Acta virologica 58: 128 – 136, 2014 doi:10.4149/av_2014_02_128

Genetic diversity, host range, and distribution of tomato yellow leaf curl virus in Iran

M. SHIRAZI[1], J. MOZAFARI[2], F. RAKHSHANDEHROO[1], M. SHAMS-BAKHSH[3]

[1]Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran; [2]Department of Genetics and National Plant Gene-Bank, Seed and Plant Improvement Institute, Mahdasht RD., Karaj, Iran; [3]Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

Received August 1, 2013; accepted May 13, 2014

Summary. – Tomato yellow leaf curl virus (TYLCV) is considered one of the most important tomato pathogens in tropical and subtropical regions including Iran. During the years 2007 to 2009, a total number of 510 symptomatic and asymptomatic vegetable, ornamental and weed samples were collected from fields and greenhouses in ten provinces of Iran. Symptoms included stunting, yellowing, leaf curl and flower senescence. PCR with specific primers showed TYLCV infection in 184 samples (36%) such as cucumber, pepper, tomato and several weeds from seven provinces. Based on the geographical origin, host range and symptoms, twenty three representative isolates were selected for phylogenetic analysis. An amplicon with a size about 608 base pair (bp) comprising partial sequence of the coat (CP) and movement protein (MP) coding regions of the viral genome was sequenced and compared with the corresponding selected sequences available in GenBank for Iran and worldwide. Phylogenetic analyses on the basis of the nucleotide sequences indicated two geographically separated clades. Isolates collected from Hormozgan, Khuzestan and Kerman provinces were grouped together with other Iranian isolates including TYLCV-Ir2, TYLCV-Kahnooj, and an isolate from Oman. It was also revealed that isolates collected from Boushehr, Fars, Tehran, and Isfahan placed close to the Iranian isolate TYLCV-Abadeh and isolates from Israel and Egypt. No correlation was found between the genetic variation and the host species, but selected Iranian isolates were grouped on the basis of the geographical origins. Results of this study indicated a high genetic diversity among Iranian TYLCV isolates.

Keywords: TYLCV; host; weed; genetic diversity; Iran

Introduction

Tomato yellow leaf curl virus (TYLCV) is one of the most important limiting factors of tomato production in tropical and subtropical regions. TYLCV was first reported from Israel in 1930s and it has since been reported in many countries in the Mediterranean basin, Africa, Asia, Europe, Australia, the Caribbean basin and the America (Czosnek *et al*., 1990; Cohen and Antignus, 1994; Hajimorad *et al*., 1996; Czosnek and Laterrot, 1997; Polston and Anderson, 1997; Nakhla and Maxwell, 1998). In the last few decades, TYLCV is expanding rapidly and new infected areas have been reported in several parts of the world especially in the New World. In Iran, since the first report of TYLCV from Southern provinces including Sistan and Baluchestan, Hormozgan, Kerman, Boushehr and Khuzestan (Hajimorad *et al*., 1996), it was reported in Khorasan, Isfahan, Markazi, Golestan, Tehran and Yazd (Shahriary and Bananej, 1998; Bananej *et al*., 1998, 2004, 2008). Although TYLCV was known as an infecting pathogen of tomato (*Solanum lycopersicum*), it can also infect several crop plants such as bean (*Phaseolus vulgaris* L.), lisianthus (*Eustomus grandiflorum*), *Mercurialis ambigua* and pepper (*Capsicum annum*) (Cohen and Gera, 1995; Navas-Castillo *et al*., 1999; Reina *et al*., 1999;

E-mail: mhishirazi@yahoo.com; phone: +98-21-44868469.

Abbreviations: CP = coat protein; IR = intergenic region; MP = movement protein; TYLCV = tomato yellow leaf curl virus

Sanchez-Campos *et al*., 2000). Infected tomato plants may show symptoms such as stunting, yellowing, leaf curl and flower senescence. The symptoms are variable in different cultivated hosts, while some wild hosts such as some weeds are symptomless. TYLCV is a single-stranded DNA virus belonging to the genus *Begomovirus* (the family *Geminiviridae*), which is transmitted by *Bemisia tabaci* in a circulative persistent manner to dicotyledonous plants. Based on the genome organization, host range and vector, geminiviruses are divided into four genera e. i. *Begomovirus*, *Curtovirus*, *Topocovirus* and *Mastrevirus* (Fauquet and Stanley, 2003). Although most of the TYLCV strains have bi-partite genomic DNA called DNA-A and DNA-B, some of them are monopartite (Stanley *et al*., 2005). In monopartites, which lack a DNA-B component, the genome has six ORFs responsible for viral replication, regulation of gene expression, coat and movement proteins, symptom expression, host range and pathogenicity (Navot *et al*., 1991). There is also an intergenic region (IR), which includes a TAATATTAC conserved sequence. Several parts of the TYLCV genome including IR, CP, Rep, and also the full-length sequence of the genome were used for analyzing the genetic diversity (Padidam *et al*., 1995; Ribeiro *et al*., 2003; Bananej *et al*., 2008). Previous studies have elucidated that TYLCV has a considerable genetic diversity in Iran, and TYLCV-Ir, TYLCV-Ir2, TYLCV-Abadeh, and TYLCV-Kahnooj have been previously reported from Southern tomato fields of Iran (Bananej *et al.,* 2004; Azizi *et al*., 2008; Pakniat *et al*., 2008; Fazeli *et al*., 2009). In this study, we aimed at determining the current status of the virus distribution as well as its genetic diversity in most important tomato growing regions of the country based on the partial sequences of the CP and MP genes.

