Molecular and Biological Characterization of *Squash Leaf Curl Virus* (SLCV) Affecting Common Beans in Egypt

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ABSTRACT

Symptomatic leaf samples obtained from common bean plants were collected from Qaliobeya governorate, Egypt, and tested for Squash Leaf Curl Virus (SLCV) infection by PCR using both degenerate and specific oligonucleotide primers. SLCV (bean isolate) was transmitted from naturally infected common bean onto twenty two species and varieties belonging to six different families *i.e. Moraceae, Solanaeae, Cucurbitaceae, Leguminoceae, Chenopodiaceae,* and *Malvaceae* using viruliferous whitefly (*Bemisia tabaci).* Results revealed that SLCV could be transmitted to 16 out of 22 species and positively back inoculated to beans from these hosts. Nucleotide sequencing of the complete genome of the virus was as follow, DNA-A (2667 bp) and DNA-B (2621 bp). The obtained sequences were submitted into the GenBank with accession numbers KJ624994 and KJ579954.The full nucleotide sequence of DNA-A, DNA-B and all open reading frames (ORF) of the SLCV-bean was aligned and compared with eleven different isolates of the SLCV available in the GenBank. The phylogenetic analysis of the complete nucleotide sequence revealed that SLCV is related to other isolates of SLCV from other governorates in Egypt (Cairo and Ismailia) as well as isolates from Lebanon (SLCV-LB2), Palestine (SLCV-Pal), Jordan (SLCV-JO) and Israel (SLCV-IL) sharing high identities ranging from (90% to 97%).

Key words: Common bean, Squash Leaf Curl Virus, PCR**,** phylogenetic analysis, Egypt

INTRODUCTION

Gemini viruses have emerged as serious pathogens of agronomic and horticultural crops (Hanley *et al.,* 2013). Members of the family *Geminiviridae* are plant viruses with circular single-stranded DNA genomes encapsidated in twin particles. Based on their genome arrangement, insect vector, and host range Geminiviruses are classified into seven genera: *Becurtovirus* (2 Species), *Begomovirus* (192 Species), *Curtovirus* (3 Species), Eragrovirus (1 Species), *Mastrevirus* (29 Species), *Topocuvirus* (1Species), and *Turncurtovirus* (1 Species) (Fauquet *et al.,* 2003; Varma and Malathi, 2003; Sopid, 2009; Varsani *et al.,* 2014). The *Begomovirus* genus is the largest genus of this family that infects dicotyledonous and monocotyledonous plants and cause devastated-crop production (Jones, 2003 and Mansoor *et al.,* 2003). *Begomoviruses* are exclusively transmitted in a persistent manner by the whitefly *Bemisia tabaci*and leafhopper insects. Most *begomoviruses* have a bipartite genome that consists of a DNA-Aand DNA-B components (Sufrin*et al.,* 2008; Nawaz-ul-Rehman and Fauquet, 2009; Jeske *et al.,* 2010).Five ORFs encoded by DNA-A, which involved in replication, regulation of gene expression, and encapsidation, while DNA-B

contains two ORFs, encoding for proteins involved in viral movement and symptom development (Lazarowitz and Lazdins, 1991; Ingham *et al.,* 1995;Gutierrez, 2002; Hanley-Bowdoin *et al.,* 2004). DNA-A, contain one ORF on the viral strand AV1 which encode for the capsid protein and four ORFs on the complementary strand: AC1 encodes for the Rep protein, which is the only viral protein essential for viral replication. AC2 encodes for a transcription activator protein (TrAP), AC3 encodes for a replication enhancer protein (REn), and a small ORF, AC4which is located within the AC1 ORF, but in a different reading frame (Gutierrez, 2002; Hanely-Bowdoin *et al.,* 2013 and Ali Shtayeh *et al,.* 2014). DNA-B contains two ORFs. These are BV1, on the viral strand, which encodes for the nuclear shuttle protein (NSP), controlling the transport of viral DNA between the nucleus and the cytoplasm, and BC1, on the complementary strand, which mediates viral cell-to-cell movement (Lazarowitz and Beachy, 1999; Mohammed *et al.,* 2014).

SLCV was isolated for the first time in Egypt from squash plants growing in Qaliobeya Governorate at 2004 (Farag *et al.,* 2005).The virus showing symptoms on squash plants as leaf curling, yellow, mottling, stunting and reduction on fruit set (Idris *et al.,* 2006 and El-Dougdoug *et al.,*2009).SLCV affects various species of cucurbits, common bean and Malva (Hill *et al.,* 1998; Al-Musa *et al.,* 2008).SLCV was recorded for the first time on *Phaseolus vulgars* plants by (Brown, 1990).

