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Emergence and diversity of *Squash Leaf Curl Virus* infecting solanaceous vegetable crops in Egypt

Amany A. Awad[1], Ahmed K. El-Attar[1], Atef M. Mohamed[2] and Mohamed Hassan[2]

[1]*Department of Virus and Phytoplasma Research, Plant Pathology Research Institute, Agricultural Research Center, Egypt*
[2]*Agricultural Botany Department, Faculty of Agriculture, Fayoum University, Egypt*

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ABSTRACT

Begomoviruses have emerged recently as a serious problem in Mediterranean countries. In 2016 through 2018, high incidence of begomovirus-like diseases was observed on solanaceous vegetable crops in Egypt. The incidence of SLCV determined in five different Egyptian governorates using specific PCR. The virus found infecting tomato, pepper, eggplant, and tomatillo with high incidence (37.4%). Also, the virus detected in four weed species which are prevailing in Egyptian fields. Two SLCV isolates, denoted as SLCV-Tom and SLCV-Pep, infecting tomato and pepper; respectively were biologically and molecularly characterized. SLCV-Tom and SLCV-Pep isolates were whitefly-transmitted to eleven plant species belonging to five different botanical families. Full genome sequences of both isolates were determined and deposited to the GenBank database. According to the best of our knowledge, this is the first report of an SLCV infecting new agriculturally important solanaceous hosts such as tomato, pepper, eggplant, and tomatillo in Egypt.

***Keywords*:** SLCV, PCR, tomato, pepper, eggplant, tomatillo, weeds, Egypt

Introduction

Solanaceae includes economically important vegetables such as potato, tomato, eggplant, capsicum and chilies. Solanaceous vegetable crops are heavily affected by viral diseases, which limit their production and quality as well. Given the economic importance of solanaceous crops in Egypt, there is a need to tackle viral diseases including their insect vectors.

Begomoviruses (genus *Begomovirus*; family *Geminiviridae*) have increased in their distribution and importance in the subtropics and tropics worldwide during recent decades and are responsible for significant yield losses in many crops (Brown, 1997; Moffat, 1999; Varma & Malathi, 2003). Begomoviruses are transmitted in a persistent manner by whiteflies of the species complex *Bemisia tabaci* (Jones, 2003). Begomoviruses native to the Americas, like SLCV, have a bipartite genome of single-stranded circular DNA, where each DNA component is approximately 2.6–2.7 kb (Sunter & Bisaro, 1991, 1992; Lazarowitz *et al.*, 1992).

The emergence of begomoviruses in a certain niche could be associated with changes in climate and cropping practices. These changes could lead to increasement of begomoviruses' vector, *Bemisia tabaci*. (Ali-Shtayeh *et al.*, 2014; Lapidot *et al.*, 2014). In the Mediterranean basin and the Middle East region, many begomoviruses have emerged during the last decade, causing a significant damage to numerous crops, including tomato, pepper, bean, and cucurbits (Abudy *et al.*, 2010; Farrag *et al.*, 2014; Ali-Shtayeh *et al.*, 2014; Lapidot *et al.*, 2014; EL-Rahmany, 2015).

SLCV infects weeds naturally such as *Malva sp*, *Datura stramonium*, *Chenopodium murale*, *Convolvulus sp* and *Prosopis farcta* in cucurbits fields (Al-Musa *et al.*, 2008; Abudy *et al.*, 2010; Ali-Shtayeh *et al.*, 2014). Syringe injection was efficient in transmitting SLCV to plant species belonging to botanical families *Cucurbitaceae, Fabaceae, Solanaceae,* and *Chenopodiaceae* (El-Dougdoug *et al.*, 2009).

In 2006, SLCV was first reported in Egypt and caused severe symptoms in squash fields (Idris *et al.*, 2006). Over the last decade, SLCV has been emerging in parallel manner in adjacent countries such as Israel, Jordan, Lebanon, and Palestine (Al-Musa *et al.*, 2008; Abudy *et al.*, 2010; Ali-Shtayeh *et al.*, 2014; Lapidot *et al.*, 2014).

Corresponding Author: Mohamed Hassan, Agricultural Botany Department, Faculty of Agriculture, Fayoum University, Egypt. E-mail: maa22@fayoum.edu.eg

Co-infection of tomato breeding lines with SLCV, WmCSV and Tomato yellow leaf curl virus (TYLCV), was reported in Jordan (Ahmad *et al*., 2013). In Egypt, SLCV extended its cucurbits host range to include common bean, causing leaf deformation, which translates into decline in yield production (Farrag *et al*., 2014).

The present study was undertaken to focus on the incidence of SLCV in major growing solanaceous crops in Egypt, and determination of the naturally infected host range of SLCV isolates. Further, molecular characterization of new SLCV isolates infecting solanaceous hosts in Egypt.

Material and methods

Source of SLCV isolates and samples collection

The incidence of SLCV was determined during (2016, 2017 and 2018). Leaf samples collected from different solanaceous crops that showed virus-like symptoms. Visited fields were in different governorates in Egypt (Qalyubiyah, Kafr El-Sheikh, Beheira, Giza, Menoufia and Fayoum). Symptomatic tomato and pepper plants collected from Qaha, Qalyubiyah Governorate exhibiting diverse symptoms (Fig. 1). Samples were tested for the presence of SLCV using a specific PCR technique as described by (Farrag *et al.*, 2014; EL-Rahmany, 2015). The infected plants which tested positive for PCR, were used as a source of the virus in the following experiments.

