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Detection and Identification of *Tomato Yellow Leaf Curl Virus*-EG Using Molecular Technique

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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 severe 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 (Thermal Inactivation Point) of 70ºC, DEP (Dilution End Point) of 10^{-7} and LIV (Longevity) 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 x 30 nm to 24 x 30 nm, respectively when negatively stained with uranyl acetate. DNA from infected plants was extracted and amplified successfully by polymerase chain reaction (PCR) using degenerate oligonucleotide primers V324(+) and C889 (-) producing ~ 500 bp fragment from infected tomato 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.

Keywords: Nucleotide sequence, TYLCV-EG, DAS-ELISA, PCR, Dot-blot hybridization,

Tomato Yellow Leaf Curl virus (TYLCV) belongs to genus begomovirus of family Geminiviridae. TYLCV is a severe viral disease of tomato (*L. esculentum*) in Egypt. Tomato plantings in the Middle East countries have been severely affected since 1960 (Czosnek & Laterrot, 1997). TYLCV disease has emerged in countries around the river 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 reached 80%.

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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 in decrease of plant growth and reduced total yield (Sinisterra *et al.,* 2000; Sider *et al*., 2001; Gafni, 2003 and 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 & Robinson, 1999 and Varma & 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-labelled digoxigenin probes (Gilbertson *et al.,* 1991)**.** This paper describes the biological and molecular identification of TYLCV-EG isolate.

Materials and Methods

Source of the virus isolate

Ninety samples of naturally infected tomato (*Lycopersicon esculentum* cv.Castle rock) plants showing symptoms suspected to TYLCV were collected from El-Behera Governorate. The collected samples were examined for the presence of TYLCV serologically by DAS–ELISA (Clark & Adams, 1977).

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 characters

Syringe injection

Healthy tomato plants cv. super marmand were inoculated by syringes using infected tomato sap as described previously (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 exposed 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). The collected insects were caged with healthy *Ipomoea batatas* (sweet potato) plants and left for two days .The adult insects were killed using selecron as systemic insecticides. These plants were kept in glass cages until the larvae developed. The adults were then transferred to healthy *I. batatas*. After consecutive transfers, the resulting virus-free insects were used as vectors in transmission experiment. About twenty insects allowed to feed on infected tomato cv. super marmand plants in insect proof cages. After 24 hr acquisition access period, the insect allow to feed for 72 hr 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.

Host range and symptomatology

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

Stability of virus isolate

Thermal Inactivation Point (tested at certain temperatures, started with 40ºC with 5 ºC intervals to 90ºC in water bath for 10 min), Dilution End point (starting from 10^{-1} to 10^{-10}) and aging **(**at room temperature of 25ºC-28 ºC for 10 days) of TYLCV was performed on healthy *L. esculentum* cv. super marmand by using infectious crude sap obtained from infected tomato plants macerated in phosphate buffer pH 7.2 (1:1w/v). 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 characters

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 Centre, Dokki, using negative staining (2 % Uranyle acetate pH 7.0) technique as described by Noordam (1973).

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 & Mackenize (1997). Samples were prepared by grinding 50 -100 mg fresh leaf tissue homogenenized in liquid nitrogen to a fine powder and 500 µl of wash buffer was added to the powdered leaves before adding CTAB buffer. The mixture was centrifuged for 5-10min. Supernatant was removed and 600 µl of CTAB buffer was added. The mixture was mixed and incubated at 60°C for 20 min with gentle agitation. After the solution has cooled down, 1volume chloroform:3 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:isoamylalcohol extraction was repeated and the mixture was centrifuged at 3,000 rpm at 10 ˚C for 25min. 2/3 volume isopropanol was added to the upper supernatant phase in a fresh tube. The DNA 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 hr. 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 20 µl of dd 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 electrophoreses 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 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 prehybridization, hybridization, and colorimetric detection procedures 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 pharmaia 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 Gene Bank.

Results and Discussion

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 reaction 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 governorate was 100%, (Table 1). These result reported by many investigators (El-Dougdoug *et al*., 1996; Czosnek & Laterrot, 1997; Sinisterra *et al.*, 2000; Sider *et al*., 2001; Polston *et al.*, 2002; Gafni, 2003; Crescenzi *et al*., 2004; Ajlan *et al*., 2006 and Zambrano *et al*., 2007). All samples collected from Nubaria, El-Behera Governorate gave positive reaction and the incidence of TYLCV was 100%. This result indicated that the presence of high population of whiteflies that transmitted TYLCV efficiently resulted in increasing TYLCV infection in open fields.