Materials and Methods

Sample collection. Symptomatic bean, cucumber, lisianthus, pepper and tomato leaves with symptoms such as stunting, yellowing, leaf curl and flower senescence along with symptomless weeds were randomly collected from tomato fields and greenhouses in ten provinces of Iran, i. e. Boushehr (Dashtestan and Khormuj), Fars (Abadeh and Eghlid), Hormozgan (Rezvan and Sarkhun), Isfahan (Isfahan), Kerman (Jiroft), Khorasan Razavi (Mashhad), Khuzestan (Dezful, Hamidiyeh and Safi Abad), Markazi (Khomein), Tehran (Islamshahr, Pakdasht and Varamin) and Alburz (Karaj). Overall, 51 fields were surveyed and 510 samples were collected during the growing seasons of the years 2007 to 2009. After taking the specific code for the regions and hosts, samples were transferred in a plastic bag to the laboratory on the ice and stored at 4°C until further use.

DNA extraction and PCR amplification. Total DNA was extracted according to the published protocol described by Dellaporta *et al*. (1983) with some modifications. About 0.1 g of each sample was grounded in liquid nitrogen and was incubated in 600 µl of extraction buffer (100 mmol/l Tris-HCl (pH 8), 50 mmol/l EDTA, 500 mmol/l NaCl, 10 mmol/l β-mercaptoethanol and 1% (w/v) SDS) at 65°C for 10 min and then mixed with 700 µl of chloroform isoamyl alcohol (24:1 v/v). The mixture was centrifuged at 13,000 rpm for 20 min and the supernatant was transferred to a new 1.5 ml micro centrifuge tube. The total DNA was precipitated by adding 40 µl of sodium acetate (5 mol/l, pH 5.2) and 600 µl isopropanol. The DNA pellet was washed with 70% (v/v) ethanol. After air drying, the pellet was dissolved in 30 µl of sterile double distilled water and stored at -20°C. Presence of TYLCV infection in collected samples was detected using PCR with specific primers to amplify a DNA fragment of about 670 bp encompassing the coat protein and the movement protein coding sequences (Pico *et al*., 1998). PCR was set with a degenerate primer pair, PBL1v2040/PCRc1 for specific amplification of the second segment of viral genome (DNA-B) (Rojas *et al*. 1993) (Table 1). The PCR mixture (final volume of 25 µl) contained 2 µl of DNA, 2 mmol/l $MgCl_2$, 0.2 mmol/l of each dNTPs, 0.4 pmol of each primer and 1 U of *Taq* DNA polymerase (CinnaGen, Iran) in the buffer recommended by the manufacturer. Amplification conditions were: 1 cycle of 94°C for 4 min, 35 cycles of 94°C for 1 min, 54°C for 1 min, 72°C for 2 min followed by 10 min at 72°C as a final extension. PCR products were analyzed by electrophoresis on a 1.5% agarose gel in 1×TAE buffer and visualized under an UV transilluminator after stained with ethidium bromide (0.5 µg/ ml).

Sequencing of amplified fragments. For every isolate, once the expected PCR product was obtained, it was subjected to purification with the Silica Bead DNA Gel Extraction Kit as described by the manufacturer (Fermentas, Germany) and used directly for sequencing with Illumina GAllx and Hiseq 2000 sequencers (Gene service, England) in forward and reverse directions. For subsequent sequencing procedure, twenty three representative PCR products were selected based on the geographical location of the original samples as well as the host plants and symptoms. Phylogenetic analysis of the 23 Iranian TYLCV isolates was conducted by comparing separately the 608 nucleotides (nt) of the partial CP and MP genes with the comparable sequences of other TYLCV isolates from GenBank. Multiple alignments were performed in the ClustalX 1.8 (Pearson and Lipman, 1988). The phylogenetic tree was constructed using the neighbor-joining algorithm (Saitou and Nei, 1987), p-distance method (Nei and Kumar, 2000) and bootstrap consisting of 1000 pseudo-replicates, and finally evaluated using the interior branch test method with Mega 5 software (Tamura *et al*., 2011).

Results

TYLCV Detection and distribution. A PCR-amplified product of approximately 670 bp, corresponding to the CP and MP genes of TYLCV, was obtained for all infected vegetable and weed samples (Fig. 1). However, no PCR products

Table 1. Primers for amplification of two parts of the genome

Primer name	Genome part	Expected size	Nucleotide sequence
Sense	DNA-A	670 Kb	5′-CGC CCG TCT CGA AGG TTC-3′
Antisense			5′-GCC ATA TAC AAT AAC AAG GC-3′
PBL1v2040	DNA-B	600 Kb	5′-GCC TCT GCA GCA RTG RTC KAT CTT CAT ACA-3′
PCRc1			5′-CTA GCT GCA GCA TAT TTA CRA RWA TGC CA-3′

K = G,T; R = A,G; W = A,T.

were amplified when the infected samples were tested for the second segment of the viral genome (DNA-B) (data not shown), which indicates that this segment of TYLCV genome is not present in Iran throughout the visited regions.

