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Molecular Characterization of the Cucumber Mosaic Virus and Cucumber Green Mottle Mosaic Virus Infecting *Allium cepa* in China

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Abstract

Onion (*Allium cepa*) belongs to the genus *Allium* in the family Liliaceae and is widely cultivated worldwide for its nutritional and medicinal value. However, in Hohhot, Inner Mongolia, China, many onion plants exhibited severe virus-like symptoms, including yellow stripes and leaf distortion. Symptomatic plants were collected, and virus identification was conducted through mechanical inoculation of *Nicotiana benthamiana* and transmission electron microscopy. Two types of virus particles, rod-shaped and spherical, were observed. Mixed infection of Cucumber mosaic virus (CMV) and Cucumber green mottle mosaic virus (CGMMV) was confirmed by high-throughput sequencing and RT-PCR. The detection rates of CMV and CGMMV in the samples were 8/161 and 1/161, respectively. Recombination analysis indicated that no recombination events were detected in the CGMMV, whereas one recombination event was identified in CMV, occurring on RNA1 from nt 59 to 171. The major parent was CMV DSMZ PV-1255 (ON013910) in Greece, and the minor parent was CMV Am (JX993909) in China. This study reports, for the first time, the complete genome sequences of CMV infecting onions in China and CGMMV infecting onions worldwide.

Keywords: cucumber mosaic virus; cucumber green mottle mosaic virus; phylogenetic analysis; recombination analysis; transmission electron microscopy

1. Introduction

Onion (*Allium cepa*), a member of the genus *Allium* in the family Liliaceae, is also commonly known as round onion and jade onion. Due to its rich nutritional content and significant medicinal value, it is often referred to as the "Queen of Vegetables". Possessing significant commercial value as the world's third most essential horticultural spice, the onion bulb is valued for its characteristic flavor [1]. Onions have a long history of cultivation, and currently onions are grown in more than 140 countries around the world. According to FAO data from the past years, China's onion (together with shallot) cultivation area and annual output are substantial (https://www.fao.org/faostat, accessed on 31 March 2026). For example, in 2023, the planting area reached 2,205,032 hectares, with a production of 48,600,783 tons; in 2024, the corresponding figures were 2,203,812 hectares and 48,568,150 tons, respectively. As a major vegetable crop in the Inner Mongolia Autonomous Region, onion production is being increasingly threatened by viral pathogens due to the expansion of large-scale planting.

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According to the comprehensive encyclopedia of plant viruses and viroids [2], at least 17 viral pathogens have been documented to naturally infect onion (*Allium cepa*). These include: Allium cepa amalgavirus 1 (AcAV1), Allium cepa amalgavirus 2 (AcAV2), *Artichoke yellow ringspot virus* (AYRSV), *Garlic common latent virus* (GarCLV), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), *Groundnut bud necrosis orthotospovirus* (GBNV), *Iris yellow spot orthotospovirus* (IYSV), *Leek yellow stripe virus* (LYSV), *Onion yellow dwarf virus* (OYDV), *Shallot latent virus* (SLV), *Shallot virus X* (ShVX), *Sint-Jan onion latent virus* (SJOLV), *Tobacco rattle virus* (TRV), *Tobacco streak virus* (TSV), *Tomato black ring virus* (TBRV), and *Tomato spotted wilt orthotospovirus* (TSWV). In addition, other viruses such as *Cucumber mosaic virus* (CMV), *Impatiens necrotic spot virus* (INSV), and *Shallot yellow stripe virus* (SYSV) have also been reported to infect onions in other studies.

Cucumber mosaic virus (CMV) belongs to the genus *Cucumovirus* of the family *Bromoviridae*. It is an important plant virus with a wide host range, infecting at least 100 plant families and 1200 plant species, both monocotyledonous and dicotyledonous [3,4]. Global distribution patterns have confirmed CMV presence across diverse geographical regions, including Asia (Japan, China, Iran) [5–7], North America (the USA), Europe (Italy, Hungary, Belarus, Bulgaria) [8–11], and Oceania (New Zealand). CMV is an icosahedral virus particle, approximately 28–30 nm in diameter, and has a segmented genome consisting of three single-stranded positive-sense RNA molecules of RNA1, RNA2, and RNA3, totaling approximately 8700 nucleotides (nt) and encoding five open reading frames (ORFs) [12]. RNA1 encodes protein 1a, RNA2 encodes proteins 2a and 2b, RNA3 encodes movement protein (MP) and coat protein (CP); the 1a and 2a are involved in genome replication, 2b is a viral suppressor of RNA silencing. CMV can cause a wide range of specific symptoms on host plants, such as leaf mosaic, yellowing, systemic necrosis, blight, ring spot, and dwarfing [4,13]. It is transmitted non-persistently by more than 80 species of aphids, seeds, and mechanical inoculation [14].

Cucumber green mottle mosaic virus (CGMMV) belongs to the genus *Tobamovirus* of the family *Virgaviridae*, and is one of the major viruses infecting cucurbit crops [15–21]. CGMMV was discovered in 1935 [22]. Its global spread progressed slowly for the next half-century but accelerated markedly from 2009 onwards [23]. It is widely distributed in 43 countries, including India [24], the Netherlands [25], Japan [26], Korea [27], China [28], Canada [29], the USA [30], and Australia [31]. It was reported in China including Liaoning, Guangxi, Gansu, and Shanghai. CGMMV has a single-stranded positive-sense RNA genome with about 6400 nt, containing four ORFs. The ORF1 encodes a 129 kDa protein, the ORF2 encodes a 186 kDa protein, the ORF3 encodes a 29 kDa protein of movement protein, and the ORF4 encodes a 17.3 kDa protein of coat protein. CGMMV can be transmitted by seed, soil, and mechanical inoculation, with symptoms of mosaic, green, and mottling appearing in the leaves of susceptible cucurbit plants. The leaves of susceptible melon plants show symptoms such as leaf blossom, greening, and mottling, and in severe cases, it causes twisted and deformed fruits, which reduces the yield and quality of melons.

