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Virus Genes

ISSN 0920-8569

Virus Genes
DOI 10.1007/s11262-013-1012-1

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Received: 15 August 2013 / Accepted: 8 November 2013
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Abstract The incidence of squash leaf curl disease and molecular characterization of the Palestinian isolate of *Squash leaf curl virus* [SLCV–(PAL)] are described in this study. Symptomatic leaf samples obtained from squash (*Cucurbita pepo*), watermelon [*Citrullus lanatus* (Thunb.)], and cucumber (*Cucumis sativus* L.) plants were tested for SLCV-[PAL] infection by PCR and RCA. SLCV was also found to occur naturally in *Chenopodium murale*, *Convolvulus* sp, and *Prosporis farcta* which showed yellowing. The disease incidence was 85 % in samples collected from Nablus in summer season, while it was 98 % in samples collected from Qalqilia in autumn. On the other hand, SLCV incidence did not exceed 25 % in winter season. The full-length DNA-A and DNA-B genomes of SLCV-[PAL] were amplified and sequenced, and the sequences were deposited in the GenBank. Sequence analysis reveals that SLCV-[PAL] is closely related to other isolates from Lebanon (SLCV-LB2), Jordan (SLCV-JO), Israel (SLCV-IL), and Egypt (SLCV-EG). DNA-A of SLCV-[PAL] showed the highest nucleotide identity (99.4 %) with SLCV-JO, and SLCV-LB2, while DNA-B had the highest nucleotide identity (99.3 %) with SLCV-IL. However, following genome sequencing, it was found that due to two separate point mutations, two viral open reading frames (ORF) were altered in some SLCV Palestinian isolates. The AC2 ORF was extended by 141 nucleotides, while the AC4 ORF was extended by 36 nucleotides.

Keywords Squash · Begomovirus · RCA · Phylogenetic analysis · Palestine

M. S. Ali-Shtayeh and R. M. Jamous have contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s11262-013-1012-1) contains supplementary material, which is available to authorized users.

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Introduction

Geminiviridae is one of the largest plant virus families; its members have a circular, single-stranded DNA (ssDNA) genome of approximately 2.7–5.2 kb encapsidated in twinned icosahedral particles [1, 2], which replicate via double-stranded replicative intermediates using a rolling circle mechanism [3, 4]. This group causes economically significant diseases in a wide range of crop plants worldwide [5, 6]. Based on their genome arrangement, insect vector, and host range, geminiviruses are classified by the International Committee on Taxonomy of Viruses (ICTV) into one of seven genera: Becurtovirus (2 Species), Begomovirus (192 Species), Curtovirus (3 Species), Eragrovirus (1 Species), Mastrevirus (29 Species), Topocuvirus (1 Species), and Turncurtovirus (1 Species) [7, 8]. The largest genus, Begomovirus has bipartite genomes, referred to as DNA-A and DNA-B components, while a few species consist of only a single genomic component, resembling DNA-A. Irrespective of their genome sizes and segmentations, all begomoviruses are transmitted by the whitefly *Bemisia tabaci* (Gennadius), and most are restricted to the phloem of the infected plants.

During the last three decades numerous whitefly-transmitted begomoviruses have emerged as devastating pathogens, particularly in the tropics and subtropics, causing huge economic losses and threatened crop

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production. The role of vector-begomovirus–plant interactions in the widespread invasion by some members of the whitefly species complex *B. tabac* is poorly understood [9]. The emergence of begomoviruses is associated with changes in crop cultivation, increased global movement of plants, and changes in cropping practices, such as the intensive use of insecticides. In particular, the emergence of begomoviruses as important pathogens is closely associated with the increased prevalence of the insect vector, the whitefly *B. tabaci* [10, 11].

Squash leaf curl disease, prevalent in the southwest United States and southern California, is caused by a whitefly-transmitted geminivirus, now designated as *Squash leaf curl virus* (SLCV) [12, 13]. The virus was reported to infect *Phaseolus vulgaris* and several species of the *Cucurbitaceae* including squash and pumpkin (*Cucurbita* species), melon (*Cucumis melo*), cucumber (*Cucumis sativus*), and watermelon (*Citrullus lanatus*) [12]. Symptoms of the disease include curled leaves with yellowed, mottled areas, and leaves have shortened petioles that cluster around the vines [14, 15].

The virus was restricted to central and North America; however, in 2003 SLCV was reported for the first time in the Mediterranean region, in Israel where the virus (SLCV-E) caused severe epidemics and the disease incidence was 100 % [14]. Four years later, the virus (SLCV-EG) was reported to cause severe symptoms observed in squash fields in Egypt [15]. In 2008, SLCV was detected in all surveyed areas in Jordan and disease incidence reached 95 % in squash samples collected from the Dir Alla area [16]. In 2010, SLCV was reported for the first time in squash fields (*Cucurbita pepo*) in Palestine [17]. More recently, in 2012, it was detected in the coastal areas of Lebanon and yield losses were reported to reach the range between 70–80 % [18].

