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Current Situation of Tomato Yellow Leaf Curl Disease (TYLCD) in Antalya, Turkey

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Abstract

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We studied the distribution of virus/viruses causing tomato yellow leaf curl disease (TYLCD) in Antalya and to characterise the partial genome sequences of the selected isolates. *Tomato yellow leaf curl virus* (TYLCV) was only detected by the triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) and polymerase chain reaction (PCR). 24 and 80 greenhouses were found to be TYLCV-infected in the spring and autumn cultivations in 2011, respectively. The rate of TYLCV infection was found to be 29% in the spring cultivation and 43.7% in the autumn cultivation. The partial nucleotide sequences of the isolates were also determined.

Keywords: *Tomato yellow leaf curl virus;* virus distribution; characterisation; diagnosis; ELISA; PCR

Turkey is ranked third globally in total tomato production. Tomatoes are produced throughout Turkey both on open fields and under cover, but greenhouse tomatoes are primarily cultivated in the Mediterranean region. Antalya, located in the western part of the Mediterranean region of Turkey, is the top greenhouse tomato producing province in the country due to its very favourable climatic conditions. In the greenhouses of Antalya, classic, cherry, beef, cocktail, and vine types of tomatoes are generally cultivated. According to 2011 statistics, 60 and 40% of tomato production in glasshouses and plastic houses of Turkey, respectively, were grown in the Antalya province (TurkStat 2013).

In Antalya, greenhouse tomatoes have been negatively affected by several pests and diseases, but the most serious are the viral diseases. Tomato yellow leaf curl disease (TYLCD) is the most destructive. The disease is one of the major virus diseases where the tomato is a primary host. TYLCD appears in many tropical and subtropical regions (Makkouk & Laterrot 1983) and the agent causes up to total yield loss if plants are infected during early growth (Czosnek & Laterrot 1997). The virus disease was first observed in the Jordan Valley of Israel in 1929 associated with outbreaks of *Bemisia tabaci* (Avidov 1944). Later, the virus was named as *Tomato yellow leaf curl virus* (TYLCV) in the early sixties by Professor I. Harpaz (Cohen & Harpaz 1964). TYLCV was firstly described in the Middle East in the 1960s and in Turkey in the early 1980s (Czosnek 2008). The TYLCD complex incorporates 15 species (Navas-Castillo *et al*. 2011), out of which four occur in Europe including TYLCV, *Tomato yellow leaf curl Axarquia virus* (TYLCAxV), *Tomato yellow leaf curl Malaga virus* (TYLCMalV), and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) (Just *et al.* 2014). In addition TYLCV-TYLCSV recombinants are also found in Spain and Italy (Urbino *et al*. 2013). TYLCV, which may cause up to 100% yield loss, is currently the most common species in Europe (Glick *et al.* 2009). The members of the complex are of the genus *Begomovirus*, family *Geminiviridae* (Navot *et al.* 1991). All of the species have a monopartite ssDNA genome and are naturally transmitted by Middle East–Asia Minor 1 (MEAM1) species of the *Bemisia tabaci* complex (formerly B biotype) in a persistent circulative manner (Abhary *et al*. 2007; De Barro *et al.* 2011).

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Today, there are not any chemicals that can be used to control the mentioned plant virus diseases, including TYLCD. Breeding for TYLCV resistance is the best strategy for combating TYLCD. This strategy depends on (*i*) introducing the virus resistance from wild tomato species into the cultivated tomatoes (Pilowski & Cohen 1990), (*ii*) introducing viral genes to cultivated tomatoes (transgene strategy) (Kunik *et al.* 1994).

The objectives were to study the distribution of virus/viruses causing TYLCD in Antalya and to characterise the partial genome sequences of the selected isolates. Because TYLCV and TYLCSV are common in the Mediterranean basin, the study was planned for these viruses. Before this study, the TYLCD disease survey had not been conducted in either Antalya specifically or Turkey in general. Antalya is the biggest greenhouse tomato producing province of Turkey.