This work described the discovery and genome characteristics of CMV and CGMMV infecting onion in Inner Mongolia, especially expanding the host plant range of the CGMMV.

2. Materials and Methods

2.1. Sample Collection

From year 2021 to 2023, a total of 161 plant samples of diseased onion were collected from the major onion-producing areas of Inner Mongolia, mainly distributed in the cities of Hohhot, Baotou, and Ordos. The plants showed typical symptoms of viral diseases, like leaf deformity, necrosis, and yellowing (Figure 1). Fresh whole plants were collected and

transported to the laboratory. The leaves were separated, frozen in liquid nitrogen, and stored at −80 °C.

Figure 1. A sample of diseased onions. The plant showed symptoms of leaf deformity, necrosis, and yellowing.

2.2. High-Throughput Sequencing of Onion Leaves

A total of 161 symptomatic onion leaf samples were collected. A small portion of each sample was pooled to obtain a composite sample, which was sent to Biomarker Technologies (Qingdao, China) for high-throughput sequencing using the Illumina NovaSeq 6000 platform (Illumina, Inc., San Diego, CA, USA). Virus-related reads were assembled into contigs using SPAdes genome assembly software (v3.15.3, accessed at https://github.com/ablab/spades, accessed on 12 November 2023), and the taxonomic status were determined through matching with the NCBI virus database (https://www.ncbi.nlm.nih.gov, accessed on 12 November 2023) by nucleotide sequence alignment (BLASTn) and translated protein sequence alignment (BLASTx) methods, respectively.

2.3. RT-PCR Validation and RACE Amplification of Viruses

Total RNA was extracted from the same pooled sample using a MiniBEST plant RNA extraction kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. cDNA was synthesized using an M5 Super plus qPCR RT kit (Mei5 Biotechnology, Beijing) following the manufacturer's protocol. The presence of three viruses (SYSV, CMV, CGMMV) identified by HTS was validated by RT-PCR using specific primers.

For SYSV, a 604 bp fragment (positions 9319–9923 nt relative to reference NC_007433) was amplified using primers SYSV-F (5′-CACCNTAYATAGCRGARACAGCTCT-3′) and SYSV-R (5′-ACTGAAATGGCGCATTATYTGYCTA-3′) with an annealing temperature of 64 °C. For CMV, a 650 bp fragment covering the 5′UTR and partial 2a gene was amplified using primers CMV-2a-F (5′-GTTTATTTACAAGAGCGTACGG-3′) and CMV-2a-R (5′-GGTTCGAARRWATAACCGGG-3′) [32] at an annealing temperature of 60 °C. For CGMMV, a 180 bp fragment of the coat protein gene was amplified using primers CGMMV-CP-F (5′-CAATCCCACGACTGCTGAGT-3′) and CGMMV-CP-R (5′-AAGCTTTCGAGGTGGTAGCC-3′) at an annealing temperature of 60 °C.

In this work, primers were designed for the amplification of CMV and CGMMV genome fragments based on the assembled genome sequences from high-throughput data, as well as primers for RACE amplification (Table 1). PCR was performed using the SuperSpeed Mix kit (MF848, Mei5 Biotechnology, Beijing, China) according to the manufacturer's instructions. RACE amplifications were conducted using a SMARTer RACE 5′/3′ kit (Takara Bio, Dalian), all steps followed the protocol provided by the manufacturer.

Table 1. Primers used for amplification of the complete genome of CMV and CGMMV.

Locations	Primers	Sequences 5′-3′	Tm (°C)	Fragment Size (bp)	Purpose
CMV RNA1	CMV-RNA1-F	AATTCCTATGGCGATGTCCTCAT	66	3300	RT-PCR
CMV RNA1	CMV-RNA1-R	GGTCTCCTTATGGAGAACCTGTG	70		RT-PCR
CMV RNA1	CMV-RNA1-5′R	TCAGCTGCAGACTTAGTGGA	60	500	5′-RACE
CMV RNA1	CMV-RNA1-3′F	ACTGCTTAGTTGCAGTTACT	56	410	3′-RACE
CMV RNA2	CMV-RNA2-F	TTATTCTCAAGAGCGTATGGTTC	64	3000	RT-PCR
CMV RNA2	CMV-RNA2-R	GGTCTCCTTATGGAGAACCTGTG	70		RT-PCR
CMV RNA2	CMV-RNA2-5′R	TTCAACGTTCGGGTGGATGT	60	521	5′-RACE
CMV RNA2	CMV-RNA2-3′F	CGTCGAAGACGAAGGTCTCG	64	560	3′-RACE
CMV RNA3	CMV-RNA3-F	CATCAGTCCACGCTGTGTGTGTG	72	2100	RT-PCR
CMV RNA3	CMV-RNA3-R	GGTCTCCTTATGGAGAACCTGTG	70		RT-PCR
CGMMV RdRp	CGMMV-1-F	AATCAACAACCAACGTGACGCCG	70	3720	RT-PCR
CGMMV RdRp	CGMMV-1-R	GCTACAAGATTCTCAAGAAGACC	66		RT-PCR
CGMMV RdRp	CGMMV-2-F	AAGCAGTCCTTGATCAAGAGTGG	68	3100	RT-PCR
CGMMV 3′UTR	CGMMV-2-R	AACCGTTCGATTTAAGTGAACGG	66		RT-PCR
CGMMV RdRp	CGMMV-5′R	CCGCCGATGTCATAACAAGG	62	320	5′-RACE
CGMMV CP	CGMMV-3′F	CGCTGTAAAGCGTACTGATG	60	320	3′-RACE