However, following detection of SLCV in 2008 in Nablus, Jenin, and Tulkarm regions in Palestine, similar disease symptoms (severe mottling, yellowing, and leaf curling) were observed in Jericho and Qalqilia regions in Palestine, the disease was always associated with an elevated whitefly population. Therefore, our aim was to identify and characterize the causal agent of Squash leaf curl disease, and to study the distribution of the disease in cucurbit-growing regions in Palestine.

Materials and methods

Samples collection

In autumn of 2009, severe geminiviruses like symptoms were observed on cucurbit crops grown in Jenin, and Nablus districts (Palestine). Infected plants showed disease symptoms like mottling, yellowing, leaf curling, and stunting (Fig. 1). In addition, high population of whitefly (*B. tabaci*) was observed in these fields. To identify the etiology of the disease, a total of 178 leaf samples were collected from symptomatic cucurbit plants. In winter, summer, and autumn of 2010, additional 1,607 leaf tissues were collected from symptomatic plants grown in 83 cucurbit fields located in the Northern and mid part of PA (Jenin, Tulkarm, Nablus, Qalqilia, and Jericho). To investigate the source of virus infection, leaf samples were also collected from weed species prevalent in cucurbit fields (Table 1). All samples were either processed directly or stored at −20 °C for further analysis.

Maintenance of virus isolates and whitefly vector

Virus isolates were maintained in a greenhouse in squash (*C. pepo*) plants by serial transmission using the whitefly vector, *B. tabaci*. Infected plants were kept in insect-proof greenhouse at ambient temperature. Nonviruliferous colonies of *B. tabaci* were reared on cotton seedlings (*Gossypium herbaceum*) in a muslin-covered (insect-proof) cage and kept in a greenhouse.

Whitefly transmission

A group of nonviruliferous *B. tabac* was allowed to feed for 48 h on symptomatic cucurbit plants [16]. The whiteflies were then transferred to 30 healthy squash seedlings at

Fig. 1 Squash plants naturally infected with SLCV showing **a** severe leaf curling and **b** chlorotic mottling

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Table 1 Plant species collected from cucurbit fields and tested for SLCV infection by polymerase chain reaction using the primer pair SLCVSTCF1F/SLCVSTCF3R

Plant name	Syptomes	PCR
Abelmoschus esculantus	−ve	−ve
Amaranthus sp.	−ve	−ve
Artemisia sp.	−ve	−ve
Chenopodium murale	−ve	−ve
Chenopodium murale	+ve	+ve
Citrullus lanatus	−ve	+ve
Citrus limon	+ve	−ve
Convolvulus sp.	+ve	+ve
Conyza bonariensis	−ve	−ve
Cucumis melo	+ve	+ve
Cucumis sativus	−ve	+ve
Cucurbita pepo	+ve	+ve
Cucurbita pepo var. melpepo	−ve	−ve
Cynodon dactylon	−ve	−ve
Datura innoxia	−ve	−ve
Lycopersicon esculentum	−ve	−ve
Malva sylvestris	+ve	−ve
Melilotus sulcatus	−ve	−ve
Prosopis farcta	+ve	+ve
Solanum melongena	−ve	−ve
Solanum tuberosum	−ve	−ve
Ziziphus spina-christi	+ve	−ve

the first leaf stage and given a 48-h inoculation access period (IAP). Following this, seedlings were sprayed with Imidacloprid (Confidor, Bayer, Basel, Switzerland) and plants were placed in an insect-proof cage and monitored for symptom development. All inoculated plants were tested for SLCV-[PAL] infection by PCR using virus-specific primers 3–4 weeks postinoculation. The primer pairs, SLCVSTCF1F/SLCVSTCF3R, were used in this study to specifically amplify a fragment of DNA-A of SLCV-[PAL] (reported by Ali-Shtayeh et al. [17] and designed by G. Anfoka, Al-Balqa' Applied University, Jordan). PCR-positive plants were kept in an insect proof cage in the greenhouse and used as inoculum source.

