SURVEY OF TOMATO YELLOW LEAF CURL DISEASE-ASSOCIATED VIRUSES IN THE EASTERN MEDITERRANEAN BASIN

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SUMMARY

Tomato production in the Mediterranean region and elsewhere is under constant threat of the whitefly-transmitted begomoviruses that cause Tomato yellow leaf curl disease (TYLCD). Sequencing has indicated that the generic 'TYLCV' includes a large number of viruses and strains. We studied the distribution of the tomato yellow leaf curl disease-associated viruses in Egypt, Israel, Jordan and Lebanon. Two simple and reliable multiplex PCR protocols (mPCR) were developed that allowed the detection of *Tomato yellow leaf curl virus* (TYLCV) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) species, in addition to *Tomato yellow leaf curl virus*-Mild (TYLCV-Mld) strain. PCR products were sequenced to confirm their identity. The full-length genomes were also sequenced of TYLCV from Egypt, Jordan and Lebanon, TYLCV-Mld strain from Jordan and Lebanon, and *Tomato yellow leaf curl Sardinia virus*-Sicily from Israel. This is the first time that TYLCSV-Sic has been detected in Israel and Jordan, and the first report of TYLCV-Mld strain from Egypt and Lebanon.

Key words: Tomato, TYLCV, geminivirus; TYLCD, survey, sequencing, multiplex PCR

INTRODUCTION

Tomato yellow leaf curl (TYLCD) is one of the most destructive viral diseases of tomato (*Lycopersicon esculentum*) worldwide. It causes up to 100% yield loss in many countries in the Mediterranean basin, tropical Africa, central and southeast Asia, the Far East, USA, and the Caribbean islands (Czosnek and Laterrot, 1997; Nakhla and Maxwell, 1998; Moriones and Navas-Castillo, 2000; Ueda *et al*., 2004; Rojas *et al*., 2007; Brown and Idris, 2008). Different virus species have been associated with TYLCD in many countries across the globe: *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Mali virus* (TYLCMLV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), *Tomato yellow leaf curl Malaga virus* (TYLCMalV), and *Tomato yellow leaf curl Axarquia virus* (TYLCAxV) (Abhary *et al*., 2007). The outburst of TYLCD probably occurred as a result of international trade of seedlings and flowers (Varma and Malathi, 2003). In several countries (e.g. Spain, Mexico), indigenous tomato-infecting begomoviruses co-exist or have been replaced by more deleterious exotic species. Hence, TYLCD-associated virus isolates belonging to two or more different species, and sometimes recombinants, have been found in the same country (Monci *et al*., 2002). Two viruses infecting tomato crops in the Middle East, *Tomato yellow leaf curl virus*-Israel (TYLCV-IL) and TYLCV-Mld, were cloned and sequenced in the 1990s. Recently, a third virus strain, *Tomato yellow leaf curl Sardinia virus*-Spain (TYLCSV-ES), has been identified in Jordan (Anfoka *et al*., 2005).

Breeding for TYLCD resistance started in the early 1970s by introgressing TYLCV resistance from wild tomato species into the domesticated tomato (Pilowski and Cohen, 1990; Lapidot and Friedmann, 2002). Resistance has also been obtained by expressing genes of TYLCV in transgenic tomato (Kunik *et al*., 1994; Yang *et al*., 2004). Recently, novel strategies based on gene silencing have produced TYLCD-resistant tomato (Abhary *et al*., 2006). To ensure the successful development of resistant tomato, it is necessary to accurately identify the virus species or strain against which the resistance is being developed, especially in the case of strategies based on viral sequences. Therefore, in this work we aimed at developing simple, rapid and reliable diagnostic tools to identify the viruses that cause TYLCD in the eastern Mediterranean basin. Using these tools, we then surveyed the major tomato-producing regions in Egypt, Israel, Jordan and Lebanon.

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MATERIALS AND METHODS

Sample collection. In Israel, a total of 259 leaf samples from tomato plants showing TYLCD symptoms (leaf curling, yellowing and stunting) were collected from 43 fields in various locations during the years 2002, 2003, and 2005. In addition, 320, 202 and 286 leaf samples from symptomatic plants were collected in 2005 and 2007 from Egypt, Jordan and Lebanon, respectively. Samples were either processed immediately or kept at -20ºC prior to analysis.

Nucleic acid extraction. Total nucleic acids were extracted from symptomatic and healthy tomato plants as previously described (Potter *et al.*, 2003). In brief, 50 mg leaf tissue were ground in 1 ml extraction buffer (50 mM EDTA, 100 mM Tris-HCl, 500 mM NaCl, 10 mM ß-mercaptoethanol) and incubated at 65°C for 10 min. After adding 1/5 volume of potassium acetate (5 M, pH 8.0), an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added. The mixture was kept on ice for 10 min and then clarified by centrifugation at 10,000 g for 20 min. An equal volume of isopropanol was added to the supernatant and the mixture was incubated for 10 min at -20°C. After a 10 min of centrifugation at 10,000 g, the pellet was washed with 70% ethanol and resuspended in sterile deionized water.

