Tropical Agricultural Research and Extension 1(2): 107-115, 1998

Molecular analysis of whitefly-transmitted tomato geminiviruses from Southeast and East Asia

Muhammad Zeidan[1], Sylvia K. Green[2], Douglas P. Maxwell[3], Medhat K. Nakhla[3], and Henryk Czosnek[1*]

[1]Department of Field, Vegetable Crops and Genetics, and the Otto Warburg Center for Biotechnology in Agriculture; Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, P.O.Box 12, Israel.
[2]Asian Vegetable Research and Development Center, P.O.Box 42, Shanhua, Tainan 74199, Taiwan, Republic of China.
[3]Department of Plant Pathology, 630 Linden Drive, University of Wisconsin, Madison, WI 53706-1598, USA.

ABSTRACT

Tomato cultures are under the constant threat of geminiviruses transmitted by the whitefly *Bemisia tabaci*. In the affected regions, yield losses often reach 100%. In Southeast and East Asia, as well as in many countries of the Old World, these viruses have been termed tomato yellow leaf curl virus (TYLCV) or tomato leaf curl virus (ToLCV). These viral diseases are becoming more widespread, usually following appearance or increases in whitefly populations. Although leaf curl-related diseases of tomato have been reported throughout Southeast and East Asia, the molecular data available on these viruses is still scarce. The genome of TYLCV and ToLCV isolates has been cloned from infected tomato plants from Bangladesh, China, India, Malaysia, Sri Lanka, Taiwan, The Philippines and Thailand, and sequenced partially or entirely. DNA and protein sequence comparisons indicate that many TYLCV and ToLCV isolates analyzed so far constitute distinct virus species and are not strains of the same species. Analysis of their coat proteins indicate that these viruses group into two branches that include the viruses from China, Malaysia, Taiwan and Thailand (together with the virus from Australia) and those from Bangladesh, India and Sri Lanka. The whitefly-transmitted tomato geminiviruses from Southeast and East Asia constitute a cluster of geminiviruses distinct from those of the Middle East, Southeast Europe and of the Americas.

Key words: geminivirus, tomato, tomato leaf curl virus, tomato yellow leaf curl virus, virus, whitefly

INTRODUCTION

Tomatoes cultivated in regions within a large tropical and subtropical belt around the globe are under the constant threat of whitefly-transmitted geminiviruses. Losses due to these viruses often reach 100% of the crop (Green and Kalloo 1994). Disease symptoms include inhibition of growth, yellowing and curling of leaves and stunting. In the Old World (Middle East, Southwest Europe, Tropical Africa, East and Southeast Asia, and Australia) these diseases are caused by a group of viruses denominated tomato yellow leaf curl virus (TYLCV) or tomato leaf curl virus (ToLCV). The geminiviruses infecting tomato from the New World are genetically different from the TYLCV/ToLCV complex (Rybicki 1994, Padidam *et al.* 1995a). The most prominent were termed as tomato mottle virus in Florida (TMoV), chino del tomate virus in Mexico (CdTV), tomato golden mosaic virus in central and south America (TGMV), tomato yellow mosaic virus in south America (TYMV) (Polston and Anderson 1997).

Geminiviruses infecting tomatoes are rapidly spreading to regions where they were unknown before, threatening tomato production. In Asia, a TYLCV has recently conquered new areas in Iran

*Corresponding author: Henryk Czosnek, Tel: 972-8-9481249 or 9481903; Fax: 972-8-9462181; E.mail: czosnek@agri.huji.ac.il

Abbreviations: aa- amino acid, PCR- polymerase chain reaction, ToLCV- tomato leaf curl virus, TYLCV - tomato yellow leaf curl virus., IR- intergenic region, CP- capsid protein, Rep- replication- associated protein

(Hajimorad *et al.* 1996), in Asian Republics of the former USSR - Azerbaidjan, Turkmenistan and Uzbekistan (Anonymous 1993), and in China (Liu *et al.* 1997). The spread of TYLCV/ToLCV-like diseases and of many other whitefly-transmitted geminiviruses has often paralleled the worldwide expansion of the B biotype of *B. tabaci* (also known as the silverleaf whitefly *B. argentifolii*, Perrring *et al.* 1993, Bedford *et al* 1994a, Brown 1995). In Asia (India, Pakistan), non-B whitefly biotypes (H, K) have been detected by esterase banding patterns; these biotypes were able to transmit TYLCV/ToLCV (Bedford *et al.* 1992).

A great deal of information has accumulated during the last decade on the molecular properties of geminiviruses in general and on that of TYLCV/ToLCV in particular (Rybicki 1994, Padidam *et al.* 1995, Czosnek and Laterrot 1997). Until the end of the eighties, it was considered that the genome of the whitefly-transmitted geminiviruses was composed of two circular single-stranded DNA genomic molecules of about 2.8 kb each (denominated DNA A and DNA B) (Lazarowitz 1992). Today, both the ToLCVs and the TYLCVs include members that have one (monopartite) (Navot *et al.* 1991, Kheyr-Pour *et al.* 1991, Dry *et al.* 1993, Noris *et al.* 1994, Chatchawankanphanich *et al.* 1995, Crespi *et al.* 1995) and two (bipartite) genomic components (Rochester *et al.* 1994, Padidam *et al.* 1995b). The monopartite TYLCV/ToLCV genome strand (or DNA-A of bipartite viruses) encodes two genes (V1 and V2), including the capsid protein (V1) (Kallender *et al.* 1988). The virus genome complementary DNA encodes four genes (C1 to C4), including the replication-associate protein (C1 or Rep) necessary for virus replication (Elmer *et al.* 1988, Laufs *et al.* 1995). The DNA-B genome encodes two genes, BR1 and BL1, involved in virus translocation (Ingham *et al.* 1995, Noueiry *et al.* 1994). A 150 to 300 nucleotide-long intergenic region (IR) (almost identical in DNA-A and B) contains a conserved stem-loop structure of 30 nucleotides bearing the origin of replication (Heyraud-Nitschke *et al.* 1995).

