New host records for *cotton leaf curl Gezira virus*: capsicum and melon in Egypt

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Abstract

Cotton leaf curl Gezira virus (CLCuGeV), in association with a DNA-beta satellite, is reported as a causal agent of cotton leaf curl disease. This study reports CLCuGeV detection in field samples of *Capsicum annuum* and *Cucumis melo* from Egypt and represents an expansion of the virus host range beyond the *Malvaceae* family to the two plant families, *Solanaceae* and *Cucurbitaceae*.

Keywords Begomovirus · Beta-satellite · DNA-A · *Capsicum annuum* · *Cucumis melo* · Potyvirus · *squash leaf curl virus* (SqLCV) · *zucchini yellow mosaic virus* (ZYMV) · *papaya ringspot virus* (PRSV) · *potato virus Y* (PVY)

Begomoviruses are considered one of the major emerging viral threats to crop production worldwide (Mansoor et al. 2006; Varma and Malathi 2003). Cotton leaf curl disease (CLCuD) is caused by a complex of different begomoviruses and DNA-beta satellite molecules. The disease was first recorded in Africa in the 1920s, in Pakistan and India during the 1990s and most recently in 2010, in China (Cai et al. 2010). The disease is reported from Pakistan, India, Sudan, Egypt, China, Tanzania, Malawi, Nigeria and South Africa (Abdel-Salam 1999; Briddon 2003; Cai et al. 2010; Mansoor et al. 1999; Mansoor et al. 2003; Malathi et al. 2003). CLCuD is responsible for serious economic losses to cotton production in many regions around the world, particularly on the Indian subcontinent. For example, CLCuD cost the Pakistan industry an estimated US\$5 billion between 1992 and 1997 (Briddon et al. 2001). In Sudan during the 1950's the disease caused yield losses of up to 40% (Malathi et al. 2003).

The number of begomoviruses causing CLCuD has risen from only a few species in 1992 to at least 30 species in 2008 (Fauquet et al. 2008). This includes *cotton leaf curl Gezira*

virus (CLCuGeV) which was first reported from Africa in 2002 (Idris and Brown 2002; Idris et al. 2005). Other begomoviruses associated with CLCuD such as *cotton leaf curl Kokhran virus* are known to have vegetable crop hosts such as tomato and capsicum (Nateshan et al. 1996). However, the host status of CLCuGeV amongst non-malvaceous vegetable crop species was unknown. This study was aimed at investigating solanaceous and cucurbit species as hosts of CLCuGeV. The detection of two new host species of CLCuGeV, capsicum and melon, is reported.

A range of vegetable and melon samples were collected from a field trial site in Ashmoun, Egypt, May 2010 and indexed for begomoviruses and associated DNA-beta satellites. The samples included those from melon (Q2540, Q2541, Q2542 and Q2545 - *Cucumis melo*), cucumber (Q2521, *Cucumis sativus*), squash (Q2517, *Cucurbita pepo*), chilli (Q2537 and Q2539, *Capsicum* spp.) and capsicum (Q2535, *Capsicum annuum*), and all showed symptoms of infection of either a begomovirus and/or a potyvirus. All samples tested positive for one or both of these virus genera. No samples had symptoms typical of the DNA-beta satellite usually associated with CLCuD (i.e severe cupping of leaves or presence of enations on leaves) (Amin et al. 2011).

To do the virus identifications, samples were first wiped clean of insects prior to processing to avoid any potential contamination, then total nucleic acid extracts (TNAEs) were prepared using a Biosprint DNA Plant Kit as per manufacturer's instructions (Qiagen, catalogue number 941557). The TNAEs were indexed by PCR using the begomovirus DNA-A genome degenerate primers Accore/Avcore (Table 1; modified, Wyatt and Brown 1996) and DNA-beta satellite

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Table 1 List of primers used in PCR amplification of viral genomes

Primer combinations	Primer Sequence (5'-3')	Cycling Parameters ^a	Product size (bp)
Avcore	GCCHATRTAYAGRAAGCCMAGRAT	95 °C 20s, 53 °C 20s and 72 °C for 40s (35 cycles), 72 °C 5 min	579
Accore	GGRITTDGARGCATGHTACANGCC		
CLCuGV705F	ACTCATCGTACTGGAAAGCG	95 °C 20s, 55 °C 20s and 72 °C for 40s (20 cycles), 72 °C 5 min	1900
CLCuGV2507R	AGCACAGCTTCAAACAAGAAATAT		
CLCuGV2637F	TGTTGGGAGGAGCCATTGAC	95 °C 15 s, 55 °C 15 s and 72 °C for 45 s (35 cycles), 72 °C 60s	371
CLCuGV262R	GCGAGCATACAACGGAAACC		
CLCuGV29F	GATGTCCAATCAGAACGCGC	95 °C 15 s, 55 °C 15 s and 72 °C for 45 s (35 cycles), 72 °C 60s	548
CLCuGV606R	CCCACTTCCCTTGGTCTACAT		
CLCuGV2315F	CCCCATTCCAGTGTGTCTCC	95 °C 15 s, 55 °C 15 s and 72 °C for 45 s (35 cycles), 72 °C 60s	569
CLCuGV78R	GCGCGTTCTGATTGGACATC		