Detection of TYLCD-associated viruses using multiplex PCR. Two sets of virus-specific oligonucleotide primers were used to detect TYLCSV-ES, TYLCV-IL, TYLCV-Mld and *Tomato yellow leaf curl Sardinia virus*-Sicily (TYLCSV-Sic) in symptomatic tomato plants (Table 1). The first set of primers, TYAlmv2516, TYAlmc115, TYv2337, TYc138, and TYv2664, previously reported to detect specifically TYLCSV-ES, but not other strains of TYLCSV species, TYLCV-IL and TYLCV-Mld in Jordan (Anfoka *et al.*, 2005), were used in a one-step multiplex PCR (mPCR) to analyze symptomatic plant samples collected in 2005 and 2007 from Jordan, Lebanon, and Egypt and samples collected in 2002, 2003 and 2005 from Israel. The second set of primers, RVC427, VP2715 and Sa2267, designed to detect all strains of TYLCSV, including the Italian TYLCSV-IT, the Sicilian TYLCSV-Sic, and the Spanish (TYLCSV-ES) strains, was used to detect TYLCV and TYLCSV in symptomatic samples collected in 2002, 2003 and 2005 in Israel and samples collected in 2007 in Jordan, Lebanon and Egypt. The locations of these primers on the virus genome and the expected lengths of the PCR products are shown in Fig. 1. The sequences of the primers are given in Table 1. The parameters for the multiplex (set 1) PCR were optimized for 25-µl reactions and the final concentrations of the reaction components were: 25 µM of each deoxynucleotide triphosphates (dNTPs), 1x PCR buffer, 2.5 mM $MgCl_2$, 5 units *Taq* DNA polymerase, 0.2 µM of each complementary and viral-sense primer (0.4 µM of primer

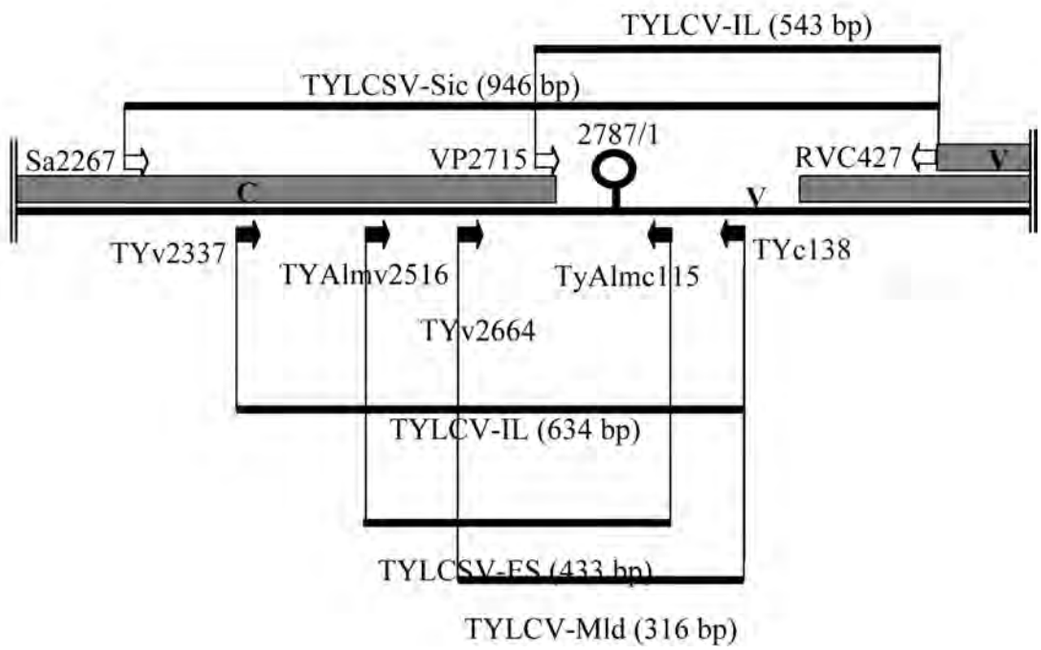

Fig. 1. Expected lengths of the PCR products obtained with the primers used in this study. Black and white arrows show the locations of primers in sets 1 and 2, respectively. Black bars represent the sizes of the amplified fragments specific for each virus. Open reading frames (ORFs) are shown as gray boxes; V denotes ORFs on the virion strand, and C denotes ORFs on the complementary strand. The hairpin loop represents the ori stem-loop sequence.

