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Identification and complete genomic sequence of a novel sadwavirus discovered in chrysanthemum (Chrysanthemum morifolium Ramat)

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Abstract

The complete genomic sequence of a putative novel member of the genus *Sadwavirus* was determined by high-throughput sequencing of a chrysanthemum from an orchard of Tongxiang Agricultural Science Institute in Tongxiang, Zhejiang province. The complete genome was further confirmed using RT-PCR and the rapid amplification of cDNA ends (RACE) method. The predicted genome of the putative virus was composed of two RNA molecules of 7016 and 6772 nucleotides in length, excluding the poly-A tails. The new virus was tentatively named “chrysanthemum sadwavirus” (ChSV). The Pro-Pol region of RNA1 and the CP region of RNA2 of ChSV shared the highest amino acid sequence identity of 53.01% and 36.40%, respectively, with the corresponding sequences of lettuce secovirus 1(LSV-1). Phylogenetic analysis showed that ChSV clustered in the subgenus *Stramovirus* (genus *Sadwavirus*). Taken together, these results suggest that ChSV is a new member of the genus *Sadwavirus.*

Full Text

Chrysanthemum (Chrysanthemum morifolium) belongs to the family *Asteraceae* and is the most economically important herbaceous perennial plant worldwide [1-2]. Virus disease-like symptoms in chrysanthemum plants in China were common and have seriously affected the high-quality development of the chrysanthemum industry [3-4]. ‘Hangbaiju’ is a cultivar of Chrysanthemum morifolium, primarily cultivated in Tongxiang city, Zhejiang Province, and has been widely used as a functional beverage in China due to its unique sensory attributes and multiple health beneficial features [5].

In October 2022, a plant of “ZaoXiaoYangJu" (a cultivated species selected from “Hangbaiju”) in an orchard of Tongxiang Agricultural Science Institute was observed showing chlorotic mottling symptoms during the field survey (Fig.1).Total RNA was extracted from the diseased leaves, and the ribosomal RNA was removed by the Epicentre Ribo-Zero rRNA Removal Kit (Epicentre, Madison, WI, U.S.A.). The ribosomal RNA-depleted RNA was then used to construct a cDNA library using a TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA, U.S.A.), and then were sequenced on an Illumina NovaSeq 6000 platform (Biomarker Biology Technology). RNA-seq sequencing resulted in 26, 443, 042 clean reads. The clean reads were de novo assembled into 116,809 contigs using the SPAdes v3.15.3 software and analyzed through BLAST analysis. The results showed that two contigs of length 6980 nt and 6684 nt had highest identity with RNA1 (46.89%) and RNA2 (38.57%) genome sequences of lettuce secovirus 1(LSV-1)(ATA66953.1 and ATA66954.1), which belong to a member in the subgenus *Stramovirus* of genus *Sadwavirus* in the family *Secoviridae*, suggest that they may represent the two RNA components of a new virus.

To confirm and obtain the complete genomes of the virus, ten primer pairs were designed based on the contig sequences and were used to amplify the sequences of RNA1 and RNA2 genomes (Supplementary Table S1). The 5’- and 3’-terminal sequences were amplified using a SMARTer RACE 5’/3’ Kit (TaKaRa, Dalian, China). Sequencing of the amplificated products showed that the complete RNA1 and RNA2 with the length 7016 nt and 6772 nt were obtained. The two sequences were deposited in GeneBank database

with accession numbers OR413567 and OR413568 respectively. The new virus was tentatively named chrysanthemum sadwavirus (ChSV).

RNA1 encodes a putative polyprotein of 1940 amino acid (aa) with a predicted molecular weight of 219 kDa, and RNA2 encodes a putative polyprotein of 1854 aa length with a predicted molecular weight of 209 kDa. Analysis of conserved domains using the NCBI Conserved Domain Search Tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) showed the presence of conserved motifs in the Pol and Hel of RNA1 polyprotein, whereas no motif was predicted for RNA2 polyprotein. Furthermore, Parallel analysis using HMMER (https://www.ebi.ac.uk/Tools/hmmer/) predicted a CP domain in RNA2 polyprotein similar to nepovirus CP. By comparing with other sadwaviruses, putative cleavage sites of the polyproteins were predicted (Fig.1B). Cleavage of RNA1 polyprotein is predicted to produce a protease cofactor (Pro-C), a helicase (Hel), a viral-genome-linked protein (VPg), a protease (Pro), and an RNA-dependent RNA polymerase (RdRp), and the cleavage of RNA2 polyprotein is predicted to produce t movement protein (MP), the large and small coat proteins (CPl and CPs, respectively).