Whitefly transmission and host range study

For whiteflies transmission of SLCV, adults of the *Bemisia tabaci* were collected from the open field in Giza Governorate. Non-viruliferous whiteflies were reared on sweet potato (*Ipomoea batatas*) for five generations in insect-proof cages. Naturally infected SLCV tomato and pepper plants were transplanted from open field into insect-proof cages and used as an SLCV source for further studies. A host range study was performed using viruliferous whiteflies, in which they were allowed for 24h acquisition access period (AAP) on systemically-infected tomato or pepper. After the AAP, 15-20 whiteflies per test plant in insect-proof cages were transferred to healthy test plants (Table 2) for a 48h inoculation access period (IAP) (Al-Musa *et al.*, 2008; El-Dougdoug *et al.*, 2009). Following IAP, insects were killed with a systemic insecticide (Malason 57% EC; KZ, Egypt), and plants were maintained in insect proof-cages within an insect-proof glasshouse until observed for symptom development. Healthy plant seedlings received the same number of non-viruliferous whiteflies, as a control. Samples from symptomatic and asymptomatic inoculated plants tested for the presence of SLCV using PCR as described by (Farrag *et al.*, 2014; EL-Rahmany, 2015).

Nucleic acid extraction and PCR amplification

Total genomic nucleic acids (TNA) were extracted from collected samples using the CTAB method as described by (Doyle, 1990). For detection of begomoviruses, degenerate universal primers were used according to a protocol described by (Wyatt & Brown, 1996). Specific detection of SLCV was performed using primer pairs and PCR protocol described by (EL-Rahmany, 2015). In order to amplify the full genome of SLCV isolated from tomato and pepper, specific primer pairs (SLCVF-SalI and SLCVR-SalI) were used to amplify the DNA-A component. Also, primer pairs (SLCVBF-HindIII and SLCVBR-HindIII) were used to amplify DNA-B components, according to (Ahmad *et al.*, 2013).

The PCR reaction mixture contained 2µl of (TNA), 0.25mM of each dNTPs, 2.5 µM of each primer, 2.5µl of 10X PCR buffer with 1.5mM MgCl and 0.5µl Taq DNA polymerase (Roche). The amplification reaction was carried out in a total volume of 25 µl using PCR thermal cycler (Biometra). The PCR program consisted of one melting cycle at 94 °C for 3 min, followed by 35 cycles of melting (45 sec at 94 °C), annealing (45 sec at 55 °C for each DNA-A and DNA-B primers) and DNA extension (2 min at 72°C). The amplified DNA product was electrophoresed on 1% agarose gel and photographed using gel documentation system from UVP-CCD.

Full genome cloning and sequence analysis

DNA-A and DNA-B from SLCV-Tom and SLCV-Pep were amplified using specific PCR primers pairs. Amplicons of expected sizes were gel purified using (PCR DNA and Gel Band Purification Kit) according to manufacture instructions (Amersham Pharmacia Biotech). Amplicons

of expected sizes were ligated into pGEM®-T Easy cloning vector (Promega) cloning vector. Aliquots from ligation reaction transformed into *E. coli* (DH5α competent cells). Recombinant Plasmids with expected inserts were sequenced using M13 primers in both directions (Macrogen, South Korea).

The full genome sequence of both isolates assembled using DNAMAN software (Lynnon BioSoft, Canada). Open Reading Frames (ORFs) were predicted using ORFinder (NCBI; https://www.ncbi.nlm.nih.gov/orffinder/). Sequence analyses were performed using the BioEdit package of sequence analysis software (Hall, 1999). Phylogenetic trees were constructed and illustrated, using MEGA7 software (Kumar *et al.*, 2016).

Results

Incidence of SLCV in solanaceous plants

In 2016, 2017 and 2018, infected leaf samples associated with geminiviruses like symptoms were collected from 52 fields of tomato, pepper, eggplant, and tomatillo located in six different governorates (Fig.1). Analysis of collected samples by PCR revealed that the infection rate is 37.4% (314 out of 840 tested samples). In tested samples, the highest infection rate was found in eggplant samples 47.6% (80 out of 168 tested samples), in tomato 38% (149 out of 392 tested samples), in pepper 28.7% (76 out of 265 tested samples), and in tomatillo 60% (9 out of 15 tested samples) (Table 1).