Table 1. Primers used to detect and amplify the full-length genomes of TYLCD-associated viruses in the eastern Mediterranean basin.

Primers		Sequence (5' to 3')	Nucleotide position	Derived from TYLCV species/isolate (accession number)
1[a]	TYAlmc115	ATATTGATGGTTTTTTCAAAACTTAGAAG	115-86	TYLCSV-ES[ES:Alm2:92] (L27708)
	TYv2337	ACGTAGGTCTTGACATCTGTTGAGCTC	2337-2364	TYLCV-IL[IL:Reo:86] (X15656)
	TYc138	AAGTGGGTCCCACATATTGCAAGAC	138-125	TYLCV-IL[IL:Reo:86] (X15656)
	TYv2664	ATTGACCAAGATTTTTACACTTATCCC	2664-2691	TYLCV-Mld[IL:93] (X76319)
2[b]	Sa2267	TGGAAAGTACCCCATTCAAGAACATC	2267-2292	TYLCSV-IT [IT:Sar:88] (X61153)
	RVC427	TGCCTTGGACA(A/G)TGGGG(A/G)CAGCAG	427-404	TYLCV-IL[IL:Reo:86] (X15656)
	VP2715	ATACTTGGACACCTAATGGCTATTTGG	2715-2741	TYLCV-IL[IL:Reo:86] (X15656)
3[c]	ILF	TGGATTGCAGAGGAAGATAGTGGGAATTCCCCCTTTAATT	1707-1746	TYLCV-IL[PR:01] (AY134494)
	ILR	TGAATGGGCTTCCCGTACTTTGTGTTGCTTTGCCAGTCCC	1747-1786	TYLCV-IL[PR:01] (AY134494
	MldF	TGGATTGCAGAGGAAGATAGTGGGAATTCCCCCTTTAATT	1707-1746	TYLCV-Mld[RE:02] (AJ865337)
	MldR	TGAATGGGCTTCCCGTACTTTGTGTTGCTTTGCCAGTCCC	1747-1786	TYLCV-Mld[RE:02] (AJ865337)
	SicF	TGGATTGCAtAGGAAGATAGTGGGAATgCCtCCTTTAATT	1743-1777	TYLCSV-IT[IT:Sar:88] (X61153)
	SicR	TGAATGGGCTTgCCaTACTTTGTGTTGCTTTGCCAaTCtC	1703-1742	TYLCSV-IT[IT:Sar:88] (X61153)

1[a] Anfoka *et al.*, 2005.
2[b] Used in this study to detect TYLCV and TYLCSV in samples collected in 2002, 2003 and 2005 from Israel and samples collected in 2007 from Jordan, Lebanon and Egypt.
3[C] Used to amplify the full-length genomes of TYLCV-IL, TYLCV-Mld and TYLCSV-Sic.

TYc138) and 3 µl (50 µg/ml) of DNA used as template. PCR cycle parameters were as follows: one cycle at 94°C for 2 min; 30 cycles at 94ºC for 1 min, 62ºC for 1.30 min, and 72ºC for 1 min, followed by one cycle at 94ºC for 1 min, 62ºC for 1 min, and 72ºC for 5 min.

The reaction components of the multiplex (set 2) PCR were as mentioned for multiplex (set 1) PCR except 0.1 µM of primer VP2715 was used. PCR cycling conditions were 95°C for 3 min, followed by 39 cycles at 95ºC for 30 sec, 52ºC for 1 min and 72ºC for 1 min; the reaction was terminated by 10 min incubation at 72ºC. The complete genomes of TYLCV-IL, TYLCV-Mld and TYLCSV-Sic were amplified using the primers shown in Table 1.

Primers were subsequently designed to sequence the full-length genomes of TYLCV-IL from Egypt (TYLCV-IL[EG:Ism], accession No. AY594174), Jordan (TYLCV-IL[JO:Tom:05], accession No. EF054893) and Lebanon (TYLCV-IL[LB:Tom:05], accession No. EF051116), of TYLCV-Mld from Jordan (TYLCV-Mld[JO:Tom:03], accession No. EF054894) and Lebanon (TYLCV-Mld[LB:LBA44:05], accession No. EF185318), and of TYLCSV-Sic from Israel (TYLCSV-Sic[IL:Hen:05], accession No. DQ845787).

The full-length viral genomes were cloned into the binary vector pCGN1547. To test the sensitivity of detection in multiplex PCRs, the complete genomes of isolates of TYLCV-IL, TYLCV-Mld and TYLCSV-Sic were amplified and then, equal amounts of amplified products were mixed together and the final concentration of the mixture was adjusted to 1 ng/µl. The virus mixture (PCR template) was subjected to serial dilutions and each dilution was mixed with 150 ng total DNA from a non-infected tomato plant. All PCRs were performed in a programmable thermal controller (model PTC-200, MJ Research Inc., USA). PCR products were visualized under UV light after gels were stained with ethidium bromide. DNA size markers (100 bp and 1 kbp DNA markers, Promega, USA) were used to estimate the sizes of the PCR products.

