MOLECULAR IDENTIFICATION AND THE COMPLETE NUCLEOTIDE SEQUENCE OF A *TOMATO YELLOW LEAF CURL VIRUS* ISOLATE FROM TURKEY

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SUMMARY

Tomato yellow leaf curl disease (TYLCD) threatens the production of tomatoes both in Turkey and other tomato-growing areas of the world. The disease is caused by several virus species belonging to the genus *Begomovirus*, family *Geminiviridae* that are transmitted by the whitefly *Bemisia tabaci*. There has been no previous information about which virus species/strains occur in tomatoes with TYLCD in Turkey. Therefore, polymerase chain reaction tests using degenerate primers were used to amplify partial sequences (579 bp) of the begomovirus coat protein gene (*V1*) from samples of diseased tomato plants collected from two major tomato-growing areas of Turkey (Mersin and Muğla). DNA sequence analyses of three isolates revealed high nucleotide sequence identities to isolates of *Tomato yellow leaf curl virus* (TYLCV, 97-98%) and tentatively identified them as belonging to that species. In addition, the complete nucleotide sequence was determined for one TYLCV isolate from Mersin (TYLCV-[TR:Mer6:04]). This isolate showed high nucleotide identity (98%) to isolates from the "prototype" strain of TYLCV, and only 92% identity to isolates of the TYLCV mild strain. A phylogenetic analysis confirmed that TYLCV-[TR:Mer6:04] belonged to the widespread "prototype" strain.

Key words: Begomovirus, Geminivirus, *Lycopersicon esculentum.*

INTRODUCTION

Tomato yellow leaf curl disease (TYLCD) was first recorded as a whitefly-transmitted disease of tomato (*Lycopersicon esculentum* L.) crops in Israel (Cohen and Harpaz, 1964; Cohen and Antignus, 1994) and it has since become a limiting factor in tomato production both in the Mediterranean region and in other parts of the world (Moriones and Navas-Castillo, 2000). The disease is caused by several virus species belonging to the genus *Begomovirus*, family *Geminiviridae* that are transmitted by the whitefly *Bemisia tabaci* (Genn.) in a persistent manner. At least three begomovirus species are associated with TYLCD in the countries around the Mediterranean Sea: *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), and *Tomato yellow leaf curl Malaga virus* (TYLCMalV) (Varma and Malathi, 2003); TYLCMalV is a recombinant between TYLCV and TYLCSV (Monci *et al*., 2002). Recently, TYLCV has also been found in the United States (Polston *et al*., 1999), the Caribbean (Polston and Anderson, 1997), Mexico (Ascencio-Ibáñez *et al*., 1999) and Japan (Kato *et al*., 1998).

TYLCV infects dicotyledonous plants and induces degeneration, crinkling, marked reduction in leaf size, yellowing of leaves and mosaic pattern in leaves, severe stunting, upward cupping, mottling, flower abscission, and drastic yield losses in tomato (Cohen and Harpaz, 1964; Antignus and Cohen, 1994). In addition to tomato, TYLCV has been found to cause disease in other crops such as common bean (*Phaseolus vulgaris* L.) (Navas-Castillo *et al*., 1999).

TYLCV and TYLCSV have a single genomic component of circular single-stranded (ss) DNA (Kheyr-Pour *et al*., 1991; Navot *et al*., 1991). TYLCV DNA (about 2.8 kb) has a molecular organization resembling the DNA-A of bipartite begomoviruses and contains six genes bidirectionally organized in two transcription units that are separated by an intergenic region (IR) of about 300 nucleotides (Navot *et al*., 1991; Gafni, 2003). Viral *cis*-acting elements involved in replication and transcription are located in the non-coding IR. The open reading frame (ORF) *V1* encodes the coat protein (CP) and is located on the virion strand DNA together with the partially overlapping ORF *V2*. The complementary strand contains four partially overlapping ORFs: *C1* (Rep), *C2* (TrAP), *C3* (REn) and *C4* (Navot *et al*., 1991; Gafni, 2003).

