International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.11 pp 278-286, 2016

Detection of *Tomato Yellow Leaf Curl Virus* TYLCV in some vegetable crops in greenhouses and identify its strains in the Syrian Coast

Aus Ali Hasan[1]* and Ahmad Mohammad Mouhanna[2]

[1]Biological Control Study and Research Center- Faculty of Agriculture– Damascus University- Syria.
[2]Department of plant protection - Faculty of Agriculture - Damascus University - Syria.

Abstract : Vegetable crops in greenhouses are susceptible to infection with large number of plant viruses belonging to Begomovirus especially *Tomato Yellow Leaf Curl Virus* which cause significant economic losses. The objective of this study to detect and identify TYLCV and its strains in the Syrian coast region.
The results showed the first report of the strains TYLCV-Mld and TYLCV-IL on tomato, pepper, cucumber and beans in Syria.
Phylogenetic tree for TYLCV-Mld strain showed very high similarity of some local samples with 95.6% nucleotide identities and 65% of bootstrap value. This indicated that the two local samples were infected with close strains of TYLCV-Mld and the Jordanian strain was the closest strain to the studied local strains. Also bootstrap value for local strain of TYLCV-IL was 65% with 95.4% nucleotide identities and the Israeli and Spain strains of TYLCV-IL were the closest strain to the studied local strains.
Keywords: Strains, *Tomato Yellow leaf Curl Virus*, Greenhouses, Syrian coast.

Introduction:

The protected cultivation of vegetables prevails widely all over the year, fact that makes it susceptible to a large number of plant pests such as viruses, which cause significant economic losses if compared to other plant pests, especially in the developing countries[1]. These viruses affect the quantity and quality of plant products and increase the sensitivity of plants toward biotic and abiotic factors[2].

The Begomovirus is considered the most economically significant group of plant viruses throughout the world particularly in tropical and sub-tropical regions, with 288 species currently recognized by the International Committee on Taxonomy of Viruses (ICTV)[3], it is the largest genus of all viral taxonomy. Begomoviruses infect a wide range of dicotyledonous plants as tomato, cotton, cassava, pepper[4].

Tomato yellow leaf curl disease (TYLCD) is one of the most devastating plant diseases caused by a group of viral species referred to as *Tomato yellow leaf curlvirus*(TYLCV). The disease was first reported in the Jordan valley in the 1930s[5]and now it affects more than 30 countries around the world that grow tomatoes[6]. The virus was isolated in 1988[5]and its genome sequenced in 1991[7] since then at least 43 isolates of TYLCV have been reported[8].

The TYLCV genome is a monopartite single-stranded DNA of approximately 2800 nucleotides encapsidated in geminate particles[7,9]. The TYLCV genome has an intergenic region (IR) of approximately 300

nucleotides involved in regulating viral replication and transcription and encompassing six open reading frames (ORFs) encoding the coat protein (V1 or CP), V2, the replication-associated protein (C1 or Rep), the transcriptional activator protein (C2), the replication enhancer protein (C3 or REn) and the C4 protein[10].

Transmitted exclusively by thewhitefly*Bemisiatabaci* biotypes (B, nonB, Q, M) in a persistent, circulative manner[11], but the nonB biotype considered the most efficient in transmitting the virus under greenhouse conditions[12].

The primary host for TYLCV is the tomato plant, and other plant hosts where TYLCV infection has been found include eggplants, potatoes, tobacco, beans, and peppers[6].

Symptoms of TYLCV infection include severe stunting, reduction of leaf size, upward cupping/curling of leaves, chlorosis on leaves and flowers, and reduction of fruit production. This virus can cause significant yield losses from 90-100%, and it is estimated that about 7 million hectares can experience TYLCV infection or mixed virus infections annually, Currently, the most effective treatments used to control the spread of TYLCV are insecticides and resistant crop varieties[6].

Phylogenetic analysis revealed that TYLCV isolates occur into two major clades, one clade including TYLCV-IL isolates and the other including TYLCV-Mld isolates[8]. These two strains found to share high DNA sequence identity up to 95%, except for the IR and the 5´ end of the AC1 gene of Replication Initiation Protein (*Rep)* that express the complementary-sense of genome between the nucleotides 2584-1496, which is used to differentiate between those two strains[13].