Many tomato diseases in Asia have been attributed to TYLCV or ToLCV (Green and Kalloo 1994). However, with the exception of a few isolates, the viruses causing these diseases have been poorly characterized, if at all. In this paper, we describe the status of the TYLCV/ToLCV diseases in Southeast and East Asia and summarize the molecular data available at the end of 1998 in relation with the other known geminiviruses affecting tomatoes.

MATERIALS AND METHODS

Comparison of sequences of TYLCV/ToLCV genes and proteins

The nucleotide sequences of the virus genomes and the amino acid sequences derived from the genes encoding the capsid (CP) and replication-associated (Rep) proteins were retrieved from the gene banks. The sequences of the geminivirus isolates infecting tomato used in this study are indicated in Table 2. Sequences were aligned using the DNAMAN software (Lynnon BioSoft, Quebec, Canada). Homology trees were set up with the distance matrix using the UPGMA method (Sneath and Sokal 1973). Phylogenetic trees were setup with the distance matrix using the Neighbor-Joining method (Saitou and Nei 1987). Bootstrap tests (5,000 replicates) were carried out to assess the confidence value of the phylogenetic trees. TYLCV/ToLCV isolates have been named according to Padidam *et al.* (1997). The sequence of the leafhopper-transmitted wheat dwarf geminivirus (WDV, MacDowell *et al.* 1985) was used as outgroup.

RESULTS AND DISCUSSION

Distribution of TYLCV/ToLCV diseases in Southeast and East Asia

Table 1 shows the spread of the TYLCV/ToLCV-related diseases as compiled from the literature. These diseases have been described in almost all countries of Southeast and East Asia, some as early as 1948 (Vasudeva and Samraj 1948), while others are recent. Everywhere, these diseases are associated with serious losses of yield, making TYLCV/ToLCV an important factor in local agricultural economies. Generally TYLCV/ToLCV has been identified by the appearance of the diseased plant associated with large whitefly populations. Geminiviral particles have been observed using the electron microscope only in a few cases (viruses from India, Taiwan, Thailand and Pakistan). In some cases, antibodies raised against heterologous geminiviruses have been used to identify or to confirm the tomato pathogen as a geminivirus (China, India, Indonesia, Malaysia, Pakistan, The Philippines, Taiwan and Thailand). Heterologous cloned geminiviral DNA have been used as probes to screen for the presence of infected tomato plants in hybridization assays (China, India, Nepal, Pakistan, Taiwan and Thailand). The genome of TYLCV/ToLCV isolates has been cloned and sequenced (entirely or partially) from China, India,

Table 1. Spread and characterization of TYLCV/ToLCV in Southeast and East Asia.

Country	First report[1]	Serology	Particles	Hybridization (H), PCR (P)	DNA Sequence
India	1948	+	+	H,P	complete A, A+B
The Philippines	1971	+	-	P	A partial
Bangladesh	1974	-	-	P	A partial
Thailand	1980	+	+	H	A+B complete
Pakistan	1982	+	+	H,P	-
Taiwan	1984	+	+	H,P	A complete
Indonesia	1985	+	-	-	-
Malaysia	1986	+	-	P	A partial
Cambodia	1989	-	-	-	-
China	1992	+	-	H	A complete
Nepal	1990	-	-	H,P	-
Sri Lanka	1995	-	-	P	A partial

[1]Based on symptoms and on the presence of large whitefly populations. See references in text.

and Thailand. Recently the polymerase chain reaction (PCR) has been applied to the diagnosis of TYLCV/ToLCV (Bangladesh, China, India, The Philippines, Sri Lanka, Taiwan and Thailand). Genomic DNA fragments of ToLCV from Bangladesh, Malaysia, Sri Lanka, Taiwan and The Philippines have been PCR-amplified using degenerate primers allowing amplification of whitefly-transmitted geminivirus DNA (Rojas *et al.* 1993), cloned and sequenced.

A whitefly-transmitted disease causing yellowing and curling of tomato leaves was reported in India as early as 1948 and termed tomato leaf curl virus disease (Vasudeva and Samraj 1948). This was the first report of the presence of ToLCV in Asia. The disease is serious in all tomato growing areas throughout India and yield losses may reach 100% (Datar 1984). Extensive investigations over the years have shown that these diseases were caused by a complex of whitely-transmitted geminiviruses (Muniyappa *et al.* 1991, Reddy *et al* 1981). Several ToLCV isolates from different regions have been cloned and sequenced: from New Delhi, Lucknow and Bangalore. ToLCV isolates from New Delhi have a bipartite genome (Padidam *et al.* 1995), while those from Bangalore seem to possess a single genomic component (Chatchawankanphanich *et al.* 1993, 1995; Hong and Harrison 1995).

In Bangladesh, ToLCV was first reported in 1974 (Talukdar 1974, Akanda 1991) and presently occurs in almost all the districts of the country. It is the most destructive disease of tomato in that country and geminivirus-related diseases may cause the loss of 100% of the crop. Many strains seem to exist but none has been identified and characterized yet. We have recently amplified by PCR a 1.4 kbp DNA fragment from ToLCV-infected tomato using the PALv1978 and PARc715 degenerate primer pairs that have been used to amplify and clone several whitefly-transmitted geminiviruses (Rojas *et al.* 1993). This fragment has been sequenced; it includes the 233 N terminal amino acids (a.a.) of the Rep protein, the IR and the 118 N-terminal a.a. of the coat protein of a DNA-A-like genomic component (Green

Table 2. Origin of TYLCV/ToLCV isolates used for sequence comparison.