In Turkey, TYLCV has been reported as the most devastating virus disease in tomato both in greenhouses and in the field (Abak *et al*., 1991). Yield losses may reach 80-100% depending on the time of infection and

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tomato variety. Hybridisation tests have been used to verify infection by TYLCV in Turkey (Navot *et al*., 1989; Czosnek and Laterrot, 1997). However, there is no information about which species/strains occur in tomatoes with TYLCD in Turkey. In this study, the first sequence information is provided for Turkish isolates of TYLCV, including the complete genome sequence of a TYLCV isolate from Mersin. With this information it was possible to deduce the relationships between Turkish TYLCV isolates and those from other countries.

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers AJ812277, AJ867486 and AJ867487.

MATERIALS AND METHODS

Collection of the plant samples. Shoot samples were collected from tomato plants grown in greenhouses in the provinces of Mersin and Muğla, Turkey in April 8-10, 2004. In all, 15 tomato plant samples with yellowing and curling disease symptoms were collected: 6 from Muğla and 9 from Mersin, representing 2 different major greenhouse tomato-growing areas in Turkey. The fresh plant samples were brought to Genetics Centre, Swedish University of Agricultural Sciences, and were stored at -20°C until analysed.

Polymerase chain reaction. Direct polymerase chain reaction (PCR) detection of begomoviruses in leaf extracts was done in microcentrifuge tubes preincubated with plant leaf extracts (Wyatt and Brown, 1996; Ala-Poikela *et al*., 2005) by using the Expand High Fidelity PCR system (Roche, Mannheim, Germany). As negative controls, PCR was done in tubes pre-incubated with leaf extracts from healthy tomato plants and in untreated tubes. Tomato samples infected with Tomato leaf curl Sinaloa virus (ToLCSinV) and *Tomato severe leaf curl virus* (ToSLCV) were used as positive controls (Rojas *et al*., 2005). The degenerate primers AV494 and AC1048 (Wyatt and Brown, 1996) were used for amplification of the core region of the coat protein (CP) gene *V1* (576-579 bp, including primers) corresponding to nt 494-1,048 in Bean golden mosaic virus-Puerto Rico (BGYMV-[PR]; accession number M10070). The nucleotide sequences determined for the cloned PCR fragments were used to design virus-specific primers. Primers TYv1 (5'-CTTCTTGGTCCGTGATAGAA-3') and TYc29 (5'-AACATGACCTGATTAGTGTG-3') were used for amplification of the complete genome of TYLCV-[TR:Mer6:04] (Mer-6 isolate).

The samples were heated to 94°C for 2 min, and then subjected to 34 cycles of amplification using a PTC-100 Programmable Thermal Controller (MJ Research Inc., Watertown, MA, USA). Each cycle consisted of 30 s at 94°C, 1 min at 55°C (degenerate primers) or 47°C (species-specific primers), and 2 min at 72°C. The last cycle was followed by 10 min of elongation at 72°C. Five µl of each PCR product was analyzed by electrophoresis in 1% agarose gels.

Cloning of the DNA and sequencing. The PCR-amplified TYLCV DNA was purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), cloned into pGEM-T Easy Vector (Promega Corporation, Madison, WI, USA), and introduced into DH5-_ high library-efficiency competent *Escherichia coli* (Invitrogen Corporation, Carlsbad, CA, USA). DNA sequences were determined by the dideoxynucleotide method by using Thermo Sequenase dye terminator cycle sequencing kit, (version 2.0; Amersham Life Sciences, Inc., Cleveland, OH, USA) on an ABI Prism 377 DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The partial sequences of three TYLCV isolates (TYLCV-[TR:Mer6:04], [TR:Mer9:04] and -[TR:Mug2:04]) were determined on both strands using synthetic oligonucleotides. Three partial clones were sequenced for each isolate. The complete genome sequence was subsequently determined for TYLCV-[TR:Mer6:04].