Two phylogenetic trees were reconstructed in order to establish the relationship between ChSV and other sadwaviruses (Fig.1). Amino acid sequences of putative Pro-Pol domain in RNA1 and CP domain in RNA2 from ChSV and 11 previously reported viruses from *Stramovirus* (4), *Satsumavirus* (1), and *Cholivirus* (6) were aligned using MEGA 11 and reconstructed tree using the maximum-likelihood (ML) algorithm with 1,000 bootstrap repetitions as branch support. The results showed that ChSV clustered together with four other stramoviruses. Sequence identity matrix revealed the maximum identities of ChSV Pro-Pol (53.01%) and CP (36.40%) with LSV-1 Pro-Pol and CP sequences, respectively. For members within the family *Secoviridae*, the species demarcation criteria are <80% identity for the aa sequence of the Pro-Pol region and <75% identity for the large and small CP together [6]. ChSV can be designated as a new member of the subgenus *Stramovirus* (genus *Sadwavirus*).

Declarations

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Data Availability All data included in this study are available upon request by contact with the corresponding author.

Conflict of Interest The authors declare no conflict of interest.

Ethical approval This article does not contain any experiments involving humans or animals, and no ethical approval was required.

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Figures

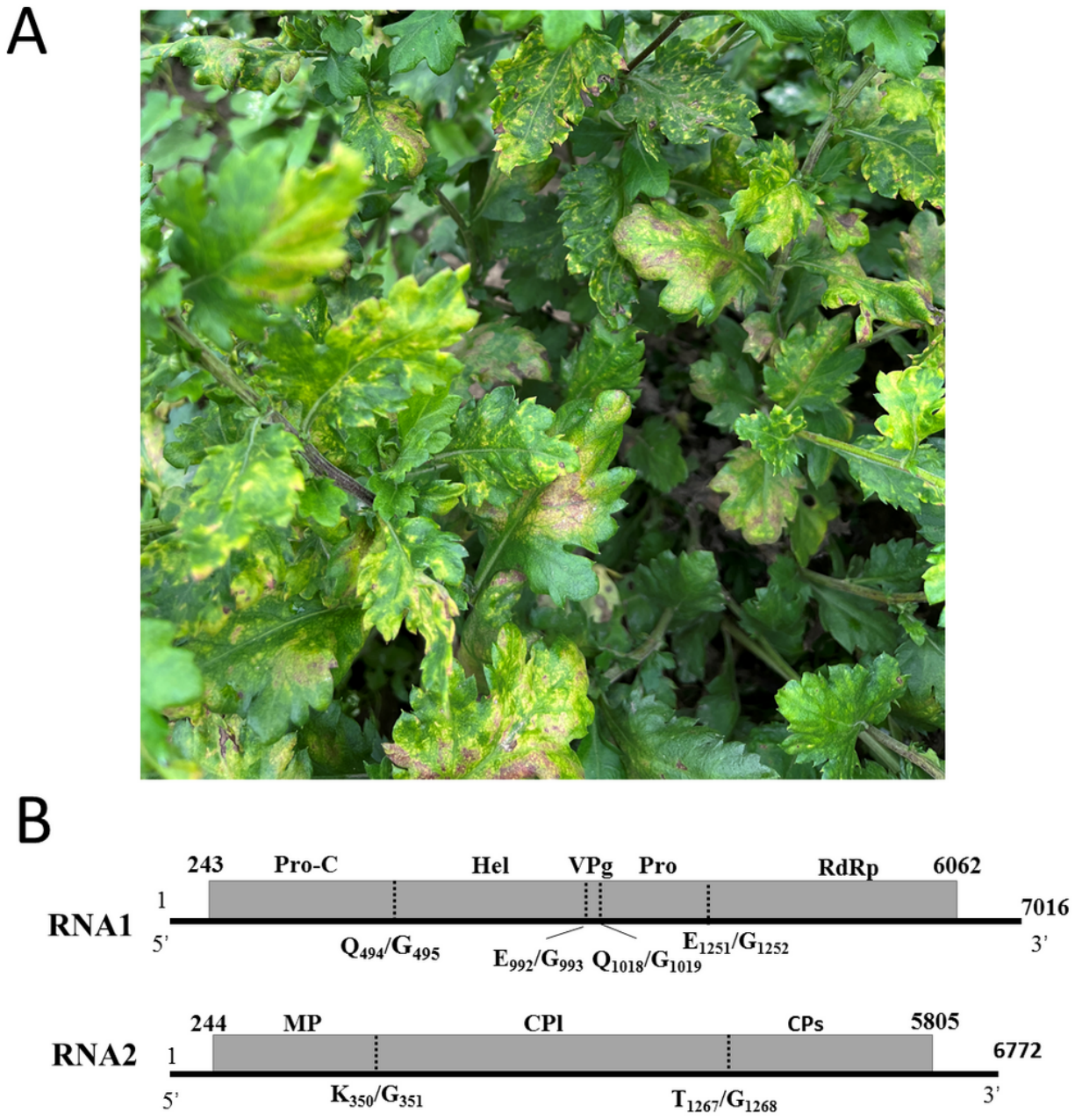


Figure 1

The symptoms observed in the sequencing chrysanthemum sample (A) and genomic organization of RNA1 and RNA2 of chrysanthemum sadwavirus (ChSV). Vertical lines within the boxes indicate the putative cleavage sites with their respective amino acid sequences.

