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A New Host Record for Cotton Leaf Curl Gezira Virus (CLCuGeV) Infecting Common Bean, (*Phaseolus vulgaris*) Plants in Egypt

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ABSTRACT

Cotton leaf curl disease is a major threat to cotton production in Africa. Some bean plants in Giza governorate, Egypt, exhibited symptoms including stunting, mottling, leaf curling, rugosity, vein enlargement, and pod malformation. Immuno-capture polymerase chain reaction (IC-PCR) using antisera for CLCuGeV and degenerate primers for begomoviruses indicated the presence of a begomovirus in infected bean plants. Analysis of the coat protein (CP) (V1 gene) of this virus indicated the presence of CLCuGeV, which was given a GenBank accession number of OQ676568.CLCuGeV-EG:Bean isolate had the highest pairwise sequence identity (PSI) of nucleotide/amino acids (Nt/AA) with an isolate from okra (USA: MN027199 [97.9/98.7]), respectively. Furthermore, CLCuGeV-EG:Bean had >94% PSI of Nt/AA CP sequences with other CLCuGeV-EG isolates from okra (Egypt:AY036010, FJ030878 [97.5/98.7]), pepper (Egypt:MK947932 [97.5/98.7]), melon (Egypt:MK947933 [97.5/98.7]), *Cucumis* sp. (Egypt:JX416187 [97.5/98.6]), cotton (Egypt:FJ030874 [97.3/97.7]), squash (Egypt:FJ030879 [97.3/-]), and other CLCuGeV isolates from Israel (KT099132 [97.7/98.1]), Jordan (GU945265 [97.5/98.7]), Pakistan (FR751145, FR751145 [97.1/97.5]), and Iran (MZ911854 [96.6/97.5]). A phylogenetic tree based on AA sequences of CPs revealed two major clusters of CLCuGeV isolates. The first cluster involved CLCuGeV isolates from the above-mentioned countries in addition to Oman, the United Arab Emirates, and Cameron. The second cluster circumvented the CLCuGeVs from Madagascar, Burkina Faso, Niger, Sudan, and Saudi Arabia. CLCuGeV from Tanzania clustered alone; suggesting that Tanzania is one of the Sahel-region countries where CLCuGeV originated. To our knowledge, this is the first report of CLCuGeV-EG:Bean naturally infecting *P. vulgaris* (*Fabaceae*) in Egypt. The *P. vulgaris* infection with CLCuGeV widens the host range of this virus and increases its biological and molecular diversity.

INTRODUCTION

Currently, cotton is cultivated in more than 80 countries, with an annual production of 27 million tons (https://www.theworldcounts.com/ challenges/consumption/ clothing/ world-cotton-production-statistics). The four worldwide cultivated cotton species are *Gossypium hirsutum* (GH), *Gossypium barbadense* (GB), *Gossypium arboreum*, and *Gossypium herbaceum* (Blaise and Kranthi, 2019).

At present, cotton production is decreasing worldwide due to several biotic and abiotic stresses. Among the biotic stresses, cotton leaf curl disease (CLCuD) is a major threat to cotton production. CLCuD in Africa was first recorded infecting GB cotton in Nigeria (Ferquharson, 1912), Sudan (Golding, 1930), Tanzania (Kirkpatrick, 1931), and Northern Africa and Tanzania (Hussain *et al.*, 1991). The whitefly vector (*Bemisia* sp.) of the disease was identified earlier (Golding, 1930). The disease etiology was attributed to whitefly-transmitted monopartite begomoviruses (*Geminiviridae*) interacting with betasatellite and alphasatellite. (Briddon *et al.*, 2003, 2004; Brown, 2017; Rahman *et al.*, 2017). The monopartite begomoviruses have a circular ssDNA (DNA A) of ~2.6-2.8 kb in size and contains the necessary genetic information for virus replication, control of gene expression, insect transmission, and movement (Rahman *et al.*, 2017).

CLCuGeV (Idris and Brown, 2002), with its associated betasatellite, is the most prevalent pathogen in cotton found throughout the cotton belt in sub-Saharan Africa, mainly in the Sahel region (Idris and Brown, 2002; Idris et al., 2005). CLCuGeV was recorded in Egypt (Abdel-Salam, 1999), the Arabian Peninsula (United Arab Emirates) (Idris *et al.*, 2014), Saudi Arabia (GenBank access. no. HG530540, unpublished), Oman (Al Shihi *et al.*, 2017), Pakistan (Tahir *et al.*, 2011), Iran (Bananej *et al.* 2021), and Iraq (Shahmohammadi *et al.*, 2023). CLCuGeV was also reported in Jordan (GenBank access. no. MT316186). Metagenomic analysis through testing *B. tabaci* (MEAM1) revealed the presence of CLCuGeV in Israel (Rosario *et al.*, 2015). Recent introductions of CLCuGeV were reported in imported Lavatera stem cuttings in both the Netherlands (Anonymous 2022a) and Germany (Anonymous 2022b). Furthermore, GLCuGeV was recorded for the first time in okra plants in the USA (Villegas *et al.*, 2019).

CLCuGeV was thought to first infect malvaceous species such as cotton, hollyhock, okra, and Sida (Idris and Brown, 2002; Idris *et al.*, 2002; Tahir *et al.*, 2011). Additional studies indicated that it can also infect common bean, soybean, and squash (Abdel-Salam, 1999), papaya (Khan *et al.*, 2012), 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).

In Egypt, a geminivirus was isolated from hollyhock plants under the name of *Hollyhock leaf crumple virus* (HLCrV) (Abdel-Salam et al., 1998). HLCrV was found to experimentally infect cotton and okra plants and transmitted by the *B. tabaci* insect vector (Abdel-Salam *et al.*, 1998). Idris *et al.* (2002) showed that HLCrV had 96.1% amino acid similarity with the *Okra leaf curl virus* (OKLCV) in the ORF of the V1 gene. Both viruses clustered in a monophyletic branch with the *Cotton leaf curl virus* from Sudan (CLCuV-SD). Another begomovirus, provisionally named Cotton Leaf Curl Mosaic Virus (CLCuMV), was isolated from GB cotton fields in Egypt (Abdel-Salam, 1999). CLCuMV was experimentally transmitted through whitefly and mechanical inoculation from infected cotton to *G. barbadense*, *G. hirsutum*, *P. vulgaris*, *Cucurbita pepo*, and *Glycine max*. However, the identification of CLCuMV remained obscure at that time since only serology and degenerate primers for the coat protein (V1 gene) were used for its identification (Abdel-Salam, 1999). DNA sequences for the coat protein (CP) of CLCuMV from cotton and squash were submitted to GenBank in 2008. Later on, the GenBank acknowledged these two sequences as isolates of CLCuGeV (access. no. FJ030874 for cotton and FJ030879 for squash). The ICTV considered a

begomovirus-infecting cotton from Gezira (Sudan), HLCrV infecting hollyhock from Egypt, and OKLCV infecting okra in Egypt as three strains of CLCuGeV (Fauquet *et al.*, 2008).

In the summer of 2022, some common bean plants in the fields of Giza governorate, Egypt, exhibited stunting, leaf rugosity, diffused mottling, downward leaf curling and enlargement of major veins and were associated with a whitefly infestation. Such symptoms were years ago observed in bean fields adjacent to cotton fields infected with CLCuGeV (CLCMV). The purpose of this study is to identify the disease-causing virus in infected bean plants at the molecular level and compare its V1 gene with other corresponding V1 genes described worldwide for CLCuGeV isolates.