Sequence analyses. Partial and complete nucleotide sequences of the Turkish TYLCV isolates were aligned with begomovirus sequences available in the GenBank database (Table 1) using the Clustal W program (Thompson *et al*., 1994). Phylogenetic analyses were carried out using Phylogenetic Analysis Using Parsimony (PAUP*) software, version 4.0 Beta (Swofford, 2002). The distance matrices for the neighbour-joining analyses were calculated using the Kimura two-parameter model. The results obtained from the neighbour-joining analyses were further assessed by maximum parsimony analyses. Essentially the same topologies were obtained with both methods. The robustness of the internal branches of the trees was estimated by bootstrap analysis using 1,000 replicates.

RESULTS

Identification of TYLCV by PCR and sequence analysis. Fifteen symptomatic tomato samples were tested for begomovirus infection by using PCR with degenerate begomovirus primers (Wyatt and Brown, 1996; Ala-Poikela *et al*., 2005). Seven tests yielded the expected band of 579 bp, indicating infection by a begomovirus. Four of these samples were from the province of Mersin and 3 samples were from the province of Muğla.

To identify the begomoviruses, PCR-amplified products were cloned and sequenced for three tomato sam-

Table 1. Virus acronyms and GenBank/EMBL/DDBJ accession numbers for TYLCV isolates used in sequence analyses.

Virus	Acronym	Acc. no.	References
Tomato yellow leaf curl virus-[Mersin 6]	TYLCV-[TR:Mer6:04]	AJ812277	This study
TYLCV-[Mersin 9]	TYLCV-[TR:Mer9:04]	AJ867486	This study
TYLCV-[Muğla 2]	TYLCV-[TR:Mug2:04]	AJ867487	This study
TYLCV (Israel)	TYLCV	X15656	Navot *et al.*, 1991
TYLCV-Mild (Israel)	TYLCV-Mld	X76319	Antignus and Cohen, 1994
TYLCV-Mild [Aichi]	TYLCV-Mld[Aic]	AB014347	Kato *et al.*, 1998
TYLCV-[Almeira]	TYLCV-[Alm]	AJ489258	Morilla *et al.*, 2005
TYLCV-[Cuba]	TYLCV-[CU]	AJ223505	Bejarano, Unpublished
TYLCV-[Dominican Republic]	TYLCV-[DO]	AF024715	Nakhla *et al.*, 1994
TYLCV-Mild [Portugal]	TYLCV-Mld[PT]	AF105975	Navas-Castillo *et al.*, 2000
TYLCV-Mild [Shizuoka]	TYLCV-Mld[Shi]	AB014346	Kato *et al.*, 1998
TYLCV-Mild [Spain 72/97]	TYLCV-Mld[ES72/97]	AF071228	Navas-Castillo *et al.*, 2000
TYLCV-[Puerto Rico]	TYLCV-[PR]	AY134494	Bird *et al.*, 2001
TYLCV-[Sudan]	TYLCV-[SD]	AY044138	Idris and Brown, 2005
TYLCV-Mild [Spain 01/99]	TYLCV-Mld[ES01/99]	AJ519441	Morilla *et al.*, 2005
Tomato yellow leaf curl Malaga virus	TYLCMalV	AF271234	Monci *et al.*, 2002
Tomato yellow leaf curl Sardinia virus	TYLCSV	X61153	Kheyr-Pour *et al.*, 1991
Tomato yellow leaf curl Thailand virus	TYLCTHV	X63015	Rochester *et al.*, 1994

ples. Sequence comparisons showed that the PCR fragment of a tomato sample from Mersin showed 98% nucleotide sequence identity to a TYLCV isolate from Egypt (accession number AY594174) and 97% identity to many TYLCV isolates of different geographic origin. The sequence identities to other begomovirus species were lower: e.g. 80% to TYLCSV and TYLCMalV. This isolate was therefore named TYLCV-[TR:Mer6:04] and is the first sequence identification of TYLCV in Turkey. Sequence analyses of the two other cloned Turkish isolates from Mersin (TYLCV-[TR:Mer9:04]) and Muğla (TYLCV-[TR:Mug2:04]) showed that their nucleotide sequences were 97-98% identical to each other and to TYLCV-[TR:Mer6:04]. These isolates could therefore also be tentatively identified as TYLCV.

