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Identification and molecular characterization of *Tomato Yellow Leaf Curl Virus*-EG

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Abstract: *Tomato Yellow Leaf Curl virus* (TYLCV-Eg) was isolated from whiteflies-infected tomato (*Lycopersicon esculentum* cv.Castle rock) plants growing in Nubaria and El-Behera Governorate. The infected plants exhibited systemic viral symptoms in the form of sever leaf curling, leaf crinkle with marginal yellowing, stem upright, twisted and stunted. TYLCV-Eg reacted positively with polyclonal antibodies specific to TYLCV using DAS-ELISA. It was transmitted by both syringe injection and whiteflies with transmission efficiency of about 80% and 100%, respectively. TYLCV-Eg isolate was transmitted to different species belonging to families *Cucurbitaceae, Fabaceae, Solanaceae* and *Chenopodiaceae.* TYLCV had TIP of 70ºC, DEP of 10^{-7} and LIV of about 6 days. Electron micrograph of the partially purified TYLCV revealed the presence of monomer and dimmer gemini particles with dimensions of 22 nm and 20 × 30 nm to 24 × 30 nm, respectively when negatively stained with uranyl acetate. Using degenerate oligonucleotide primers, the viral coat protein gene was amplified successfully by PCR, producing ~ 500 bp fragment from tomato infected plants. The viral genome was detected by specific DNA probe using dot blot hybridization technique. Comparative nucleotide sequence analysis showed a similarity of 98% between TYLCV-Eg and other isolates.

Key words: TYLCV-EG, DAS-ELISA, PCR, Dot-blot hybridization, and Nucleotide sequence

التعريف والتوصيف الجزيئى لفيروس تجعد واصفرار اوراق الطماطم – العزلة المصرية

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الملخص:سجلت هذه الدراسة عزل وتعريف فيروس تجعد واصفرار أوراق الطماطم احد اخطر الفيروسات المنقولة بحشرات الذبابة البيضاء و وقد ظهرت الأعراض الفيروسية الجهازية معروضة في شكل تجعد واصفرار بين العروق والتواء حواف الأوراق والتواء الساق وتقزم نباتات الطماطم المنزرعة في محافظتي النوبارية والبحيرة بجمهورية مصر العربية و أظهرت النتائج رد فعل إيجابي باستخدام الأجسام المضادة المتعددة التخصصية specific polyclonal antibodies للكشف عن الفيروس بالطريقة السير ولوجيه DAS-ELISA وقد أشارت الدراسة إلى أن الفيروس يمكن أن ينتقل ميكانيكيا بالمحقن وأيضا عن طريق حشرة الذبابة البيضاء بكفاءة نقل حوالي 80% و100% على التوالي. وقد أوضحت الدراسة أن الفيروس يصيب أنواع نباتية مختلفة تابعه للعائلات القرعيه ,البقولية, الباذنجانية والزربيحيه وبدراسة الخواص الفيزيائية للفيروس قيد الدراسة تبين أن درجة الحرارة المثبطة لنشاطه هي 70ºCونقطة التخفيف التي تفقده القدرة على إحداث العدوى هي 10^{-7} وكذلك يستطيع البقاء حيا في درجة حرارة الغرفة لمدة أربعة أيام. وقد اظهر الفحص بالميكروسكوب الالكتروني وجود جزيئات مفرده وتوأميه أبعادها 22 نانوميتر و 30 X 20 نانوميتر إلى 30 X 24 نانوميتر على التوالي عند صباغتها بصبغة خلات اليورانيل السالبة و باستخدام البادئات المتخصصة لجين الغلاف البروتينى لجينوم الفيروس تم تضخيم جزء من جين الغلاف البروتينى بنجاح من خلال تقنية تفاعل البلمرة المتسلسل PCR وتم الحصول على شظية من الحمض النووي DNA حجمها حوالي 500 نيوكليوتيدة من نباتات الطماطم المصابة. وقد تم الكشف عن الجينوم الفيروسي في النباتات المصابة بواسطة مجس متخصص من DNA باستخدام طريقه التهجين النقطي Dot blot hybridization. وبدراسة مقارنة تشابه التتابع النيوكليوتيدى لعزلة الفيروس المصرية مع العزلات الأخرى ,أظهرت النتائج تشابها يصل إلى 98% بين العزلة المصرية وغيرها من عزلات نفس الفيروس.

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Introduction

Tomato Yellow Leaf Curl virus (TYLCV) belongs to genus begomovirus of family *Geminiviridae*. TYLCV is a severe viral disease of tomato (*Lycopersicon esculentum*) in Egypt. The first report of damage caused by this virus were from Israel in the late of 1930 and tomato plantings in the Middle East countries have been severely affected since 1960 (Czosnek and Laterrot, 1997). TYLCV disease has emerged in countries around Nile and Mediterranean Basins in the last 20 years (Fauquet et al., 2005). Moustafa (1991) recorded that 100% of the fall- grown tomato plants are usually infected with TYLCV and production losses may reach 80%.