Of the 510 collected vegetable, ornamental and weed samples tested by PCR, TYLCV was detected in 184 samples (36%) including cucumber (*Cucumis sativus*), *Daucus* sp. (*Asteraceae*), *Malva* sp. (*Malvaceae*), *Melilotus officinalis* (*Legominosae*), pepper (*Capsicum annum*), tomato (*Solanum lycopersicum*) and *Trigonella* sp. (*Legominosae*) from seven provinces including Boushehr (Dashtestan and Khormuj), Fars (Abadeh), Hormozgan (Rezvan and Sarkhun districts), Isfahan (Isfahan), Kerman (Jiroft), Khuzestan (Dezful) and Tehran (Islamshahr, Pakdasht and Varamin) (Fig. 2 and Table 2). Our results showed that *Tomato yellow leaf curl virus* is not limited to the southern regions and its geographical distribution has been expanded into the central regions of the country such as Tehran province (Fig. 3), in which tomato cultivations showed a high percentage of TYLCV infection. Infection rates ranged from no infection in Alburz, Khorasan Razavi and Markazi provinces in central and eastern regions to 66% in Hormozgan province in the southern region of Iran.

Genetic diversity. Blast analyses of the sequences of the twenty three TYLCV isolates from Iran disclosed 94–100% identities at the nucleotide level with other TYLCV sequences from GenBank. Pairwise nucleotide comparisons of Iranian isolates showed low levels of divergence (6%) amongst them.

Blasting of sequences in GenBank showed close relationship between the sequences of Iranian isolates and other isolates from Iran, Oman and Israel (Table 3).

When the sequences of the isolates collected from Abadeh district with difference in symptoms expression in their hosts were compared, low level of nucleotide variations was observed and the identity was 99%. This result indicated that nucleotide variation in the CP and MP genomic region cannot much affect the symptom expression, indicating to the role of other genomic regions of the virus in pathogenicity and symptom expression. Our results also revealed minimum similarity between TYLCV-Ir with TYLCV-Ir2 and D2 (94.7%) and maximum identity between TYLCV Ir2, D2, MZ, and ES isolates (100%). According to phylogenetic analyses, Iranian isolates were divided into two major groups and three subgroups in group I (Fig. 4). Interestingly, Iranian isolates were scattered in both groups and three subgroups

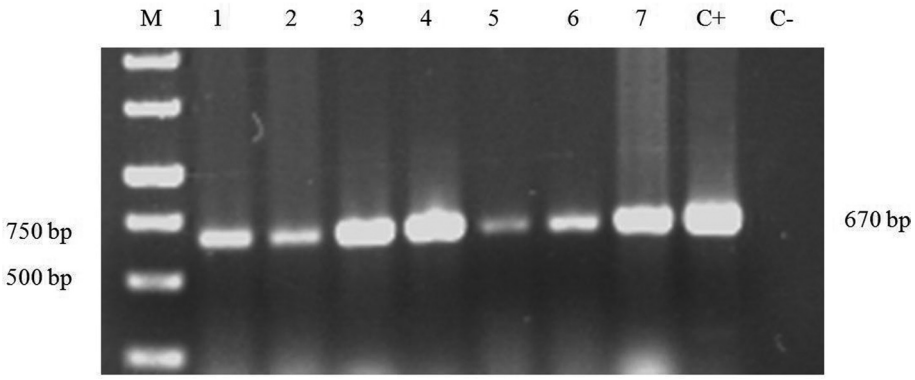

Fig. 1
Electrophoresis analysis of PCR products for some of the detected TYLCV isolates in Iran
Lanes 1 to 7 represent amplified products for naturally infected cucumber, pepper, tomato, *Daucus* sp., *Malva* sp., *Melilotus officinalis* and *Trigonella* sp. samples, respectively. Lane M, 1 Kb DNA ladder (Fermentas, Germany); Lane C+, infected tomato material used as a positive control; Lane C-, negative control (PCR conducted using healthy plant material).

Table 2. Incidence of TYLCV on vegetable crops and weed plants collected from the main cultivation areas in Iran

Sampled plants						Infection ratio	Region	Province
Weed	Lisianthus	Bean	Cucumber	Pepper	Tomato			
ND	ND	ND	ND	4/13	5/25	9/38	Sarkhun	Hormozgan (66.1%)[a]
ND	ND	ND	ND	ND	69/80	69/80	Rezvan	
ND	ND	ND	ND	ND	3/5	3/5	Dezful	Khuzastan (10%)
ND	ND	ND	ND	ND	0/10	0/25	Safi abad	
ND	ND	0/15	ND	ND	ND	ND	Hamidiyeh	
ND	ND	ND	ND	2/4	4/6	6/10	Jiroft	Kerman (60%)
ND	ND	ND	ND	3/6	0/26	3/32	Isfahan	Isfahan (9.4%)
ND	0/15	0/17	ND	2/10	23/32	25/74	Pakdasht	Tehran (47.6%)
ND	ND	ND	ND	NDt	11/19	11/19	Islamshahr	
2/2	ND	ND	ND	2/2	19/27	23/31	Varamin	
ND	ND	0/7	ND	ND	0/11	0/18	Karaj	Alborz (0%)
0/2	ND	0/5	ND	ND	0/18	0/25	Mashhad	Khorasan Razavi (0%)
2/9	ND	ND	7/10	ND	17/17	26/36	Abadeh	Fars (27.6%)
ND	ND	0/58	ND	ND	ND	0/58	Eghlid	
6/8	ND	ND	ND	ND	2/4	8/12	Dashtestan	Boushehr (36%)
0/4	ND	ND	ND	1/5	0/4	1/13	Khormuj	
ND	ND	0/34	ND	ND	ND	0/34	Khomein	Markazi (0%)
10/25 (40%)	0/15 (0%)	0/136 (0%)	7/10 (70%)	14/40 (35%)	153/284 (53.9%)	184/510 (36%)	– –	Total