The amplified products were purified using a gel extraction kit (MF029, Mei5 Biotechnology, Beijing, China) and ligated onto a pTOPO-TA vector (Aidlab Biotechnology, Beijing, China), followed by transformation of *E. coli* DH5α. Positive colonies were identified by PCR using primers same above and sent to Sangon Biotech (Shanghai, China) for Sanger sequencing.

2.4. Sequence-Based Analyses

The whole genome sequences of CMV and CGMMV published in NCBI GenBank were retrieved for analyses. MEGA X software (version 10.2.6) was used to perform multiple sequence comparison with the MUSCLE algorithm and to construct a phylogenetic tree using the maximum likelihood method with a bootstrap test of 1000. Pairwise nucleotide sequence and amino acid identity matrices were generated using the tool BioEdit version 7.0.5. Detection of potential recombination events in full coding sequence of CMV and CGMMV genome were conducted using RDP, GENECONV [33], BOOTSCAN [34], CHIMEARA [35], MAXCHI [36], SISCAN [37], and 3Seq packaged in the software RDP version 4 [38]. The recombination events were accepted by at least four methods with p value < 0.05.

2.5. Mechanical Inoculation and Transmission Electron Microscopy

The samples that were positive for both the CMV and CGMMV were frozen and ground in liquid nitrogen and then suspended in a modified phosphate-based inoculation buffer (containing 0.1 M Na_2HPO_4-KH_2PO_4, 0.01% sodium diethyldithiocarbamate, and 0.1% sodium thioacetate) at a ratio of 1:10 (*m*/*v*). Six plants of *N. benthamiana* were mechanically inoculated using the inoculum, and symptom development was continuously observed after inoculation. The presence of CMV and CGMMV infections in *N. benthamiana* was verified by RT-PCR, as described above. The positive samples were tested for virus particles using a transmission electron microscope at the institute of Yunnan Academy of Agricultural Sciences.

3. Results

3.1. RT-PCR and Biological Detection of CMV and CGMMV in Plants

Preprocessing with Trimmomatic v0.39 generated 34,172,316 high-quality clean reads (Q30 score: 93.95%). Blastn alignment results show that 16 contigs aligned to CMV and two contigs aligned to CGMMV. Based on the high-throughput sequencing results, onion yellow dwarf virus, leek yellow stripe virus, CMV, and CGMMV were detected in the mixed pool of the 161 collected onion samples; due to lack of data in Inner Mongolia, we focused on the occurrence of CMV and CGMMV in this work.

By RT-PCR, using the primers CMV-2a-F/R, eight out of the 161 samples tested positive for CMV infection, while using primers CGMMV-CP-F/R, only one sample was confirmed to be positive for CGMMV infection. It was also positive for CMV infection. The sample was marked as onion Y156.

Using the onion Y156 as inoculum source, six *N. benthamiana* plants were mechanically inoculated and successfully infected with CMV and CGMMV. The infected *N. benthamina* developed obvious symptoms of leaf rolling downward (Figure 2). Under a transmission electron microscope, typically spherical and rod-shaped particles were observed (Figure 3), suggestive of the existence of the CMV and CGMMV virions in the infected *N. benthaminana*.

Figure 2. *Nicotiana benthamiana* showing main symptom of leaf rolling downward. (**A**): a plant mechanically inoculated using the infected onion as inoculum. (**B**): a mock plant.

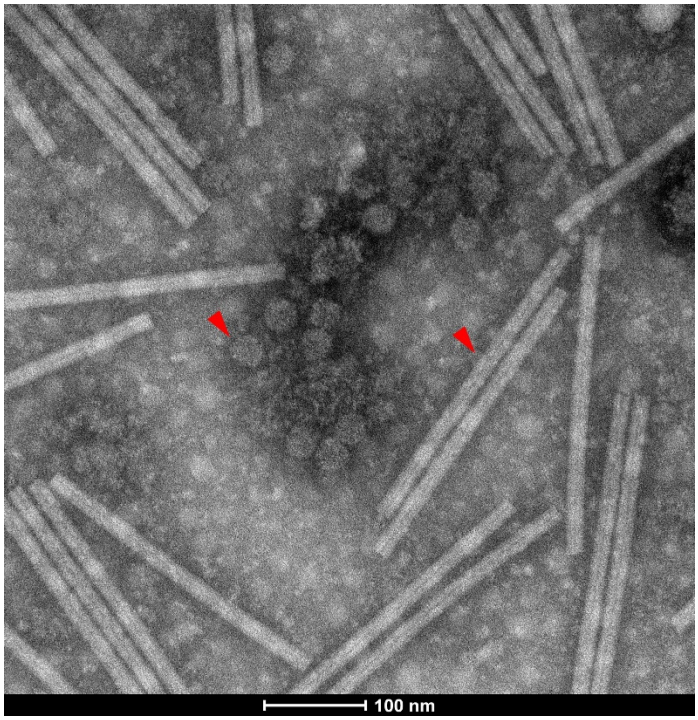

Figure 3. Rod and spherical particles observed in the inoculated *Nicotiana benthamia*. Triangles indicated the particles.