The symptoms of disease become visible in tomato 2-3 weeks after infection and consist of upward curling of leaflet margins, yellowing of young leaves and abortion of flowers. Those leaflets that appear soon after inoculation are cupped down and inwards. Infected plants are severely stunted and resulting decrease of plant growth reduces total yield (Sinisterra et al., 2000; Sider et al., 2001; Gafni, 2003; Crescenzi et al., 2004).

The morphology of geminivirus particles is unique and they are characterized by twin icosaheaderal capsid approximately 20×30 nm in size encapsidating a single molecule of covalently closed circular single stranded DNA (ssDNA) genomes of 2500 to 3000 bp that replicate in the nuclei of the infected cells via a double stranded DNA (dsDNA) intermediate (Harrison and Robinson, 1999; Varma and Malathi, 2003)**.**

Polymerase chain reaction (PCR) using specific or degenerate primers have proved to be a rapid, accurate and efficient method of detecting and determining genetic diversity among geminiviruses (Aref et al., 1994). Sequencing of PCR fragments has contributed to the classification and phylogeny of geminiviruses (Rojas, 1992). The DNA genome of geminiviruses can be easily detected by nucleic acid hybridization visualizing geminiviral DNA-labeled digoxigenin probes (Gilbertson et al., 1991). This paper aimed to isolate, identify and characterize the tomato yellow leaf curl virus which is prevalent in Egypt (Nubaria and El-Behera Governorates.

Materials and Methods

Source of the virus isolate

About ninety samples of naturally infected tomato (*Lycopersicon esculentum* cv. Castle rock) plants showing symptoms suspected to TYLCV was collected from Nubaria and El-Behera Governorate. Collected samples were detected for the presence of TYLCV serologically by using double antibody sandwich enzyme linked immunosorbent assay (DAS–ELISA) as described by (Clark and Adams, 1977**)** using TYLCV specific polyclonal antibodies (LOEWE Biochemica, GmbH, DSMZ, Germany).

Isolation and Propagation of TYLCV

The infected plants which gave positive results with DAS-ELISA were used as a source of the TYLCV under study. The virus isolate was inoculated on healthy tomato cv. Super marmand plants using virus free whiteflies, *Bemisia tabaci* biotype B. Insect inoculated plants were kept in insect-proof cages under greenhouse conditions at the faculty of Agriculture, Ain Shams University for 3-6 weeks. The new symptoms appeared similar to the original symptoms were examined by dot blot hybridization to confirm the existence of the original virus isolate.

Biological characteristics

Mode of transmission

Syringe injection

Healthy tomato plants cv.super marmand were inoculated by syringes using infected tomato sap according to (Allam et al., 1994). The inoculated plants as well as uninoculated ones were kept under greenhouse conditions and symptoms were observed daily up to 60 days. Syringe transmission efficiency was recorded as a number of infected plants / total number of inoculated plants.

Insect transmission

Whiteflies *Bemisia tabaci* biotype B belongs to family *Aleroididae* were collected from tomato plants grown in open fields and identified by the Department of Plant

Protection, Faculty of Agriculture, Ain Shams University. Virus-free whiteflies were used as vectors in transmission experiment and insect transmission was done as previously described by (Ghanem et al., 2001). About twenty insects allowed feed on infected tomato cv.super marmand plants in insect proof cages. After 24 hrs acquisition access period, the insect allow to feed for 72 hrs on healthy tomato plants then the whiteflies were removed by spraying the tomato plants by 0.5% selecron and left for symptoms development. Insect inoculated plants were observed daily for a period of about 60 days. Insect transmission efficiency was recorded as number of infected plants / total number of inoculated plants. The experiment was repeated four times.

Host range and symptomology

Nineteen species and varieties belonging to six families (Solanaeae, Cucurbitaceae, Leguminosae, Chenopodiaceae, Compositae and Graminae) were inoculated with the studied virus isolate under greenhouse conditions. External symptoms were observed for a long period of time (up to 60 days) and confirmed by ELISA and dot blot hybridization assay.

Stability of virus isolate

Thermal Inactivation Point, Dilution End point and aging of TYLCV was performed on healthy *L. esculentum* cv. super marmand by using infectious crude sap obtained from infected tomato plants. The injected seedlings were kept under greenhouse conditions and observed daily up to 60 days for symptoms development. Stability of TYLCV was recorded as number of infected plants / total number of inoculated plants.