Aim of this studyis to detect, identify and characterize the SLCV that can infect bean plants using both biological and molecular tools.

MATERIALS AND METHODS

Isolation and propagation of virus:

Leaf samples were collected from infected bean plants (*Phaseolus vulgariscv.* Bronco) Cultivated in Qaliobeya governorate. Samples were tested by PCR using Geminivirus degenerate primers and Specific primers for *Squash Leaf Curl Virus* (SLCV) (Table 1). Infected plants, those gave positive results by PCR, were served as a source for the virus. The virus isolate was inoculated on 15 days-old healthy bean seedlings using virus free whiteflies, *Bemisia tabaci* then Plants were kept in insect proof greenhouse

Whitefly maintenance and plant inoculation

Whiteflies *Bemisia tabaci* were collected from the open field and purified from Geminivirus in glass cages held in an insect proof greenhouse through rearing on SLCV- resistant plants *i.e.* mulberry and Tomato. Whiteflies were tested by PCR to confirm that they are virus free. Non-viruliferous whiteflies were allowed a 48-hrs acquisition access period (AAP)on SLCV infected beans followed by a 48-hrs inoculation access period (IAP) using 20 whiteflies per plant. Plants were kept in insect proof greenhouse for one month after inoculation.

Twenty two species and varieties belonging to six families (Moraceae, Solanaeae, Cucurbitaceae, Fabaceae, Chenopodiaceae, and Malvaceae) were inoculated using viruliferous insects (Table 2). Plants, those inoculated by non-viruliferous whiteflies, were used as negative control. All treated plants were sprayed with insectside “Actellic ® 5E” and kept under insect proof greenhouse conditions for symptoms development, The Viral-infection occurrence was

confirmed by PCR. Back inoculation to bean plants was followed.

PCR detection of SLCV

Total DNA was extracted from collected symptomatic and non-symptomatic bean samples using Dellaporta method (Dellaporta *et al.,* 1983). Polymerase chain reaction (PCR) was used for the detection of SLCV in bean tissues using degenerate primers AV-core and AC-core (Abdel-Salam *et al.* 2006) shown in Table 1. Molecular diagnosis of SLCV was performed using a specific PCR primer pair;v268/c1166 (Table 1). All PCR reactions were performed in 25 µl volume containing 1 µl of DNA, 1.25 UTaq DNA Polymerase, 200 µM of each dNTP. 2.5 µl 10x Dream Taq Buffer, 25 pmol of each primer, 2.5 mM MgCl2 and 15.25 µl of sterile water. PCR tubes containing DNA, extracted from healthy squash plants, were used as negative controls. PCR was designed as 94°C for 3min, followed by 35 cycles, 94°C for 1 min, 60°C for 1min,, 72°C for 2min, and final extension at 72°C for10 minutes. The PCR products were stained with gel star (Lonza, USA) and separated on 1% agarose gel with 1x TBE buffer then analyzed using (Gel Doc 2000 Bio.RAD).. All PCR amplifications were performed in T-Gradient thermal cycler (Biometra, Germany).

Amplification of DNA-A and DNA-B of SLCV

Full-length DNA-A and DNA-B components were amplified from infected bean tissues using two sets (Haj Ahmad *et al.,* 2013). SLCVF-SalI and SLCVR-SalI pair was used to amplify component A while SLCVBF-HindIII and SLCVBR-HindIII were used to amplify component B (Table 1). The template DNA was denatured for 3 min at 94°C followed by 35 cycles starting with denaturation at 94°C for 45 sec., primer annealing at 55°C for 45 sec and extension at 72°C for 4 minutes. Final extension at the end of the 35th cycle was performed at 72°ċ for 10 minutes. The PCR products were analyzed on 0.7 % agarose gel electrophoresis.

Cloning and sequencing

Five µl of the PCR products representing the full-length component A and/or component B were ligated into pGEM-T Easy vector (Promega, USA) using T4 DNA ligase enzyme according to the manufacturer's instructions. The cloning reaction mixture was transformed into DH5α *Escherichia coli* competent cells by heat shock at 42° C for 1 min. Transformed competent cells were cultured on LB liquid media for one hour then grown on LB agar plates containing (100 µg/ml) ampicillin, coated with IPTG and X-Gal then incubated at 37°C overnight. The plasmid DNA constructs were extracted from the selected *E. coli* white colonies using alkaline lysis method (Maniaties *et al.,* 1982).The purified plasmids were digested using *EcoRI* restriction enzyme. The DNA-A and DNA-B carrying constructs were directly sequenced (Macrogen Inc.; South Korea). The full nucleotide sequences were analyzed utilizing the DNAMAN Sequence Analysis Software (Lynnon BioSoft. Quebec, Canada) and compared with those of SLCV different isolates available in GenBank. All open reading frames (ORFs) of components A and B were identified and compared with the corresponding ORFs of other SLCV isolates available in GenBank.

