Genetic Relatedness among Iraqi, Jordanian and Iranian TYLCV Based on the Identity Analysis

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ABSRACT--- ***To look for genetic relatedness among a new iraqi Tomato Yellow Leaf Curl Virus (TYLCV) and the Jordanian and Iranian ones, identity coefficient was calculated. Amplification Chain Reaction (PCR) technique was used for this purpose from degenerated primers of the begomoviruses coat protein gene. A identity from 94 to 95% was calculated, indicating that the Iraqi isolate is effectively a TYLCV. From the viewpoint of the phyletic relationships, the identity between Iraqi and Iranian TYLCV isolates was 95%, as against 85% for those from Iraq and Jordan. However, it was showed that DNA from Iraqi is not of recombinant origin, implying that these three isolates separately evolve.***

***Keywords* ---** Begomovirus, Geminivirus, PCR amplification products, Basrah, electorphoresis profile

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is the most extensively grown horticultural crop in the world after potato. Present world productions about 100 million tons fresh fruit produced on 3.7 million hectares (FAOSTAT Database, 2004). However, due to its continuous large-scale production throughout the year, it has become susceptible to a number of pathogens, limiting its production. Apart from a number of bacterial and fungal pathogens which cause severe infections on tomato, it is also infected by a number of viruses. Among the virus carriers white fly, have become the most important in the tropics and subtropics.

All members of the geminiviridae family have circular, single-stranded DNA genomes that are approximately 2.7 kb in length. This encapsidated within twinned (geminate) icosahedral particles. Geminiviruses can be either monopartite or bipartite (Stanley *et al.,* 2005*).* The most devastating disease of tomato is caused by the tomato yellow leaf curl virus (TYLCV). TYLCV is the generic name given to a complex of viral species occurring in tropical and subtropical regions that cause severe disease. In the world, the yielding losses, caused by this virus, in the order of 100% are very economically important (Glicke *et al.*,2009) .

TYLCV symptoms appear several weeks after infection. They include severe stunting, a marked reduction leaf size, upward cupping and chlorosis of the leaf margins mottling, flower abscission and significant yield reduction. The disease was first reported in the Jordan Valley in the 1930s and it is now a serious problem to cultivation of tomato world-wide. TYLCD is associated with outbreaks of the whitefly *Bemisia tabaci*, the insect vector that transmits the virus in a persistent and circulative manner (Cohen and Nitzany, 1966). In 1988, TYLCV was isolated, characterized and identified as a member of the family Geminiviridae, genus Begomovirus (Czosnek *et al*., 1988). In the early 1990s, the TYLCV genome was sequenced and characterized (Navot *et al*., 1990).

And since then several TYLCV strains and isolates have been identified.

Because TYLCV and other geminiviruses are distributed worldwide in tropical and subtropical regions. They cause serious damages to tomato plantations. The screening and early diagnoses of viral diseases are therefore very necessary to avoid enormous yield losses.

The current approaches to detect viral infection are based on indicator plants or serological assays which in some cases like whitefly- transmitted geminiviruses have not been sufficiently effective. The vast difficulties to obtain sufficient quantities of viral antigens for the production of antiserum, together with the imprecise evaluation of symptoms, makes molecular procedures an important tool for an accurate detection of plant viruses. Using PCR-based

methods, many difficulties associated with serological methods can be overcome (Lisset Herrera *et al.*, 1999). The analysis of DNA sequences has become the tool of choice, allowing the accurate identifying of the virus and evaluating of its relationship with other TYLCV isolates.

Comparisons of the sequence of geminiviral products have been used to construct phylogenetic trees. These analyses have shown that new world and old world whitefly-transmitted geminiviruses are of two distinct groups. Among them, the TYLCVs were grouped according to a geographical gradient comprising isolates from the Eastern Mediterranean basin, the middle east and south east Asia.

Sequence comparisons have also suggested that name TYLCV may cover different virus species such as TYLCV-ITA-Sar, TYLCV-THA etc. (Czosnek and latterrot, 1997).

