama virus (PYMPV)	*Tomato leaf curl Bangalore virus* (ToLCBV)
Potato yellow mosaic Trinidad virus (PYMTV)	*Tomato leaf curl Bangladesh virus* (ToLCBdV)
Potato yellow mosaic virus (PYMV)	*Tomato leaf curl Gujarat virus* (ToLCGV)
Squash leaf curl virus (SLCV)	*Tomato leaf curl Karnataka virus* (ToLCKV)
Squash mild leaf curl virus (SMLCV)	*Tomato leaf curl Laos virus* (ToLCLV)
Squash yellow mild mottle virus (SYMMoV)	*Tomato leaf curl Malaysia virus* (ToLCMV)
Tomato chlorotic mottle virus (ToCMoV)	*Tomato leaf curl New Delhi virus* (ToLCNDV)
Tomato chlorosis virus (ToCV)	*Tomato leaf curl Sri Lanka virus* (ToLCSLV)
Tomato golden mosaic virus (TGMV)	*Tomato leaf curl Taiwan virus* (ToLCTWV)
Tomato golden mottle virus (ToGMoV)	*Tomato leaf curl Vietnam virus* (ToLCVV)
Tomato mosaic Havana virus (ToMHV)	*Tomato leaf curl virus* (ToLCV)
Tomato mosaic Taino virus (ToMoTV)	*Tomato yellow leaf curl China virus* (TYLCCnV)
Tomato mottle virus (ToMoV)	*Tomato yellow leaf curl Gezira virus* (TYLCGV)
Tomato rugose mosaic virus (ToRMV)	*Tomato yellow leaf curl Malaga virus* (TYLCMalV)
Tomato severe leaf curl virus (ToSLCV)	*Tomato yellow leaf curl Sardinia virus* (TYLCSV)
Tomato severe rugose virus (ToSRV)	*Tomato yellow leaf curl Thailand virus* (TYLCTHV)
*Tomato yellow leaf curl virus** (TYLCV)	*Tomato yellow leaf curl virus** (TYLCV)

(continued)

Table 1. (continued)

New World	Old World
Tentative species	
Melon leaf curl virus (MLCV)	Pepper yellow leaf curl virus (PepYLCV)
Pepper mild tigré virus (PepMTV)	Tomato curly stunt virus (ToCSV)
Tomato Chino La Paz virus (ToCHLPV)	Tomato leaf curl India virus (ToLCIV)
Tomato chlorotic vein virus (ToCVV)	Tomato leaf curl Indonesia virus (ToLCIDV)
Tomato crinkle virus (ToCrV)	Tomato leaf curl Philippines virus (ToLCPV)
Tomato dwarf leaf curl virus (TDLCV)	Tomato leaf curl Senegal virus (ToLCSV)
Tomato leaf curl Barbados virus (ToLCBBV)	Tomato leaf curl Tanzania virus (ToLCTZV)
Tomato leaf curl Nicaragua virus (ToLCNV)	Tomato yellow dwarf virus (ToYDV)
Tomato leaf curl Sinaloa virus (ToLCSinV)	Tomato yellow leaf curl Kuwait virus (TYLCKWV)
Tomato mosaic Barbados virus (ToMBV)	Tomato yellow leaf curl Nigeria virus (TYLCNV)
Tomato mottle leaf curl virus (ToMoLCV)	Tomato yellow leaf curl Saudi Arabia virus (TYLCSAV)
Tomato Uberlandia virus (ToUV)	Tomato yellow leaf curl Tanzania virus (TYLCTZV)
Tomato yellow dwarf virus (ToYDV)	Tomato yellow leaf curl Yemen virus (TYLCYV)
Tomato yellow mosaic virus (ToYMV)	
Tomato yellow mottle virus (ToYMoV)	
Tomato yellow vein streak virus (ToYVSV)	

[a]Source of virus names: Fauquet *et al*., (2003); *Species from the Old World causing serious disease in the New World.

Recombination of geminiviruses is a very frequent and widespread phenomenon and occurs between species as well as within and across genera, and is a significant contributor to begomovirus evolution. The high rate of recombination may be contributing to the recent emergence of new begomovirus diseases (Zhou *et al*., 1997; Padidam *et al*., 1999; Saunders and Stanley, 1999; Navas-Castillo *et al*., 2000; Sanz *et al*., 2000; Unseld *et al*., 2000; Berrie *et al*., 2001; Berry and Rey, 2001; Jeske *et al*., 2001; Schnippenkotter *et al*., 2001; Chatchawankanphanich and Maxwell, 2002; Kirthi *et al*., 2002; Saunders *et al*., 2002; Ramos *et al*., 2003; Revill *et al*., 2003; Ribeiro *et al*., 2003).

Genome organization of bipartite begomoviruses

The bipartite genome comprises two single-stranded DNA (ssDNA) components of similar size (2.5-2.8 kb), referred to as DNA-A and DNA-B. The nucleotide

sequences of DNA A and DNA B are quite different, except for a short "common region" of ≈ 200 nucleotides that is very similar or identical in the two DNAs. The common region includes a stem-loop structure, with the loop containing the nonanucleotide TAATATTAC, which is conserved in the genomes of all four geminivirus genera. It also includes the origin for rolling circle replication (Eagle *et al*., 1994; Laufs *et al*., 1995; Padidam *et al*., 1996; Orozco *et al*., 1998; Harrison and Robinson, 1999, Harrison and Robinson, 2002; Zhou *et al*., 2003). Both DNA components contain protein-coding regions in the viral strand and in the complementary strand. Six such genes seem to be universally present. DNA A contains one gene (*AV1*) in the viral strand and three genes (*AC1*, *AC2*, and *AC3*) in the complementary strand. DNA B contains one gene (*BV1*) in the viral strand and one gene (*BC1*) in the complementary strand (Fig. 1). Some of the known functions of those proteins are summarized in Table 2.

Table 2. Some known functions of the mature begomovirus proteins

Gene	Protein	Function	References[a]
AV1	**CP**	whitefly-mediated transmission and virion capsid assembly	4, 7, 9
AC1	**Rep**	viral DNA replication	1, 2, 3
AC2	**TrAP**	transcriptional activator for the virus-sense genes, suppresses RNA silencing and other host defence responses	16, 17, 19, 20
AC3	**REn**	increases viral replication	14, 15
AC4		hypersensitive response-like reaction initiated by Rep	18
BV1	**NSP**	transport of viral DNA between the nucleus and cytoplasm and host range properties of the virus	10, 11, 12
BC1	**MPB**	mediates the cell-to-cell movement and viral pathogenic properties	5, 6, 8, 13

[a]References: 1) Fontes *et al*., 1994; 2) Gutierrez, 2003; 3) Hanley-Bowdoin *et al*., 1999; 4) Höhnle *et al*., 2001; 5) Ingham *et al*., 1995; 6) Jeffrey *et al*., 1996; 7) Kheyr-Pour *et al*., 2000; 8) Lazarowitz and Beachy, 1999; 9) Noris *et al*., 1998; 10) Noueiry *et al*., 1994; 11) Sanderfoot and Lazarowitz, 1995; 12) Sanderfoot *et al*., 1996; 13) Schaffer *et al*., 1995; 14) Settlage *et al*., 1996; 15) Settlage *et al*., 2001; 16) Sunter and Bisaro, 1992; 17) Sunter and Bisaro, 2003; 18) van Wezel *et al*., 2002a; 19) van Wezel *et al*., 2002b; 20) Voinnet *et al*., 1999.

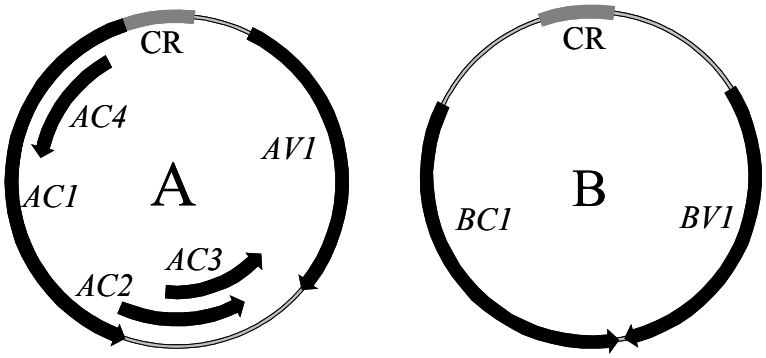


Fig 1. Genome organization of bipartite begomoviruses showing the position of the genes (*AV1, AC1, AC2, AC3, AC4, BC1*, and *BV1*) and the common region (CR) in the A and B components.