Detection of SLCV-[PAL] by polymerase chain reaction using degenerate primers

Total nucleic acids were extracted from the collected leaf samples as described by Dellaporta et al. [19]. The degenerate primer pairs PAL1v1978/PAR1c496 and PBL1v2040/PCRc1 [20] were used to detect SLCV in leaf samples collected from symptomatic cucurbits plants. These primers amplify fragments of DNA-A (~1.1 kb) and DNA-B (0.5 kb) of whitefly-transmitted geminiviruses, respectively [20]. Nucleic acids obtained from healthy squash plants were used as negative control. PCR reactions were performed in Gene Amp PCR System 9700 Thermal Cycler (Applied Biosystems). Each reaction contained 100 ng of sample DNA, 0.25 mM dNTPs, 0.25 mM MgCl2, 2.5 µM of each primer, 0.5 U of Taq DNA Polymerase, and 1× enzyme buffer in 25 µl final volume. The amplification program was as follows: an initial denaturing step at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 40 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min. Amplified PCR products were resolved on 1 % agarose gel stained with ethidium bromide (0.5 mg/ml).

Rolling circle amplification (RCA)

Total nucleic acids extracted from symptomatic squash plants were subjected to TempliPhi amplification essentially according to the manufacturer's instructions (Amersham Biosciences, NJ, USA). Briefly, 2 µl (10–20 ng) of total DNA was dissolved in 5 µl of sample buffer, denatured for 3 min at 95 °C and cooled down on ice for 1 min followed by the addition of 5 µl reaction buffer and 0.2 µl enzyme mix. Amplification was performed for 16–20 h at 30 °C and the reaction was stopped by heating for 10 min at 65 °C to inactivate the enzyme. The amplified DNA was used as a template for the PCR to amplify the full length genome of SLCV-[PAL].

Restriction fragment length polymorphism (RFLP)

The RFLP analysis was performed by digesting 1 µl (~300 ng DNA) of the RCA product with the restriction enzyme *HpaII* (New England Biolabs, Frankfurt, Germany). The reaction was carried out at 37 °C for 2 h, followed by treatment for 20 min at 65 °C for enzyme inactivation, according to supplier's recommendation. DNA fragments were separated on 2 % agarose gel following standard protocols [21] and visualized by ethidium bromide staining. Molecular weight marker with known reference fragments was used to the estimation of fragment sizes (Genedirex, www.genedirex.com).

Amplification of DNA-A and DNA-B full genomes of SLCV–PAL

The DNA-A and DNA-B of SLCV-[PAL] were amplified by PCR using the primer pairs SLCVA2295F/Xho-SLCV-R (1004-987) and SLCVA2314R/Xho-SLCVA-F (988-1012) for DNA-A and SLCV-DNA-B1-F/SLCV-DNA-B1-R and SLCV-DNA-B2-F/SLCV-DNA-B2-R for DNA-B (see Supplementary information Table S1). The PCR

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reaction contained 1 µl of RCA product, 0.25 mM dNTPs, 0.25 mM MgCl2, 2.5 µM of each primer, 0.5 U of Taq DNA Polymerase, and 1x enzyme buffer in a total volume of 25 µl. Viral DNAs were amplified in a Gene Amp PCR System 9700 Thermal Cycler (Applied Biosystems) by one cycle of melting at 94 °C for 3 min followed by 35 cycles of melting, annealing, and DNA extension conditions of 1 min at 94 °C, 1 min at 65 °C (for DNA-A primers), and 58 °C (for DNA-B primers), 2 min at 72 °C. For the last cycle, the extension time was increased to 10 min. Amplified DNA fragments were electrophoresed in 1 % agarose gel in TAE buffer (Tris-Acetate EDTA) and visualized using UV transilluminator after staining in ethidium bromide.

Cloning and sequencing of the full length genomes (DNA-A and DNA-B) of SLCV–PAL

The amplified fragments for DNA-A and DNA-B were immediately ligated into pTZ57R/T cloning vector (Thermo Scientific, USA) and cloned according to the manufacturer's instructions. After transformation into the JM109 strain of *E. coli*, white colonies were screened for the gene of interest by PCR and restriction digestion with *HindII* and *XbaI*. Clones of DNA-A and DNA-B fragments amplified using specific primers (SLCVA2295F/Xho-SLCV-R (1004-987) and SLCVA2314R/Xho-SLCVA-F (988-1012) for DNA-A and SLCV-DNA-B1-F/SLCV-DNA-B1-R and SLCV-DNA-B2-F/SLCV-DNA-B2-R for DNA-B) were sequenced in both directions using the following primers: M13/pUC #SO100 5′GTAAAACGACGGCCAGT3′ and M13/pUC#SO101 5′CAGGAAACAGCTATGAC3′ (Macrogen Inc., South Korea). Twenty-five clones of SLCV-[PAL]-A and one clone of SLCV-[PAL]-B were subjected to sequencing. Complete sequences of 31 geminiviruses infecting cucurbit crops (see Supplementary information Table S2) were retrieved from the GenBank (http://www.ncbi.nlm.nih.gov) and used for comparison using CLCmainworkbench 5.6 software (CLC bio A/S, Denmark). ORF finder (http://www.ncbi.nlm.nih.gov/projects/gorf/) was used to identify the ORFs of DNA-A and DNA-B. After multiple sequence alignments, phylogenetic trees have been constructed using the UPGMA algorithm of CLC workbench. A consensus dendrogram was generated using bootstrap value of 1,000 replicates for these algorithms.