MATERIALS AND METHODS

Sample Collection:

Leaves of *P. vulgaris* plants showing symptoms of stunting, rugosity, and leaf curling were collected from the Experimental Farm of the Faculty of Agriculture, Cairo University, in the summer of 2022.

Detection and Identification of The Virus: IC-PCR:

A modified procedure for that described by Abdel-Salam (2006) for the detection of begomovirus presence in mucilaginous plant sap, such as cotton and okra, was followed. A mixture of two polyclonal antisera, prepared for the CP of CLCuGeV-external and internal epitopes was used (Abdel-Salam, 1999). Sterile polypropylene thin-walled 0.2 ml microfuge tubes were coated overnight at 4ºC with 25 µl of the cocktail-polyclonal antisera mixture, diluted to 1/100, in ELISA coating buffer (pH 9.6). Tubes were then washed three times, each with 50 µl of PBST (pH 7.4), and incubated overnight at 4ºC with 25 µl of sap extract. The sap extract was prepared by grinding 0.5 g/sample of fresh tissue in a sterile mortar and pestle in the presence of liquid nitrogen. Tissues infected with CLCuGeV from GB cotton or *P. vulgaris*, were then suspended in a buffer composed of 100 mM Na_2HPO_4-NaH_2PO_4, 20 mM Na_2SO_3, 20 mM EDTA. 1.5% Triton X -100, pH 8.3. The extracts were subsequently clarified with low-speed centrifugation (8000 rpm for 10 min at 5ºC) and each sample was diluted 1/100 in the same suspending buffer. After overnight incubation, tubes were washed twice, each with 50 µl of PBST, and left to dry at 37ºC for 15 min. PCR mixture (void of Taq polymerase) and containing 5 µl of 5X GoTaq DNA polymerase reaction buffer (Cat No. M8301, Promega, Madison, WI, USA), 2.5 mM $MgCl_2$, 0.2 mM for each dNTP base, 0.4 µmol for each forward and reverse primers, and sterile bi-distilled water-containing 5% Triton X-100 was added to make a final volume of 24.5 µl. The tubes were heated up to 65ºC for 10 minutes and cooled in ice for 2 minutes. 0.5 µl of 1.25 U Taq polymerase (M8301, Promega) was added to each tube, and the tubes were subjected to PCR analysis later. Information about degenerate primers for the detection of DNA A core CP of begomoviruses, specific primers for the full-length DNA-A CP of squash leaf curl virus (SLCV), primers for the DNA-B component of begomoviruses, and PCR cycling parameters are described in Table 1. Ten microliters per sample of PCR amplicons were analyzed by electrophoresis (80 V) in 1% agarose gel prepared in TAE buffer and stained with 0.5 µg/ml ethidium bromide. PCR bands were visualized using a UV illuminator. DNA bands at the proper size were cut and purified according to the method of Borodina et al. (2003). Purified DNAs were sequenced using the chain termination method (Sanger's method) at Macrogen Inc., Seoul, South Korea.

Table 1. List of primers and cycling parameters used in PCR amplification of begomoviral genomes**.**

Primers	Primer Sequence (5′-3′)	Cycling Parameters*	Product size (bp)	References
Avcore	GCCHATRTAYAGRAAGCCMAGRAT	95 °C 20s, 53 °C 20s and 72 °C for 40s (35 cycles), 72 °C 5 min	579	Gambley et al. (2020)
Accore	GGRTTDGARGCATGHGTACANGCC			
SLCV-CPF	CCACGTTCCGCCTGACGAG	92 °C 60s, 60 °C 20s and 72 °C for 30s (35 cycles), 72 °C 5 min	900	Abdel-Salam et al. (2006)
SLCV-CPR	AATTATGTACTCGAGAATCATGAA			
BV1855	AC(A/G) CAA(A/G) TG(A/G) TC(A/T/G) AT(C/T) TTCAT	95 °C 60s, 50 °C 60s and 72 °C for 60s (30 cycles), 72 °C 7 min	665	Idris &Brown (1998)
BC2571	GGTAATATTATA(A/C/T)CGGATGG			Idris &Brown (2004)

*All reactions received an initial denaturation at 95 °C/3 min prior to the cycling steps described above

Pairwise Sequence Identity (PSI) Studies:A PSI of the CP-V1 gene of 33 GLCuGeV isolates and five other begomovirues was involved in this study (Table 2). DNA nucleotide sequences of the ORF of the V1 gene of these begomoviruses were extracted from the GenBank (https://www.ncbi.nlm.nih.gov/) according to their accession numbers using Editseq software (DNASTAR). Nucleotide sequences were aligned by both Sequence Demarcation Tool Version 1.2 (SDTv1.2) (Muhire et al., 2014) and ClustalW-MegAlign software (DNASTAR). The PSI of AA in some selected CPs was measured with the NCBI BLASTP software using the capsid protein id # of the AA sequences mentioned in Table 2.

Table 2. CLCuGeV isolates and other begomoviruses used in pairwise sequence identity, and phylogeny in the present study**.**