MATERIAL AND METHODS

Sample collection. Tomato leaves exhibiting TYLCD-like symptoms were collected from greenhouses of six districts (the most important tomato growing areas) of Antalya (Figure 1). The symptomatic leaves of three tomato plants were individually analysed per greenhouse. Because there are two cultivation seasons in the Antalya region, the survey was conducted in two seasons (spring and autumn of 2011). In the spring and autumn surveys, 117 and 183 greenhouses were checked, respectively. The leaf samples were randomly collected in the greenhouses. The collected leaves were placed in paper towels in plastic bags, stored at 4°C, and tested by TAS-ELISA and PCR.

TAS-ELISA. All tomato samples were screened for the presence of TYLCV and TYLCSV by TAS-ELISA, using a commercial kit (DSMZ GmbH, Braunschweig, Germany) based on the manufacturer's instructions. Absorbance values were read at 405 nm. Samples were evaluated positive when the mean absorbance of duplicate wells exceeded twice the mean absorbance of the healthy controls.

DNA isolation. Each leaf sample (50 mg) was squeesed with sterile mortar and pestle in liquid nitrogen. DNA isolation was performed by i-genomic Plant DNA Extraction Mini Kit (Intron Biotechnology Inc., Seongnam, South Korea). DNA quantities were measured by nanodrop spectrophotometer device BioSpec-nano (Schimdzu-Biotech, Kyoto, Japan).

Primers and duplex PCR amplification. TYLCV and TLYCSV were screened in this research, because these viruses and their recombinants were only observed in the Mediterranean region. Hence, three different oligonucleotide primers (AV632, AC950, and AC1048), which amplified the coat protein gene, were used to determine TYLCV and TYLCSV. The primers and the expected size of the amplicons are presented in Table 1. Each tomato leaf sample was tested to determine both TYLCV and TYLCSV (Martinéz-Culebras *et al.* 2001) by the duplex PCR technique. Primers were commercially purchased (Iontek-Turkey and Ella-Biotech., Martinsried, Germany). Duplex PCR reaction mixture was prepared as follows: 25 µl total volume containing a final concentration of 1X Taq buffer, 2mM $MgCl_2$, 0.15mM dNTP mix, 0.2 µm of each primer, 1.24 U*Taq* DNA polymerase, and 100 ng DNA extract (modified from Accotto *et al.* 2000). The PCR condition was performed as pre-denaturation at 95°C, 1 min, in 35 of total cycles at 95°C, 30 s (denaturation), at 65°C, 1 min (annealing), at 72°C, 1 min (elongation), and finally at 72°C, 10 min (last elongation). The PCR products were analysed by agarose gel (1.5%) electrophoresis with TAE buffer and stained with red safe DNA dye (Intron Biotechnology Inc., Seongnam, South Korea). DNA bands were visualised under UV transillumina-

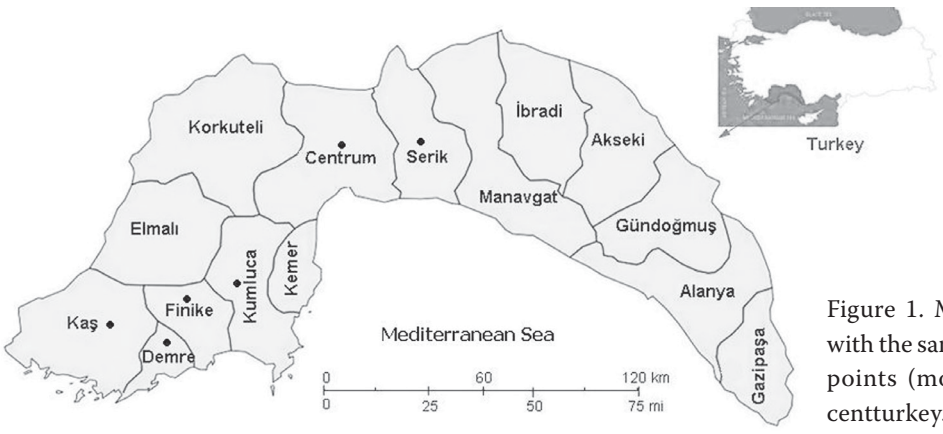

Figure 1. Map of the Antalya province with the sampling sites indicated by black points (modified from http://magnificentturkey.weebly.com/antalya.2html)