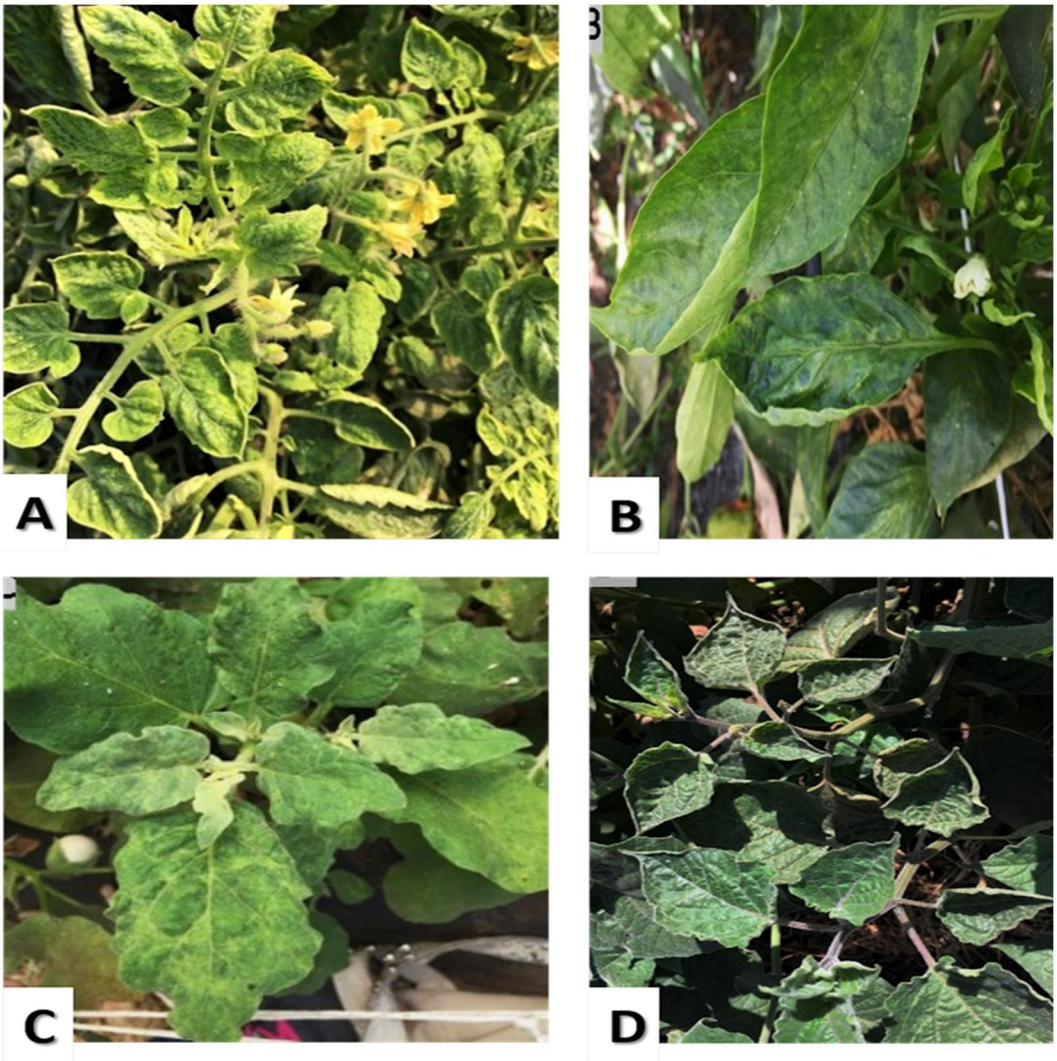

Fig. 1 Symptoms exhibited on solanaceous crops naturally infected with SLCV. (A) leaf curling in tomato plant collected from Giza Governorate; (B) leaf mottling in pepper plant from Menoufia Governorate; (C) leaf mottling eggplant and (D) Upward cupping of tomatillo plants collected from Qalyubiyah Governorate.

Table 1: Detection of *Squash Leaf Curl Virus* in naturally infected solanaceous crops using PCR.

Governorate	Crop	Season	No. of	No. of infected/	Infection
Qalyubiyah	Tomato	Summer/2017	3	90/150	60
	Pepper	Autumn/2017	3	27/120	22.5
	Eggplant	Summer/2017	3	40/60	66.6
	Tomatillo	Summer/2017	1	9/15	60
Giza	Tomato	Autumn/2017	5	23/87	26.4
	Pepper	Summer/2017	5	22/70	31.4
	Eggplant	Summer/2017	5	27/55	49
Kafr Elsheikh	Tomato	Autumn/2016	2	12/45	27
	Pepper	Autumn/2018	2	12/33	36.4
	Eggplant	Autumn/2017	3	6/33	18.2
Menoufia	Tomato	Autumn/2017	3	17/40	42.5
	Pepper	Autumn/2017	4	10/25	40
	Eggplant	Summer/2018	3	7/20	35
Fayoum	Tomato	Summer/2018	4	7/45	15.5
	Pepper	Summer/2018	3	5/17	29.4
Beheira	Tomato	Winter/2018	3	0/25	-
Total			**52**	**314/840**	**37.4**

Host range study

Both SLCV isolates (SLCV-Tom and SLCV-Pep) successfully transmitted back by whiteflies *Bemisia tabaci*, to tomato and pepper virus-free seedlings, respectively. After 3 weeks of post inoculation (DPI), inoculated plants showed the same symptoms observed earlier on the original plants in the open field (Fig 2 A -I). SLCV was detected in symptomatic plants using specific PCR as described in (Farrag *et al.*, 2014; EL-Rahmany, 2015).

To ascertain the experimental host range of SLCV-Tom and SLCV-Pep isolates, fourteen plant species belonging to five families were tested (Table 2). In two independent experiments, eleven out of 14 species found to be infected by SLCV-Tom and SLCV-Pep (Table 2).

Table 2. Plant hosts tested against SLCV-Tom and SLCV-Pep isolates.

Host	English name	Symptoms*	PCR Test
Family: *Chenopodiaceae*			
Chenopodium album	Lamb's Quarters	S, Cr	+
Family: *Cucurbitaceae*			
Citrullus lanatus L.	Watermelon	Lc, De, M, S	++
Cucumis sativus L.	Cucumber	Lc, De, Cr, Dw	++
Cucurbita pepo L.	Zucchini	Lc, Bl, Vb	++
Family: *Fabaceae*			
Phaseolus vulgaris cv. Bronco	Common Bean	Lc, Y, Bl, Cr	+
Family: *Solanaceae*			
Capsicum annum L.	Pepper	Lc	+
Solanum esculantum cv. Castel Rock	Tomato	Ns	+
Solanum nigrum	Black nightshade	Vc	+
Solanum melongena	Eggplant	Vc, Lr	+
Family: *Malvaceae*			
Abelmoschus esculentus	Okra	Ns	-
Gossypium herbaceum	Lavant cotton	Ns	+
Malva neglecta L.	Hollyhocks Marshmallow	Cr, S	+
Althaea officinalis L.		Cr, S, Lr	+
Family: *Convolvulaceae*			
Ipoma batatas L.	Sweet potato	Ns	-

Lc, leaf curl; Vb, vein banding; M, mosaic; Lr, leaf rolling; Vc, vein clearing; Mo, mottling; S, stunting; Y, yellowing; Dw, downward; De, deformation; Ns, No symptoms; Cr, crumpling; Uw, upward; Bl, blisterin

Infected plants exhibited different virus-like symptoms, which associated with both isolates. Symptoms observed ranged from leaf curling, dwarfing, interveinal yellowing, crinkling and crumpling, vein clearing to upward curling and stunting. All symptomatic plants were found positive using SLCV-specific PCR as described by (Farrag *et al.*, 2014; EL-Rahmany, 2015).