Table (1): Primers used in PCR detection and Full genome organization

	Primer name	Primer sequence	Expected size
1	AV-core	GCCHATRTAYAGRAAGCCMAGRAT	~ 550 bp
2	AC-core	GGRTTDGARGCATGHGTACANGCC	
3	v268	CGGGGACCACACACAGCAC	900 bp
4	c1166	ACAATGGATACGCGCGCC	
5	SLCVF-SalI	TATAGTCGACGTTGAACCGGATTTGAATG	2667 bp
6	SLCVR-SalI	TATAGTCGACCTGAGGAGAGCACTAAATC	
7	SLCVBF-HindIII	ATTAAAGCTTAGTGGTTATGCAAGGCGT	2621 bp
8	SLCVBR-HindIII	ATTAAAGCTTGGCTGCACCATATGAACG	

RESULTS

Virus isolation:

SLCV was isolated and propagated on inoculated 15 days-old healthy beans cv. Bronco using whiteflies. After 2-3 weeks from inoculation plant showed Leaf malformation, mosaic and leaf curling and gave positive results with PCR test.

Host range study:

Twenty two species belonging to six families were tested for its susceptibility to SLCV. Data presented in (Table 2) revealed that SLCV could be transmitted to 16 out of 22 species (Fig.1) and could be back inoculated to beans from these hosts .SLCV caused mottling and growth stunting on *Chenopodium quinoa*, mosaic and leaf curling on *Cucumissativus* cv. Beitalpha, *Citrullus lanatus* cv. Giza1, *Cucurbita pepo* cv. Eskandrany. All *Phaseolus vulgaris* cultivars developed mosaic and downward leaf curling except the" Top Crop" cltivar that showed sever upward leaf curling, vein distortion and stunted growth .Youngest leaves of Cowpea (*Vigna unguiculata* subsp. *unguiculata*) developed Leaf malformation, mosaic and leaf curling. Upward leaf curling symptoms were developed on the emerging leaves of marshmallow (*Althaeaofficinalis*). Eggplant (*Solanum melongena*) developed mosaic and crinkle on young leaves, Mosaic and leaf curling on pepper (*Capsicum annuum*) leaves, mosaic and yellowing on *Physalis peruviana.* No symptoms were observed on the following inoculated species: *Morusnigra*, *Luffaa egyptiaca, Solanum esculentum* cv., Castel Rock, *Datura stramonium* and *Solanum nigrum* which gave also negative results with PCR test.

Molecular detection of SLCV by polymerase chain reaction:

All infected bean samples as well as the viruliferous whiteflies showed a fragment at the expected size of approximately ~550 bp when the degenerate primer pair AV-core/AC-core was used (Fig.2A). The SLCV specific primer pair v268/c1166 was used successfully to amplify 900 bp fragments (Fig.2B) from infected hosts as well as from the naturally infected bean samples collected from different fields.

Amplification of the SLCV full genome:

Electrophoresis analysis showed clear bands at the expected size for the complete SLCV DNA-A and –DNA-B

components, where DNA-A fragment found to be ~ 2,667 bp and for DNA-B was ~2,621 bp (Fig.3A). PCR products of the full length components A and B were cloned successfully in pGEM-T Easy vector. The restriction digestion analysis using *EcoRI* enzyme confirmed the successful cloning. Electrophoresis analysis on 0.7% agarose gel separated the digested fragments representing the full length genome (Fig.3B).

Table 2: Host range for SLCV Bean Isolate by whitefly-mediated transmission employing a 48-hrs followed by a 48-hrs inoculation access period

Tested Host	Days for symptoms expression	Reaction	Infectivity Infected/tested	PCR Test
Family *Moraceae*				
Morusnigra		N.S.	0/10	-
Family *Chenopodiaceae*				
Chenopodium quinoa	15	Mo.	4/10	+
Family *Cucurbitaceae*				
Cucumissativuscv.Beitalpha	17	M &LC	9/10	+
Citrulluslanatus cv. Giza 1	20	M &LC	7/10	+
Cucurbitapepo cv. Eskandrany	19	M &LC	9/10	+
Luffaaegyptiaca		N.s.	0/10	-
Family *Fabaceae*				
Phaseolus vulgaris cv. Top Crop	15	L.C.,V.D.	10/10	+
cv. Neprasca	16	L.C.	9/10	+
cv. Bronco	15	L.C.	7/10	+
cv. Tema	20	L.C.	10/10	+
cv. Paulista	15	L.C.	8/10	+
cv. Smenta	16	L.C.	10/10	+
cv. Giza 6	19	L.C.	5/10	+
Vignaanguiculata subsp.unguiculata	19	L.C.	4/10	+
Family *Malvaceae*				
Althaeaofficinalis	20	L.C.	8/10	+
Gossypiumherbaceum		N.S.	0/10	-
Family *Solanaceae*				
Solanumesculentumcv. Castel Rock.		N.s.	0/10	-
Solanummelongena	18	M &L.C.	8/10	+
Capsicum annuum	17	M &L.C.	6/10	+
Physalisperuviana	20	M &Y	4/10	+
Daturastramonium		N.s.	0/10	-
Solanumnigrum		N.s.	0/10	-