Results

Field survey

In 2009 and 2010, leaf samples were collected from different cucurbit crops that showed disease symptoms similar to those previously described to be caused by SLCV. Analysis of collected samples by PCR and RCA revealed that 57.8 % (1,031 out of 1,781) of the samples were infected with SLCV-[PAL]. In samples collected in 2009 from Nablus and Jenin, 71 squash out of 168 samples were infected with the virus (42.3 %) (Table 2). Disease

Table 2 Detection of Squash leaf curl virus-[PAL] in cucurbit fields in Palestine in 2009-2010 using PCR and RCA

Season	Site	No. of fields	Crop	No. of +ve sample (deg. primers)	No. of infected[a]/ no. of tested	Infection rate (%)
Autumn/2009	Nablus	5	Squash	57	36/57	63.2
		1	Cucumber	2	0/2	0
	Jenin	9	Squash	100	35/109	32.1
Winter/2010	Nablus	5	Squash	17	1/38	2.6
		2	Cucumber	10	2/15	13.3
	Jericho	17	Squash	36	2/214	0.9
		1	Cucumber	3	0/10	0
	Qalqilia	8	Cucumber	85	2/63	3.2
	Tulkarm	12	Cucumber	80	20/80	25
		7	Squash	39	10/62	16.1
Summer/2010	Nablus	5	Squash	80	68/80	85
	Tulkarm	3	Watermelon	39	15/62	24.20
		1	Cucumber	0	0/20	0
Autumn/2010	Jenin	8	Squash	ND	196/296	66.2
	Qalqilia	4	Squash	ND	148/151	98
	Tulkarm	5	Squash	ND	249/276	90.2
	Jericho	5	Squash	ND	229/240	95.4
	Total	98			1,013/1,775	57.1

[a] No. of positive samples using PCR and RCA techniques

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incidence in 2010 ranged between 0 % in Jericho in winter and 98 % in Qalqilia in autumn season (Table 2). Total nucleic acids extracted from symptomatic plants using the minipreparation method provided good DNA quality with sufficient template for reliable and robust amplification of the full-length genomes (DNA-A and DNA-B) of SLCV-[PAL]. The expected fragment size of DNA-A (1300 bp) was amplified from collected cucurbit plants using SLCVSTCF1F/SLCVSTCF3R primer pair.

Amplification of the full genome of SLCV-[PAL] using the TempliPhi™ amplification kit

Total nucleic acids extracted from squash plants infected with SLCV-[PAL] were used as template to amplify the full-length genomes (DNA-A and DNA-B). The expected sizes of DNA-A (2,638 bp) and DNA-B (2,611 bp) were successfully amplified using the bacteriophage Ø DNA polymerase enzyme (not shown) and the identity of the amplified products were verified by digestion with *HpaII* enzyme (New England Biolabs, www.neb.com) producing eight bands (Fig. 2). The full-length DNA-A and DNA-B of SLCV-[PAL] were amplified from squash plants collected in 2010 from Nablus, Jenin, and Qalqilia by PCR using the primers pairs SLCVA2295F/Xho-SLCV-R (1004-987) and SLCVA2314R/Xho-SLCVA-F (988-1012) for DNA-A and SLCV-DNA-B1-F/SLCV-DNA-B1-R and SLCV-DNA-B2-F/SLCV-DNA-B2-R for DNA-B. Amplified PCR products were cloned and sequenced, sequenced fragments for each DNA were assembled together using CLCmainworkbench 5.6 software to obtain the full-length

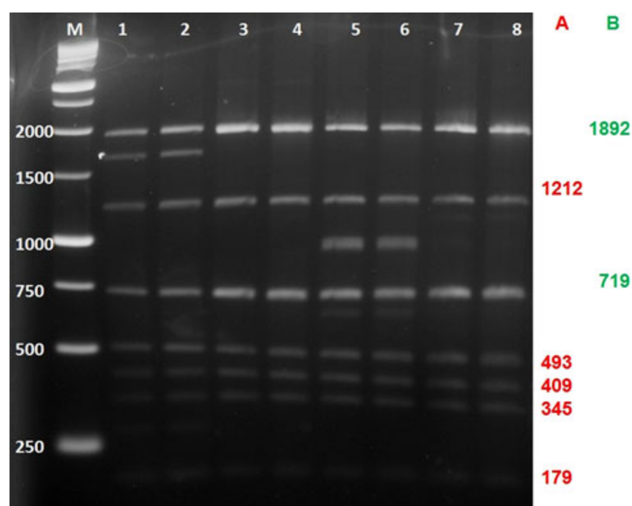

Fig. 2 Gel electrophoresis of RCA followed by RFLP products using *Hpa*II. The expected fragments sizes (nts) for DNA-A (*red*) and DNA-B (*green*) shown obtained by in silico digestion of the retrieved sequences are shown at the right side of gel. *M* molecular weight marker, *1–8* squash samples infected with SLCV

