Complete genome sequence of a tentative novel capillovirus isolated from Gerbera jamesonii

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Abstract

The currently named gerbera virus A (GeVA) has been identified as a novel capillovirus with a complete genome of 6929 nucleotides (nt) (GenBank accession no. OM525829.1). GeVA was detected in *Gerbera jamesonii* using high-throughput RNA sequencing analysis. The complete genome represents a single linear RNA with two open reading frames (ORF). The large ORF encodes a polyprotein contains four domains, while the smaller ORF encodes a movement protein. The addition of find two UUAGGU promoters for subgenomic RNA (sgRNA) transcription was also identified in this study. BLAST analysis demonstrated that it shared the highest identity with the rubber tree capillovirus 1 (MN047299.1) (complete nucleotide: 68.54%; polyprotein amino acid: 44.53%). Phylogenetic analysis of complete genome and replication protein sequences place GeVA alongside other genus *Capillovirus* in the family *Betaflexiviridae*. These data suggest that GeVA is a new member of the genus *Capillovirus*.

Full Text

Capilloviruses are members of the family *Betaflexiviridae*, which possess flexuous filaments particles 640-700 nm in length and a 6.5-7.4 kb, linear positive-sense ssRNA genome. The genomic RNA has two open reading frames (ORF) encoding a large replication-associated protein fused with the coat protein (CP) and a putative movement protein (MP), and is polyadenylated at its 3’ ends [1]. Currently, four virus species (*Apple stem grooving virus*, *Cherry virus A*, *Currant virus A*, and *Mume virus A*) are known to belong to the genus *Capillovirus*. They are known to occur mainly in woody plants, but a strain of ASGV has been reported in lily plants [2]. Moreover, no vectors have been clearly identified.

Gerbera plants (*Gerbera* spp.) are exceptionally important cut flowers in the current global floricultural trade. They are also widely grown in many countries as flowering potted or garden plants [3]. Several species of viruses were reported in gerbera plants worldwide including, *Chrysanthemum stem necrosis virus* (CSNV), *Cucumber mosaic virus* (CMV), *Impatiens necrotic spot virus* (INSV), *Tobacco rattle virus* (TRV), *Tobacco ringspot virus* (TRSV), and *Tomato spotted wilt virus* (TSWV) [4–9]. However, capilloviruses have not been identified in gerbera plants.

In August 2019, leaves (n=32) with viral symptoms such as chlorosis, ringspot, leaf distortion, yellowing and yellow spot were collected from *G. jamsonii* in seven greenhouses within Gyeongsangbuk-do, South Korea. To identify the causal agent of the virus, high-throughput RNA sequencing (HTS) was performed. An equal amount (10 mg) of the leaf was taken from the collected samples, pooled, and grounded using liquid nitrogen. The total RNA was extracted from the subsequently grounded powder using a Maxwell® 16 LEV Plant RNA Kit (Promega, Madison, USA). A cDNA library was constructed using a TruSeq Standard Total RNA with Ribo – Zero Plant (Illumina, San Diego, USA). Subsequently, paired-end sequencing was performed on 101 bp using the Illumina HiSeq 4000 platform (Macrogen, Seoul, Korea). The acquired reads were assembled *de novo* into transcript contigs using Trinity software as described previously [10]. The assembled contigs were subjected to BLASTx searches against the non-redundant NCBI protein database. These revealed a tentative novel capillovirus-like contig of 6908 nt that shares a

