Molecular characterization of a putative alphapartitivirus from Impatiens balsamina L.

Yun Zheng
Yangtze University
Daoshun Zhang
Yangtze University
Haoran Wang
Yangtze University
Yan Wei
Yangtze University
Hongliu An
Yangtze University
Jianyu Gou
Zunyi City Company, Guizhou Tobacco Company
Shouguo Fang
Yangtze University
Xiaoyan Wang
Zunyi City Company, Guizhou Tobacco Company
Songbai Zhang (yangtze2008@126.com)
Yangtze University https://orcid.org/0000-0002-8425-1434

Research Article

Keywords: Partitiviridae, alphapartitivirus, impatiens cryptic virus 1

Posted Date: April 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1533276/v1

Abstract

Two double stranded RNAs (dsRNAs) that likely represent the genome of an alphapartitivirus tentatively named impatiens cryptic virus 1 (ICV1) were recovered from a symptomless *Impatiens balsamina* L.. DsRNA1 (2008 bp) codes for the RNA-dependent RNA polymerase (RdRp) of ICV1, which shares <83% amino acid sequence identities with the RdRp of other alphapartitiviruses. DsRNA2(1906 bp) codes for the capsid protein (CP) of ICV1, which shares <60% amino acid sequence identities with the CP of other alphapartitiviruses. Phylogenetic analysis suggested that ICV1 is closely related to plant alphapartitiviruses including vicia cryptic virus, beet cryptic virus 1, carrot cryptic virus and white clover cryptic virus 1. Reverse-transcription polymerase chain reaction with primers specific for dsRNA1 or dsRNA2 showed that ICV1 is common in *I. balsamina* from a wide range of places of China.

Full Text

The family *Partitiviridae* includes five genera. Members of *Alphapartitivirus* and *Betapartitivirus* infect either plants or fungi, whereas those of *Cryspovirus*, *Deltapartitivirus* and *Gammapartitivirus* have been found only from protozoa, fungi and plants, respectively [1]. The genome of a partitivirus consists of two essential double stranded RNAs (dsRNA) totaling 3–4.8 kbp in size. Each dsRNA is encapsidated separately in a non-enveloped isometric virion measuring 25–43 nm in diameter.

Plant partitiviruses show several features different from “conventional” viruses. First, partitiviruses lack a movement protein. Their spread within the host occurs only during cell division. Secondly, partitiviruses cannot be transmitted by mechanical means, nor by grafting or any vectors. Thirdly, partitiviruses do not cause apparent symptoms [3]. Owing to these properties, plant partitiviruses had been considered of little importance. In recent years, however, several lines of evidence suggest that partitiviruses may confer selective advantages to their host plants: (a) the capsid protein (CP) of white clover cryptic virus 1 (WCCV1) seems to suppress nodule formation [4-6]; (b) the RNA-dependent RNA polymerase (RdRp) of a probable deltapartitivirus may be involved in salinity tolerance in ryegrass [7]; (c) Pepper cryptic virus 1 (PCV-1) affects the transmission a “conventional” virus named cucumber mosaic virusby manipulating the behavior of aphids [8].

Here, we describe two dsRNAs from *Impatiens balsamina* L. The two dsRNAs likely represent the genome of an alphapartitivirus, for which the name impatiens cryptic virus 1 (ICV1) was tentatively given.

A healthy *I. balsamina* was collected from Jingzhou, Hubei Province of China, in September 2018. A procedure described by Morris and Dodds was used to extract dsRNAs from the plant [9]. After sequential treatment with DNase I and S1 nuclease (TAKARA Dalian, China), the dsRNAs were electrophorized in a 1% TBE gel, which revealed two bands very similar in size (about 2 Kbp, Fig. 1A). The two bands were recovered as a mixture with the TIANgel Midi Purification Kit (Tiangen, China). Complementary DNA (cDNA) fragments obtained with a single primer amplification (SPAT) procedure were cloned and sequenced as described previously [10]. Contigs were assembled with DNAstar and gaps were filled by

PCR with specific primers. The 5’ and 3’ termini of the dsRNAs were determined by RLM-mediated rapid amplification of cDNA ends. The sequences of the two dsRNAs (dsRNA1 and dsRNA2) were deposited in GenBank under the accession numbers MW553845 and MW553846, respectively.