[a]Percentage of TYLCV infections in each province. ND = not determined.

indicating to a high genetic diversity among them. In the first group, isolates from Fars, Tehran, Isfahan, and Boushehr provinces were closely stood beside the TYLCV-Mild and TYLCV-Egypt. The second group consisted of Iranian isolates from the southern regions including Hormozgan, Khuzestan and Kerman that were closely categorized with the other Iranian strains that had been previously reported from the southern regions, like TYLCV-Kahnooj, TYLCV-Ir2 and also TYLCV-Albitaneh. In this group, TYLCV-Ir was located on a separate tree branch. Iranian TYLCV isolates from distinct geographical locations were divided into three subgroups in group I, pointing to the correlation between geographical origin of the isolates and their phylogenetic positions at the regional scale (Fig. 4). Isolates from Abadeh district of Fars province in the south of Iran were categorized in subgroup I, isolates from Pakdasht and Varamin districts of Tehran province in the central parts of Iran were distinct from others in subgroup II, and isolates from Dashtestan district of Boushehr province in the south were stood close to each other in subgroup III (Fig. 4).

Discussion

Our results showed a high incidence of TYLCV in major tomato production areas of Iran, especially in central and southern regions (36%). This incidence can be considered low when compared with that of other countries. For example, the incidence of TYLCV in tomato fields in Saudi Arabia, neighboring the southern region of Iran, was found to be 89% (Ajlan *et al.*, 2007). The extraordinary outbreak of TYLCV in these regions could be due to the warm weather

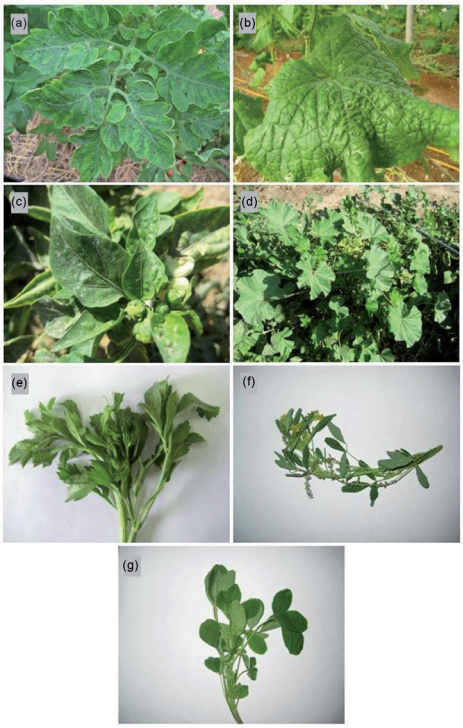

Fig. 2
Symptomatic and asymptomatic TYLCV infected tomato, cucumber, pepper and weed samples
(a) Tomato *(Solanum lycopersicum)*, (b) Cucumber *(Cucumis sativus)*, (c) Pepper *(Capsicum annum)*, (d) Malva sp., (e) *Daucus* sp., (f) *Melilotus officinalis*, (g) *Trigonella* sp.

conditions to upper latitude (Morales and Jones, 2004; Jones, 2009) that may favor the vector activity, virus replication or host susceptibility. It is also possible that TYLCV was introduced to the Northern provinces by the exchanges of TYLCV-infected seedlings, plant materials or by viruliferous whiteflies from infected countries in the Middle East region to the southern regions of Iran (Navas-Castillo *et al.*, 1999; Kim *et al.*, 2011). It has been recently elucidated that Mediterranean basin and Middle East are the main launch-pad of global TYLCV movements (Lefeuvre *et al.*, 2010). Results also showed that the occurrence of TYLCV in central regions of the country, pointing to the distribution of this virus through the country, which may expand to the northern regions, where numerous kinds of host are available for the virus as the potential infection sources.

In this research, the infection of four weeds with TYLCV was confirmed by PCR. To our knowledge this is the first report of *Daucus* sp., *Trigonella* sp., and *Melilotus officinalis* infection by TYLCV in Iran. Infected weeds are the virus sources for whiteflies throughout the year. Natural reservoirs may harbor the virus and give a primary source of inoculum to newly established tomato crops, when virus reservoirs are present in the same area with the tomato crops. Thus, the crop was considered a secondary host plant that could potentially act as a natural reservoir for TYLCV (Sawalha, 2012). The removal of reservoir weed plants from tomato fields before the appearance of whiteflies may limit the spread of the virus into tomato fields and is necessary to control the disease. We also detected the virus in cucumber samples. Detection of TYLCV in cucumber plants near the tomato cultivations could be because of the viruliferous vector and exchange of the infected materials between tomato and cucumber cultivations in visited regions in Iran and is a new threat for cucumber production in these regions. Despite the presence of potential vectors for TYLCV in the major geographical regions, no TYLCV infection in Mashhad and Khomein could be due to the absence of the viral inoculum in these regions. It could be also due to weather conditions unsuitable for the potential vector and its activity at sampling time. However a larger number of suspicious samples need to be tested. Despite the fact that bean and lisianthus plants have been reported to be the host of TYLCV (Cohen and Gera, 1995; Navas-Castillo *et al.*, 1999), we were not be able to detect TYLCV infection in bean throughout the visited regions, which are considered as the main bean plantations of Iran (Khomein and Eghlid). One probable reason is that bean is cultivated as a monoculture crop and there is no neighboring or alternation between bean and tomato fields. Bean infections have taken place where bean cultivation was in rotation with tomato (Navas-Castillo *et al.*, 1999). During our inspections in Khomein and Eghlid, co-cultivation of bean with other vegetable crops was not evident at all, which may favor virus-free cultivations in these regions and it seems this fact can be true for TYLCV-free lisianthus plants as well.

