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Molecular characterization of cucumber mosaic virus isolates infecting Sugar beet (*Beta vulgaris*) and other crops in three governorates of Egypt

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Sugar beet (*Beta vulgaris*) is one of the principal sources for sugar production in Egypt. Viral infections could seriously impact its cultivation and development. This study was performed to investigate genetic diversity of cucumber mosaic virus (CMV) isolates infecting sugar beet and other crops growing nearby such as pepper, cowpea, common bean and sweet potato. A total number of nine CMV isolates were characterized at the molecular level, according to coat protein gene sequence analysis (6 isolates from sugar beet and other three isolates from other crops). Four isolates showed highest identity to CMV subgroup IA. The other five isolates showed the highest levels of nucleotide identity to CMV group II. While group IA and 1B were known to occur in Egypt, to our knowledge, this is the first report of CMV group II from sugar beet and sweet potato in Egypt which indicate recent introduction of new CMV genotypes.

Keywords: Sugar beet, *Cucumoviruses*, coat protein, genetic diversity and Subgroup I and II

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INTRODUCTION

Sugar beet (*Beta vulgaris L.*) is an essential crop belonging to the *Chenopodiaceae* family (Banager et al. 2022, Yashwant 2015). It is the second most significant crop for sugar production worldwide, reaching up to 266.8 million tons. It is an economically important crop that provides nearly one third of the global sugar production. (Ghaemi et al. 2020). It provides 42 million tons of global white sugar need. Sugar beet farmland has grown from 57,000 hectares in 2000 to 237,000 hectares in 2017 (FAOSTAT 2020). A major problem associated with sugar beet is the susceptibility to at least sixteen different viruses including Cucumber Mosaic Virus (CMV) (Mokbel et al. 2020), Alfalfa Mosaic Virus (AMV), (kamel et al. 2023), Beet Curly Top Virus (BCTV), (Yildrim et al. 2022) Beet Necrosis Yellow Vein Virus (BNYVV), Beet Soil Born Mosaic Virus (BSBMV), (Wetzel et al. 2021, Fernado et al. 2020,), Beet Mild Yellow Virus (BMYV), Beet Mosaic Virus (BtMV), Beet yellow virus (BYV), Beet Chlorosis Virus (BChV), (Hossain et al. 2021), Tomato Black Ring Virus (TBRV) (Hassan et al. 2011), Beet Necrosis Ring Spot Virus (BNRSV) , Beet Severe Curly Top Virus (BSCTV) (Mabrouk et al. 2019), Beet Western Yellow Virus (BWYV) (Yoshida and Tamada 2019), Tomato Yellow Leaf Curl Virus (TYLCV) (Mabrouk et al. 2019), Beet Virus Q (BVQ) (Moradi and Mehrvar 2021), Tomato Bushy Stunt Virus (TBSV) (Mayo et al. 2005, Novak and Lanzova 1982), and seven of these have been recorded in Egypt that include AMV, CMV, BNYVV, BCTV, BYV, BNRSV, BtMV (Kamel et al. 2023, Mokbel et al. 2020, Sheshata et al. 2023, Mabrouk et al. 2019, Megahed et al. 2015, El Helaly et al. 2021, El Gaied et al. 2019, Sheshata et al. 2023).

CMV belongs to the *Bromoviridae* family and the genus *Cucumovirus* (Mrkvova et al. 2022, Sastry et al. 2019). CMV is considered as one of the most important and destructive pathogens of vegetables and fruits, reported all over the world (Yoon et al. 2019, Ayo-John 2014). CMV is easily transmitted through mechanical inoculation of plant sap and is also transmitted in a non-persistent, and non-circulative manner by approximately 80 species of aphids in 33 genera. The symptoms attributed to a suspected CMV infection on pepper were filiform leaves, stunting, severe mosaic and yellowing (Deloko et al. 2022). Naturally occurring sugar beet leaves with CMV infection showed signs of mild mosaic (Mokbel et al. 2020). CMV was isolated from naturally infected Cowpea plants showing different symptoms of mosaic; mottle, dwarfing, and vein clearing (Hamdy and Aly, 2019). According to Aziz et al. (2014), common bean displayed a range of symptoms, from severe deformity and necrosis to mosaic. Necrosis and mosaic are also reported as two signs of CMV infection in sweet potato plants (Opiyo et al. 2010). CMV has a relatively wide host range that includes both wild and cultivated plants, containing several dicotyledons and a few monocotyledons. High viral population diversity and the virus genome tolerance for mutations probably explain this extraordinary adaptability to various host species (Yoon et al. 2021, Mauck et al. 2015,). The type and severity of symptoms can vary greatly depending on the host species, specific combination of plant host and virus

