Molecular Characterization of a Novel Virga-like Virus Associated to Wheat

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Abstract

In this work, we report the detection of a novel single strand RNA virus from wheat tentatively named as *Triticum aestivum*-associated virga-like virus 1 (TaAVLV1). Further characterization revealed that the complete genome of TaAVLV1 can be divided into two segments, RNA1 and RNA2. Each fragment excluding their respective polyA tails has 3530 and 3466 nt in length and contains only one open reading frame (ORF). The ORF in RNA1 encodes an RNA-dependent RNA polymerase (RdRp), while the other in RNA2 encodes a putative protein carrying MET and HEL domains. Phylogenetic analysis showed that the RdRp of TaAVLV1 was closely related to the RdRp protein members of the unclassified virga-like virus group in the family *Virgaviridae*. Therefore, we have identified TaAVLV1 as a putative novel virga-like virus belonging to the family *Virgaviridae*.

Introduction

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

Virgaviridae is a family of plant viruses consisting of rod-shaped virions with genomes 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 contains seven genera such as *Furovirus*, *Hordeivirus*, *Goravirus*, *Pecluvirus*, *Pomovirus*, *Tobamovirus*, and *Tobravirus*. However, in recent years many new viruses which couldn't be recognized belonging to any above known genera in the *Virgaviridae* family have been discovered in many different plant hosts [10–12] and these new viruses were therefore assigned as unclassified *Virgaviridae*.

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

Provenance Of The Virus Material

The sample was collected from a wheat plant that had brown spots on the yellow leaves 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 (Illumina, San Diego, CA, USA). A total of 59,733 contigs were generated from the clean reads (44667466) 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. 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 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]. Total 8945 viral reads of TaAVLV1 were detected and accounted for 0.02% in the dataset through the company's subsequent analysis. Moreover, we found that two contigs displayed no significant similarity using BLASTn. Therefore, we considered it a potentially new virus closely related to the family *Virgaviridae*, and tentatively named it as TaAVLV1.

The sequences of this potentially novel virus were further characterized using the extracted Total RNA returned from Novogene company. 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 10 min final renaturation at 10°C. 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) for 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 was divided into RNA1 and RNA2, which were 3530 and 3466 nt in length, excluding their respective polyA tails. Only one putative open reading frame (ORF) was identified in the RNA1 or the RNA2, respectively 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), corresponding to the RdRp domains at positions 2180–3463 nt (Fig. 1a). The other ORF in RNA2 was predicted to encode a putative 125.87 kDa protein with 1122 aa, corresponding to the methyltransferase (MET) domains at positions 374-1141nt and the Helicase (HEL) domains at positions 2564–3334 nt (Fig. 1a). Regarding the levels of sequence similarity, 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 for each RNA segment revealed conserved sequences at the 5'- and 3'-ends, which were GAGAA and CATGG, respectively (Fig. 1b, c). Additionally, this type of genome organization is common to be seen in members of unclassified *Virgaviridae* such as in Plasmopara viticola lesion associated virga-like virus 1–4 [12].

As we showed above that TaAVLV1 genome has two RNA segments RNA1 and RNA2, and the RdRp encoded by RNA2 is a conserved viral protein, we used this RdRp to evaluate the relationship of TaAVLV1 with other members of each genus of *Virgaviridae*. Based on the RdRp protein sequences downloaded from the NCBI database, a phylogenetic tree was constructed by the maximum likelihood methodology with 1000 bootstrap replicates using MEGA v6.0 and showed that TaAVLV1 could be grouped as the recognized members of unclassified *Virgaviridae* and was similar to Sisal-associated Virgavirus A. These results indicated that TaAVLV1 was a new discovered viral species related to the family *Virgaviridae* (Fig. 2).

Over the last decade, HTS has been widely applied to plant virology, leading to a significant increase in the discoveries of novel plant viruses [13] as well as in wheat new emerging plant viruses are constantly being identified [14–16]. In our study, the genome organization of TaAVLV1 detected from wheat leaves hasn't evolved to obtain any sequences for encoding any coat protein (CP) or movement protein (MP), but shared a lot of similarity to fungal viruses. However, a coexistence relationship between plant virus and fungal virus could occur by cross-kingdom infection and cross-kingdom RNA trafficking [17–18]. Currently, our findings couldn't be able to clarify whether the wheat plant is the primary or secondary host of TaAVLV1 because the association of this wheat virus TaAVLV1 with fungal hosts has not been ruled out. Interestingly, the existence of BSMV was also found in our study, and whether TaAVLV1 and BSMV had the cross-kingdom communication, as described above, still needs to be further investigated.