Infection cycle of begomoviruses

Begomoviruses are inoculated to plant cells by the vector *Bemisia tabaci* but a precise virus-host interaction is needed for begomovirus infection to occur (Lazarowitz, 1999). The second step is the movement of the virus to the nucleus were the replication and transcription of the genome occurs. The virus particle movement apparently is entirely dependent on the coat protein (CP) trough interactions with the host transport network. A complex between the single-stranded ssDNA and the CP is formed which enters the nucleus (Gafni and Epel, 2002). The third step is the replication process, which for begomoviruses follows a rolling circle strategy and the viral proteins required for the process are encoded by the A component of the virus genome (Gutierrez, 2000). The *AC1* gene (Rep) is responsible for initiating DNA replication during the rolling-circle amplification stage, but also *AC3* (REn) has been proposed to be important for viral DNA replication (Fontes *et al*., 1994a; Fontes *et al*., 1994b; Sunter *et al*., 1994; Laufs, *et al*., 1995; Settlage *et al*., 1996; Orozco *et al*., 1997; Gutierrez, 2002). Another replication strategy has been reported by Jeske *et al*., (2001) named recombination-dependent replication (RDR) where the host factors alone or in combination with the Rep protein are necessary or sufficient for replication. The fourth step of the process is cell-to-cell and systemic spread of the single-stranded form of the viral genome produced during replication and this movement depends on proteins encoded by the B component of the virus genome. Two movement proteins (MPs), NSP and MPB, are essential for virus movement and systemic infection of host plants (Schaffer *et al*., 1995; Gilbertson and Lucas, 1996; Jeffrey *et al*., 1996; Sanderfoot and Lazarowitz, 1996; Sanderfoot *et al*., 1996; Guevara-Gonzales *et al*., 1999; Lazarowitz, 1999; Lazarowitz and Beachy, 1999; Gafni and Epel, 2002; Hehnle *et al*., 2004). *BV1* encodes as a nuclear shuttle protein (NSP) and *BC1* a movement protein (MPB). NSP forms a complex with the virus genome and transports it from the nucleoplasm to the cytoplasmic domains where interacts with *BC1* and they function cooperatively in cell-to-cell movement of the viral DNA through the plasmodesmata. *BC1* also has been reported to be responsible for pathogenicity of bipartite begomoviruses. The next step occurs when, via

short-distance movement, a virus reaches the vascular system and the host plant becomes systemically infected (long distance movement). In *Squash leaf curl virus* (SLCV), *BV1* has been implicated in the host range properties and *BC1* in viral pathogenic properties (Ingham *et al*., 1995). Begomovirus infection produces alterations of plant cells and organelles and the appearance of virus-associated structures in infected plants. These structures show phloem limitation. Some begomoviruses are restricted to cells of the vascular system, whereas others can invade mesophyll tissue (Morra and Petty, 2000). The loss of tissue specificity could, in some cases, be due to co-infection of the begomovirus with another virus (Brown, 1997).

Begomovirus transmission

Begomoviruses are transmitted in a circulative persistent manner by the whitefly *Bemisia tabaci,* which is an insect of the family Aleyrodidae, order Homoptera, (Idris *et al*., 2001; Brown and Czosnek, 2002). About 1300 whitefly species in over 120 genera have been described, but relatively few transmit plant viruses. Only whiteflies in the genera *Bemisia* and *Trialeurodes* are virus vectors. In the genus *Bemisia*, only *B. tabaci* has been shown to be a vector and has an extremely wide host range. It attacks more than 500 species of plants from 63 families (Jones, 2003). The existence of *B. tabaci* biotypes and numerous whitefly-transmitted begomoviruses are the most important constraint to agricultural development in tropical and subtropical regions of the world (Brown, 1994). In Mesoamerica and the Caribbean *B. tabaci* acts as a pest and a virus vector (Morales and Jones, 2004). Some of the main crops affected by the begomoviruses are: tomato, pepper, potato, chili peppers, tobacco, eggplant, cucurbit (melon, watermelon, squash, and others), cotton, common bean, and papaya. *B. tabaci* is a vector of 111 recognized plant virus species in the genera *Begomovirus* (*Geminiviridae*), *Crinivirus* (*Closteroviridae*), *Carlavirus,* and *Ipomovirus* (*Potyviridae*). Of the whitefly-transmitted virus species, 90 % belong to the genus *Begomovirus*, 6 % to the genus *Crinivirus* and the remaining 4 % are in the genera *Closterovirus, Ipomovirus* or *Carlavirus* (Jones, 2003). No replication of those viruses has been found in their whitefly vector with exception for *Tomato yellow leaf curl virus* (TYLCV), which can be transovarially transmitted through at least two generations. Up to 20% of the insects in each generation were able to inoculate tomato plants (Ghanim *et al*., 1998). Another case is *Tomato yellow leaf curl Sardinia virus* (TYLCSV). Its DNA has been detected in eggs, nymphs, and to a lesser extent in adults, of the first-generation progeny. Inheritance of TYLCSV DNA was found until the third generation, but not the infectivity (Bosco *et al*., 2004).

Virus-vector relationships between begomoviruses and *B. tabaci* have been studied for transmission characteristics. Minimum acquisition access period (AAP) and inoculation access period (IAP) have been reported for many begomoviruses, from the Old and New World, and in general ranged from 10 to 60 min and from 10 to 30 min, respectively (Idris and Brown, 1998; Brown and Czosnek, 2002; Muniyappa *et al*., 2003). After acquisition, begomoviruses can be transmitted by whiteflies for 5 to 20 days, i.e sometimes for the entire life time of the whitefly

(Costa, 1976; Stenger *et al*., 1990; Brown and Bird, 1992; Nateshan *et al*., 1996; Rubinsten and Czosnek, 1997; Idris & Brown 1998; Idris *et al*., 2001; Brown and Czosnek, 2002; Muniyappa *et al*., 2003; Rojas *et al*., 2004).

In Nicaragua, *B. tabaci* was recorded as a pest of cotton in the 1960s and the country became a testing ground for many new insecticides against cotton pests (Swezey *et al*., 1986). In 1977, *B. tabaci* became one of the major pests and virus vectors in cotton (Morales and Anderson, 2001). In the early 1980s, the large scale production of cotton ended and new crops such as tomato, melons, chili pepper and soybean became intensively cultivated. The Sebaco Valley was a place for intensive tomato production and subsequently the appearance of high whiteflies populations, which were managed with heavy synthetic insecticide applications. In the mid 1980s, disease epidemics associated with whiteflies affected all the tomato crops, and others crops like peppers and cucurbits were also heavily attacked. The response was an overuse of insecticide application with high negative impacts on the environment, health of farmers and consumers, as well as the build-up of insecticide resistance in the whiteflies. Field populations of *B. tabaci* collected from tomato and cucurbits in four localities of the country showed moderate to high levels of resistance to bifentrin (Talstar), metamidofos (Tamaron 600), and endosulfan (Thiodan 35 EC)(Perez *et al*., 2000). Changes in this cropping system (extensive and intensive) and overuse of insecticides were ideal conditions for the appearance of a new and more aggressive biotype B of *B. tabaci* (Morales and Anderson, 2001). This biotype arrived in America in the mid 1980s and was found in Nicaragua in 1992 (Brown, 1993). However, it was probably introduced earlier according to the begomovirus epidemics observed in many crops around the country.

Begomoviruses infecting crop plants

In Meso America and the United States many different begomoviruses have been found in several important food crops, including beans, tomatoes, peppers and cucurbits (Brown and Bird, 1992; Polston and Anderson, 1997; Morales and Anderson, 2001; Jones, 2003). Many of those begomoviruses have been identified in tomato, but also in peppers, chili peppers, and cucurbits (Table 3)

Table 3. Some begomoviruses reported infecting tomato, peppers and cucurbits in American countries.