Cloning, sequencing and alignment analysis To confirm the identity of the amplified fragments, PCR products obtained with samples collected from different countries were cloned into pGEM-T Vector (Promega, USA) according to manufacturer's instructions, and sequenced. Alignment analysis was performed using the online BLAST service of the National Center for Biotechnology Information (URL:http://www.ncbi.nih.gov/BLAST/). Phylogenetic tree was constructed from the multiple alignments using the DNAMAN software (Lynnon, Canada) using the neighbor-joining method (Saitou and Nei, 1987) and the Jukes-Cantor distance-correction method (Jukes and Cantor, 1969).

RESULTS

Detection and identification of TYLCD-associated viruses by multiplex PCR. Isolates of TYLCV-IL, TYLCV-Mld, TYLCSV-Sic, and TYLCSV-ES, were detected using two sets of primers (Table 1). The specificity of the primers was tested in standard and multiplex PCRs using DNA templates obtained from clones of each virus and/or from tomato plants infected with virus clones (not shown). As shown in Fig. 2 (A, B, C, D), the expected sizes of TYLCV-Mld (316 bp), TYLCSV-ES (433 bp), and TYLCV-IL (634 bp) were amplified from

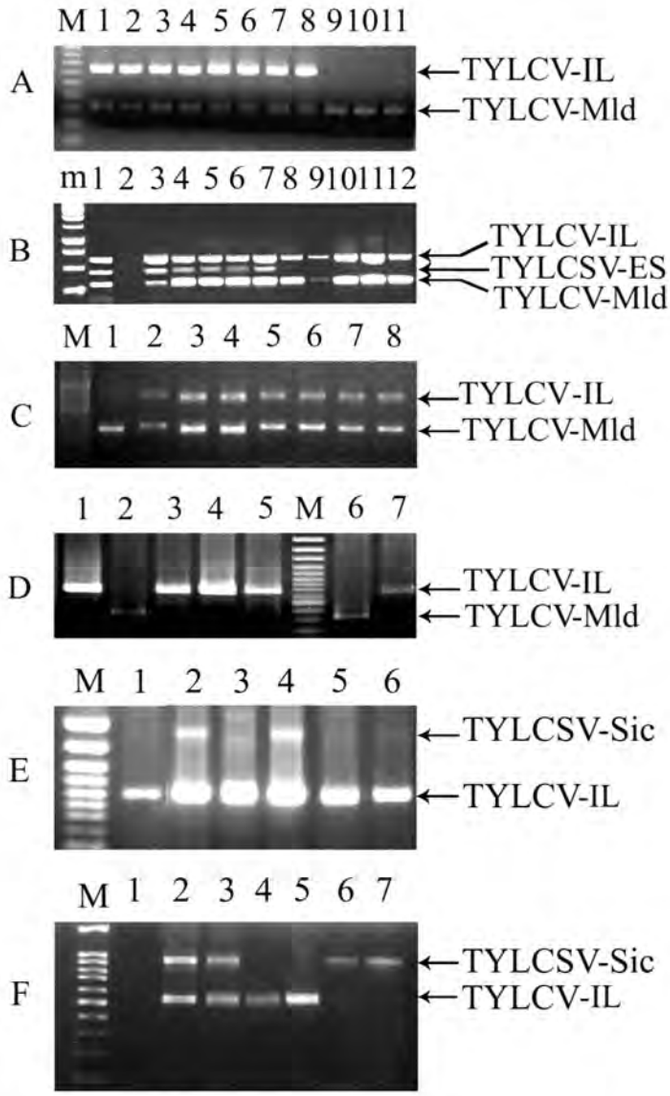

Fig. 2. Detection of TYLCD-associated viruses in symptomatic tomato plants collected from Lebanon (A), Jordan (B, F), Egypt (C) and Israel (D, E). The agarose gels show the PCR products obtained with primer set 1 (TYv2337, TYc138, TYv2664, TYAlmv2516, 440 and TYAlmc115) (A, B, C and D) and primer set 2 (Sa2267, VP2715, RVC427) (E, F). Lanes 1-12; samples collected from symptomatic tomato plants. Lane M, 100 bp DNA marker. Lane m, 1 Kbp DNA marker.

Table 2. Survey of TYLCD-associated viruses in Egypt.