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)
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.

Isolation and propagation of virus isolates

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 post infection, 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 (Fig. 1). This result was agreement with other investigation (Abouzid *et al*., 2002)**.** High ELISA readings indicated high virus concentration in naturally infected tomato plants.

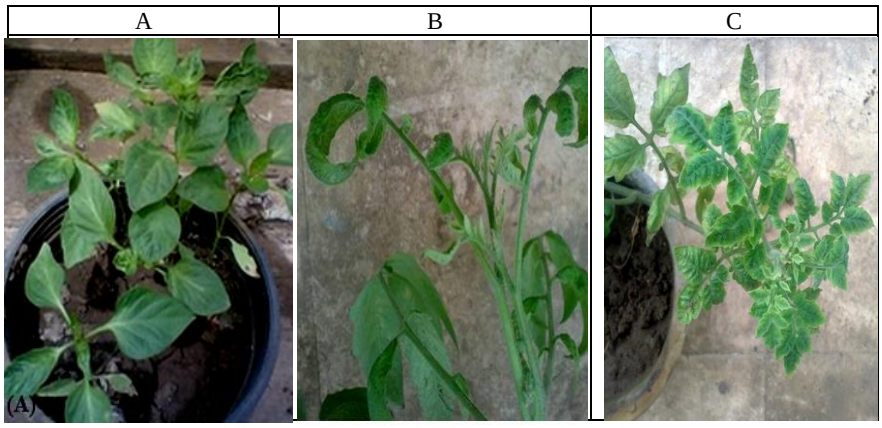

Fig. 1. Symptoms of TYLCV on *L. esculentum* cv. Super marmand whitefly inoculated showing Leaf curling, leaf crinkle (A), Cup shape leaves (B). Leaf curling, yellowing and stem upright and stunted plant growth (C).

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.

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	2-4	16/20	80%
		Marginal yellowing and stunting	4		
	Whitefly (*B. tabaci* biotype B)	Leaf curling	3-5	20/20	100%
		Marginal yellowing, stem twisting and stunting	5		

A/B=Number of infected plants / total number of inoculated plants.

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 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 weeks at 28-30°C under greenhouse. These results are in agreement with that obtained by Abdel Salam (1991b), Allam *et al.* (1994) and El-Dougdoug & 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.

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 illustrated the different symptoms produced on the different plant species when inoculated with TYLCV by both syringe injection and whitefly (*B. tabaci* biotype B) transmission. These results are in agreement with that obtained by Abdel Salam (1991b), Allam *et al.* (1994) and El-Dougdoug & 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.

Morphology of virus particles

Electron microscopic examination of partially purified preparation of TYLCV revealed the presence of isometric and pentagonal in shape, 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, (Fig. 2). These results were similar with that reported in other studies (Abdel-Salam, 1991a; Lazarwaitz, 1992; Argüello-Astorga *et al.,* 1994; El-Dougdoug *et al.,* 1996; Harrison & Robinson, 1999; Varma & Malathi, 2003 and Ajlan *et al.,* 2006).

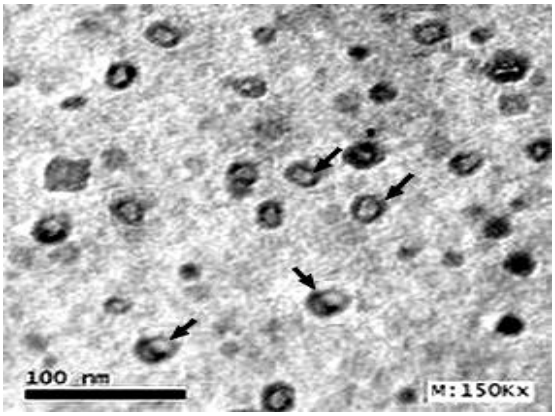

Fig. 2. Electron micrographs showing the partially purified squash leaf curl gemiviruse negatively stained with 2 % Uranyl acetate, Bar represents 100 nm .