Of all the known TYLCV strains, TYLCV-IL and TYLCV-Mld have the broadest geographical ranges stretching in the Old world from Japan in the east to Spain in the west and the Indian Ocean island of Reunion and Australia in the south[14]. In the Middle East, the two strains TYLCV-IL and TYLCV-Mld, were cloned and sequenced in the 1990s. Recently, two other virus strains, Tomato yellow leaf curl Sardinia virus-Spain (TYLCSV-ES) and Tomato yellow leaf curl Sardinia virus-Sicily (TYLCSV-Sic) has been identified in this area[15].

Material and Methods:

Sample collection:

Number of leaf samples from vegetables plants showing TYLCD symptoms (leaf curling, yellowing and stunting) were collected from greenhouses in Syrian Coast area. Samples were immediately put in liquid nitrogen then kept at -20ºC prior to analysis (table 1).

Table1. Collected samples (species, symptoms and location).

Symptoms	Symbol	Plant	Location		Sample
Leaf curling	SY-Tom1	Tomato	Baniyas	Tartous	1
Leaf curling, yellowing	SY-Cuc2	Cucumber	Alqadmus		2
Plant dwarfing	SY-Pep3	Pepper	AshaykhBadr		3
Leaf curling, yellowing	SY-Cuc4	Cucumber	Safita		4
Leaf curl	SY-Tom5	Tomato	Draykish		5
Leaf crumple	SY-Bea6	Common Bean	Hosain Al Bahr		6
Leaf curl	SY-Pep7	Pepper	As-Sifsafeh		7
Plant dwarfing	SY-Tom8	Tomato	Bait Yashoot	Lattakia	8
Plant dwarfing	SY-Pep9	Pepper	Besaysin		9
Leaf crumple	SY-Bea10	Common Bean	Al Qardahah		10
Plant dwarfing	SY-Pep11	Pepper	JawbatBurghal		11
Leaf curling, yellowing	SY-Cuc12	Cucumber	Al Bahloliah		12
Plant dwarfing	SY-Tom13	Tomato	Al Hinnadi		13
Leaf curling, yellowing	SY-Cuc14	Cucumber	Fidyu		14
Plant dwarfing	SY-Tom15	Tomato	Bouqa		15
Leaf crumple	SY-Bea16	Common Bean	Ash Shabatliah		16

Leaf curling	SY-Tom17	Tomato	Burj Islam		17
Leaf curling yellowing	SY-Cuc18	Cucumber	Rasyun		18
Leaf curling	SY-Pep19	Pepper	Qatriyah		19

DNA extraction:

The Sodium Dodecyl Sulfate(SDS) method provides a good and efficient option for detection of *Begomovirus*in plants for molecular studies[16]. So DNA was extracted using this method [50 mM-EDTA, 500 mM-NaC1, 10 mM β-mercaptoethanol, 20% SDS, 5 M-potassium acetate (pH 4.5)][17]with some modification by adding 0.1 mg/ml Proteinase K.

Polymerase chain reaction (PCR):

PCR was carried out in a TC-5000 Thermocyclertechne Ltd. (UK)thermocycler. The parameters for the PCR reaction were optimized for 25 µl. All components of the PCR reaction were obtained from Promega Co. (Madison, WI, USA). The final concentrations of reaction components were: 0.25 mMdeoxynucleotide triphosphate (dNTPs), 1x *Taq*DNA polymerase buffer, 0.25 mM $MgCl_2$, 0.5 units *Taq*DNA polymerase, 2.5 µM of each complementary and virus-sense primers and 2.5 µl of DNA, and RNAse free water up to 25 µl. The PCR mixture was subjected to an initial cycle of denaturalization for 5 min at 94ºC, 40 cycles for 30 s at 94ºC for denaturation, 45 s for annealing with different temperatures due to primer (table 2), 1 min at 72ºC for the extension, and a final extension for 10 min at 72°C and PCR products were resolved by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

Table 2.Description of primers and PCR conditions used in this study.

Virus	Primer	Sequence 5'→3'	Ann.Tem	Gene	DNA Size	Reference
Geminiviridae	AV494[a]	GCCYATRTAYAGRAAGCCMAG	52°C	CP	550-bp	18
	AC1048[a]	GGRTTDGARGCATGHGTACATG				
TYLCV	TycpV369[a]	ACGCCCGYCTCGAAGGTTCG	53°C	CP	650-bp	19
	TycpC1023[a]	GTACAWGCCATATACAATAACAAGGC				
TYLCV-Mld	TYMF[b]	AAGCGCTTCCAAATAAATTG	47°C	Rep	450-bp	13
	TYMR[b]	TACTAATTCTTTAATGATTC				
TYLCV-IL	TYSF[b]	CGTTTATTTAAAATATATGCC	47°C	Rep	336-bp	13
	TYSR[b]	GGAAACTCCAAAATCAATGA				
TYLCSV-ES	TYAlmv2516[b]	TTTTATTTGTTGGTGTTTGTAGTTGAAG	62°C	MP-Rep	433-bp	20
	TYAlmc115[b]	ATATTGATGGTTTTTTCAAAACTTAGAAG				
TYLCSV-Sic	Sa2267[b]	TGGAAAGTACCCCATTCAAGAACATC	52°C	MP-Rep	946-bp	15
	RVC427[b]	TGCCTTGGACAATGGGGACAGCAG				

