



First report of cotton leaf curl Gezira virus infecting *Malva parviflora* and in Iraq

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Abstract

In the current study, the complete genome of an isolate of cotton leaf curl Gezira virus (CLCuGeV), identified for the first time from *Malva parviflora* in Iraq, was amplified using rolling circle amplification and sequenced. The Iraqi isolate of CLCuGeV shared highest nucleotide identity at 98.2% with an Israeli isolate and clustered with isolates of the Egyptian and Cameroon strains in phylogenetic analysis.

Keywords Begomovirus · Cheeseweed · Geminivirus · Reservoir host · Weed

Weeds commonly grow along with cultivated plants and may act as reservoir hosts of various vectors and plant viruses (Varma and Malathi 2003; Hull 2014). Cotton leaf curl Gezira virus (CLCuGeV, genus *Begomovirus*, family *Geminiviridae*) is one of the begomoviruses causing cotton leaf curl disease (CLCuD), which is as a serious threat to cotton production around the world (Varma and Malathi 2003; Sattar et al. 2013). CLCuGeV was first reported from Africa in 2002 (Idris and Brown 2002) and has a circular single-stranded monopartite DNA genome of 2.7 kb (Brown et al. 2015). The natural hosts of CLCuGeV are mostly limited to wild or crop species of Malvaceae (Tahir et al. 2011; Leke et al. 2013; Bananej et al. 2021b; Salari et al. 2021). CLCuGeV in association with tomato leaf curl betasatellite is recently reported from *Malva sylvestris* plants in Iran (Bananej et al. 2021b). However, CLCuGeV has been shown to infect also papaya (Khan et al. 2012; Bananej et al. 2021a), tomato

(Al-Shihi et al. 2017), pepper and melon (Gambley et al. 2020), sunflower (Salari et al. 2021) and *Amaranthus* sp. (GenBank Accession no. MN381116; unpublished). Like other begomoviruses, CLCuGeV is transmitted by whiteflies of the *Bemisia tabaci* species complex (Ghanim 2014; Shahmohammadi et al. 2022). To date, 12 strains of CLCuGeV, including CLCuGeV-Egypt (-EG), -Niger (-NE), -Sudan (-SD), -Cameroon (-CM), -Cairo (-Ca), -Burkina Faso (-BF), -Hollyhock (-Ho), -Lysoka (-Ly), -Madagascar (-MG), -Mali (-ML), -Okra (-OK), and -Al-Batinah (-AB), have been identified (Al-Shihi et al. 2017; ICTV Online 2021), but merging of some strains according to the demarcation criterium of higher than 94% nucleotide identity for strains is proposed (Al-Shihi et al. 2017).

In 2015, nine Cheseseeweed mallow (*Malva parviflora*) samples showing leaf curling and yellowing were collected close to tomato fields in Dhi-Qar, Iraq, and subjected to total DNA isolation by a CTAB method (Doyle and Doyle 1987). In order to identify the begomoviruses associated with the symptoms in the collected samples, PCR was performed using the begomovirus-specific degenerate primers PAL1v1978/PAR1c496 (Rojas et al. 1993) and a fragment of 1.4 kb was amplified for four of the samples. To test the possible presence of tomato yellow leaf curl virus (TYLCV) in PCR-positive samples, a subsequent PCR was performed using a TYLCV-specific primer pair (V1 (CP) Forward/V1 (CP) Reverse), which revealed TYLCV infection of three samples (Al-Waeli et al. 2017). For the sample testing negative for TYLCV, circular viral DNA was amplified by rolling circle amplification (RCA) using a Templiphi RCA Kit