We observed symptom variations on tomato plants. It could be considered as heterogeneity among Iranian TYLCV isolates as it had been mentioned before (Hajimorad *et al.*, 1996). Naturally infected tomatoes were infected with heterogenic TYLCV populations (Ge *et al.*, 2007). Genetic variation in TYLCV genome either through mutation or DNA recombination is responsible for most of variations (Hanley-Bowdin *et al.*, 1999). Genetic diversity had been reported in wild and cultivated hosts (Ueda *et al.*, 2008; Mubin *et al.*, 2010). On the basis of partial sequences of CP and MP, studied isolates were categorized into two major groups. Iranian isolates had previously been divided into two groups on the basis of IR (Intergenic Region) sequences (Bananej *et al.*, 2008). These data emphasized that studied isolates were distinct from each other's based on geographical distribution and not by host range. Comparison of the CP sequences of TLCV isolates from India with TYLCV isolates from North Africa and Saudi Arabia had also revealed three geographically distinct groups (Hong and Harrison, 1995). Based on these results TYLCV has a high genetic diversity

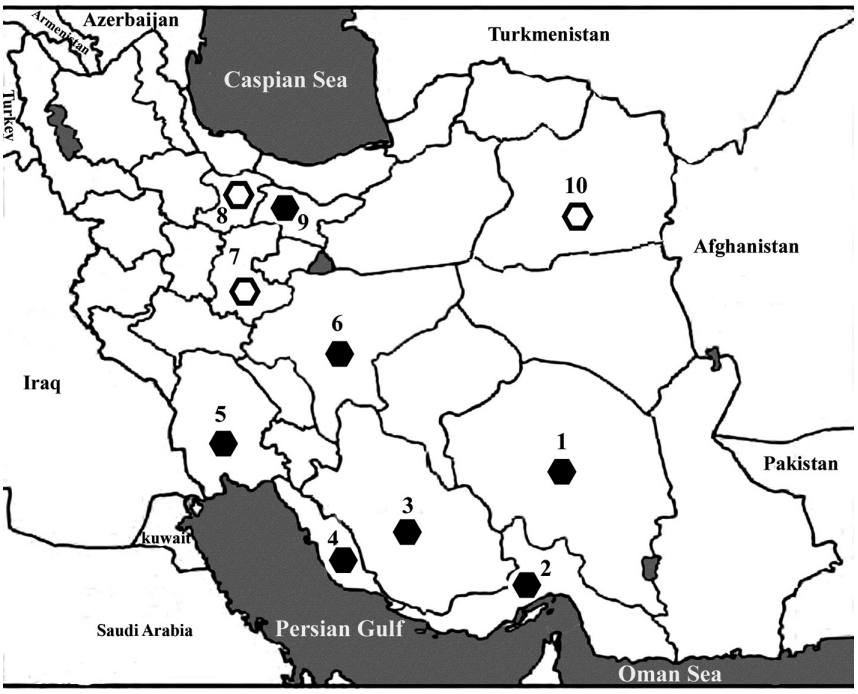

Fig. 3
Map of Iran showing the distribution of TYLCV in 10 provinces
Provinces, in which TYLCV was not detected are indicated as (⬡) and TYLCV-infected provinces are shown with (⬢) on the map. 1) Kerman, 2) Hormozgan, 3) Fars, 4) Boushehr, 5) Khuzestan, 6) Isfahan, 7) Markazi, 8) Alburz, 9) Tehran, 10) Khorasan Razavi.

Fig. 4
Phylogenetic tree constructed using MEGA 5 with 1000 bootstrapping iteration of 608 bp of CP and MP genes
Only bootstrap values greater than 60% are shown. Bootstrap percentages of clades are shown along internal branches of trees derived from bootstrap-resembled data sets. Branch lengths represent bootstrap values. The tree was rooted with the CP-MP nucleotide sequences of Indian cassava mosaic virus (ICMV) as an out-group species.