(**D:** A, G, T; **H:** A, C, T; **M:** A, C; **R:** A, G; **W:** A, T; **Y:** C, T)
a=Degenerate Primers b=Specific Primers

Sequencing and alignment analysis:

To confirm the identity of the amplified fragments of the 5' end of Rep gene, PCR products of TYLCV-Mld (samples 5 and 10) and TYLCV-IL (Samples 4, 7 and 15) were sequenced at National Commission for Biotechnology (Damascus, Syria) using the Big Dye sequencing kit (Applied Biosystems, Foster City, CA, USA).

These samples were chosen for sequencing depending on TYLCV-Mld produces milder disease symptoms than TYLCV-IL[21].

Pairwise sequence comparisons to determine the taxonomic position of each isolate were performed including 12 isolates of TYLCV-Mld and 15 of TYLCV-IL sequences available in GenBank as well as sequences of the most closely related viruses according to a BLAST search (Table 3).

Phylogenetic analysis was performed through multiple alignment with MegAlign (version 7) (DNASTAR,2007) using the default parameters. A neighbour-joining tree was constructed and bootstrapped using 1,000 bootstrap trials[22]. The tree was visualizedusingNJplot software. For comparison of sequence identity, an identity matrix was generated using MegAlign (version 7).

Table 3 - List of TYLCV-IL and TYLCV-Mld compared in the present study, their origin and NCBI gene accession numbers

Accession Number	Origin	Isolate
EU143745	Jordan	TYLCV-Mld
HF548825	Sweden	TYLCV-Mld
KJ913683	Dominican Republic	TYLCV-Mld
GQ861427	Jordan	TYLCV-Mld
EF054894	Jordan	TYLCV-Mld
AF071228	Spain	TYLCV-Mld
X76319	Palestine	TYLCV-Mld
AJ865337	Reunion	TYLCV-Mld
EF185318	Lebanon	TYLCV-Mld
AJ519441	Spain	TYLCV-Mld
AF105975	Poland	TYLCV-Mld
DQ302033	Venezuela	TYLCV-Mld
GU076448	Iran	TYLCV-Mld
X15656	Palestine	TYLCV-IL
LN846610	Palestine	TYLCV-IL
EF433426	Jordan	TYLCV-IL
KT099158	Spain	TYLCV-IL
AJ489258	Spain	TYLCV-IL
AY594174	Egypt	TYLCV-IL
KT921313	Egypt	TYLCV-Ty
HG969256	Oman	TYLCV-Hp
HG969287	Oman	TYLCV-Tm
GU076452	Iran	TYLCV-OM
KF356163	China	TYLCV-IL
KT852577	China	TYLCV-IL
LC099965	China	TYLCV-IL
KP685598	China	TYLCV-IL
KP684146	China	TYLCV-IL

Results:

Detection of Geminiviruses and strains of the TYLCV:

All the nineteen tested samples yielded begomovirus specific (550 bp) products when amplified by Generic-PCR using the degenerate primer pair AV494/AC1048.

The expected size of Generic-PCR products (650 bp) was amplified from all samples except samples (1, 2, 3) using the TYLCV specific degenerate primerpairs TycpV369/TycpC1023.

Two fragments of TYLCV-Mld(450 bp) and TYLCV-IL (336 bp) were amplified from tissues obtained from samples (6, 8, 11, 12, 13, 14, 16, 17, 18), whereas one band of TYLCV-Mld (450 bp) was amplified from

DNA of samples (5, 9, 10), also samples (4, 7, 15, 19) yielded one band of TYLCV-IL (336 bp), and no PCR products were amp1ified from samples (1,2 ,3) by multiplex PCR using two sets of primers TYMF/TYMR and TYSF/TYSR (fig.1).