N0.	Begomovirus Names	Isolate/strain/Clone	Hosts	Country Origin	GenBank Access. #	Capsid Protein id #	Collection Date
AFRICA							
1	CLCuGeV	Okra:BFA	Okra	Burkina Faso	FN554541	CBG23014	2009
2	CLCuGeV	BF/Djeri/Sida690BE	Sida	Burkina Faso	MH794666	QEL50672	2015
3	CLCuGeV	OBKG1	Okra	Cameroon	MN372225	QHN70219	2009
4	CLCuGeV	CLCMV(H4)	Cotton	Egypt	FJ030874	ACJ12889	2005
5	CLCuGeV	OLCV(H1)	Okra	Egypt	FJ030878	ACJ12891	2005
6	CLCuGeV	SLCV(H9)	Squash	Egypt	FJ030879	NF CP***	2005
7	CLCuGeV	OkLCV/EG okra	Okra	Egypt	AY036010	AAK64553	2001
8	CLCuGeV	Egypt	Hollyhock	Egypt	AF014881	AAD01546	1997
9	CLCuGeV	CLCGV.Q2535	Pepper	Egypt	MK947932	QEQ90663	2010
10	CLCuGeV	CLCGV.Q2545	Melon	Egypt	MK947933	QEQ90669	2010
11	CLCuGeV	CLCuGeV/bean-GZ	Bean	Egypt	OQ676568	WGU13594	2023
12	CLCuGeV	Q2545	*Cucumis* sp	Egypt	JX416187	AGI62933	2010
13	HLCrV	Cairo	Hollyhock	Egypt	NC_004071	NP_665671	2002
14	HLCrV	HLCrV hollyhock	Hollyhock	Egypt	AY036009	AAK64546	2001
15	CLCuGeV	Okra:Niger	Okra	Niger	FJ469626	ACK77806	2007
16	CLCuGeV	CLCuV-S	Cotton	Sudan	NC_038444	YP_009506401	2000
17	CLCuGeV	Okra/Gezira	Okra	Sudan	AY036006	AAK64528	2001
18	CLCuGeV	FLATZ016_I7	Amaranth	Tanzania	MN381116	QJA07411	2009
19	BLCMV**	Madagascar	Bean	Madagascar	AM701757	CAM91887	2001
20	ToLCMV	Mali	Tomato	Mali	AY502936	AAR89448	2003
21	ToLCSDV	ToLCSDV-Gez	Tomato	Sudan	NC_005855	YP_006466	2004
ASIA							
22	CLCuGeV	Okra	Okra	Iran	MZ911857	UYH99768	2019
23	CLCuGeV	IR:Anb:1M:Mar:19	Hollyhock	Iran	MZ911854	UYH99759	2019
24	CLCuGeV	P4-3:Pap:10	Papaya	Iran	MN328257	QJP24290	2010
25	CLCuGeV	IsSq4	? PLANT	ISRAEL	KT099132	ALK03653	2011
26	CLCuGeV	Hollyhock/Jordan	Hollyhock	Jordan	GU945265	ADF56037	2009
27	CLCuGeV	J3-17	Okra	Jordan	MT316186	QJP24348	2013
28	CLCuGeV	Tom 94	Tomato	Oman	HG969199	CDO50009	2013
29	CLCuGeV	NT31	Cotton	Pakistan	FR751145	CBY85328	2005
30	CLCuGeV	NT28	Cotton	Pakistan	FR751146	CBY85336	2005
31	CLCuGeV	KSA27	Okra	Saudi Arabia	HG530540	CDI44961	2013
32	CLCuGeV	Al-Ain	Okra	UAE	KJ939446	AIQ77734	2014
33	OELCuV	OELCuV_IR_P7_2010	Papaya	Iran	KJ397529	AHN60584	2010
34	TYLCV-MLD	TYLCV-MiLD	Cucumber	Jordan	EF158044	ABM52986	2006
35	BYVINV	OY66	Okra	India	GU112025	ADO40656	2005
36	CLCUBV	Bangalore	Cotton	India	AY705380	AAW28990	2004
37	MaLCV	Fujian	Malvastrum	China	FJ712189	ACO53436	2006
North America							
38	CLCuGeV	OK02A-18	Okra	USA	MN027199	QGN03702	2018

** BLCMV= *Bean leaf curl Madagascar virus* (a synonymous name for CLCuGeV from Madagascar), BYVINV= *Bhendi yellow vein India virus*, CLCUBV= *Cotton leaf curl Bangalore virus*, CLCuGeV= *Cotton leaf curl Gezira virus*, MaLCV= *Malvastrum leaf curl virus*, OELCuV=*Okra enation leaf curl virus*, ToLCMV= *Tomato leaf curl Mali virus*, ToLCSDV= *Tomato leaf curl Sudan virus*, TYLCV-MLD= *Tomato yellow leaf curl virus-Mild*,

***NF CP= non-functional coat protein

Phylogenic study:

The phylogenetic relationships between the AA of the CP sequences of begomoviruses (Table 2) were measured using the neighbor-joining (NJ) analysis. Analysis of the AA CPs of the tested viruses, including alignment with the Clustal W algorithm (Thompson et al., 1994), and the NJ tree was made using the Mega 11 program (Tamura et al., 2021).

RESULTS AND DISCUSSION

Symptomatology:

Natural symptoms developed on bean plants infected with CLCuGeV in the field (Fig. 1) mimic those described on cotton upon CLCuGeV infection (Brown, 2017). These include stunting in most of the infected plants. The primary symptoms on leaves involve leaf chlorosis, followed by diffused mottling or systemic necrosis, according to bean varieties. Some leaflets show marginal waving, inward leaf curling, and rugosity. Vein enlargement and leaf enations can be seen on the lower surface of some leaves. Developed pods are curled and carry smaller seeds, whereas other pods are rudimentary and stop developing into mature pods. Such described symptoms are typical of an infection of beans with the CLCuMV isolates of CLCuGeV reported before upon whitefly and mechanical inoculation (Abdel-Salam, 1999). However, such identification with serology and even PCR, using degenerate primers, may not be the ultimate judgment for the presence of CLCuGVe in bean plants. It is known that several different begomoviruses, and even some curtoviruses, share common epitopes for the CP (Abdel-Salam et al., 2017). Further degenerate primers can also amplify several begomoviruses, thus increasing ambiguity in virus identification.

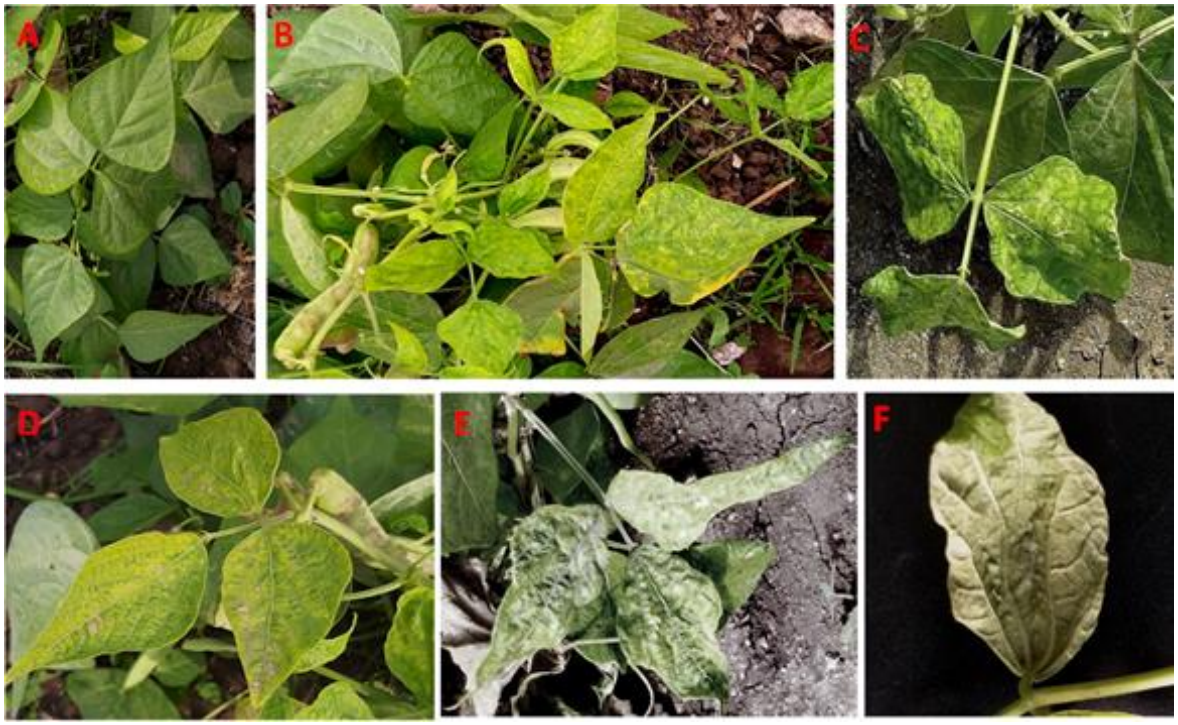

Fig. 1. Symptoms developed upon natural infection of CLCuGeV-EG to bean plants in Egypt. A, healthy bean plants; B, an infected bean plant showing chlorosis, rugosity, downward leaf curling, curled and underdeveloped pods; C, diffused mottling; D, systemic necrosis (Swiss Blanc variety); E, leaf malformation (Giza 3 variety); F, underside view of a bean leaflet showing vein enlargement and enation.