In iraq, the yield losses caused by TYLCV on tomato, in the order of 80%, are very high. In Iraq the total tomato-cultivating areas increase gradually so there are many Agricultural pests that infect tomato plants, among them plant viruses, especially tomato yellow leaf curl virus (TYLCV) which has become a limiting factor in tomato production in Basra, South of Iraq. Moreover, no information is available about TYLCV strains which attack tomatoes in Basra.

It seems that Iraq-TYLCV might be a recombinant of that from Iran or Jordan. The knowledge of the genetic relatedness among Iraqi, Jordanian and Iranian TYLCV could allow the tracing of evolutionary TYLCV history in the region.

The goal of this study was to provide information for Basra isolate of TYLCV and to establish the genetic relatedness among Iraqi, Jordanian and Iranian TYLCV using identity coefficient.

2 MATERIALS AND METHODES

Experimental site and collection of the plant samples

Shoot samples were collected from tomato plants grown in plastic houses in the province of Basra – south of Iraq. In all, 17 tomato plant samples with yellowing and curling disease symptoms were collected, representing 17 varieties of tomato from different major tomato-growing areas in Basra. The fresh plant samples were brought to molecular genetics lab. College of agriculture – university of Basra, and were stored at -20˚c until analyzed .

Total DNA was extracted from the collected leaves using a CTAB method (Doyle and Doyle, 1987). Seventeen symptomatic tomato samples were tested for begomovirus infection by using PCR with degenerate begomovirus primer (Navot *et al.*,1991) .
This test was done in the faculty of medicine and Biological Science – university of Leicester - United Kingdom.

Viral DNA Detection using PCR

To confirm the presence of TYLCV DNA in infected plants, polymerase chain reaction (PCR) was run using specific TYLCV primer. The DNA extracts were used as template for PCR amplification of about 400pb long DNA fragment.

The primer was commercially obtained from Bioneer corporation, Korea(http://eng.bioneer.com/) .

The optimized PCR procedure was carried out in a 20ml reaction volume containing 1µl of each primer, 5µl of plant DNA, 5µl master mix, and an appropriate volume of deionized H_2O to make up to 20ml. The optimal conditions for amplification were as follows :

Initial denaturation at 95˚c for 4min, followed by 35 cycles of denaturation at 95˚c for 30 sec, annealing at 60˚c for 30 sec, extension at 72˚c for 30 sec, and a final extension at 72˚c for 10 min. PCR was carried out in a thermocycler machine (CS cleaver GTC). PCR products were fraction-ated and assessed on 1%(w/v) TEB agarose gels. (Navot *et al.*, 1991) .

V61 F:5-ATA CTT GGA CAC CTA ATG G-3

R:5- AGT CAC GGG CCC TTA CAA -3

Sequence and phylogenetic Analysis

Sequence was compared with the GenBank database using the BLAST programs available at the National center for biotechnology information (NCBI)., The translation of sequence was done by using the serial cloner program.

Phylogenetic analysis was carried out using Clustal W program

3. RESULT AND DISCUSSION

Identification of Iraqi TYLCV by PCR and sequence analysis

Six tests achieved from six samples yielded the expected band of ~400bp, indicating infection by a begomovirus. These six samples were from Basra – south of Iraq (Fig 1,2) .

To identify the begomoviruses, PCR amplified products was cloned and sequenced for one tomato sample, representing the supermarimond variety of tomato which was widespread in the south of Iraq.