44.62% (query cover: 93%) amino acid (aa) sequence identity with the rubber tree virus 1 (RTV1) (GenBank no. QGR26011). To validate the presence of the novel capillovirus, total RNA was extracted from each sample (n = 32) using an easy-spin™ Total RNA Extraction Kit (iNtRON Bio, Seongnam, Korea). RT-PCR was then performed using a primer pair specifically designed against the acquired contig. Consequently, the novel capillovirus was confirmed in a sample showing yellow spots on leaf (Fig. 1). Then, cDNA was synthesized from the total RNA of the detected novel capillovirus using Oligo dT(18) as an initiator of the reaction, following which, PCR was performed with nine pairs of primer designed from the contig (Fig. 2, Supplementary Table 1). The obtained amplicon was cloned using an All in One™ PCR Cloning Kit (BioFact, Daejeon, Korea) and sequenced by Macrogen. To determine the complete genome sequence, the 5’ and 3’ ends were amplified using the 5’ and 3’ RACE System for Rapid Amplification of cDNA Ends (Invitrogen, Carlsbad CA, USA) with gene specific primers (Supplementary Table 1). The 5’ and 3’ RACE amplicons were cloned and sequenced in the same manner as other fragments. The resulting nucleotide sequences were assembled using the DNAMAN version 7.0.8.2 software (Lynnon Biosoft, Quebec, Canada). Additionally, the infected gerbera leaf tissue used sap inoculate gerbera plant which novel capillovirus was not infected and 15 species of indicator plants (*Chenopodium amaranticolor*, *C. quinoa*, *Citrullus lanatus*, *Cucumis melo* var. *makuwa*, *Cu. sativus*, *Glycine max*, *Capsicum annuum*, *Datura stramonium*, *Nicotiana benthamiana*, *N. occidentalis*, *N. rustica*, *N. tabacum*, *Physalis angulata*, *Solanum lycopersicum*). All plants were observed for 28 days after inoculation, but no symptoms were observed. Meanwhile, in RT-PCR assay, all indicator plants were negative, but only gerbera plant reacted positively. Therefore, it was confirmed that novel capillovirus was derived from the gerbera plant and that gerbera plant was included in the host range.

The complete genome of the virus consists of 6929 nucleotides (nt) of which 40.68% is G/C content (Fig. 2), while it is predicted, via the NCBI ORF Finder, to contain two ORFs like other known capilloviruses [1]. The 5’ and 3’ untranslated regions (UTR) are composed of 37 and 357 nt, respectively. The complete genome nucleotide shares a 68.54% identity (query: 22%) with the rubber tree capillovirus 1 (MN047299.1). The ORF1 (nt 38-6532) encodes a large replication-associated protein fused to the coat protein that is 2164 aa long. Additionally, it shares a 44.53% and 43.25% aa sequence identity (99% and 76% query cover) with a polyprotein in the rubber tree capillovirus 1 (QGR26011.1) and *Currant virus A* (YP_009229912.1), respectively. The ORF2 encodes a putative movement protein, 425 aa in length, which shares 40.00% and 41.85% aa sequence identity (97% and 52% query cover) with a movement protein of the rubber tree caillovirus 1 (QGR26012.1) and *Mume virus A* (QIM55854.1), respectively.

A simple modular architecture research tool (SMART) analysis revealed that the ORF1 encoded polyprotein contains four domains: methyltransferase (Mtr, Pfam01660; nt 161-1141), helicase (Hel, Pfam01443; nt 2669-3154), RNA-dependent RNA polymerase (RdRP, Pfam00978; nt 3527-4579, and coat protein (CP, Pfam05892; nt 5810-5815). In contrast, the ORF2 contains a single domain: movement protein (MP, Pfam01107; nt 4918-5475). The coat protein (CP) cistron of the genus *Capilloviruses* is located in the C-termiunal end of ORF1, and ORF2 (MP) is nested within ORF1 [1]. In this virus, the CP cistron was also located at the C-terminal end of ORF1 and it shares a 62.33% aa sequence identity (99% query cover) with a CP cistron in the rubber tree capillovirus 1 (QGR26011.1). In addition, the sequences,

which appear as promoters (UUAGGU) and direct the subgenomic RNA (sgRNA) transcription of capilloviruses [11] were found at the 5' terminus (4841 nt and 5810 nt) of the MP and CP domains, respectively. The MP and CP of this virus are expected to be expressed through sgRNA transcription. Accordingly, the novel capillovirus was tentatively named “Gerbera virus A” (GeVA).

A phylogenetic tree was constructed using aa sequences of the polyprotein of GeVA along with 41 members of 10 genera in the family *Betaflexiviridae* (Supplementary Table 2). In the phylogenetic analysis, GeVA clearly placed with the capilloviruses. Also, pairwise comparisons of GeVA and 41 betaflexiviruses showed 31.4%-40.4% similarity in the nt of the complete genome, 37.9-48.2% for aa of the ORF1, and 5.2%-24.5% for aa of the movement protein (MP). The taxonomic position and sequence comparisons identified GeVA as a new member of the genus *Capillovirus* according to the ITCV species demarcation criteria [1].