Mo= Mottling, M=Mosaic,L.C. =Leaf curling,V.D=vein distortion, Y= yellowing, N.S, = no symptoms

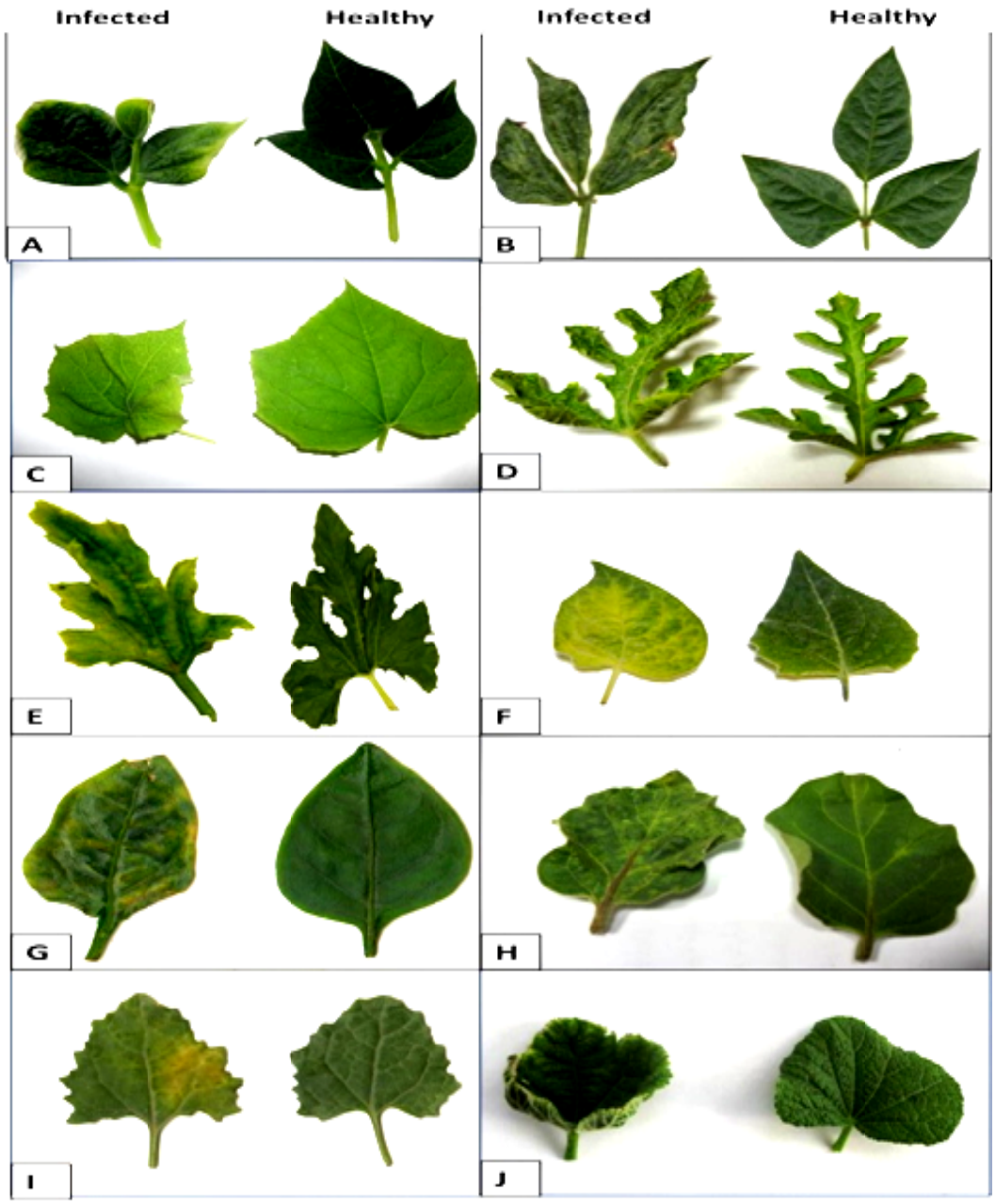
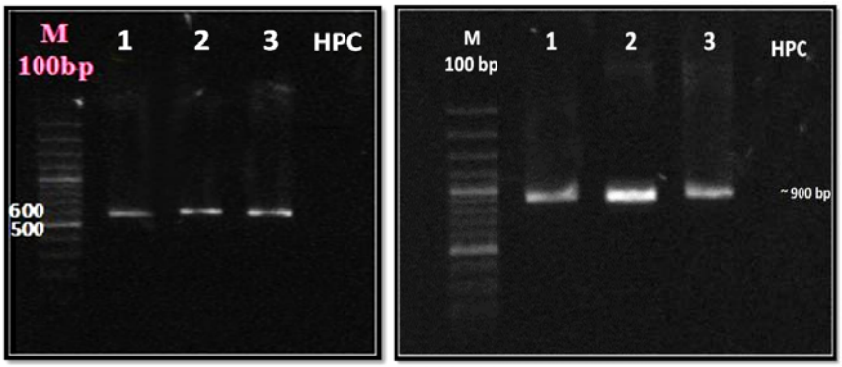
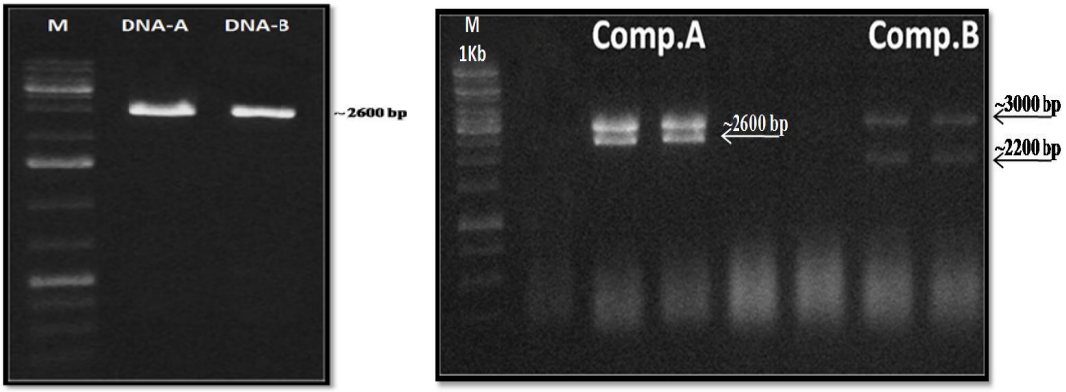