sequence of DNA-A and DNA-B of the Palestinian isolate of SLCV. Sequences of one isolate DNA-A and DNA-B obtained from the field-collected squash sample were deposited in the GenBank under accession numbers KC441465 and KC441466, respectively.

Sequence comparison and analysis of SLCV-[PAL]

Comparison analysis was carried out between sequences of DNA-A and DNA-B of SLCV-[PAL] and those of geminiviruses known to infect cucurbits, and available in the GenBank (Table 3). High degree of nucleotide identity (99.4 and 99.0 % in DNA-A and DNA-B, respectively, was detected between SLCV-[PAL] and SLCV-LB (HM36873.1) from Lebanon, 99.3 and 99.2 % nucleotide identity with SLCV-H [JO] (JX444577.1) from Jordan, and 99.2 and 99.3 % nucleotide identity with SLCV-IL (HQ184437.1) from Israel. A lower nucleotide identity (59.9; 43.9 %) was observed with Squash mild leaf curl virus (EU479710) from Taiwan.

A more detailed analysis of DNA-A sequences from Palestine showed them to encompose five open reading frames (ORFs) which are positionally conserved with those of other begomoviruses; one ORFs, AV1 (753 nt), in viral sense and four ORFs, AC1 (1,044 nt), AC2 (396 and 540 nt), AC3 (402 nt), and AC4 (345–381 nt), in complementary sense. Three of the 25 DNA-A isolates sequenced have a mutation that affect the ORF of the AC2. Due to a deletion of (g) at position 1380 the ORF of AC2 was extended by 141 nucleotides. Thus the AC2 of some SLCV–PAL isolates should be longer by 47 amino acids. Another mutation, this time in the AC4, is the change of the stop codon (TAA) into codon (TCA). This change resulted in extending the AC4 by 12 amino acids. DNA-B was 2,611 nucleotides in length, containing two ORFs, including BV1 (749 nt) in viral sense and BC1 (1,728 nt) in complementary sense.

Database searches conducted with the complete sequences of DNA-A and DNA-B revealed high degree of sequence identity (i.e., 99 %) with other SLCV isolates and low sequence identity with other begomoviruses. For example, DNA-A of SLCV-[PAL] and its intergenic region have the highest sequence identity (99.4 %) with the SLCV-[LB], and SLCV-JO (Table 3). In addition, AV1 ORFs shared 99.6 % amino acid identity with those of SLCV-[LB], SLCV-[IL], and SLCV-[JO]. The four other ORFs (AC3, AC2, AC4, and AC1) showed more than 97.6 % amino acid identity with those of SLCV-[LB2], and SLCV-H [JO] from Lebanon and Jordan, respectively (Table 3). Comparison of the complete DNA-B sequence of SLCV-[PAL] with other bipartite begomoviruses revealed 99.3 % identity with SLCV-[IL], 99.2 % identity with SLCV-H [JO], and most distantly related (43.9 %) to SLCV-[YL] from Taiwan. The BV1

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Table 3 Percentage nucleotide and amino acid sequence identities (%) between SLCV-[PAL] and other begomoviruses