3.2. Genome Organization of CMV and CGMMV

The CMV and CGMMV from the onion Y156 were amplified and sequenced, and designated as CMV isolate 23IM_AlCe2 and CGMMV isolate 23IM_AlCe2.

The genome segments of CMV 23IM_AlCe2 were amplified using primers listed in Table 1. The RNA1 consists of 3376 nucleotides (nt) and has open reading frame (ORF) 1a, and nt 493–3060, encode a helicase or methyltransferase protein with 855 amino acids (aa). The RNA2 has 3039 nt, ORF 2a (nt 94–2616), and ORF 2b (nt 2414–2716), and encodes an RNA polymerase of 840 aa and 2b protein of 100 aa. The 2b is an RNA silencing suppressor. The RNA3 has 2171 nt, two ORFs of 3a (nt 66–905) and 3b (nt 1117–1848), and encodes movement protein of 279 aa and coat protein of 243 aa, respectively. The full-length sequences of the RNA1, RNA2, and RNA3 of CMV 23IM_AlCe2 were submitted and deposited in NCBI GenBank under accession numbers PX056295, PX056296, and PX056297, respectively.

The full-length genome fragments of CGMMV 23IM_AlCe2 were amplified using primers listed in Table 1. The genome is 6342 nt in length and has four ORFs. The ORF1 (nt 133–3426) encodes a 129 kDa protein of 1097 aa, the ORF2 (nt 133–4938) encodes a 186 kDa protein via a leaky stop codon at the end of ORF1, the ORF3 (nt 4925–5719) encodes a movement protein of 264 aa, and the ORF4 (nt 5694–6179) encodes a coat protein of 161 aa. The full-length genome of CGMMV 23IM_AlCe2 was submitted to NCBI GenBank under accession number of PX056294.

3.3. Phylogenetic and Sequence Identity Analysis of CMV

Based on the complete nucleotide sequences of RNA1, RNA2, and RNA3 of CMV and other isolates published in NCBI GenBank (Supplementary Tables S1–S3), three phylogenetic trees were constructed (Figures 4–6), onto which all CMV isolates were clearly clustered into three groups. They were designed as IA, IB, and II, and CMV 23IM_AlCe2 was classified into group II. Moreover, CMV 3IM_AlCe2 was closely related to CMV WSJ on the three phylogenetic trees, and CMV WSJ occurred in marigold plants in China. The

accession numbers for its RNA1, RNA2, and RNA3 were MW556587, MW556588, and MW556589, respectively.

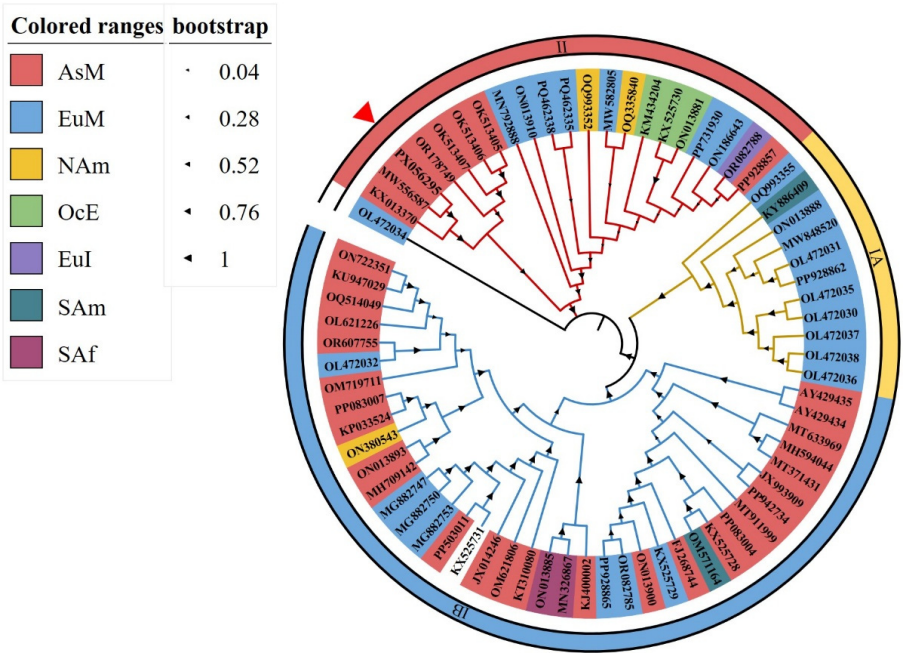

Figure 4. The phylogenetic tree constructed based on the full-length genome nucleotide sequences of RNA1 of CMV. The red triangle indicates the NCBI GenBank accession number of the CMV 23IM_AlCe2 isolate. AsM: Mainland Asia; EuM: Mainland Europe; NAm: North America; OcE: Oceania; EuI: European Islands; Sam: South America; SAf: South Africa.