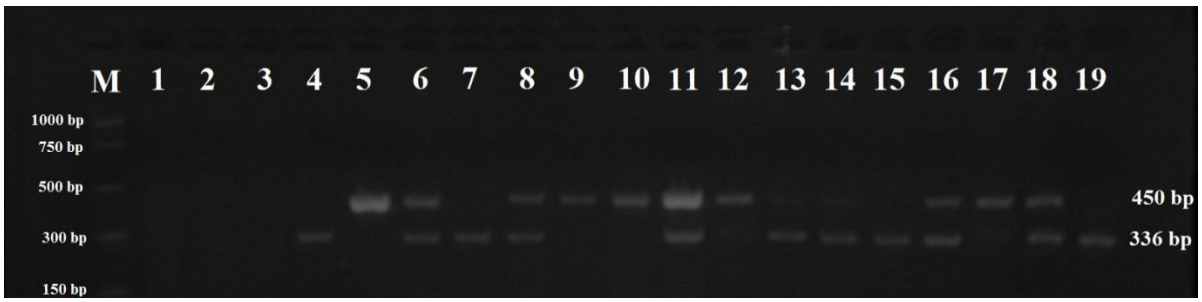

Fig (1) Theagarose gels show the Multiplex PCR products obtained with two sets of primers TYMF/TYMR and TYSF/TYSR.

No PCR products were amplified from all tested samples using the specific TYLCSV-ES primer pair TYAlmv2516/TYAlmc115 and specific TYLCSV-Sic set of primers Sa2267/RVC427.

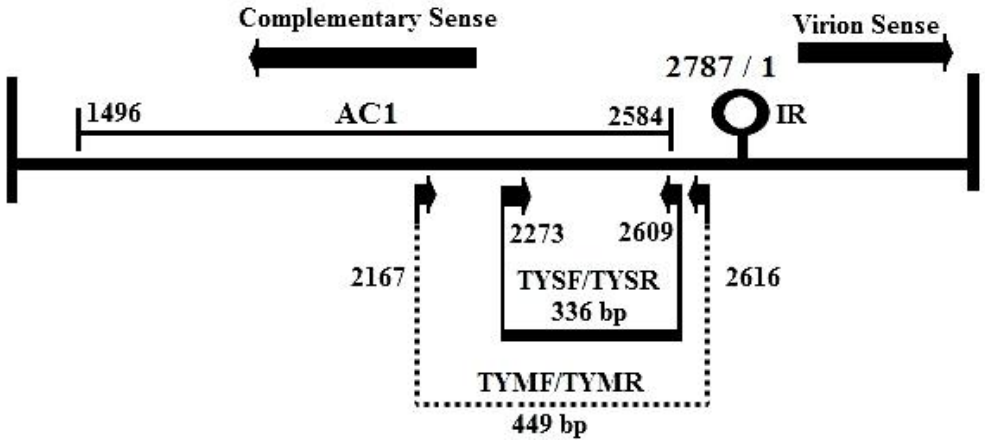

Fig.2 Relative positions of amplified fragments using two sets of primers TYMF/TYMR and TYSF/TYSR on genome of *Begomovirus*.

Sequence of Rep Gene and Phylogenetic Analysis of TYLCV-Mld:

Nucleotide sequence of 450 bp fragment amplified using set of primers TYMF/TYMR showed that this fragment extends over portion of the *Rep* 5´ end and IR from the nucleotide 2167 to 2616 (www.ncbi.nlm.nih.gov) (fig.2).

Phylogenetic analysis based on Neighbour-joining method for samples (5, 10) showed that the two samples are together in one subgroup, and this subgroup exists in same group with the Jordanian [EU143745,EF054894], Lebanese [EF185318] and Israeli [X76319] isolates. Bootstrap value on the common nude of the two samples was 65%. Due to Phylogenetic tree the Jordanian [EU143745] strain was the closest strain to the studied local strains (Fig.3).