Begomovirus[a]	**Crop**[b]	**Countries**[c]	**References**[d]
Chino del tomate virus (CdTV)	t, p	U, M	5, 18, 46
Cucurbit leaf curl virus (CuLCuV)	c	U, M	6, 9, 13
Melon chlorotic leaf curl virus (MCLCuV)	c	G	7
Pepper golden mosaic virus (PepGMV)	p, t, c	U, M, G, H, N, CR	1, 4, 15, 22, 28, 46
Pepper huasteco yellow vein virus (PHYVV)	p, t	U, M	8, 16, 18, 28, 45
Potato yellow mosaic virus (PYMV)	t, p	PR, Gu, Mt, V	29, 33, 38, 49, 50
Potato yellow mosaic Panama virus (PYMPV)	t	P	10
Potato yellow mosaic Trinidad virus (PYMTV)	t, p	TT	46
Squash leaf curl virus (SLCV)	c	U, M, G, H, N, P	9, 21
Squash yellow mild mottle virus (SYMMoV)	c	CR, N	20, **II**
Tomato chino La Paz virus (ToChLPV)	t	M	14
Tomato chlorotic mottle virus (ToCMoV)	t	B	37
Tomato dwarf leaf curl virus (TDLCV)	t, p	J	41
Tomato golden mosaic virus (TGMV)	t	B	26
Tomato golden mottle virus (TGMoV)	t	G	27
Tomato mosaic Havana virus (ToMHV)	t	C, H, J	23, 27
Tomato mottle Taino virus (ToMoTV)	t	C	36
Tomato mottle virus (ToMoV)	t	U, M	12, 32
Tomato leaf curl Barbados virus (ToLCBBV)	t, p, c	Bb	42
Tomato leaf curl Sinaloa virus (ToLCSinV)	t, p	U, M, G, N, CR	7, 17, 19, 27, 39, 40
Tomato severe leaf curl virus (ToSLCV)	t, p, c	M, G, H, N	27, 30, 39, 40
Tomato yellow mottle virus (ToYMoV)	t	C R	27
Tomato yellow leaf curl virus (TYLCV)	t, p, c	U, M, C, DR, Bh, Ha, Gu, J	2, 3, 24, 25, 27, 31, 34, 35, 43, 44, 48, 51
Tomato yellow vein streak virus (ToYVSV)	t	B	11

continued

[a]Begomovirus names in italics are recognized as species, whereas begomovirus names not italixed are tentative species according to Fauquet *et al*., 2003.
[b]Crop: t= tomato; p= pepper; c= cucurbits
[c]Countries: B= Brazil; Bb= Barbados; Bh= Bahamas; C= Cuba; CR= Costa Rica; DR= Dominican Republic; G= Guatemala; Gu= Guadeloupe; H= Honduras; Ha= Haiti; J= Jamaica; M= Mexico; Mt= Martinique; N= Nicaragua; P= Panama; PR= Puerto Rico; TT= Trinidad and Tobago; U= USA; V= Venezuela.
[d]References: 1) Ala-Poikela *et al*., 2004; 2) Ascencio-Ibañez *et al*., 1999; 3) Bird *et al*., 2001; 4) Brown and Poulos, 1990; 5) Brown *et al*., 2000a; 6) Brown *et al*., 2000b; 7) Brown *et al*., 2001a; 8) Brown *et al*., 2001b; 9) Brown *et al*., 2002; 10) Engel *et al*., 1998; 11) Faria *et al*., 1997; 12) Garrido-Ramirez and Gilbertson, 1998; 13) Guzman *et al*., 2000; 14) Holguin-Peña *et al*., 2003; 15) Holguin-Peña *et al*., 2004; 16) Hou *et al*., 1996; 17) Idris and Brown, 1998; 18) Idris and Brown, 1999; 19) Idris *et al*., 1999; 20) Karkashian *et al*., 2002; 21) Lazarowitz, 1991; 22) Lotrakul *et al*., 2000; 23) Martinez *et al*., 1997; 24) Martinez-Zubiaur *et al*., 1996; 25) Martinez-Zubiaur *et al*., 2004; 26) Matyis *et al*., 1975; 27) Maxwell *et al*., 2002; 28) Mendez-Lozano *et al*., 2001; 29) Morales *et al*., 2001; 30) Nakhla *et al*., 1994; 31) Polston *et al*.,, 1999; 32) Polston *et al*., 1993; 33) Polston *et al*., 1998; 34) Polston *et al*., 1999; 35) Quiñonez *et al*., 2002; 36) Ramos *et al*., 1997; 37) Ribeiro *et al*., 2003; 38) Roberts *et al*., 1986; 39) Rojas *et al*., 2000; 40) Rojas *et al*., 2004; 41) Roye *et al*., 1999; 42) Roye *et al*., 2000; 43) Salati *et al*., 2002; 44) Sinisterra *et al*., 2000; 45) Torrez-Pacheco *et al*., 1993; 46) Torrez-Pacheco *et al*., 1996; 47) Umaharan *et al*., 1998; 48) Urbino and Tassius, 2003; 49) Urbino *et al*., 2003; 50) Uzcategui and Lastra, 1978; 51) Wernecke *et al*., 1995.

The whitefly-transmitted viruses are among the most destructive plant viruses. Early virus infection often results in total crop loss. Because losses in many vegetable crops have been so large, the common response has often been the massive overuse of insecticides at considerable cost but without significant benefit (Hilje and Arboleda, 1993). Applications are often made every 2-3 days or even daily. A good management of the disease could be through the understanding of the interactions between the begomovirus pathogen, the whitefly vector, and plant species that serve as hosts of both begomoviruses and/or the insect vector (Brown, 1997). More biological and molecular research is needed to establish clear taxonomic distinctions for many of the begomoviruses infecting horticultural crops (Polston and Anderson, 1997). Understanding the epidemiology of begomoviruses may help to establish efficient control measures and improve procedures for breeding virus-resistant cultivars (Zeidan and Czosnek, 1991). In Central America, as in many other tropical countries, there is an urgent need to develop a good strategy (IPM program) to avoid the problems of begomoviruses. For this strategy to be successful, it has to be developed under an epidemiological approach and should be considering the vector, begomovirus, crop, alternative hosts for vector and virus, and the environment.

The most important economic activity in Nicaragua has been agriculture. Traditional crops like cotton and coffee have been used as the major export crops during decades. Cotton production collapsed at the end of 1970s while coffee still persists as the most important export crop. However, coffee production decreases every year due to the low price in the international market. As many other American countries Nicaragua started with a non-traditional crop diversification at the beginning of the 1980s. Crops like tomato, pepper (chilli Jalapeño) and cucurbit (melon) were planted at a large scale (extensive and intensive) for

exporting. In addition, these crops are important components of the crop systems used by farmers of small holdings around the country, for local markets. The crop systems used by those farmers are very simple and consist basically of five crops: maize and beans as consumptions crops, and tomato, peppers and cucurbits as cash crops. These crops are present in the field almost all the time during the rain season and in some places during the dry season under irrigation. Begomoviruses appeared in the country probably in the early 1980s in the tomato production areas of Sebaco Valley. The first detection of a begomovirus in Central America was recorded in Nicaragua in tomato samples from Sebaco by Brown and Anderson in 1986 (Polston and Anderson, 1997). Nakhla *et al*., (1994) later on reported two begomoviruses associated with tomatoes in Central America (TomGV1 and TomGV2), from which only the first one was found in Nicaragua. By the middle of 1980s, all the tomato production areas of Sebaco were affected and by the 1990s the epidemic affected the whole country with drastic reductions of yield and tomato production virtually vanished. The epidemic also affected peppers and cucurbits. These virus epidemics are causing catastrophic economical and social problems.

The basic condition in management of any disease is a precise understanding of the pathogens involved. This study is the first step towards understanding the molecular aspects of the most common begomoviruses found infecting horticultural crops in Nicaragua.

Aims of the study

This thesis focuses on the study of the begomoviruses naturally infecting tomato, pepper and cucurbit crops in Nicaragua.

The specific aims of this study were:

- Identification of the begomoviruses in tomato, and their possible, alternative pepper and cucurbit hosts in Nicaragua.
- The genomic characterization of two begomoviruses, ToLCSinV and ToSLCV, and studies on some of the biological properties of these viruses.
- To define the relationship between ToLCSinV, ToSLCV and other begomoviruses.
- To contribute to the understanding of begomovirus evolution.

Results and discussion

Identification of begomoviruses in common cropping systems in Nicaragua

The objectives of this study were to confirm the relation between the observed diseases in tomato field and the presence of begomoviruses, to have an idea about the distribution of the problem around the country (**I**), and to determine the genetic diversity of begomoviruses infecting tomato, pepper, cucurbit and the Mexican fire-plant weed (*Euphorbia heterophylla*) (**II**).