To make the begomovirus identification more complicated is the introduction of the bipartite begomovirus SLCV into Egypt and its infection of fabaceous hosts as common beans (Abdel-Salam *et al.,* 2006; Idris *et al.,* 2006; El-Dougdoug *et al.,* 2009). Therefore, more stringent measures were necessary to confirm that symptoms developed on bean plants were due to the presence of CLCuGeV as the sole pathogen in our case. Of these measures, there is the use of specific primers for SLCV, primers for the DNA B components of begomoviruses, and finally, DNA sequencing and phylogeny of the amplicons were amplified from infected bean plants through IC-PCR.

IC-PCR:

Results in Figure 2, showed positive amplification of 579 bp of CLCuGeV from infected cotton and bean plants upon using specific cocktail antisera for capturing CLCuGeV. In comparable IC-PCR gels (results not shown), no amplification was observed in gels upon using the CP F/R-specific primers for the full-length CP of DNA-A of SLCV or the BV1855/BC2571 primer pairs for the DNA-B component of bipartite begomoviruses. Such results indirectly indicate the absence of SLCV or any other associated bipartite begomovirus from the tested bean plants infected with the monopartite CLCuGeV. It is worth mentioning that IC-PCR is a very swift and effective technique for diluting out the PCR mucilaginous inhibitors present in cotton or okra extracts, per se that inhibits PCR reactions (Abdel-Salam, 2006).

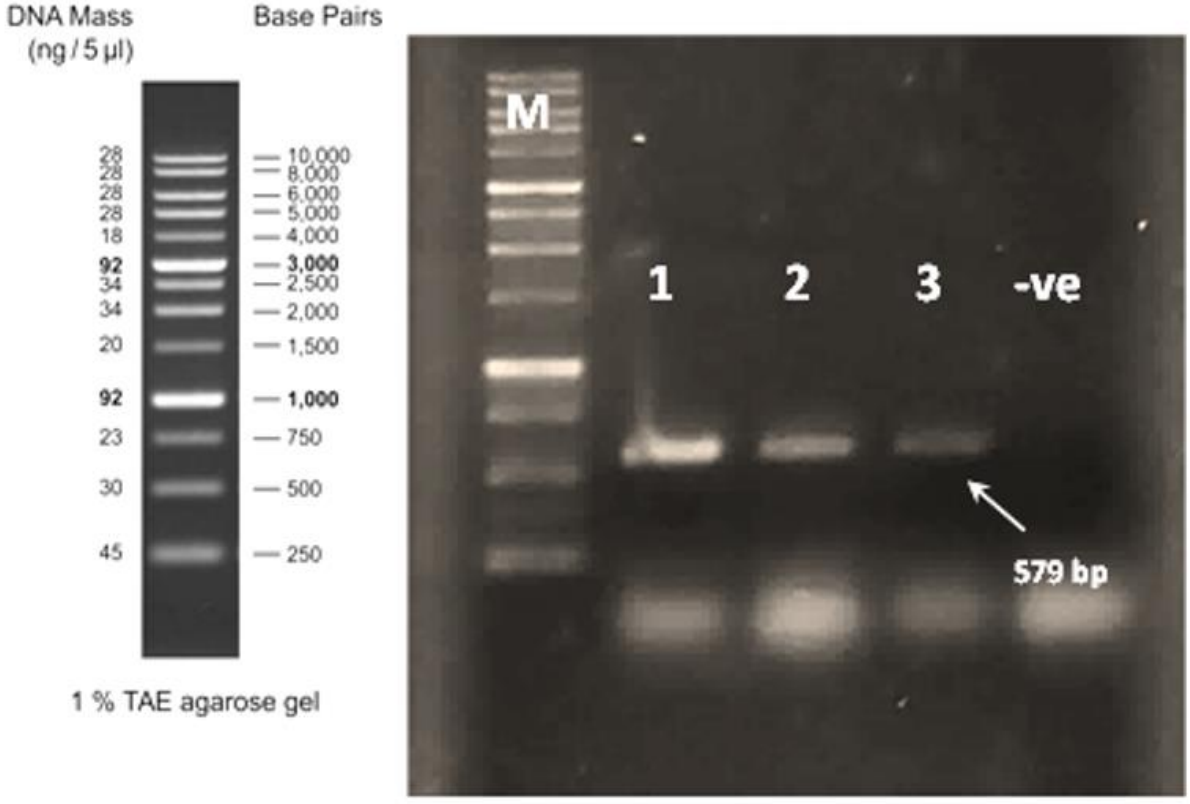

Fig. 2. Agarose Gel electrophoresis showing the migration of DNA amplicons amplified with the Avcore/Accore primer pairs. 1, +ve control of cotton infected with CLCuGeV; 2 & 3 bean plants infected with CLCuGeV; -Ve heathy bean. Arrow points out to the 579 bp position of the amplified amplicons.

PSI and Phylogeny:

The use of the ORF of the CP (V1 gene) in pairwise sequence comparison and phylogeny has already drawn the attention of several investigators, as protein-coding DNA sequences are more advantageous in terms of speed and accuracy than comparable DNA sequence alignment (Bininda-Emonds, 2005). Further, the reason for this choice is that the CP is the only structural protein in begomovirus particles. It is responsible for virion integrity, serologic-particle identity, vector transmission, shuttling of viral DNA into and out of the nucleus in monopartite begomoviruses, cell-to-cell and systemic spread of virus, and may intervene indirectly in viral DNA replication (Fondong, 2013; Bahder *et al.*, 2016; Saunders et al., 2020). Furthermore, though all CPs of begomoviruses and other geminiviruses are highly conserved, they also have variable regions that can be used to correlate phylogenic differences with biotic and geographic characteristics (Padidam *et al.*, 1995; Fondong, 2013; Bahder et al., 2016). PSI analysis using SDTv1.2 (Fig. 3) indicated that CLCuGeV-Bean (Egypt: OQ676568) shared the highest nucleotide percentages of 96.9–97.1 PSI with CLCuGeV-EG isolates from cotton (FJ030874) and okra (FJ030878), respectively.