Morphological characteristics

Partially purified suspension of TYLCV was prepared according to (Black et al., 1963) and examined by electron microscope at the Electron Microscope Unit, National Research Center, Dokki, using negative staining (2% Uranyle acetate pH 7.0) technique as described by (Noordam, 1973).

Observation of molecular characters

Extraction of Viral DNA

Genomic DNA was extracted from TYLCV infected *L. esculentum* plants using cetyl trimethyl ammonium bromide method (CTAB) as described by (Gibbs and Mackenzie, 1997). Samples were prepared by grinding 50-100 mg fresh leaf tissue homogenized in liquid nitrogen to a fine powder and 500 µl of CTAB buffer was added to the powdered leaves. The mixture was centrifuged for 5-10min. Supernatant was removed and 600 µl of CTAB buffer was added. The mixture was incubated at 60°C for 20 min. with gentle agitation. After the solution has cooled down, an equal volume of chloroform : isoamylalcohol were added. The tubes were centrifuged at 3,000 rpm for 25 min at 10°C. The upper aqueous phase was transferred to a fresh tube and re-extracted with 2 ml of 10% CTAB and the mixture was incubated at 65°C. Chloroform: isoamyl-alcohol extraction was repeated and the mixture was centrifuged at 3,000 rpm at 10°C for 25 min. 2/3 volume isopropanol was added to the upper supernatant phase in a fresh tube. The DNA was collected by centrifugation at 10,000 rpm for 20 min. The liquid was drained carefully and the DNA pellets were washed with 70% ethanol and the tubes were centrifuged at 5,000 rpm for 5 min. DNA pellets were dried and re-suspended in 200 µl TE buffer. Four µl RNase A (10mg/ml) was added and incubated at 65°C for 1 hour. The DNA was precipitated again by adding 0.1 volume 3M sodium acetate and 0.7 volume isopropanol and left overnight at 4°C. The tubes were centrifuged at maximum speed for 15 min at 4°C and the DNA pellets were washed with 500µl 70% ethanol, centrifuged for 5 min. then air dried and resuspended in 20µl of deionized, sterile and dist. H_2O. The nucleic acid was stored at -20°C.

Oligonucleotide primers

The oligonucleotide primers used to amplify the coat protein gene of TYLCV was commercially obtained from Operon, (Qiagen Company, 1000 Atlantic Avenue, Suite 108. A lameda, CA., 94501). Oligonucleotide

degenerate primers were selected according to (Brown et al., 2001). V324 (+) primer corresponding to 5' GCC YAT RTA YAG RAA GCC MAG 3' and C889 (-) primer corresponding to 5' GGR TTD GAR GCA TGH GTA CAT G 3'.

PCR amplification

PCR reaction mixture of 2.5 µl (200 ng) of extracted DNA, 10 mM of each dNTPs (0.5 µl), 1 µl of 25 pmole from each amplification primer, 2.5 µl of 10X PCR buffer with 1.5mM $MgCl_2$ and 0.5 µl Taq DNA polymerase (Roche). The amplification reaction was carried out in a total volume of 25 µl using PCR thermal cycler, UNOII from Biometra and using 0.2 ml micro Amp PCR tubes with denaturation at 94^{o}C for 30 sec, annealing at 50^{o}C for 45 sec, and extension at 72^{o}C for 1 min. A single tailing cycle of long extension at 72^{o}C for 7 min was carried out in order to ensure flush ends on the DNA molecules. Finally, the amplification reactions were hold at 4°C. The amplified DNA was electrophoresed on 1% agarose gel and photographed using gel documentation system from UVP-CCD Camera, Laboratory products, Epichemi, 11 Darkroom, 3 UV Transilluminator, Pharmacia.

Dot Blot Hybridization Assay

Digoxigenin-11-dUTP–labeled DNA probe, corresponding to TYLCV/CPs were prepared by using 10X DNA labeling nucleotide mix (Roche, Boehringer Mannheim, Indianapolis). Digogxigenin-11-dUTP nucleotide mix was incorporated into the PCR cocktail instead of the normal nucleotide mix using the protocol described under the technical bulletin (Roche, Boehringer Mannheim, Indianapolis).

Non-radioactive DNA hybridization was used to detect TYLCV-DNA in infected plant tissues with typical symptoms of TYLCV and/or without symptoms. The nucleic acid of infected samples was extracted as described by Loebenstein et al. (1997) and 5 µl of each extract was spotted directly on the nitrocellulose membrane. The DNA was fixed on the membranes by ultraviolet (U.V) cross linked for 3 min.

Membrane was subjected to hybridization according to Boehringer Mannheim corp. protocol. The prehybridization, hybridization, and colorimetric detection procedures were carried out according to the protocol described by "Genius II DNA labeling and detection kit" (Boehringer Mannheim IN).