Sequence analysis of SLCV isolates infecting tomato and pepper

The complete nucleotide sequence of DNA-A and DNA-B were obtained for both SLCV isolates (SLCV-Tom and SLCV-Pep). Sequences of SLCV-Tom and SLCV-Pep were deposited in GenBank under accession numbers (MG763920 - MG763921; MH346454 - MH346455).

Sequence analysis revealed that SLCV-Tom and SLCV-Pep isolates had a typical genome organization of new world bipartite begomoviruses (Idris & Abedl-Salam, 2006; Lapidot *et al.*, 2014). In DNA-A of both isolates, four genes (AC1, AC2, AC3, and AC4) in complementary-sense and one gene (AV1) in virus-sense encode for coat protein. DNA-B component encoded two major ORFs; BC1 on complementary-sense and encoding movement protein (MP) and BV1 on virion-sense and encoding nuclear shuttle protein (NSP). DNA-A and DNA-B of SLCV-Tom and SLCV-Pep isolates shared a common region (CR), which encompass the putative nona-nucleotide sequence present in the CRs of all characterized begomoviruses (Ali-Shtayeh *et al.*, 2014; Lapidot *et al.*, 2014).

Based on pairwise nucleotide comparisons, SLCV-Tom and SLCV-Pep shared 94.9% and 96% between each other in DNA-A and DNA-B, respectively (Tables 3 & 4).

Blast search conducted with the complete sequences of DNA-A and DNA-B for SLCV-Tom and SLCV-Pep revealed high degree of nucleotide sequence identity with other SLCV isolates (i.e., >92%) (Tables 3 & 4). The highest degree of nucleotide identity in DNA-A of SLCV-Tom (96.1%) and SLCV-Pep (97.2%) was found with DNA-A of SLCV-JO2-214 (KM595191) infecting tomato in Jordan. In DNA-B, the highest nucleotide identity of SLCV-Tom (95.8%) and SLCV-Pep (97.8%), were found with the same isolate from SLCV-JO2-2014 (JX444574) (Table 3).

DNA-A of SLCV-Tom and SLCV-Pep isolates encompassed five ORFs, which are positionally conserved with other new world begomoviruses. AV1 ORFs of SLCV-Tom and SLCV-Pep isolates shared the highest amino acid identity (96.9 %) with SLCV- Sq-EG3 isolate (KC895398) from Egypt. Interestingly, ORFs (AC1 and AC4) in the SLCV-Tom isolate were highly diverse. AC1 ORFs highly matched (89.9%) SLCV-Sq-PAL. Also, ORFs AC4 shared 77.8% identity with SLCV-Sq-EG1 (MK284929). Whereas, ORFs (AC1 and AC4) of SLCV-Pep were more conservative and showed the highest amino acid identity with isolate SLCV-Sq-LB infecting squash in Lebanon (Table 3 & 4).

In DNA-B of SLCV-Tom and SLCV-Pep isolates, two ORFs were encoded. The BV1 ORFs shared 94.9 % amino acid identity with SLCV-To-JO (JX444574), while BC1 ORFs shared the highest amino acid identity (92.6%) with SLCV-Sq-EG1 isolate (MK284930) from Egypt (Tables 3 & 4).

Phylogenetic analyses based on a complete nucleotide sequence of DNA-A showed that SLCV-Tom and SLCV-Pep grouped together in a distinct clade with high bootstrap value (99%) from other SLCV isolates (Fig. 4 A). Whereas DNA-B of both isolates were closer to other SLCV isolates infecting squash from Egypt (Fig. 4 B).

Table 3: Percentage nucleotide and amino acid sequence identities between SLCV-Tom and other SLCV isolates from different hosts and countries.