Sequence comparisons showed that the PCR fragment of tomato sample for supermarimond variety- Basrah showed 95% nucleotide sequence identity to TYLCV isolated from Iran (accession number GU076441.1) Lefeuvre *et al*., (2010) and 94% identity to many TYLCV isolates of different geographic origin EU834061.1 from Iran, and FJ956705, DQ644565.1 from Oman (Fazeli *et al*., 2009; Idris & Brown, 2010 ; Khan *et al*. 2007 respectively). and 89% identity to TYLCV isolated access. JN6800353.1 from Mexico (Banuelos-Hemandez *et al.*, 2012) and 87% identity to isolate AB1166353.1 from Japan (Uedo, S. *et al*., 2004) and 86% identity to isolate DQ317747.1from Mediterranean basin (Garcia –Andress, *et al*., 2007;Table 1)

This isolate was therefore named TYLCV-Bsr and is the first sequence identification of TYLCV in Iraq. Fig. 4,5

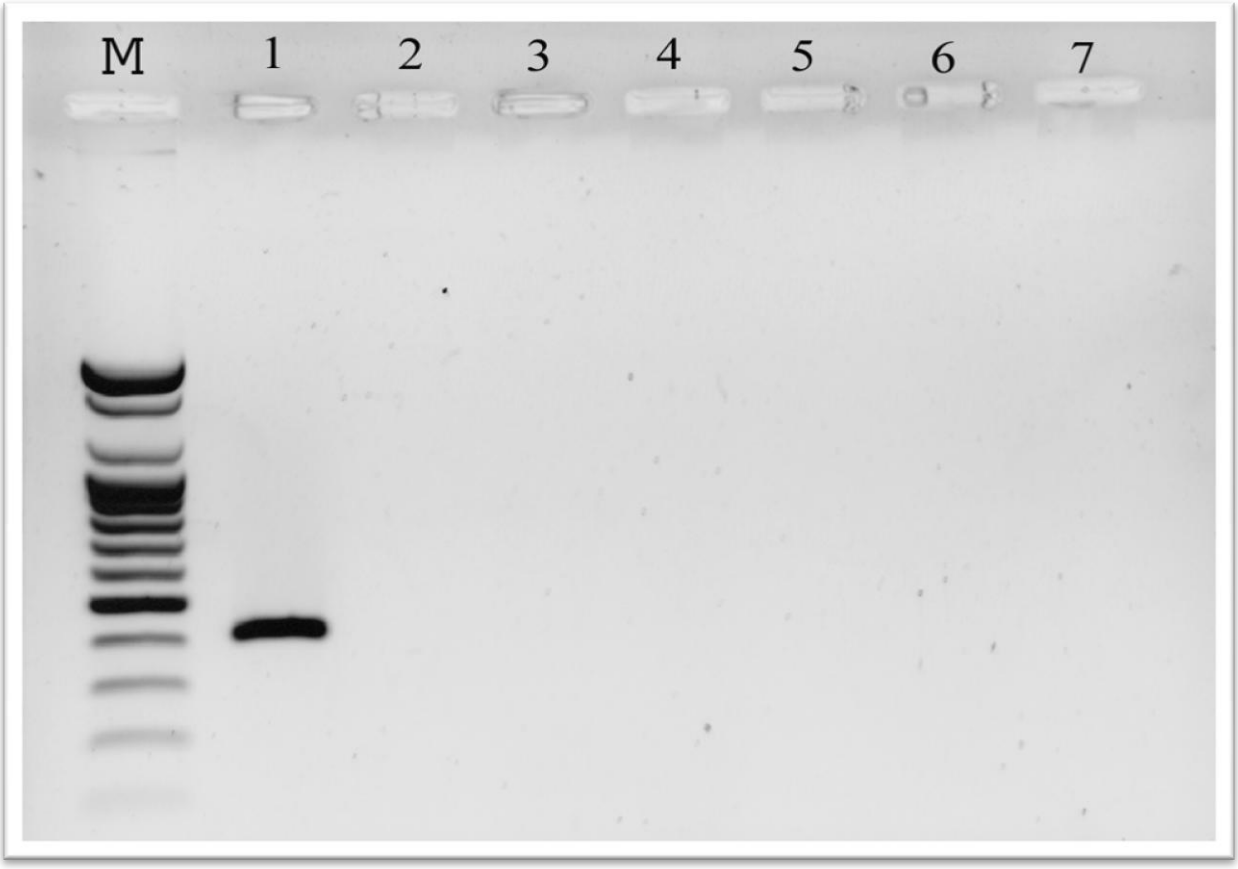

Figure 1 : Electrophoresis profile of the PCR amplification products achieved from leaf samples to detect TYLCV.