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	LK,LC,M	3.950	++	LK ,LC, MY,	1.940	++
cv. super marmand	Y, SU,S			SU, S		
C. annum cv. Chilli	LK,M	0.582	+	Lk, M,S	0.548	+
D. stramonium	LK,E, M,S	2.083	++	LK,E,M,S	1.966	++
D. metel	Mild LK	0.682	+	Mild LK	0.500	+
N. glutinosa	LC,R,B,M,	1.885	++	B	0.796	++
N. rustica	S	0.204	-ve	(0)	0.222	-ve
N. tabacum	(0)	(Na)	(Na)	(0)	(Na)	(Na)
cv. whiteBurley	VC	0.510	+	VC	0.644	+
Samson	VC	0.488	+	VC	0.500	+
Cucurbitaceae						
C. pepo cv. Eskandrani.	Mild LK	1.200	+	Mild LK	0.506	+
C. maxima	(0)	0.274	-ve	(0)	0.157	-ve
C. sativus	(0)	0.210	-ve	(0)	0.232	-ve
Fabaceae						
P. vulgaris	LK,M	0.844	++	LK,N,M	0.570	++
G. max	LC,R,NM,	2.516	++	LC,R	1.500	++
P.sativum	VC	0.242	-ve	(0)	0.234	-ve
V. faba	(0)	0.224	-ve	(0)	0.250	-ve
	(0)	(Na)	(Na)	(0)	(Na)	(Na)
Chenopodiaceae						
Ch. amaranticcolor	(0)	0.252	-ve	-ve	0.250	-ve
B. vulgaris	LC,E	0.538	+	LC	0.490	+
Graminea						
Z. mays	(0)	0.238	-ve	-ve	0.790	-ve
Compositae						
L. sativa	(0)	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 as reported by Brown *et al*. (2001). 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 ladder as shown in Fig. 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 was 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.

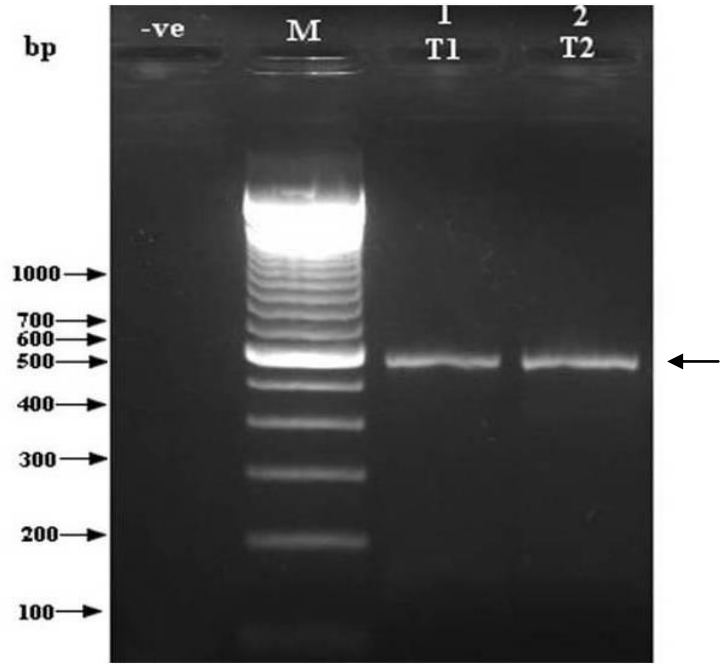

Fig. 3. 1.5% agarose gel electrophoresis showing the PCR products of TYLCV coat protein gene using Begomoviruses specific primers V324 (+) & C889 (-). Genomic DNAs were extracted from naturally infected tomato leaves (T1) and syringe injected tomato plants (T2). The arrow pointed to the amplified PCR products (~500 bp) (Lanes 1 to 2). M: Molecular weight DNA ladder (100 bp ladder, BRL). –ve: Negative control (No DNA template).

Dot blot hybridization assay

Membrane hybridization result 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 (Fig.4).