A phylogenetic analysis was carried out, including the partial nucleotide sequences (CP core region) of the three Turkish virus isolates and representative sequences of TYLCV isolates and other tomato-infecting begomoviruses from the Old World (Table 1). The TYLCV isolates formed a well-supported group (bootstrap value 100%) and included the Turkish TYLCV isolates (not shown). In general, the CP core region did not give a clear grouping within the TYLCV clade. Still, the analysis showed a close relationship between TYLCV-[TR:Mer6:04] and TYLCV-[TR:Mer9:04] (bootstrap value 98%), while TYLCV-[TR:Mug2:04] grouped separately from the two other Turkish TYLCV isolates.

Sequence analysis of a complete sequence for a Turkish TYLCV isolate. Recombination events have been shown to be frequent in the genomes of tomato-infecting begomoviruses from both the Old World and the New World (Padidam *et al.*, 1999; Navas-Castillo *et al.*, 2000; Monci *et al.*, 2002; Bananej *et al.*, 2004; Fauquet *et al.*, 2005; Idris and Brown, 2005; Rojas *et al.*, 2005). Also, within the TYLCV species there are several different genotypes of recombinant origin. Therefore, it is necessary to sequence the complete genome to obtain certain virus identification. The complete genome sequence of TYLCV-[TR:Mer6:04] was deduced by using primers designed against the previously determined partial sequence. It was found to be 2,781 nucleotides in length and contained the six ORFs found in monopartite begomoviruses: *V1*, *V2*, *C1*, *C2*, *C3* and *C4* as well as an IR (Varma and Malathi, 2003).

The nucleotide and deduced amino acid sequences for the predicted ORFs and the nucleotide sequence for IR of TYLCV-[TR:Mer6:04] were compared with those available in GenBank (Table 1). The complete genome sequence of TYLCV-[TR:Mer6:04] showed the highest identity with isolates of the "prototype" strain of TYLCV (Fauquet *et al.*, 2005): TYLCV-[Alm] at 98.4%, TYLCV-[DO] at 98.3%, TYLCV-[CU] at 98.2%, TYLCV at 98.2% and TYLCV-[PR] at 97.9%. The identities to isolates of the TYLCV mild strain (TYLCV-Mld, TYLCV-Mld[ES01/99], TYLCV-Mld[Aic], TYLCV-Mld[PT], TYLCV-Mld[Shi] and TYLCV-Mld[ES72/

97]) were lower (~92%), and lower still to TYLCV-[SD] and isolates of other begomovirus species (<90%). The different ORFs (*V1*, *V2*, *C1*, *C2*, *C3* and *C4*) of TYLCV-[TR:Mer6:04] all had high nucleotide and amino acid identities with the isolates of the TYLCV "prototype" strain (97-99% at nucleotide sequence level). Also the IR of TYLCV-[TR:Mer6:04] had a high identity to the TYLCV "prototype" strain (96-98%). There were also high identities (97-99%) for *V1*, *V2*, *C2* and *C3* of TYLCV-[TR:Mer6:04] and the mild strain of TYLCV. However, the identities to *C1*, *C4* and IR of the mild strain were lower at 87%, 78% and 77-81%, respectively.

A phylogenetic analysis was done for the complete genome sequences of virus isolates causing TYLCD (Fig. 1). All TYLCV isolates in the analysis formed one clade, within which the TYLCV mild strain and TYLCV "prototype" strain made up two separate well-supported clades (bootstrap values 100%). The TYLCV-[SD] isolate had an intermediate position between these two strains. The analysis confirmed that TYLCV-[TR:Mer6:04] belongs to the TYLCV "prototype" strain together with TYLCV, TYLCV-[CU], TYLCV-[DO], TYLCV-[PR] and TYLCV-[Alm].