M : Marker 1000bp .
Lane 1: super marimond variety .
Lane 2,3,4,5,6,7 : other cultivars.

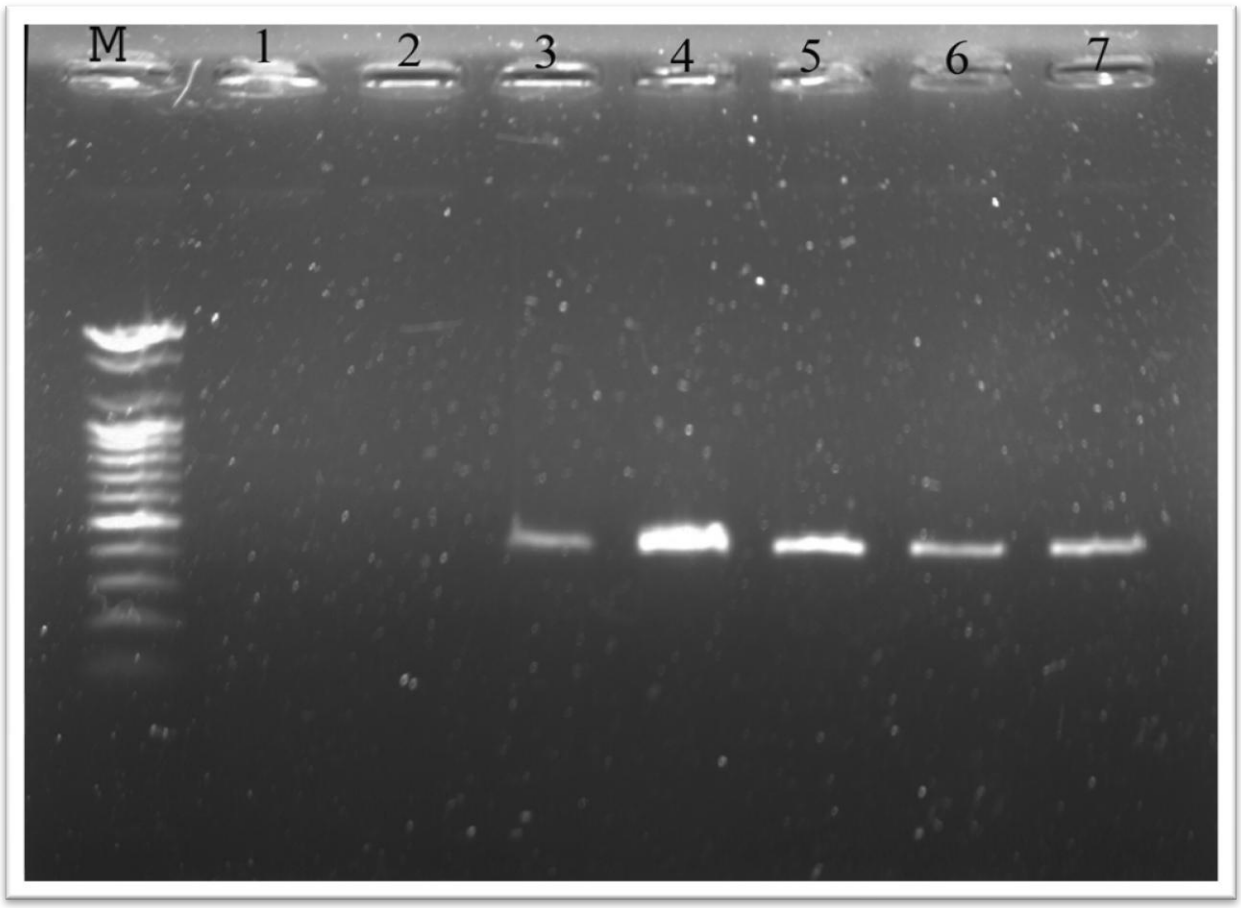

Figure (2) : Electrophoresis profile of the PCR amplification products achieved from leaf samples to detect TYLCV.