	Country (region)	Abbreviation	GenBank accession[1]	Reference
TYLCV	China	TYLCV-CN	(C) D88773	Liu *et al.* 1997
	Cuba	TYLCV-CU	(C) U65089	Martinez Zubiaur *et al.* 96
	Dominican Republic	TYLCV-DO	(C) AF024715	Nakhla *et al.* 1994
	Egypt	TYLCV-EG	(C) L12219	Nakhla *et al.* 1993
	Israel, virulent	TYLCV-IL	(C) X15656	Navot *et al.* 1991
	Israel, mild	TYLCV-IL/Mild	(C) X76319	Antignus & Cohen, 1994
	Italy (Sardinia)	TYLCV-IT/Sar	(C) X61153	Kheyr-Pour *et al.* 1991
	Italy (Sicily)	TYLCV-IT/Sic	(C) Z28390	Crespi *et al.* 1995
	Senegal	TYLCV-SN	(C) D88800	Liu *et al.* 1996
	Saudi Arabia (North)	TYLCV-NSA	(P)	Hong and Harrison 1995
	Saudi Arabia (South)	TYLCV-SSA	(P)	Hong and Harrison 1995
	Spain	TYLCV-ES	(C) Z25751	Noris *et al.* 1994
	Thailand	TYLCV-TH	(C) X63016	Rochester *et al.* 1994
	Yemen	TYLCV-YE	(P) X79429	Bedford *et al.* 1994b
ToLCV	Bangladesh	ToLCV-BD1	(P)	Shih *et al.*, 1998
	Bangladesh	ToLCV-BD2	(P)	Shih *et al.*, 1998
	India (New Delhi) mild	ToLCV-Nde/Mld	(C) U15016	Padidam *et al.* 1995b
	India (New Delhi) severe	ToLCV-Nde/Svr	(C) U15015, U15017	Padidam *et al.* 1995b
	India (Lucknow)	ToLCV-Luc	(P) X78956, X89653	Srivastava *et al.* 1995
	India (Bangalore)	ToLCV-Ban1	(P) L12738, L11746, L12739,	Chatchawankanphanich *et al.* 1993
	India (Bangalore)	ToLCV-Ban2	(C) Z48182	Hong and Harrison 1995
	India (Bangalore)	ToLCV-Ban3	(C) U38239	Chatchawankanphanich *et al.* 1995
	India (Bangalore)	ToLCV-Ban4	(C)	Muniyappa *et al.*, unpublished
	Malaysia	ToLCV-MY	(C)	Shi *et al.*, 1998b
	Sri Lanka	ToLCV-SL	(P)	Green *et al.*, unpublished
	The Philippines	ToLCV-PH	(P)	Shih *et al.*, 1997
	Taiwan	ToLCV-TW	(C) U88692	Chiang *et al.* 1997
	Australia	ToLCV-AUS	(C) S53251	Dry *et al.* 1993
Others	Brazil	TGMV	(C) K02029	Hamilton *et al.* 1984
	USA (Florida)	ToMoV	(C) L14460	Abouzid *et al.* 1992
	Czechoslovakia	WDV	(C) X02869	MacDowell *et al.* 1985

[1]Viruses have been sequenced completely (C) or partially (P).

et al. unpublished).

In Pakistan tomato plants showing ToLCV-like symptoms were identified in the early 1980s. Now symptoms are present in all tomato-growing regions in Sind and Punjab. Extracts of diseased plants reacted with an antiserum against TYLCV (Solangi *et al.* 1983, Rana *et al.* 1992). An isolate of ToLCV was obtained in 1993 and maintained in graft-inoculated tomato (Harrison *et al.*,1997). No molecular data are available on this isolate. Universal DNA primers for whitefly-transmitted geminiviruses were used to amplify a full-length DNA A-like geminiviral genome from ToLCV-diseased tomato plants. Similarly, degenerate primers pBLv2040 and PCRc1, which amplify DNA B, were used to amplify a 650 bp fragment. The amplified DNA A and DNA B were used as probes in Southern blot hybridization to detect ToLCV in diseased plants from Punjab. Therefore a bipartite geminivirus is associated with tomato leaf curl disease in Pakistan (Mansoor *et al.* 1997).

In Thailand whitefly-transmitted diseases of tomato were known from the late 1970s and the causal agent was named tomato yellow leaf curl virus (TYLCV-TH) (Giatgong 1980, Thanapas *et al.* 1983). TYLCV has become a serious problem in the mid-1980s. The virus was isolated from infected tomato samples (Attathom *et al.* 1990) and its genome has been completely sequenced (Rochester

et al. 1994). Contrary to the TYLCV isolates from the Middle East and South-Western Europe, TYLCV-TH has a genome composed of two molecules, DNA A- and DNA-B. Agroinoculation of cloned viral genomes has indicated that DNA-A is sufficient to induce disease symptoms, while DNA-B enhances them (Rochester *et al.* 1990).

In Taiwan, ToLCV-related disease symptoms in tomato were first observed in 1981 (AVRDC 1984, Green *et al.* 1987). The disease that was sporadic then became suddenly critical in 1993 following a dramatic increase in the whitefly populations in southern and central Taiwan. Electron microscopic examination revealed geminate virus particles. DNA-A clones of the Taiwan tomato leaf curl virus (TLCV-TW) have been sequenced (Chiang *et al.* 1997). It is not known whether the cloned viral genome is infective and whether TLCV-TW has a DNA-B genomic component.