genotype, phenological stage of infection, environmental conditions, and the presence of other biotic factors such as satRNAs and other viruses (Hirsch and Moury 2020). Based on the genomic sequence similarity and serological relationships, CMV isolates are categorized into two major groups, group I and II. Group I can be further separated into subgroups IA and IB (Mrkvova et al. 2022, Yousef et al. 2022, Jacquemond 2012). group I CMV strains produce a mild green mosaic phenotype, whereas group II CMV strains cause a severe chlorotic phenotype (Li et al. 2020, Mochizuki and Ohki 2012). Not much is known about the CMV infecting sugar beet crops in Egypt so, the overall objective of the present study was undertaken to determine the molecular characteristics of selected CMV isolates from sugar beet and other nearby crops in three governorates of Egypt.

MATERIALS AND METHODS

Field survey

Surveys and collection of samples from three governorates of Egypt namely El- Beheira, Kafr El-Sheikh and Alexandria were performed during the winter season (December and January) of 2019 and 2020. Leaf samples from 60 sugar beet plants were collected. In addition, samples were collected from other crops growing nearby that included 18 peppers (*Capsicum annuum*), 14 cowpea (*Vigna unguiculata*), 12 common bean (*Phaseolus vulgaris*) and 13 sweet potato (*Ipomoea batatas*). All the collected samples showed various virus-like symptoms such as mosaic, chlorosis and deformation of leaves and necrosis (Figure 1).

RT-PCR for the detection of BCTV, BNYVV, BWYV, BMYV and BtMV

Total RNA was extracted using Gene JET Plant RNA Purification Mini Kit (Thermo Scientific). First strand cDNA was generated using a Thermo Scientific™ Revert Aid ™ first strand cDNA kit (Thermo Scientific, USA). Primer pairs and thermal conditions that were specific to coat protein gene (CP) are given (Supplementary Table S1).

RT-PCR for the detection of CMV

Total RNA was extracted using Gene JET Plant RNA Purification Mini Kit (Thermo Scientific). First strand cDNA was generated using a Thermo Scientific™ Revert Aid ™ first strand cDNA kit (Thermo Scientific, USA). Primer pair that was specific to CP gene CMV F (5` GCG CGA AAC AAG CTT CTT ATC 3`) and CMV R (5` GTA GAC ATC TGT GAC GCG A 3`) were used to amplify a product of 540 bp (De Blass 1994).

DNA sequencing and phylogenetic analyses

Amplicons were sequenced and submitted to the GenBank database. The phylogenetic tree was generated by CLUSTAL W using MEGA version 11. (Tamura et al., 2021) by using the maximum likelihood method for nucleotide and amino acid sequence. Reference sequences retrieved from GenBank database are included as representatives of CMV (Supplementary Table S2).

Molecular characterization of CMV EM173 isolate by Two Step RT-PCR of the movement protein gene (MP), 2a and 2b genes and the Non-Translated Region (NTR)

Viral cDNAs (2.5 µl) were amplified by PCR (25 µl final volume) in a reaction mixture of 12.5 µl of Dream Taq green PCR Master mix (2x), 0.5 µl (20 pmol) each primer (Lin et al., 2004), and 9 µl water nuclease free were added to the mixture. The sequences of the primers and PCR cycle parameters as described are in (Supplementary Table S4). Sequence analysis was performed as described above. Reference sequences retrieved from GenBank database are included as representatives (Supplementary Tables; S3- S7).