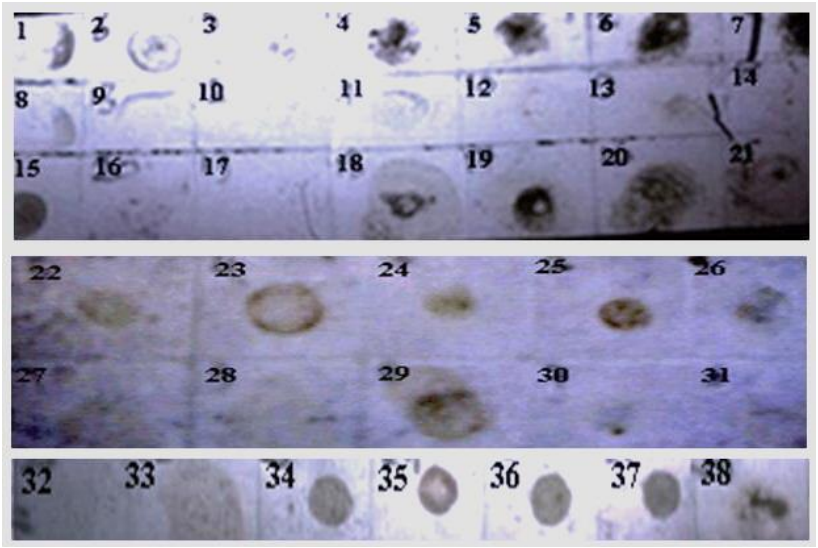

Fig. 4. Dot blot hybridization of syringe and whitefly inoculated plants using TYLCV- DNA probe.

Sequence analysis of TYLCV/CP genes

A multiple sequence alignment of TYLCV/Cp nucleotide sequence (current study) 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 (Fig. 5 A&B). 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 & 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 Fig. 5B revealed high degree of similarity (~98%) to the other four isolates sequences of TYLCV.

To study the molecular characters of the isolated virus, purified TYLCV-DNA was used in PCR 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 from infected samples. The Dig-labelled probe was capable of detecting TYLCV-DNA with different degrees of sensitivity.