In the Philippines, a ToLCV-like disease has been described in the early 1980s (AVRDC 1985). We have recently amplified by PCR a 1.4 kbp TLCV DNA fragment from infected tomatoes using the PALv1978 and PARc715 primer pairs, and sequenced it (Shi *et al.*, 1997). It comprises the 272 N terminal a.a. of the Rep protein, the IR and the 82 N-terminal a.a. of the coat protein

In Nepal, tomato with leaf curl symptoms associated with the presence of whiteflies was first reported in the late 1980s in the western part of the country. ToLCV was identified in 1990 (Dahal *et al.* 1993, Ghimire and Pradhanang 1996). The disease started to be of economical importance after 1994 and has been increasing every year. The identity of ToLCV was confirmed by hybridization using DNA probes from ToLCV-Ban1-3 and TYLCV-TH (Green *et al.* unpublished).

In China the occurrence of yellow leaf curl disease in tomato was first discovered in GuangXi Province in 1992 where losses reached 20% (Y. Liu and P. Tien, personal communication). The genome of a TYLCV-CN DNA-A like component has been cloned and sequenced (Liu *et al.* 1997). It is not known whether TYLCV-CN has a monopartite or a bipartite genome.

Diseases with TYLCV/ToLCV-related symptoms have also been described on tomato from Cambodia (Rowell *et al.* 1989, Marom 1993), Indonesia (AVRDC 1985) and Malaysia (Abu Kassim and Abu Bakar 1986). These diseases have not been characterized and molecular data is lacking. Recently we have used the PALv1978 and PARc715 primer pairs to PCR-amplify 1.4 kbp viral DNA fragment from infected tomatoes from Malaysia (Shi *et al.* 1998b) and Sri Lanka (Green *et al.*, unpublished). The sequence of the ToLCV-SL and ToLCV-MY fragments comprise the N terminal moiety of the Rep protein, the IR and the N-terminal moiety of the coat protein.

In Japan a disease called tomato yellow dwarf, has been reported from tomato (Osaki and Inouye 1978). The disease now occurs mainly in southwestern Japan as a result of a surge of whitefly populations but it is not considered of economical importance. The disease agent has been referred to tobacco leaf curl geminivirus (Kobatake *et al.* 1981).

Although formally not part of Southeast Asia, Australia is sufficiently close to interrelate with Asia. A monopartite whitefly-transmitted geminivirus, denominated ToLCV-AU, causes severe crop damage to tomato in the northern part of Australia. An infectious clone has been sequenced (Dry *et al.* 1993).

Relationship between TYLCV/ToLCV isolates based on comparisons of the intergenic region (IR), capsid (CP) and replication-associated (Rep) proteins

Up to date the complete nucleotide sequences of eleven isolates of TYLCV and of eight isolates of ToLCV have been published or are available in databases. In addition, sequences of genes and gene fragments from many other isolates have been published in the literature and in databases. We have compared the nucleotide and protein sequences of TYLCV/ToLCV isolates from Eastern and Southeastern Asia among them and with isolates from other regions of the world. The comparison was based on three genetic elements of the virus: (1). The IR, the least conserved region of the geminiviruses (Rybicki 1994, Padidam *et al.* 1995), except for the 30 nucleotide stretch forming a stem-loop structure and containing the origin of replication (Laufs *et al.* 1995); (2). the CP, assembled to form the capsid and necessary for transmission by the whitefly vector (Azzam *et al.* 1994, Briddon *et al.* 1990); (3). the Rep, necessary for replication in the infected plant (Elmer *et al.* 1988, Laufs *et al.* 1995).

Figure 1 shows homology trees obtained by aligning nucleotide sequences of the IR and the amino acid sequences of the CP and Rep of TYLCV/ToLCV isolates from Asia and of tomato geminiviruses from other regions of the world. All the TYLCV/ToLCV isolates from East and Southeast Asia analyzed so far constitute different species, not strains from the same virus as are, for example, TYLCV from Israel and from Egypt (Nakhla *et al.*

1993). Even the four ToLCV isolates from Bangalore, India, cannot be considered as strains of the same virus, and are different from the ToLCVs from New Delhi and from Lucknow. For example, CP comparisons indicated that the Indian ToLCVs share between 84 to 94 % homology; the four isolates from Bangalore share 87 to 90% homology. The Indian ToLCVs share 69 to 77 % homology with the Thai TYLCV. Similarly TYLCV from China is different from TYLCV/ToLCV from neighboring Thailand and Taiwan, sharing 78 to 83 % homology with these viruses, about the same level of homology it shares with the Mediterranean TYLCV isolates. ToLCV from Australia is different from those of Southeast and East Asia. Comparisons of IR and Rep provide identical conclusions.

Figure 2 shows phylogenetic trees based on nucleotide sequences of the IR and amino acid sequences of the CP and Rep of TYLCV/ToLCV isolates from Asia and from other regions of the world. As observed before (Kheyr-Pour *et al.* 1991, Nakhla *et al.* 1993, Rybicki 1994, Hong and Harrison 1995, Padidam *et al.* 1995) the relationships based on the CP reflects the geographical origin of the various isolates. The tree presents three main branches representing isolates from the Eastern Mediterranean basin (Italy, Spain), the Middle East (Israel, Egypt, North Saudi Arabia) and the Far East. The latter divides into two subgroups: the first comprises isolates from Australia, China, Malaysia, Taiwan and Thailand; the second isolates from Bangladesh, India and Sri Lanka. ToLCV from the Philippines behaves as an outgroup because only the sequence of a small stretch of the CP is available. Tomato geminiviruses from the New World constitute a clear distinct group. The Caribbean TYLCV groups with the Middle Eastern isolates, indicating that they are recent imports. The geographical grouping of the whitefly-transmitted tomato geminiviruses according to similarities of their capsid protein may reflect the co-evolution of local virus-whitefly complexes (McGrath and Harrison 1995).