DsRNA1 and 2 comprise 2008 and 1906 nucleotide base pairs (bp), respectively. Both dsRNA1 and 2 contain a large open reading frame (ORF). The dsRNA1 ORF starts from nucleotide position 94 and ends at position 1944, whereas the dsRNA2 ORF spans nucleotide positions 119-1944 (Fig. 1B). The 5’ non-translated regions (NTRs) preceding the dsRNA1 or 2 ORFs are almost identical, with the exception that the latter is 25 nucleotides longer than the former (Fig. 1C). They also show apparent similarities to the 5’ NTRs of vicia cryptic virus (VCV) [11], beet cryptic virus 1 (BCV1) [12] and carrot cryptic virus (CCV) [13]. The 3’ NTRs differ substantially: while that of dsRNA1 consists of only 64 nucleotides, that of dsRNA2 is as long as 321 nucleotides (Fig. 1C). Nevertheless, both 3’ NTRs have a long stretch of A at their 3’ termini, which mimics the poly(A) tract of eukaryotic mRNAs and seems to be common to alphapartitiviruses [1, 2].

In searches of the NCBI non-redundant protein database, the top 100 Blastp hits of the protein predicated from the dsRNA1 ORF can be divided into two groups. Group 1 hits share 80%-83% amino acid sequence identities, whereas group 2 hits share <68% amino acid sequence identities with the predicated ICV1 protein. With few exceptions, group 1 hits are the RdRp of plant alphapartitiviruses, whereas group 2 hits are the RdRp of fungal alphapartitiviruses. These observations suggest that the protein encoded by the dsRNA1 ORF is the RdRp ICV1. In line with this, a conserved domain search found a RNA-dependent DNA polymerase domain (cl02808, E-value, 3.97e-14) from amino acid residue 243 to 548 of this protein [14].

Like the situation for dsRNA1, the top 100 Blastp hits of the protein predicated from the dsRNA2 ORF can be divided into two groups. Group 1 hits share 48% to 60% acid sequence identities with the ICV1 protein, whereas for Group 2 hits, the values are smaller than 48%. Group 1 and 2 hits are dominated by the CP of plant and fungal alphapartitiviruses, respectively. Noteworthily, several “non-viral” proteins are included in Group 2 hits of ICV1 CP, of particular interest is a *Helianthus annuus* protein named IAA-leucine resistant 2 (XP_022025252).

An additional ORF was predicted from nucleotide 735 to 1331 of dsRNA2. This ORF is in the same strand with the major ORF but in a different frame. Blastp searches with the protein encoded by this ORF revealed no similarity. Additional ORFs are not uncommon for plant alphapartitiviruses [11, 12]. As these ORFs are not conserved, they may be artificial. However, a virus-specific role of these ORFs deserves further consideration.

Given the features described above and according to the criteria suggested by the International Committee on Taxonomy of Viruses (RdRp identity ≤90%, CP identity ≤80%), ICV1 can be considered as a novel species of the genus *Alphapartitivirus* (www.ictv.global/report/partitiviridae). Supporting this, ICV1 was placed in a clade comprised by alphapartitiviruses in a phylogeny inferred using the aligned RdRp amino acid sequences of ICV1 and selected partitiviruses (Fig. 2). Within this clade, ICV1 formed a subclade together with 4 plant alphapartitiviruses, namely BCV1, CCV, VCV and WCCV. It is interesting to

note that the 5’ NTRs of ICV1, BCV1, CCV and VCV, but not that of WCCV, share a 5’-GAUCAAAA sequence at the 5’ extremity.

To see whether ICV1 is common, *I. balsamina* seeds were commercially obtained from 4 different suppliers from Beijing, Shaoyang (Hunan Province China), Shenyang (Liaoning Province of China) and Hohhot (the Nei Monggol Autonomous Region of China), respectively. ICV1 was detected from 28 seedlings from the seeds of each supplier by RT-PCR with 2 ICV1 specific primer pairs. The results showed that 100% of the seeds from Beijing, ShaoYang and Shenyang contain ICV1. However, seeds from Hohhot were tested negative for ICV1.

As far as we know, this is the first report of a probable alphapartitivirus from *I. balsamina*. The data here may be valuable in understanding the diversity and evolution of plant partitiviruses.

Declarations

Compliance with Ethical Standards:

Funding: This work was supported by the Science and Technology Project of Guizhou tobacco company （No. 201921) and the National Natural Science Foundation of China (No. 31972243). We are grateful to Dr. Fangluan Gao and Zhenguo Du from Fujian Agriculture and Forestry University for their help in preparing the manuscript.