The main cropping system used by small-holding farmers is based on five crops, which can be grown in different combinations at three times during the year (Fig. 2). All these crops, except maize, have been reported as begomovirus hosts. Previously, no molecular research has been conducted on begomoviruses in Nicaragua and almost nothing was known about the begomoviruses infecting the crops. *Euphorbia heterophylla* is a common weed that can be found almost everywhere in Nicaragua and it often shows virus-like symptoms. This study also aimed to find out if pepper, cucurbits and weed plants could function as reservoir hosts for the begomoviruses infecting tomatoes, or vice versa. It is important to know the relation between the begomoviruses found infecting tomato and the other crops of the system. The knowledge of this relationship will be an important component in the attempts to control the diseases.

Top leaves showing symptoms were sampled from tomato, pepper, cucurbit, and euphorbia in different locations in Nicaragua during August 1998, January 1999 and January to March 2003. The symptoms found in the fields were diverse and included heavy to mild mosaic, yellowing, downward curling, leaf distortion, veinal chlorosis and severe stunting (**I**, **II**). The samples were processed and direct PCR detection of begomoviruses in leaf extracts was carried using degenerate primers (Wyatt and Brown, 1996). PCR products of 576 bp were obtained, cloned and sequenced (**I**). Analyses of the genetic diversity of begomoviruses were also carried out using large scale sequencing of cloned PCR fragments from single plants (**II**). These sequences were compared with sequences from other begomoviruses available in GenBank.

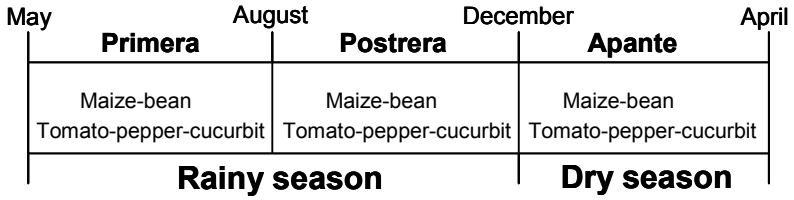


Fig 2. Cropping system used by small-holding farmers in Nicaragua.

The results of the initial studies on tomato crops showed that begomovirus diseases are widespread in Nicaragua as in many countries of the region (Brown and Bird, 1992; Brown, 1997; Polston and Anderson, 1997; Morales and Anderson, 2001). Begomoviruses were detected in eleven of the twelve locations where samples were collected (**I**). According to the comparisons and phylogenetic analyses of the partial *AV1* sequences they were grouped into four groups in relation with the previously described begomoviruses. They were found to be widely distributed (present in the most important areas) and belonged to at least four species (Fig. 3) (**I**).

A second study including more samples of tomato and also other species showed that the samples contained begomoviruses (Table 4). The sequence analyses of the cloned PCR fragments (533 bp) revealed that they corresponded to five previously described viruses: ToSLCV, ToLCSinV, PepGMV, SYMMoV, and EuMV. In addition, a new tentative species, Tomato leaf curl Las Playitas virus (ToLCLPV), was detected (**II**).

Fig 3. Distribution of the four begomovirus groups (1-4) detected in tomato crops in Nicaragua.

The incidence of begomoviruses, in the symptomatic plant species evaluated, was 100% for tomato plants, 43% for peppers, 30% for chili peppers, and 46% for cucurbits but no tested potato plant was begomovirus-infected (Table 4). Two strains of ToSLCV were detected: ToSLCV-NI was found infecting tomato and pepper, and ToSLCV-[GT96-1] was found infecting tomato. ToLCSinV infected tomato and pepper; PepGMV infected tomato, pepper and cucurbit. SYMMoV infected cucurbits, whereas EuMV infected euphorbia. Mixed infections in single plants with two or three begomovirus were commonly found. ToSLCV, ToLCSinV and PepGMV were the most common viruses causing mixed infections in tomato and pepper. PepGMV was found together with SYMMoV in a mixed infection in a cucurbit plant (**II**). Infections of pepper and tomato by the same begomovirus have previously been reported (Torres-Pacheco *et al*., 1996; Roye *et al*., 1999; Reina *et al*., 1999; Quiñones *et al*., 2002), but some begomoviruses also infect both tomato and cucurbits (Mansoor *et al*., 2000; Samretwanich *et al*., 2000). Mixed infections may involve different begomovirus strains or species, but they also can occur between begomoviruses and other viruses, for example in cassava (Berry and Rey, 2001; Were *et al*., 2004), tobacco (Paximadis *et al*., 2001), cotton (Sanz *et al*., 2000), cucurbits (Yuki *et al*., 2000), peppers and tomato (Brown and Nelson, 1988; Paplomatas *et al*., 1994; Nakhla *et al*., 1994). Sometimes the mixed infections cause synergistic effects and more severe diseases (Fondong *et al*., 2000; Pita *et al*., 2001). However, mixed infections can also have some antagonist effects. Nevertheless, the most important role of mixed infections is that they allow recombination to occur and more virulent variants of viruses may evolve. This is very important for virus epidemiology and evolution (Harrison and Robinson, 1999; Padidam *et al*., 1999; Varma and Malathi, 2003; Kitamura *et al*., 2004; Bananej *et al*., 2004).

Table 4. Detection of begomoviruses in samples collected in fields in Nicaragua. Viruses were detected by PCR with degenerate primers.

Field	Plant specie	Number of samples	Number (%) of PCR positive samples
CNIA	*Lycopersicon esculentum*	37	37(100)
	Capsicum annuum	64	32(50)
	Cucurbita argyrosperma	18	9(50)
Las Playitas	*Lycopersicon esculentum*	4	4(100)
	Cucurbita argyrosperma	8	3(37.5)
Sebaco	*Lycopersicon esculentum*	10	10(100)
	Capsicum annuum	26	14(53.8)
	Euphorbia heterophylla	3	3(100)
Tecolostote	*Lycopersicon esculentum*	10	10(100)
	Capsicum annuum	9	3(33.3)
	Euphorbia heterophylla	1	1(100)
UNA	*Capsicum spp.*	56	17(30.3)
Jinotega	*Solanum tuberosum*	17	0(0)

When comparing sequence identities (%) between all begomovirus isolates in different plants there is about the same amount of variability as when comparing sequence identities within single plants (**II**). PepGMV clones were 99-100% identical and only one clone was different, showing 91% sequence identity. ToLCSinV clones were 98-100% identical and only one clone was different with 96% identity to the other clones. ToSLCV clones were 98-100% identical and only one clone was different with 95% identity to the others. In one of the plants infected with ToSLCV-NI the clones can be divided in two groups with high sequence identity to ToSLCV-NI[H11] or ToSLCV-NI[Ti21]. In general the genetic variability of these begomoviruses was 1-2% in nucleotide sequence (**II**). This variability can be considered as low and shows genetic stability of the virus (Garcia-Arenal *et al*., 2001; Roossinck, 2003; Garcia-Arenal *et al*., 2003). However, there were some variants that significantly differ in some plants. In one of the plants, the ToSLCV-NI clones were only 92-95% identical and constituted two genetic subtypes. In addition, this plant contained two deviant sequence variants (**II**). This virus maybe is a result of recombination and it could be speculated that the variability found has been influenced by the recombination detected.

Partial genomic and biological characterization of two begomoviruses

The aims of this study were to carry out genomic characterization of the most important begomoviruses identified, to compare transmissibility of those begomoviruses and also test whether their host range was potentially broader than the tomato crops in which they were found in the field.

Samples were collected in three locations of the country (Condega, Santa Lucia, and Sebaco) from tomato plants showing typical symptoms of begomovirus infection. The symptoms found in the fields were diverse and included severe to mild mosaic, yellowing, downward curling, leaf distortion, veinal chlorosis and severe stunting. The samples were transferred to Sweden and direct PCR detection of begomoviruses in leaf extracts was carried out as described (Wyatt and Brown, 1996). Degenerate PCR primers were used for the amplification of begomovirus DNA. PCR products of ≈550 bp, ≈600bp, 1.1kb and 1.3kb were obtained. Those fragments were cloned and sequenced and the sequences obtained were used to design sequence (virus) specific sets of primers for the amplification of the complete DNA-A and DNA-B of the begomovirus under study (Fig 4). The sequences determined in this study were compared with sequences from other begomoviruses reported in GenBank (**III**).

Analyses of the partial sequences showed that these plants were infected with ToSLCV and ToLCSinV (**III**). Complete sequences of the DNA-A component in one isolate and DNA-B component in two isolates of ToLCSinV, as well as the complete sequence of DNA-A of two ToSLCV-NI isolates were determined. Sequence analysis revealed that each component contained the ORFs found in bipartite American begomoviruses (Brown, 1997; Harrison and Robinson, 1999). The length of the single-stranded DNA-A and DNA-B components of ToLCSinV-

[SaL] were 2611 and 2561 nt, respectively. The length of the single stranded DNA-A component of ToSLCV-NI[Con] was 2593 nt (Fig 5) (**III**).

