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Identification of Squash Leaf Curl Virus (Egyptian Isolate)

[1]Kh. A. El-Dougdoug; [2]Hayam S. Abd El-Kader;
[3]Ibtisam A. Hamad; [3]Entsar A. Ahmed and [3]Asmaa F. Abd El-Monem

[1]Microbiology Department, Faculty of Agriculture, Ain Shams University, Cairo,
[2]Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agriculture Research Center (ARC), Giza,
[3]Botany and Microbiology Department, Faculty of Science, Helwan University, Egypt.

Abstract: Squash leaf curl virus (SqLCV-Eg) was isolated from whiteflies-infected squash (*Cucurbita pepo* cv. Eskandrani) plants growing in Nubaria, El-Behera Governorate. The infected plants exhibited leaf curling, leaf crinkle, leaf narrowing, blistering, chlorosis, fruit malformation and stunting. SqLCV-Eg reacted positively with polyclonal antibodies specific to SqLCV using DAS-ELISA. It was transmitted by both syringe injection and whiteflies with transmission efficiency of about 35% and 100%, respectively. SqLCV-Eg isolate was transmitted from naturally infected squash to different species belonging to families *Cucurbitaceae, Fabaceae, Solanaceae* and *Chenopodiaceae.* SqLCV had TIP of 60ºC, DEP of 10^{-6} and LIV of about 8 days. Electron micrograph of the partially purified SqLCV revealed the presence of monomer and dimmer gemini particles with dimensions of 22 nm and 20 X 30 nm, respectively when negatively stained with uranyl acetate. Using degenerate oligonucleotide primers, the viral coat protein gene was amplified successfully by PCR, producing ~ 550 bp fragment from squash infected plants. The viral genome was detected by specific DNA probe using dot blot hybridization technique. Comparative nucleotide sequence analysis showed a similarity of 99% between SqLCV-Eg and other isolates.

Key words: Squash, Leaf Curl Virus, Egyptian isolate, identification.

INTRODUCTION

Squash leaf curl virus (SqLCV) belongs to genus begomovirus of family *Geminiviridae*. SqLCV is a severe viral disease of squash (*Cucurbita pepo* L.) in Egypt. It was isolated for the first time in Egypt from squash (*Cucurbita pepo* L. cv. Eskansarani) plants growing in Qaluobiya Governorate (Farag *et al.*, 2005). In the spring of 2005, Idris *et al.* (2006) observed that squash plants showing leaf curling, yellow mottling, stunting and reduced fruit set in Giza, Egypt. SqLCV disease affects various species of cucurbits e.g. squash, pumpkin and watermelons (*Citrullus* sp.). *P.vulgaris* is the non-cucurbitaceous host affected by SqLCV (Hill *et al.*, 1998). Al-Musa *et al.* (2008) reported that SqLCV was found to occur naturally in *Malva parviflora*. SqLCV virus is transmitted efficiently by whitefly, *Bemesia tabaci* (Genn) (Cohen *et al.,* 1983 and Mc Creight, 1984). 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).

The present study demonstrates the identification of Squash leaf curl virus (SqLCV-Egyptian isolate) through biological and molecular studies.

Corresponding Author: Khaled El-Dougdoug, Microbiology Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
E-mail: drdougdoug@yahoo.com

MATERIALS AND METHODS

Source of the Virus Isolate:

About twenty nine samples of naturally infected squash (*Cucurbita pepo* cv. Eskandrani) plants showing symptoms suspected to SqLCV was collected from Nubaria , El-Behera Governorate. Collected samples were detected for the presence of SqLCV serologically by using double antibody sandwich enzyme linked immunosorbent assay (DAS–ELISA) as described by Clark & Adams (1977) using SqLCV specific polyclonal antibodies (LOEWE Biochemica, GmbH, DSMZ, Germany).

Isolation and Propagation of Virus Isolate:

The infected plants which gave positive results with DAS-ELISA were used as a source of the SqLCV under study.The virus isolate was inoculated on healthy squash cv. *Eskandrani* plants using virus free whiteflies, *Bemisia tabaci* biotype B.

Insect inoculated plants were kept in insect-proof cages under green house conditions at the faculty of Agriculture, Ain Shams University for 3-8 weeks. The new symptoms appeared similar to the original symptoms and they were examined by dot blot hybridization to confirm the existence of the original virus isolate.