The tree based on the Rep protein reveals a geminivirus grouping similar to that obtained with the CP, although less stringent. The Rep protein of geminiviruses from the same geographical group shows a much lesser degree of homology than that exhibited by the CP of the same viruses. Nonetheless, it is clear that the viruses from Asia and Southeast Asia constitute a homogenous group, separated from those of Southeast Europe and the Middle East. Variability of the CP is constrained to assure virion

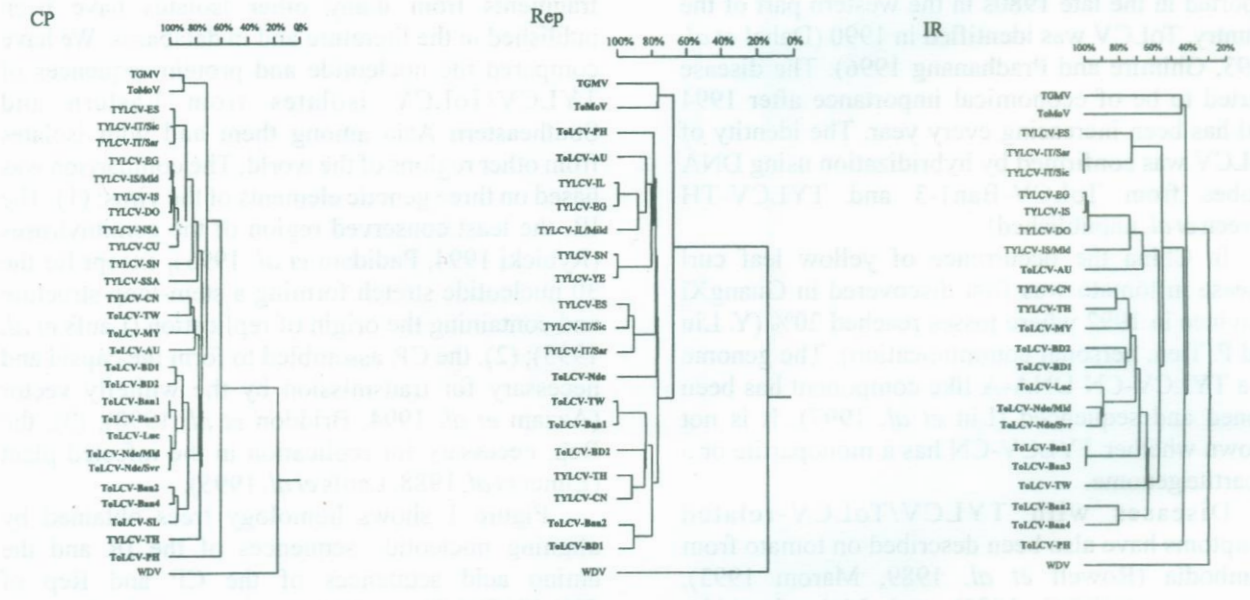

Figure 1. Dendograms showing homologies among the capsid proteins (CP), the replication-associated protein (Rep) and the intergenic region (IR) of TYLCV/ToLCV isolates from Southeast and East Asia (in bold capitals) with other whitefly-transmitted tomato geminiviruses. Scale represents percent homology. The sequences of the CP, Rep and IR of WDV are included as outgroups. Only the 118, 108, 82 and 114 N-terminal amino acids from ToLCV-BD, TYLCV-CU, ToLCV-PH and ToLCV-SL CP are available, respectively. Only 233, 229 and 132 N-terminal amino acids of ToLCV-BD, ToLCV-PH and TYLCV-YE Rep proteins are available, respectively. The sequences of the CP, Rep and IR of WDV are included as out group. See Table 2 for virus nomenclature.

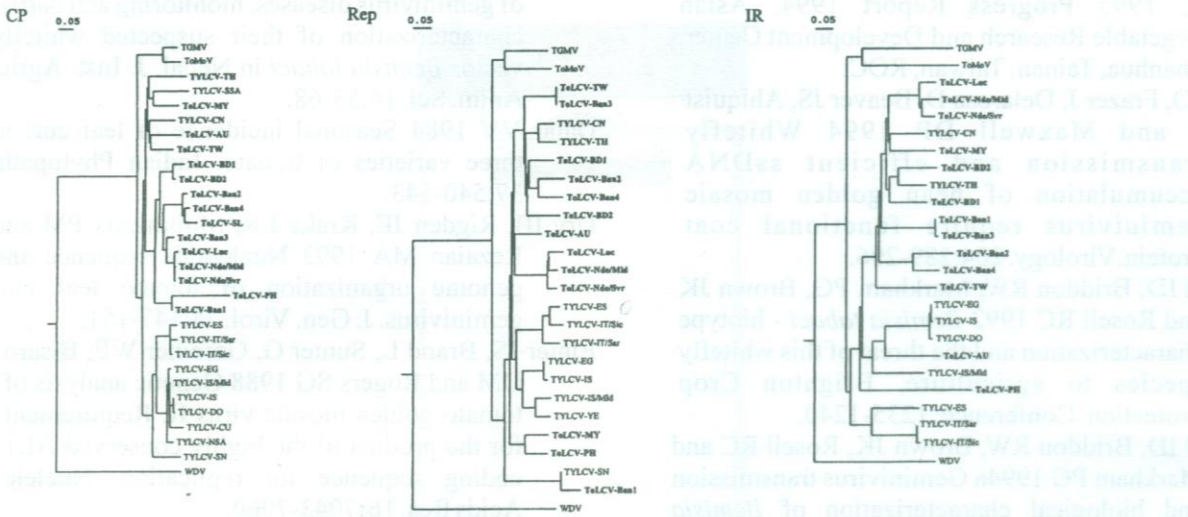

Figure 2. Phylogenetic trees showing relationships among the capsid proteins (CP), the replication-associated protein (Rep) and the intergenic region (IR) of TYLCV/ToLCV isolates from Southeast and East Asia (in bold capitals) with other whitefly-transmitted tomato geminiviruses. The sequences of the CP, Rep and IR of WDV are included as outgroup. The trees are rooted. The scale refers to branch lengths (percent of substitutions). See Table 2 for virus nomenclature.

assembly and recognition by the insect vector, hence most of the diversity of this protein is confined to its N-terminus (Padidam *et al.* 1995). In contrast the Rep protein seems to allow a higher degree of change, even though the domains ensuring binding to the virus origin of replication and replication are conserved (Jupin *et al.* 1995).