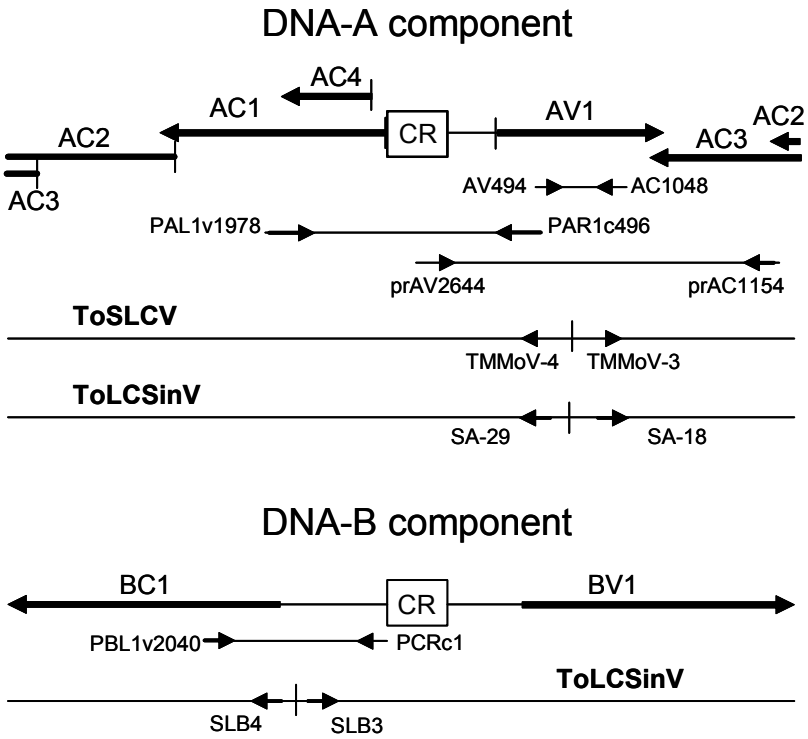
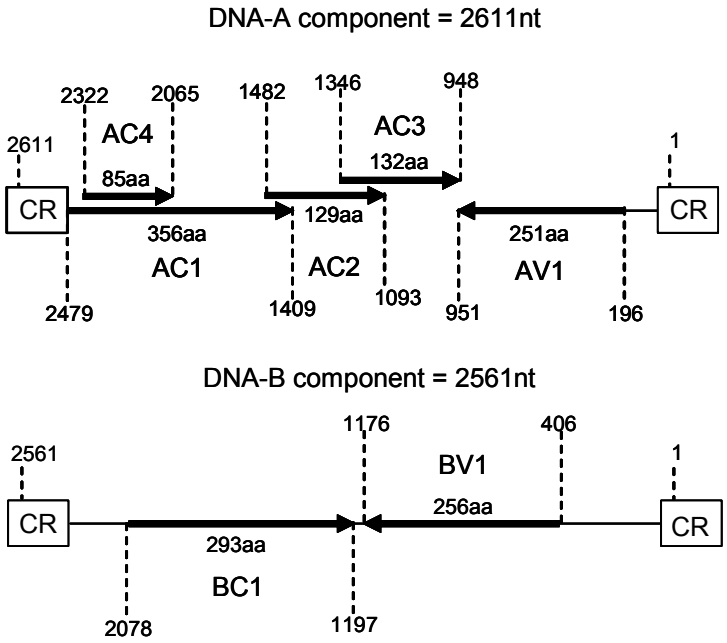


Fig 4. Linearized genomic maps of DNA-A (ToSLCV and ToLCSinV) and DNA-B (ToLCSinV) showing the annealing sites of the PCR primers used for the amplification of the corresponding begomovirus species. Degenerate primer pairs AV494 and AC1048; PAL1v1978 and PAR1c496; prAV2644 and prAC1154, were used for DNA-A of ToLCSV and ToLCSinV. Specific primer pairs TMMoV-3 and TMMoV-4; SA-18 and SA-29, were used for the complete DNA-A of ToSLCV and ToLCSinV, respectively. Degenerate primer pair (PBL1v2040 and PCRc1) and specific primer pair SLB3 and SLB4 were used for the DNA-B component of ToLCSinV. Solid arrows indicate the 5' – 3' orientation on the viral (+) strand and the complementary (-) strand.

A. ToLCSinV-[SaL]



B. ToSLCV-NI[Con]

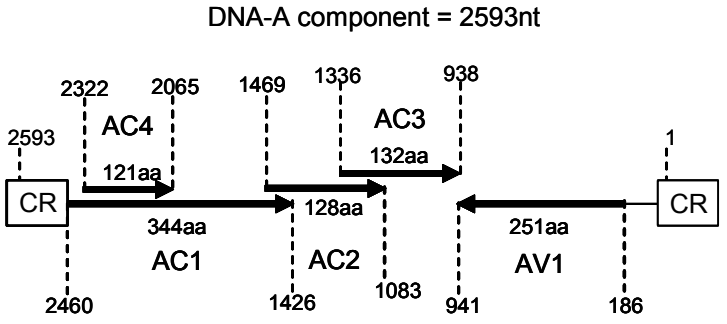


Fig 5. Linearized genomic maps of the DNA-A and DNA-B components for ToLCSinV-[SaL] and DNA-A component for ToSLCV-NI[Con]. Numbers indicate the first and last nucleotide of each open reading frame (ORF) and the number of amino acids (aa) for each one. Solid arrows indicating the 5' – 3' orientation on the viral (+) strand and the complementary (-) strand.

Sequence comparisons of the complete genomes confirmed that they belonged to the species ToLCSinV and ToSLCV. Those analyses showed that the highest identity of both components of ToLCSinV-[SaL] were with *Chino del tomate virus* (CdTV) (Brown *et al*., 2000), *Sida golden mosaic Honduras virus* (SiGMHV) and *Sida yellow vein virus* (SiYVV) (Frischmuth *et al*., 1997) (**III**). Similar results were obtained when using nt or aa sequences for the different ORFs (Table 5 and 6). Phylogenetic analyses based on the complete DNA-A or DNA-B components showed that these begomoviruses were placed to the AbMV clade (Brown *et al*., 1999).

Previously, only partial sequences have been available for ToLCSinV (Idris and Brown, 1998; Idris *et al*., 1999) and it was considered as a tentative species (Fauquet *et al*., 2003; Fauquet and Stanley, 2003). Now with the complete sequence of both components, ToLCSinV could be considered as a recognized species (**III**).

Table 5. Percent identities in nucleotide and predicted amino acid sequences for DNA-A of ToLCSinV-[SaL] compared to American isolates of the genus *Begomovirus*. The three highest identities are shown in bold.