Biological Characters:

-Mode of Transmission:

Syringe Injection:

Healthy squash plants cv. Eskandrani were inoculated by syringes using infected squash sap according to Allam *et al.* (1994).

-Insect Transmission:

Whiteflies *Bemisia tabaci* biotype B belongs to family *Aleroididae* were collected from squash plants grown in open fields and identified by the Department of Plant Protection, Fac. of Agric., 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 to feed on infected squash cv. *Eskandrani* plants in insect proof cages. After 24 hrs acquisition access period, the insect allow to feed for 72 hrs on healthy squash plants then the whiteflies were removed by spraying the squash plants by 0.5% selecron and left for symptoms development.

Both in syringe injection and whitefly transmission, 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 number of infected plants / total number of inoculated plants.

-Host Range and Symptomatolog:

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

-Stability of Virus Isolate:

Thermal Inactivation Point, Dilution End point and aging of SqLCV was performed on healthy *C. pepo* cv. Eskandrani by using infectious crude sap obtained from infected squash plants. The injected seedlings were kept under green house conditions and observed daily up to 60 days for symptoms development. Stability of SqLCV was recorded as number of infected plants / total number of inoculated plants.

Morphological Characters:

Partially purified suspension of SqLCV 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).

Molecular Characters.

-Extraction of Viral DNA:

Genomic DNA was extracted from SqLCV infected *C. pepo* cv. Eskandarani plants using cetyl trimethyl ammonium bromide method (CTAB) as described by Gibbs and Mackenize (1997). The nucleic acid was stored at -20˚C.

-Oligonucleotide Primers:

The oligonucleotide primers used to amplify the coat protein gene of SqLCV 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 2.5µl (200ng) of extracted DNA, 10mM of each dNTPs (0.5µl), 1µl of 25 pmole from each amplification primer, 2.5µl of 10X PCR buffer with 1.5mM $MgCl_2$ and 0.5µl 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°C for 30 sec, annealing at 50°C for 45 sec, and extension at 72°C for 1 min. A single tailing cycle of long extension at 72°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 SqLCV/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 SqLCV-DNA in infected plant tissues with typical symptoms of SqLCV 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 SqLCV was purified by using GFX column and Gel Band purification kit (Amersham pharmaia Biotech, GmbH, Germany). The SqLCV coat protein genes (407 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 SqLCV coat protein genes from GeneBank.

RESULTS AND DISCUSSION

Field Inspection and Serological Detection:

Whiteflies-infected squash plants with SqLCV showed viral symptoms of leaf curling, leaf crinkle, leaf narrowing blistering, chlorosis, fruit malformation and stunted plant growth (Fig. 1). All sample gave positive reaction and were susceptible to squash leaf curl viral infection with nearly the same degree of sensitivity. These results indicated that the incidence of SqLCV in Governorate was 100%.

Isolation and Propagation of Virus Isolate:

SqLCV was isolated and propagated on healthy squash plants cv. Eskandrani from the selected ELISA positive squash samples by whitefly (*B.tabaci* biotype B) transmission. After 3-5 weeks symptoms of leaf curling, leaf crinkle and blistering produced till it gives stunting of squash plants after 5-6 weeks.

Biological Characters of Virus Isolate:

Mode of Transmission:

Results in Table (1) showed that both syringe and whitefly inoculation methods transmitted SqLCV from infected squash plants cv. Eskandrani 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 blistering developed after 3-6 weeks while in case of whitefly (*B.tabaci* biotype B) transmission, leaf curling, leaf crinkle and blistering were first developed after 3-5 weeks till it gives stunting after 5-6 weeks at 28-30°C under green house conditions. In addition, virus transmission efficiency in case of syringe injection was 35% while in case of whitefly (*B.tabaci* biotype B) transmission was 100%.

Fig. 1: Infected Cucurbita pepo cv. Eskandrani under field conditions showing: (A) and (B) leaf curling, leaf crinkle, leaf narrowing, blistering, chlorosis and stunting, (C) Fruit malformation.

Table 1: Mode of transmission of SqLCV.