Automated DNA Sequencing

The resulting PCR product of TYLCV was purified by using GFX column and Gel Band purification kit (Amersham pharmacia Biotech, GmbH, Germany). The TYLCV coat protein genes (~360 bp) were sequenced on one direction using V324 (+) primer. The sequence was carried out using ABI PRISM model 310, version 5.3.1 at gene analysis unit, VACSERA, Cairo, Egypt. Nucleotide sequence analyses were performed using the published nucleotide sequences of TYLCV coat protein genes from GenBank.

Results

Field inspection and **serological detection**

Whiteflies-infected tomato plants with TYLCV showed viral symptoms of sever leaf curling, leaf crinkle with marginal yellowing, stem upright, twisted and stunted. All samples gave positive reactions and were susceptible to tomato yellow leaf curl viral infection with different degrees of disease severity. These results indicated that the incidence of TYLCV in El Behera Governorate was 100% (Table 1).

Table 1. Detection of TYLCV in different samples of naturally infected tomato cv. Castle rock plants by DAS-ELISA using specific polyclonal antibody.

Location	Symptoms	ELISA-reading (O.D)	Reactivity
Nubaria (El Behera Governorate)	LC,MY, SU, S	2.08	++
	LC, MY	1.953	++
	LC, MY,ST, S	2.317	++
	LC, MY	1.468	++
	LK	0.142	+
	LC, MY	0.149	+
	LC, MY	0.226	+
	LC, MY	0.133	+
	LC, MY	0.123	+
	LK, MY	0.123	+
	LC, MY	0.133	+
	LC, MY	0.185	+
	LC, MY, ST, S	2.423	++
	LC, MY	0.135	+
	LK, MY	0.105	+
	LC, MY	0.109	+
	LC, MY	0.122	+
	LC, MY	0.164	+
	LC, MY	2.375	++

O.D= Optical density, LC=leaf curling, LK=leaf crinkle, MY=marginal yellowing, SU=stem upright, ST=stem twisting, S=stunting. Negative control=0.011, positive control=3.18, + Weak positive reaction, ++strong positive reaction

Isolation and propagation of virus isolate

TYLCV was isolated and propagated on healthy tomato plants cv. super marmand from the selected ELISA positive tomato samples by whitefly (*B. tabaci* biotype B) transmission. After 3-5 weeks, typical external symptoms of leaf curling, leaf crinkle with marginal yellowing produced till it gives deformation and stunted plant growth after 5-6 weeks from insect inoculation (Figure 1).

Figure 1. Symptoms of TYLCV on *L. esculentum* cv. Super marmand whitefly inoculated showing Leaf curling and leaf crinkle (A), Cup shape leaves (B). Leaf curling, yellowing and stem upright and stunted plant growth (C).

Table 2. Mode of transmission of TYLCV.

Virus isolate	Transmission mode	Symptoms	Incubation period (Weeks)	A/B	% Virus transmission efficiency
TYLCV	Syringe Injection	leaf curling and leaf crinkle Marginal yellowing and stunting	2-4 4-8	16/20	80%
	whitefly (*B. tabaci* biotype B)	leaf curling Marginal yellowing, stem twisting and stunting.	3-5 5-6	20/20	100%

A/B=Number of infected plants / total number of inoculated plants. Each experiment was repeated for four times.

Biological characters of virus isolate

Mode of transmission

Results in table 2 showed that both syringe and whitefly inoculation methods transmitted TYLCV from infected tomato plants cv.super marmand to healthy ones but the efficiency of whitefly transmission was higher than the efficiency of syringe injection.

In case of syringe injection, symptoms of leaf crinkle and leaf curling were first developed after 2-4 weeks till it gives marginal yellowing and stunting of tomato plants after 4-8 weeks while in case of whitefly (*B. tabaci* biotype B) transmission, leaf curling with marginal yellowing were first developed after 3-5 weeks till it gives cup shape leaves, stem twisted and sever stunting after 5-6 weeks at 28-30°C under greenhouse. In addition, virus transmission efficiency in case of syringe injection was 80% while in case of whitefly (*B. tabaci* biotype B) transmission was 100%.

Host range and Symptomology

Results showed that TYLCV isolate infected large number of species from family Solanaceae. In addition, TYLCV infected a few species of family Cucurbitaceae, Fabaceae and Chenopodiaceae. On the other hand, no symptoms were observed on Compositae and Graminae.

Table 3 shows the different symptoms produced on the different plant species when inoculated with TYLCV by both syringe injection and whitefly (*B. tabaci* biotype B) transmission.