		AV1		AC1		AC2		AC3	
Begomovirus	**DNA-A**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**
CdTV-[IC]	**85.5**	**83.2**	89.7	**86.7**	**85.7**	**87.4**	80.0	**89.5**	**87.2**
SiGMHV	**85.2**	**85.1**	88.9	**86.3**	**87.1**	**87.2**	**82.3**	86.5	77.4
SiYVV	**83.6**	83.1	88.9	84.4	82.6	86.4	**83.1**	86.5	78.2
PYMTV-[TT]	83.3	81.0	88.9	**85.6**	**86.0**	85.4	76.9	89.0	82.7
SiGMV	83.1	**83.6**	89.3	82.7	81.8	**87.2**	76.9	85.0	80.5
BDMV	82.2	82.8	89.3	84.8	85.4	84.9	**81.5**	86.7	78.9
AbMV	81.8	82.0	86.9	83.0	82.1	84.9	75.4	85.5	80.5
ToMHV-[Qui]	81.6	81.2	88.1	82.9	82.6	85.6	76.2	85.2	78.9
ToMoTV	81.6	81.2	88.5	81.7	79.3	84.9	73.1	87.5	**84.2**
SiGMCRV	81.6	81.1	88.1	83.0	82.1	83.3	77.7	87.5	79.7
ToMoV-[FL]	80.3	82.3	86.5	81.7	81.2	85.1	74.6	86.0	82.7
SiGYVV-[A11]	79.8	80.0	86.1	81.0	79.0	82.1	77.7	81.2	72.9
PYMPV	79.7	81.7	89.7	77.2	78.4	85.4	76.2	**88.0**	**85.0**
PYMV-VE	78.7	81.2	89.3	78.7	79.8	85.1	79.2	**87.7**	81.2
ToSRV	78.4	82.8	88.1	75.4	74.1	77.9	65.4	82.7	76.7
TYMLCV	77.7	81.5	**90.4**	79.0	82.9	73.6	60.5	79.7	72.9
ToRMV-[Ube]	77.4	82.3	86.9	74.6	74.5	79.0	69.2	82.0	79.7
TGMV-YV	77.3	83.1	89.9	76.4	76.8	77.2	67.7	79.9	73.7
AbMV-HW	77.2	81.7	84.5	81.9	79.8	84.9	76.9	85.0	79.7
MaYMFV	76.9	79.6	85.7	78.3	78.0	73.6	62.3	76.4	68.4
SiGMFV-[A1]	76.8	81.0	87.4	83.4	83.2	84.6	77.7	83.7	77.4
SiMoV-[BR]	76.8	80.4	87.3	75.1	66.7	81.0	70.8	83.0	77.4
BGMV-[BR]	76.5	79.6	87.3	74.4	74.2	76.4	68.5	79.9	73.7
DiYMoV	76.4	80.7	86.1	75.4	77.6	73.1	61.5	74.4	63.9
ToCMoV-[BZ]	76.4	81.3	86.9	74.1	75.3	79.2	69.2	83.2	83.5
ToMLCV	76.4	78.3	88.8	77.4	80.1	74.1	61.5	78.7	71.4
SiYMV-[BR]	76.1	79.6	82.1	73.6	72.5	80.3	58.5	83.5	63.2
BGYMV-[PR]	75.9	79.9	86.8	75.5	74.3	73.6	63.8	77.4	70.7
SiMMV	75.8	79.4	87.3	76.1	76.5	78.5	70.0	82.0	78.9
MaYMV-[CU]	75.6	79.2	86.5	76.3	76.8	71.8	59.2	75.4	66.2
CLCrV	74.7	80.6	88.1	69.6	64.8	82.8	72.3	82.7	78.9
RhGMV	74.3	77.6	85.3	72.1	70.3	72.3	56.2	75.2	65.4
ToGMoV-[GT94-R2]	73.8	80.7	89.3	75.4	74.4	73.3	63.1	78.2	69.9
ToChLPV	73.7	75.8	81.0	75.7	76.4	74.7	63.6	81.0	71.4
MaMPRV	73.3	79.5	88.4	70.0	66.4	69.0	60.0	75.7	66.9
ToSLCV-[GT96-1]	72.8	82.9	88.5	70.1	66.4	73.4	60.5	78.4	69.2
PHYVV	72.1	78.6	86.1	71.5	69.7	69.0	53.1	73.4	64.7
BCaMV	71.6	81.2	88.4	67.4	64.0	74.1	62.3	77.9	69.9
ToSLCV-NI[SaL]	71.2	76.9	81.0	69.4	66.1	74.4	62.8	80.5	70.7
ToSLCV-NI[Con]	71.1	77.0	81.0	69.2	65.8	74.4	62.8	80.5	70.7
CaLCuV	71.1	81.6	89.7	66.3	63.7	74.9	60.0	76.9	65.4
SMLCV-[IV]	70.2	81.3	90.1	66.7	63.1	72.3	57.7	73.7	64.7
SYMMoV-[CR]	69.9	80.3	90.1	65.2	58.8	71.5	60.8	74.7	67.7
MCLCuV-[GT]	69.4	81.0	89.3	65.5	59.4	72.6	59.2	74.9	68.4
CuLCuV	68.4	82.0	**90.4**	65.0	61.1	70.5	64.6	75.9	66.9
PepGMV	68.4	79.8	87.7	64.2	59.9	71.5	60.8	75.2	66.2
SLCV	68.2	81.5	**91.3**	66.1	60.3	71.5	62.3	76.4	70.7
TYLCV-[DO]	61.4	62.8	67.9	69.7	64.4	69.7	48.5	71.4	54.1
TYLCV-[PR]	60.8	62.4	67.9	69.1	64.1	67.7	48.5	71.7	54.9

Table 6. Percent identities in nucleotide and predicted amino acid sequences for DNA-B of ToLCSinV-[SaL] compared to American isolates of the genus *Begomovirus*. The three highest identities are shown in bold.

		BV1		BC1	
Begomovirus	**DNA-B**	**nt**	**aa**	**nt**	**aa**
ToLCSinV-[Con]	**90.9**	**90.7**	**84.4**	**94.2**	**96.3**
SiGMHV	**79.0**	**82.7**	**86.8**	**86.6**	**93.5**
CdTV-[IC]	**75.3**	79.2	82.9	84.5	91.5
SiYVV	74.8	**82.9**	**85.2**	**85.5**	**93.2**
SiGMCRV	72.4	80.7	83.3	84.0	90.8
ToMoV-[FL]	70.0	73.8	76.3	84.1	92.5
BDMV	69.4	79.6	82.1	81.7	91.8
SiGMV	69.4	77.7	79.1	84.2	92.2
ToMoTV	67.7	77.0	76.3	84.0	92.2
AbMV	67.4	58.2	78.2	67.8	89.8
ToMHV-[Qui]	66.6	75.5	76.2	80.2	75.2
AbMV-HW	66.3	74.8	77.0	81.1	86.7
PYMTV-[TT]	63.7	71.2	68.1	81.7	89.5
PHYVV	62.9	71.2	69.6	74.6	81.6
ToMLCV	61.0	64.4	62.0	77.0	82.2
DiYMoV	60.8	72.0	72.4	77.9	83.7
PYMPV	60.6	69.6	66.9	81.1	89.1
BGMV-[BR]	60.5	72.8	74.4	76.2	78.6
MaMPRV	60.0	70.6	73.0	77.4	83.3
MaYMFV	59.5	67.1	69.8	75.9	80.6
TYMLCV	59.5	73.4	70.4	75.2	83.3
ToCMoV-[BZ]	58.5	71.1	71.2	74.1	79.9
PYMV-VE	54.0	70.7	66.5	82.0	88.4
SiMMV	52.0	72.5	74.3	77.4	83.7
CLCrV	51.5	69.8	71.2	77.2	85.0
ToRMV-[Ube]	50.9	72.6	71.6	76.2	81.3
CaLCuV	49.8	71.2	72.8	74.7	80.3
BGYMV-[PR]	49.2	72.4	73.9	75.6	83.3
BCaMV	48.3	70.7	71.2	75.0	79.9
PepGMV	48.2	71.3	73.5	74.4	82.4
TGMV-YV	48.0	65.8	69.3	79.6	88.6
SMLCV-[IV]	47.6	71.5	67.5	73.6	78.9
SLCV	47.0	67.1	63.8	72.8	78.9
CuLCuV	46.9	70.3	65.8	72.1	76.2
SYMMoV-[CR]	46.6	68.1	66.9	71.0	77.6

The complete nt sequence for the DNA-A component of ToSLCV-NI[SaL] was compared to all available complete sequences of begomoviruses (**III**). Sequence analyses showed that the highest similarity of ToSLCV-NI[SaL] was with ToSLCV from Guatemala (ToSLCV-[GT96-1]) (Nakhla *et al*., 2002), *Tomato chino La Paz virus* (ToChLPV) (Holguin-Peña *et al*., 2003), and *Bean calico mosaic virus* (BCaMV) (Brown *et al*., 1999)(Table 8). Similar results were obtained when using nt or amino acid (aa) sequences for the differents ORFs except for *AC1* and *AV1*. The similarities were within a similar range with ToSLCV-[GT96-1] except for *AV1* and *AC1* in the case of ToChLPV (Table 7).

Table 7. Percent identities in nucleotide and predicted amino acid sequences for DNA-A of ToSLCV-NI[SaL] compared to American isolates of the genus *Begomovirus*. The three highest identities are shown in bold.