The tree based on the IR present the same general patterns as those obtained with the CP and Rep, although clustering is less pronounced probably because of a greater diversity of the IR, except for the conserved stem-loop sequence, some promoter elements and Rep binding sites (Fontes *et al.* 1994).

The great variability of the TYLCV/ToLCV complex worldwide should be considered when breeding programs for virus resistance are established. A tomato line tolerant/resistant to a particular TYLCV/ToLCV isolate may not be as effective against another distantly related virus isolate.

ACKNOWLEDGMENTS

The authors thank Ms Zehavit Regev for help in sequence analysis. Supported in part by a grant from the Israel Ministry of Science. M.Z. is the recipient of the Postdoctoral Fellowhip Award No. FI-0182-94 from the United States-Israel Binational Agricultural Research and Development Fund (BARD).

REFERENCES

Abouzid AM, Polston JF and Hiebert E 1992 The nucleotide sequence of tomato mottle virus, a new geminivirus isolated from tomatoes in Florida. J. Gen. Virol. 73:3225-3229.

Abu Kassim B and Abu Bakar 1986 Virus diseases of horticultural crops in Malaysia. In: Plant Virus Diseases of Horticultural Crops in Tropics and Subtropics. FFTC Book Series No 33. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taipei, Taiwan, ROC, p.3-6.

Akanda AM 1991 Virus and mycoplasma diseases of crops in Banglasdesh. Ph.D. Thesis, Kyushu University, Japan. 190 pp.

Anonymous 1993 TYLCV conquers new areas. Tomato Leaf Curl Newsl. 4:1.

Antignus T and Cohen S 1994 Complete nucleotide sequence of an infectious clone of a mild isolate of tomato yellow leaf curl virus (TYLCV). Phytopathology 84:707-712.

Attathom S, Chiemsombat P, Sutabutra T and Pongpanitanoud R 1990 Characterization of nucleic acid of tomato yellow leaf curl virus. Kasetsart J. (Nat. Sci. Suppl.) 24:1-5.

AVRDC 1984 Progress Report 1982. Asian Vegetable Research and Development Center. Shanhua, Tainan, Taiwan, ROC.

AVRDC 1985 Progress Report 1983. Asian Vegetable Research and Development Center. Shanhua, Tainan, Taiwan, ROC.

AVRDC 1995 Progress Report 1994. Asian Vegetable Research and Development Center. Shanhua, Tainan, Taiwan, ROC.

Azzam O, Frazer J, Delarosa D, Beaver JS, Ahlquist P and Maxwell DP 1994 Whitefly transmission and efficient ssDNA accumulation of bean golden mosaic geminivirus require functional coat protein. Virology. 204:289-296.

Bedford ID, Briddon RW, Markham PG, Brown JK and Rosell RC 1992 *Bemisia tabaci* - biotype characterization and the threat of this whitefly species to agriculture. Brighton Crop Protection Conference, 1235-1240.

Bedford ID, Briddon RW, Brown JK, Rosell RC and Markham PG 1994a Geminivirus transmission and biological characterization of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. Annals Applied Biol. 125:311-325.

Bedford ID, Briddon RW, Jones P, Alkaff N and Markham PG 1994b Differentiation of three whitefly-transmitted geminiviruses from the Republic of Yemen. Eur. J. Plant. Pathol. 100:243-257.

Briddon RW, Pinner MS, Stanley J and Markham PG 1990 Geminivirus coat protein gene replacement alters insect specificity. Virology 177:85-94.

Brown JK 1995 The Sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex? Annals Entomol. Soc. America 40: 511-534.

Chatchawankanphanich O, Chiang BT, Green SK, Singh SJ and Maxwell DP 1993 Nucleotide sequence of a geminivirus associated with tomato leaf curl from India. Plant Dis. 77:1168.

Chatchawankanphanich O, Chiang BT, Green SK, Singh SJ and Maxwell DP 1995 Tomato leaf curl virus strain Bangalore, India isolate. GenBank accession U38239.

Chiang BT, Green SK, Tsai WS, Nakhla MK and Maxwell DP 1997 Tomato leaf curl geminivirus (TwToLCV) from Taiwan, sequence of full-length clone, pTW1. GenBank accession U88692.

Crespi S, Noris E., Vaira AM and Accotto GP 1995 Molecular characterization of cloned DNA from a tomato yellow leaf curl virus isolate from Sicily. Phytopath. Medit. 34:93-99.

Czosnek H and Laterrot 1997 A worldwide survey of tomato yellow leaf curl viruses. Arch. Virol. 142: 1391-1406.

Dahal G, Thapa RB and Dangol DR 1993 Epidemics of geminivirus diseases, monitoring and partial characterization of their suspected whitefly vector *Bemisia tabaci* in Nepal. J. Inst. Agric. Anim. Sci. 14:55-68.

Datar VV 1984 Seasonal incidence of leaf curl in three varieties of tomato. Indian Phytopath. 37:540-543.