Virus isolate	Transmission mode	Symptoms	Incubation period (Weeks)	A/B	Virus transmission efficiency (%)
SqLCV	Syringe Injection	leaf crinkle and blistering	3-6	7/20	35%
	whitefly (*B. tabaci* biotype B)	leaf curling , leaf crinkle, blistering	3-5	20/20	100%
		stunting	5-6		

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

Host Range and Symptamology:

Results showed that SqLCV isolate had nearly a wide host range between members of family *Cucurbitaceae*, *Fabaceae* and *Chenopodiaceae*. In addition, SqLCV had a limited host range and showed mild symptoms between members of family *Solanaceae*. On the other hand, no symptoms were observed on members of family *Compositae* and *Graminae.* Table (2) illustrated the different symptoms produced on the different plant species when inoculated with SqLCV by both syringe injection and whitefly (*B. tabaci* biotype B) transmission.

Virus Stability:

Results indicated that SqLCV isolate under test was completely inactivated in undiluted crude sap of infected squash leaves at 60ºC, inactivated when diluted to 10^{-6} and completely inactivated after storage after 8days at room temperature (25-28ºC).

Morphology of Virus Particles:

Electron microscopic examination of partially purified preparation of SqLCV revealed the presence of isometric and pentagonal in shape, with single and paired Gemini virus, (monomers and dimmers) with dimension of 22nm and 20 X 30 nm, respectively when negatively stained with 2% Uranyl acetate pH 7.0 (Fig. 2).

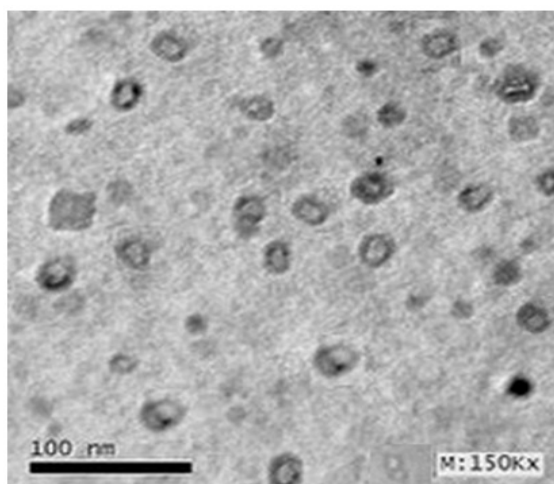

Fig. 2: Electron micrographs showing the partially purified squash leaf curl gemiviruse negatively stained with 2 % Uranyl acetate, Bar represents 100 nm.

Table 2: Differential host of SqLCV as determined by syringe injection and whitefly (*B. tabaci*) transmission. Existence of virus was confirmed by DNA hybridization.

Host plants	Syringe injection Symptoms	Dot blot hybridization	Whitefly inoculation Symptoms	Dot Blot Hybridization
Solanaceae				
L. esculentum	--	-		
cv. super marmand			--	-
C. annum cv. Chilli	Mild LC	+		
D. stramonium	Mild LK,E	+	Mild LC	+
D. metel	--	-	Mild LK,E	+
N. glutinosa	Mal.	+	--	-
N. rustica	--	-	Mal.	+
N. tabacum	--	-	--	-
cv. whiteBurley	--	-	--	-
Samson			--	-
Cucurbitaceae				
C. pepo	LK,B	++	Lk, B, Mal., S	++
cv. Eskandrani.				
C. maxima	LK	++	LK, B	++
C. sativus	--	-	--	-
Fabaceae			LC, LK, Mal	++
P. vulgaris	LC, VN, SN	++	LC, R	++
G. max	LC, R, NM,	++		-
	VC,VN		--	-
P. sativum	--	-	--	-
V. faba	--	-		
Chenopodiaceae			Mild LK	+
Ch. amaranticcolor	Mild LK	+	LC,E	+
B. vulgaris	LC, E	+		
Graminea			--	-
Z. mays	--	-		
Compositae			--	-
L. sativa	--	-		

LC=leaf curling, LK=leaf crinkle, E=epinosity, Mal=malformation, B=blistering S=stunting, VC=vein clearing, R=rugosity, VN=vein necrosis, SN=stem necrosis, NM=net mosaic. Negative control of syringe injection=0.149, negative control of whitefly transmission=0.139. ++ Strong positive reaction, +weak positive reaction,-negative reaction, -- symptomless.

Molecular Characterization of Virus Isolate:

SqLCV DNA prepared from naturally infected squash plants was 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 naturally infected squash plants was estimated by comparing its electrophoretic mobility with those of standard DNA marker as shown in Fig. (3). The amplified DNAs were in the expected size calculated (~480 bp) from the positions of the primers. The authenticity of the resulting PCR products (~480 bp) was verified by direct DNA sequencing after purification of the DNA fragments from agarose gel using rapid and efficient kit (QiAquick Gel Extraction Kit) from Qiagen.