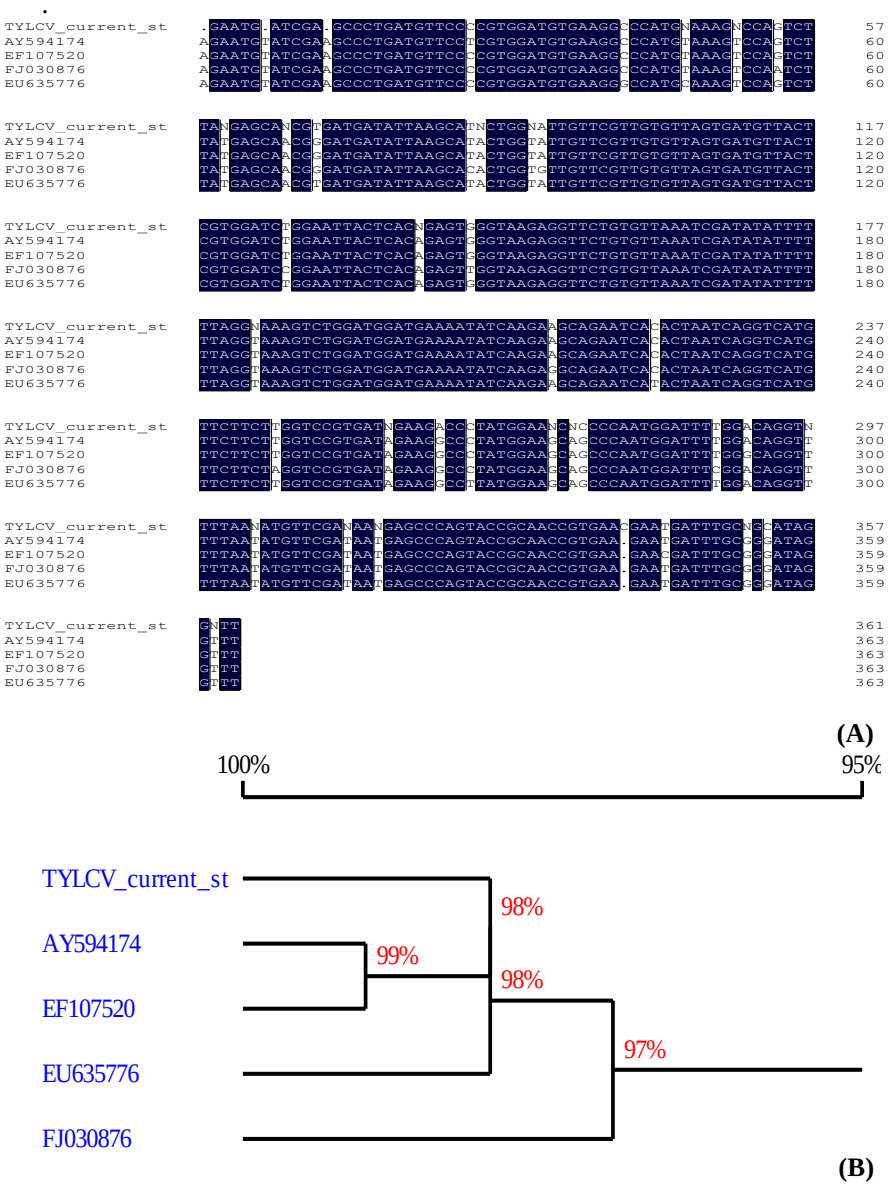

Fig. 5. (A&B). Multiple sequence alignment and homology tree of TYLCV isolates based on the nucleotide sequences of the CP gene. Accession numbers indicated above were as following: TYLCV-current study, AY594174 (TYLCV- Egyptian isolate) reported by Abhary *et al.* (2006). EF107520 (TYLCV-Nob) reported by Abdallah *et al.* (2000), EU635776 (TYLCV Iranian isolate) reported by Fazeli *et al.* (2009), FJ030876 (TYLCV - H11) reported by Abdel-Salam & Rehman (2008).

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 Fig. 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 & 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 Fig. 5B revealed high degree of similarity (~98%) to the other four isolates sequences of TYLCV.

The obtained results of biological and molecular study of TYLCV-EG (current isolate) showed some different characters from the other begomovirus strains reported. Further research should be done to identify the species of begomoviruses infecting the Egyptian tomatoes. Identification through sequencing is necessary in order to map the diversity and evolution of begomoviruses and to understand the appearing of reemerging new isolates.

References

Abdallah, N.A., Fauquet, C.M., Beachy R.N. and Madkour, M.A. (2000) Cloning and constructing infectious clones of an Egyptian isolate of *Tomato Yellow Leaf Curl Virus. Arab J. Biotechnol*. **3** (1), 35-54.

Abdel-Salam, A.M. (1991a) *Tomato Yellow Leaf Curl Virus* in Egypt:1-Characterization, partial purification and anti-serum production. *Bull. Fac. Agric., Univ. Cairo*, **42**, 507-520.

Abdel-Salam, A.M. (1991b) *Tomato Yellow Leaf Curl Virus* in Egypt: 2-The use of its locally induced antiserum for the detection of its incidence in economic and wild plants in the field. *Bull. Fac. Agric., Cairo, Univ*. **42**(2), 521-532.

Abdel-Salam, A.M. and Rehman, M.M. (2008) Diversity of begomoviruses in Egypt (Unpublished).

Abhary, M.K., Anfoka, G.H., Nakhla, M.K. and Maxwell, D.P. (2006) Post-transcriptional gene silencing in controlling viruses of the *Tomato Yellow Leaf Curl Virus* complex. *Arch. Virol*. **151** (12), 2349-2363.

Abouzid, A.M. , Freitas-Astua, J., Purcifull, D. E., Polston, J. E., Beckham, K. A., Crawford, W.E., Petersen, B., Peyser, M.A., Patte, C. and Hiebert, E. (2002) Serological studies using polyclonal antisera prepared against the viral coat protein of four begomoviruses expressed in *Escherichia coli. Plant Disease*, **86**(10), 1109-1114.

Ajlan, A.M., Ghanem, G.A.M. and Abdul Salam, K.S. (2006) *Tomato Yellow Leaf Curl Virus* (TYLCV) in Saudi Arabia: Identification, partial characterization and virus-vector relationship. *Arab J. Biotech*. **10** (1), 179-192.

Allam, E.K., Abo El – Nasr, M. A., Othman B.A. and Thabeet, S.A. (1994) A new method for mechanical transmission of *Tomato Yellow Leaf Curl Virus*. *Egyptian Phytopathol. Soc*. **7**, 91.

Aref, N.M., Abdallah, N.A., Allam, E.K. and Madkour, M.A. (1994) Use of polymerase chain reaction and radiolabelled specific probe to identify *Tomato Yellow Leaf Curl Virus* DNA from infected plants. *Egyptian Phytopathol. Soc*. **93**, 109.