Conflict of Interest: All authors declare that they have no conflict of interest.

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

References

1. Vainio EJ, Chiba S, Ghabrial SA, Maiss E, Roossinck M, Sabanadzovic S, Suzuki N, Xie J, Nibert M (2018) ICTV Virus Taxonomy Profile: *Partitiviridae*. J Gen Virol 99(1):17–18& Ictv Report Consortium
2. Nibert ML, Ghabrial SA, Maiss E, Lesker T, Vainio EJ, Jiang D, Suzuki N (2014) Taxonomic reorganization of family *Partitiviridae* and other recent progress in partitivirus research. Virus Res 188:128–141
3. Boccardo G, Lisa V, Luisoni E, Milne RG (1987) Cryptic plant viruses. Adv Virus Res 32:171–214
4. Boccardo G, Candresse T (2005) Complete sequence of the RNA2 of an isolate of *white clover cryptic virus 1*, type species of the genus *Alphacryptovirus*. Arch Virol 150(2):403–405
5. Suzuki A, Kobayashi F, Abe M, Uchiumi T, Higashi S (2001) Cloning and expression of a down-regulated gene (TrEnodDR1) of white clover responded by thenod genes derived from *Rhizobium leguminosarum bv. trifolii* strain 4S. 266:77–84Gene1

6. Nakatsukasa-Akune M, Yamashita K, Shimoda Y, Uchiumi T, Abe M, Aoki T, Kamizawa A, Ayabe S, Higashi S, Suzuki A (2005) Suppression of root nodule formation by artificial expression of the TrEnodDR1 (coat protein of *white clover cryptic virus 1*) gene in *Lotus japonicus*. Mol Plant Microbe Interact 18:1069–1080
7. Li H, Hu T, Fu J (2012) Identification of genes associated with adaptation to NaCl toxicity in perennial ryegrass (*Lolium perenne* L.). Ecotoxicol Environ Saf 79:153–162
8. Safari M, Ferrari MJ, Roossinck MJ (2019) Manipulation of aphid behavior by a persistent plant virus. J Virol 93(9):e01781–e01718
9. Morris TJ, Dodds JA (1979) Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69:854–858
10. Guo L, Yang X, Wu W, Tan G, Fang S, Zhang S, Li F (2016) Identification and molecular characterization of *Panax notoginseng virus A*, which may represent an undescribed novel species of the genus *Totivirus*, family *Totiviridae*. Archives of virology, 161(3), 731–734
11. Blawid R, Stephan D, Maiss E (2007) Molecular characterization and detection of *vicia cryptic virus* in different *Vicia faba* cultivars. Arch Virol 152(8):1477–1488
12. Szegő A, Enünlü N, Deshmukh SD, Veliceasa D, Hunyadi-Gulyás É, Kühne T, Ilyés P, Potyondi L, Medzihradszky K, Lukács N (2010) The genome of *beet cryptic virus 1* shows high homology to certain cryptoviruses present in phylogenetically distant hosts. Virus Genes 40(2):267–276
13. Willenborg J, Menzel W, Vetten HJ, Maiss E (2009) Molecular characterization of two alphacryptovirus dsRNAs isolated from *Daucus carota*. Arch Virol 154(3):541–543
14. Lu S, Wang J, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Marchler GH, Song JS, Thanki N, Yamashita RA, Yang M, Zhang D, Zheng C, Lanczycki CJ, Marchler-Bauer A (2020) CDD/SPARCLE: the conserved domain database in 2020. Nucleic Acids Res 48(D1):D265–D268
15. Nguyen LT, Schmidt HA, Von Haesele A, Quang MB (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular biology and evolution, 32(1), 268–274

Figures

Figure 1

Characterization of two dsRNAs from *I. balsamina* (A) Agarose gel electrophoresis of the two dsRNAs. M, molecular maker. (B) ORF arrangement of the dsRNA1 and dsRNA2. (C) the 5’ and 3’ non-translated regions (NTR) of dsRNA1 and dsRNA2.

Figure 2

A phylogenetic tree showing the relationship between ICV1 and other partitiviruses. The maximum likelihood phylogenies were inferred using the viral RdRp amino acid sequences with IQ-TREE under the VT+I+G4+F model for 10000 ultrafast bootstraps [15].

Supplementary Files

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

- renamede85b3.pdf