		Total nt		DNA-A										DNA-B			
				AV1		AC1		AC2		AC3		AC4		BV1		BC1	
		DNA-A	DNA-B	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
1	SLCV-LB2	**99.4**	99.0	**99.4**	**99.6**	99.4	98.3	**99.7**	**99.2**	**99.5**	**98.5**	**99.2**	**97.6**	99.5	**99.6**	99.0	98.6
2	SLCV-Homra	99.3	99.2	99.3	99.2	**99.5**	**98.6**	99.5	**99.2**	99.3	97.8	**99.2**	**97.6**	**99.6**	98.8	99.3	98.6
3	SLCV-JO-2	99.2	98.8	99.2	**99.6**	99.0	98.0	**99.7**	**99.2**	**99.5**	**98.5**	98.9	96.8	97.8	98.9	**99.5**	99.0
4	SLCV IL	99.2	**99.3**	99.2	99.6	99.1	98.3	99.2	97.0	99.0	97.0	98.2	96.5	99.5	99.2	99.4	98.3
5	SLCV-EG	99.2	75.1[a]	99.2	98.8	99.3	98.3	**99.7**	**99.2**	99.3	97.8	98.9	96.8	99.3	98.9	**99.5**	99.0
6	SLCV-AZ-Isolate10	98.0	96.1	98.0	98.4	98.1	97.7	98.5	98.5	98.3	98.5	97.1	92.0	97.3	95.5	98.3	99.0
7	SLCV-WAZ	96.9	95.4	96.9	97.6	97.5	97.4	97.5	96.2	97.5	96.2	96.3	91.2	96.3	95.5	97.6	98.6
8	SLCV-JO-Mal-DoI	97.0	96.1	97.0	97.2	95.1	89.1	99.2	98.5	98.8	96.3	88.3	67.2	97.3	95.5	98.3	99.0
9	SLCV-E	97.8	95.9	97.8	98.4	97.8	97.4	98.5	97.7	98.8	97.8	–	–	97.5	95.5	98.1	**99.3**
10	MChLCV-Gua	86.2	73.8	86.2	96.4	86.3	85.2	88.1	77.9	91.4	92.5	–	–	77.6	70.2	82.2	85.7
11	SYMMV-R-98-631	85.1	79.9	85.1	95.2	84.3	85.8	88.7	81.7	90.6	91.0	–	–	76.6	67.8	81.1	92.5
12	SMLCV-IV	83.7	75.3	83.7	93.6	84.3	85.4	85.4	74.8	87.8	84.1	–	–	74.9	69.4	86.4	92.8
13	SMLCV	83.7	75.1	83.7	93.2	84.2	85.4	85.1	74.0	88.0	84.1	87.4	72.5	75.1	69.4	86.4	92.5
14	EuMV-Me	82.0	75.5	82.0	93.2	83.7	82.5	79.5	69.5	81.3	78.8	–	–	73.7	60.5	75.1	79.8
15	CuLCV-Cal	81.2	76.8	81.2	91.2	83.3	82.7	80.9	74.0	81.0	77.3	–	–	72.1	59.2	80.7	85.0
16	CulCrV-US-Cal-98	81.2	76.6	81.2	91.6	83.5	82.7	80.7	74.0	80.2	77.3	–	–	72.8	58.2	80.5	84.3
17	TMYLCV-VE	81.6	76.5	81.6	92.8	83.4	83.0	80.1	67.9	80.7	77.3	–	–	70.9	65.7	76.5	85.3
18	BCMV	80.2	76.2	80.2	90.4	79.6	81.3	81.3	69.2	80.2	74.2	84.7	66.3	73.7	62.0	76.2	83.6
19	WmCSV-IL	58.7	46.1	65.4	71.7	57.1	53.1	58.2	46.0	58.4	49.6	55.2	8.8	46.3	28.4	42.9	41.9
20	WmCSV-JO	58.6	45.9	66.0	71.7	57.0	52.3	58.1	46.7	58.2	50.4	55.2	8.8	46.8	28.4	42.6	41.5

The numbers in bold refer to the highest percentages of sequence identities

[a] Partial sequence

ORFs shared 99.5 % amino acid identity with those of SLCV-[JO], while BC1 ORFs shared 99 %, amino acid identity with those of SLCV-[JO], and SLCV-[EG], 98.6 % with those of SLCV-[LB].

Phylogenetic trees based on the alignment of the complete nucleotide sequences of DNA-A and DNA-B of SLCV-[PAL] with selected begomoviruses sequences available in the GenBank database are shown in Fig. 3 This shows SLCV-[PAL] to cluster together with other SLCV isolates.

Discussion

Distribution of SLCV in Palestine

Whitefly-transmitted begomoviruses cause major crop losses in vegetable, grain legume, and cotton crops throughout tropical, subtropical, and Mediterranean regions. The whitefly, *B. tabaci*, is the exclusive transmitter for a large group of emerging plant viruses that infect several hundred plant species worldwide [22]. A large number of viruses (114 virus species) are transmitted by whiteflies (family *Aleyrodidae*), 90 % of these viruses belong to the begomovirus genus [23, 24]. This whitefly and the plant viruses it transmits are no longer restricted to their native habitats or contained by natural geographic boundaries. In Palestine, information on the occurrence of whiteflies-transmitted geminiviruses is lacking.

The virus was reported for the first time in three districts in Palestine (Jenin, Nablus, and Tulkarm) [18]. Data presented here demonstrate the distribution of SLCV on cucurbits fields in Palestine. The introduction of this virus to Palestine might have occurred through transplant movement between Palestine and neighboring countries (Jordan and Israel) or through viruliferous *B. tabaci* that moved from infected cucurbit fields in Israel and the Jordan Valley to the fields in Palestine. The occurrence of SLCV in the region was previously reported in Israel, Egypt, Jordan, and Lebanon [14, 18]. Although most cucurbit samples tested in this study were obtained from symptomatic plants, SLCV was not detected in all plant samples. This might suggest that SLCV-negative symptomatic cucurbits, were infected with other viruses.