RESULTS

Detection of CP gene of BNYVV, BCTV, BWYV, BMYV and BtMV by PCR

None of the 60 samples tested were positive for the above viruses

Detection of CMV CP gene by RT-PCR and phylogenetic analysis

CMV was detected in 87% of the collected symptomatic samples (Supplementary Figure S1). Isolates were sequenced and their GenBank accession numbers are included in Table 1. Nucleotide and amino acid sequence comparisons indicated variability in CMV population. Four Egyptian isolates (EM166, EM31, EM29, ENM), grouped together in the same clade showed a higher degree of relatedness to the four isolates (MY from Japan, NY from Australia, Behera-EG from Egypt and MF from South Korea), (Figure 2), and showed close relatedness to known CMV subgroup IA isolates (Figure 2A,B). Two of those isolates (EM31, EM29) infected sugar beet, whereas the other two (EM166, ENM) were from Cowpea growing in a nearby field. Of the five isolates, EM52, EM27, EM53, EM49 and EM170, four of them infected sugar beet and EM170 was isolated from sweet potato. They all were grouped together in the same

Table 1. Local Egyptian isolates of Cucumber Mosaic Virus and their NCBI accession numbers.

Isolate	Gene	Accession number	Location	Host
EM166	CP	OL310177	Alexandria	*Vigna unguiculata*
EM170	CP	OL343772	Alexandria	*Ipomoea batatas*
EM31	CP	MW023062	Kafr El-Sheikh	*Beta vulgaris*
EM49	CP	MW023066	kafr El-Sheikh	*Beta vulgaris*
EM29	CP	MW023063	Kafr El-Sheikh	*Beta vulgaris*
EM27	CP	MW023064	kafr El-Sheikh	*Beta vulgaris*
ENM	CP	MW023065	El Beheira	*Vigna unguiculata*
EM53	CP	MW602806	kafr El-Sheikh	*Beta vulgaris*
EM52	CP	MW602805	kafr El-Sheikh	*Beta vulgaris*
EM52	MP	OL343767	Kafr El- Sheikh	*Beta vulgaris*
EM170	MP	OL343768	Alexandria	*Ipomoea batatas*
EM53	MP	MW674918	kafr El-Sheikh	*Beta vulgaris*
EM173	MP	MW674919	El Beheria	*Vigna unguiculata*
EM173	NTR	MW674920	El Beheria	*Vigna unguiculata*
EM173	2a	MW674921	El Beheria	*Vigna unguiculata*
EM173	2b	MW674922	El Beheria	*Vigna unguiculata*

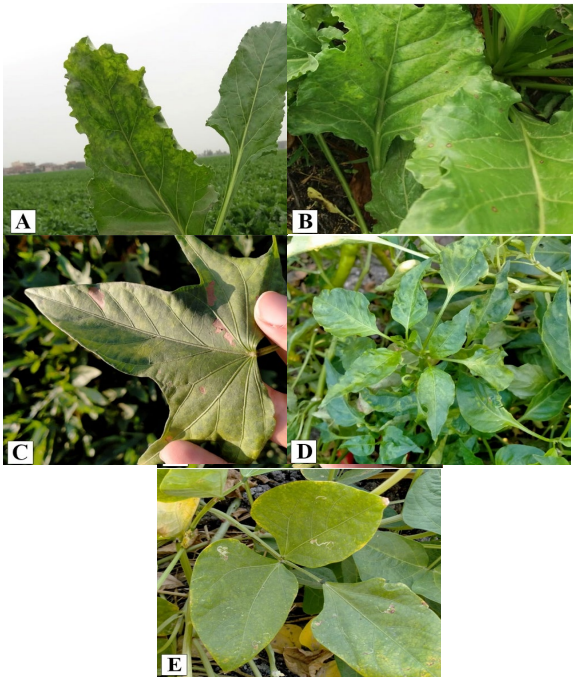

Figure 1. Symptoms of virus infection in different hosts. A& B: *B. vulgaris*, C: *I. batatas, D: C. annuum* and E: *p. vulgaris* respectively observed in different governorates in Egypt. A. Mosaic, B. Mosaic and deformation, C. Mosaic and necrosis, D& E. Mosaic.

clade and showed a higher degree of relatedness to isolates Q and LY from Australia, and S from South Africa. All belonged to CMV group II.