Virus stability

Results indicated that TYLCV isolate under test was completely inactivated in undiluted crude sap of infected squash leaves at 70ºC, inactivated when diluted to 10^{-7} and completely inactivated after storage after 6 days at room temperature (25-28ºC).

Morphology of virus particles

Electron microscopic examination of partially purified preparation of TYLCV revealed the presence of isometric and pentagonal, with single and paired Gemini virus, (monomers and dimmers) with dimension of 22nm and 20 × 30nm to 24×30 nm, respectively when negatively stained with 2% Uranyl acetate pH 7.0 (Figure 2).

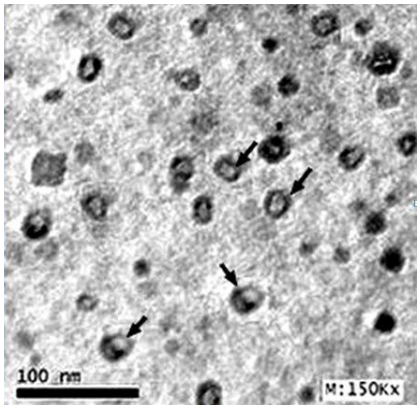

Figure 2. Electron micrographs showing the partially purified Tomato yellow leaf curl gemiviruse negatively stained with 2% Uranyl acetate, Bar represents 100 nm.

Table 3. Host range of TYLCV as determined by syringe injection and whitefly (*B. tabaci*) transmission. Presence of virus was confirmed by DAS-ELISA and DNA hybridization.

Host plants	Syringe injection			Whitefly inoculation		
	Symptoms	O.D.	D.B.H.	Symptoms	O.D.	D.B.H.
Solanaceae						
L. esculentum cv. super marmand	LK,LC,MY, SU,S LK,M	3.950	++	LK ,LC, MY, SU, S	1.940	++
C. annum cv. Chilli	LK,E, M,S	0.582	+	Lk, M,S	0.548	+
D. stramonium	Mild LK	2.083	++	LK,E,M,S	1.966	++
D. metel	LC,R,B,M,S	0.682	+	Mild LK	0.500	+
N. glutinosa	(0)	1.885	++	B	0.796	++
N. rustica	VC	0.204	-ve	(0)	0.222	-ve
N. tabacum	VC	(Na)	(Na)	(0)	(Na)	(Na)
cv. whiteBurley		0.510	+	VC	0.644	+
Samson		0.488	+	VC	0.500	+
	Mild LK					
Cucurbitaceae	(0)					
C. pepo cv. Eskandrani.	(0)	1.200	+	Mild LK	0.506	+
C. maxima		0.274	-ve	(0)	0.157	-ve
C. sativus		0.210	-ve	(0)	0.232	-ve
	LK,M					
Fabaceae	LC,R,NM,VC					
P. vulgaris	**(0)**	0.844	++	LK,N,M	0.570	++
G. max	**(0)**	2.516	++	LC,R	1.500	++
P.sativum		0.242	-ve	(0)	0.234	-ve
V. faba	(0)	0.224	-ve	(0)	0.250	-ve
	LC,E	(Na)	(Na)	(0)	(Na)	(Na)
Chenopodiaceae						
Ch. amaranticcolor		0.252	-ve	-ve	0.250	-ve
B. vulgaris	(0)	0.538	+	LC	0.490	+
Gramineae						
Z. mays	(0)	0.238	-ve	-ve	0.790	-ve
Compositae						
L. sativa		0.254	-ve	-ve	0.200	-ve

O.D.=optical density ,D.B.H=dot blot hybridization, LK=leaf crinkle , LC=leaf curling, MY=marginal yellowing, SU, stem upright, S=stunting, E=epinosity, M=malformation, R=rugosity, B=blistering, VC=vein clearing, NM=net mosaic , N= necrosis. Negative control of sap inoculation=0.149, negative control of whitefly transmission=0.139, ++= strong positive reaction,+= weak positive reaction, -ve = negative reaction, (0) symptomless, and (Na)= not applicable .

Molecular characterization of virus isolate

TYLCV DNA prepared from infected tomato plants were amplified by PCR using the oligonucleotides V324 (+) and C889 (-) as PCR primers. The size of the PCR product of coat protein gene (CP) amplified from infected tomato plants was estimated by comparing its electrophoretic mobility with those of standard DNA marker as shown in figure 3. The amplified DNAs were in the expected size calculated (~500 bp) from the positions of the primers. The authenticity of the resulting PCR products were verified by direct DNA sequencing after purification of the DNA fragments from agarose gel using rapid and efficient gel purification kit from Amersham Pharmaia Biotech, GmbH, Germany.