Location (Year)	No. of fields	Infected/ tested	Single infection				Mixed infection				
			A	B	C	D	A+B	A+C	A+D	A+B+C	A+B+D
Fayoum (2005)	4	19/20	2	4	0	-	13	0	-	0	-
Fayoum (2007)	7	38/40	16	0	0	0	22	0	0	0	0
Giza (2005)	4	18/20	2	5	0	-	11	0	-	0	-
Giza (2007)	5	34/40	10	2	0	0	22	0	0	0	0
Ismailia (2005)	4	19/20	0	1[b]	0	-	18	0	-	0	-
Ismailia (2007)	5	35/40	5[a]	6	0	0	24	0	0	0	0
Kalubia (2005)	4	16/20	0	10	0	-	6	0	-	0	-
Kalubia (2007)	6	40/40	15	5	0	0	20	0	0	0	0
Sinai (2005)	2	20/20	1	1	0	-	18	0	-	0	-
Sinai (2007)	3	33/40	2	8	0	0	23	0	0	0	0
Suez (2005)	4	20/20	1	0	0	-	19	0	-	0	-
Total	48	292/320	54	42	0	-	196	0	-	0	-

A: TYLCV, B: TYLCV-Mld, C: TYLCSV-ES, D: TYLCSV-Sic.
[a] TYLCV-IL[EG-Ism] (AY594174); [b] TYLCV-Mld[EG:07] (DQ845786). -: Not tested.

tissues obtained from symptomatic tomato plants using primer set 1. Similarly, fragments of TYLCV-IL (543 bp) and TYLCSV-Sic (946 bp) were amplified using primer set 2 (Fig. 2E, F). No PCR product was detected when DNA extracted from healthy tissues was used as template (not shown).

The PCR amplicons of TYLCSV-ES and TYLCSV-Sic from Jordan and TYLCV-Mld from Egypt, were cloned and their sequences determined, compared with known TYLCV/TYLCSV species and deposited in the GenBank under accession No. EF198465 (TYLCSV-ES[JO:Anf:06]), EU307940 (TYLCSV-Sic[JO:Anf:06]) and DQ845786 (TYLCV-Mld[EG:07]). PCR results showed that the lowest template concentration that could be used to detect the three viruses was 54 x 10^{-7} ng/µl (not shown).

This was the first time that an isolate of TYLCSV-Sic strain was detected in Israel and Jordan, and the first report of TYLCV-Mld from Egypt and Lebanon. The phylogenetic relationship between the different TYLCV/TYLCSV species studied is shown in Fig. 3. As expected, each of the three viral species/strains, TYLCV-IL, TYLCV-Mld, and TYLCSV-Sic, grouped together, suggesting a common origin.

Distribution of members of the TYLCD-associated viruses in the eastern Mediterranean basin. Primers developed in this study were used to investigate the distribution of TYLCD-associated viruses in four eastern Mediterranean countries. Fig. 2 shows examples of PCR products obtained with symptomatic plants collected in Israel, Jordan, Egypt and Lebanon. The results are summarized in Tables 2-5.

TYLCV-IL and TYLCV-Mld were present in all surveyed countries, whereas TYLCSV-ES isolate was only found in Jordan. TYLCSV-Sic could be detected in Israel and Jordan. In Egypt, TYLCV-Mld was detected, for the first time, in all of the regions surveyed. Mixed infections of TYLCV-Mld and TYLCV-IL were prominent (196 samples) compared to single infections with TYLCV-IL (54 samples) or TYLCV-Mld (42 samples). TYLCSV-ES was not detected in any sample (Table 2). In Israel, single infections with TYLCV-IL were the rule (204 out of 231 samples), independent of the year of sampling. TYLCV-Mld was the only virus detected in samples collected in a single field in the Arava in 2005 (3 samples). The occurrence of mixed infections by TYLCV-IL and TYLCV-Mld was also low (4 samples), and again, these were all detected in the Arava in 2005. TYLCSV-Sic was detected in Israel in samples collected in 2002 as a mixed infection with TYLCV-IL and in samples collected in subsequent years (altogether 16 samples out of 231). In only two instances was TYLCSV-Sic found alone, in northern Negev in 2003 and in the central coastal plain of Israel in the same year. Before the present survey, TYLCSV species had never been detected in Israel. A full-length copy of the viral DNA was PCR-amplified, cloned and sequenced (TYLCSV-Sic[IL:Hen:05]). It showed 98% sequence homology with the TYLCSV-Sic[IT:Sic]; Z28390, and was assigned the GenBank accession No. DQ845787. TYLCV-IL, TYLCV-Mld and TYLCSV-Sic were not found together in the same plant (Table 3).

In Jordan, four members of TYLCD-associated

Table 3. Survey of TYLCD-associated viruses in Israel.

