Molecular Characterization of a Novel Virga-like Virus Isolated From Wheat

Hua Li

Ningbo University

Jun Guo

Ningbo University <https://orcid.org/0000-0002-4456-7231>

ZhongHua Zhao

National agro-technical Extension and Service Center

Zhuangxin Ye

Ningbo University

Jianping Chen

Ningbo University

Jian Yang (✉ nather2008@163.com)

Ningbo University

Research Article

Keywords: Triticum aestivum-associated virga-like virus 1 (TaAVLV1), Molecular characterization, Wheat (Triticum aestivum L), RNA1 and RNA2

Posted Date: December 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1177163/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

In this work, we report the isolation of a novel positive-sense 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 was divided into two segments, RNA1 and RNA2, which were 3530 and 3466 nt long, excluding the polyA tail. These segments contained two open reading frames (ORFs). The ORF in RNA1 encoded an RNA-dependent RNA polymerase (RdRp), while the ORF in RNA2 encoded a putative protein carrying MET and HEL domains. Phylogenetic analysis based on the RdRp protein of each representative genus of *Virgaviridae* placed TaAVLV1 in the unclassified *Virgaviridae* clade of the *Virgaviridae* family. To our knowledge, this is the first report of virga-like virus isolated from wheat. Future studies will be conducted to examine its effect on host growth and development.

Introduction

Wheat (*Triticum aestivum* L), which is an important staple food worldwide, provides energy, nutrients, and numerous bioactive components, thus contributing to a healthy diet for human [1]. The demand for wheat is increasing worldwide as the global population rises. Thus, wheat production needs to be improved; however, viral diseases pose a significant threat to grain yield and quality [2–4]. China is the world's largest wheat producer and has been struggling with viral disease for a long time. Wheat yellow mosaic disease, which is mainly caused by *Wheat yellow mosaic virus* (WYMV) and *Chinese wheat mosaic virus* (CWMV), severely damages wheat production in China [5–7].

Virgaviridae is a family of plant viruses consisting of rod-shaped virions with positive-sense single strand RNA (+ssRNA) genomes [8–9]. According to the 2020 International Committee on Taxonomy of Viruses (ICTV) taxonomy ([http:// ictv.global/report](http://ictv.global/report)), the family includes the genera *Furovirus*, *Hordeivirus*, *Goravirus*, *Pecluvirus*, *Pomovirus*, *Tobamovirus*, and *Tobravirus*. In recent years, many viruses belonging to the *Virgaviridae* family but different from any of the known genera in this family have been characterized in different hosts [10–12]. Therefore, those viruses were temporarily assigned to unclassified *Virgaviridae*.

Here, we report a novel +ssRNA virus isolated from 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 sequence in the GenBank database under the following accession numbers: RNA1 OL519585, RNA2 OL519586.

Provenance Of The Virus Material

The samples were collected in a wheat field by the Tibet plant protection station, China, 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 annotation were carried out by Novogene (Tianjin, China). Paired-end (150 bp) sequencing of the RNA library was performed on the Illumina HiSeq 4000 platform. A total of 59,733 contigs were generated from the clean reads (22,333,733) through de novo assembly. By comparing NT and NR databases, as well as Pfam and Swissprot databases, two assembled contigs were found to be homologous to viral RNA-directed RNA-polymerase. A BLASTx search against the two selected contigs revealed that one contig (3479 nt) shared 52.62% amino acid pairwise identity with Sisal-associated virgavirus A (a virus found in *Agave sisalana* that belongs to unclassified Virgaviridae)[6], while the other contig (3436 nt) shared 48.63% amino acid pairwise identity with Sisal-associated virgavirus C [11]. Moreover, we found that the contigs displayed no significant similarity using BLASTn. Therefore, we considered it a potentially new virus closely related to plant virgaviruses, and tentatively named it TaAVLV1.