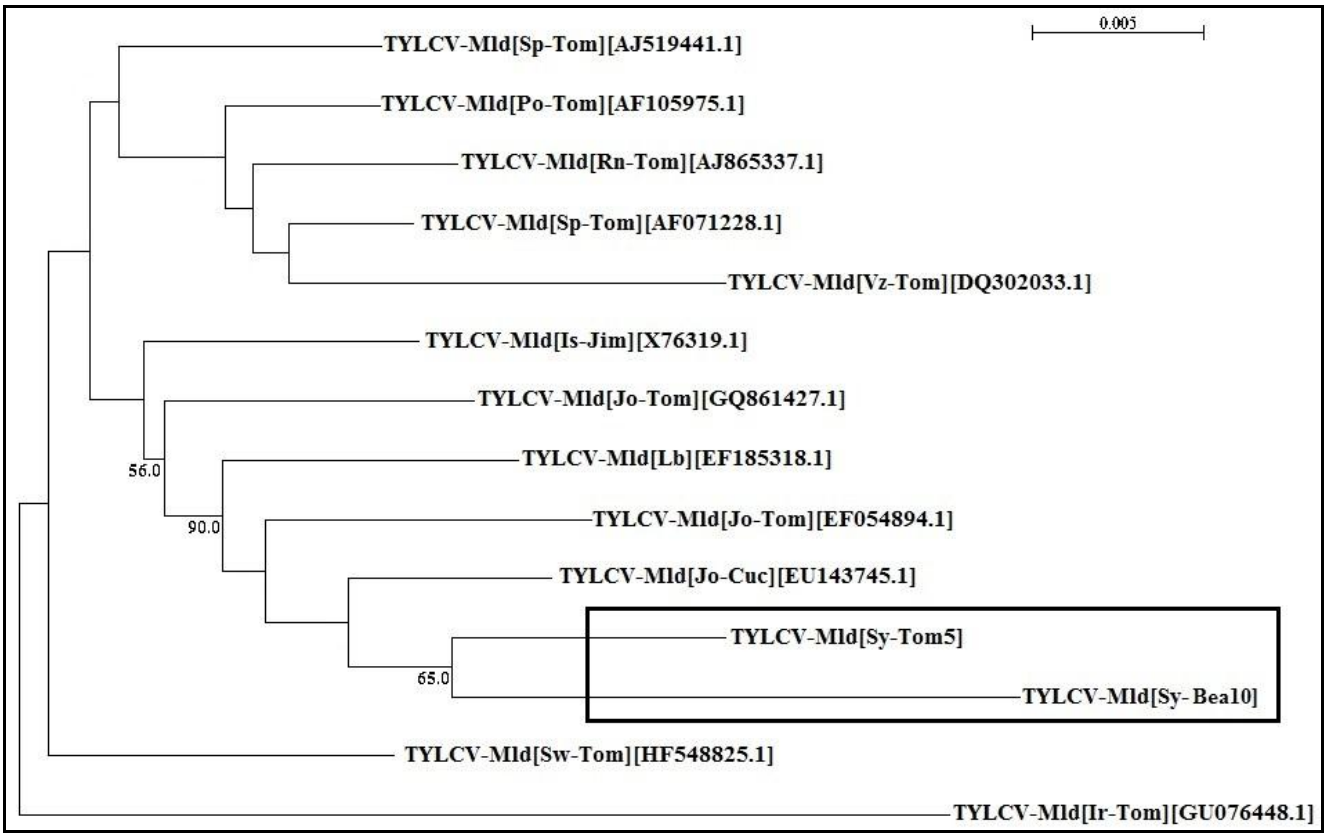

Fig.3 Phylogenetic tree showing predicted relationships between samples (5, 10) to other TYLCV-Mld isolates based on the nucleotide sequences of the Rep gene.

The sequence of samples 5 and 10 was aligned with the sequences from different geographical locations available in GenBank using MegAlign (DNASTAR). The percent nucleotide identities between the two samples was 95.6% and the Divergence was 0.0%. also The percent nucleotide identities between samples 5 and 10 with the Jordanian isolate [EU143745] was 94.4% and 97.1% respectively.

Sequence of Rep Gene and Phylogenetic Analysis of TYLCV-IL:

Nucleotide sequence of 336 bp fragment amplified using set of primers TYSF/TYSR showed that this fragment extends over portion of the *Rep* 5´ end and IR from the nucleotide 2273 to 2609(www.ncbi.nlm.nih.gov) (fig.2).

Phylogenetic analysis based on Neighbour-joining method for samples (4, 7, 15) was together in one subgroup, and this subgroup exists in same group with the Israeli [LN846610] and Spain [KT099158, AJ489258] isolates. Bootstrap value on the common nude of samples (4, 7) was 50.4% and 60.9% in the common nude with sample (15). Due to Phylogenetic tree the Israeli [LN846610] strain was the closest strain to the studied local strains (Fig.4).