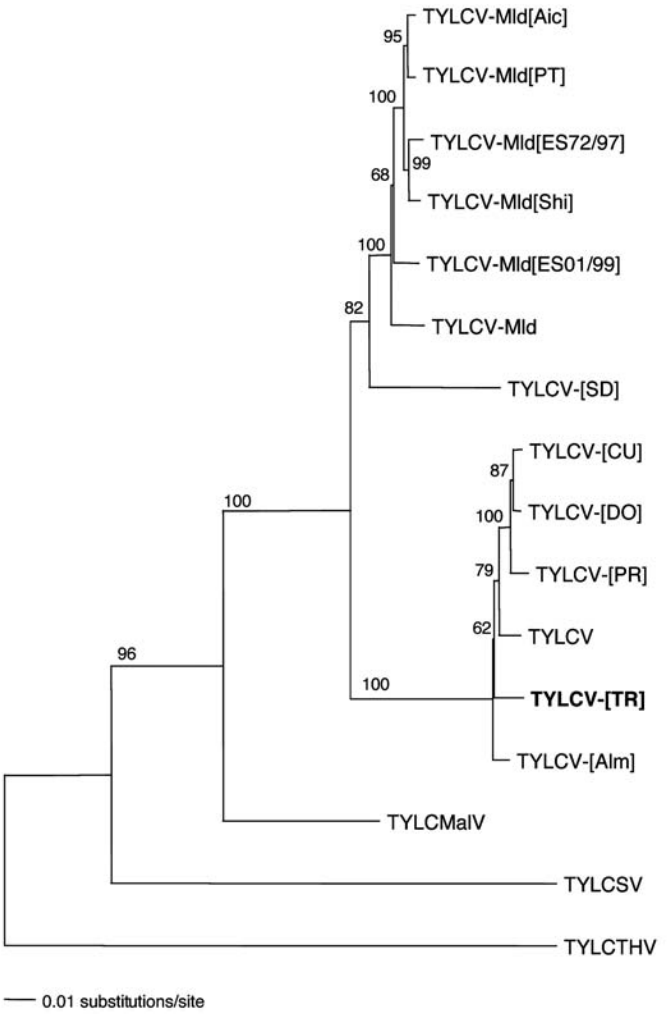

Fig. 1. Neighbour-joining analysis showing predicted relationships between TYLCD-causing begomoviruses based on complete genomic nucleotide sequence. The Turkish isolate determined in this study (TYLCV-[TR:Mer6:04]) is indicated in bold. Horizontal lines are in proportion to the number of nucleotide differences between branch nodes. Numbers represent the percentages of bootstrap replicates that support each node (1,000 replicates). Only bootstrap values higher than 50% are shown. For abbreviations of virus names and accession numbers, see Table 1.

DISCUSSION

TYLCD threatens the production of tomatoes both in Turkey and other tomato-growing areas of the world (Abak *et al.*, 1991; Cohen and Antignus, 1994; Moriones and Navas-Castillo, 2000). It is one of the main reasons for yield losses of tomato, especially in greenhouses. The recent emergence of begomoviruses in tropical and subtropical regions has been associated with the spread of the B biotype of *B. tabaci* (Polston and Anderson, 1997; Moriones and Navas-Castillo, 2000). Hosted by cotton, tobacco, and eggplant crops as well as wild plants in Turkey, *B. tabaci*, the only vector of TYLCV, is available throughout the vegetative period and transmits the virus easily. The B biotype of *B. tabaci* has recently been detected in vegetable fields in the region of Mersin, and its prevalence has increased year by year (Ulusoy and Bayhan, 2003). Insecticide control of *B. tabaci* is complicated by the development of resistance, which has occurred also in Turkey (Dittrich *et al.*, 1990).

Therefore, the management of TYLCD is dependent on crop management practices and the development of TYLCV-resistant tomato cultivars (Moriones and Navas-Castillo, 2000).

Identification of the viruses involved is fundamental for controlling TYLCD. PCR tests with degenerate primers revealed the presence of begomoviruses in seven out of 15 symptomatic tomato samples from the provinces of Mersin and Muğla in Turkey. The reason why not all of the samples tested positive for begomoviruses may be infections by RNA viruses or by other begomoviruses. For example, sequence comparisons suggested that the degenerate primers (Wyatt and Brown, 1996) used by us were not likely to amplify DNA of TYLCSV (not shown).