SLCV Isolates	Country	Host	Accession numbers		Total nt.		DNA-A					DNA-B	
			DNA-A	DNA-B	DNA-A	DNA-B	AV1	AC1	AC2	AC3	AC4	BV1	BC1
SLCV-Tom	Egypt	Tomato	MG763920	MG763921	ID	ID	ID	ID	ID	ID	ID	ID	ID
SLCV-Pep	Egypt	Pepper	MH346454	MH346455	94.9	96.0	92.8	85.3	99.3	100	77.6	87.6	83.9
SLCV-Sq-EG1	Egypt	Squash	MK284929	MK284930	95.6	95.0	95.6	89.6	97.7	97.7	77.8	82.3	84.6
SLCV-Sq-EG2	Egypt	Squash	MK284931	MK284932	95.7	94.8	95.2	89.3	97.7	98.5	76.8	81.9	84.3
SLCV- Sq-EG3	Egypt	Squash	KC895398	KF030954	95.8	91.1	96.2	89.6	97.7	100	76.9	85.3	75.5
SLCV- Bea-EG6	Egypt	Bean	KJ624994	KJ579954	93.5	93.2	94.4	NA[a]	NA	NA	NA	83.4	83.6
SLCV-Sq-OM	Oman	Squash	HG969277	HG941652	95.8	95.2	95.6	89.0	97.7	98.5	76.8	90.6	84.6
SLCV-Sq-PAL	Palestine	Squash	KC441465	KC441466	95.9	95.7	96.0	89.9	98.5	97.0	75.2	82.3	84.3
SLCV-To-JO	Jordan	Tomato	JX444577	JX444574	96.1	95.8	95.2	89.6	99.2	97.7	77.6	94.9	84.9
SLCV-Sq-LB	Lebanon	Squash	HM368373	HM368374	96.0	95.4	96.0	89.6	99.2	98.5	77.6	94.4	83.6
SLCV-Sq-IL	Israel	Squash	KM595114	HQ184437	95.6	95.2	95.2	88.2	97.0	98.5	77.6	94.8	83.9
SLCV-Cot-PAK	Pakistan	Cotton	MF504010	MF504013	88.5	92.4	84.0	84.4	97.0	96.2	73.6	79.5	84.3
SLCV-Mal-Jo	Jordan	*Malva sp*	EF532620	EF532621	93.9	92.4	93.6	89.3	99.2	96.2	60.0	79.1	84.3
SLCV-Sq-US1	USA	Squash	DQ285016	DQ285018	93.7	92.4	94.0	88.7	95.5	98.5	72.8	79.1	83.6
WmCSV-WM-PAL	Palestine	Watermelon	KC462552	KC462553	55.5	41.1	69.7	48.6	50.3	48.9	8.8	25.5	83.9

NA[a] unverified sequence according to GenBank database

Table 4: Percentage nucleotide and amino acid sequence identities between SLCV-Pep and other SLCV isolates from different hosts and countries.

SLCV Isolates	Country	Host	Accession numbers		Total nt.		DNA-A					DNA-B	
			DNA-A	DNA-B	DNA-A	DNA-B	AV1	AC1	AC2	AC3	AC4	BV1	BC1
SLCV-Tom	Egypt	Tomato	MG763920	MG763921	94.9	96.0	92.8	85.3	99.2	100	77.6	87.6	83.9
SLCV-Pep	Egypt	Pepper	MH346454	MH346455	ID	ID	ID	ID	ID	ID	ID	ID	ID
SLCV-Sq-EG1	Egypt	Squash	MK284929	MK284930	96.6	97.2	96.4	94.5	98.5	97.7	99.2	79.1	92.6
SLCV-Sq-EG2	Egypt	Squash	MK284931	MK284932	96.8	97.0	96.0	94.2	98.5	98.5	99.2	78.7	91.4
SLCV- Sq-EG3	Egypt	Squash	KC895398	KF030954	96.9	89.7	96.9	94.9	98.5	100	99.2	81.7	64.2
SLCV- Bea-EG6	Egypt	Bean	KJ624994	KJ579954	93.7	95.3	96.4	NA[a]	NA	NA	NA	86.7	92.1
SLCV-Sq-OM	Oman	Squash	HG969277	HG941652	96.8	97.2	96.4	93.9	98.5	98.5	97.6	79.1	92.1
SLCV-Sq-PAL	Palestine	Squash	KC441465	KC441466	97.0	97.7	96.8	94.8	99.2	97.0	100	89.6	92.1
SLCV-To-JO	Jordan	Tomato	JX444577	JX444574	97.2	97.8	96.4	94.5	100	97.7	100	89.2	91.1
SLCV-Sq-LB	Lebanon	Squash	HM368373	HM368374	96.9	97.4	96.4	94.8	100	98.5	100	89.2	91.8
SLCV-Sq-IL	Israel	Squash	KM595114	HQ184437	96.8	97.4	96.0	92.8	97.7	98.5	95.2	76.3	91.8
SLCV-Cot-PAK	Pakistan	Cotton	MF504010	MF504013	89.6	94.5	84.4	85.6	97.7	96.2	69.6	75.9	91.8
SLCV-Mal-Jo	Jordan	*Malva sp*	EF532620	EF532621	94.2	94.6	95.2	93.9	100	96.2	94.4	75.9	91.4
SLCV-Sq-US1	USA	Squash	DQ285016	DQ285018	94.8	94.5	96.4	93.3	96.2	98.5	9.6	22.8	38.9
WmCSV-WM-PAL	Palestine	Watermelon	KC462552	KC462553	56.4	41.6	70.1	50.0	50.3	48.9	77.6	87.6	83.9

