Molecular characterization of a novel virga-like virus associated with wheat

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Received: 24 February 2022 / Accepted: 31 March 2022 / Published online: 25 June 2022
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Abstract
In this work, we report the detection of a novel single-strand RNA virus from wheat, tentatively named "Triticum aestivum-associated virga-like virus 1" (TaAVLV1). Further characterization revealed that the complete genome of TaAVLV1 is divided into two segments, RNA1 and RNA2, which are 3530 and 3466 nt in length, excluding their respective polyA tails, and each contains only one open reading frame (ORF). The ORF of RNA1 encodes an RNA-dependent RNA polymerase (RdRp), while the ORF of RNA2 encodes a putative protein with methyltransferase and helicase domains. Phylogenetic analysis showed that the RdRp of TaAVLV1 is closely related to those of members of the unclassified virga-like virus group in the family Virgaviridae. Thus, we have identified TaAVLV1 as a putative novel virga-like virus belonging to the family Virgaviridae.

Wheat (Triticum aestivum L.), an important staple food crop worldwide, provides energy, nutrients, and numerous bioactive components, contributing greatly to a healthy diet for humans [1]. The demand for wheat production continues to increase with the expansion of the global population. However, viral diseases pose a significant threat to wheat grain yield and quality [2–4]. China is one of the world’s largest wheat producers and has been challenged with viral diseases for a long time. For example, wheat yellow mosaic disease, caused by both wheat yellow mosaic virus (WYMV) and Chinese wheat mosaic virus (CWMV), has severely damaged wheat production in China [5–7].

Virgaviridae is a family of plant viruses with rod-shaped virions and a genome consisting of a positive-sense single strand RNA (+ ssRNA) [8, 9]. According to the 2020 International Committee on Taxonomy of Viruses (ICTV) taxonomy (https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/virgaviridae), this virus family includes seven genera: Furovirus, Hordeivirus, Goryavirus, Pecluvirus, Pomovirus, Tobamovirus, and Tobravirus. However, in recent years, many new viruses that could not be assigned to any of the established genera in the family Virgaviridae have been discovered in various plant hosts [10–12], and these new viruses form a separate group of unclassified virga-like viruses.

Here, we report a novel + ssRNA virus detected in symptomatic leaf samples that were collected in a cultivated wheat field in Tibet, China. We have tentatively named it "Triticum aestivum-associated virga-like virus 1" (TaAVLV1) and deposited its full-length genome sequence in the GenBank database under the accession numbers OL519585 (RNA1) and OL519586 (RNA2).

Provenance of the virus material
The sample was collected from a wheat plant that had brown spots on yellow leaves in a wheat field near the Tibet plant protection station during the 2021 wheat disease survey. To obtain accurate and comprehensive information, high-throughput sequencing (HTS) was performed on symptomatic leaf tissue. Sequencing library construction, RNA sequencing (RNA-seq), quality trimming, assembly, and gene functional

Handling Editor: Robert H. A. Coutts.

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annotation were carried out by Novogene (Tianjin, China). Paired-end (150 bp) sequencing of the RNA library was performed on an Illumina HiSeq 4000 platform (Illumina, San Diego, CA, USA). A total of 59,733 contigs were generated from the clean reads (44,667,466) through de novo assembly. By comparing the NT and NR databases, as well as the Pfam and Swissprot databases, two assembled contigs were found to be homologous to viral RNA-directed RNA polymerases. In addition, we also detected three contigs related to barley stripe mosaic virus (BSMV), which are not relevant to this study. A BLASTx search revealed that one of the contigs (3479 nt) shared 52.62% pairwise amino acid sequence identity with sisal-associated virgavirus A (a member of the family *Virgaviridae* found in *Agave sisalana* that belongs to the group of unclassified virga-like viruses) [6], while the other contig (3436 nt) shared 48.63% pairwise amino acid sequence identity with sisal-associated virgavirus C [11]. A total of 8945 viral reads of TaAVLV1 were detected and accounted for 0.02% in the dataset according to the company’s subsequent analysis. Moreover, using BLASTn, we found that two contigs displayed no significant similarity to other sequences. Therefore, we considered it a potentially new virus closely related to members of the family *Virgaviridae* and tentatively named it TaAVLV1.

