Complete genome sequence of Aconitum amalgavirus 1, a distinct member of the genus Amalgavirus

Jie Yang · Ping-Xiu Lan · Jin-Ming Li · Xiao-Jiao Chen · Guan-Lin Tan · Tai-Yun Wei · Ru-Hui Li · Fan Li

Received: 22 January 2022 / Accepted: 22 May 2022 / Published online: 17 July 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2022

Abstract
A novel virus named Aconitum amalgavirus 1 (AcoAV-1) was identified in Chinese aconite (Aconitum carmichaelii) plants. The complete genome of AcoAV-1 is 3,370 nucleotides long, containing two partially overlapping open reading frames encoding a putative coat protein and a RNA-dependent RNA polymerase, respectively. Its fusion protein shares 34.9%-50.7% amino acid sequence identity with other amalgaviruses. Phylogenetic analysis showed that this virus formed a clade with blueberry latent virus and four other related viruses, suggesting that it belongs to the genus Amalgavirus in the family Amalgaviridae.

Chinese aconite (Aconitum carmichaelii), also known as fuzi or chuanwu, is a member of the family Ranunculaceae that is native to East Asia and an important medicinal plant used for treating inflammation and arrhythmia. Three viruses, Aconitum latent virus (AcLV), aconite virus A (AcVA), and cucumber mosaic virus (CMV), have been reported to infect Aconitum spp. [1–3].

Members of the genus Amalgavirus, family Amalgaviridae, are monopartite, double-stranded RNA viruses [4]. Their genomes are approximately 3.5 kb in length and contain two partially overlapping open reading frames (ORFs) [5]. The upstream ORF1 encodes a putative coat protein (CP), and the downstream ORF2 encodes an RNA-dependent RNA polymerase (RdRp). In addition, a fusion protein, ORF1+2, is predicted to be expressed via a +1 programmed ribosomal frameshifting (PRF) event during the translation of ORF1 [6]. The putative +1 PRF motif sequence UUU_CGN (underline indicates the codon boundary for ORF1; N, any nucleotide) is highly conserved in amalgaviruses and induces a codon boundary change from UUU_CGN (ORF1) to U_UUC_GNN (ORF2) in translation [7]. Members of the genus Amalgavirus are transmitted vertically via seeds and are not thought to be capable of efficient extracellular transmission unless assisted by an unknown vector [8].

In 2017, symptoms of foliar mosaic, mottle, necrosis, and chlorosis were observed on some Chinese aconite plants in a field in Zhanyi County, Yunnan Province, China (Fig. 1c). Symptomatic leaves were collected from each of 17 diseased plants, and a pooled sample of leaf tissues was subjected to high-throughput sequencing (HTS) on an Illumina HiSeq X Ten platform (BioMarker Biotech Co., Ltd, Beijing, China). The RNA reads of 150 bp were analyzed using CLC Genomics Workbench 15 (QIAGEN, Germantown, USA). A total of 13,635,451 paired-end reads were obtained after trimming and used for de novo assembly, which generated 14,423 contigs (>200 nucleotides [nt]). A BLASTx search revealed the presence of several large contigs (>1,000 nt) with different degrees of amino acid (aa) sequence similarity to five viruses, including CMV (99%), AcVA (99%), tobacco vein distorting virus (TVDV, 99%), tomato spotted wilt orthotospovirus (TSWV, 97%) and a novel virus (54%). The novel virus contig of 3,282 nt shared the highest sequence identity...
Characterization of the novel amalgavirus Aconitum amalgavirus 1 (AcoAV-1), isolated from Chinese aconite plants in China. (a) Genome organization of AcoAV-1 and the predicted +1 programmed ribosomal frameshifting (PRF) motif. (b) Maximum-likelihood tree based on the ORF1+ORF2 fusion protein amino acid sequences of AcoAV-1 and other related plant amalgaviruses. The fungus-infecting virus Zygosaccharomyces bailii virus Z (ZbV-Z) of the genus Zybovirus, family Almagaviridae, was used as an out-group. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The scale bar represents a genetic distance of 0.5. AcoAV-1, characterized in this study, is indicated by a solid triangle. (c) Symptomatic Chinese aconite plant infected with AcoAV-1 in the field. (d) Seedling geminated from seed of the infected plants of 54% with blueberry latent virus (BBLV, genus *Amalgavirus*) in the fusion protein.

To verify the presence of the amalgavirus in the diseased plants, total RNA was prepared from each of the 17 HTS samples using an EasyPure Plant RNA Kit (TransGen Biotech, Beijing, China), and a positive sample was selected for complete genome sequence amplification. Four pairs of overlapping primers were designed based on the contig sequence (Supplementary Table S1). RT-PCR was performed using a PrimeScript™ One-Step RT-PCR Kit Ver. 2 (TaKaRa Biotechnology Co. Ltd., Dalian, China). Each of the four amplicons was cloned into the vector pMD19-T (TaKaRa Biotechnology Co. Ltd., Dalian, China). At least three clones of each amplicon were sequenced at both directions (BGI, Guangzhou, China). The 5′ end sequence was amplified using a SMARTer RACE 5′/3′ kit, whereas the 3′ end sequence was obtained using the method described by Lan et al. [9]. The genome sequence was assembled and analyzed using SeqMan in the Lasergene v7.1 package (DNASTAR Inc., Madison, WI, USA). Phylogenetic analysis was performed in MEGA7 by the maximum-likelihood method with 1000 bootstrap replicates. Conserved domains in protein sequences were predicted using the NCBI website https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi.