M : Marker 1000bp
Lane 1 : Semona .
Lane 2 : 942 .
Lane 3 : AL-Majid .
Lane 4 : Samara .
Lane 5 : Shedy Lady .
Lane 6 : Sultana .
Lane 7 : Aula .

PCR tests with degenerated primer revealed the presence of begomoviruses in six out of 17 symptomatic tomato varieties from the south of Iraq. The reason why not all of the varietiessamples tested positive for begomoviruses may be infections by RNA viruses or by other begomoviruses . The results of this study confirmed the presence of TYLCV in south of Iraq (Basra). And this is the first sequence identification of TYLCV in Iraq. The completely sequenced isolate (TYLCV-BSR) showed a close relationships with other virus isolates from the TYLCV "prototype" strain Fig 3. The high sequence identity to TYLCV throughout the genome shows that TYLCV-BSR is not of recombinant origin.

For the future, it will be important to sequence more isolates of TYLCV – causing viruses of Iraq to monitor the viral genotypes, and to be able to follow possible changes in the virus population structure. It would be interesting to study the genetic diversity of all TYLCV infecting tomato farms in this region.

Table (1) : results of search by BLAST program

Tylcv Strain	Accession	Percent	Author	Country
TYLCV-IR1	GU076441.1	%95	Lefeuvre,P., *et al.,*	Iran
TYLCV-IR2	EU834061.1	%94	Fazeli,R., *et al.,*	Iran
TYLCV-OM1	FJ956705.1	%94	Idris,A.M. and Brown,J.K.	Oman
TYLCV-OM2	DQ644565.1	%94	Khan,A.J., *et al.*	Oman
TYLCV-IR3	EU085423.2	%92	Azizi,A., *et al.,*	Iran
TYLCV-OM3	FJ956703.1	%92	Idris,A.M. and Brown,J.K.	Oman
TYLCV-ETH	DQ358913.1	%91	Shih,S.L., *et al.,*	Ethiopia
TYLCV-SU	AY044137.1	%90	Idris,A.M. and Brown,J.K.	central Sudan
TYLCV-REU	AJ842307.1	%90	Delatte,H., *et al.,*	Reunion Island
TYLCV-WA	AB669434.1	%90	Chung,B.N., *et al.,*	West of Asia
TYLCV-CH	FN256256.1	%90	Zhang,H., *et al.,*	China
TYLCV-KO	AB636410.1	%90	Chung,B.N., *et a.,l*	Korea
TYLCV-POR	AF105975.1	%90	Navas-Castillo,J., *et al.,*	Portugal
TYLCV-MEX	JN680353.1	%89	Banuelos-Hernandez,B. *et al.,*	Mexico
TYLCV-JAP	AB116636.1	%87	Ueda,S., *et al.*	Japan
TYLCV-MED	DQ317747.1	%86	Garcia-Andres,S., *et al.,*	Mediterranean basin
TYLCV-JO	EU143754.1	%85	Anfoka,G., *et al.,*	Jordan

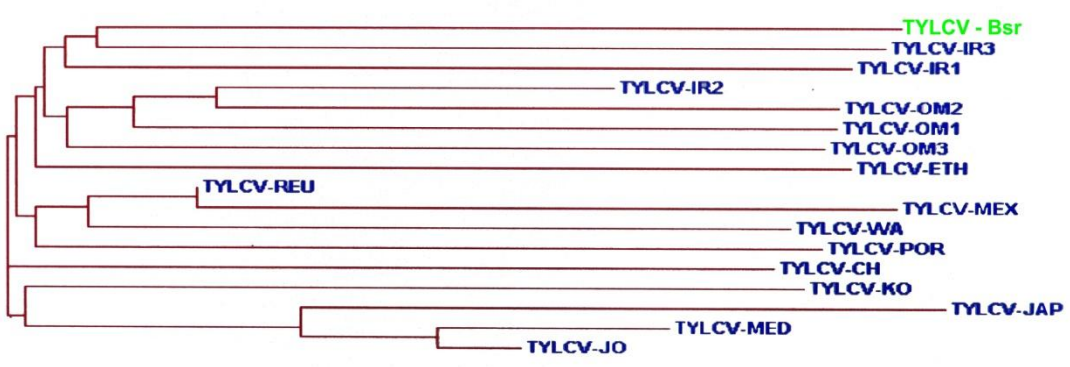

Fig 3 (Phylogenic tree)