The sequences of the potentially novel virus were further characterized. Total RNA was extracted from approximately 100 mg of leaf tissue using TRIzol reagent (Invitrogen, Waltham, USA) according to the manufacturer's instructions. To confirm sequence-derived 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 (Fig. S1) under the following conditions: 5 min at 95°C, 30 s at 95°C, 30 s at 60°C, 1 min at 72°C, 6 min final elongation at 72°C, and final renaturation at 10°C for 10 min. To determine the 5'- and 3'-terminal sequences, we employed rapid amplification of cDNA ends (RACE) to obtain terminal sequences using the SMARTer® RACE 5'/3' kit (Takara, Dalian, China) with Universal Primer A Mix (UPM) and sequence-special primers (Fig. S1), following the manufacturer's instructions. All the products were then cloned into the pEASY-Blunt Zero Cloning Vector (TransGen Biotech, Beijing, China), and the positive clones were selected for Sanger sequencing at Ykang (Hangzhou, China) at least three times. 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 were 3530 and 3466 nt long, excluding the polyA tail. These comprised two putative open reading frames (ORFs), which were identified 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) protein composed of 1135 amino acids (aa), containing RdRp domains at positions 2180–3463 nt (Fig. 1a). The ORF in RNA2 encoded a putative 125.87 kDa protein of 1122 aa, containing methyltransferase (MET) domains at positions 374–1141 nt and Helicase (HEL) domains at positions 2564–3334 nt (Fig. 1a). Regarding the levels of sequence identity, the results of a BLASTp search against the two putative proteins were consistent with those of BLASTx. Moreover, 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 of each RNA segment revealed conserved sequences at the 5'- and 3'-ends, which were GAGAA and CATGG, respectively (Fig. 1b, c). Thus, we concluded that RNA1 and RNA2 are different segments of TaAVLV1. RdRp is a conserved

viral protein, therefore, we used it to evaluate the relationship of TaAVLV1 with representative members of each genus of *Virgaviridae*. Based on RdRp protein sequences downloaded from the NCBI database, a phylogenetic tree was constructed by the maximum likelihood model with 1000 bootstrap replicates using MEGA v6.0. Phylogenetic analysis showed that TaAVLV1 was grouped with recognized members of unclassified *Virgaviridae* and close to Sisal-associated virgavirus A. These results indicated that TaAVLV1 is a newly discovered viral species that belongs to unclassified *Virgaviridae* (Fig. 2).

Over the last decade, HTS has been widely applied to plant virology, leading to a significant increase in the number of novel plant viruses discovered [13]. Moreover, emerging plant viruses are constantly being identified in wheat [14–15]. To the our best knowledge, this is the first report of virga-like virus isolated from wheat. We characterized its genome and revealed its genetic relationship with the family *Virgaviridae*. In the future, we will examine whether this virus affects host growth and development.

Declarations

Acknowledgments

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 Funding, Ningbo University.

Compliance with ethical standards

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 that were performed by any of the authors.

References

1. Shewry PR, Hey SJ (2015) The contribution of wheat to human diet and health. Food and energy security 4:178-202 <https://doi.org/10.1002/fes3.64>
2. Kühne T (2009) Soil-borne viruses affecting cereals: known for long but still a threat. Virus research 141:174-183 <https://doi.org/10.1016/j.virusres.2008.05.019>
3. Chen JJAoAB (2010) Occurrence of fungally transmitted wheat mosaic viruses in China. 123:55-61 <https://doi.org/10.1111/j.1744-7348.1993.tb04072.x>