The complete genome sequence of the new amalgavirus isolate YZYi-WT is 3,370 nt in length (GenBank accession no. MZ389237) and contains two partially overlapping ORFs on its positive strand (Fig. 1a). The 5′ and 3′ untranslated regions (UTR) are 160 nt and 62 nt long, respectively. ORF1 was predicted to start at nt 161 and end at nt 1,262-1,264, encoding a putative CP of 367 aa with a molecular mass of 40.6 kDa. ORF2 was predicted to start at nucleotide position 990, where the conserved +1 PRF (989UUU_CGA 994) motif is located. A frameshift at the +1 PRF motif is predicted to produce a 1,049-aa fusion protein with a molecular mass of 118.7 kDa, whose coding region includes nt 161-988 and nt 990-3,308. The fusion protein contains signature polymerase motifs (active, nucleic acid binding, and NTP binding sites) between residues 542 and 751 with an E-value of 2.76e-12.

Pairwise comparison of fusion protein sequences showed that the new virus shares 34.9% (capsicum annuum amalgavirus 1, NC_040662) to 50.7% (BBLV, NC_014593) sequence identity with those of other amalgaviruses (Supplementary Table S2), which is below the species demarcation threshold of 65-70% [6]. A phylogenetic tree was constructed using the maximum-likelihood method, based on the fusion protein sequences of this virus and other related amalgaviruses. The analysis placed the new virus in the same clade with BBLV and four other related viruses (Fig. 1b). Therefore, this virus should be considered a member of a new species of the genus *Amalgavirus*, and we have tentatively named it “Aconitum amalgavirus 1” (AcoAV-1).
To study the host range and transmission mode of AcoAV-1, seedlings of Chinese aconite, cowpea (*Vigna sinensis*), pepper (*Capsicum annuum*), tomato (*Solanum lycopersicum*), *Nicotiana glutinosa*, *N. rustica*, *N. tabacum* var. K326, and *N. tabacum* var. Xanthi nc were mechanically inoculated with crude sap from the infected plants. Twelve asymptomatic seedlings gminated from seeds obtained from the infected plants were also maintained in an insect-proof greenhouse. These plants were tested by RT-PCR using specific primers of AcoAV-1 and four other viruses detected by HTS. Eight of the twelve aconite seedlings (66.7%) were positive for AcoAV-1 (Fig. 1d), but none tested positive for the four other viruses. Therefore, AcoAV-1 is transmitted by seed, and infected plants are asymptomatic. These characteristics have been observed for other members of the genus *Amalgavirus* [10, 11].

The occurrence of AcoAV-1 in the field was also investigated using the initial 17 HTS samples and an additional 71 symptomatic aconite samples collected from Wuding County. Two of the initial 17 samples and 25 of the additional 71 samples tested positive for AcoAV-1 (30.7%), revealing a high incidence of the virus. The effect of virus infection on crop production is unknown, and the impact of single and mixed infections with the virus and other viruses needs to be examined in the future.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00705-022-05521-8.

**Acknowledgements** This study was funded by the Yunnan Academician Expert Workstation (202005AF150040).

**Declarations**

**Conflict of interest** The authors declare no competing interests.

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

**References**

1. Cohen J, Zeidan M, Rosner A, Gera A (2000) Biological and molecular characterization of a new carlavirus isolated from an *Aconitum* sp. Phytopathology 90(4):340–344
2. Wang R, Chen BW, Li Y, Cao MJ, Ding WL (2021) Complete nucleotide sequence of a new carlavirus infecting *Aconitum carmichaelii* in China. Arch Virol 166(5):1513–1515
3. Fumiyoshi F, Shin-ichi F, Kouichi S, Masahide I (2008) *Cucumber mosaic virus* isolated from *Aconitum* spp. in Japan. J Gen Plant Pathol 74(1):88–905
4. Goh CJ, Park D, Lee JS, Sebastiani F, Hahn Y (2018) Identification of a novel plant amalgavirus (*Amalgavirus, Amalgaviridae*) genome sequence in *Cistus incanus*. Acta Virol 62(2):122–128
5. Sabanadzovic S, Nibert ML, Krupovic M, Tzanetakis IE, Valverde RV (2018) Reorganization of the family *Amalgaviridae* by recognizing five new species in the genus *Amalgavirus* and creating a new genus *Zybavirus*. ICTV. https://talk.ictvonline.org/taxonomy/taxonomy-history?taxnode_id=202106725
6. Nibert ML, Pyle JD, Firth AE (2016) A +1 ribosomal frameshifting motif prevalent among plant amalgaviruses. Virology 498:201–208
7. Dongbin P, Chul JG, Hyein K, Yoonsoo H (2018) Identification of two novel amalgaviruses in the common eelgrass (*Zostera marina*) and *in Silico* analysis of the amalgavirus +1 programmed ribosomal frameshifting sites. Plant Pathol J 34(2):150–156
8. Sabanadzovic S, Valverde RA, Brown JK, Martin RR, Tzanetakis IE (2009) Southern tomato virus: the link between the families *Totiviridae* and *Partitiviridae*. Virus Res 140(1–2):130–137
9. Lan PX, He P, Zhang YK, Zhang S, Zhang ZB, Chen XJ, Tan ST, Luo HM, Cao MJ, Li F (2019) Molecular characterization of a novel potyvirus infecting noni. Arch Virol 164(12):3099–3102
10. Martin RR, Zhou J, Tzanetakis IE (2010) Blueberry latent virus: an amalgam of the *Partitiviridae* and *Totiviridae*. Virus Res 155(1):175–180
11. Park D, Hahn Y (2017) Genome sequences of Spinach delta-partitivirus 1, Spinach amalgavirus 1, and Spinach latent Virus identified in spinach transcriptome. J Microbiol Biotechnol 27(7):1324–1330

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.