Location (Year)	No. of fields	Infected/ tested	Single infection				Mixed infection				
			A	B	C	D	A+B	A+C	A+D	A+B+C	A+B+D
Arava (2005)	12	70/89	63	3	0	0	4	0	0	0	0
Central Costal Plain (2002)	3	20/20	17	0	0	0	0	0	3	0	0
Central Costal Plain (2003)	7	41/45	34	0	0	1[a]	0	0	6	0	0
Central Costal Plain (2005)	3	19/20	19	0	0	0	0	0	0	0	0
North Jordan Valley (2002)	5	22/25	22	0	0	0	0	0	0	0	0
North Negev (2003)	8	39/40	32	0	0	3	0	0	4	0	0
North Negev (2005)	3	10/10	8	0	0	0	0	0	2	0	0
Southern Coastal Plain (2005)	2	10/10	9	0	0	0	0	0	1	0	0
Total	43	231/259	204	3	0	4	4	0	16	0	0

A: TYLCV, B: TYLCV-Mld, C: TYLCSV-ES, D: TYLCSV-Sic.
[a]TYLCSV-Sic[IL:Hen:05] (DQ845787); -: Not tested.

Table 4. Survey of TYLCD-associated viruses in Jordan.

Location (Year)	No. of fields	Infected/ tested	Single infection				Mixed infection				
			A	B	C	D	A+B	A+C	A+D	A+B+C	A+B+D
Central Jordan Valley (2005)	17	52/56	22	7	0	-	15	0	-	8	-
Southern Jordan Valley (2005)	9	63/66	18	3	2[c]	-	27	4	-	9	-
Central Jordan Valley (2007)	1	7/7	3[a]	0	0	0	2	0	1	0	1
Sroo (2007)	4	19/24	7	5	0	3	4	0	0	0	0
Madaba (2007)	2	8/11	3	0	0	0	5	0	0	0	0
Baqa' (2007)	3	14/22	5	0	0	1[d]	8	0	0	0	0
Homret Al-Sahen (2007)	1	16/16	2	3[b]	0	0	11	0	0	0	0
Total	37	179/202	60	18	2	4	72	4	1	17	1

A: TYLCV, B: TYLCV-Mld, C: TYLCSV-ES, D: TYLCSV-Sic.
[a] TYLCV-IL[JO:Tom:05] (EF054893); [b] TYLCV-Mld[JO:Tom:03] (EF054894); [c] TYLCSV-ES[JO:Anf:06] (EF198465)
[d] TYLCSV-Sic[JO:Anf:06] (EU307940); -: Not tested.