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Table 1. Primer pairs used for identification of TYLCD by duplex PCR

Primer	Description (specific primer)	Sequence 5' → 3'	Amplicon size	Reference
AV 632	TYLCV	CYG GTG TTG TKC GTT GTG TTA G	462 bp AV 632-AC1048 primers	Martinéz-Culebras *et al.* (2001)
AC1048	*Begomovirus*	GGA TTA GAG GCA TGC GTA CAT	135 bp	Wyatt and Brown (1996)
AV 950	TYLCSV	TGA AGG AGC AGT GTY TGY TG	AV 950-AC1048 primers	Martinéz-Culebras *et al.* (2001)

tor. Sizes were estimated with 100 bp DNA ladder (Thermo-Scientific, Waltham, USA).

Purification and sequencing of PCR products. Six PCR products which were selected randomly, belonging to six sampling sites, were purified using High Pure PCR Product Purification kit (Roche Diagnostic, Indianapolis, USA). Purified PCR products were sequenced using BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies) with AV632 and AC1048 primers and then, they were run by ABI 3130xL Genetic Analyzer tools. Sequence analysis products were purified with NaOAC-EtOH method.

Six oligonucleotide sequences were obtained and checked for homologous sequences available from Gapped BLAST and PSI program in the NCBI database (Altschul *et al.* 1997). The oligonucleotide sequences are available in the NCBI database under the Accession Nos. KC489095–KC489100.

RESULTS

The size of the surveyed greenhouses ranged between 1 000 and 120 000 m^2. TYLCD symptoms were observed in 114 out of 300 greenhouses. In the disease-suspected greenhouses, the disease symptoms ranged from moderate (slight curling of leaves, slight leaf chlorosis) to severe (severe curling of leaves, extensive chlorosis, stunting, and greatly reduced fruit set). In these greenhouses, the infection rates (visually) varied from 0.05% to 100%.

Based on TAS-ELISA tests, 24 and 34 greenhouses (from a total of 58) were found to be TYLCV-positive in the 1st and 2nd surveys, respectively. However, in the 1st and the 2nd surveys, 36 and 80 greenhouses (from a total of 114), respectively, were found to be infected with TYLCV (462 bp) by PCR. As seen in the results, the rate of TYLCV infection in autumn cultivation was higher than in spring cultivation. TYLCSV was detected in the greenhouses by neither TAS-ELISA nor PCR (Figure 2). In the PCR tests, the expected size of the primer triplets (AC950, AV632, and AC1048) that were designed from the viral coat protein genes were 462 bp (TYLCV) and 462 + 135 bp (TYLCSV) (Table 1). No band was observed in the duplex PCR results of DNA from healthy tomato leaf extracts (Figure 3).

In the spring cultivation survey, the rate of TYLCV infection in the districts was found out (in descending order) as follows: Demre (66.7%), Kas (38.9%), Centrum (33.3%), Serik (31%), Finike (18.8%), and Kumluca (8.3%). Similarly, on the province-wide basis, the rate of TYLCV infection was: Demre (8.6%), Serik (7.7%), Kas (6%), Centrum (4.3%), Finike (2.6%), and Kumluca (1.7%) (Table 2). In the autumn cultivation survey, the detected infection rate was: Kumluca (66.7 %), Kas (55.2%), Centrum (41.9%), Serik (38.2%), Finike (33.3%), and Demre (31.7%). Similarly, on a province-wide basis, the rate of TYLCV infection was: Kumluca (9.8%), Kas (8.8%), Centrum (7.1%), Serik (7.1%), Demre (7.1%), and Finike (3.8%) (Table 2).

As a result, the rate of TYLCV infection in Antalya was found to be 30.9% in spring cultivation and 43.7% in autumn cultivation.