NA[a] unverified sequence according to GenBank database

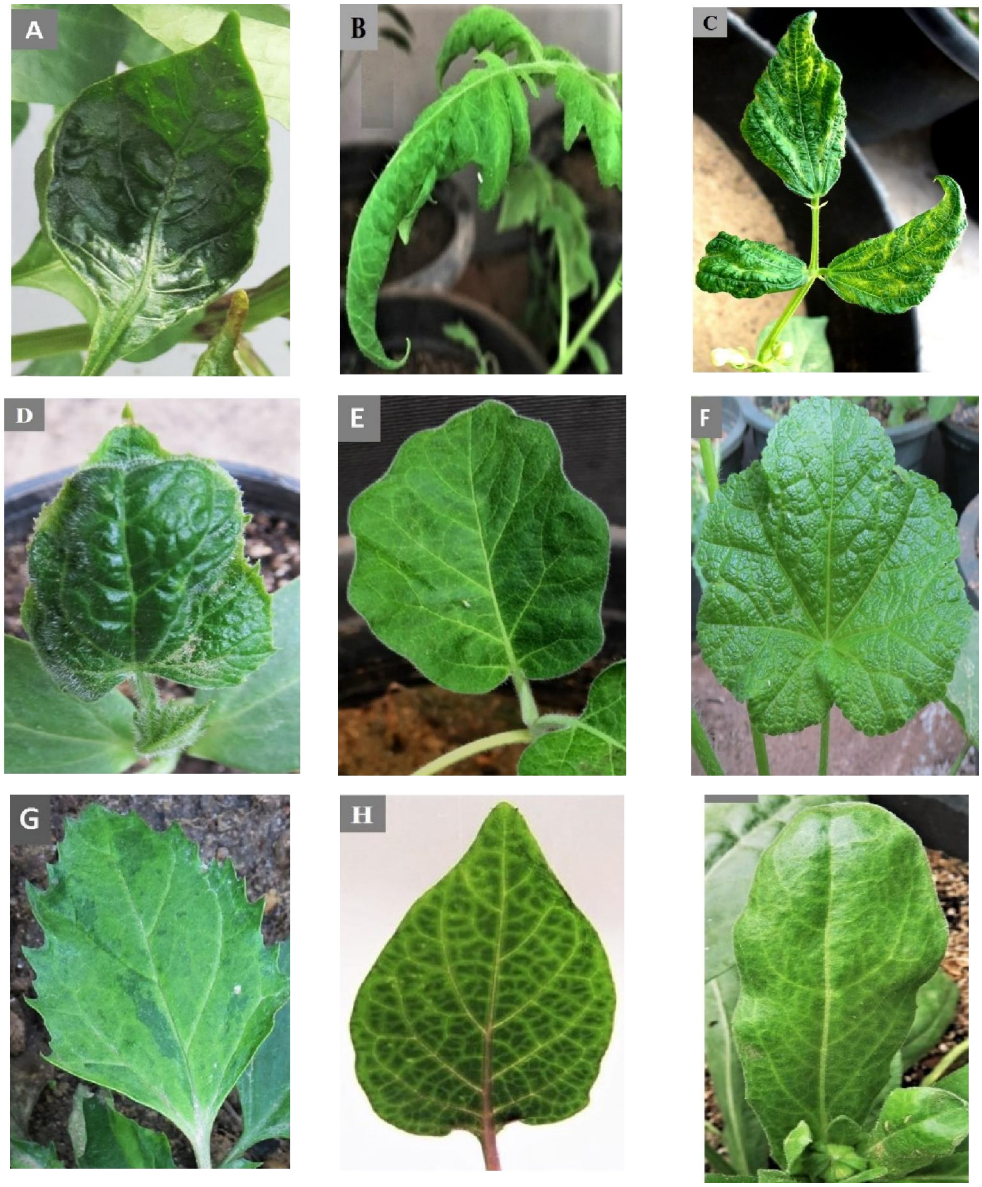

Fig. 2: Symptoms associated with SLCV infection upon whitefly transmission to experimental host range. (A) leaf curl and upward in pepper, (B) leaf narrow, leaf rolling, vein clearing and stunting in tomato, (C) downward leaf rolling, crumpling, and vein clearing in common bean, (D) leaf curl, downward and crumpling in cucumber, (E) mosaic, crumpling and vein clearing in eggplant, (F) venial chlorosis clearing and leaf rolling in *Althaea officinalis*, (G) vein clearing and stunting in *Chenopodium album*, (H) vein clearing and stunting in *Solanum nigrum*, (I) vein clearing in marigold.

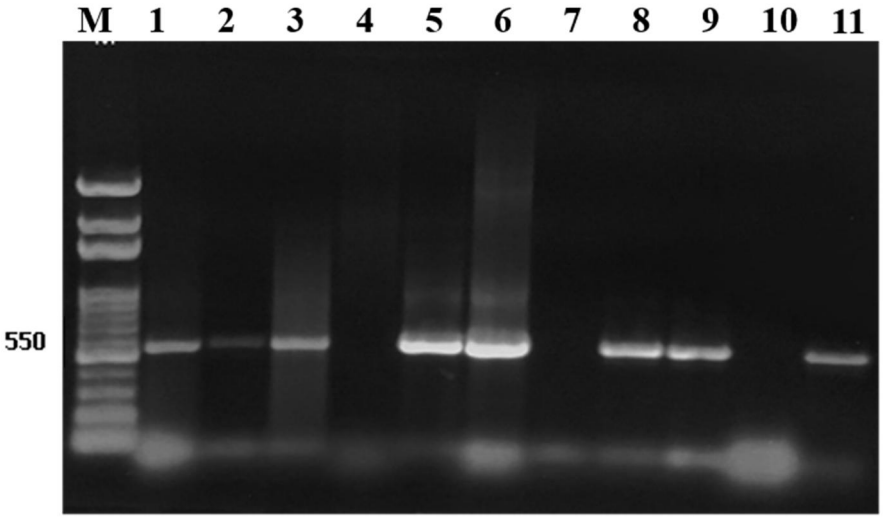

Fig. 3. Molecular evidence of a begomovirus present in symptomatic solanaceous vegetable crops. Agarose gel electrophoresis of PCR products obtained by PCR using begomovirus genus specific degenerate primers (Wyatt and Brown 1996) from infected and healthy leaf samples. Lanes (1–3) tomato samples, lanes (4-6) pepper samples, lanes (7-9) eggplant samples. Lanes (10 and 11) negative and positive control; respectively. **M** is 100bp molecular marker.