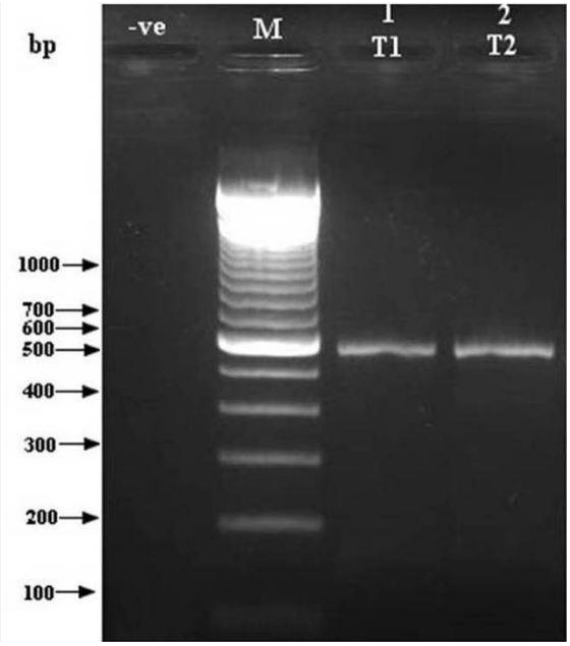

Figure 3. 1.5% agarose gel electrophoresis showing the PCR products of TYLCV coat protein gene using begomoviruses specific primers V324 (+) & C889 (-).

Genomic DNAs were extracted from naturally infected tomato leaves (T1) and syringe injected tomato plants (T2). The arrow pointed to the amplified PCR products (~500 bp) (Lanes 1 to 2). M: Molecular weight DNA marker (100 bp ladder, BRL). –ve: negative control (No DNA template).

Dot blot hybridization assay

Membrane hybridization results of TYLCV infected plants showed that *L. esculentum, D. stramonium, N. glutinosa, P. vulgaris* and *G. max* gave a strong positive reaction while *C. annum*, *D.metel, N. tobacum* cv. whiteberly, *N. tobacum* cv. samson, *C. pepo* and *B. vulgaris* gave a mild positive reaction. On the other hand, *N. rustica C. maxima*, *C. sativus, P. sativum, V. faba, Ch. amaranticolor, Z. mays* and *L. sativa* gave negative reaction (Figure 4).

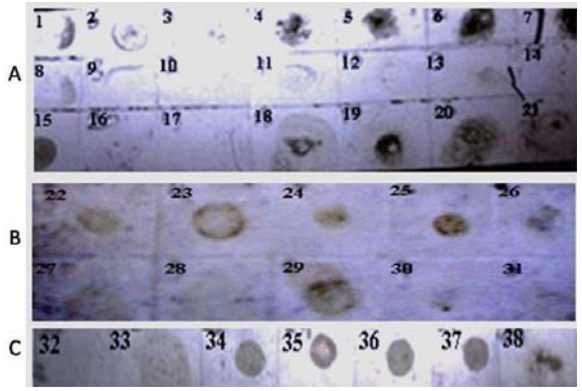

Figure 4. Dot blot hybridization of syringe and whitefly inoculated plants using TYLCV- DNA probe.

Sequence analysis of TYLCV/CP genes

A multiple sequence alignment of TYLCV/Cp nucleotide sequence (current study) was carried out with four TYLCV sequences published in the GenBank. Sequence comparison showed that TYLCV/Cp of the current study had sequence homology of about 98% with other TYLCV isolates (Figure 5A and B).