The results of this study confirmed the presence of TYLCV in two regions of Turkey and this is the first sequence identification of TYLCV in this country. The completely sequenced isolate (TYLCV-[TR:Mer6:04]) showed a close relationships with other virus isolates from the TYLCV "prototype" strain. The high sequence identity to TYLCV throughout the genome shows that TYLCV-[TR:Mer6:04] is not of recombinant origin. This strain was first identified in Israel (Navot *et al.*, 1991) and has also been found more recently in Spain (Morilla *et al.*, 2005), Japan (Ueda *et al.*, 2004),

Réunion (Delatte *et al*., 2005), the United States (Polston *et al*., 1999), Mexico (Ascencio-Ibáñez *et al*., 1999) and the Caribbean (Polston and Anderson, 1997). The other isolate from Mersin (TYLCV-[TR:Mer9:04]) is likely to belong to this strain because of its close relationship with TYLCV-[TR:Mer6:04].

However, for TYLCV-[TR:Mug2:04], it was not possible to assign the isolate to a specific strain of TYLCV and more sequence information is required. Detailed surveys in Spain have demonstrated the replacement of TYLCSV by TYLCV (Sánchez-Campos *et al*., 1999) and virus evolution by recombination (Monci *et al*., 2002). For the future, it will be important to sequence more isolates of TYLCD-causing viruses of Turkey to monitor the viral genotypes, and to be able to follow possible changes in the virus population structure.

ACKNOWLEDGEMENT

This study was partly supported by TUBITAK.

REFERENCES

Abak K., Yılmaz M.A., Kesici S., 1991. Problème de TYLCV en Turquie et moyens utilisés pour diminuer son effet. *Proceeding of Seminar on Resistance to TYLCV, Avignon* 1991, pp. 28-30.

Ala-Poikela M., Svensson E., Rojas A., Horko T., Paulin L., Valkonen J.P.T., Kvarnheden A., 2005. Genetic diversity and mixed infections of begomoviruses infecting tomato, pepper and cucurbit crops in Nicaragua. *Plant Pathology* **54**: 448-459.

Antignus Y., 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.

Ascencio-Ibáñez J.T., Diaz-Plaza R., Méndez-Lozano J., Monsalve-Fonnegra Z.I., Argüello-Astorga G.R., Rivera-Bustamante R.F., 1999. First report of tomato yellow leaf curl geminivirus in Yucatán, México. *Plant Disease* **83**: 1178.

Bananej K., Kheyr-Pour A., Hosseini Salekdeh G., Ahoonmanesh A., 2004. Complete nucleotide sequence of Iranian tomato yellow leaf curl virus isolate: further evidence for natural recombination amongst begomoviruses. *Archives of Virology* **149**: 1435-1443.

Bird J., Idris A.M., Rogan D., Brown J.K., 2001. Introduction of the exotic *Tomato yellow leaf curl virus*-Israel in tomato to Puerto Rico. *Plant Disease* **85:** 1028.

Cohen S., Antignus Y., 1994. Tomato yellow leaf curl virus, a whitefly-borne geminivirus of tomatoes. *Advances in Disease Vector Research* **10**: 259-288.

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

Czosnek H., Laterrot H., 1997. A worldwide survey of tomato yellow leaf curl viruses. *Archives of Virology* **142**: 1391-1406.

Delatte H., Holota H., Naze F., Peterschmitt M., Reynaud B., Lett J.M., 2005. The presence of both recombinant and nonrecombinant strains of *Tomato yellow leaf curl virus* on tomato in Réunion Island. *Plant Pathology* **54**: 262.

Dittrich V., Ernst G.H., Ruesch O., Uk S., 1990. Resistance mechanisms in sweetpotato whitefly (Homoptera: Aleyrodidae) populations from Sudan, Turkey, Guatemala and Nicaragua. *Journal of Economic Entomology* **83**: 1665-1670.

Fauquet C.M., Sawyer S., Idris A.M., Brown J.K., 2005. Sequence analysis and classification of apparent recombinant begomoviruses infecting tomato in the Nile and Mediterranean Basins. *Phytopathology* **95**: 549-555.

Gafni Y., 2003. *Tomato yellow leaf curl virus*, the intracellular dynamics of a plant DNA virus. *Molecular Plant Pathology* **4**: 9-15.