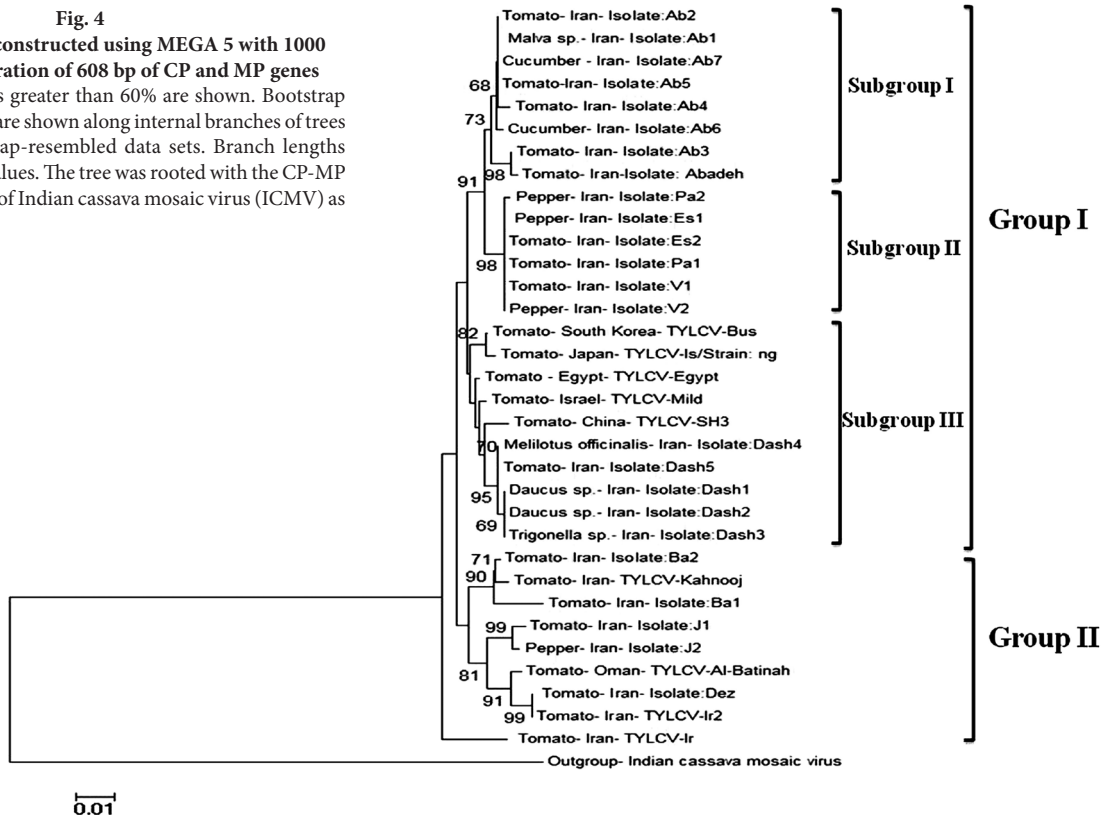

Table 3. Characteristics of the sequenced isolates and their identities with GenBank Acc. used in this study for phylogenetic comparison

Isolate name	Acc. No.	Host	Variety	Collested region	Symptom	The closest accessions	Identity[a]
Ab1	GU325649	*Malva* sp.	–	Abadeh	Symptomless		
Ab2	GU585383	*Solanum lycopersicum*	Falcato	Abadeh	Leaf curl, small leaves with slight yellowing		
Ab3	GU585384	*Solanum lycopersicum*	Falcato	Abadeh	End compression, small leaves with slight yellowing	FJ355946	99%
Ab4	GU585385	*Solanum lycopersicum*	Falcato	Abadeh	Leaf rolling with slight yellowing	AY594174 GQ141873	98% 98%
Ab5	GU585386	*Solanum lycopersicum*	Falcato	Abadeh	Leaf curl with yellow spots on common leaflets		
Ab6	GU585387	*Cucumis sativus*	–	Abadeh	Slight leaf curl		
Ab7	GU585388	*Cucumis sativus*	–	Abadeh	Slight leaf curl		
Ba1	GU585381	*Solanum lycopersicum*	PS	Rezvan	Yellowing, leaf curl and severe stunting	EU635776	98%
Ba2	GU585382	*Solanum lycopersicum*	PS	Sarkhun	Yellowing, leaf curl and severe stunting	AB110217 AY594174	97% 97%
J1	GU585397	*Solanum lycopersicum*	PS	Jiroft	Yellowing, leaf curl and severe stunting	DG644565 EU635776	98% 97%
J2	GU585398	*Capsicum annum*	–	Jiroft	Yellowing and rough leaves	AY594174	97%
Dez	GU585394	*Solanum lycopersicum*	PS	Dezful	Yellowing, leaf curl and severe stunting	EU085423 DQ644565 X76319	100% 99% 97%
Dash1	GU585389	*Daucus* sp.	–	Dashtestan	Symptomless		
Dash2	GU585390	*Daucus* sp.	–	Dashtestan	Symptomless		
Dash3	GU585391	*Trigonella* sp.	–	Dashtestan	Symptomless	X76319 AY594174	99% 99%
Dash4	GU585392	*Melilotus officinalis*	–	Dashtestan	Symptomless	FN256258	98%
Dash5	GU585393	*Solanum lycopersicum*	Royal	Dashtestan	Severe yellowing with slight leaf curl		
Es1	GU585395	*Capsicum annum*	–	Isfahan	Yellowing and rough leaves		
Es2	GU585396	*Solanum lycopersicum*	Banemi	Islamshahr	Yellowing, leaf curl, stunting and end compression		
Pa1	GU585399	*Solanum lycopersicum*	Y	Pakdasht	Leaf curl, stunting with small leaves	FJ355946 AY594174	98% 98%
Pa2	GU585400	*Capsicum annum*	–	Pakdasht	Small leaves and leaf deformation	GQ141873	98%
V1	GU585401	*Solanum lycopersicum*	Razan	Varamin	Yellowing, leaf curl, stunting and flower senescence		
V2	GU585402	*Capsicum annum*	–	Varamin	Yellowing and rough leaves		