Declarations

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Author Contributions

All authors contributed to the study’s conception and design. Material preparation, data collection, and analyses were performed by Sangmin Bak, San Yeong Kim, Minhui Kim, and Wonyoung Jeong. The first draft of the manuscript was written by Sangmin Bak and all authors provided comments to the previous versions of the manuscript. Also, San Yeong Kim were supervised during the research and all related work. All the authors have read and approved the final manuscript.

Data Availability

The complete genome sequence of gerbera virus A (GeVA) was deposited in GenBank under the accession number OM525829.1.

Competing Interests

No potential conflict of interest relevant to this article was reported.

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Figures

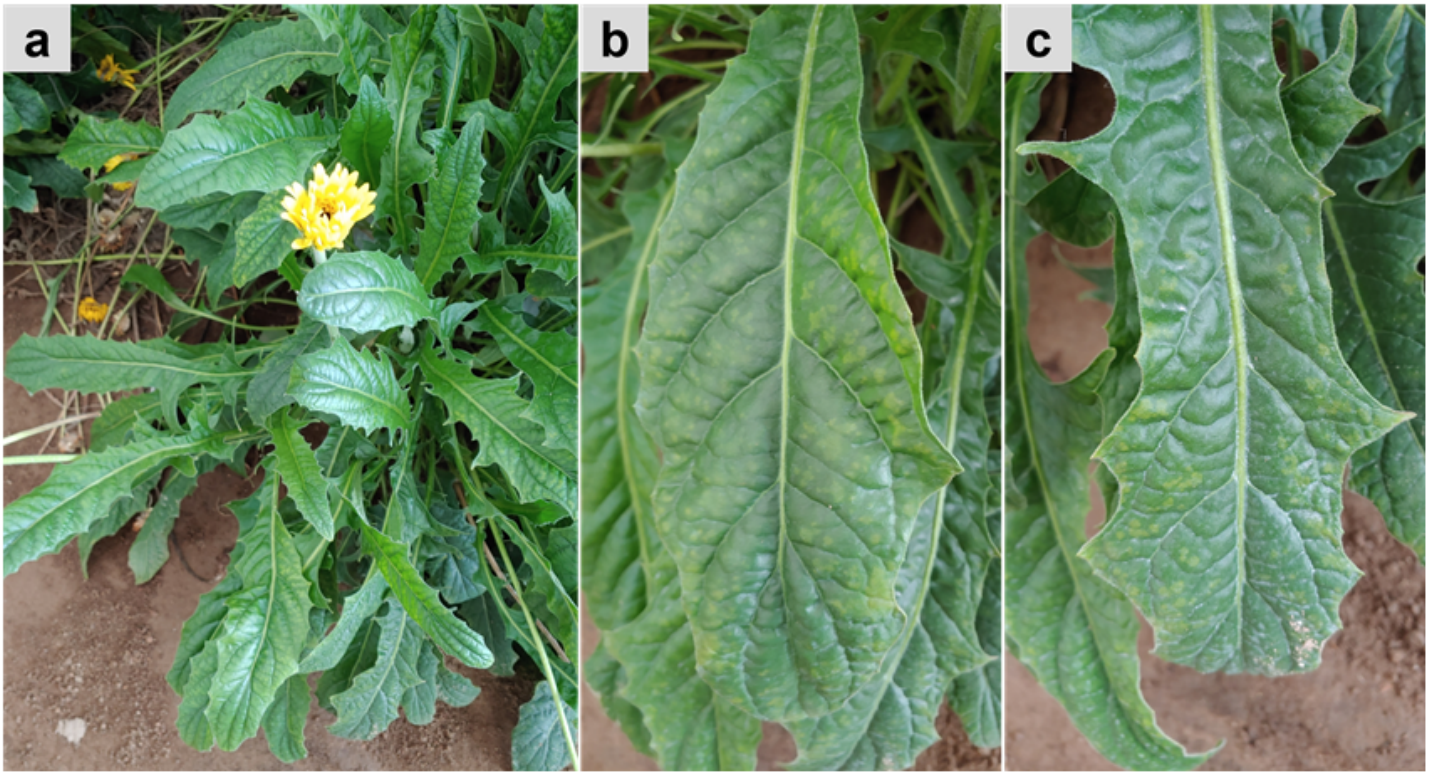

Figure 1

(a) Gerbera plant in which novel capillovirus was detected. (b-c) Leaves showing yellow spots.