		AV1		AC1		AC2		AC3	
Begomovirus	**DNA-A**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**
ToSLCV-NI[Con]	**99.8**	**99.9**	**99.6**	**99.7**	**99.1**	**100**	**99.2**	**100**	**99.2**
ToSLCV-[GT96-1]	**91.0**	**80.2**	**85.3**	**96.8**	**97.4**	**96.4**	**95.3**	**93.5**	**90.2**
ToChLPV	**82.7**	**93.8**	**98.0**	72.9	67.8	**94.6**	**93.8**	**95.0**	**94.7**
BCaMV	78.9	76.8	83.6	**83.1**	**85.8**	79.1	68.2	83.7	82.0
SYMMoV-[CR]	78.3	77.8	81.3	81.9	80.6	76.7	64.3	78.4	73.7
SLCV	75.6	76.7	82.9	79.4	79.7	76.7	64.3	79.4	74.4
CaLCuV	75.2	78.4	83.7	82.7	84.1	77.8	65.1	81.5	77.4
SMLCV-[IV]	74.8	78.0	84.1	83.0	84.6	77.5	65.1	79.4	72.2
PepGMV	74.4	77.9	83.3	76.8	79.4	76.5	65.1	79.7	74.4
CuLCuV	74.3	78.5	83.2	81.4	83.5	74.7	67.4	80.5	72.9
MCLCuV-[GT]	74.1	78.2	84.1	81.4	80.6	76.0	62.8	78.4	75.2
CLCrV	73.3	77.4	82.1	79.4	79.4	73.6	59.7	78.2	75.2
PYMPV	73.1	79.2	82.9	67.7	64.6	76.0	62.8	82.7	75.2
ToCMoV-[BZ]	73.0	78.7	81.7	69.5	63.5	73.9	62.0	79.7	73.7
PYMTV -[TT]	72.9	78.8	82.1	67.5	64.3	75.2	62.8	81.7	74.4
BGMV-[BR]	72.6	78.0	81.7	67.7	65.5	75.2	63.6	82.7	73.7
TYMLCV	72.6	77.1	83.5	68.6	62.3	77.8	65.9	81.0	76.7
ToGMoV-[GT94-R2]	72.5	78.6	84.9	67.3	61.7	78.0	66.7	79.7	74.4
SiGMCRV	72.1	76.9	82.5	67.5	63.5	74.4	62.8	81.2	72.2
PYMV-VE	71.9	78.4	82.5	68.0	61.7	75.2	65.1	81.5	75.9
ToMLCV	71.8	77.5	81.9	70.6	63.5	80.1	67.4	80.5	76.7
ToLCSinV-[SaL]	71.2	76.9	81.0	69.4	66.1	74.4	62.8	80.5	70.7
BDMV	70.9	78.0	82.5	69.5	63.8	73.9	62.8	79.7	69.2
SiGMV	70.9	79.0	81.7	69.1	61.7	75.2	62.8	77.9	73.7
ToSRV	70.7	78.6	81.7	67.3	59.7	74.4	60.5	82.2	75.9
CdTV-[IC]	70.7	78.6	82.1	68.5	63.2	72.6	58.1	79.7	71.4
MaYMV-[CU]	70.7	77.3	82.9	69.1	62.0	75.2	61.2	79.7	73.7
AbMV	70.4	78.7	81.3	69.4	62.6	73.9	62.8	78.9	72.2
ToMoTV	70.4	77.8	81.3	69.6	60.9	70.5	59.7	79.2	69.9
TGMV-YV	70.3	78.8	83.9	71.5	63.5	77.0	66.7	80.2	76.7
ToMoV-[FL]	70.3	77.6	80.6	66.8	60.9	73.9	58.9	79.7	74.4
SiYVV	70.2	77.5	80.6	69.0	62.0	73.6	62.0	80.2	71.4
MaYMFV	70.1	77.0	82.1	67.2	62.0	76.5	63.6	79.7	75.9
ToRMV-[Ube]	70.0	78.3	81.3	68.1	62.6	75.2	65.1	80.2	72.9
SiGMHV	69.9	77.0	80.6	69.6	62.6	73.9	62.0	80.2	72.2
ToMHV-[Qui]	69.6	79.0	80.2	67.1	62.0	72.4	61.2	77.9	68.4
SiGYVV-[A11]	69.5	75.4	79.0	68.3	60.9	72.6	60.5	76.4	68.4
SiMoV-[BR]	69.5	77.0	82.9	70.1	58.3	74.9	61.2	76.2	69.9
SiMMV	68.1	77.1	82.5	68.2	60.0	76.2	61.2	76.9	71.4
SiYMV-[BR]	67.1	76.3	76.6	69.3	61.4	75.2	53.5	76.7	59.4
AbMV-HW	66.9	78.4	79.8	70.0	62.0	74.2	62.0	78.2	69.9
BGYMV-[PR]	66.6	76.7	80.2	64.5	60.6	79.1	69.0	83.2	78.9
DiYMoV	66.6	77.6	80.2	66.6	64.1	73.9	60.5	75.7	68.4
MaMPRV	66.3	80.1	82.8	62.0	50.7	68.7	57.4	75.7	69.9
SiGMFV-[A1]	66.3	76.0	79.1	68.8	64.1	74.9	61.2	77.7	70.7
RhGMV	63.6	76.7	80.6	61.9	54.5	70.5	59.7	77.4	66.9
PHYVV	63.4	76.6	79.8	60.4	51.3	66.1	49.6	75.2	63.9
TYLCV-[DO]	55.9	61.5	69.4	61.2	51.9	61.0	52.7	70.4	52.6
TYLCV-[PR]	55.5	61.8	66.3	60.8	51.6	60.5	52.7	69.7	53.4

Phylogenetic analyses of the complete DNA-A component of ToSLCV-NI[Con] and ToSLCV-NI[SaL] and other begomovirus sequences from GenBank showed that the aforementioned Nicaraguan begomoviruses belonged to the SLCV clade (Brown *et al*., 2001) (**III**). The same results were observed when the ORFs *AC2* and *AC3* were used for the analysis. Nevertheless, when only the ORFs *AV1* or *AC1* were analyzed different results were obtained. In the case of *AV1*, ToSLCV-[GT96-1] was placed to the AbMV clade and, interestingly, ToSLCV-NI[Con], ToSLCV-NI[SaL] and ToChLPV grouped separate from the AbMV and SLCV clades. In the case of *AC1*, ToSLCV from Nicaragua and Guatemala was placed to the SLCV clade, while ToChLPV belonged to the Brazil clade (Fig. 6). Based on differences in the *AV1* region, two different strains of ToSLCV can be distinguished: ToSLCV and ToSLCV-NI.

The data suggest that *AV1* of ToSLCV-[GT96-1] and *AC1* of ToChLPV may be derived from other begomoviruses as a result of recombination. This result is consistent with several other studies indicating that recombination among virus strains or species is an important driving force in the evolution and appearance of new begomoviruses (Torres-Pacheco *et al*., 1993; Briddon *et al*., 1996; Harrison and Robinson, 1999; Padidam *et al*., 1999; Garrido-Ramirez *et al*., 2000; Navas-Castillo *et al*., 2000; Berrie *et al*., 2001; Jeske *et al*., 2001; Martin *et al*., 2001; Pita *et al*., 2001; Galvão *et al*., 2003). The recombination hypothesis was tested using software specifically designed for this purpose (RDP version 1.8) (Martin and Rybicki, 2000). The results of the recombination analyses predicted that ToSLCV-NI was generated by recombination between the *AV1* genes of ToSLCV and ToChLPV, or that ToSLCV was generated from ToChLPV through subsequent recombinations in the *AC1* region with a virus of the SLCV clade forming ToSLCV-NI and then in the *AV1* region with a virus in the AbMV clade (**III**).