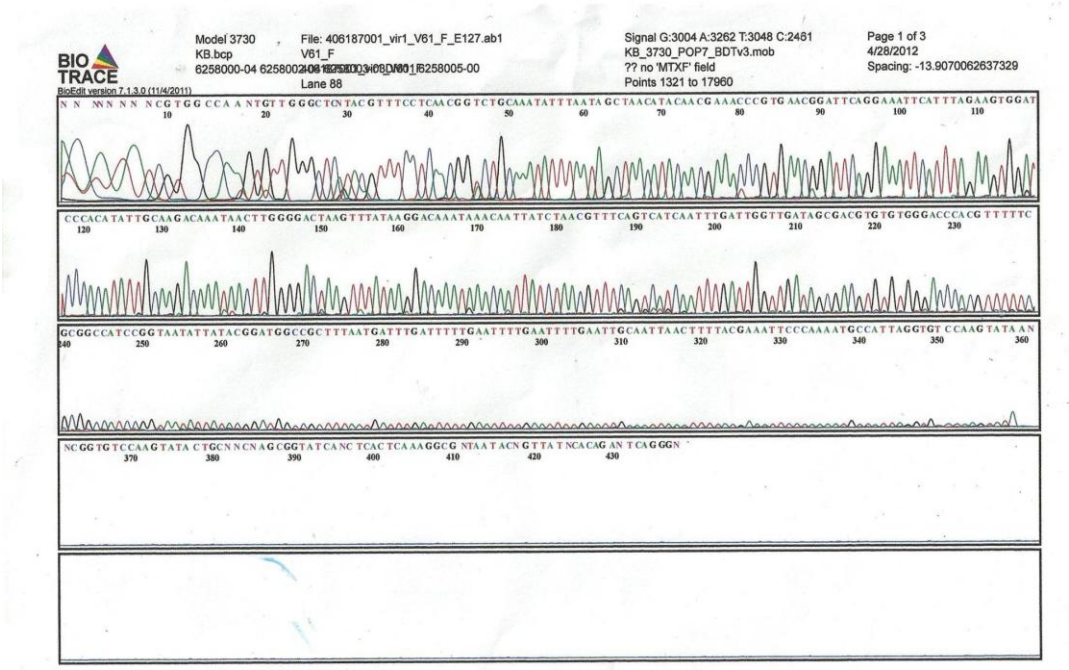

Fig 4 (Chart of TYLCV Sequences)

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Alignment: E:\sequence london\406187001_vir1_V61_F_E12.seq

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        10         20         30         40         50
406187001_      NNNNNNNNCG TGGCCAANTG TTGGGCTCNT ACGTTTCCTC AACGGTCTGC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        60         70         80         90        100
406187001_      AAATATTTAA TAGCTAACAT ACAACGAAAC CCGTGAACGG ATTCAGGAAA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       110        120        130        140        150
406187001_      TTCATTTAGA AGTGGATCCC ACATATTGCA AGACAAATAA CTTGGGGACT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       160        170        180        190        200
406187001_      AAGTTTATAA GGACAAATAA ACAATTATCT AACGTTTCAG TCATCAATTT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       210        220        230        240        250
406187001_      GATTGGTTGA TAGCGACGTG TGTGGGACCC ACGTTTTTCG CGGCCATCCG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       260        270        280        290        300
406187001_      GTAATATTAT ACGGATGGCC GCTTTAATGA TTTGATTTTT GAATTTTGAA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       310        320        330        340        350
406187001_      TTTTGAATTG CAATTAACTT TACGAAATTC CCAAAATGCC ATTAGGTGTC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       360        370        380        390        400
406187001_      CAAGTATAAN NCGGTGTCCA AGTATACTGC NNCNAGCGGT ATCANCTCAC