[a]Nucleotide sequence identity of the partial sequences for the CP and MP genes of selected TYLCV strains in this study.

and there are at least two major groups of TYLCV in different geographical regions of Iran. In conclusion, we report here on the natural infection of new weeds as a host of TYLCV that increases the maintenance of the virus, the chance of recombination and also genetic diversity. According to the results, expansion of TYLCV and observation of newly infected areas emphasize the establishment of an effective quarantine in order to prevent further distribution of TYLCV into other northern regions. Future studies are needed to evaluate the genetic diversity of Iranian TYLCV isolates based on the full length sequences of the viral genome.

Acknowledgement. This work was conducted in Department of Genetics and National Plant Gene-Bank, Seed and Plant Improvement Institute and financially supported by Agricultural Research, Education and Extension Organization of Iran (Proj. No. 0-100-12-25-8311-83003).

References

Ajlan AM, Ghanem GAM, Abdulsalam KS (2007): Tomato yellow leaf curl virus (TYLCV) in Saudi Arabia: Identification, partial characterization and virus-vector relationship. Arab. J. Biotech. 10, 179–192.

Azizi A, Mozafari J, Shams-bakhsh M (2008): Phenotypic and molecular screening of tomato germplasm for resistance to tomato yellow leaf curl virus. Iran. J. Biotech. 6, 199–206.

Bananej K, Ahoonmanesh A, Shahraeen N (1998): Occurrence and identification of Tomato yellow leaf curl virus from Khorasan province of Iran. Proc. XIII Iranian Plant Prot. Cong. Agricultural Research and Education Organization (AREO), Karaj, p. 193.

Bananej K, Kheyr-Pour A, Salekdeh GH, Ahoonmanesh A (2004): Complete nucleotide sequence of Iranian tomato yellow leaf curl virus isolate: further evidence for natural recombination amongst begomoviruses. Arch. Virol. 149, 1435–1443. http://dx.doi.org/10.1007/s00705-004-0308-9

Bananej K, Vahdat A, Hoseini-salekdeh G (2008): Begomoviruses associated with yellow leaf curl disease of tomato in Iran. J. Phytopathol. 10, 434–439.

Cohen S, Antignus Y (1994): Tomato yellow leaf curl virus, a whitefly-borne geminivirus of tomatoes. Adv. Dis. Vector Res. 10, 259–288. http://dx.doi.org/10.1007/978-1-4612-2590-4_10

Cohen J, Gera A (1995): Lisianthus Leaf Curl a New Disease of Lisianthus Caused by Tomato yellow leaf curl virus. Plant Dis. 79, 416–420. http://dx.doi.org/10.1094/PD-79-0416

Czosnek H, Laterrot H (1997): A worldwide survey of tomato yellow leaf curl viruses. Arch. Virol. 142, 1391–1406. http://dx.doi.org/10.1007/s007050050168

Czosnek H, Navot N, Laterrot H (1990): Geographical distribution of tomato yellow leaf curl. A first survey using a specific DNA probe. Phytopathol. Mediterr. 29, 1–6.

Dellaporta SL, Wood JY, Hicks JB (1983): A plant DNA minipreparation: version II. Plant Mol. Biol. Rep. 1, 19–21. http://dx.doi.org/10.1007/BF02712670

Fauquet CM, Stanley J (2003): Geminivirus Classification and Nomenclature: progress and problems. Ann. Appl. Biol. 142, 165–189. http://dx.doi.org/10.1111/j.1744-7348.2003.tb00241.x

Fazeli R, Heydarnejad J, Massumi H, Shaabanian M, Varsani A (2009): Genetic diversity and distribution of tomato-infecting begomoviruses in Iran. Virus Genes 38, 311–319. http://dx.doi.org/10.1007/s11262-008-0310-5

Ge L, Zhang J, Zhou X, Li H (2007): Genetic structure and population variability of Tomato yellow leaf curl china virus. J. Virol. 81, 5902–5907. http://dx.doi.org/10.1128/JVI.02431-06

Hajimorad MR, Kheyr-Pour A, Alavi V, Ahoonmanesh A, Bahar M, Rezaian MA, Gronenborn B (1996): Identification of whitefly transmitted tomato yellow leaf curl geminivirus from Iran and a survey of its distribution with molecular probes. Plant Pathol. 45, 418–425. http://dx.doi.org/10.1046/j.1365-3059.1996.d01-151.x

Hanley-Bowdoin L, Settlage SB, Orozoo BM, Nagar S, Robertson D (1999): Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. Crit. Rev. Plant Sci. 18, 71–106. http://dx.doi.org/10.1080/07352689991309162

Hong YG, 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. http://dx.doi.org/10.1099/0022-1317-76-8-2043

Jones RAC (2009): Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. Virus Res. 141, 113–130. http://dx.doi.org/10.1016/j.virusres.2008.07.028