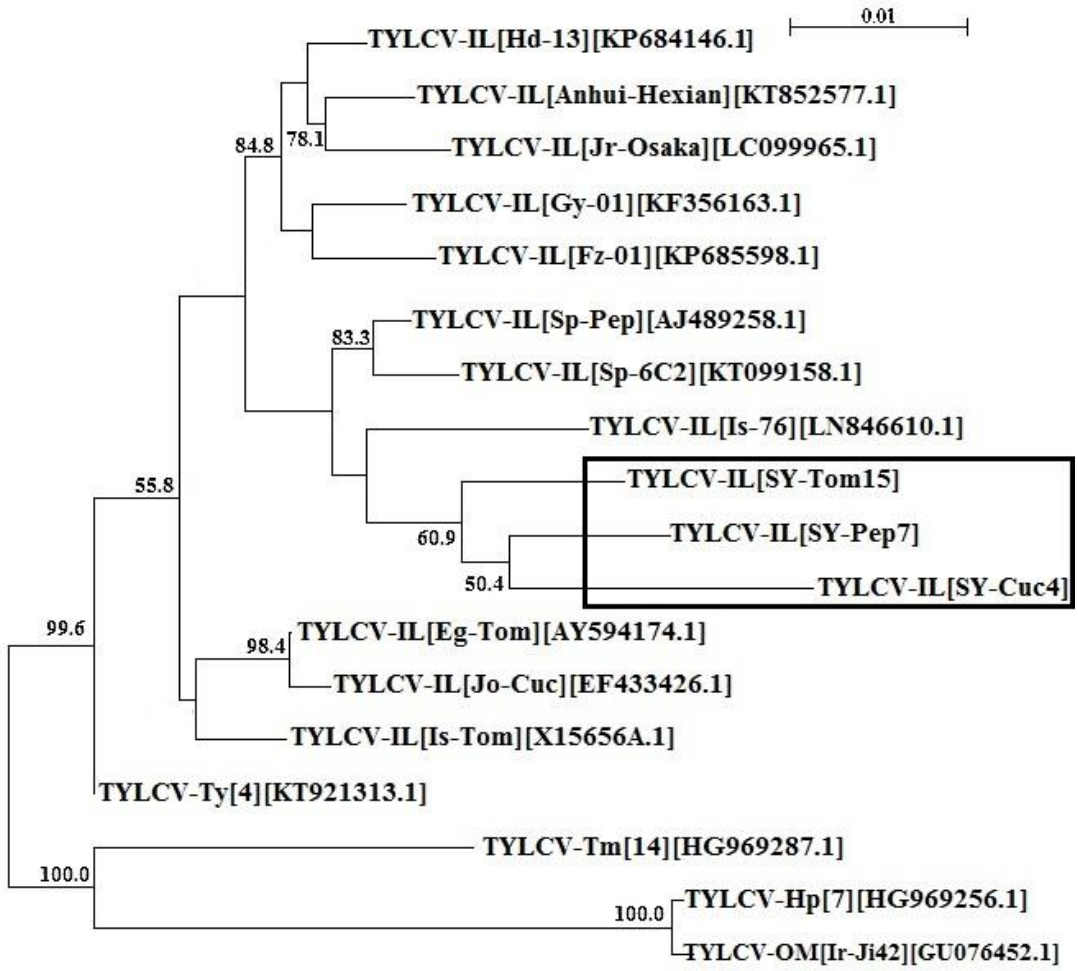

Fig.4 Phylogenetic tree showing predicted relationships between samples (4, 7, 15) to other TYLCV-IL isolates based on the nucleotide sequences of the Rep gene.

The sequence of samples (4, 7, 15) was aligned with the sequences from different geographical locations available in GenBank using MegAlign (DNASTAR). The percent nucleotide identities between the samples 4 and 7 was 91.2% and the Divergence was 3.0%. also the identities between samples 4 and 15 was 92.9% and the Divergence was 3.3%. whereas the identities between samples 7 and 15 was 95.4% and the Divergence was 2.1%.and the identities between samples (4, 7, 15) with the Israeli [LN846610] was 93.6%, 94.9% and 94.9% respectively.

Discussion:

This study aimed at detecting the strains of *tomato yellow leaf curl virus* (TYLCV) in the Syrian coast region using Polymerase chain reaction (PCR) and the study of its genetic diversity. The study was performed on 19 vegetable samples collected from greenhouses in several areas in the Syrian coast.

The Generic-PCR Results using set of degenerate primer AV494/AC1048 showed that all the nineteen samples taken from symptomatic plants are infected with viruses belonging to the family Geminiviridae. While, sixteen of the nineteen samples resulted in an amplification product in Generic-PCR using the specific degenerate primers TycpV369/TycpC1023for TYLCV. And we could say that samples [1 (tomato), 2 (cucumber), 3 (pepper)], which in turns showed similar symptoms to TYLCV symptoms, could be infected with some other viruses belonging to the family Geminiviridae.