Dry IB, Rigden JE, Krake LR, Mullineaux PM and Rezaian MA 1993 Nucleotide sequence and genome organization of tomato leaf curl geminivirus. J. Gen. Virol. 74:147-151.

Elmer JS, Brand L, Sunter G, Gardiner WE, Bisaro DM and Rogers SG 1988 Genetic analysis of tomato golden mosaic virus II. Requirement for the product of the highly conserved AL1 coding sequence for replication. Nucleic Acids Res. 16:7043-7060.

Fontes EPB, Luckow VA and Hanley-Bowdoin (1994) A geminivirus replication protein is a sequence-specific DNA binding protein. The Plant Cell 4:597-608.

Giatgong P 1980 Host index of plant diseases in Thailand (2nd edition) Dept. of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand, 118p.

Gilbertson RL, Faria JC, Hanson SF, Morales FJ, Ahlquist P, Maxwell DP and Russell DR 1991 Cloning of the complete DNA genomes of four bean-infecting geminiviruses and determining their infectivity by electric discharge particle acceleration. Phytopathology. 81:980-985.

Ghimire SR and Pradhanang PM 1996 Plant diseases monitoring and disease pest diagnostis 1993/94. LARC Working Paper No. 96/46. Kaski, Nepal: Lumle Agric. Res. Centre. 13 pp.

Green SK and Kalloo G 1994 Leaf curl and yellowing viruses of pepper and tomato: An overview. Technical Bulletin No 21. Asian Vegetable Research and Development Center, Tainan, ROC.

Green SK, Sulyo Y and Lesemann DE 1987 Leaf curl virus on tomato in Taiwan Province. FAO Plant Prot. Bull. 35:62.

Grimsley N, Hohn B, Hohn T and Walden R 1986 "Agroinfection," an alternative route for viral infection of plants by using the Ti plasmid. Proc. Natl. Acad. Sci. USA 83:3282-3286.

Hajimorad MR, Kheyr-Pour A, Alavi V, Ahoonmanesh A, Bahar M, Rezaian MA and Gronenborn B 1996 Identification of whitefly transmitted tomato yellow leaf curl geminivirus from Iran and a survey of its

distribution with molecular probes. Plant Pathology 45:418-425.

Hamilton WDO, Stein VE, Coutts RHA and Buck KW 1984 Complete nucleotide sequence of the infectious cloned DNA components of tomato golden mosaic virus: Potential coding regions and regulatory sequences. EMBO J. 3: 2197-2205.

Harrison BD, Liu YL, Khalid S, Hameed S, Otim-Nape GW and Robinson DJ 1997 Detection and relationships of cotton leaf curl virus and allied whitefly-transmitted geminiviruses occuring in Pakistan. Ann. Appl. Biol. 130:61-75.

Heyraud-Nitschke F, Schumacher S, Laufs J, Schaefer S, Schell J and Gronenborn B 1995 Determination of the origin cleavage and joining domain of geminivirus Rep proteins. Nucleic Acids Res. 23:910-916.

Hong YG and Harrison BD 1995 Nucleotide sequences from tomato leaf curl viruses from different countries: evidence for three geographically separate branches in the evolution of the coat protein of whitefly-transmitted geminiviruses. J. Gen. Virol. 76:2043-2049.

Ingham DJ, Pascal E and Lazarowitz SG 1995 Both bipartite geminivirus movement proteins define viral host range, but only BL1 determines viral pathogenicity. Virology 207:191-204.

Jupin I, Hericourt F, Benz B and Gronenborn B 1995 DNA replication specificity of TYLCV geminivirus is mediated by the amino-terminal 116 amino acids of the Rep protein. FEBS Lett. 362:116-120.

Kallender H, Petty ITD, Stein VE, Panico M, Blench IP, Etienne AT, Morris HR, Coutts RHA and Buck KW 1988 Identification of the coat protein gene of tomato golden mosaic virus. J. Gen. Virol. 69:1351-1357.

Kheyr-Pour A, Bendahmane M, Matzeit M, Accotto GP, Crespi S and Gronenborn B 1991 Tomato yellow leaf curl virus from Sardinia is a whitefly-transmitted monopartite geminivirus. Nucl. Acids Res. 19:6763-6769.

Kobatake H, Osaki T and Inouye T 1981 Ecology and control of yellow dwarf disease of tomato caused by tobacco leaf curl virus. Proc. Kansai Plant Prot. Soc. 23:8-14.

Laufs J, Traut W, Heyraud F, Matzeit V, Rogers SG, Schell J and Gronenborn B 1995 *In vitro* cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Proc. Natl. Acad. Sci. USA 92:3879-3883.

Lazarowitz SG 1992 Geminiviruses: genome structure and gene function. Crit. Rev. Plant Sci. 11:32-349.

Liu YL, Cai JH, Li DL, Qin BX and Tien P 1997 Tomato yellow leaf curl virus in China. GenBank accesssion D88773

Liu Y, Li D, Tien P and Harrison BD 1996 DNA cloning of tomato yellow leaf curl geminivirus from Senegal. GenBank accession D8800.

MacDowell SW, Macdonald H, Hamilton WDO, Coutts RHA and Buck KW (1985) The nucleotide sequence of cloned wheat dwarf virus DNA. EMBO J. 4:2173-2180.

Mansoor S, Khan SH, Saeed M, Bashir A, Zafar Y, Malik KA and Markham PG (1997) Evidence for the association of a bipartite geminivirus with tomato leaf curl disease in Pakistan. Plant Dis. 81:958.

Marom U 1993 Tomato leaf curl virus in kandal Province, Cambodia, Tomato Leaf Curl Newsl. 3:2-3.