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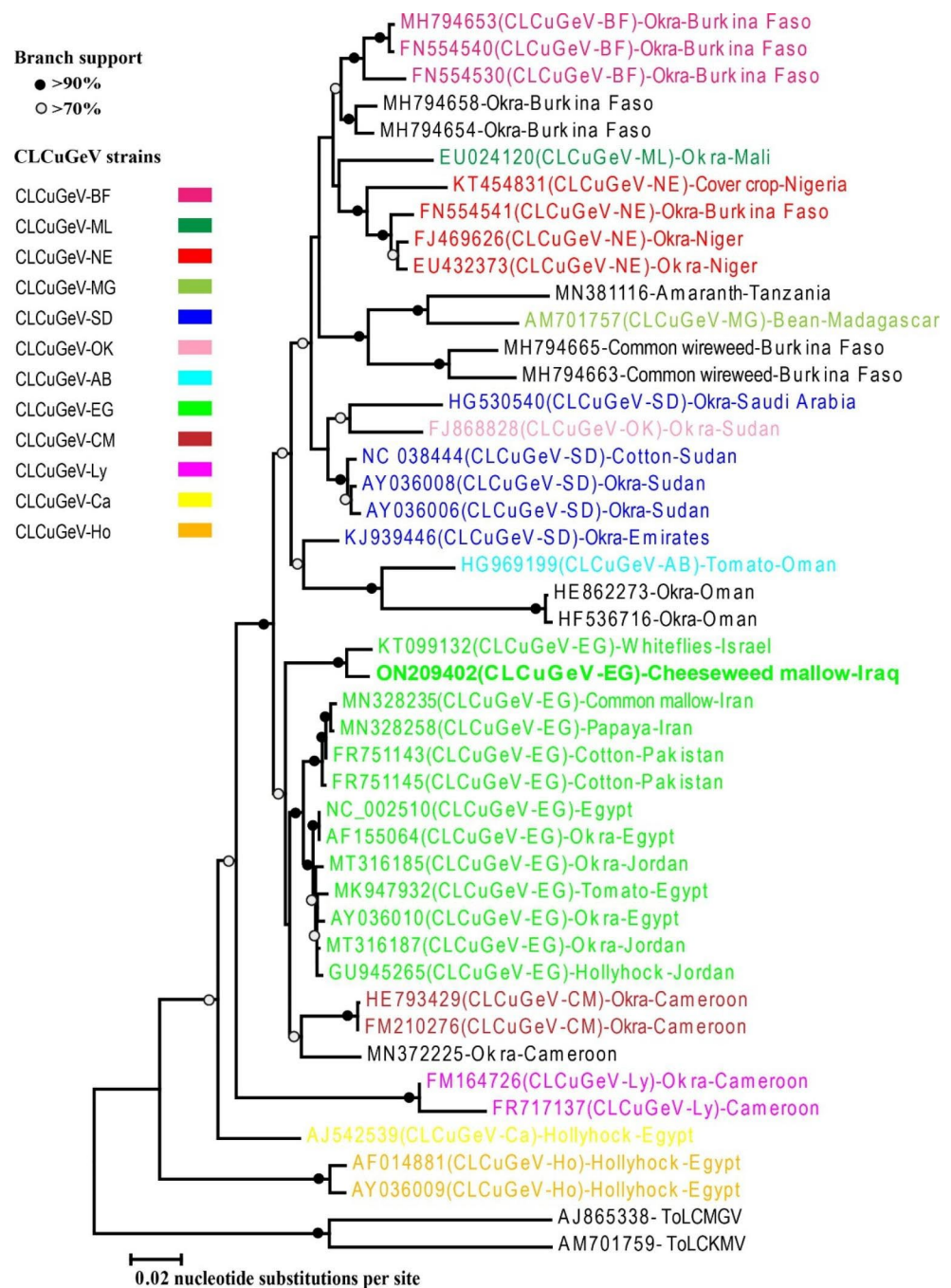
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Fig. 1 Neighbour-joining phylogenetic tree reconstructed using MEGA 7 software based on complete nucleotide sequences of isolates of different strains of cotton leaf curl Gezira virus (CLCuGeV). The strain of each isolate is shown in parenthesis. The host and origin of isolates are shown in the tree. The close relatives tomato leaf curl Madagascar virus (ToLCMGV, AJ865338) and tomato leaf curl Comoros virus (ToLCKMV, AM701759) were used as out-groups. Bootstrap values higher than 70 are shown at each node. The Iraqi isolate is shown in bold green text