Phylogenetic analysis of CMV MP gene, NTR, 2a and 2b genes

The nucleotide sequence of the MP gene of EM173 isolate (MW674919) grouped in the same clade and showed a higher degree of relatedness to three isolates (Ri-8, PV0187 and Fny) from Spain, Germany and USA belonging to subgroup IA and all had the highest levels of nucleotide identity with CMV subgroup IA isolates. Three Egyptian isolates EM53, EM170, EM52 (Table1) grouped together in the same clade and showed a higher degree of relatedness to two isolates, LS and Trk7 from the USA and Hungary, respectively, and grouped within CMV group II (Figure 3).

The nucleotide sequence of the NTR of EM173 Egyptian isolate and sequences of 18 selected isolates available in GenBank revealed that EM173 (MW674920) grouped in the same clade and showed a higher degree of relatedness to three isolates Ri-8, O and Fny from Spain, Japan and USA, respectively and formed a cluster in CMV subgroup IA isolates (Fig. 4). Nucleotide sequence comparisons based on the 2a and 2b gene with those of other isolates revealed that EM173 isolate (MW674921, MW674922) clustered in the same clade with isolates from Spain in subgroups IA (Figures 5,6).

DISCUSSION

CMV is considered among the most destructive viruses affecting the sustainability of crops in countries of the Mediterranean basin (Radouane et al. 2021, Jacquemond 2012). During the survey of sugar beet and several other overlapping crops, CMV was initially detected using a specific primer pair (CMV F/R) for the CP. Following the identification of CMV, nine CMV isolates were characterized from sugar beet and other plants growing nearby. Phylogenetic assessment using both nucleotide and amino acid sequences of the CP gene showed that