The results of Multiplex PCR confirmed that samples [common bean (6, 16), tomato (8, 13, 17), cucumber (12, 14, 18), pepper (11)] have mixed infection with both strains TYLCV-Mld and TYLCV-IL, while none of the samples resulted any PCR fragments using the specific TYLCSV-ES primer pair TYAlmv2516/TYAlmc115 and specific TYLCSV-Sic set of primers Sa2267/RVC427. This may indecate that strains TYLCV-Mld and TYLCV-IL are the most common strains in Syrian coast region and this agrees with what Anfoka[15]confirmed that both strains TYLCV-Mld and TYLCV-IL are the most common strains of TYLCV in the Middle East region.

The determination of nucleotide sequences of virus strains and its comparing to other isolates available on GenBank give more accuracy and reliability for the identification of virus strains, and its genetic divergence to the other local and global strains for the purpose of determination of the source of viral infection and identification of whether the infection caused by new emerge strains of virus or just spread out from neighboring countries by geographical distribution of its insect vector *Bemisiatabaci.*

For studying the genetic diversity of the two local strains the nucleotide sequences ofportion of the *Rep* 5´ end and IR were chosen; which are very different among the strains of TYLCV-Mld and TYLCV-IL. Phylogenetic analysis showed a very high similarity of the two local samples 5 and 10 with 95.6% nucleotide identities and 0.0% of divergence.

Bootstrap value of the two local samples was 65%. This indicates that the two local samples are infected with close strains of TYLCV-Mld. By comparing the nucleotide sequences of the two samples to some isolates available on GenBank the Jordanian [EU143745] strain was the closest strain to the studied local strains (Fig.3). The nucleotide percentage of identities was also the highest for the Jordanian [EU143745] strain with 94.4% and 97.1% for samples 5 and 10 respectively.

Phylogenetic tree showed substantial covariance predicted between the three samples of the local strain (4, 7, 15). But there was a strict group between samples 4 and 7 with 91.2% percent nucleotide identities and 50.4% bootstrap value. This subgroup was in same group with sample 15 and bootstrap value was 60.9%. the strict grouping could be found according to geographic origin because both samples 4 and 7 originated from nearby places (Safita and As-Sifsafeh in Tartous governorate whereas sample 15 collected from relatively farther place (Boka in Lattakia governorate) (Table.1). By comparing the nucleotide sequences of local strains to other strains available in GenBankthe Israeli [LN846610] strain was the closest strain to the studied local strains.

This suggests that the two PCR fragments of the strains TYLCV-Mld and TYLCV-IL are amplified from relatively conserved regions on the genome of virus. This agrees with what Al-Abdallat[20]mentioned that rely on the end 5' of Rep gene can be used as a suitable indicator for the studying of divergence of nucleotide sequences as well as the identification of strains of TYLCV originated from different geographical areas.

This is the first study in Syria for the detection and identification of TYLCV and its strains, and this is the first report of the strains TYLCV-Mld and TYLCV-IL on tomato, pepper, cucumber and common bean in Syria. However, it is essential for this study to be continued to determine the complete nucleotide sequences for the genome of local strains in order to compare with other strains in spread in the neighboring countries like Lebanon, Jordan and other countries.

Also it is necessary to perform future studies aiming to identify and detect all strains of TYLCV that infect a large number of plants in Syria, both in greenhouses or open fields, causing huge economic losses.

References.

1. Rybicki ED, Pietersen G. Plant Virus Disease Problems in The Developing World. Advances in virus research., 1990, 50; 183-234.
2. Mauck KE. Infection of host plants by Cucumber mosaic virus increases the susceptibility of *Myzus persicae* aphids to the parasitoid *Aphidius colemani*. *Sci. Rep.* **5**,10963; doi: 10.1038/srep10963, 2015.
3. King AM, AdamsQ, Carstens MJ, Lefkowitz EB, E. J. Virus Taxonomy: Classification and Nomenclature of Viruses – Fifth Version of Ninth Report of the International Committee on Taxonomy of Viruses., 2015, In: Elsevier Academic Press, USA. http://www.ictvonline.org/virusTaxonomy.asp