Fig.1.Symptoms induced by SLCV on the different hosts (infected) compared with the healthy plant controls. A: mosaic and downward leaf curling on *Phaseolus vulgaris* cultivars Bronco. B: Leaf malformation, mosaic and leaf curling on Cowpea. C:Mosaic and leaf curling on cucumber leaves cv Betaalpha, D: Mosaic and leaf curling on watermelon leaves var Giza 1,E:Mosaic and leaf curling on Squash leaves var. Eskandrany, F:mosaic and yellowing on *Physalis peruviana, G:*Mosaic and leaf curling on pepper (*Capsicum annuum*) leaves, H:developed mosaic and crinkle on young leaves of eggplant, I:mottling and growth stunting on *Chenopodium quinoa,* J:.Upward leaf curling symptoms were developed on the emerging leaves of marshmallow.



[A] [B]

Fig. 2. PCR detection of SLCV using the AV/AC core degenerate primer pair specific for Gemini viruses [A]; and v268/c1166 primer pair specific for SLCV [B]. M= 100 bpDNAladder.L1, L2 and L3 common bean samples infected with the SLCV.HPC: healthy plant control.



[A] [B]

Fig.3: Amplification of DNA-A and DNA-B full genome of SLCV [A]. Digestion analysis of the DNA-A and DNA-B carrying pGEM-T Easy cloning vector using *EcoRI* restriction enzyme [B]. M: 1 kb DNA ladder.

Sequence analysis and Genomic organization of SLCV

To generate the full-length sequence of DNA-A and DNA-B of the SLCV–Qaliobeya isolate; the sequenced fragments for each component were assembled together and analyzed using DNAMAN software. Accordingly, the complete nucleotide sequence of the DNA-A component was determined to be 2667 bp in length and contains five open reading frames (ORFs), AV1, AC1, AC2, AC3 and AC4. While the complete nucleotide sequence of the DNA-B component was determined to be 2621 bp encoding two ORFs BV1 and BC1.Sequences of the SLCV DNA-A and DNA-B (Qaliobeya isolate)were submitted, individually, into the GenBank with accession numbers KJ624994 and KJ579954, respectively.