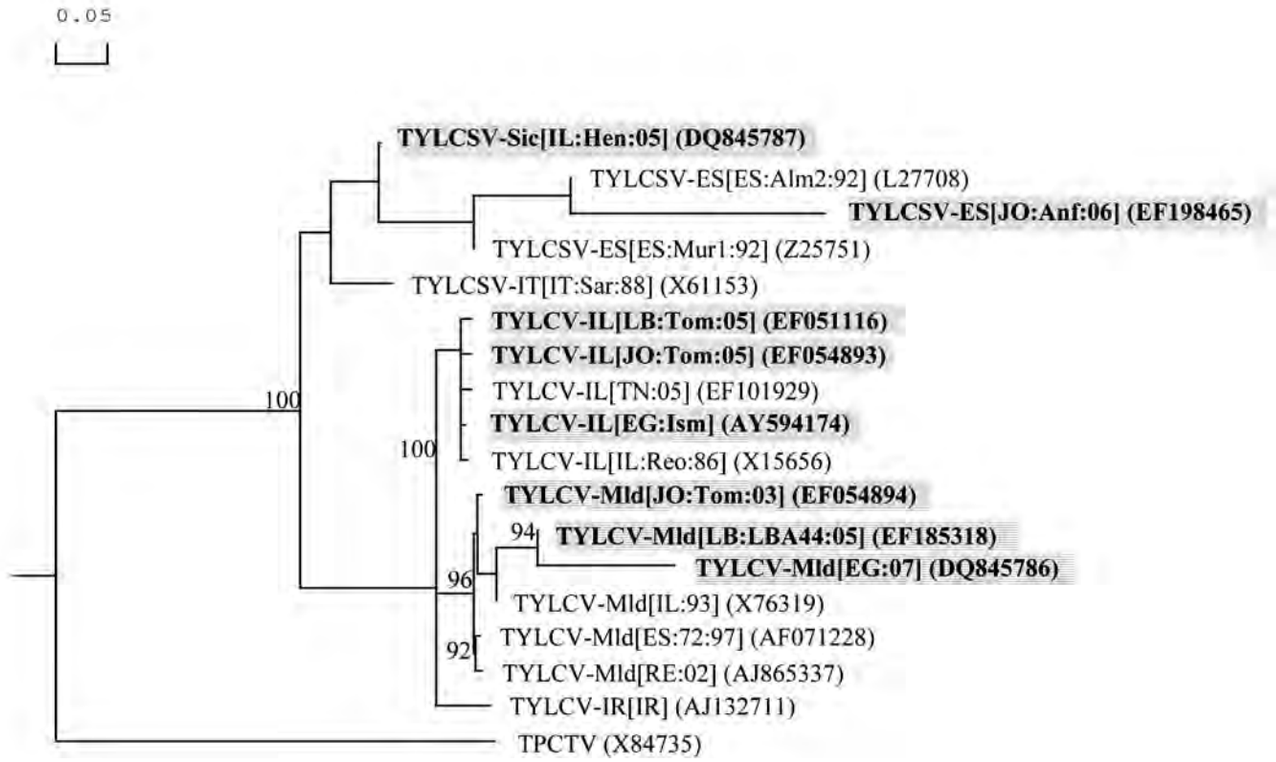

Fig. 3. Phylogenetic tree based on multiple alignments of different TYLCV/TYLCSV sequences. The GenBank accession numbers are indicated. All sequences are from complete genomes, except TYLCSV-ES[JO:Anf:06] and TYLCV-Mld[EG:07]. Only bootstrap values above 90 from 1000 replicates were shown. Isolates reported in this study are shaded. Bar represents the genetic distance between sequences. *Tomato pseudo-curly top virus* (TPCTV) was used as the outgroup.

viruses were detected in the surveys conducted in 2005 and 2007. TYLCV-IL and TYLCV-Mld were the two dominant viruses found in the infected plants, either as single infections (60 samples containing only TYLCV-IL and 18 samples containing only TYLCV-Mld) or as mixed infection (72 samples). Only 2 and 4 plants were infected solely with TYLCSV-ES, and TYLCSV-Sic, respectively. Four plants were infected with a mixture of TYLCV-IL and TYLCSV-ES, and 17 plants were infected with three viruses TYLCV-IL, TYLCV-Mld and TYLCSV-ES. One plant was mixed infected with TYLCV-IL, TYLCV-Mld and TYLCSV-ES (Table 4).

In Lebanon, only TYLCV-IL and TYLCV-Mld were detected in the four regions surveyed. Of a total 254 infected plants, 68 were infected solely with TYLCV-IL and 63 only with TYLCV-Mld. Mixed infections of these two viruses were detected in 123 plants (Table 5).

DISCUSSION

This study has shown that multiplex PCR with virus-specific primers can be used to detect four members of TYLCD-associated viruses in symptomatic tomato samples collected from infected fields in Egypt, Israel, Jordan and Lebanon. The different viruses cannot be distinguished by the symptoms they induce in tomato. Moreover, in the field, the symptoms were so severe (even with TYLCV-Mld) that it was not possible to distinguish between single and mixed infections based on symptoms. The viruses were identified by the size of the PCR amplification products and confirmed by sequencing.

The PCR results were the first step in the cloning and sequencing of TYLCV-IL from Egypt, Jordan and Lebanon, of TYLCV-Mld from Jordan, Lebanon and Egypt, and of TYLCSV-Sic from Israel and Jordan. TYLCSV-ES was first found in samples collected in 2002 in the central, but not southern Jordan Valley, as a mixed infection with TYLCV-IL (Anfoka *et al*., 2005). In these samples, TYLCSV-ES was detected with a low frequency compared to TYLCV-IL (27 vs. 73%). In samples collected in 2003, the incidence of TYLCSV-ES reached 99 to 100% of the samples tested in the central and southern Jordan Valley, while TYLCV-IL was detected at lower rates, as a mixed infection with TYLCSV-ES (Anfoka *et al*., 2005). However, in samples collected in 2005, TYLCSV-ES had almost disappeared. It was not found in the central Jordan Valley and only 6 of the 63 infected tomato samples contained this virus, 4 as a mixed infection with TYLCV-IL. Since none of samples collected in 2007 were infected with TYLCSV-ES, it seems that TYLCSV-ES was imported to Jordan

Table 5. Survey of TYLCD-associated viruses in Lebanon.

Location (Year)	No. of fields	Infected/ tested	Single infection				Mixed infection				
			A	B	C	D	A+B	A+C	A+D	A+B+C	A+B+D
Mountains (2005)	27	87/113	37	26	0	-	24	0	-	0	-
South coast (2005)	10	11/17	1[a]	3[b]	0	-	7	0	-	0	-
South coast (2007)	14	106/106	18	21	0	0	67	0	0	0	0
North coast (2007)	8	50/50	12	13	0	0	25	0	0	0	0
Total	59	254/286	68	63	0	0	123	0	0	0	0

A: TYLCV B: TYLCV-Mld; C: TYLCSV-ES, D: TYLCSV-Sic.
[a] TYLCV-IL[LB:Tom:05] (EF051116); [b] TYLCV-Mld[LB:LBA44:05] (EF185318). -: Not tested

(presumably on infected seedlings) but was unable to establish itself and is being displaced by TYLCV-IL.