(GE Healthcare, USA). The RCA product was digested with a range of restriction enzymes, including *EcoRI*, *BamHI*, *SalI* and *PstI*, yielding a fragment with the expected size of ~3 kb by *BamHI*. The purified fragment was cloned into pBluescript II KS (+) followed by transformation into *Escherichia coli* DH5 α competent cells and sequencing of the complete viral genome in both directions. The presence of a betasatellite was proven by PCR amplification of a 1.3 kb fragment using the primer pair Beta01/Beta02 (Bridson et al. 2002), while no amplicon was obtained for alphasatellites

when PCR was performed using the primer pairs DNA101/DNA102 and UN101/UN102 (Bull et al. 2003).

BLASTn searches of the assembled full genome sequence of 2777 nucleotides (nts) showed highest nucleotide identities with isolates of CLCuGeV. Pairwise nt sequence comparisons of the identified Iraqi CLCuGeV isolate (IQ:Dhi:Malva-90:15; GenBank Accession no. ON209402) with other previously reported CLCuGeV isolates using SDT 1.2 software (Muhire et al. 2014) revealed the highest nt identity at 98.2% with an Israeli whitefly isolate of CLCuGeV collected from squash plants (GenBank Accession no.

KT099132). High nt identities were also shared with isolates of the strains CLCuGeV-Egypt (95.6–95.9%), including isolates from Pakistan and Iran (FR751143, FR751145, MN328258, MN175235) and CLCuGeV-Cameroon (HE793429, FM210276) (94.0–94.7%; Fig. S1). A neighbour-joining phylogenetic analysis based on the complete nucleotide sequence of IQ:Dhi:Malva-90:15 and isolates of different CLCuGeV strains using MEGA 7 (Kumar et al. 2016) revealed that IQ:Dhi:Malva-90:15 grouped with the isolate from Israel (KT099132) in a distinct branch closely related to the Egypt and Cameroon strain groups (Fig. 1). In a previous study, a merge of the strains Sudan, Cairo, Egypt, Cameroon, Okra, Burkina Faso and Niger was proposed (Al-Shihi et al. 2017). Our results confirmed the proposal as they all have identity values exceeding 94%. Analysis of recombination in the genome of IQ:Dhi:Malva-90:15 using RDP4 (Martin et al. 2015) showed that the Iraqi isolate has the evidence of a recombination event (supported by all seven used methods) in its genome, in which CLCuGeV-Hollyhock and an isolate from Oman (HF536716) were identified as putative major and minor parents, respectively ($P = 3.20 \times 10^{-10} - 8.29 \times 10^{-20}$). The recombinant region is of ~450 nts (nucleotides 1005–1450) covering the gene region of *cp/ren/trap*. While it seems that CLCuGeV is circulating in many countries in the Middle East (Tahir et al. 2011; Khan et al. 2012; Idris et al. 2014), to our knowledge, this is the first report of CLCuGeV from Iraq. As this virus was identified from a weed close to a tomato field, further studies are required to investigate the incidence of CLCuGeV on other crop hosts, such as tomato, as well as the geographic distribution.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13314-023-00498-y>.

Declarations

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication Publication has been approved by all co-authors.