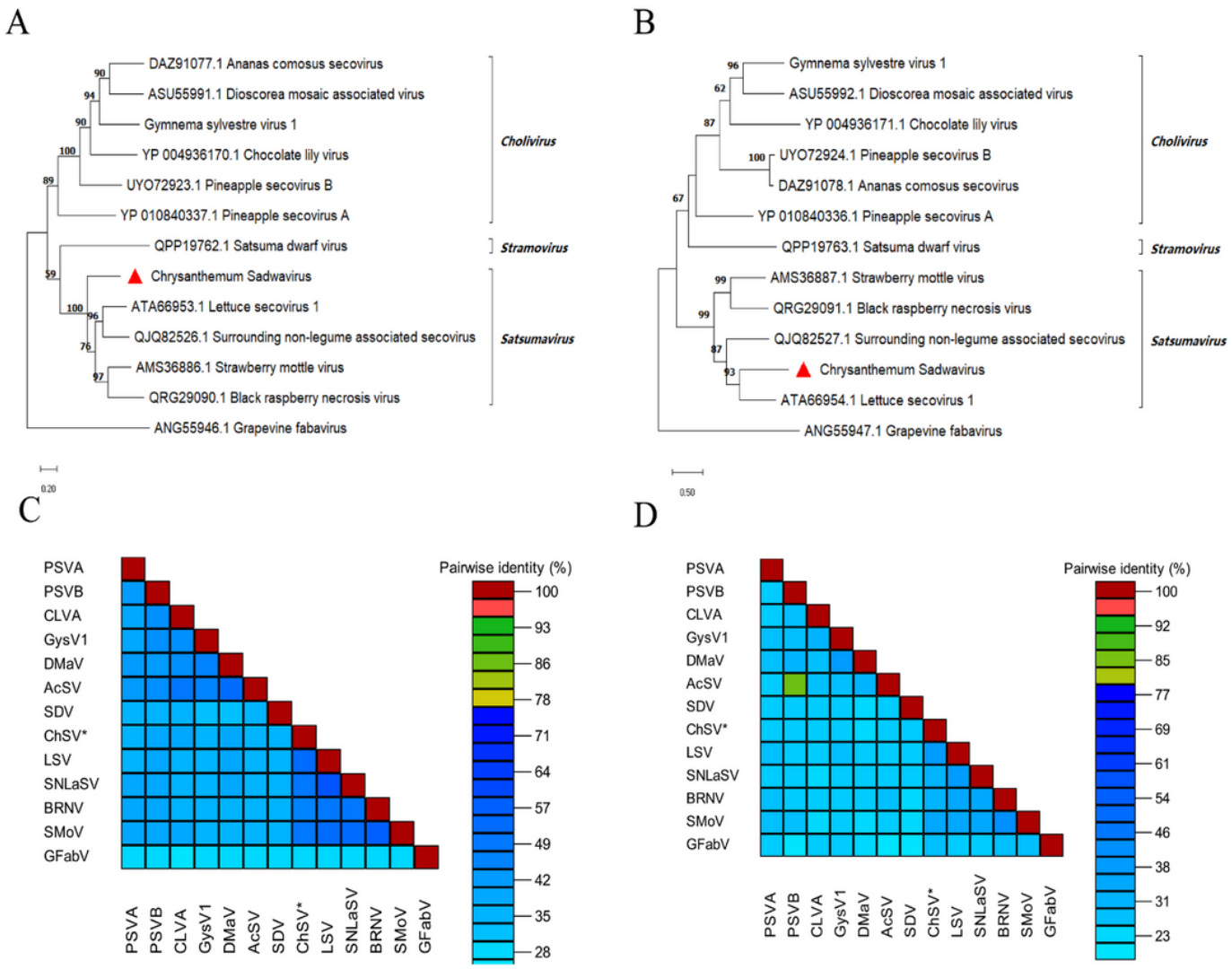


Figure 2

Phylogenetic trees (**a**, **b**) and sequence identity matrices (**c**, **d**) showing the relationship of Chrysanthemum sadwavirus with other sadwaviruses based on the conserved Pro-Pol (a, c) and CP (b, d) regions. Phylogenetic trees were constructed using maximum-likelihood (ML) method with 1000 bootstrap replicates. Bootstrap values >50% are only indicated. Grapevine fabavirus sequences were used as outgroups. AcSV: ananas comosus secovirus, BRNV: black raspberry necrosis virus, CLVA: chocolate lily virus A, DMaV: dioscorea mosaic associated virus, GFabV: grapevine fabavirus, GysV1: Gymnema sylvestre virus 1, LSV: lettuce secovirus 1, PSVA: pineapple secovirus A, PSVB: pineapple secovirus B, SDV: satsuma dwarf virus, SMoV: strawberry mottle virus, SNLaSV: Surrounding non-legume associated secovirus.