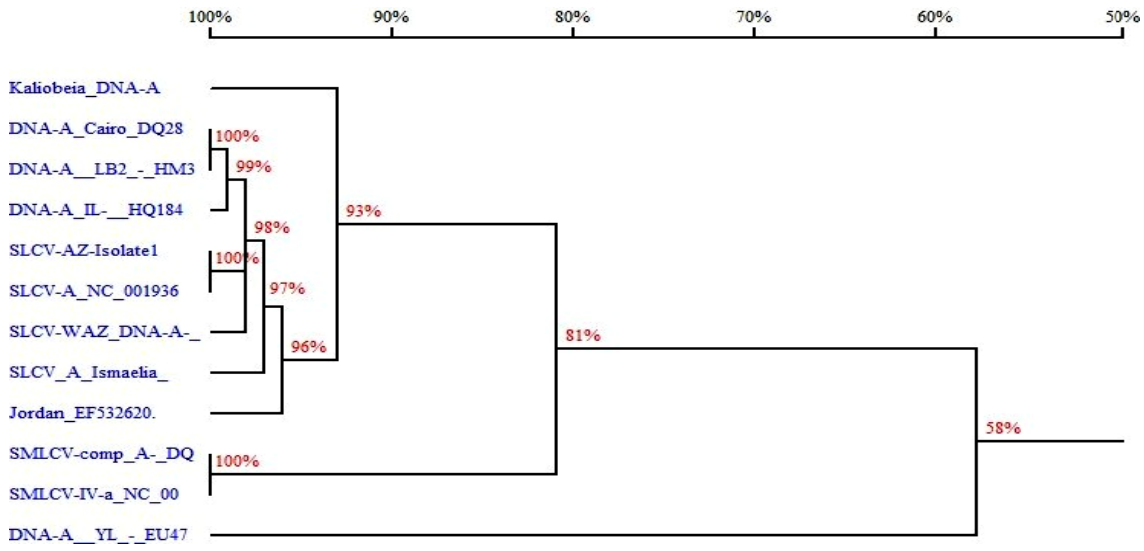
Multiple sequence alignments and phylogenetic analysis (Fig.4A,B) were carried out between sequences of DNA-A and DNA-B, including all open reading frames, of the SLCV-Qaliobeya and other

SLCV isolates available in the GenBank(Tables 3 and 4). The highest degree of identity in DNA-A, ranged from 92.26% up to 93.26 %,was detected between Qaliobeya isolate and Cairo (DQ285019) from Egypt (93.26%), IL(HQ184436)from Israel (93.26%), LB(HM368373) from Lebanon (93.18%), Homra (JX444577)from Jordan, 93.07%, Pal (KC441465) from Palestine (92.89%) and Ismailia(KC895398) from Egypt (92.62%).A lower nucleotide identity (77.15 %) was observed with Squash mild leaf curl virus (DQ285014 and NC_004645) from USA: Imperial Valley, California. The comparison of the complete DNA-B showed a relatively high degree of nucleotide identity detected between Qaliobeya and Pal (KC441466) from Palestine (**97.03%),** IL (HQ184437) from Israel (96.99%), LB (HM368374) from Lebanon (96.72%) and Homra (JX444574) from Jordan (96.72%).While a lower degree of nucleotide identity (89.85%) was observed with Ismailia (KF030954) from Egypt. Similarly, like DNA-A, the lowest degree of identity (68.45% and 68.54%) was detected with Squash mild leaf curl virus (DQ285015 and NC_004646) from USA.