References

- Al-Shihi AA, Al-Sadi AM, Deadman M, Briddon RW, Shahid MS (2017) Identification of a distinct strain of cotton leaf curl Gezira virus infecting tomato in Oman. *J Phytopathol* 166(3):199–205
- Al-Waeli M, Dizadji A, Mossahebi GH, Ahangaran A (2017) Natural occurrence and phylogeny of *Tomato yellow leaf curl virus* on *Malva parviflora* and *Melilotus indicus* from Iraq. *Life Sci J* 14:111–119
- Bananej K, Shafiq M, Shafiq Shahid M (2021a) Association of cotton leaf curl Gezira virus with tomato leaf curl betasatellite infecting *Carica papaya* in Iran. *Australas Plant Dis Notes* 16:1–4
- Bananej K, Shafiq Shahid M, Shafiq M (2021b) Evidence that leaf curl disease of *Malva sylvestris* in Iran is associated with cotton leaf curl Gezira virus and associated betasatellite. *J Plant Pathol* 103:671–672
- Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG (2002) Universal primers for the PCR-mediated amplification of DNA b: a molecule associated with some monopartite begomoviruses. *Mol Biotechnol* 20:315–318
- Brown JK, Zerbini FM, Navas-Castillo J, Moriones E et al (2015) Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. *Arch Virol* 160:1593–1619
- Bull SE, Briddon RW, Markham PG (2003) Universal primers for the PCR-mediated amplification of DNA 1: a satellite-like molecule associated with begomovirus-DNA β complexes. *Mol Biotechnol* 23:83–86
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bull* 19:11–15
- Gambley C, Cremer J, Campbell P, Roach R, Abdel-Salam MA (2020) New host records for cotton leaf curl Gezira virus: capsicum and melon in Egypt. *Australas Plant Dis Notes* 15(3). <https://doi.org/10.1007/s13314-019-0372-3>
- Ghanim M (2014) A review of the mechanism and component that determine the transmission efficiency of tomato yellow leaf curl virus (*Geminiviridae*, *Begomovirus*) by its whitefly vector. *Virus Res* 189:47–54
- Hull R (2014) *Plant Virology*, 5th edn. Academic Press
- Idris AM, Brown JK (2002) Molecular analysis of cotton leaf curl virus-Sudan reveals an evolutionary history of recombination. *Virus Genes* 24:249–256
- Idris A, Al-Saleh M, Amer M, Abdalla O, Brown J (2014) Introduction of cotton leaf curl Gezira virus into the United Arab Emirates. *Plant Dis* 98:1593
- Khan AJ, Akhtar S, Al-Matrushi AM, Al-Shihi AA, Al-Hinai FM, Briddon RW (2012) Identification of cotton leaf curl Gezira virus in papaya in Oman. *Plant Dis* 96:1704
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Leke WN, Sattar MN, Ngane EB, Ngeve JM, Kvarnheden A, Brown JK (2013) Molecular characterization of begomoviruses and DNA satellites associated with okra leaf curl virus in Cameroon. *Virus Res* 174:116–125
- Martin DP, Murrell B, Golden M, Khoosal A, Muhire B (2015) RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evol* 1:vev003
- Muhire BM, Varsani A, Martin DP (2014) SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS ONE* 9:e108277
- Rojas MR, Gilbertson RL, Russell DR, Maxwell DP (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Dis* 77:340–347
- Salari K, Heydarnejad J, Massumi H, Hasanvand V (2021) First report of cotton leaf curl Gezira virus incidence and the associated betasatellite in marshmallow, okra and sunflower in Iran. *Iran J Plant Pathol* 56(4):405–408 (abstract)
- Sattar MN, Kvarnheden A, Saeed M, Briddon RW (2013) Cotton leaf curl disease - an emerging threat to cotton production worldwide. *J Gen Virol* 94:695–710
- Shahmohammadi N, Mansourpour M, Golnaraghi A (2022) Current challenges and future perspectives on detection of geminiviruses. In: Gaur RK, Sharma P, Czosnek H (eds) *Geminivirus: detection,*

diagnosis and management, 1st edn. Academic Press (Elsevier, pp 3–24

Tahir MN, Amin I, Briddon RW, Mansoor S (2011) The merging of two dynasties- identification of an african cotton leaf curl disease-associated *begomovirus* with cotton in Pakistan. PLoS ONE 6:e20366

Varma A, Malathi V (2003) Emerging geminivirus problems: a serious threat to crop production. Ann Appl Biol 142:145–116

<https://ictv.global/report/chapter/geminiviridae/geminiviridae/begomovirus>

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