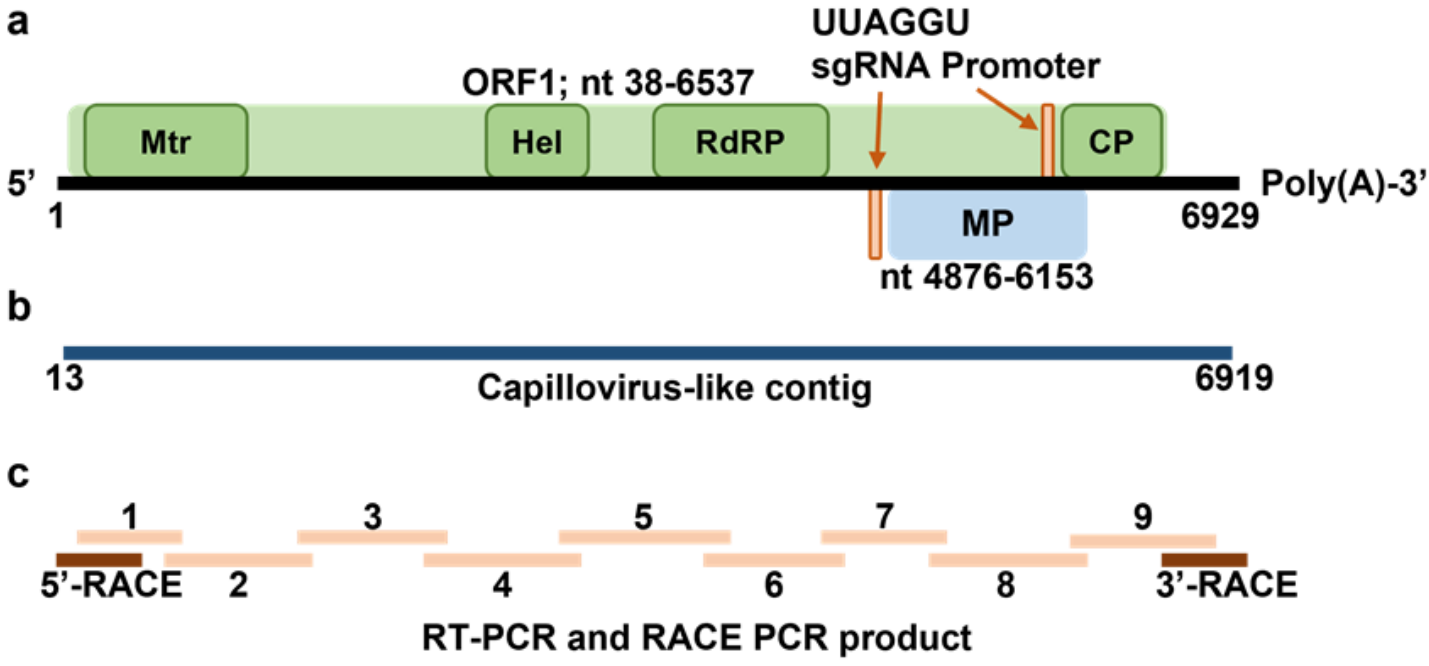

Figure 2

(a) Schematic representation of the genome organization for the gerbera virus A. The complete genome consists of two ORFs and is polyadenylated at the 3' end. ORF1 consists of four domains already located in previous capilloviruses: methyltransferase (Mtr), helicase (Hel), RNA-dependent RNA polymerase (RdRP), coat protein (CP). ORF2 encodes a putative movement protein (MP). The promoter sequence (UUAGGU), like in additional capilloviruses, was located in two regions and would be involved in subgenomic RNA (sgRNA) transcription for MP and CP expression. (b) The blue bar indicates the novel capillovirus-like contig, which are composed of 6908 nt, and *de novo* assembled from 5286 reads. (c) The 11 fragments represent the amplicon used to determine the complete genome (see also Supplementary Table S1).