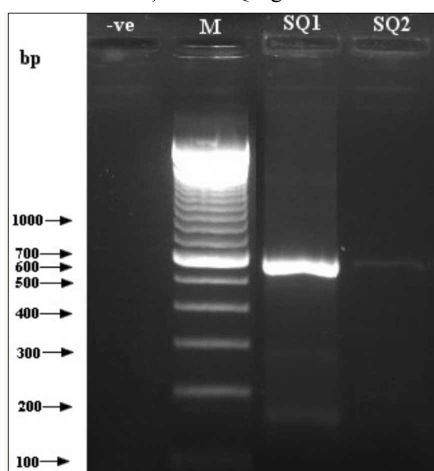

Fig. 3: 1.5% agarose gel electrophoresis showing the PCR products of SqLCV coat protein gene using Begomoviruses specific primers V324 (+) & C889 (-). Genomic DNAs were extracted from naturally infected squash leaves (SQ1) and syringe injected squash plants (SQ2). The arrow pointed to the amplified PCR products (550 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 of SqLCV syringe and whiteflies infected plants showed that *C.pepo, C.maxima, P.vulgaris* and *G. max* gave a strong positive reaction while *C. annum, D.stramonium*, *N. glutinosa* and *Ch. amaranticolor* and *B.vulgaris* gave a mild positive reaction. *L. esculentum*, *D. metel, N.rustica, N. tobacum* cv. whiteberly, *N. tobacum* cv. samson, *C. sativus*, *P. sativum, V. faba, Z. mays* and *L. sativa* gave negative reaction (Fig. 4).

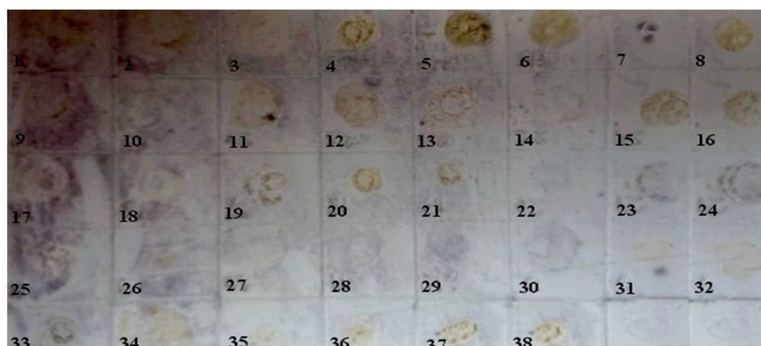

Fig. 4: Dot blot hybridization of syringe and whitefly inoculated plants using SqLCV- DNA probe.

Sequence Analysis of SqLCV/CP Genes:

A multiple sequence alignment of SqLCV/Cp nucleotide sequence (current study) with six SqLCV sequences published in the GenBank. Sequence comparison showed that SqLCV/Cp of the current study had sequence homology of about 99% with other SqLCV isolates (Fig. 5 A&B).