Martinez Zubiaur Y, Zabalgogeazcoa I, De Blas C, Sanchez F, Peralta EL, Romero J and Ponz F 1996 Geminiviruses associated with diseased tomatoes in Cuba. J. Phytopathology. 144:277-279.

McGrath PF and Harrison BD 1995 Transmission of tomato leaf curl geminivirus by *Bemisia tabaci*: effects of virus isolate and vector biotype. Ann. Appl. Biol. 126:307-316.

Muniyappa V, Swanson MM, Duncan GH and Harrison BD 1991 Particle purification, properties and epitope variability of Indian tomato leaf curl geminivirus. Ann. Appl. Biol. 118:595-604.

Nakhla MK, Mazyad HM and Maxwell DP 1993 Molecular characterization of four tomato yellow leaf curl virus isolates from Egypt and development of diagnostic methods. Phytopath. Medit. 32:163-173.

Nakhla MK and Maxwell DP 1994 Widespread occurrence of the eastern Mediterranean strain of tomato yellow leaf curl geminivirus in tomatoes in the Dominican Republic. Plant Dis. 78:926 (abst).

Navot N, Pichersky E, Zeidan M, Zamir D and Czosnek H 1991 Tomato yellow leaf curl virus: a whitefly-transmitted geminivirus with a single genomic component. Virology. 185:151-161.

Noris E, Hidalgo E, Accotto GP and Moriones E 1994 High similarity among the tomato yellow leaf

curl virus isolates from the western Mediterranean basin: The nucleotide sequence of an infectious clone from Spain. Arch. Virol. 135:165-170.

Noueiry AO, Lucas WJ and Gilbertson RL 1994 Two proteins of a plant DNA virus coordinate nuclear and plasmodesmatal transport. Cell 76:1-20.

Osaki T and Inouye T 1978 Resemblance in morphology and intranuclear appearance of viruses isolated from yellow dwarf diseased tomato and leaf curl diseased tobacco. Ann. Phytopath. Soc. Japan 44:167-178.

Padidam M, Beachy RN and Fauquet CM 1995a Classification and identification of geminiviruses using sequence comparisons. J. Gen. Virol. 76:249-263.

Padidam M, Beachy RN and Fauquet CM 1995b Tomato leaf curl from India has a bipartite genome and coat protein is not essential for infectivity. J. Gen. Virol. 76:25-35.

Padidam M, Maxwell DP and Fauquet CM 1997 A proposal for naming geminiviruses. Arch. Virol. 142:2553-2561.

Perring TM, Cooper AD, Rodriguez RJ, Farrar CA and Bellows TSJ 1993 Identification of a whitefly species by genomic and behavioral studies. Science 259: 74-77.

Polston JE and Anderson PK 1997 The mergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. Plant Dis. 81:1358-1369.

Rana NH, Rao SA, Qureshi MA AND Jilani MB 1992 Occurrence and identification of viruses on tomato plants in Sindh, Pakistan. Pakphyton 4:57-63.

Reddy KS, Yaraguntaia RC and Sastry KS 1981 Strains of leaf curl virus in India. I. Plant Dis. Prot. 88:400-404.

Rochester DE, Kositratana W and Beachy RN 1990 Systemic movement and symptom production following agroinoculation with a single DNA of tomato yellow leaf curl geminivirus (Thailand). Virology 178:520-526.

Rochester DE, DePaulo JJ, Fauquet CM, and Beachy RN 1994 Complete nucleotide sequence of the geminivirus tomato yellow leaf curl virus, Thailand isolate. J. Gen. Virol. 75:477-485.

Rojas MR, Gilbertson RL, Russell DR and Maxwell DP 1993 Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis. 77:340-347.

Rowell AB, Lay TE and Chum B 1989 Tomato production in Cambodia and screening for leaf curl disease tolerance. In: Tomato and Pepper Production in the Tropics. Green SK, Griggs TG and McLean BT (ed.) p.406-415. AVRDC, Shanhua, Taiwan, ROC.

Rybicki EP 1994 A phylogenetic and evolutionary justification for three genera of *Geminiviridae*. Arch. Virol. 139:49-78.

Saitou N and Nei M 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Ecol. 4:406-425.

Shi SL, Dolores LM, Nakhla MK, Maxwell DP and Green SK 1998a A new geminivirus associated with a leafcurl disease in the Philippines. Plant Protection Bull. (ROC) 39:394.

Shi SL, Roff MMN, Nakhla MK, Maxwell DP and Green SK 1998b A new geminivirus associated with a leafcurl disease of tomato in Malaysia. Plant Prot. Bull. (In press).

Shi SL, Tsai WS, Nakhla MK, Maxwell DP, Rashid H and Green SK 1998c Molecular comparison of two tomato yellow leaf curl virus isolates from Bangladesh. Plant Prot. Bull. (In press).

Sneath PH and Sokal RR 1973 Numerical Taxonomy. WH Freeman, San Francisco, USA.

Solangi GR, Moghal SM and Khanzada 1983 Identification of some viruses infecting solanaceous hosts in Sind. Pak. J. Bot. 15: 19-24.

Srivastava KM, Vipin Hallan, Raizada RK, Govind Chandra, Singh BP and Sane PV 1995 Molecular cloning of Indian tomato leaf curl virus genome following a simple method of concentrating the supercoiled replicative form of viral DNA. J. Virol. Methods 51:297-304.

Talukdar MJ 1974 Plant diseases in Bangladesh. Bangladesh J. Agric. Res. 1:61-84.

Thanapas VJ, Poolpol T, Sutabutra T and Attathom S 1983 Causal agent and some important proterties of tomato leaf curl disease. Kasetsart J. 17:76-73.

Vasudeva RS and Samraj J 1948 A leaf curl disease of tomato. Phytopathology 38:364-369.