In conclusion, we have identified TaAVLV1 as a putative novel virga-like virus from wheat with its genome sequenced, showing a close genetic relationship with the family *Virgaviridae.*

Declarations

Acknowledgments

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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-mJJoI (2019) Chinese wheat mosaic virus: A long-term threat to wheat in China. 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/10.3389/fmicb.2021.715545
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/10.1007/s00705-021-05175-y
16. Redila C D, Prakash V, Nouri S (2021) Metagenomics Analysis of the Wheat Virome Identifies Novel Plant and Fungal-Associated Viral Sequences. Viruses 13: 2457 https://doi.org/10.3390/v13122457
17. Andika I B, Wei S, Cao C (2017) Phytopathogenic fungus hosts a plant virus: A naturally occurring cross-kingdom viral infection. Proceedings of the National Academy of Sciences 114: 12267-12272 https://doi.org/10.1073/pnas.1714916114
18. Bian R, Andika I B, Pang T (2020) Facilitative and synergistic interactions between fungal and plant viruses. Proceedings of the National Academy of Sciences 117: 3779-3788 https://doi.org/10.1073/pnas.1915996117

Figures

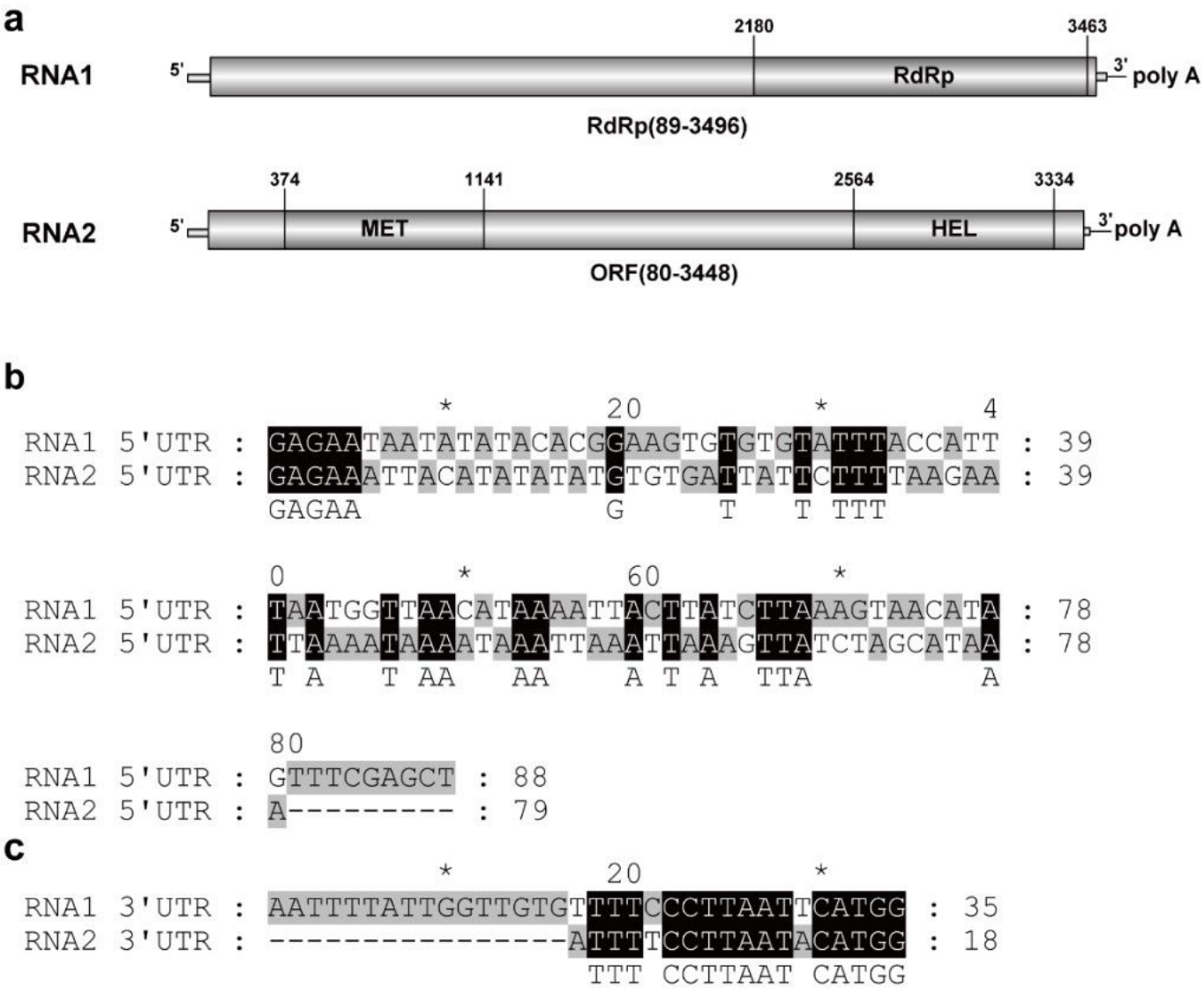

Figure 1

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.