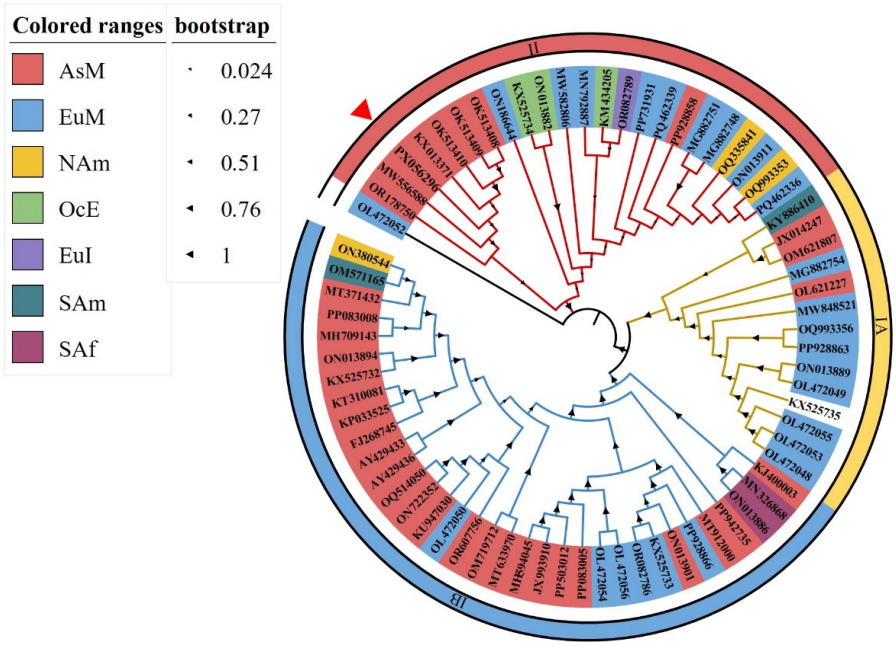

Figure 5. The phylogenetic tree constructed based on the full-length genome nucleotide sequences of RNA2 of CMV. The red triangle indicates the NCBI GenBank accession number of the CMV 23IM_AlCe2 isolate. AsM: Mainland Asia; EuM: Mainland Europe; NAm: North America; OcE: Oceania; EuI: European Islands; Sam: South America; SAf: South Africa.

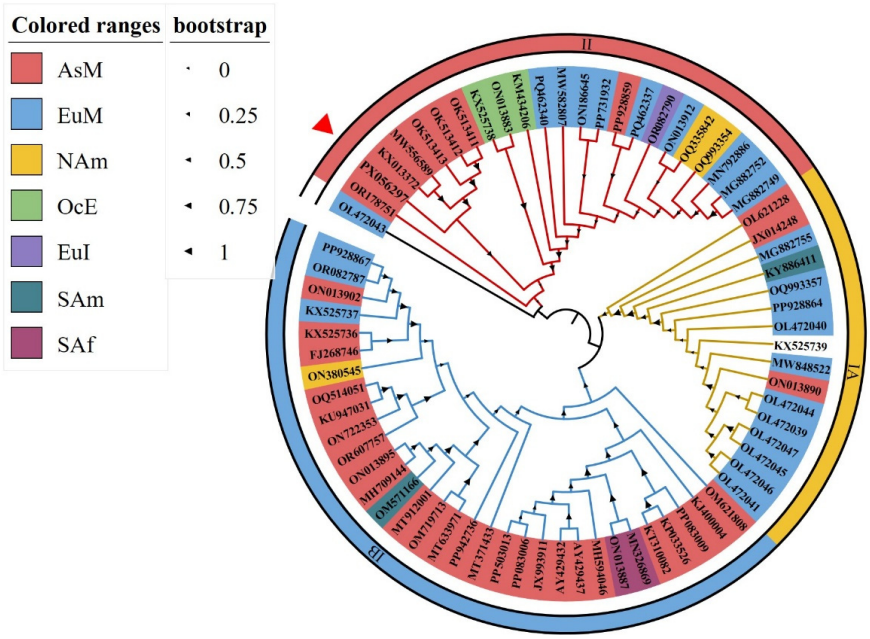

Figure 6. The phylogenetic tree constructed based on the full-length genome nucleotide sequences of RNA3 of CMV. The red triangle indicates the NCBI GenBank accession number of the CMV 23IM_AlCe2 isolate. AsM: Mainland Asia; EuM: Mainland Europe; NAm: North America; OcE: Oceania; EuI: European Islands; Sam: South America; SAf: South Africa.

Sequence identity analysis was conducted between CMV 23IM_AlCe2 with other isolates published in NCBI GenBank. The results (Supplementary Tables S1–S3) indicated that the nucleotide sequence identity of RNA1 between CMV 23IM_AlCe2 and other isolates ranged from 73.5% to 97.9%. Specifically, the identity ranged from 74.5% to 75.1% with subgroup IA isolates, 73.5% to 75.4% with subgroup IB isolates, and 87.5% to 97.9% with subgroup II isolates. The highest nucleotide sequence identity (97.9%) was observed with CMV WSL (MW556587), whereas the lowest identity (73.5%) was observed with CMV IN:Kar:Cucumber:21 (OQ514049) in India.

On RNA1, the nucleotide sequence identity of the 1a gene between CMV 23IM_AlCe2 and other isolates ranged from 76.1% to 98.2%. In particular, the identity ranged from 76.1–76.7% with subgroup IA isolates, 75.6–77.3% with subgroup IB, and 96.2–98.2% with subgroup II. The highest nucleotide sequence identity (98.2%) was observed with CMV WSL (MW556587), whereas the lowest identity (75.6%) was observed with CMV B (OL621226). At the amino acid level, the identity ranged from 83.1–83.8% with subgroup IA isolates, 81.8–84.0% with subgroup IB, and 96.3–97.3% with subgroup II. The highest sequence identity (97.3%) was observed with CMV WSL (MW556587), whereas the lowest identity (81.8%) was observed with CMV IN:Kar:Cucumber:21 (OQ514049).