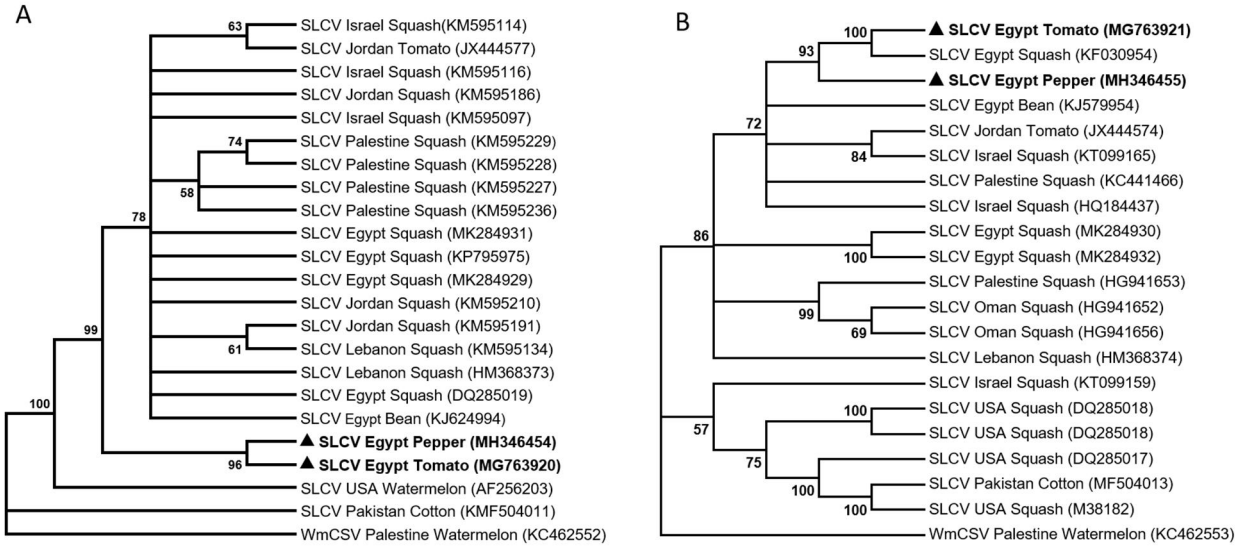

Fig. 4. Phylogenetic trees of DNA-A (A) and DNA-B (B) sequence from *Squash Leaf Curl Virus* (SLCV) sequences and other closely related begomoviruses. The DNA sequence of *Watermelon Chlorotic Stunt Virus* (WmCSV), a bipartite begomovirus from Palestine, was used as an outgroup. The black triangle denoted to isolates, SLCV-Tom and SLCV-Pep, infecting tomato and pepper; respectively. The trees were constructed by the neighbor-joining method using Mega 7.0 program and condensed to show only clusters with bootstrap value more than 50%. Bootstrap values (1000 replicates) are given at the branch nodes. Viruses acronym, country, host, and GenBank accession numbers are shown in the trees.

Discussion

SLCV is considered as a typical ‘‘New World’’ bipartite begomovirus that has been introduced during the last decade by an ambiguous route in the Mediterranean countries (Antignus *et al.*, 2003; Idris & Abedl-Salam, 2006; Abudy *et al.*, 2010; Ali-Shtayeh *et al.*, 2014; Lapidot *et al.*,

2014). The virus constitutes one of the major constraints for cucurbit crops in the region wherever whitefly *B. tabaci* exists (Lecoq & Desbiez, 2012).

In Egypt, SLCV was reported infecting cucurbits and common bean (Abdel-Salam *et al.*, 2006; El-Dougdoug *et al.*, 2009; Farrag *et al.*, 2014; EL-Rahmany, 2015). However, the knowledge about occurrence of SLCV in solanaceous crops is scarce. Results from PCR tests indicated a high incidence of SLCV in tomato, pepper, eggplant, and tomatillo in all surveyed Governorates, Qalyubiyah, Giza, Menoufia, and Fayoum. Occurrence of SLCV in solanaceous vegetable crops is comparable with those reported in cucurbits and common bean in Egypt and nearby countries (Abdel-Salam *et al.*, 2006; Farrag *et al.*, 2014; Ali-Shtayeh *et al.*, 2014; Lapidot *et al.*, 2014).

The high incidence of SLCV observed in samples collected from Qalyubiyah, Giza, Menoufia, and Fayoum Governorates during summer and autumn seasons, where *B. tabaci* whitefly population is high in surveyed areas. However, low SLCV incidence observed only in Baheira Governorate, during winter season. The positive correlation between the activity of whiteflies and disease incidence was reported previously (Ali-Shtayeh *et al.*, 2014). It worth to mention that, a number of plants exhibited begomovirus-like symptoms in the field, however, they were SLCV-negative. This result indicates that these plants may be infected with other viruses, therefore there is a need for more investigation to ascertained the causal agent associated with these diseased plants.

Interestingly, fifteen tomato samples found co-infected with SLCV and TYLCV in Qalyubiyah Governorate, similar to results reported previously by (Ahmad *et al*., 2013). Other reports showed that SLCV prone to produce reassortants with related viruses, that may modify both pathogenicity and host range (Brown *et al*., 2002; Sufrin-Ringwald & Lapidot, 2011).

SLCV was detected in four common weed species grown within solanaceous crops in surveyed areas in Egypt. SLCV-infecting weeds such as *Convolvulus sp*, *Chenopodium album*, *Solanum nigrum,* and *Malva parviflora* are prevailed in Egypt and could serve as a reservoir of SLCV throughout the year. Similarly, SLCV detected in *Malva parviflora* in Jordan*, Malva nicaeensis* and *Ecballium elaterium* in Israel (Antignus *et al.*, 2003; Al-Musa *et al.*, 2008). These findings are important from epidemiological point of view because, in the presence of SLCV-susceptible weeds and high population of whitefly *B. tabaci,* SLCV management will became more challenging. SLCV-Tom and SLCV-Pep isolates, transmitted by whitefly *B. tabaci* to eleven plant species belonging to five botanical families, indicated that SLCV is a potential threat to other economically important crops (Antignus *et al.*, 2003; Al-Musa *et al.*, 2008; Abudy *et al.*, 2010).