Results of the survey using the PCR and RCA (Table 2) indicated high SLCV incidence in all surveyed areas

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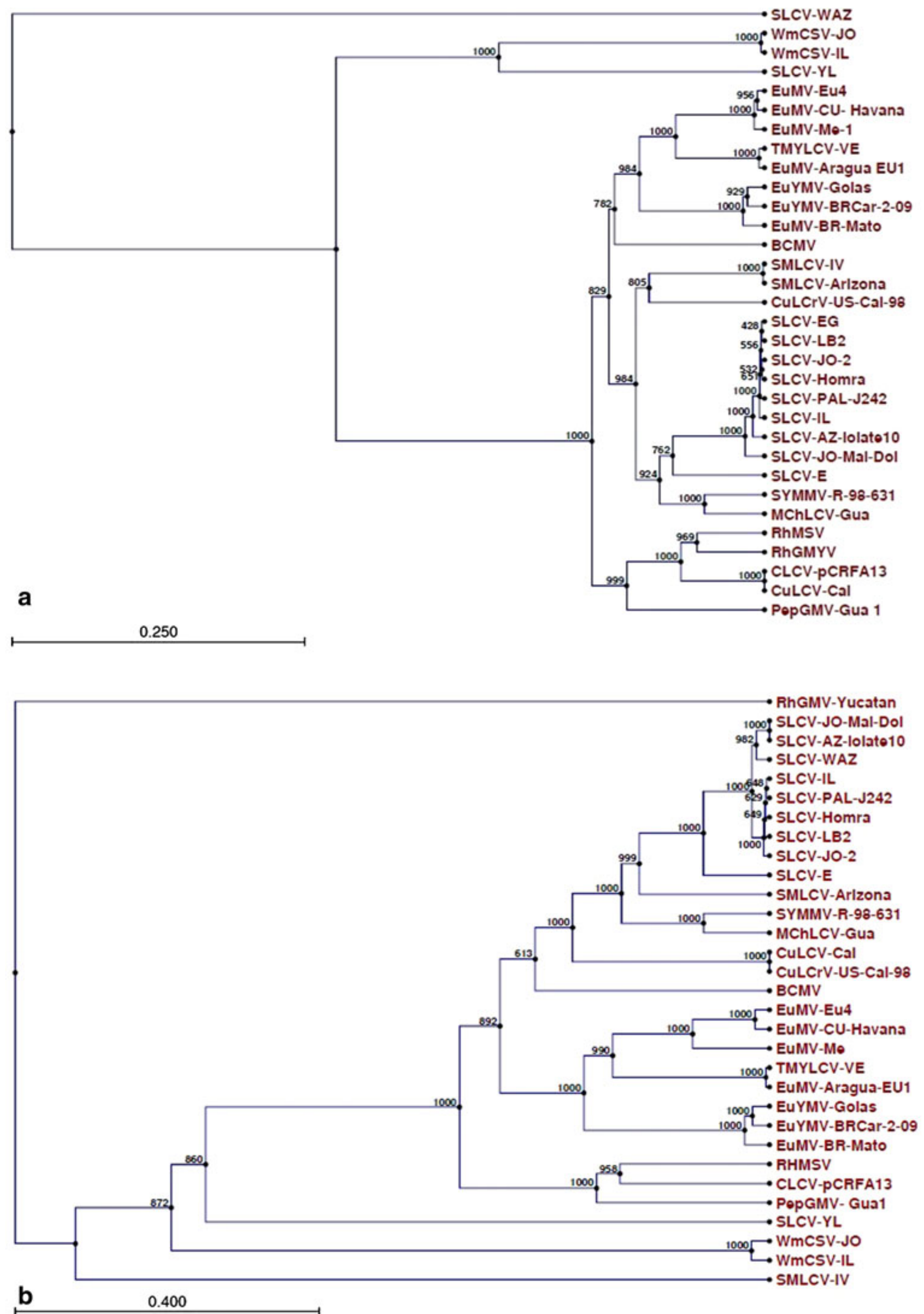

Fig. 3 Phylogenetic trees based on a multiple sequence alignment of the complete DNA-A (**a**) and DNA-B (**b**) components of selected begomoviruses with SLCV-[PAL]. Trees were constructed by the UPGMA method, and branches were bootstrapped with 1,000 replications. As outgroups, isolates of SLCV-WAZ and RhGMV-Yucatan were used. Acronyms and accession numbers are Supplementary information Table S2)

specially in summer and fall seasons where high population of whitefly were observed. For example, the disease incidence was 85 %, and 98 % in samples collected from Nablus and Qalqilia in summer and fall seasons, respectively. On the other hand, SLCV incidence did not exceed 25 % in winter season. However, the high incidence of disease in all surveyed areas, might be because of the poor knowledge of the farmers about the etiology of the disease, inefficient control measures against the whitefly vector and the implementation of improper cultural practices, such as crop overlapping, which provided continuous source of SLCV.