The nucleotide sequence identity of RNA2 between CMV 23IM_AlCe2 and other isolates ranged from 65.5% to 99.0%. Specifically, the identity ranged from 68.7% to 69.9% with subgroup IA isolates, 65.5% to 70.0% with subgroup IB isolates, and 88.2% to 99.0% with subgroup II isolates. The highest nucleotide sequence identity (99.0%) was observed with CMV SL in China (KX013371), whereas the lowest identity (65.5%) was observed with CMV TabEC in Ecuador (OM571165) (Supplementary Table S2).

On RNA2, the nucleotide sequence identity of the 2a gene between CMV 23IM_AlCe2 and other isolates ranged from 70.4% to 99.2%. In particular, the identity ranged from 70.9% to 71.2% with subgroup IA isolates, 70.4% to 71.8% with subgroup IB isolates, and 97.7% to 99.2% with subgroup II isolates. The highest nucleotide sequence identity (99.2%) was observed with CMV SL (KX013371), whereas the lowest identity (70.4%) was observed with

CMV 21 in Poland (MG882754) and CMV NGSTPS18 in Germany (MW848521). At the amino acid level, the identity ranged from 74.3–74.8% with subgroup IA isolates, 73.1–74.7% with subgroup IB, and 97.7–99.4% with subgroup II. The highest sequence identity (99.4%) was observed with CMV SL (KX013371), and the lowest identity (73.1%) was observed with CMV ZS in China (MT912000).

On RNA2, the nucleotide sequence identity of the 2b gene between CMV 23IM_AlCe2 and other isolates ranged from 49.1% to 99.3%. In particular, the identity ranged from 55.8% to 57.0% with subgroup IA isolates, 49.1% to 59.4% with subgroup IB isolates, and 98.0% to 99.3% with subgroup II isolates. The highest nucleotide sequence identity (99.3%) was observed with CMV WSJ in China (MW556588) and DSMZ PV-1307 in Germany (MW582806), whereas the lowest identity (49.1%) was observed with CMV EP-1 in Zambia (MN326868). At the amino acid level, the identity ranged from 45.9% to 47.7% with subgroup IA isolates, 39.0% to 50.0% with subgroup IB, and 97.0% to 100.0% with subgroup II. The highest sequence identity (100%) was observed with CMV WSJ (MW556588) and SL (KX013371) in China, CMV VD (ON186644) in Russia, CMV DSMZ PV-1307 (MW582806) in Germany, CMV ES210111 (PP731931) in France, CMV DSMZ PV-0359 (PP928858) in Iran, and CMV 7 (MG882748) in Poland. The lowest identity (39.0%) was observed with CMV EP-1 (MN326868).

The nucleotide sequence identity of RNA3 between CMV 23IM_AlCe2 and other isolates ranged from 67.1% to 95.5%. Specifically, the identity ranged from 71.3% to 93.5% with subgroup IA isolates, 67.1% to 72.2% with subgroup IB isolates, and 72.2% to 95.5% with subgroup II isolates. The highest nucleotide sequence identity (95.5%) was observed with CMV PV-0184 (KX525738) in Australia, while the lowest identity (67.1%) was observed with CMV ELI (ON380545) in Jamaica (Supplementary Table S3).

On RNA3, the nucleotide sequence identity of the MP gene between CMV 23IM_AlCe2 and other isolates ranged from 78.4% to 98.8%. In particular, the identity ranged from 93.2% to 94.1% with subgroup IA isolates, 92.1% to 98.8% with subgroup IB isolates, and 78.4% to 79.3% with subgroup II isolates. The highest nucleotide sequence identity (98.8%) was observed with CMV JN-Cu (PP083006) in China, whereas the lowest identity (78.4%) was observed with CMV DSMZ PV-0418 (OQ335842) in the USA. At the amino acid level, the identity ranged from 94.9–96.4% with subgroup IA isolates, 92.1–99.6% with subgroup IB, and 83.1–84.2% with subgroup II. The highest sequence identity (99.6%) was observed with CMV JN-Cu (PP083006) and CMV DSMZ PV-0505 (PP503013) in China, and the lowest identity (83.1%) was observed with CMV Trk7 (PQ462340) in Hungary.

On RNA3, the nucleotide sequence identity of the CP gene between CMV 23IM_AlCe2 and other isolates ranged from 57.6% to 90.9%. In particular, the identity ranged from 67.8% to 70.7% with subgroup IA isolates, 57.6% to 70.3% with subgroup IB isolates, and 87.7% to 90.9% with subgroup II isolates. The highest nucleotide sequence identity (90.9%) was observed with CMV ScL (PQ462337) in Hungary, whereas the lowest identity (57.6%) was observed with CMV ELI (ON380545) in Jamaica. At the amino acid level, the identity ranged from 72.5–74.1% with subgroup IA isolates, 64.6–74.5% with subgroup IB, and 87.2–94.7% with subgroup II. The highest sequence identity (94.7%) was observed with CMV SL (PQ462337) in Hungary, and the lowest identity (64.6%) was observed with CMV EP-1 (MN326869) in Zambia.

3.4. Phylogenetic and Sequence Identity Analysis of CGMMV

The phylogenetic tree constructed based on the complete genome sequence of CGMMV showed that the CGMMV 23IM_AlCe2 was closely related to the CGMMV CG002 (MH271408) and CG045 (MT184941) in the USA (Figure 7).