Another two isolates of TYLCSV were discovered in Israel and Jordan. Isolates TYLCSV-Sic[IL:Hen:05] and TYLCSV-Sic[JO:Anf:06] were detected with a low frequency in Israel and Jordan. Of samples collected in Israel, 20 were proved to be infected with TYLCSV-Sic out of 259 tested. A lower infection rate with TYLCSV-Sic was found in samples collected from Jordan (6 out of 202 tested samples). These results suggest that TYLCSV-Sic[IL:Hen:05] and TYLCSV-Sic[JO:Anf:06] have been unable to establish themselves in Israel and Jordan. It is interesting to note that TYLCSV-ES and TYLCSV-Sic strains were not found in Lebanon and Egypt, despite the short distances between the four countries in question.

Strains TYLCV-IL and TYLCV-Mld occurred in all four countries surveyed. In Egypt, Jordan and Lebanon, the two viruses were predominantly found as mixed infections (respectively, 196 out of 320, 72 out of 202 and 123 out of 286 infected plants). Compared to Egypt, Jordan and Lebanon, TYLCV-Mld was less represented in Israel (7 out of 231 infected plants, 3 as a single infection, all found in the Arava in 2005).

TYLCV-Mld appears to have been the original virus present in tomato fields when first described in Israel in the 1960s and to have been maintained in the crops since then (Antignus and Cohen, 1994). In Israel, this virus has been replaced by the more virulent TYLCV-IL and is seldom found today in the field. The high incidence of TYLCV-Mld in Egypt, Jordan and Lebanon suggests that TYLCV-Mld was also the original virus present in these countries, while today it is found mainly as mixed infections with TYLCV-IL. Future surveys may indicate that in these countries, as in Israel, TYLCV-Mld is in the process of being replaced by TYLCV-IL. It is interesting to note that in Spain, recombinants between these two viruses which are present in mixed infections in a large number of tomato plants have not yet been found (Monci *et al*., 2002).

The history of TYLCV's discovery has always assumed that the Middle East was the origin of the worldwide spreading of this virus. TYLCV-IL outbreaks, which were sporadic in the 1960s (Cohen and Harpaz, 1964), have become a serious economic problem, from the early 1970s, when yield losses often reached 100%. By the end of the 1970s, all tomato-growing regions of the eastern Mediterranean basin were affected by the virus (Makkouk, 1978; Makkouk *et al*., 1979; Mazyad *et al*., 1979). In the late 1980s, TYLCV-IL particles were isolated (Czosnek *et al*., 1988) and the viral genome was cloned and sequenced (Navot *et al*., 1991). TYLCV-IL was thought to be the sole causal agent of the whitefly-transmitted disease of tomato. TYLCV-IL has spread worldwide to East Asia, Western Europe and the Americas (Moriones and Navas-Castillo, 2000). However, in the 1990s, TYLCSV and related virus strains were discovered in Sardinia and Sicily (Kheyr-Pour *et al*., 1991; Crespi *et al*., 1995) and have since spread to Spain, Morocco and Tunisia (Sánchez-Campos *et al*., 1999; Gharsallah Chouchane *et al*., 2006; Tahiri *et al*., 2006). TYLCV and TYLCSV recombinants have also been found (Monci *et al*., 2001). The spread of TYLCV-IL westward, causing the displacement of the endogenous TYLCSV, especially in Spain (Sánchez-Campos *et al*., 1999), and the invasion of the Americas (Polston *et al*., 1999; Rojas *et al*., 2007), led to the assumption that TYLCV-associated diseases had radiated from the Middle East.

However, TYLCSV-ES has recently been reported from Jordan, showing that TYLCSV has spread eastward (Anfoka *et al*., 2005). The detection of TYLCSV-Sic in Israel and Jordan confirms the two-way expansion of tomato begomoviruses, as this was not an isolated observation. In recent years, two bipartite begomoviruses that infect a variety of cucurbits, *Squash leaf curl virus* (SLCV) which originated in North America, and *Watermelon chlorotic stunt virus* (WCSV) which originated in Yemen, have also been found in eastern Mediterranean (Al-Shahwan *et al*., 2002; Antignus *et al*., 2003; EPPO, 2003; Idris *et al*., 2006). Hence, movement and invasion of exogenous begomoviruses is a phenomenon for which farmers and breeders must be prepared, to be able to cope with it in the near future.

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