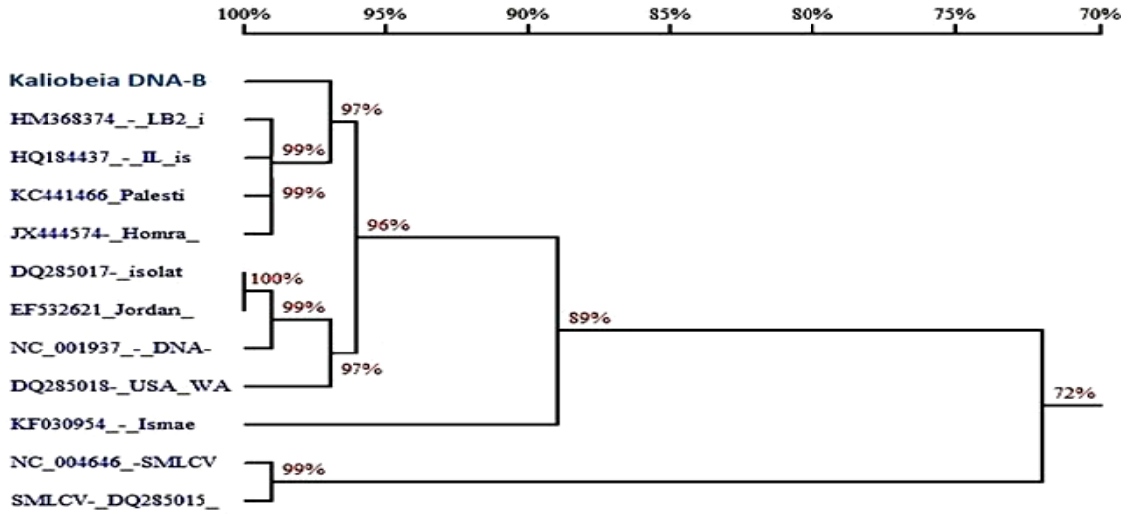
The sequence comparison of the five open reading frames of DNA-A with those of other *begomoviruses* showed that; the ORF, AV1 (251 aa), was found in the viral sense and the other four ORFs, AC1 (346 aa), AC2 (133 aa), AC3 (129 aa), and AC4 (124 aa), in complementary sense. While the DNA-B encodes two ORFs, BV1 (254 aa) in viral sense and BC1 (293 aa) in complementary sense. Multiple alignments and sequence Comparison between ORFs of the Qaliobeya isolate and other SLCV isolates showed that, AV1 ORFs shared high identity (more than 96 and up to 99.47 %) with those of SLCV- Cairo, SLCV- Ismailia, SLACV-Jordan, SLACV- USA, SLCV-[Palestine], SLCV-[Lebanon] and SLCV-[Israel] and lower identity (~ 87 %) with the SMLCV. The three ORFs AC1, AC2 and AC3 shared identity lower than that of AV1 among the same isolates. In contrast, AC4 showed a very low identity (56 up to 67 %) with all other SLCV isolates. Comparison of the DNA-B showed that; the BV1 ORFs shared identity more than 90% with those of SLCV-Ismailia [Egypt], SLCV-[IL], SLCV- Homra and SLCV-[Palestine] and shared lower identity (less that 80%)with SLCV- Jordan, SLCV-[Lebanon] and SLCV-USA. While the BC1 ORFs shared high identity(97 to 99 %) with those of SLCV-[Lebanon], SLCV-[Israel], SLCV-Jordan, SLCV- Homra, SLCV- USA and SLCV-[Palestine] but it shared unexpectedly, low identity (80%) with SLCV-Ismailia [Egypt].

DISCUSSION

The occurrence of SLCV in the region was previously reported in Israel and Egypt on cucurbits (Antignus *et al.,* 2003; Idris *et al.,* 2006 and Sobh *et al.* 2012).Data presented here demonstrate the occurrence of SLCV on common bean fields in Egypt. PCR successfully detected SLCV in Beans tissue, either through the natural infection or the artificial inoculation by whitefly insect. These results showed that, SLCV can infect common bean naturally or artificially using the high population of whitefly (20whitefly/plant) which agreed with (Navas *et al.*, 2011).The data reflected the broader host range of SLCV including agricultural, ornamental, and



[a]



[b]

Fig. 4.Phylogenetic trees based on a multiple sequence alignment of the complete DNA-A (a) and DNA-B (b) components of Bean isolate (Qaliobeya) and other SLCV isolates available in the GenBank. The tree was generated using the DNAMAN. Horizontal distances are proportional to sequence distances (see scale bar).

Table (3): Percentage of nucleotide sequence identities (%) between SLCV-Qaliobeya [Egypt] DNA-A and other SLCV isolates available in the GenBank.

	ISOLATE	ACC. NUMBER	COMPLETE NT. %	DNA-A CDS				
				AV1	AC1	AC2	AC3	AC4
1	Cairo	DQ285019	**93.26**	**99.47**	86.95	95.29	91.38	67.00
2	Ismailia	KC895398	**92.62**	**99.07**	85.37	95.78	92.12	65.52
3	Jordan	EF532620	**92.43**	**98.81**	85.18	94.79	90.89	56.65
4	Lebanon-LB	HM368373	**93.18**	**99.21**	87.5	95.29	91.63	67.73
5	Israel-IL	HQ184436	**93.26**	**99.07**	87.33	69.84	91.13	61.82
6	USA-IV	DQ285016	91.91	**98.02**	85.18	94.04	90.64	64.29
7	USA-WAZ	AF256203	90.68	**96.43**	84.81	93.05	88.42	63.05
8	SMLCV	DQ285014	77.15	87.17	73.98	80.65	79.06	57.72
9	SMLCV-IV	NC_004645	77.15	87.30	74.07	80.89	79.06	---
10	Palestine-Pal	KC441465	**92.89**	**99.12**	86.86	95.04	91.38	67.00
11	Jordan- Homra	JX444577	**93.07**	**99.07**	87.14	71.99	91.38	67.73

Table (4): Percentage of nucleotide sequence identities (%) between SLCV-Qaliobeya [Egypt] DNA-B and other SLCV isolates available in the GenBank.