4. Liu Y, Khine MO, Zhang P, Fu Y, Wang X (2020) Incidence and Distribution of Insect-Transmitted Cereal Viruses in Wheat in China from 2007 to 2019. *Plant disease* 104:1407-1414 <https://doi.org/10.1094/PDIS-11-19-2323-RE>
5. Han C, Li D, Xing Y, Zhu K, Tian Z, Cai Z, Yu J, Liu Y (2000) Wheat yellow mosaic virus Widely Occurring in Wheat (*Triticum aestivum*) in China. *Plant disease* 84:627-630 <https://doi.org/10.1094/PDIS.2000.84.6.627>
6. Zhang F, Liu S, Zhang T, Ye Z, Han X, Zhong K, Yang J, Chen J, Liu P (2021) Construction and biological characterization of an infectious full-length cDNA clone of a Chinese isolate of Wheat yellow mosaic virus. *Virology* 556:101-109 <https://doi.org/10.1016/j.virol.2021.01.018>
7. GUO, Liu-ming, HE, Jing, LI, Jing, CHEN, Jian-ping, ZHANG, Agriculture H-mJJol (2019) Chinese wheat mosaic virus: A long-term threat to wheat in China. [https://doi.org/10.1016/S2095-3119\(18\)62047-7](https://doi.org/10.1016/S2095-3119(18)62047-7)
8. Adams MJ, Antoniw JF, Kreuze J (2009) Virgaviridae: a new family of rod-shaped plant viruses. *Archives of virology* 154:1967-1972 <https://doi.org/10.1007/s00705-009-0506-6>
9. Adams MJ, Adkins S, Bragard C, Gilmer D, Li D, MacFarlane SA, Wong SM, Melcher U, Ratti C, Ryu KH, Ictv Report C (2017) ICTV Virus Taxonomy Profile: Virgaviridae. *The Journal of general virology* 98:1999-2000 <https://doi.org/10.1099/jgv.0.000884>
10. Thekke-Veetil T, Lagos-Kutz D, McCoppin NK, Hartman GL, Ju HK, Lim HS, Domier LL (2020) Soybean Thrips (Thysanoptera: Thripidae) Harbor Highly Diverse Populations of Arthropod, Fungal and Plant Viruses. *Viruses* 12 <https://doi.org/10.3390/v12121376>
11. Quintanilha-Peixoto G, Fonseca PLC, Raya FT, Marone MP, Bortolini DE, Mieczkowski P, Olmo RP, Carazzolle MF, Voigt CA, Soares ACF, Pereira GAG, Góes-Neto A, Aguiar E (2021) The Sisal Virome: Uncovering the Viral Diversity of Agave Varieties Reveals New and Organ-Specific Viruses. *Microorganisms* 9 <https://doi.org/10.3390/microorganisms9081704>
12. Chiapello M, Rodríguez-Romero J, Ayllón MA, Turina M (2020) Analysis of the virome associated to grapevine downy mildew lesions reveals new mycovirus lineages. *Virus evolution* 6:veaa058 <https://doi.org/10.1093/ve/veaa058>
13. Hadidi A, Flores R, Candresse T, Barba M (2016) Next-Generation Sequencing and Genome Editing in Plant Virology. *Frontiers in microbiology* 7:1325 <https://doi.org/10.3389/fmicb.2016.01325>
14. Kondo H, Yoshida N, Fujita M, Maruyama K, Hyodo K, Hisano H, Tamada T, Andika IB, Suzuki N (2021) Identification of a Novel Quinvirus in the Family Betaflexiviridae That Infects Winter Wheat. *Frontiers in microbiology* 12:715545 <https://doi.org/10.1007/s00705-021-05175-y>
15. Fu S, Zhang T, He M, Sun B, Zhou X, Wu J (2021) Molecular characterization of a novel wheat-infecting virus of the family Betaflexiviridae. *Archives of virology* 166:2875-2879 <https://doi.org/10.3389/fmicb.2021.715545>

Figures

Figure 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>). Then, conserved sequences in the 5'- (b) and 3'- (c) ends of the RNA segments from TaAVLV1 were identified using Gene Doc 2.7.

Figure 2

Phylogenetic analysis of TaAVLV1 and representative members of seven subfamilies of *Virgaviridae*. Maximum likelihood tree was conducted based on the amino acid sequences of the conserved RdRp proteins using MEGA v6.0. The identified virus is highlighted in a red circle. Viral names and GenBank accession numbers of the proteins are displayed on the tree.

Supplementary Files

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

- [FigS1.docx](#)
- [ElectronicSupplementaryMaterial.docx](#)