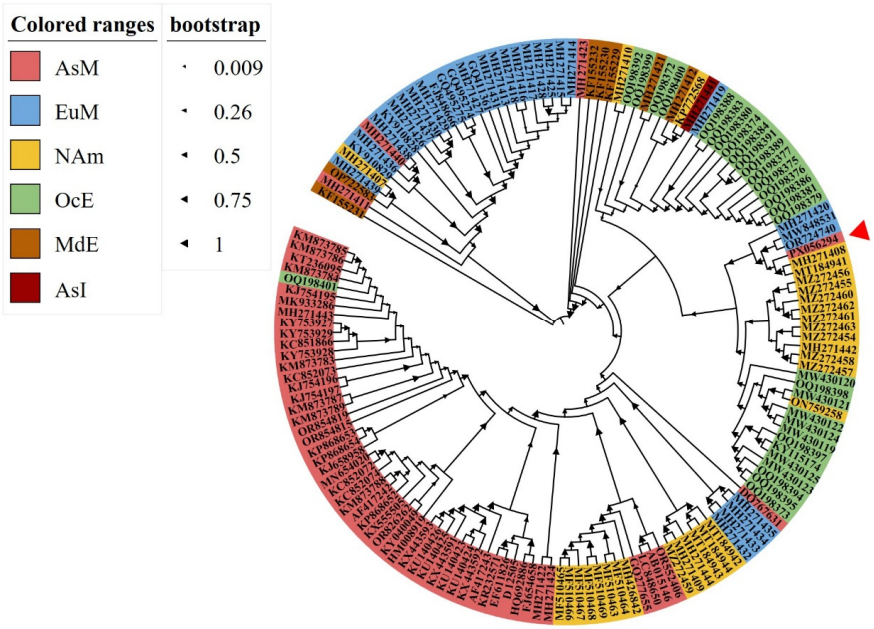

Figure 7. The phylogenetic tree constructed based on the full-length genome nucleotide sequences of RNA of CGMMV. The red triangle indicates the NCBI GenBank accession number of the CGMMV 23IM_AlCe2 isolate. AsM: Mainland Asia; EuM: Mainland Europe; NAm: North America; OcE: Oceania; EuI: European Islands; Sam: South America; SAf: South Africa.

The nucleotide sequence identity between CGMMV 23IM_AlCe2 and other isolates ranged from 88.3% to 98.7%. The highest nucleotide sequence identity (98.7%) was observed with CGMMV SI-2016-02 (OQ198400) and CGMMV QLD-2015-01 (OQ198392), NT-2019-10 (OQ198386) and NT-2020-02 (OQ198388) in Australia, while the lowest identity (88.3%) was observed with CGMMV DSMZ PV-1338 (OP722583) in Israel (Supplementary Table S4).

The nucleotide sequence identity of 186 kDa protein gene between CGMMV 23IM_AlCe2 and other isolates ranged from 88.6% to 99.4%, the highest nucleotide sequence identity (99.4%) was observed with CGMMV CG045 (MT184941), CG002 (MH271408), CG014 (MH271420), ABCA13-01 (KP772568), SI-2016-01 (OQ198399), CG006 (MH271412), CG015 (MH271421), NT-2014-02 (OQ198374), NT-2019-07 (OQ198383), NT-2020-02 (OQ198388), and Ec (KF155231). The lowest nucleotide sequence identity (88.6%) was observed with CGMMV DSMZ PV-1338 (OP722583) and CG001 (MH271407). At the amino acid level, the identity ranged from 94.7% to 99.7%. The highest sequence identity (99.7%) was observed with CGMMV CG045 (MT184941), CG002 (MH271408), SA-2020-01 (OQ198398), and 2019-26A-BE (OR724740), while the lowest sequence identity (99.7%) was observed with CGMMV CG005 (MH271411).

The nucleotide sequence identity of MP gene between CGMMV 23IM_AlCe2 and other isolates ranged from 91.9% to 100%, the highest nucleotide sequence identity (100%) was observed with 17 isolates of CGMMV (highlighted in yellow in Supplementary Table S4). The lowest nucleotide sequence identity (91.9%) was observed with CGMMV DSMZ PV-1338 (OP722583) in Israel. At the amino acid level, the identity ranged from 96.9% to 100%. The highest sequence identity (100%) was observed with 50 isolates of CGMMV (highlighted in yellow in Supplementary Table S4), while the lowest sequence identity (96.9%) was observed with CGMMV EU1 (KY910828) in Germany.

The nucleotide sequence identity of the CP gene between CGMMV 23IM_AlCe2 and other isolates ranged from 90.5% to 100%. The highest nucleotide sequence identity (100%) was observed with 25 isolates of CGMMV (highlighted in yellow in Supplementary Table S4). The lowest nucleotide sequence identity (90.5%) was observed

with CGMMV DSMZ PV-1338 (OP722583) in Israel. At the amino acid level, the identity ranged from 95.6% to 100%. The highest sequence identity (100%) was observed with 105 isolates of CGMMV (highlighted in yellow in Supplementary Table S4), while the lowest sequence identity (95.6%) was observed with CGMMV Alm08 (GQ411361) in Spain.

3.5. Recombination Analysis

Genetic recombination analyses were performed based on the whole genome sequences of CMV between the isolate23IM_AlCe2 those published in NCBI GenBank database. Additionally, the same analyses were conducted for CGMMV. Recombination events were not found in CGMMV genome sequences. The results indicated that a recombination region from nt 59 to nt 171 was detected, with the major parent of CMV DSMZ PV-1255 (ON013910) in the Greece isolate, and the minor parent of CMV Am (JX993909) in China (Table 2).

Table 2. Recombination event of RNA1 in CMV 23IM_AlCe2.