^a All reactions were incubated at 95 °C for 1 min prior to cycling through incubations steps as described above

Fig. 1 Genome map of cotton leaf curl Gezira virus DNA-A isolate Q2535 showing location of primers used in genome walking

Table 2 Distance matrix of the percent nucleotide identities of the two new begomovirus isolates, Q2535 and Q2545, with a range of begomoviruses using the complete DNA-A genomes. Virus acronyms are as follows: BG MV – *bean golden mosaic virus*, CLCuAIV – *cotton leaf curl Alabad virus*, CLCuBaV – *cotton leaf curl Bangalore virus*, CLCuGeV – *cotton leaf curl Gezira virus*, CLCuKoV – *cotton leaf curl Kokhran virus*, CLCuMuV – *cotton leaf curl Multan virus* and TYLCV – *tomato yellow leaf curl virus*. Distances were calculated using a multiple sequence alignment and the software program <http://www.cbio.uct.ac.za/SDT> as described by Brown et al. (2015)

Virus	GenBank code	TYLCV	CLCuKoV	CLCuAIV	CLCuMuV	CLCuBaV	CLCuGeV	
TYLCV	GU178815	100.0						
CLCuKoV	AJ496286	75.7	100.0					
CLCuAIV	AJ002452	71.4	74.5	100.0				
CLCuMuV	AJ132430	73.1	79.1	82.0	100.0			
CLCuBaV	AY705380	73.8	81.2	79.0	85.0	100.0		
CLCuGeV	FM164726	73.9	75.7	69.7	73.4	73.9	100.0	
	AJ542539	74.3	76.2	70.4	73.2	74.5	90.4	100.0
	EU024120	73.0	75.6	70.5	74.0	74.2	88.9	88.8
	FN554540	73.0	75.6	70.2	73.8	74.4	89.6	89.7
	EU432373	73.3	75.8	70.2	73.8	74.3	89.5	88.8
	FJ868828	74.1	75.5	69.8	74.1	74.5	87.6	88.5
	AF260241	73.6	76.1	70.4	73.7	74.2	90.8	91.7
	FM210276	73.9	75.5	70.2	73.8	75.4	89.4	91.7
	MK947932 (Q2535)	74.4	76.2	70.7	73.6	74.2	91.0	92.2
	MK947933 (Q2545)	74.4	76.2	70.7	73.6	74.2	91.0	92.2
	AF155064	74.3	75.9	70.5	73.4	74.2	91.1	94.2
BGMV	JN419006	67.8	67.3	66.2	67.7	67.7	66.5	68.1
Virus	CLCuGeV							BGMV
	FN554540	EU432373	FJ868828	FM210276	MK947932 (Q2535)	MK947933 (Q2545)	AF155064	JN419006
TYLCV								
CLCuKoV								
CLCuAIV								
CLCuMuV								
CLCuBaV								
CLCuGeV								
	100.0							
	96.9	100.0						
	92.9	93.6	100.0					
	95.0	94.5	95.9	100.0				
	96.2	93.2	90.7	100.0				
	93.4	92.6	92.1	96.1	100.0			

Table 2 (continued)

	93.4	92.6	92.1	95.2	96.1	100.0	100.0	100.0	100.0
	93.7	92.9	92.1	95.4	96.3	99.2	99.2	100.0	100.0
BGMV	67.0	67.6	66.9	67.5	66.9	66.9	66.9	67.1	100.0

primers $\beta 01/\beta 02$ (Briddon et al. 2002). The ca. 600 bp amplicon expected for the DNA-A PCR was amplified from several samples. This included Q2517, Q2521, Q2535 and Q2545. The remaining samples Q2537, Q2539, Q2540, Q2541 and Q2542, tested negative for DNA-A by PCR. All samples tested negative using the $\beta 01/\beta 02$ primers and lacked the typical severe CLCuD symptoms of leaf rolling, cupping and enations (Amin et al. 2011), thus indicating an absence of a DNA-beta satellite molecule. Amplification of circular genome components from the samples was attempted using rolling-circle amplification techniques, however, these were unsuccessful due to the effect of gamma-irradiation of samples required as a post-entry quarantine treatment for their importation into Australia.