Idris A.M., Brown J.K., 2005. Evidence for interspecific-recombination for three monopartite begomoviral genomes associated with the tomato leaf curl disease from central Sudan. *Archives of Virology* **150**: 1003-1012.

Kato K., Onuki M., Fuji S., Hanada K., 1998. The first occurrence of Tomato yellow leaf curl virus in tomato (*Lycopersicon esculentum* Mill.) in Japan. *Annals of the Phytopathological Society of Japan* **64**: 552-559.

Kheyr-Pour A., Bendahmane M., Matzeit V., Accotto G.P., Crespi S., Gronenborn B., 1991. Tomato yellow leaf curl virus from Sardinia is a whitefly-transmitted monopartite geminivirus. *Nucleic Acids Research* **19**: 6763-6769.

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

Morilla G., Janssen D., García-Andrés S., Moriones E., Cuadrado I.M., Bejarano E.R., 2005. Pepper (*Capsicum annuum*) is a dead-end host for *Tomato yellow leaf curl virus*. *Phytopathology* **95**: 1089-1097.

Moriones E., Navas-Castillo J., 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Research* **71**: 123-134.

Nakhla M.K., Maxwell D.P., Martinez R.T., Carvaeho M.G., Gilbertson R.L., 1994. Widespread occurrence of the Eastern Mediterranean strain of Tomato yellow leaf curl geminivirus in tomatoes in the Dominican Republic. *Plant Disease* **78**: 926.

Navas-Castillo J., Sánchez-Campos S., Díaz J.A., Sáez-Alonso E., Moriones E., 1999. Tomato yellow leaf curl virus-Is causes a novel disease of common bean and severe epidemics in tomato in Spain. *Plant Disease* **83**: 29-32.

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

Navot N., Ber R., Czosnek H., 1989. Rapid detection of tomato yellow leaf curl virus in squashes of plants and insect vectors. *Phytopathology* **79**: 562-568.

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

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

Polston J.E., Anderson P.K., 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. *Plant Disease* **81**: 1358-1369.

Polston J.E., McGovern R.J., Brown L.G., 1999. Introduction of tomato yellow leaf curl virus in Florida and implications for the spread of this and other geminiviruses of tomato. *Plant Disease* **83**: 984-988.

Rochester D.E., DePaulo J.J., Fauquet C.M., Beachy R.N., 1994. Complete nucleotide sequence of the geminivirus tomato yellow leaf curl virus, Thailand isolate. *Journal of General Virology* **75**: 477-485.

Rojas A., Kvarnheden A., Marcenaro D., Valkonen, J.P.T., 2005. Sequence characterization of Tomato leaf curl Sinaloa virus and *Tomato severe leaf curl virus*: Phylogeny of New World begomoviruses and detection of recombination. *Archives of Virology* **150**: 1281-1299.

Sánchez-Campos S, Navas-Castillo J., Camero R., Saria C., Díaz J.A., Moriones E., 1999. Displacement of Tomato yellow leaf curl virus (TYLCV)-Sr by TYLCV-Is in tomato epidemics in Spain. *Phytopathology* **89**: 1038-1043.

Swofford D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (and other methods). Version 4. Sinauer Associates, Sunderland, Mass, USA.

Thompson J.D., Higgins D.G., Gibson T.J., 1994. CLUSTAL W: Improving the sensitivity of the progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673-4680.

Ueda S., Kimura T., Onuki M., Hanada K., Iwanami T., 2004. Three distinct groups of isolates of *Tomato yellow leaf curl virus* in Japan and construction of an infectious clone. *Journal of General Plant Pathology* **70**: 232-238.

Ulusoy M.R., Bayhan E., 2003. A new whitefly species on vegetable fields in the east Mediterranean region of Turkey: silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae). *Turkiye Entomoloji Dergisi* **27**: 51-60.

Varma A., Malathi V.G., 2003. Emerging geminivirus problems: A serious threat to crop production. *Annals of Applied Biology* **142**: 145-164.

Wyatt S.D., Brown J.K., 1996. Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. *Phytopathology* **86**: 1288-1293.

Received 19 September 2005
Accepted 13 December 2005