                ....|....| ....|....| ....|....| ....|.
                       410        420        430
406187001_      TCAAAGGCGN TAATACNGTT ATNCACAGAN TCAGGG
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Fig 5 (TYLCV Sequences)

REFERENCES

1. Ban˜uelos-Herna´ndez B., Mauricio-Castillo, J.A., Cardenas-Conejo, Y., Guevara-González, Arguello Astorga. , G.R. (2012). A new strain of tomato severe leaf curl virus and a unique variant of tomato yellow leaf curl virus from Mexico. Arichives of Virology ,158(9):1835-1841.
2. Cohen, S., and Nitzany, F. E. (1966). Transmission and host range of the tomato yellow leaf curl virus. Phytopathology 56:1127-1131.
3. Czosnek H, Ber R, Antignus Y, Cohen S, Navot N and Zamir D (1988) Isolation of tomato yellow leaf curl virus, a geminivirus. Phytopathology 78:508-512.
4. Czosnek, H. and Laterrot, H. (1997). A worldwide survey of tomato yellow leaf curl viruses. *Archives of Virology* 142, 1391-1406.
5. Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. Phytochemical Bulletin. *1*9: 11-15.
6. FAOSTAT Database (2004) Tomato world production statistics http:///www.growtomatoes.com/world_production_statistic s. htm.
7. Fazeli, R., J. Heydarnejad, H. Massumi, M. Shaabanian and A. Varsani. (2009). Genetic diversity and distribution of tomato infecting begomoviruses in Iran. Virus Genes 38(2):311-9.
8. García-Andrés, S., Acotto,G.P., Navas-Castillo, J., and Moriones E. (2007).Founder effect , plant host , and recombination shape the emergent population of Begomoviruses that cause the tomato yellow leaf curl disease in Mediterranean basin. Virology 359 (2), 302-312.
9. Glick E., Levy Y., Gafni Y. (2009): The viral etiology of tomato yellow leaf curl disease – a review. Plant Protect. Sci., 45: 81–97.
10. Herrera, L., Guerra, O., Ramos, P., Peral, R., Echemendia, A, L., Ramirez,N.,Dreste,V.,Oramas,P.,(1999). Molecular Techniques for the Detection of Tomato Yellow Leaf Curl Geminivirus in Infected Plants and Viruliferous Whiteflies Biotecnología Aplicada 16:237-241.
11. Idris,A.M.and Brown,J.K.(2010). Anovel recombinant species of Begomovirus is associated with Tomato yellow leaf curl disease in Oman : Molecular characterization and infectivity analysis .J. Gen. Virol. In press
12. Lefeuvre,P., Martin,D.P., Harkins,G., Lemey,P., Gray,A.J., Meredith, S., Lakay, F., Monjane, A., Lett, J.M., Varsani, A. and Heydarnejad, J. (2010).The spread of Tomato yellow leaf curl virus from the Middle East to the world . PLoS Pathog. 6 (10), E1001164.
13. Navot N, Pichersky E, Zeidan M, Zamir D and Czosnek H (1991) Tomato yellow leaf curl virus: a whitefly-transmitted geminivirus with a single genomic component. Virology 185: 151–161.
14. Stanley J., Bisaro D.M., Briddon R.W., Brown J.K., Fauquet C.M., Harrison B.D., Rybicki E.P. Stenger D.A. (2005): Geminiviridae: Virus Taxonomy. In: Ball L.A. (ed.): VIIIth Report International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London: 301–326.
15. Ueda S., Kimura T., Onuki M., Hanada K., Iwanami T., 2004. Three distinct groups of isolates of Tomato yellow leaf curl virus in Japan and construction of an infectious clone. Journal of General Plant Pathology. 70: 232-238.