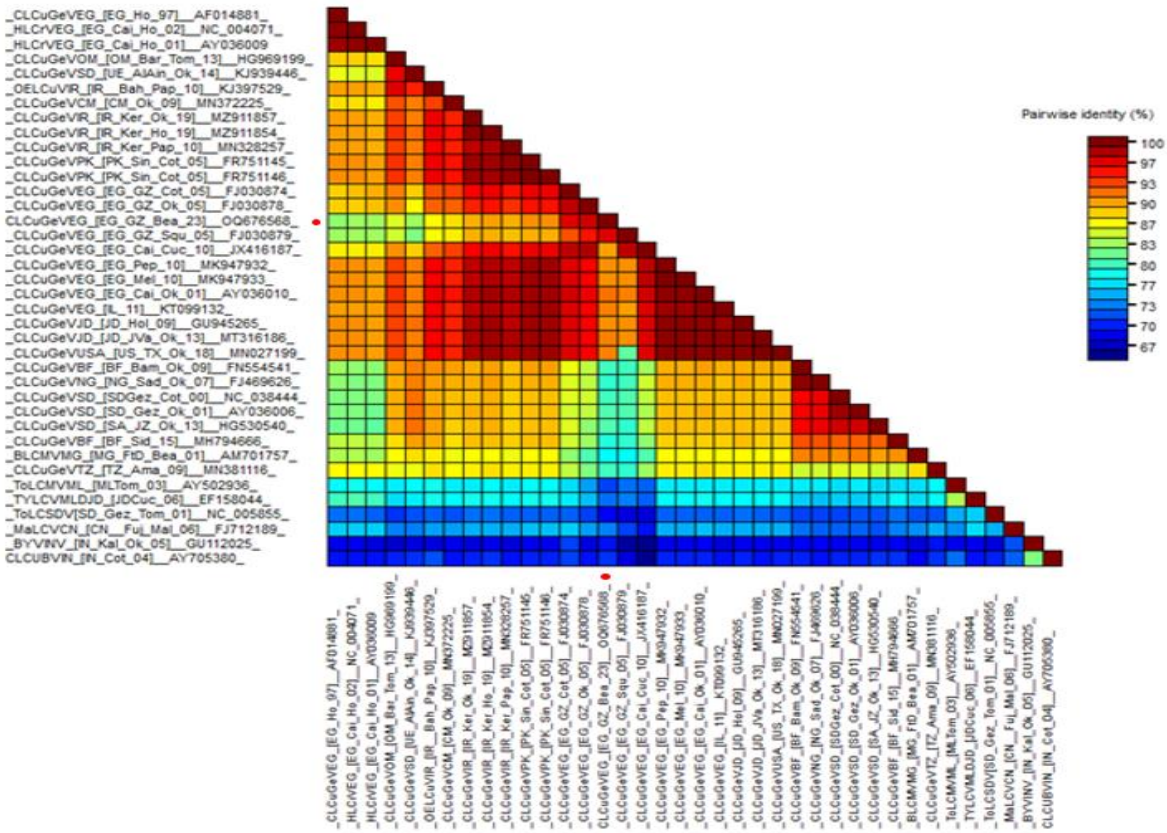

Fig. 3. A graphical representation of percentage pairwise genome scores and nucleotide identity plot of 38 coat protein-V1 genes (see Table 2 for virus acronym) using (Species Demarcation Tool, SDTv1.2 l) (Muhire et al., 2014). For illustration, CLCuGeV-EG: GZ: Bea_OQ676568 was marked with red filled circle.

On the other hand, upon using MegAlign-DNA and the NCBI BLASTP software respectively for Nt and AA analysis of the CP of several isolates of CLCuGeV (Tables 3 & 4), results showed that CLCuGeV-Bean (Egypt:OQ676568), shared, in descending orders, the highest percentage of Nt/AA identities,

respectively, with isolates from okra (USA: MN027199[97.9/98.7]), okra (Egypt: AY036010, FJ030878[97.5/98.7]), pepper (Egypt:MK947932[97.5/98.7] and melon (Egypt:MK947933[97.5/98.7]), *Cucumis* sp. (Egypt:JX416187[97.5/98.6]), squash (Egypt:FJ030879[97.3/-]), hollyhock (Jordan:GU945265[97.5/98.7]), okra (Jordan:MT316186[97.7/98.1]), Whitefly insects collected from squash (Israel: KT099132[97.7/98.1]), cotton (Egypt: FJ030874 [97.3/97.7]), cotton (Pakistan: FR751145, FR751145[97.1/97.5]), hollyhock (Iran:MZ911854[96.6/97.5]), papaya (Iran:MN328257[96.2/95.5]), and okra (Iran: MZ911857 [96.0/96.2]). The above-mentioned CLCuGeV isolates share an Nt/AA PSI > 94%. According to the strain demarcation cut-off value of ≥94% for nucleotide PSI (Fauquet et al., 2008; Brown et al. 2015), the above-mentioned isolates from the USA, Jordan, Israel, Pakistan, and Iran are therefore considered variants of the Egypt strain of CLCuGeV.

Table 3: Paiwise DNA sequence of 33 CLCuGeV isolates and 5 other begomoviruses using MegAlign-DNASTAR analysis