SQLCV-current
GCAGCGGCATGATATCTCCCATCTTGGCAAGGTAATGTGTATTTCGGACGTGACGCGTGG
DQ468655 GCAGCGGCATGATATCTCCCATCTTGGCA-GGTAATGTGTATTTCGGNCGTGACGCGTGG
AY206998 GCAGCGGCATGATATCTCCCATCTTGGCAAGGTAATGTGTATTTCGGACGTGACGCGTGG
GQ273922 GCAGCGGCATGATATCTCCCATCTTGGCAAGGTAATGTGTATTTCGGACGTGACGCGTGG
FJ455514 GCAGCGGCATGATATCTCCCATCTTGGCAAGGTAATGTGTATTTCGGACGTGACGCGTGG
DQ364057 GCAGCGGCATGATATCTCTCATCTTGGCAAGGTAATGTGTATTTCGGACGTGACGCGTGG
DQ285019 GCAGCGGCATGATATCTCCCATCTTGGCAAGGTAATGTGTATTTCGGACGTGACGCGTGG
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SQLCV-current
TAATGGCATTACTCACCGCGTCGGCAAGCGCTTTTGTGTTAAGTCTGTATATATTTTAGG
DQ468655 TAATGGCATTACTCACCGCGTCGGCAAGCGCTTTTGTGTTAAGTCTGTATATATTTTAGG
AY206998 TAATGGCATTACTCACCGCGTCGGCAAGCGCTTTTGTGTTAAGTCTGTATATATTTTAGG
GQ273922 TAATGGCATTACTCACCGCGTCGGCAAGCGCTTTTGTGTTAAGTCTGTATATATTTTAGG
FJ455514 TAATGGCATTACTCACCGCGTCGGCAAGCGCTTTTGTGTTAAGTCTGTATATATTTTAGG
DQ364057 TAATGGCATTACTCACCGCGTCGGCAAGCGCTTTTGTGTTAAGTCTGTATATATTTTAGG
DQ285019 TAATGGCATTACTCACCGCGTCGGCAAGCGCTTTTGTGTTAAGTCTGTATATATTTTAGG
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SQLCV-current
CAAGATATGGATGGACGAAAATATCAAGTTGAAGAATCACACGAACAGTGTGATATTTTG
DQ468655 CAAGATATGGATGGACGAAAATATCAAGTTGAAGAATCACACGAACAGTGTGATGTTTTG
AY206998 CAAGATATGGATGGACGAAAATATCAAGTTGAAGAATCACACGAACAGTGTGATGTTTTG
GQ273922 CAAGATATGGATGGACGAAAATATCAAGTTGAAGAATCACACGAACAGTGTGATGTTTTG
FJ455514 CAAGATATGGATGGACGAAAATATCAAGTTGAAGAATCACACGAACAGTGTGATGTTTTG
DQ364057 CAAGATATGGATGGACGAAAATATCAAGTTGAAGAATCACACGAACAGTGTGATGTTTTG
DQ285019 CAAGATATGGATGGACGAAAATATCAAGTTGAAGAATCACACGAACAGTGTGATGTTTTG
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SQLCV-current
GTTAGTTCGAGATCGTAGACCATATGGCACTCCGATGGATTTCGGCCAGGTGTTCAACAT
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SQLCV-current
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DQ364057 GTTTGACAACGAGCCCAGCACGGCAACTATCAAGAACGATC-TACGTGATCGTTATCAAG
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SQLCV-current
TCATGCACAGGTTCTACGCGAAGGTCACTGGTGGTCAGTATGCTAGCAACGAACAAGCCT
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GQ273922 TCATGCACAGGTTCTACGCGAAGGTCACTGGTGGTCAGTATGCTAGCAACGAACAAGCCT
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DQ364057 TCATGCACAGGTTCTACGCGAAGGTCACTGGTGGTCAGTATGCTAGCAACGAACAAGCCT
DQ285019 TCATGCACAGGTTCTACGCGAAGGTCACTGGTGGTCAGTATGCTAGCAACGAACAAGCCT
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SQLCV-current
TGGTTAGGCGTTTCTGGAAGGTCANCACCCATGTTGTGTATAATCACC
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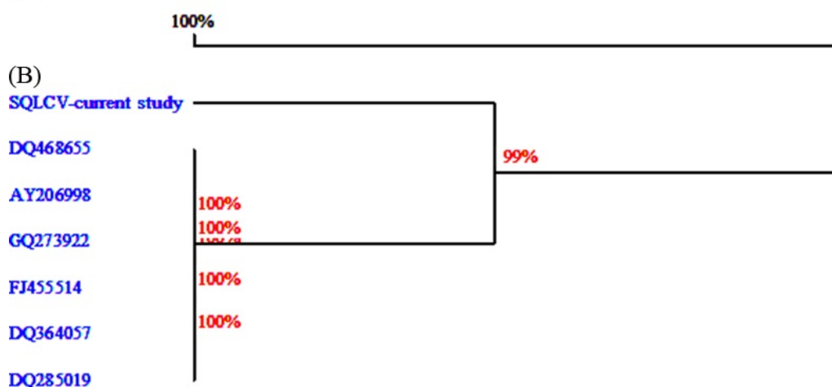

Fig. 5: (A&B): Clustal multiple sequence alignment and homology tree of SqLCV isolates based on the nucleotide sequences of the CP gene. Accession numbers indicated above were as following: SQLCV-current study, GQ273922 (SqLCV- Syrian isolate) reported by Al-Tamimi *et al.* (2009). FJ455514 (Giza-CA H10) reported by Abdel-Salam and Rehman (2008), DQ364057 (Giza CP gene) reported by Abdel-Salam *et al* (2006), DQ285019 (Cairo segment DNA-A) reported by Idris *et al*. (2006) DQ468655 (SqLCV-EG) reported by Farag *et al*. (2005) and AY206998 (Israel isolate) reported by Antignus *et al*. (2002).