Methods	Average *p*-Value
RDP	7.076×10^{-26}
GENECONV	1.215×10^{-20}
BootScan	7.458×10^{-18}
MaxChi	2.942×10^{-6}
Chimaera	1.797×10^{-6}
SiScan	–
3Seq	1.324×10^{-11}

4. Discussion

Allium cepa, a biennial plant belonging to the genus *Allium*, has a relatively long growth cycle and is highly susceptible to viral pathogens, which can severely impact its yield and quality. The occurrence of viral diseases in onions is often caused by complex infections. However, for onions infected with viral diseases, there is a significant exacerbation of symptoms, or there may be a certain synergistic relationship between different viruses.

This study reports the occurrence of viruses infecting onions in Inner Mongolia, China. It was found that one onion plant (designated Y156) exhibiting severe mosaic, puckering, and stunted growth symptoms was co-infected with CMV and CGMMV. Under natural conditions, it is common for a single plant to be infected with two or more viruses simultaneously. In theory, high-throughput sequencing can detect several viruses present in the tested plant tissues [39]. There are a few reports of it infecting *Allium* species. The first record of CMV infecting *Allium* species may have been when it was serologically detected in a garlic sample from Zagreb, Croatia (former Yugoslavia) [40]. To date, CMV Ankara Onion 14.3Po (accession numbers MN070136 and MN864792) and CMV Ankara Onion 15.5Po (MN070137 and MN864793) from Turkey were reported infecting onions. In this work, we confirmed the occurrence of the isolate CMV 23IM_AlCe2 on onions in China.

To date, complete genome sequences of CGMMV isolated from plants in the families Cucurbitaceae, Solanaceae, Vitaceae, and Apocynaceae have been reported (Supplementary Table S4). Here we reported the first complete sequence of CGMMV infecting onion.

Based on the reports by Sialer, Cillo, Barbarossa, and Gallitelli [32], this study designed primers to amplify and obtain the complete genome of CMV infecting onions, analyzed the nucleotide and amino acid sequence consistency of CMV, and conducted a phylogenetic analysis of its evolution. From the phylogenetic analysis of RNA1, 2, and 3 nucleotide sequences, the CMV 23IM_AlCe2, along with some isolates from Asia, Europe, Oceania,

and North America, were classified into Subgroup II. The geographical distribution pattern of CMV isolates was not obvious, and each RNA had an independent evolutionary history, similar to previous reports [41]. Sequence analysis also showed that the CP nucleotide and amino acid sequences of CMV 23IM_AlCe2 had the highest consistency with the sequence of the *Scopolia carniolica* isolate reported from Hungary (PQ462337). The sequence of CMV 23IM_AlCe2 also had a significantly high consistency with the sequence of the marigold isolate WSJ reported from Beijing, China. This may be due to the spread of CMV by viral vectors (such as aphids) between onion fields and marigold fields. In future studies, it is worth investigating how aphids and other viral vectors spread CMV between onion fields and marigold fields. Nucleotide sequence analysis of CGMMV isolates showed that the CGMMV 23IM_AlCe2 had significant high consistency with the CGMMV CG045 (MT184941) of *Citrullus lanatus* (Cucurbitaceae) in the USA. The phylogenetic tree indicated that CGMMV isolates had obvious geographical distribution differences, with most European isolates classified into Subgroup 1 and most Asian, North American, and Oceanian isolates classified into Subgroup 2. The CMV 23IM_AlCe2 isolate obtained in this work was classified into Subgroup 2.

In this study, recombination was only detected in the 5′UTR region of CMV RNA1. It is known that RNA recombination can promote the diversity of plant viruses and their adaptation to new hosts, often generating new variants and virus isolates that can overcome resistance. Therefore, the observed recombination in the 5′UTR of RNA1 may be a result of CMV adapting to a new host. The possibility of host expansion may be due to host shifting via vectors (aphids) during winter. Similar to this study, recombination in non-coding regions, which are more prone to recombination, is relatively rare compared to ORF regions. Among the three RNAs, RNA1 is the most susceptible to recombination. Generally, intrasubgroup recombination (within IA and IB subgroups) is more likely to occur than intersubgroup recombination [12].

5. Conclusions

In this study, symptomatic onion plants from Hohhot, Inner Mongolia, were analyzed to identify associated viral infections. High-throughput sequencing, RT-PCR, and RACE revealed the presence of *cucumber mosaic virus* (CMV) in eight out of 161 samples and *cucumber green mottle mosaic virus* (CGMMV) in a single sample (1/161). Mixed infection of CMV and CGMMV was confirmed only in the CGMMV-positive plant, as further supported by transmission electron microscopy showing both rod-shaped (CGMMV) and spherical (CMV) particles. Notably, this study reports the first complete genome sequences of CGMMV infecting onion worldwide and the first complete genome sequences of CMV infecting onion in China. A recombination event was identified within the CMV RNA1 segment between nucleotides 59 and 171, providing new insights into the genetic diversity and evolutionary characteristics of these viruses. However, given the low detection rates of CMV and CGMMV (8/161 and 1/161, respectively), the primary causal agent(s) responsible for the widespread symptoms in most of the sampled plants remain to be further investigated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae12050607/s1, Table S1: Analysis of sequence identity between RNA1 of CMV 23IM_AlCe2 and other isolates; Table S2: Analysis of sequence identity between RNA2 of CMV 23IM_AlCe2 and other isolates; Table S3: Analysis of sequence identity between RNA3 of CMV 23IM_AlCe2 and other isolates; Table S4: Analysis of sequence identity between isolate 23IM_AlCe2 and other different CGMMV isolates.

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