Kim SH, Oh S, Oh TK, Park JS, Kim SC, Kim SH, Kim YS, Hong JK, Sim SY, Park KS, Lee HG, Kim KJ, Choi CW (2011): Genetic diversity of tomato-infecting Tomato yellow leaf curl virus (TYLCV) isolates in Korea. Virus Genes 42, 117–127. http://dx.doi.org/10.1007/s11262-010-0541-0

Lefeuvre P, Martin DP, Harkins G, Lemey P, Gray AJA, Meredith S, Lakay F, Monjane A, Lett JM, Varsani A, Heydarnejad J (2010): The spread of Tomato yellow leaf curl virus from the Middle East to the world. PLoS Pathog. 6, 1–12. http://dx.doi.org/10.1371/journal.ppat.1001164

Morales FJ, Jones PG (2004): The ecology and epidemiology of whitefly-transmitted viruses in Latin America. Virus Res. 100, 57–65. http://dx.doi.org/10.1016/j.virusres.2003.12.014

Mubin M, Shahid MS, Tahir MN, Briddon RW, Mansoor S, (2010): Characterization of begomovirus components from a weed suggests that begomoviruses may associate with multiple distinct DNA satellites. Virus Genes 40, 452–457. http://dx.doi.org/10.1007/s11262-010-0470-y

Nakhla MK, Maxwell DP (1998): Epidemiology and management of tomato yellow leaf curl disease. In: Hadidi A, Khetarpal RK, Koganezawa H (Eds): Plant Virus Disease Control. APS Press, USA, pp. 565–583.

Navas-Castillo J, Sánchez-Campos S, Díaz JA, 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 Dis. 83, 29–32. http://dx.doi.org/10.1094/PDIS.1999.83.1.29

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. http://dx.doi.org/10.1016/0042-6822-(91)90763-2

Nei M, Kumar S (2000): Molecular evolution and phylogenetics. Oxford University Press, USA, p. 333.

Padidam M, Beachy RN, Fauquet CM (1995): Classification and identification of geminiviruses using sequence comparisons. J. Gen. Virol. 76, 249–263. http://dx.doi.org/10.1099/0022-1317-76-2-249

Pakniat Jahromy A, Behjatnia SA, Izadpanah K (2008): A new strain of Tomato yellow leaf curl virus (TYLCV) in Fars province. Proc. XVIII Iranian Plant Prot. Cong. Hamedan, p. 491.

Pearson WR, Lipman DJ (1988): Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. (PNAS) 85, 2444–2448. http://dx.doi.org/10.1073/pnas.85.8.2444

Pico B, Diez MJ, Nuez F (1998): Evaluation of whitefly-mediated inoculation techniques to screen Lycopersicon esculentum and wild relatives for resistance to tomato yellow leaf curl virus. Euphytica 101, 259–271. http://dx.doi.org/10.1023/A:1018353806051

Polston JE, Anderson PK (1997): The emergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. Plant Dis. 81, 1358–1369. http://dx.doi.org/10.1094/PDIS.1997.81.12.1358

Reina J, Morilla G, Bejarano ER, Rodríguez MD, Janssen DY, Cuadrado IM (1999): First report of Capsicum annuum plants infected by Tomato yellow leaf curl virus. Plant Dis. 83, 1176. http://dx.doi.org/10.1094/PDIS.1999.83.12.1176D

Ribeiro SG, Ambrozevicius B, Avil AC, Bezerra IC, Calegario RF, Fernandes JH, Lima MF, de Mello RN, Rocha H, Zerbini FM (2003): Distribution and genetic diversity of tomato infecting begomoviruses in Brazil. Arch. Virol. 148, 281–295. http://dx.doi.org/10.1007/s00705-002-0917-0

Rojas MR, Gilbertson RL, Russell DR, Maxwell DP (1993): Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis. 77, 340–347. http://dx.doi.org/10.1094/PD-77-0340

Saitou N, Nei M (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sanchez-Campos S, Navas-Castillo J, Monci F, Diaz JA, Moriones E (2000): Mercurialis ambigua and Solanum luteum: two newly discovered natural hosts of tomato yellow leaf curl geminiviruses. Eur. J. Plant Pathol. 106, 391–394. http://dx.doi.org/10.1023/A:1008758622582

Sawalha H (2012): Epidemiology of Tomato yellow leaf curl virus in the northern regions of the west bank, Palestine. Adv. Life Sci. Appl. (ALSA) 1, 6–12.

Shahriary D, Bananej K (1998): Occurrence of Tomato yellow leaf curl virus (TYLCV) in tomato fields of Varamin. Appl. Entomol. Phytopathol. 65, 29–30.

Stanley J, Bisaro DM, Briddon RW, Brown JK, Fauquet CM, Harrison BD, Rybicki EP, Stenger DC (2005): Family Geminiviridae. In Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (Eds): Virus Taxonomy: The Eighth Report of the International Committee on Taxonomy of Viruses. Academic Press. New York, USA, pp. 301–326.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011): MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739. http://dx.doi.org/10.1093/molbev/msr121

Ueda S, Onuki M, Hanada K, Takanami Y (2008): Unique grouping of the Far East Asian begomovirus complex based on sequence analyses of the DNA-A genome and associated DNA-ß satellite molecules isolated from tomato, honeysuckle and Eupatorium plants in Japan. Arch. Virol. 153, 417–426. http://dx.doi.org/10.1007/s00705-007-0011-8