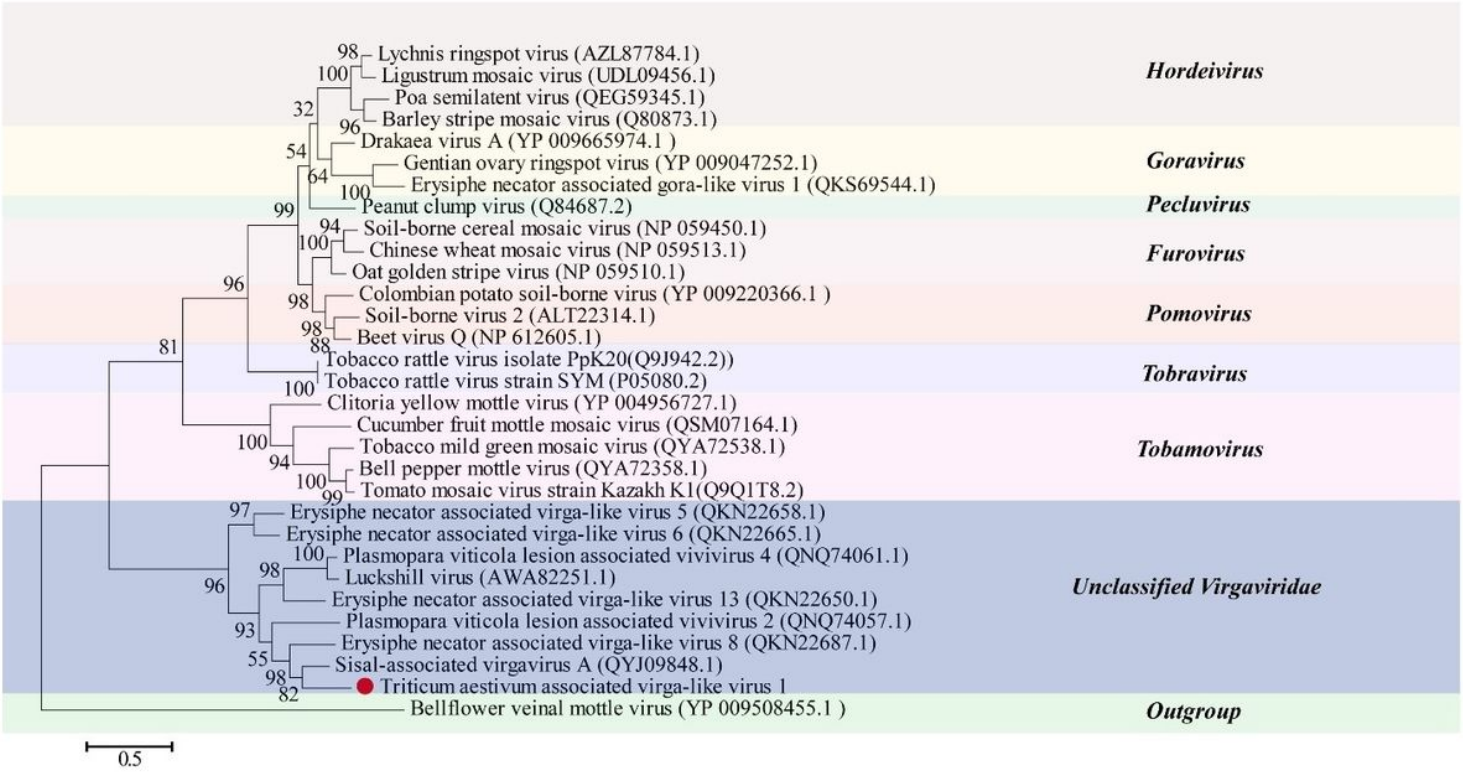

Figure 2

Phylogenetic analysis of TaAVLV1 and representative members of seven subfamilies of *Virgaviridae*. Phylogenetic tree was conducted with Maximum likelihood method (Jones-Taylor-Thornton (JTT) model) based on the amino acid sequences of the conserved RdRp proteins using MEGA v6.0. The identified virus is highlighted in a red circle. Bellflower veinal mottle virus was used as the outgroup. Viral names and GenBank accession numbers of the proteins are displayed on the tree.

Supplementary Files

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