Similar to all ‘‘New World’’ begomoviruses, the genome organization of SLCV-Tom and SLCV-Pep shares similarities to that of WmCSV except that, SLCV lacks ORF AV2 (Fauquet *et al.*, 2005; Briddon *et al.*, 2010). The high nucleotide sequence identity between SLCV-Tom and SLCV-Pep indicate that they are variants of SLCV, based on species demarcation criteria (Fauquet *et al.*, 2008).

In conclusion, the occurrence of SLCV on tomato and pepper is a new report on new hosts. Also, occurrence and survival of SLCV on common weeds and other commercially important hosts during offseason when solanaceous vegetable crops are not grown may be considered as a threat to such commercially important crops in Egypt.

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References

Abdel-Salam, A.M., N.N. Abdallah, D.Z. Soliman and A.A. Rezk, 2006. The incidence of squash leaf curlbegomovirus in Egypt. Arab journal of Biotechnology, 9, 375–388.

Abudy, A., T. Sufrin-Ringwald, Dayan-Glick C *et al.*, 2010. Watermelon chlorotic stunt and Squash leaf curl begomoviruses—New threats to cucurbit crops in the Middle East. Israel Journal of Plant Sciences, 58, 33–42.

Ahmad, F.H., W. Odeh and G. Anfoka, 2013. First Report on the Association of Squash leaf curl virus and Watermelon chlorotic stunt virus with Tomato Yellow Leaf Curl Disease. Plant Disease, 97, 428–428.

Ali-Shtayeh, M.S., R.M. Jamous, E.Y. Hussein, O.B. Mallah and S.Y. Abu-Zeitoun, 2014a. Squash leaf curl virus (SLCV): a serious disease threatening cucurbits production in Palestine. Virus Genes, 48, 320–328.

Ali-Shtayeh, M.S., R.M. Jamous, O.B. Mallah and S.Y. Abu-Zeitoun, 2014b. Molecular characterization of Watermelon chlorotic stunt virus (WmCSV) from Palestine. Viruses, 6, 2444–2462.

Al-Musa, A., G. Anfoka, S. Misbeh, M. Abhary and F.H. Ahmad, 2008. Detection and molecular characterization of Squash leaf curl virus (SLCV) in Jordan. Journal of phytopathology, 156, 311–316.

Antignus, Y., O. Lachman, M. Pearlsman, O. Uko, S. Omer, Y. Hisham, A .Koren, 2003. Watermelon chlorotic stunt virus (WmCSV) a new virus disease of watermelons and melons. Gan Sadeh Vameshek, October, 53-54. (Hebrew)

Briddon, R.W., B.L. Patil, B. Bagewadi, M.S. Nawaz-ul-Rehman and C.M. Fauquet, 2010. Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses. BMC Evolutionary Biology, 10, 97.

Doyle, J.J., 1990. Isolation of plant DNA from fresh tissue. Focus, 12, 13–15.

El-Dougdoug Kh.A., H.S. AbdEl-Kader, I.A. Hamad, E.A. Ahmed and A.F. Abd El-Monem, 2009. Identification of Squash Leaf Curl Virus (Egyptian Isolate). Australian Journal of Basic and Applied Sciences 3(4): 3470–3478.

EL-Rahmany, R., 2015. Molecular studies on some whitefly transmitted viruses affecting cucurbits. Thesis, Submitted in Partial Fulfillment of the Requirements for the Degree of. In: MSc in Agricultural Sciences.

Farrag, A.A., A.K. El-Attar, Om-Hashem M. El-Banna, A. I. Ibrahim, H.M. Mazyad, 2014. Molecular and biological characterization of squash leaf curl virus (SLCV) affecting common beans in Egypt. Egyptian journal of Virology, 11, 14–27.

Fauquet, C.M., R.W. Briddon, J.K. Brown *et al.*, 2008. Geminivirus strain demarcation and nomenclature. Archives of Virology, 153, 783–821.

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

Hall, T.A., 1999. Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic acids symposium series*. [London]: Information Retrieval Ltd., c1979-c2000., 95–98.

Idris, A. M., A. Abdel-Salam, and J. K. Brown, 2006. Introduction of the New World Squash leaf curl virus to Squash (*Cucurbita pepo*) in Egypt: a potential threat to important food crops. Plant Disease, 90(9). https://doi.org/10.1094/PD-90-1262B

Kumar, S., G. Stecher and K. Tamura, 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33, 1870–1874.

Lapidot, M., D. Gelbart, A. Gal-On *et al.*, 2014. Frequent migration of introduced cucurbit-infecting begomoviruses among Middle Eastern countries. Virology Journal, 11, 181.

Lazarowitz, S.G., L.C. Wu, S.G. Rogers and J.S. Elmer, 1992. Sequence-specific interaction with the viral AL1 protein identifies a geminivirus DNA replication origin. The Plant Cell, 4, 799–809.

Lecoq, H. and C. Desbiez, 2012. Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. In: Advances in Virus Research. Elsevier, 67–126.

Sunter, G. and D.M. Bisaro, 1991. Transactivation in a geminivirus: AL2 gene product is needed for coat protein expression. Virology, 180, 416–419.

Sunter, G. and D.M. Bisaro, 1992. Transactivation of geminivirus AR1 and BR1 gene expression by the viral AL2 gene product occurs at the level of transcription. The Plant Cell, 4, 1321–1331.

Wyatt, S.D. and J.K. Brown, 1996. Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathology, 12.