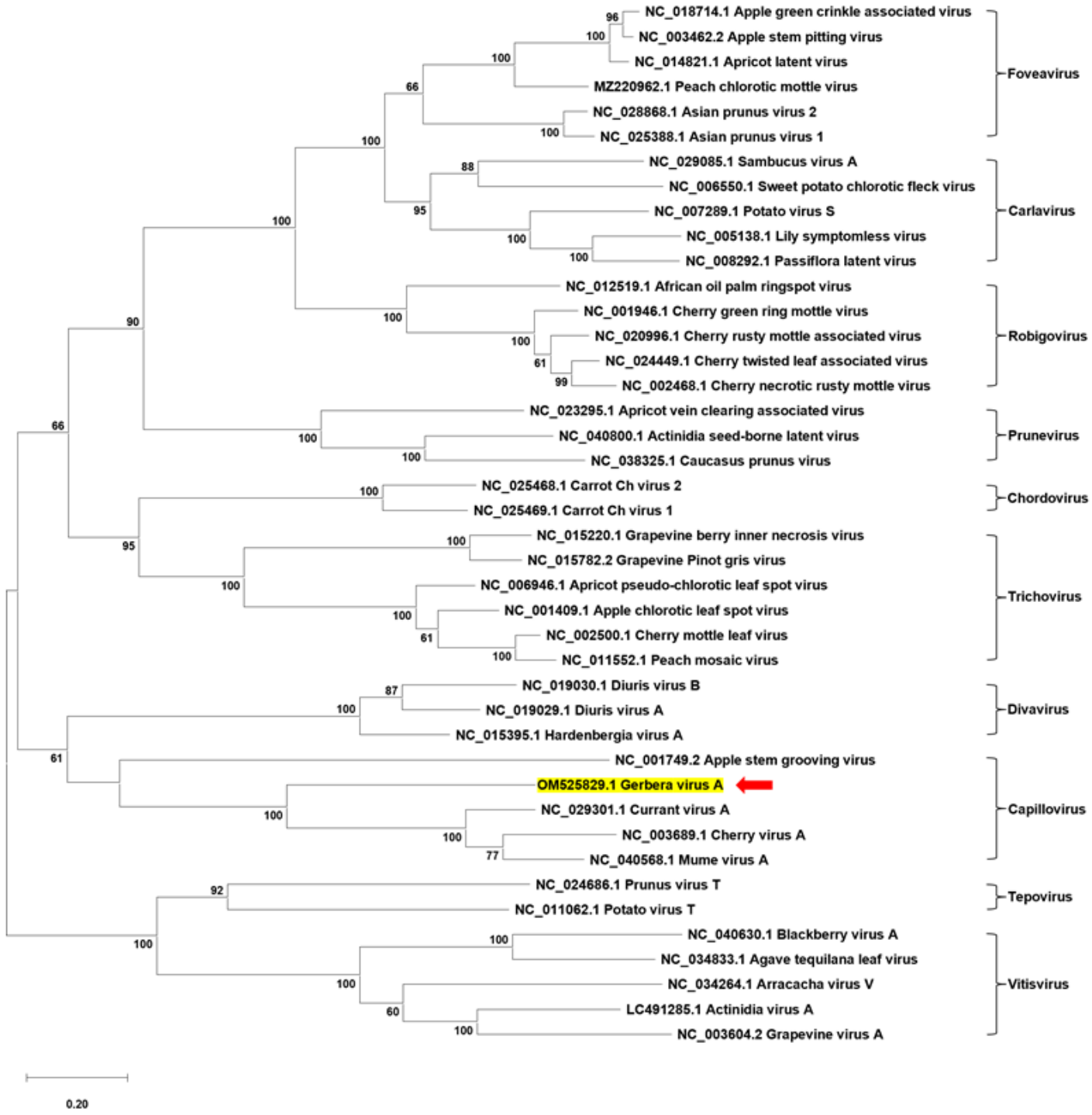

Figure 3

Phylogenetic analysis of the gerbera virus A (GeVA) and 41 members of the family *Betaflexiviridae*. A phylogenetic tree was constructed using the amino acid sequences of the complete polyprotein. The phylogenetic analysis was performed with the maximum likelihood method using MEGA (software version 11.0.10) with the Jone-Taylor-Thornton (JTT) model, and the bootstrap replicates at 1000. All viral sequences were obtained through NCBI, and GeVA is indicated by the red arrow.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- OM525829.1GerberacapillovirusAcompletegenome.fasta
- Supplementarytables.docx
- c95439g1i1Capilloviruslikecontig.fasta