	ISOLATE	ACC. NUMBER	COMPLETE NT. %	DNA-B CDS	
				BV1	BC1
1	Ismailia	KF030954	89.85	91.28	80.05
2	Jordan Al Musa	EF532621	93.93	81.00	**98.07**
3	Lebanon-LB	HM368374	**96.72**	82.62	**98.75**
4	Israel - IL	HQ184437	**96.99**	92.95	**99.89**
5	USA - IV	DQ285017	93.93	81.11	**98.07**
6	USA - WAZ	DQ285018	93.25	79.95	**97.39**
7	SMLC	DQ285015	68.54	67.14	85.71
8	SMLC - IV	NC_004646	68.45	68.45	85.71
9	Palestine-Pal	KC441466	**97.03**	92.95	**99.32**
10	Jordan- Homra	JX444574	**96.72**	92.82	**99.09**

weed species commonly found in Egypt; These results agreed with Ghanim *et al,*.(2007); Al-Musa *et al.*,(2008) and El-Dougdoug *et al.*,(2009)who reported that SLCV had a wide host range between members of family *Cucurbitaceae*, *Fabaceae, Solanaceae*, *Malvaceae* and *Chenopodiaceae*. Moreover; the study of the host range showed that; common bean, pepper, eggplant and *physalis*, can work as a reservoir host and cause an increase in SLCV epidemics in Cucurbits crops. These results agreed with Navas-Castillo *et al.*,(1999).Molecular diagnosis based on the specific PCR detection of the virus, provided clear evidence on the infection of common bean plants with SLCV. The PCR detection of the virus was applied for all infected samples. The results presented in figures 2A and 2B showed bands at ~ 550 bp, when using degenerate primers, and 900 bp when

using specific primers, these results agreed with Sobh *et al.*(2012). All results achieved here showed the successful molecular characterization of the SLCV full genome isolated at Qaliobeya, Egypt from common bean plants. As described by Dellaporta et al. *(*1981); the Dellaporta method for DNA extraction provided sufficient template for a successful amplification of the full-length genomes (DNA-A and DNA-B) of SLCV utilizing the primers specific for DNA-A and DNA-B components (Ali-Shtayeh *et al.,* 2010). The results of the electrophoresis analysis for the PCR products, that showed sharp bands characteristic for the DNA-A and DNA-B at ~ 2,667 bp and ~2,621 respectively, confirmed the successful PCR amplification for the complete genome of SLCV components. The results obtained from the digestion analysis using EcoRI restriction enzyme as well as DNA sequencing confirmed the successful cloning of the full-length SLCV genomes. The nucleotide blast at NCBI data base was verified the identity of the sequenced fragments. The sequence analysis for the complete SLCV DNA-A and DNA-B of the Qaliobeya isolate reflected the typical arrangement of the open reading frames of all other SLCV isolates that matches the results obtained by Ali-Shtayeh*et al.,* 2014.The phylogenetic analysis which represented the sequence comparison between DNA-A and DNA-B of the Qaliobeya isolate with other SLCV isolates in the GenBank showed that, the degree of nucleotide identity was relatively low. While, the SLCV Qaliobeya isolate was found to be, relatively, highly homologous (93% to 97%) to SLCV from other governorates in Egypt (Cairo and Ismaielia) as well as the neighboring countries (Jordan, Lebanon, Palestine and Israel).The deeper analysis of the DNA-A and DNA-B sequences provided a clear evidence on that; the five open reading frames of DNA-A and the two ORFs of DNA-B have the same conserved genomic organization as well as the same coding orientations; but not the genes' size (numbers of amino acids); with those of other *begomoviruses* (Al-Musa *et al.,* 2008). Therefore; it's not known if there is any effects, due to the change in gene size, on the activities of the AC2 and AC4, where the AC2 protein is a transcription activator of the capsid protein [Abudy *et al.*,2010and Ali-Shtayeh *et al.*,2014], and AC4 protein is a pathogenicity determinant, that may act as a suppressor of post transcriptional gene silencing (PTGS) [Imran *et al.*,2011, Jie Zhang *et al.*,2012].Further researches are needed to study the distribution of the virus in Egypt as well as deeper sequence analysis and molecular characterization are required in the future to focus on the gene function of each ORF in the full genome of the SLCV, due to the economic importance of the disease caused by SLCV.

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