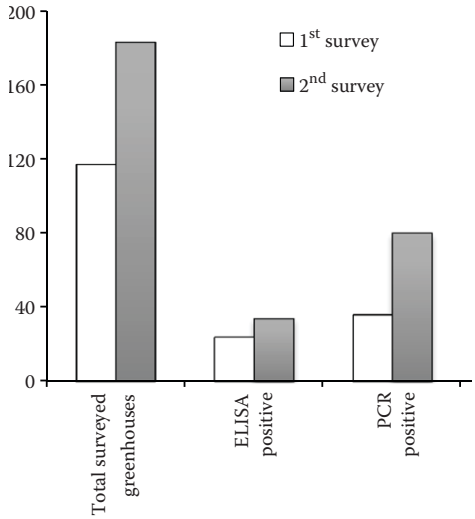

Figure 2. Comparison of results obtained by TAS-ELISA and duplex PCR

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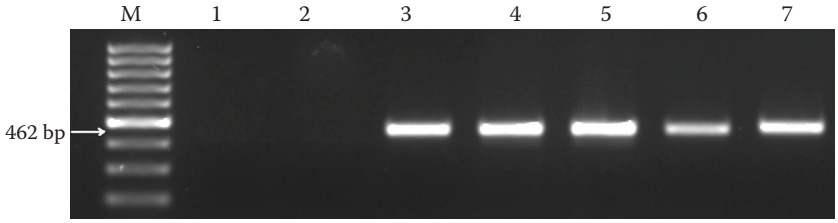

Figure 3. Results of duplex PCR for TYLCV

M – 100 bp DNA Ladder; 1 – negative control (dH_2O); 2 – negative control (healthy tomato sample); 3–7 – leaf samples infected with TYLCV

The BLAST search of the sequence analysis verified the results of duplex PCR. The results of the query of partial sequences indicated that PCR products from Centrum, Kumluca, Finike, Demre, and Kas had the 99% sequence identity with AJ867487.1 (Mugla2 from Turkey), JQ928349.1 (Bojnord from Iran), JQ928348.1 (Dargaz from Iran), JQ928347.1 (Cherry-Fadisheh from Iran) although only Serik (Figure 4) had the 99% sequence identity with AY594174.1 (from Egypt), EF433426.1 (from Jordan), EF051116.1 (from Jordan), and AJ867487.1 (first four accessions were considered).

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  1 ATGGATGAAA ATATCAAGAA GCAGAATCAC ACTAATCAGG TCATGTTCTT CTTGGTCCGT
 61 GATAGAAGGC CCTATGGAAA CAGCCCAATG GATTTTGGAC AGGTTTTTAA TATGTTCGAT
121 AATGAGCCCA GTACCGCAAC CGTGAAGAAT GACTTGCGGG ATAGGTTTCA AGTGATGAGG
181 AAATTTCATG CTACAGTTAT TGGTGGGCCC TCTGGAATGA AGGAACAGGC ATTAGTTAAG
241 AGATTTTTTA AAATTAATAG TCATGTAACT TATAATCATT AGGAGGCAGC CAAGTATGAG
301 AACCATACTG AAAACGCCTT GTTATTGTAT ATGGCATGTA C
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Figure 4. Nucleotide sequence of the PCR product of TYLCV Serik isolate coat protein gene (V1)

DISCUSSION

In Antalya, nearly all growers have used commercial tomato varieties and purchased as seedlings from seedling companies. Hundreds of tomato varieties are available in the Turkish market for both spring and autumn cultivations. However, we observed that about fifty tomato varieties have been dominantly cultivated. Yield, colour, shape, and hardness of tomato are the first preferences in variety of tomato growers. The virus resistance or tolerance in preference of variety has not been generally considered by growers.