Argüello-Astorga, G., Guevara-González, R.G., Herrera-Estrella, L.R. and Rivera-Bustamante, R.F. (1994) Geminivirus replication origins have a group-specific organization of iterative elements: A model for replication. *Virology*, **203**, 90-100.

Black, L.M., Brakke, K. and Vatter, A.E. (1963) Purification and electron microscopy of *Tomato Spotted Wilt Virus*. *Virology*, **20**, 120–130.

Brown, J. K., Idris, A.M., Torres-Jerez, I., Banks, G.K. and Wyatt, S.D. (2001) The core region of the coat protein gene is highly useful for establishing the provisional. Identification of begomoviruses. *Arch. Virol*. **146**, 1581-1598.

Clark, M.F. and Adams, N.E. (1977) Characterization of the microtitre plate method of enzyme – linked immunoassay (ELISA), for the detection of plant viruses. *J. Gen. Virol.* **37**, 475-483.

Credi, R., Betti, L. and Canova, A. (1989) Association of a geminivirus with a severe disease of tomato in Sicily. *Phytopath. Medit*. **28**, 223-226.

Crescenzi, A., Comes, S., Napoli, C., Fanigliulo, A., Pacella, R. and Accotto, G.P. (2004) Severe outbreaks of *Tomato Yellow Leaf Curl Sardinia Virus* in Calabria, outhern Italy. *Communications in Agricultural and Applied Biological Sciences*, **69**(4), 575-580.

Czosnek, H. and Laterrot, H. (1997) A worldwide survey of *Tomato Yellow Leaf Curl Viruses*. *Arch Virol* .**142**, 1391-1406.

El-Dougdoug, K.A. and Aref, N.M. (1996) Biological and molecular diagnosis of three different symptoms of TYLC-disease in open field. *Ann. Agric. Sci. Cairo*, **41** (1), 173-185.

Fauquet, C., Sawyer, M., Idris, S. and Brown, J.K. (2005) Sequence analysis and classification of apparent recombinant begomoviruses infecting tomato in the Nile and Mediterranean Basins. *Phytopathology*, **95**, 549–555.

Fazeli, R., Heydarnejad, J., Massumi, H., Shaabanian, M. and Varsani, A. (2009) Genetic diversity and distribution of tomato-infecting begomoviruses in Iran. *Virus Genes.* **38** (2), 311-9.

Gafni, Y. (2003) *Tomato Yellow Leaf Curl Virus*, the intracellular dynamics of a plant DNA virus. *Molecular Plant Pathology*, **4**(1), 9–15.

Ghanem, M., Morin, S. and Czosnek, H. (2001) Rate of *Tomato Yellow Leaf Curl Virus* pathway at its vector, the whitefly *Bemisia tabaci*. *Phytopathology*, **91**, 188- 196.

Gibbs, A. and Mackenzie, A.A (1997) primer pair for amplifying part of the genome of all potyvirids by RT–PCR. *J. of Virological Methods*, **63**, 9–16.

Gilbertson, R.L., Haidayat, S.H., Martinez, R.T., Leong, S.A., Faria, J.C., Morrales F. and Maxwell, D.P. (1991) Differentiation of bean-infecting geminiviruses by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. *Plant Dis*. **75**, 336-342.

Harrison, B. D. and Robinson, D. J. (1999) Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). *Annual Review of Phytopathology*, **37**, 369-398

Ioannou, N. (1995) Yellow leaf curl and other disease of tomato in Cyprus. *Plant Pathol.* **34**, 428-434.

Lazarowitz, S. and Geminiviruses, G. (1992) Genome structure and gene function. *Critical Reviews in Plant Sciences*, **11**, 327-349.

Loebenstein, G., Akad, F., Filatov, V., Sadvakasova, G., Manadilova, A., Bakelman, H., Teverovsky, E., Lachmann, O. and Davis, A. (1997) Improved detection of *Potato leaf roll Luteovirus* in leaves and tubers with a digoxigenin-labeled cRNA probe. *Plant Disease*, **81**, 489-491.

Moustafa, S. E. (1991) Tomato cultivation and breeding programme for *Tomato Yellow Leaf Curl Virus*. pp. 6-8 in: resistance of the tomato to TYLCV, *Proceedings for the seminar of EEC contract DGXII-TS2-A-055 F (CD) partners.* H. Latterrot, and C. Trousse, (Ed). INRA-Station de' Amelioration des plantes Maraicheres, Montfavet-Avignon, France.