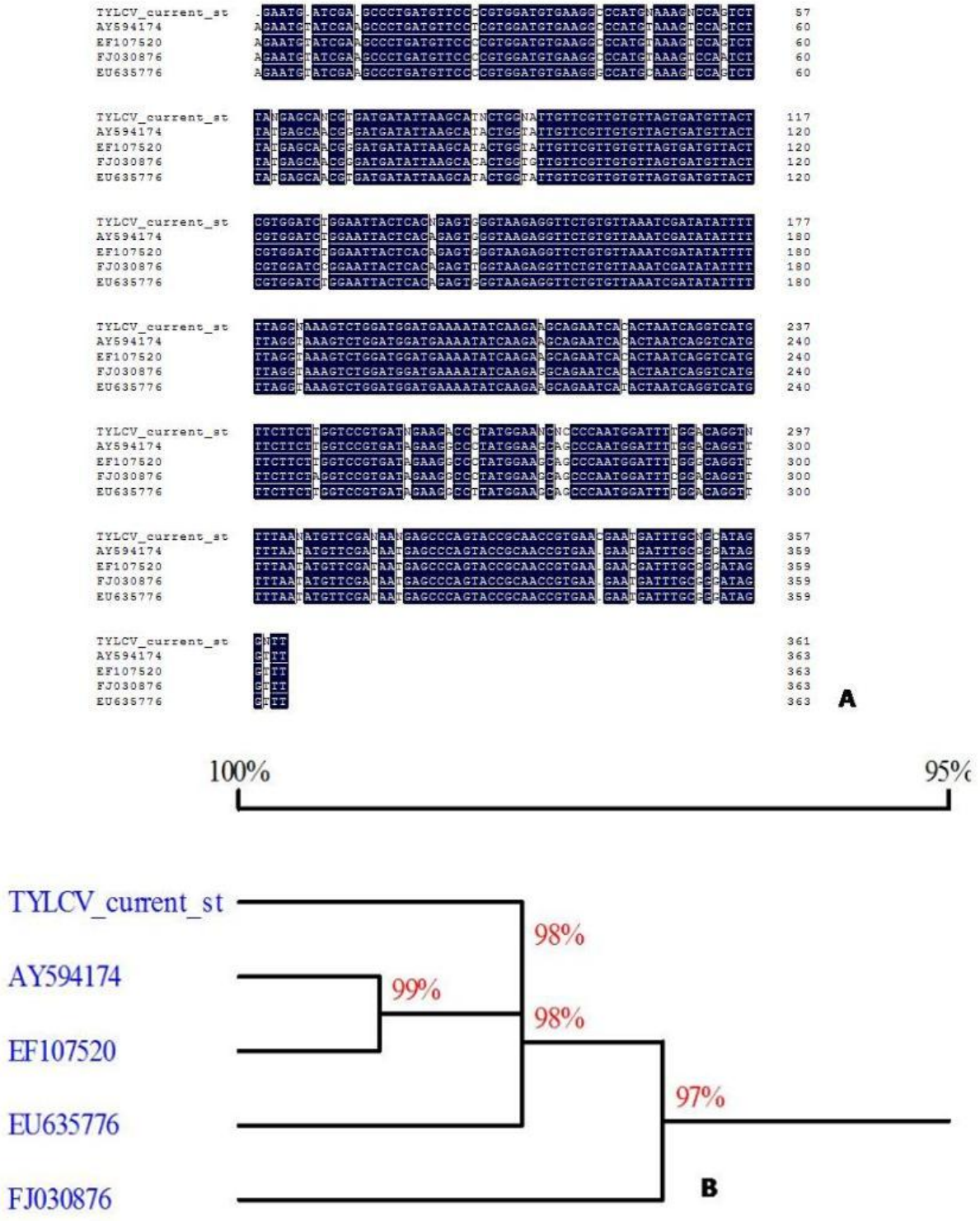

Figure 5 (A & B). Multiple sequence alignment and homology tree of CP gene of TYLCV isolates.

Accession numbers indicated above were as following: TYLCV-current study, AY594174 (TYLCV- Egyptian isolate) reported by Abhary *et al*. (2006). EF107520 (TYLCV-Nob) reported by Abdallah et al. (2000), EU635776 (TYLCV Iranian isolate) reported by Fazeli et al. (2009), FJ030876 (TYLCV - H11) reported by Abdel-Salam and Rehman (2008). Scale represents percent homology.

Discussion

The present study demonstrates the identification of *Tomato Yellow Leaf curl virus* (Egyptian isolate) through biological and molecular studies.

Tomato plants infected with TYLCV showed viral symptoms of sever leaf curling, leaf crinkle with marginal yellowing, stem upright, twisted and stunted as reported by many investigators (Czosnek and Laterrot, 1997; Sinisterra et al., 2000; Sider et al., 2001; Polston et al., 2002; Gafni, 2003; Crescenzi et al., 2004; Ajlan et al., 2006; Zambrano et al., 2007). All samples collected from Nubaria, El-Behera Governorate gave positive reaction and the incidence of TYLCV in was 100%. This results indicated that the presence of high population of whiteflies that transmitted TYLCV efficiently.

DAS-ELISA using TYLCV antiserum confirmed the identity of the isolated TYLCV from tomato plants. This result was in agreement with other investigation (Abouzid et al., 2002). High ELISA readings indicated that high specificity between antibody and virus coat protein as well as high virus concentration in naturally infected tomato plants.

Tomato (*L. esculentum*) used as propagative hosts for TYLCV, systemic symptoms produced firstly after 3-5 weeks in the form of typical external symptoms of leaf curling, leaf crinkle with marginal yellowing produced till it gives deformation and stunted plant growth after 5-6 weeks from insect inoculation. These results are in agreement with that obtained by (Abdel Salam 1991b; Allam et al. (1994) and El-Dougdoug and Aref (1996) while Ioannou (1985) and Credi et al. (1989) reported that first TYLCV symptoms on tomato plants appear 2-4 weeks after inoculation and become fully developed after a period of up to 2 months**.**

TYLCV was sap-transmitted by syringe injection as described by (Allam et al., 1994). Insect transmission by the whitefly *Bemisia tabaci,* which is an insect of the family *Aleyrodidae*, order Homoptera, clearly demonstrated that TYLCV successfully transmitted persistently by this insects. These results are in agreement with that obtained by (Ghanem et al., 2001; Idris et al., 2001; Brown and Czosnek, 2002).