DNA-A amplicons were obtained from four samples which were sequenced and analysed by BLASTN (National Centre for Biotechnology Information) to give putative virus identifications. Amplicons from two of the samples were identical to CLCuGeV and the amplicons from the remaining two sample were identical to *squash leaf curl virus* (SqLCV) in BLASTN searches. GenBank accession references for the SqLCV isolates are Q2517 - MN422079 and Q2521 - MN422078. The GenBank accession references of the two CLCuGeV isolates are of their full DNA-A genomes as described below (Q2535 - GenBank MK947932 and Q2545 - MK947933).

As CLCuGeV has not previously been reported from melon and capsicum, further work was done to confirm the virus presence in these two samples. Sequence of full-length DNA-A was achieved by sanger sequencing of PCR amplicons for the samples, Q2535 and Q2545. The primer pair 705F/2507R was used first and generated 1765 bp of sequence, overlapping the original Avcore/Accore sequence by 282 bp. Sequence analyses and additional primers were designed using the Geneious Bioinformatics software package (Geneious). The newly designed primers were used to amplify and sequence the remaining DNA-A component from freshly prepared TNAEs (Table 1, Fig. 1). Cycling parameters for the different PCRs are listed in Table 1 and reactions contained 5 pmol of forward and reverse primer, 1.75 mM $MgCl_2$, 100 μM dNTPS, and 0.2 U *MangoTaq* Polymerase (Bioline) in a total volume of 25 μl . The full DNA-A nucleotide sequence of the two isolates (Q2535 - GenBank MK947932 and Q2545 - MK947933) were 100% identical to each other and 99.5% to CLCuGeV (GenBank AF155064) by BLASTN searches. Using the criteria set by Brown et al., (2015) which states the demarcation for begomovirus species is <91% for full DNA-A nucleotide sequences the isolates are identified as CLCuGeV (Table 2). Translation of the full DNA-A sequences generated the expected ORFs in the expected order for CLCuGeV (Fig. 1).

As many of the samples had symptoms similar to that expected for a potyvirus, and not all tested positive for begomovirus, the samples were also indexed for potyvirus. This was done using

Fig. 2 **a** Symptoms of virus infection in the melon sample Q2545. The leaf distortion and mosaic is typical of a potyvirus infection and the sample was found to be co-infected with zucchini yellow mosaic virus and cotton leaf curl Gezira virus **(b)** Symptoms of virus infection in the capsicum sample Q2535. The upward cupping of the capsicum leaves is consistent with infection by a begomovirus

the primers U341/Poty1 (Langeveld et al. 1991; Gibbs and Mackenzie 1997) which amplify the 3' end of the coat protein and the 3'UTR. Seven of the nine samples tested positive in this assay and the amplicons sequenced to determine virus identity. Based on BLASTN searches using the partial genome sequences, *zucchini yellow mosaic virus* (ZYMV), was detected in three of the melon samples (Q2540, Q2542 and Q2545) and the squash sample (Q2517), *papaya ringspot virus* (PRSV) was detected in the fourth melon sample (Q2541) and *potato virus Y* (PVY) was detected in the chilli sample (Q2537). The GenBank accession references for ZYMV are Q2540 - MN422075, Q2542 - MN422077, Q2545 - MK981542 and Q2517 - MN422073, for PRSV Q2541 - MN422076 and for PVY Q2537 - MN422074.

The symptoms observed in the samples of these new hosts of CLCuGeV are shown in Fig. 2. The melon sample, Q2545, had symptoms more typical of a potyvirus infection (Fig. 2a) and was confirmed co-infected with ZYMV. The presence of the potyvirus has masked any potential begomovirus induced symptoms. Further work with a single virus infection is required to elucidate what symptoms CLCuGeV causes in melon, if any. The capsicum sample, Q2535, was from a plant with upward leaf cupping (Fig. 2b), typical of a begomovirus

infection, although not as severe as expected for co-infection with a DNA-beta satellite. As only one capsicum plant with these symptoms was sampled, again further work is needed to determine if this symptom was induced by CLCuGeV infection.

This is the first report of CLCuGeV infecting melon and capsicum which expands the known host range for the virus. This report also furthers the research on genetic diversity of begomoviruses in Egypt (Abdel-Salam et al. 2016).

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