Noordam, D. (1973) "*Identification of Plant Viruses. Methods and Experiments*". Center of Agriculture Publishing and Demonstration (Pudoc), Wageningen.

Polston, J.E., Rosebrock, T.R, Sherwood, T., Creswell, T. and Shoemaker, P.J. (2002) Appearance of *Tomato Yellow Leaf Curl Virus* in North Carolina. *Plant Disease*, **86** (1), 73.

Rojas, M. R. (1992) Detection and characterization of whitefly-transmitted geminiviruses by the use of polymerase chain reaction. *M. Sc. Thesis*. Departement of Plant Pathology. University of Wisconsin-Madison at Madison. 92 pp.

Sider, M.M.F., Franco, A. D., Vovlas, C. and Gallitelli, D. (2001) First report of *Tomato Yellow Leaf Curl Virus* in Apulia (Southern Italy). *J. of Plant Pathol*. **83** (2), 148.

Sinisterra, X., Patte, C.P., Siewnath, S. and Polston, J. E. (2000) Identification of *Tomato Yellow Leaf Curl Virus*-Is in the Bahamas. *Plant Disease*, **84** (5), 592.

Varma, A. and Malathi, V.G. (2003) Emerging geminivirus problems: A serious threat to crop production. *Annals of Applied Biology*, **142**, 145-164.

Zambrano, K., Carballo, O., Geraud, F., Chirinos, D., Fernandez, C. and Marys, E. (2007) First report of *Tomato Yellow Leaf Curl Virus* in Venezuela. *Plant Disease*, **91**(6), 768.

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الكشف والتعرف على فيروس تجعد واصفرار أوراق الطماطم باستخدام تقنية PCR

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سجلت هذه الدراسة عزل وتعريف فيروس تجعد واصفرار أوراق الطماطم احد اخطر الفيروسات المنقولة بحشرات الذبابة البيضاء وقد ظهرت الأعراض الفيروسية الجهازية معروضة فى شكل تجعد واصفرار بين العروق وإلتواء حواف الأوراق والتواء الساق وتقزم نباتات الطماطم المنزرعة فى محافظتى النوبارية والبحيرة بجمهورية مصر العربية وأظهرت النتائج رد فعل إيجابى بإستخدام الأجسام المضادة المتعدد التخصصية specific polyclonal antibodies للكشف عن الفيروس بالطريقة السيرولوجية DAS-ELISA وقد أشارت الدراسة إلى أن الفيروس يمكن أن ينتقل ميكانيكيا بالمحقن وأيضاً عن طريق حشرة الذبابة البيضاء بكفاءة نقل حوالى ٨٠ ٪ و ١٠٠ ٪ على التوالى . وقد أوضحت الدراسة أن الفيروس يصيب أنواع نباتية مختلفة تابعة للعائلات القرعية ، البقولية ، الباذنجانية والزربيحية وبدراسة الخواص الفيزيائية للفيروس قيد الدراسة تبين أن درجة الحرارة المثبطة لنشاطه هى ٧٠°س ونقطة التخفيف التى تفقده القدرة على إحداث العدوى هى ١٠-٧ وكذلك يستطيع البقاء حيا فى فى درجة حرارة الغرفة لمدة أربعة أيام. وقد أظهر الفحص بالميكروسكوب الإلكترونى وجود جزيئات مفردة وتوأميه أبعادها ٢٢ نانوميتر و ٣٠ x ٢٠ نانوميتر إلى ٣٠ x ٢٤ نانوميتر على التوالى عند صباغتها بصبغة خلات اليورانيل السالبة و بإستخدام 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 النووى DNA حجمها حوالى ٥٠٠ نيوكليوتيدة من نباتات الطماطم المصابة . وقد تم الكشف عن الجينوم الفيروسى فى النباتات المصابة بواسطة مجس متخصص من DNA بإستخدام طريقة التهجين النقطى Do blot hybridization وبدراسة مقارنة تشابه التتابع النيوكليوتيدى لعزلة الفيروس المصرية مع العزلات الأخرى ، أظهرت النتائج تشابهاً يصل إلى ٩٨ ٪ بين العزلة المصرية وغيرها من عزلات نفس الفيروس .