Percent Identity

CLCuGeV-BF [BF:Sid:15] (MH794666)	CLCuGeV-CM [CM:Ok:09] (MN372225)	CLCuGeV-EG [EG:GZ:Cot:05] (FJ030874)	CLCuGeV-EG [EG:GZ:Cot:01] (AY036010)	CLCuGeV-EG [EG:Pep:10] (MK947932)	CLCuGeV-EG [EG:GZ:Bea:23] (OQ676568)	CLCuGeV-EG [EG:GZ:Cuc:10] (JX416187)	HLCuV-EG [EG:Cal:Hib:02] (NC_004071	HLCuV-EG [EG:Cal:Hib:01] (AY036009)	CLCuGeV-SD [SD:GezCot:00] (NC_038444)	CLCuGeV-SD [SD:GezOk:01] (AY036006)	CLCuGeV-TZ [TZ:Ama:09] (MN381116)	BLCMV-MG [MG:FD:Bea:01] (AM701757)	CLCuGeV-YR [YR:KerOk:19] (MZ911857)	CLCuGeV-YR [YR:KerHo:19] (MZ911854)	CLCuGeV-YR [YR:Ker:Pap:19] (MN328257)	CLCuGeV-EG [EG:11] (KT099132)	CLCuGeV-JD [JD:Hol:09] (GU945265)	CLCuGeV-JD [JD:Vac:Ok:13] (MT316186)	CLCuGeV-OM [OM:Bar: Tom:13] (HG969199)	CLCuGeV-PK [PK:Sin:Cot:05] (FR751145)	CLCuGeV-SD [SD:JAZ:Ok:13] (HG530540)	CLCuGeV-SD [UE:Al-Ain: Ok:16] (KJ939446)	OELCuV-YR [IR: BahPap: 18] (KJ939529)	CLCuGeV-USA [US:TX:Ok:18] (MN027199)	TYLCV-MLD-JD [JD-Cuc:06] (EF138044)	CLCuGeV-BF [BF:Bam:Ok:09] (FN554541)	CLCuGeV-EG [EG:GZ:Ok:05] (FJ030878)	CLCuGeV-EG [EG:Hor:97] (AF014881)	CLCuGeV-EG [EG:Mal:10] (MK947933)	CLCuGeV-NG [NG:Sud:Ok:07] (EF469626)	ToLCSDV-[SD:GezTom:01] (NC_005855)	CLCuGeV-PK [PK:Sin:Cot:05] (FR751145)	CLCUDV-IN [IN:Cot:04] (AY705380)	MaLCV-CN [CN: Fuj:Mal:06] (FJ712189)	BYVINV [IN:Kal:Ok:05] (GU112025)	CLCuGeV-EG [EG:GZ:Squ:05] (FJ030873)	ToLCMV-ML [ML: Tom:01] (AY502936)	DNA sequence description and Genbank accession numbers	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38		
100.0	87.4	85.3	88.0	88.0	84.0	85.5	83.5	83.5	92.4	92.1	84.6	90.1	88.3	88.4	87.8	87.6	88.3	87.9	87.4	88.2	91.9	89.3	89.1	87.9	75.9	92.7	85.4	83.7	88.0	92.7	73.5	88.2	74.3	48.6	72.2	84.5	76.3	1	MH794666
	100.0	94.1	94.3	94.3	93.5	95.2	87.0	87.0	88.7	88.4	86.7	85.6	94.2	94.7	94.2	94.5	94.3	94.5	92.3	94.9	87.9	90.7	92.8	94.5	76.7	89.4	94.6	87.0	94.3	98.2	72.9	94.9	73.8	49.4	71.4	93.8	76.6	2	MN372225
		100.0	97.6	97.6	97.3	99.0	89.8	89.8	86.2	85.8	84.8	83.2	96.4	96.9	96.0	97.4	97.6	97.8	92.1	96.9	86.0	89.1	93.8	97.8	77.7	96.5	98.9	89.5	97.6	86.2	72.5	96.9	71.3	46.8	71.3	98.4	77.2	3	FJ030874
			100.0	100.0	97.5	100.0	90.1	90.1	88.8	88.5	86.7	86.2	97.9	98.5	97.9	99.1	99.2	99.4	93.3	98.6	88.3	90.7	95.5	99.4	77.0	89.1	97.7	90.1	100.0	88.8	73.9	98.6	73.5	49.5	71.9	96.4	76.4	4	AY036010
				100.0	97.5	100.0	90.1	90.1	88.8	88.5	86.7	86.0	97.9	98.5	97.9	99.1	99.2	99.4	93.3	98.6	88.3	90.7	95.5	99.4	77.0	89.1	97.9	90.1	100.0	88.8	73.9	98.6	73.5	49.5	71.9	96.4	76.4	5	MK947932
					100.0	97.5	88.8	88.8	86.1	85.7	85.5	82.9	96.0	96.6	96.2	97.7	97.5	97.7	91.4	97.1	85.7	88.6	92.4	97.9	77.5	85.3	97.5	88.6	97.5	84.8	70.9	97.1	67.2	38.3	68.2	97.3	76.8	6	**OQ676568**
						100.0	90.3	90.3	87.1	86.7	85.3	83.5	97.8	98.4	97.4	98.8	99.0	92.8	92.8	98.2	86.3	89.3	94.8	99.2	76.7	86.9	99.2	89.9	100.0	86.5	71.2	98.2	69.4	50.0	70.0	99.0	76.9	7	JX416187
							100.0	100.0	81.6	813	86.4	83.1	89.4	89.6	89.6	89.7	90.0	90.1	87.9	90.0	81.0	85.2	89.3	90.1	79.7	82.2	90.0	99.2	90.1	81.7	74.3	90.0	73.8	50.4	71.7	88.6	77.6	8	NC_004071
								100.0	81.6	81.3	86.4	83.1	89.4	89.6	89.6	89.7	90.0	90.1	87.9	90.0	81.0	85.2	89.3	90.1	79.7	82.2	90.0	99.2	90.1	81.7	74.3	90.0	73.8	50.4	71.7	88.6	77.6	9	AY036009
									100.0	99.5	85.6	90.5	88.8	89.3	88.3	88.7	88.8	88.7	89.3	88.8	97.3	91.5	89.6	88.7	75.5	95.8	86.5	81.7	88.8	96.3	73.1	88.8	73.2	48.8	72.0	85.7	76.2	10	NC_038444
										100.0	85.2	90.6	88.9	89.1	88.0	88.4	88.5	88.4	89.1	88.5	97.0	91.4	89.3	88.4	75.2	95.6	86.1	81.5	88.5	96.1	73.3	88.5	72.9	48.7	71.0	85.3	76.1	11	AY036006
											100.0	87.0	86.5	87.0	86.5	86.5	86.7	86.9	85.7	86.7	84.6	85.3	87.1	86.9	77.1	84.4	85.1	86.4	86.5	84.2	73.5	86.7	74.1	49.9	72.6	84.1	76.7	12	MN381116
												100.0	86.0	86.4	85.7	85.8	86.1	86.0	86.2	86.1	89.7	87.1	86.9	86.0	76.1	90.5	83.3	83.1	86.0	90.5	73.4	86.1	72.9	49.6	71.3	82.0	75.8	13	AM701757
													100.0	992	97.9	97.9	98.2	98.1	93.3	98.6	88.4	90.9	95.9	98.1	77.0	89.2	96.7	89.4	97.9	88.9	73.8	98.6	73.7	49.7	71.4	95.2	76.6	14	MZ911857
														100.0	98.5	98.5	98.7	98.6	93.8	99.1	88.8	91.2	96.1	98.6	77.0	89.6	97.2	89.6	98.5	89.3	73.9	99.1	74.2	49.7	72.1	95.7	76.7	15	MZ911854
															100.0	97.9	97.9	98.1	93.3	99.4	87.8	90.6	95.1	98.1	76.7	88.7	96.3	89.6	97.9	88.2	73.6	99.4	73.4	49.2	71.7	94.8	76.1	16	MN328257
																100.0	99.1	99.2	93.2	98.6	88.3	90.7	95.2	99.2	77.0	88.9	97.7	89.8	99.1	88.7	73.6	98.6	73.7	49.6	71.6	96.2	76.3	17	KT099132
																	100.0	99.6	93.3	98.6	88.3	91.2	95.5	99.6	76.8	89.1	97.9	90.0	99.2	88.8	74.0	98.6	73.8	49.2	71.9	96.4	76.1	18	GU945265
																		100.0	93.2	98.7	88.2	90.9	95.4	99.7	76.8	88.9	98.1	90.1	99.4	88.7	73.6	98.7	73.4	49.4	71.9	96.5	76.2	19	MT316186
																			100.0	93.7	88.8	95.8	96.8	93.2	76.1	89.3	92.3	87.6	93.3	89.1	72.4	93.7	73.4	48.1	69.9	90.8	76.4	20	HG969199
																				100.0	88.3	91.0	95.8	98.7	77.3	89.3	97.2	90.0	98.6	88.8	73.8	100.0	73.9	49.6	71.9	95.7	76.7	21	FR751146
																					100.0	91.5	89.3	88.2	75.8	95.2	85.8	81.1	88.3	95.8	73.0	88.3	73.2	48.1	71.2	85.1	75.9	22	HG530540
																						100.0	94.5	90.9	76.6	91.0	88.9	85.3	90.7	90.7	73.7	91.0	73.7	47.9	70.8	88.3	76.7	23	KJ939446
																							100.0	94.5	77.3	90.0	94.0	89.3	95.5	89.7	73.3	95.8	75.1	49.4	70.8	92.6	77.6	24	KJ897529
																								100.0	77.1	88.8	98.1	90.1	99.4	88.7	73.9	98.7	73.7	49.5	72.2	96.5	76.4	25	MN027199
																									100.0	75.0	77.2	79.0	77.0	74.5	77.4	77.3	73.5	51.0	71.6	76.9	83.4	26	EF158044
																										100.0	86.5	82.4	89.1	99.5	72.6	89.3	73.0	48.7	71.4	85.7	76.2	27	FN554541
																											100.0	89.6	97.1	86.1	72.3	97.2	71.1	46.1	69.8	99.1	77.2	28	FJ030878
																												100.0	90.1	81.9	74.3	90.0	73.7	50.3	71.7	88.3	77.3	29	AF014881
																													100.0	88.8	73.9	98.6	73.5	49.5	71.9	96.4	76.4	30	MK947933
																														100.0	72.4	88.8	72.8	48.7	71.5	85.3	76.1	31	EJ469626
																															100.0	73.6	72.5	48.4	71.2	71.7	75.3	32	NC_005855
																																100.0	73.9	49.6	71.9	95.7	76.7	33	FR751145
																																	100.0	49.7	81.8	70.6	75.2	34	AY705380
																																		100.0	50.5	46.1	48.7	35	FJ712189
																																			100.0	70.8	71.7	36	GU112025
																																				100.0	76.9	37	FJ030879
																																					100.0	38	AY502936