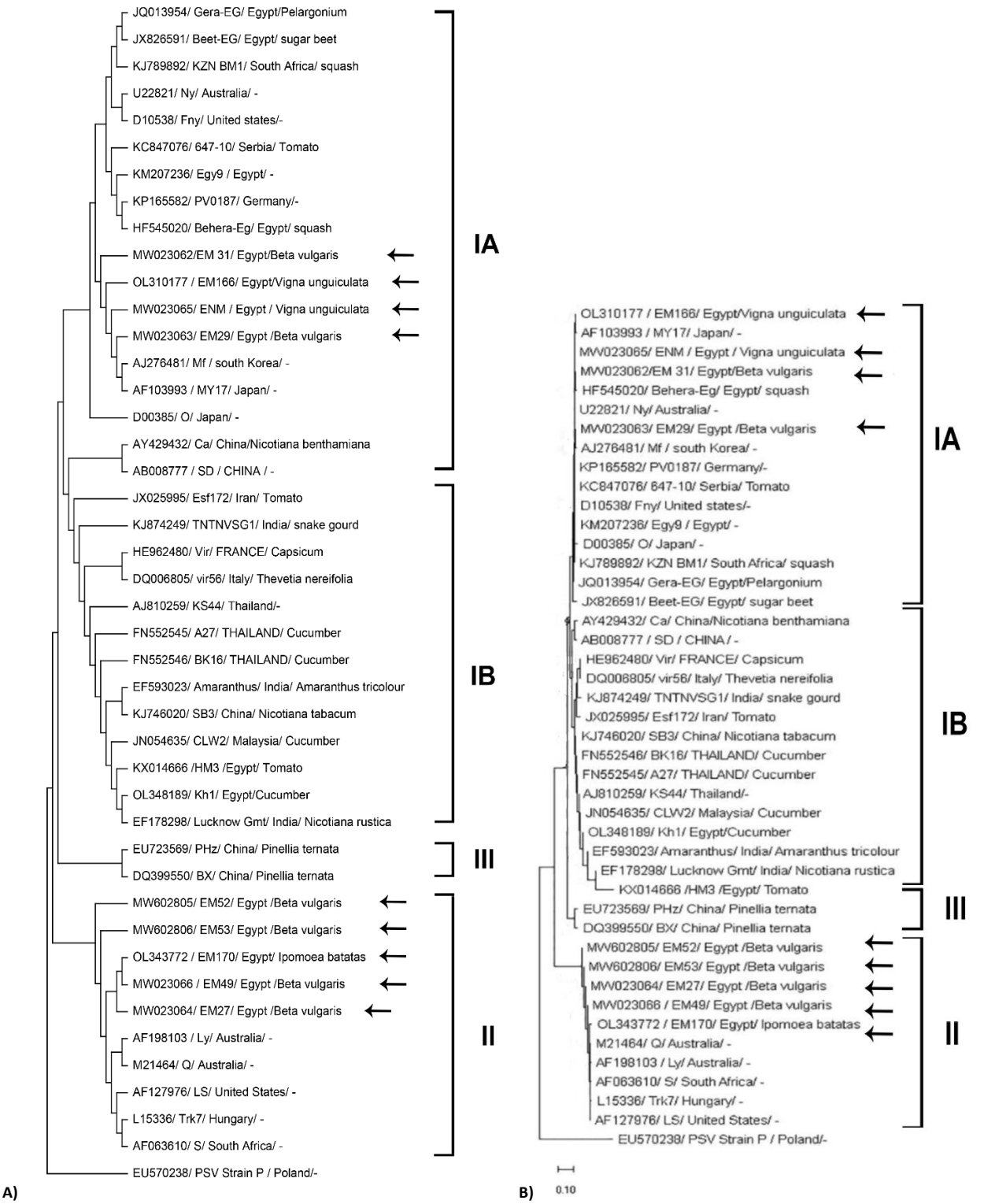

Figure 2. Maximum-likelihood phylogenetic trees of *Cucumber mosaic virus* based on (A) the nucleotide sequences of the coat protein (CP) gene (B) amino acid sequences of CP gene of the Egyptian CMV isolates (black arrows) compared to those of previously reported CMV isolates. The name of the isolate, host and the geographic origin (if available) are indicated. Roman numerals indicate respective CMV subgroups.