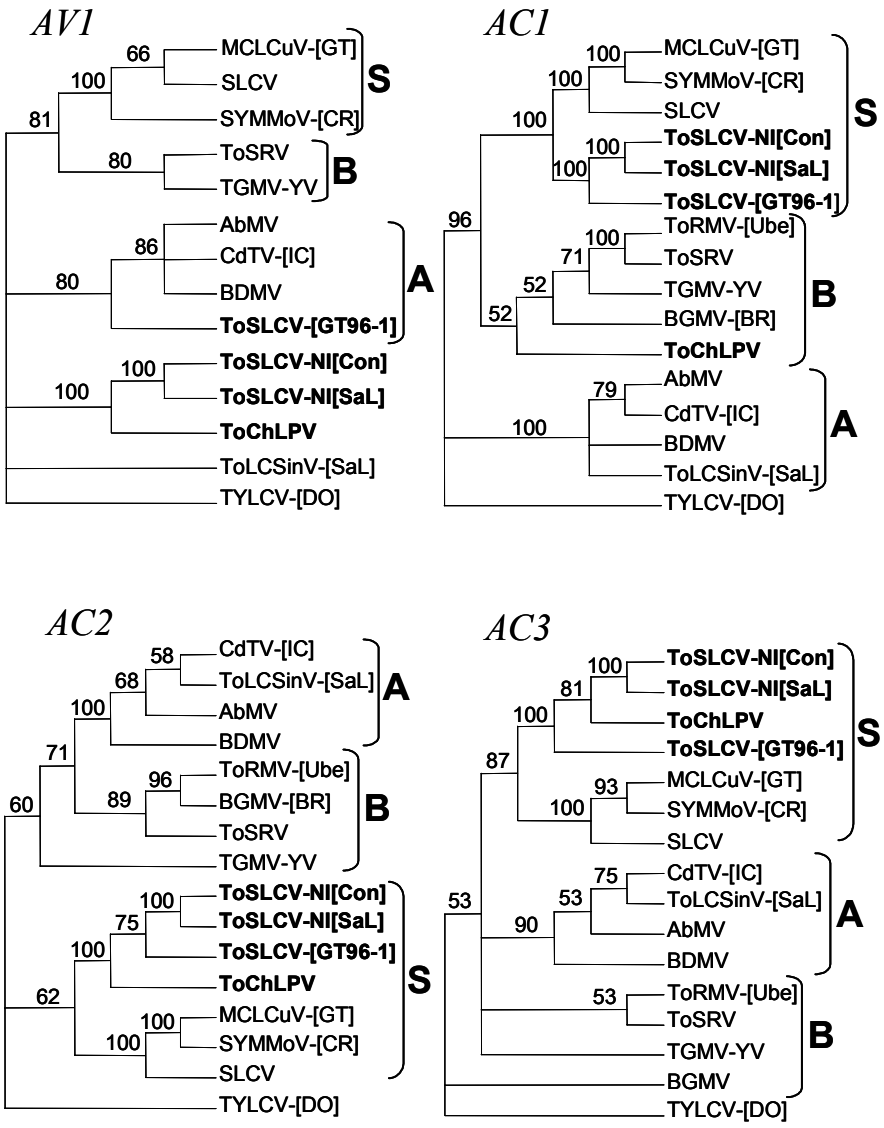


Fig 4. Phylogenetic trees based on the *AV1, AC1, AC2*, and *AC3* nucleotides sequences showing the predicted relationships between ToSLCV-NI isolates Con and SaL, *Tomato chino La Paz virus* (ToChLPV; accession number AY339618), ToSLCV-[GT96-1](AF130415) and other begomoviruses. Numbers represent the bootstrap values out of 1000 replicates. Only bootstrap values higher than 50 are shown. AbMV, Brazil and SLCV begomovirus clades are indicated with the letters **A**, **B**, and **S**, respectively.

Transmission of ToSLCV and ToLCSinV by whiteflies

The whitefly *B. tabaci* is a very important component of the problems caused by begomoviruses because of its capacity to transmit all begomoviruses and its ability to feed on a large number of plant species. In our study, working with the same plants as in study **III** (Con, SaL, Seb), we found that the acquisition access period (AAP) and inoculation access period (IAP) of *B. tabaci* for begomoviruses can be only 10 min, and the whitefly remains infective between five to seven days without a new virus acquisition (**IV**). Longer AAP and IAP in general resulted in higher acquisition and inoculation rates of the viruses and significant differences could be observed between 10 min and 24h treatments. An AAP and IAP exceeding 24h did not improve the rate of transmission (**IV**). The efficiency for acquisition, inoculation and retention period of begomoviruses depend on many factors like whitefly biotype, plant species, virus strain, and the environment, but in general it has been found that the acquisition and inoculation could be done in some minutes or hours and the retention after acquisition could be as long as 20 days (Costa, 1976; Stenger *et al*., 1990; Brown and Bird, 1992; Nateshan *et al*., 1996; Idris and Brown, 1998; Idris *et al*., 2001; Jones, 2003; Muniyappa *et al*., 2003).

Sequence analyses of the viruses present in virus source plants revealed mixed infections with ToSLCV and ToLCSinV in Con and SaL, and single infection with ToLCSinV in Seb. Alignment of the CP amino acid sequences showed a high identity between the isolates of each begomovirus species, 98.8-99.6% in the case of ToLCSinV isolates (Con, SaL, Seb), and 99.6% in the case of ToSLCV isolates (Con, SaL). Nevertheless, some amino acid differences could be observed within a region essential for transmissibility of begomoviruses (Noris *et al*., 1998; Kheyr-Pour *et al*., 2000). According to previous studies (Höhnle *et al*., 2001) the amino acids at positions 124, 149, and 174 play an important role for vector transmission, and higher or lower efficiency depends on the amino acids combination at these positions. One efficient combination reported is K, Q, M (lysine, glutamine, methionine). In our study, this combination can be observed in ToLCSinV isolates, but not in the ToSLCV isolates, which showed the combination K, H, M (lysine, histidine, methionine). The histidine at that positions has been reported to be essential for the non-transmissibility of AbMV (Höhnle *et al*., 2001). It can be speculated that ToSLCV is affected in its transmission efficiency and in some way needs the assistance of another virus for improve the efficiency. This virus has mainly been found in mixed infections with ToLCSinV (**II, III**, **and IV**). Further studies on transmissibility of those viruses will be required to address this issue and to understand the relationship between ToSLCV and ToLCSinV causing epidemics in mixed infections.

Conclusions

- Tomato, pepper and cushaw in Nicaragua are infected by several begomoviruses, of which ToSLCV, ToLCSinV and PepGMV were found in both tomato and pepper. PepGMV was also found in cushaw. Mixed infections with begomoviruses seem to be common in horticultural crops in Nicaragua.

- ToLCSinV and ToSLCV commonly infect tomato crops in Nicaragua and they are widely distributed in the country. The short acquisition and inoculation access period of begomoviruses by the vector *B. tabaci*, the high populations of the vector, and the cropping system used by the farmers are probably significantly contributing to the severe disease epidemics caused by begomoviruses in Nicaragua.

- Phylogenetic analyses using the DNA-A and DNA-B components of ToLCSinV, and the DNA-A component of ToSLCV (two strains) grouped them differently from what was observed when a single gene (*AV1*) from ToSLCV was used. Recombination was predicted in ToSLCV. Recombination could be one of the main factors behind the appearance of more destructive begomovirus strains or even species causing epidemics in the field. Common occurrence of mixed infections and the intraspecific viral variability found in this study further support this possibility.

- Taken together these results clearly show that the disease caused by begomoviruses in tomato plants cannot be "controlled" by conventional methods like insecticide applications. An IPM (Integrated Pest Management) program is necessary for the management of the problem. Resistant varieties are needed, as a component of the IPM program, but the high diversity of the begomoviruses could be make this approach very difficult to obtain.

Future perspectives

- Single and mixed infections by begomoviruses and their recombinants are probably responsible for new epidemics in horticultural crops. Little information is available about identity and relationships between the viruses involved in these epidemics in most crops and more studies would allow an increase of our knowledge on the variability and evolution of the begomoviruses in the New World.

- More studies are necessary on the occurrence of begomoviruses in wild species and the role of those plants in the epidemiology and evolution of begomoviruses.

- The vector capacity for transmission of begomoviruses needs also to be further investigated in order to understand the effects of the disease when different combinations of viruses are inoculated at the same time or at different times.

- Resistant tomato varieties seem to be necessary for slowing down the epidemics, but production of such varieties will be difficult due to the high diversity of begomoviruses infecting the tomato crops.

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Acknowledgements

I would like to express my gratitude to all people who helped and encouraged me to start, continue and finish this thesis. My heartfelt thanks to:

My supervisors, Jari Valkonen and Anders Kvarnheden, for their invaluable support, close supervision and guidance throughout my study. But more important than the thesis, you have been teaching me the way to continue with research in my country in the future.

Lars Ohlander, who has been working very hard during the last twenty-two years helping the Nicaraguan people through the UNA-SLU-PhD Programme, supported by Sida/SAREC. Lars, only your everlasting patience has made this programme successful.

My colleagues and friends of the Virology Group: Carl “el cholo”, Igor, Hector, Minna, Tuija, Hannele, Jan, Jaana, Robert, Settumba, Fred, Anna, Elin, Jon, Ingela, Eugene, Andrey.

The National Agrarian University (UNA) for giving me the opportunity and the educational leave for my PhD study.

De manera muy especial a mis seres mas queridos, mis hijos, mi mujer, mi madre. Quienes me han apoyado todos estos años y me han dado motivos y fuerzas para seguir adelante.

Finalmente, a todos aquellos que han tratado de bloquearme en mí trabajo. A ustedes también les agradezco por permitirme conocerlos mejor y seguirme convenciendo que estamos muy lejos de llegar a donde debemos llegar.