*Virus acronym includes virus name followed by country/city, isolate, host, collection date, and GenBank accession number as detailed in Table 2. BLCMV= *Bean leaf curl Madagascar virus*, BYVINV= *Bhendi yellow vein India virus*, CLCUBV= *Cotton leaf curl Bangalore virus*, CLCuGeV=*Cotton leaf curl Gezira virus*, MaLCV=*Malvastrum leaf curl virus* OELCuV=*Okra enation leaf curl virus*, ToLCMV= *Tomato leaf curl Mali virus*, ToLCSDV=*Tomato leaf curl Sudan virus*, TYLCV-MLD=*Tomato yellow leaf curl virus-Mild*.

In addition, CLCuGeV-Bean (Egypt:OQ676568) shared Nt PSI <94% with the following virus isolates from hollyhock (Egypt:FJ030873, AF014881, NC_004071[88.2, 88.6, 88.8, respectively]), cotton (Sudan: NC_ 038444[86,1]), okra (Sudan: AY036006 [85.5]), okra (Cameron: MN372225[93.5]), and tomato (Oman: HG969199[91.4]), indicating that these CLCuGeV isolates are considered different strains from the Egypt strain of CLCuGeV according to Fauquet *et al.* (2008) and Shahmohammadi *et al.* (2023). Tahir *et al.* (2011) showed that the Sudan strain of CLCuGeV (AY036006) clustered separately from the Egypt strains of CLCuGeV from okra (AY036010) and hollyhock (AF014881). Furthermore, Idris and Brown (2002) indicated that CLCuGeV-SD (AY036006) had a history of CP recombination with other begomoviruses. Such results probably explain its lower PSI when compared with CLCuGeV-Bean (Egypt: OQ676568) per

se. Similarly, CLCuGeV-MG (=BLCMV) is a begomovirus with a history of recombinant CP (Lefeuvre *et al.*, 2007) and only PSI of N/AA identities of 82.9/90.5, respectively, with CLCuGeV-Bean (Egypt: OQ676568).

Table 4. Nucleotide and amino acid sequence identities of the coat protein (V1 gene) of CLCuGeV-EG:GZ:Bean (GenBank accession no. OQ676568) with other comparable strains of CLCuGeV

GenBank access. /Country*: host	% identities N** /AA***	GenBank access. /Country*: host	% identities N** /AA***
AY036010. EG:Okra	97.5 / 98.7	MT316186. JD:Okra	97.7 / 98.1
FJ030874. EG:Cotton (GB)	97.3 / 97.7	GU945265. JD:Hollyhock	97.5 / 98.7
AF014881. EG:Hollyhock	88.6 / 95.5	KT099132. IL:Whitefly	97.7 / 98.1
NC_004071. EG:Hollyhock	88.8 / 95.4	FR751145. PK:Cotton (GH)	97.1 / 97.5
FJ030873. EG:Hollyhock	88.2 / 95.5	FR751146. PK:Cotton (GH)	97.1 / 97.5
FJ030878. EG:Okra	97.5 / 98.7	MN328257.IR: Papaya	96.2 / 95.5
FJ030879. EG:Squash	97.3 / --NFCP	MZ911854.IR: Hollyhock	96.6 / 97.5
MK947932.EG: Pepper	97.5 / 98.7	MZ911857.IR: Okra	96.0 / 96.2
MK947933.EG: Melon	97.5 / 98.7	MN372225. CM:Okra	93.5 / 93.0
JX416187. EG:*Cucumis* sp.	97.5 / 98.6	HG969199.OM: Tomato	91.4 / 93.6
NC_038444. SD:Cotton (GB)	86.1 / 89.8	AM701757. MG:Bean****	82.9 / 90.5
AY036006. SD:Okra	85.5 / 89.2	MN027199.US: Okra	97.9 / 98.7

* CM=Cameron, Eg=Egypt, IR=Iran, IL= Israel, JD=Jordan, MG= Madagascar, OM=Oman, PK=Pakistan, SD=Sudan, US=USA.

**% nucleotide sequence identity of the coat protein (CP) gene measured with ClustalW-MegAlign software; DNASTAR (see Table 3).

***% Amino acid (AA) sequence identity was measured with NCBI blastp using the capsid protein id # of the AA sequences mentioned in Table 2.

**** BLCMV= CLCuGeV-MG = CLCuGeV Madagascar.

NFCPNon-functional coat protein due to a mutation.

A variation in some values of nucleotide PSI upon using SDTv1.2 and MegAlign software is understood. Brown et al. (2015) pointed out such variations when using different programs for PSI analysis for given begomoviruses. Results of BLASTP analysis for AA PSI of the different begomovirus-CPs, however, could represent an invaluable tool for solidifying nucleotide PSI results, as shown in the present study as well as by other investigators using PSI of AA sequences for the CP V1 gene (Idris and Brown, 2002; Bahder *et al.*, 2016; Villegas *et al.*, 2019).

As shown in Table 5, comparisons between six nucleotide PSI of CLCuGeV isolates using the capsid V1 gene, versus the PSI of their corresponding full-length DNA-A yielded minor differences ranged between 0.2 (R1) up to 0.6 % (R3). Both analyses were equal in PSI (R2). Such results validate the use of PSI of the CLCuGeV-capsid proteins should the full length of the DNA-A is not available.

Table 5. Comparisons between nucleotide PSI of three CLCuGeV isolates upon using full genome DNA-A and coat protein V1 gene.

CLCuGeV Accession Numbers*	PSI	
	DNA-A**	Capsid V1 gene***
AY036006 vs. FJ030878	86.3	86.1
GU945265 vs. MN027199	99.6	99.6
MK947933 vs. AY036010	99.4	100.0

*AY036006=CLCuGeV-SD:Okra, FJ030878=CLCuGeV-EG:Okra, GU945265=CLCuGeV-JD:Hollyhock, MN027199=CLCuGeV-US:Okra, MK947933=CLCuGeV-EG:Melon, AY036010=CLCuGeV-EG:Okra

** PSI values were extracted from the full-length DNA-A GenBank accession numbers using Blastn analysis software

***PSI for the capsid V1 gene of CLCuGevs were extracted from Table 3.

Phylogenetic analysis of the predicted AA sequence of the CP of CLCuGeV isolates was built upon using Clustal W to improve the sensitivity of multiple sequence alignment (Thompson et al., 1994). Results in Figure 4, revealed that the CP of the CLCuGeV-Bean (Egypt: OQ676568) was most similar to the okra isolate (USA: MN027199) and complies with the PSI results in Tables 3 and 4 in the sense of the presence of an inverse correlation between the values of genetic distances, between taxa, and PSI, as previously suggested by Abdel-Salam (2020). A general outlook at the evolutionary relationships of CLCuGeV taxa indicates that two major branches were circumventing CLCuGeV isolates. The first one included CLCuGeV from Tanzania (MN381116), whereas the second major branch engulfed two separate monophyletic CLCuGeV strains, or variants, from Egypt and Sudan. Such results may refer to the African Sahel region countries, including Tanzania, as the origin of CLCuGeV (Idris and Brown, 2002; Brown, 2017). The CLCuGeV-EG strains, or variants, from Egypt, Israel, Jordan, Iran, Pakistan, Oman, the UAE, and Cameron clustered together and separately from the CLCuGev-SD strains from Madagascar, Burkina Faso, Niger, Saudi Arabia, and Sudan. These latter results agree with the phylogenetic analysis by Tahir *et al.* (2011) and Shahmohammadi *et al.* (2023) that referred to the separate clustering between the CLCuGeV variants and strains from Egypt and Sudan.