Table 2. Rate of TYLCV infection in greenhouses of the Antalya province in survey 1 and survey 2 in 2011 (based on duplex PCR results)

Sampling site	Number of greenhouses	Infection rate (%) (according to the sampling sites)	Infection rate (%) (general)	Number of TYLCV infected/healthy greenhouses
Survey 1				
Serik	29	31.0	7.7	9/20
Centrum	15	33.3	4.3	5/10
Kumluca	24	8.3	1.7	2/22
Finike	16	18.8	2.6	3/13
Demre	15	66.7	8.6	10/5
Kaş	18	38.9	6.0	7/11
Total	117	–	30.9	36/81
Survey 2				
Serik	34	38.2	7.1	13/21
Centrum	31	41.9	7.1	13/18
Kumluca	27	66.7	9.8	18/9
Finike	21	33.3	3.8	7/14
Demre	41	31.7	7.1	13/28
Kaş	29	55.2	8.8	16/13
Total	183		43.7	80/103

number of greenhouses = number of samples

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Fifty-eight and 114 greenhouses were found to be TYLCV-infected by TAS-ELISA and PCR, respectively. However, PCR results are definitely more reliable than ELISA results due to its high sensitivity. TYLCSV was not detected by either test. Some TYLCV symptomatic leaf samples gave negative results by TAS-ELISA. However, all of these samples were found to be TYLCV-infected by PCR. This result is not surprising, because of the fact that PCR is a much more sensitive detection method than ELISA in detecting TYLCV (Accotto & Noris 2007). Although TAS-ELISA is very useful for large-scale detection, it lacks the differentiating capacity and sensitivity in the stages of infection in which the infected plant has a low virus titre.

As mentioned before, the rate of TYLCV infection was significantly greater in autumn tomato cultivation than in spring cultivation. In Antalya, spring and autumn plantings were done in January–February and August–September, respectively. Since temperature and humidity are very suitable for whitefly reproduction and activity in August–September, the high rate of TYLCV infection in autumn cultivation is corresponding.

On the Antalya province basis, TYLCV was detected in about 37% of total of the surveyed greenhouses in autumn and spring of 2011 (Table 2). Studies on the rate of TYLCV infection have not previously been conducted in Antalya. Hence, it is not possible to make a comparison with the past. The results have shown that because of the fact that some resistant and tolerant commercial varieties are available in the market; the disease still maintains its importance and is common. TYLCV is the most serious viral agent of greenhouse tomatoes in Antalya. *B. tabaci* biotype B, the only vector of TYLCV, is predominant type in Turkey (Bayhan *et al.* 2006). The success of insecticides to control the insect has been limited by the development of resistance to many insecticides (Erdogan *et al.* 2008). Pesticide resistance usually results from the overuse and/or misuse of pesticides (Denholm 1988). The best recommendation for chemical control of whiteflies in Turkey is to use different classes of insecticide for each application (Erdogan *et al.* 2008).

It is observed that in well-managed greenhouses with the absence of whiteflies and weeds, the virus has not been detected. Additionally, conscious tomato producers in Antalya prefer using TYLCV-resistant tomato varieties and effective chemical methods against whiteflies. It has already been reported that the integration of whitefly control methods with the use of TYLCV-resistant varieties is the best solution for TYLCV control (Moriones & Navas-Castillo 2000; Erdogan *et al.* 2008).

Sequencing results with Centrum, Kumluca, Finike, Demre, and Kas isolates showed an extremely high degree of identity (99%) with AJ867487.1 (Mugla2 from Turkey), JQ928349.1 (Bojnord from Iran), JQ928348.1 (Dargaz from Iran), and JQ928347.1 (Cherry-Fadisheh from Iran). Additionally, Serik isolate showed an extremely high degree of identity (99%) with AY594174.1 (from Egypt), EF433426.1 (from Jordan), EF051116.1 (from Jordan), and AJ867487.1 (Mugla2 from Turkey). TYLCV has been found as the only strain in Egypt (Anfoka *et al.* 2008) and Iran (Azizi *et al.* 2011), whereas in Jordan, both TYLCV and TYLCSV have been known to exist (Anfoka *et al.* 2008). Iran is an eastern neighbour country of Turkey, and Egypt and Jordan are not far from Turkey (Czosnek & Laterrot 1997). Therefore, this very close identity is quite common.

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