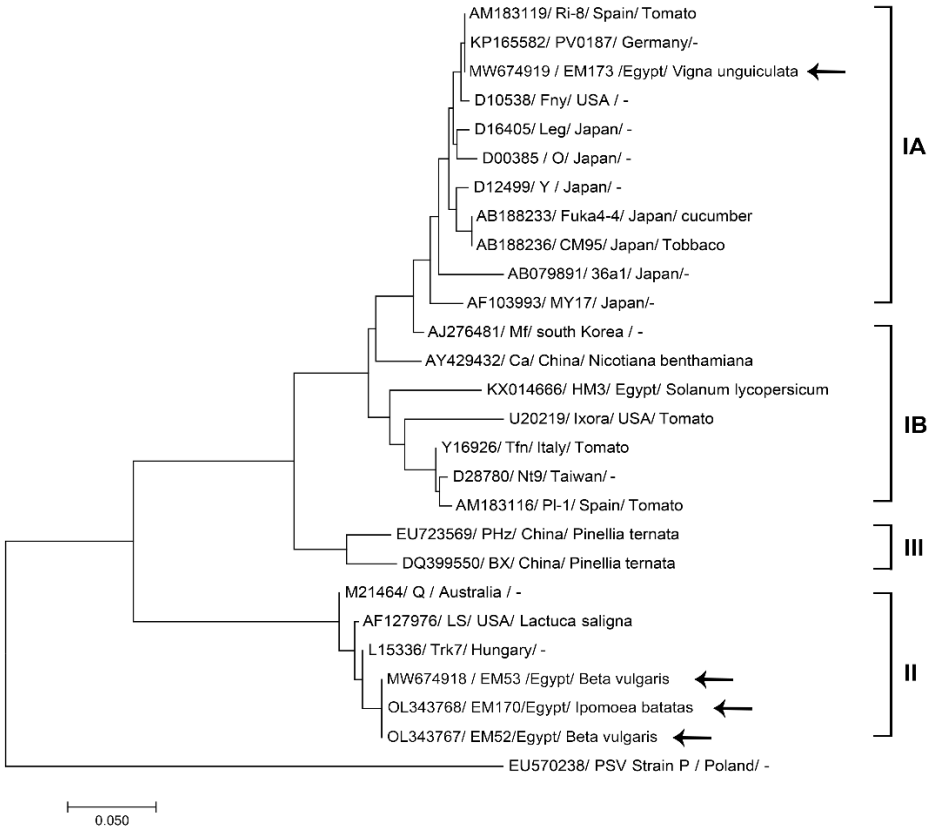

Figure 3. Maximum-likelihood phylogenetic trees of CMV based on the nucleotide sequences of the MP gene (black arrows) compared to some other previously reported CMV isolates. The name of the isolate, host and the geographic origin (if available) are indicated. Roman numerals indicate respective CMV subgroups.

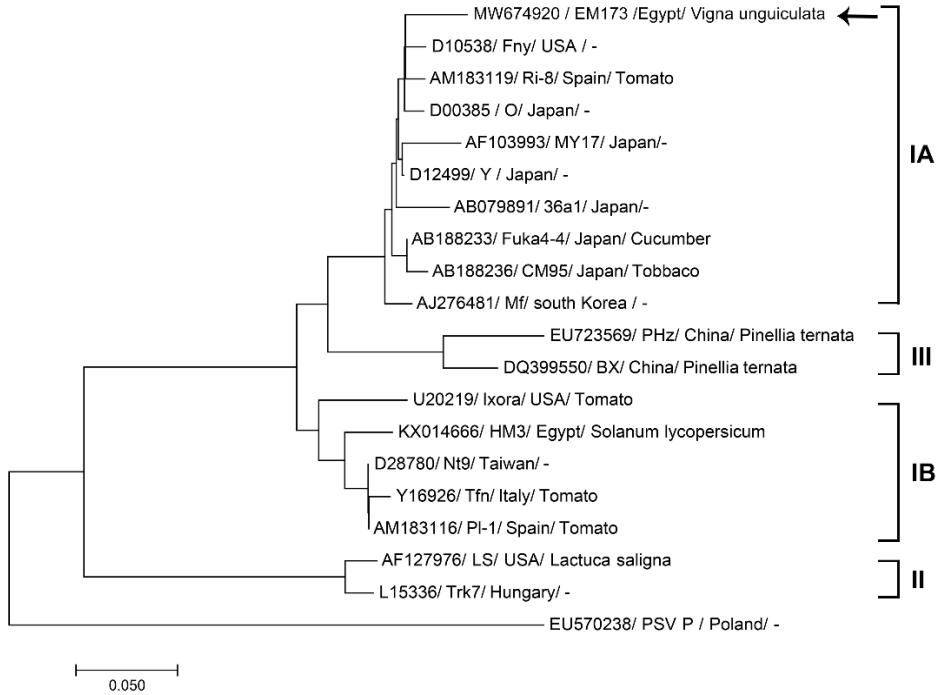

Figure 4. Maximum-likelihood phylogenetic trees of CMV based on the nucleotide sequences of the untranslated region (NTR) (black arrows) compared to some other previously reported CMV isolates. The name of the isolate, host and the geographic origin (if available) are indicated. Roman numerals indicate respective CMV subgroups.