The sequences of this potentially novel virus were further characterized using the extracted total RNA returned from Novogene. To confirm sequence derived from HTS data, random reverse transcription PCR (RT-PCR) was performed to construct a cDNA library using a first-strand cDNA synthesis kit (Toyobo, Osaka, Japan) under the following conditions: 10 min at 30°C, 20 min at 42°C, 5 min at 99°C, and 5 min at 4°C. Then, PCR amplification was carried out using two sets of specifically designed primers (Supplementary Fig. S1) under the following conditions: 5 min at 95°C, cycles of 30 s at 95°C, 30 s at 60°C, and 1 min at 72°C, 6 min final elongation at 72°C, and 10 min final renaturation at 10°C. To determine the 5’- and 3’-terminal sequences, we employed rapid amplification of cDNA ends (RACE), using a SMARTer® RACE 5/3’ kit (Takara, Dalian, China) with Universal Primer AMix (UPM) and sequence-specific primers (Supplementary Fig. S1), following the manufacturer’s instructions. All of the products were then cloned into the pEASY-Blunt Zero Cloning Vector (TransGen Biotech, Beijing, China), and at least three positive clones were selected for Sanger sequencing at Ykang (Hangzhou, China). The final full-length sequence was assembled by segment concatenation using DNAMAN v6.0.

**Sequence properties**

The complete genome of TaAVLV1 is divided into RNA1 and RNA2, which are 3530 and 3466 nt in length, excluding their respective polyA tails. Only one putative open reading frame (ORF) was identified in each of the segments using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). The ORF in RNA1 was predicted to encode a 127.90-kDa RNA-dependent RNA polymerase (RdRp) composed of 1135 amino acids (aa), with RdRp domains encoded at nt 2180–3463 (Fig. 1a). The ORF in RNA2 was predicted to encode a putative 125.87-kDa protein of 1122 aa, with a methyltransferase (MET) domain encoded at nt 374–1141 and a helicase (HEL) domain encoded at nt 2564–3334 (Fig. 1a). The results of a BLASTp search using the amino acid sequences of the putative proteins were consistent with those obtained using BLASTx. RNA1 had 5’ and 3’ untranslated regions (UTRs) of 88 and 34 nt, respectively, while those in RNA2 were 79 and 18 nt, respectively. Multiple alignments of the UTRs for each RNA segment revealed conserved sequences at the 5’ and 3’ ends, which were GAGAA and CATGG, respectively (Fig. 1b and c). This type of genome organization is common in unclassified members of the family *Virgaviridae* such as Plasmopara viticola lesion associated virga-like virus 1–4 [12].

Since the RdRp encoded by RNA2 is a conserved viral protein, we used its amino acid sequence to evaluate the relationship of TaAVLV1 to the members of each genus of the family *Virgaviridae*. Using RdRp protein sequences downloaded from the NCBI database, a phylogenetic tree was constructed by the maximum-likelihood method with 1000 bootstrap replicates using MEGA v6.0. The results showed that TaAVLV1 grouped with other unclassified members of the family *Virgaviridae* and was most similar to sisal-associated virgavirus A. These results indicate that TaAVLV1 is a newly discovered member of the family *Virgaviridae* (Fig. 2).

Over the last decade, HTS has been widely applied to plant virology, leading to a significant increase in the rate of discovery of novel plant viruses [13], and previously unknown plant viruses are continually being identified in wheat [14–16]. The genome of TaAVLV1 detected in wheat leaves does not encode a coat protein (CP) or movement protein (MP), but it otherwise exhibits similarity to some fungal viruses. The coexistence of plant viruses and fungal viruses might have led to cross-kingdom infection or cross-kingdom RNA trafficking [17, 18]. Currently, we do not know if the wheat plant was the primary or secondary host of TaAVLV1, because its association with fungal hosts has not been ruled out. Interestingly, the presence of BSMV was also detected in our study, and the question of cross-kingdom communication between TaAVLV1 and BSMV, as described above, still needs to be investigated.

In conclusion, we have identified a putative novel virga-like virus from wheat that shows a close genetic relationship to members of the family *Virgaviridae*. ❘ Springer
Fig. 1 Genomic characterization of Triticum aestivum-associated virga-like virus 1 (TaAVLV1). (a) Genome organization of TaAVLV1. The conserved domains of the viral proteins were identified using the Conserved Domain Search Service (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Conserved sequences in the 5′- (b) and 3′- (c) ends of the RNA segments from TaAVLV1 were identified using Gene Doc 2.7.
### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00705-022-05473-z.

### Acknowledgments

The authors thank Professor K.Q. Tang (USA) for manuscript correction.

### Funding

This work was supported by the National Key R&D Plan in China (2018YFD0200408, 2018YFD0200507, 2017YFD-0201701), the China Agriculture Research System from the Ministry of Agriculture of the P.R. China (CARS-03), the National Key Project for Research on Transgenic Biology (2016ZX08002-001), the International Science and Technology Cooperation Program of China (2012DFA30900), and the K.C. Wong Magna Fund of Ningbo University.

### Declarations

#### Conflict of interest

The authors declare that they have no conflicts of interest.

#### Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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