The complete genomes of SLCV-[PAL] were successfully amplified by the RCA technique. RCA has shown to be a very convenient and efficient method for molecular characterization of viruses with circular genomes [25]. It has been used for the amplification of the full genomes of other geminiviruses including *Maize streak virus, Tomato*

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yellow leaf curl virus, Tomato yellow leaf curl Thailand virus, and *Tobacco leaf curl Thailand virus* [26–29], and has been proposed to be a practical routine geminivirus diagnostic tool bearing advantages compared with other diagnostic techniques [26]. Results of the present study confirm these advantages, leading to identification, and cloning of SLCV-[PAL]. The full length DNA-A and DNA-B of SLCV-[PAL] were cloned and sequenced. Sequence analysis showed that SLCV-[PAL] had a high sequence homology (98.8–99.4 %) to different known isolates of the virus from Jordan, Lebanon, Israel, and Egypt.

A high incidence of SLCV was recorded in all surveyed areas. To investigate the source of SLCV infection several weed species that prevailed in cucurbit fields were tested for SLCV infection. However, SLCV could be detected in *Convolvulus* sp., *Chenopodium murale*, *Prosopis farcta.* In 2008 Al-Musa et al. [16] reported that SLCV could infect *Malva parviflora* in Jordan, and in 2003, Antignus et al. [14] reported that SLCV could infect *Malva nicaeensis* and *Ecballium elaterium* (Cucurbitaceae) in Israel. These findings are important from epidemiological point of view as *Convolvulus* sp., *C. murale*, and *P. farcta* are among the most common weed species prevailing in cucurbits crops in Palestine and subsequently it can be speculated that they might serve as reservoir for SLCV throughout the year. Therefore, a high incidence of SLCD could be expected in Palestine especially during the period of the year where high populations of *B. tabaci* occur.

Comparisons of the DNA-A sequences obtained showed 25 of the sequences to share 99–100 % nucleotide sequence identity, indicating that they are isolates of a single species, based on presently applicable species demarcation criteria [30]. SLCV–PAL was found to be highly homologous (99 %) to SLCV from neighboring countries (Jordan, Lebanon, and Israel). However, some isolates of SLCV–PAL had two sets of point mutations that affected two viral ORFs, the AC2 ORF being extended by 129 nucleotides, while the AC4 ORF was extended by 36 nucleotides. The Israeli and the Egyptian isolates were recorded to have the same mutations in AC2 as the Palestinian isolate [31] (see Supplementary Information Fig. S3). For the AC4 point mutation, it was similar to the point mutation in the Jordanian isolate (JX444577) (see Supplementary Information Fig. S4). Taking in consideration that the SLCV–PAL isolates has AC2 and AC4 with and without the point mutations (similar to the Israeli and Jordanian isolates), this indicates that SLCV was transmitted to Palestine from neighboring counties Jordan and Israel either by whiteflies, or by exchange of seeds between these countries, where several viruses and viroids have been, and undoubtedly still are, disseminated worldwide through exchange of seeds having undetected infection [32].

The AC2 protein is a transcription activator of the capside protein [31, 33], while AC4 protein is pathogenicity determinant, and may act as a suppressor of RNA-mediated gene silencing, also known as posttranscriptional gene silencing (PTGS) [34, 35], it's not known, if any, the effects of the additions of 47 and 12 aa may have on the activities of AC2 and AC4.

Given the wide distribution of SLCV in the Mediterranean countries and the endemic population of the whitefly vector in the region, particular attention should be paid to this virus. Otherwise, it could invade and cause remarkable yield losses to other crops. Since the incidence of SLCV is low in some regions surveyed in this study, intensive efforts have to be done to avoid further spread of the disease to other cucurbit-growing regions specially during the spring growing season where the population of the insect vector is usually high. Furthermore, to reduce yield losses caused by SLCV collaborative efforts among scientists in the region, should be initiated to search for resistance sources against SLCV in wild cucurbit species, and to develop SLCV-resistant cucurbit crops via classical breeding or through genetic engineering.

Acknowledgments This research was funded by the German Research Council's trilateral (German-Israeli-Palestinian ref. no. JE 116/12-2) Project and by USAIDMERC Grant no. M26-063.

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