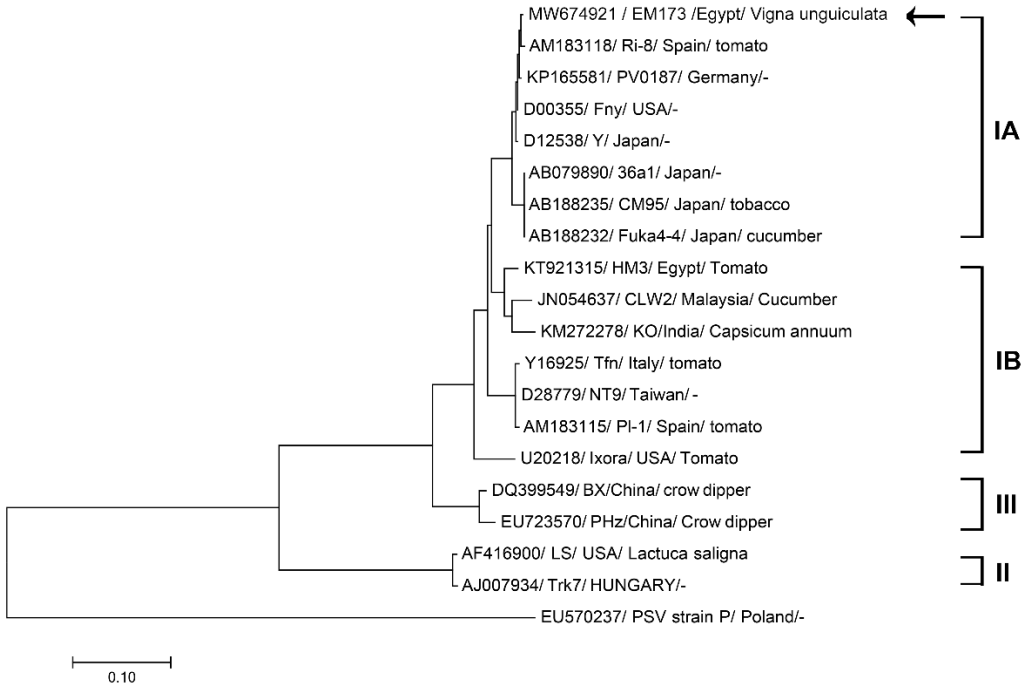

Figure 5. Maximum-likelihood phylogenetic trees of CMV based on the nucleotide sequences of the 2a gene (black arrows) compared to some other previously reported CMV isolates. The name of the isolate, host and the geographic origin (if available) are indicated. Roman numerals indicate respective CMV subgroups.