Discussion:

Squash plants infected with SqLCV showed viral symptoms of leaf curling, leaf crinkle, leaf narrowing, blistering, chlorosis, fruit malformation and stunted plant growth as described by (Cohen *et al.*, 1983; Farag *et al.*, 2005 and Idris *et al.*, 2006). All samples collected from Nubaria, El-Behera Governorate gave positive reaction and the incidence of SqLCV in was 100%. This results indicated that the presence of high population of whiteflies that transmitted SqLCV efficiently.

DAS-ELISA using SqLCV antiserum was confirmed the identity of the isolated SqLCV from squash plants. This result was agreement with other investigation (Cohen *et al.*, 1983). High ELISA readings indicated that high specificity between antibody and virus coat protein as well as high virus concentration in naturally infected squash plants.

C.pepo used as propagative host for SqLCV, after 3-5 weeks symptoms of leaf curling, leaf crinkle and blistering produced till it gives stunting of squash plants after 5-6 weeks.

SqLCV 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 SqLCV successfully transmitted persistently by this insects. These results are in agreement with that obtained by (Ghanem *et al.,* 2001; Idris *et al*., 2001 and Brown & Czosnek, 2002).

SqLCV infected *C. annum* cv. Chili, *D. stramonium* and *N. glutinosa* belonging to family *Solanaceae, C. pepo* cv. Eskandrani and *C. maxima* belonging to family *Cucurbitaceae, P. vulgaris, G. max* and belonging to family *Fabaceae* and *Ch. amaranticolor* and *B. vulgaris* belonging to family *Chenopodiaceae* and not reacted systemically with *L. esculentum* cv.Super marmand *D. metel, N. rustica, N. tabacum* cv.whiteberly and *N. tabacum* cv.samson belonging to family *Solanaceae, C. sativus* belonging to family *Cucurbitaceae, P. sativum* and *V.faba* belonging to family *Fabaceae, L. sativa* belonging to family *Compositae* and *Z. may* belonging to family *Gramineae.* These results are in agreement with that obtained by Cohen *et al*. (1983); Brown (1994) and Hill *et al*. (1998) with exception that Cohen *et al*. (1983) reported that *B.vulgaris* apparently immune species.

SqLCV isolate have thermal inactivation point 60ºC, dilution End Point 10^{-6} -10^{-7} and was completely inactivated after 8 days at room temperature (25-28 ºC) These results are not agreed with that obtained by Cohen *et al*. (1983).

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 respectively, when negatively stained with 2 % Uranyl acetate pH 7.0. These results were similar with that obtained by (Cohen *et al*., 1983; Sunter *et al.,* 1994; Harrison & Robinson, 1999 and Varma & Malathi, 2003).

To study the molecular characters of the isolated virus, purified SqLCV-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 squash plants was (550 bp), these result was not agreed with that obtained by Farag *et al.* (2005) that amplified fragment with expected size (~419 bp). This is due to the difference in primers and PCR conditions used.

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

Partial nucleotide sequence (407s nt) of SqLCV-CP-EG of the current study was aligned with other published CP sequences of SqLCV as shown in Fig. (5 A). SqLCV-CP was found to display 99 % sequence homology with DQ468655 (SqLCV-EG) reported by Farag *et al*. (2005), 97.8 % with AY206998 (Israel isolate) reported by Antignus *et al*. (2002), 98.0 % with GQ273922 (SqLCV- Syrian isolate) reported by Al-Tamimi *et al*. (2009), 97.9 % with FJ455514 (Giza-CA H10) reported by Abdel-Salam and Rehman (2008), 98.0 % with DQ364057 (Giza CP gene) reported by Abdel-Salam *et al*. (2006), and 95.8 % with DQ285019 (Cairo segment DNA-A) reported by Idris *et al*. (2006). Multiple sequencing alignments were generated using (DNAMAN V 5.2.9 package, Madison, Wisconsin, USA). The homology tree of SqLCV-EG presented in Fig. (5 B) revealed high degree of similarity to the other six isolates sequences of SqLCV.

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