TYLCV infected *L. esculentum* cv. Super marmand, *C. annum* cv. Chili, *D. stramonium, D. metel, N. glutinosa, N. tabacum* cv. whiteberly and *N. tabacum* cv. samson belonging to family *Solanaceae, C. pepo* cv. Eskandrani, belonging to family Cucurbitaceae. *G. max* and *P. vulgaris* belonging to family Fabaceae and *B. vulgaris* belonging to family Chenopodiacea and not reacted systemically with *N. rustica* belonging to family Solanaceae*, C. maxima and C. sativus* belonging to family Cucurbitaceae*, P. sativum* and *V. faba* belonging to family Fabaceae*, Ch. amaranticolor* belonging to family Chenopodiaceae and *L. sativa* belonging to family Compositae and *Z. may* belonging to family Gramineae. These results were in agreement with that of many investigators (Allam et al., 1994; El-Dougdoug and Aref, 1996; Youssef, 1998; Ajlan et al., 2006) and not agreed with that obtained by Nakhla et al. (1978), Brunt et al. (1990) and Abdel-Salam (1991b).

TYLCV isolate have thermal inactivation point 70ºC which were not agreed with (Abdel-Salam 1991a) results, Dilution End Point 10^{-7} -10^{-8} which agreed with Allam et al. (1994) and not agreed with Abdel-Salam (1991a) results and was completely inactivated after 6 days at room temperature (25-28 ºC) which were also not agreed with Abdel-Salam (1991a).

The examination with the electron microscope of the isolated virus particle revealed the presence of isometric and pentagonal in shape, with single and paired Gemini virus, (monomers and dimmers) with dimension of 22 nm and 20 x 30 nm to 24X30nm respectively, when negatively stained with 2% Uranyl acetate pH 7.0. These results were similar with that obtained by Abdel-Salam (1991a), Lazarwaitz (1992), Argüello-Astorga et al. (1994), El-Dougdoug et al. (1996), Harrison and Robinson (1999), Varma and Molathi (2003) and Ajlan et al. (2006).

To study the molecular characters of the isolated virus, purified TYLCV-DNA was used in PCR was performed using degenerate oligonucleotide primers V324 (+) and C889 (-) as reported by Brown et al. (2001). The size of the PCR product of coat protein gene (CP) amplified from naturally infected tomato plants was (~500 bp).

Non-radioactive DNA hybridization method using Digoxigenin-11-dUTP–labeled DNA probe, corresponding to TYLCV/CPs was used to detect TYLCV in infected samples. The Dig-labelled probe was capable of detecting TYLCV-DNA with different degrees of sensitivity.

Partial nucleotide sequence (~360 nt) of TYLCV-CP-EG of the current study was aligned with other published CP sequences of TYLCV as shown in figure 5A. TYLCV-CP was found to display 95.6% sequence homology with EF107520 (TYLCV-Nob) reported by Abdallah et al. (2000), 92.4% with AY594174 (TYLCV- Egyptian isolate) reported by Abhary et al. (2006), 88.7% with FJ030876 (TYLCV - H11) reported by Abdel-Salam and Rehman (2008), 90.0% with EU635776 (TYLCV Iranian isolate) reported by Fazeli et al. (2009). Multiple sequencing alignments were generated using (DNAMAN V 5.2.9 package, Madison, Wisconsin, USA). The homology tree of TYLCV-EG presented in figure 5B. revealed high degree of similarity (~98%) of TYLCV-eg with other four TYLCV isolates.

Conclusions

The host range of TYLCV includes several solanaceous and cucurbitaceous plants with economically important crop plants as sweet pepper, common weeds such as *Datura stramonium and Cucurbita pepo*. TYLCV transmission efficiency in case of syringe injection was 80% while in case of whitefly (*B. tabaci* biotype B) transmission was 100%. Based on NASH technique TYLCV infected tomato, and *D. stramonium,* gave a strong positive reaction while *C. annum*, and *D. metel,* gave a mild positive reaction. On the other hand, *N. rustica, C. maxima*, *C. sativus, P. sativum, V. faba, Ch. maranticolor, Z. mays* and *L. sativa* gave negative reaction. Based on sequence comparisons and phylogenetic analysis, and in accordance with current ICTV criteria, TYLCV (current study) revealed high degree of similarity (~98%) to the four begomovirus species illustrated during this study.

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