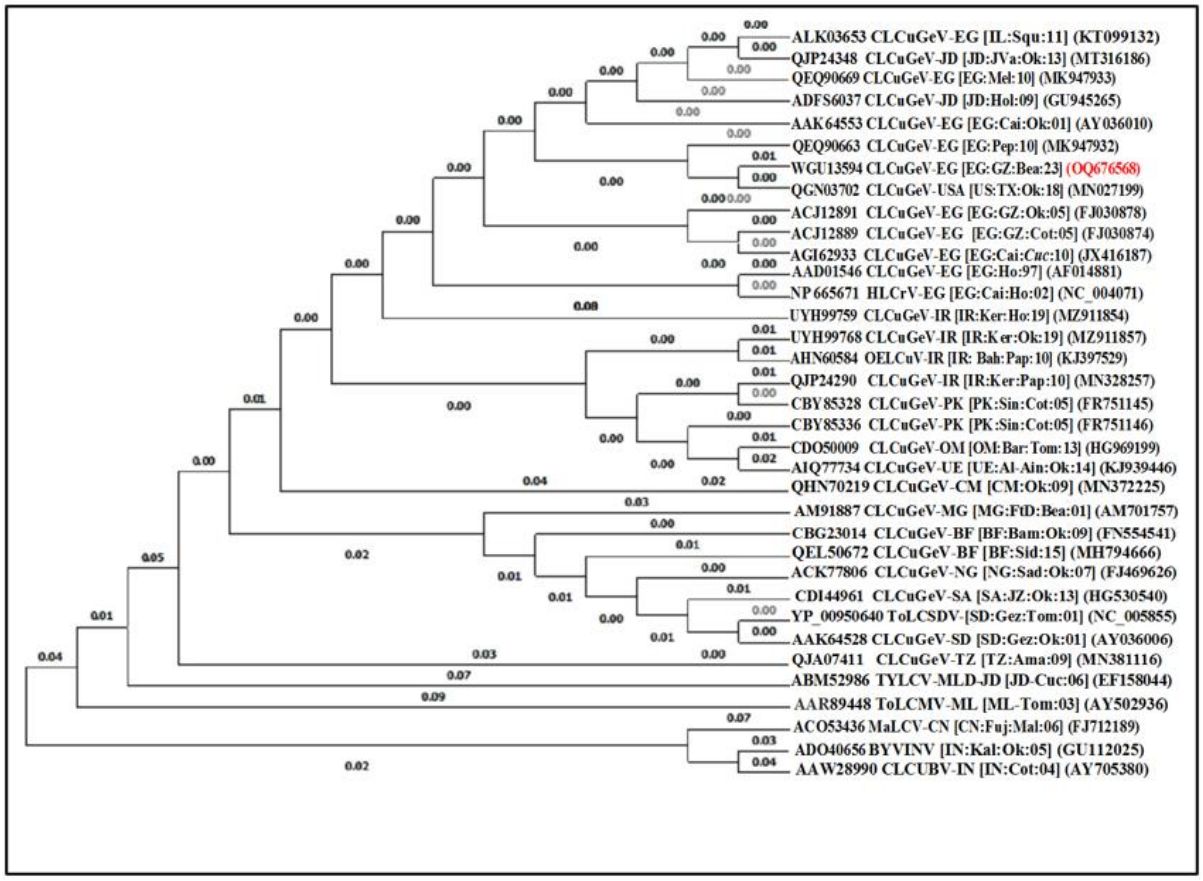

Fig. 4. The evolutionary history of CLCuGeV taxa (see Tables 2 & 3 for virus acronym) was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree is shown (next to the branches). The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and are in the units of the number of amino acid substitutions per site. This analysis involved 35 amino acid sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 258 positions in the final dataset. The evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021). GenBank accession number (OQ676568) for CLCuGeV-EG:Giza:Bea was marked with red color. Each sequence description was preceded with its CP id. number and followed by its GenBank access. no. TYLCV-MLD, ToLCMV-ML, MaLCV-CN, BYVINV, and CLCUBV-IN were used as outgroups.

Based on molecular identification, this is the first report of CLCuGeV naturally infecting *P. vulgaris* plants in Egypt. This expands the known host range of CLCuGeV in Egypt from malvaceous hosts (Abdel-Salam, 1999), solanaceous and cucurbitaceous hosts (Gambley *et al.*, 2020), to fabaceous hosts (the present study). With previous reports on the infection of bean plants with the bipatite begomovirus, viz., SLCV in Egypt (Abdel-Salam et al., 2006; Idris *et al.*, 2006; El-Dougdoug *et al.*, 2009), and the monopartite CLCuGeV (the present study), there is a great chance of increasing the diversity of these two whitefly-transmitted viruses through both genetic recombination and pseudo-recombination (Lefeuvre et al., 2007). Also, the possible association of betasatellite DNA of ClCuGeV with SLCV in mixed infection in bean plants may lead to new trigenomic relationships that modify virus virulence and fitness (Sivalingam *et al.*, 2012; Jyothsna *et al.*, 2013; Abdel-Salam *et al.*, 2017).

Molecular and statistical analysis methods based on the coat protein (V1) genes enabled comparisons between the

different isolates and strains of CLCuGeV especially in building phylogeny. This, in turn, narrowed the clustering of CLCuGeVs into the Sudanese and Egyptian groups detected worldwide.

Control of *B. tabaci* whitefly as the major vector responsible for the spread of CLCuGeV worldwide (Golding, 1930; Brown, 2017) must be followed by stringent quarantine rules for importing and exporting ornamental stem cuttings from countries where CLCuGeV was reported. For example, CLCuGeV can infect ornamentals such as hollyhock (Abdel-Salam *et al.*, 1998), lavatera (Annonymous 2022 a, b), and amaranth (GenBank access. # MN381116) with unnoticeable virus symptoms and may act as vehicles for spreading CLCuGeV worldwide.

Declarations:

Ethical Approval: Not applicable.

Author contributions: Dr. Aly M. Abdel-Salam (first author): Contributed to virus-filed isolation, serologic identification (through IC-PCR), DNA submission to GenBank, DNA molecular and phylogenetic analysis, and writing and reviewing the manuscript. Dr. Doaa Z. Soliman (coauthor): Contributed to PCR studies and DNA purification of the coat protein gene of the virus understudy.

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