4. Polston JE, Anderson PK. The emergence of whitefly-transmitted *Geminiviruses* in tomato in the Western Hemisphere, Plant Dis., 1997, 81; 1358 – 1369.
5. Czosnek H, Ber R, Antignus Y, Cohen S, Navot N, Zamir D. Isolation of Tomato Yellow Leaf Curl Virus, a Geminivirus. The American Phytopathological Society. Phytopathology., 1988, 78; 508-512.
6. Glick M, Levy Y, Gafni Y. The Viral Etiology of Tomato Yellow Leaf Curl Disease-A Review. Plant Protection Sciences., 2009, **3**; 81–97.
7. Navot N, Pichersky E, Zeidan M, Zamir D, Czosnek H. *Tomato yellow leaf curl virus*: a whitefly-transmitted geminivirus with a single genomic component. Virology., 1991, 185; 151-161.
8. Fauquet, CM, Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X. Geminivirus strain demarcation and nomenclature. Archives of Virology., 2008, 153; 783-821.
9. Gronenborn B. *Tomato yellow leaf curl virus,* genome and function of its proteins. In, Tomato yellow leaf curl virus disease, edited by H. Czosnek, Springer., 2009, P; 329-342.
10. Gafni, Y. *Tomato yellow leaf curl virus,* the intracellular dynamics of a plant DNA virus. Molecular Plant Pathology., 2003, 4; 9-15.
11. Wei J, Zhao J, Zhang T, Li FF, Ghanim M, Zhou XP, Ye GY, Liu SS. Specific Cells in the Primary Salivary Glands of the Whitefly *Bemisiatabaci* Control Retention and Transmission of *Begomoviruses*, Journal of Virology., 2014, 88; 13460–13468.
12. Yousef A, Mouhanna AM, Barhum HS. Efficiency of different Biotypes of *Bemisia tabaci* Genn. that spread in the Syrian coast in the transmission of mild isolate of TYLCV. International Journal of ChemTech Research., 2016, 9.
13. Lefeuvre P, Hoareau M, Delatte H, Reynaud B, and Lett JM. A multiplex PCR method discriminating between the TYLCV and TYLCV-Mld clades of *tomato yellow leaf curl* virus. Journal of Virologica lMethods., 2007, 144; 165-168.
14. Lefeuvre P, Martin DP, Harkins G, Lemey P, Gray AJA, Meredith S, Lakay F, Monjane A, Lett J, Varsani A, Heydarnejad J. The Spread of Tomato Yellow Leaf Curl Virus from the Middle East to the World. PLoS Pathogens., 2010, 6. e1001164
15. Anfoka GH, Abhary M, Haj Ahmad F, HusseinAF, Rezk A, Akad F, Abou-Jawdah Y, Lapidot M, Vidavski F, Nakhla MK, Sobh H, Atamian H, Cohen L, Sobol I, Mazyad H, Maxwell DP, Czosnek H. Survey of Tomato Yellow Leaf Curl Disease-Associated Viruses in the Eastern Mediterranean Basin. Journal of Plant Pathology., 2008, 90; 313-322.
16. Hasan AA, Mohanna AM. Evaluating of several methods to DNA extract from pepper plants *Capsicum annuum* L. to detect viruses belonging to the genus *Begomovirus*. Journal of Al Baath University.2016, 38.
17. Gilbertson R, Rojas M, Russell D, Maxwell D. Use of the asymmetric polymerase chain reaction and DNA sequencing to determine genetic variability of bean golden mosaic *Geminivirus* in the Dominican Republic. Journal of General Virology., 1991, 72; 2843- 2848.
18. Khan AJ, Al-Saady NA, Al-Mahruki S, Al-Oufi M, Al-SubhiAM. Molecular characterization of *Begomovirus* infecting sweet pepper in Oman. Indian Journal of Biotechnology., 2007, 6; 45-51.
19. Wyatt SD, Brown JK, Detection of subgroup III *Geminivirus* isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathology., 1996, 86; 1288-1293.
20. Anfoka GH, Abhary M, Nakhla MK. Molecular Identification of Species of the Tomato Yellow Leaf Curl Virus Complex in Jordan. Journal of Plant Pathology., 2005, 87; 65-70**.**
21. Al-Abdallat, AM, Al-Debei H, Akash M, Misbeh S, Kvarnheden A. Complete Nucleotide Sequences and Construction of Infectious Clones of Two Jordanian Isolates of Tomato Yellow Leaf Curl Virus. Jordan Journal of Agricultural Sciences., 2011, 7; 273-283.
22. Saitou N, Nei M. The neighbor joining method, a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution., 1987, 4; 406-425.

*****