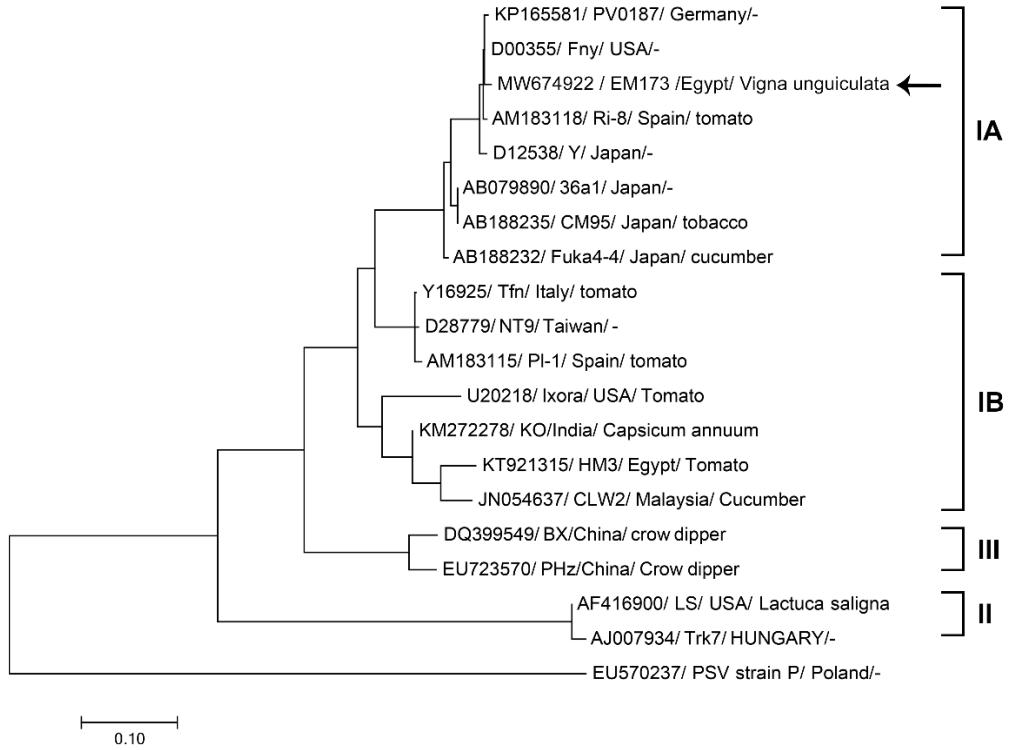

Figure 6. Maximum-likelihood phylogenetic trees of CMV based on the nucleotide sequences of the 2b gene (black arrows) compared to some other previously reported CMV isolates. The name of the isolate, host and the geographic origin (if available) are indicated. Roman numerals indicate respective CMV subgroups.

four Egyptian isolates (EM166, EM31, EM29, ENM) showed high levels of sequence identity to CMV subgroup IA isolates. While the remaining five Egyptian isolates (EM52, EM27, EM53, EM49, EM170) grouped with CMV group II. Isolates of subgroup I often prevail in tropical and subtropical zones, while subgroup II dominates in temperate regions (Singhal et al. 2023, Hord et al. 2001). In previous studies in Egypt, subgroup IA was reported to be more dominant than subgroup IB and group II (Wagih et al. 2021, Rabie et al. 2017, Megahed et al. 2014). In addition, the overall greater similarity at the amino acid level among all CMV isolates may indicate the restrictions on the virus' ability to move within the host (Guiu 2015). Usually, members of Subgroup I induce more severe symptoms in the field than subgroup II and thus they are easily visually recognized (Parrella and Sorrentino 2009). Results indicate that CMV isolates of subgroup II are present in Egypt, denoting genetic variation among CMV isolates in different geographic areas. Besides sugar beet, CMV was detected in, cowpea and sweet potato in both genotypes belonging to subgroups I and II. Such variability imposes a big threat to field crops as more virulent genotypes may arise and spread among different host species. Growing non-hosts of CMV in the vicinity could lead to reduced incidence of the virus. The phylogenetic analysis based on the nucleotide sequence of the MP gene showed that EM173 belongs to CMV subgroup IA. However, the three other Egyptian isolates (EM53, EM170, EM52) belong to CMV group II. Subgroup IA has a worldwide distribution as reported before (Vinodhini et al. 2020, Garcia 2009, Hong 2007,), while subgroup IB strains were previously reported (Ali et al. 2022, Esfraq et al. 2021, Ahsan et al. 2020, Rabie et al. 2017, Jacquemond 2012, Aramburu 2007). Phylogenetic analysis based on the nucleotide sequence of the NTR, 2a and 2b genes showed that isolate EM173 belongs to subgroup 1A.

Evidence of the occurrence of CMV genotypes belonging to Group II could be due to recombination and an alternative cause may be due to introduction of new genotypes from other geographic regions through exchange of plant materials. Changes in genetic composition of a virus population in addition to new phenotypes that can arise because of genetic exchanges, can compromise effectiveness of disease control strategies (Ehsan et al. 2023, Paul et al. 2022, Wageh et al. 2022, Ahsan et al. 2020, Acosta 2011, Chen 2007). Therefore, a better understanding of genetic structure and selective forces driving CMV evolution will be useful for developing disease management strategies. This supports the imperative need to develop a strategy to improve the use of virus-tested sugar beet plants for national and international exchange. In view of detection of viruses in new areas and incidence of mixed strain infection along with the genetic diversity of the virus species, rigorous implementation of reduction of the incidence of viral infections would prevent new disease outbreaks in sugar beet-growing regions under diverse environmental conditions.

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DATA AVAILABILITY STATEMENT

Sequence data generated during the current study are available as nucleotide sequences in the NCBI GenBank.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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