Molecular characterization of a putative alphapartitivirus from *Impatiens balsamina* L

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Received: 7 April 2022 / Accepted: 6 June 2022 / Published online: 13 July 2022
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Abstract

Two double stranded RNAs (dsRNAs) that likely represent the genome of an alphapartitivirus, tentatively named “impatiens cryptic virus 1” (ICV1), were recovered from *Impatiens balsamina* L. RNA1 (2008 bp) codes for the RNA-dependent RNA polymerase (RdRp) of ICV1, which shares <83% amino acid sequence identity with the RdRps of other alphapartitiviruses. RNA2 (1906 bp) codes for the coat protein (CP) of ICV1, which shares <60% amino acid sequence identity with the CPs 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. Using primers specific for RNA1 or RNA2, ICV1 could be detected in *I. balsamina* from various parts of China.

Partitiviruses constitute a family of double-stranded RNA (dsRNA) viruses that infect plants, fungi, or protozoa [1]. The genome of a partitivirus consists of two essential dsRNAs, each of which is encapsidated separately in a non-enveloped isometric virion with a diameter of 25–43 nm [1, 2]. The two dsRNAs differ slightly in size. Normally, the larger one codes for the RNA-dependent RNA polymerase (RdRp), whereas the smaller one codes for the coat protein (CP) [1, 2]. In plants, partitiviruses do not cause apparent symptoms [2, 3]. However, the CP of white clover cryptic virus 1 (WCCV1) seems to regulate nodule formation in white clover and *Lotus japonicus* [4–6]. Moreover, pepper cryptic virus 1 (PCV-1) was recently found to affect the transmission of cucumber mosaic virus, an important disease-causing virus for pepper, by altering aphid behavior [7]. These observations suggest that the importance of plant partitiviruses may have been underestimated.

Here, we describe a putative novel partitivirus from *Impatiens balsamina* L., which was tentatively named "impatiens cryptic virus 1" (ICV1).

A non-symptomatic *I. balsamina* plant was collected in Jingzhou, Hubei Province, China, in September 2018. dsRNA was extracted from this plant using the procedure described by Morris and Dodds [8] and subjected to electrophoresis in a 1% TBE agarose gel after treatment with DNase I and S1 nuclease (TAKARA Dalian, China). The two dsRNA bands (both about 2 kbp in size, Fig. 1A) were recovered from the gel using a TIANgel Midi Purification Kit (Tiangen, China) and reverse transcribed using the primer 5'-CGA TCG ATC ATG ATG CAA TGC, and selected PCR fragments were cloned in *Escherichia coli* and sequenced by the Sanger method as described previously [9]. Sequence contigs (Supplementary Fig. S1) were assembled using DNAAstar. The 5' and 3' termini of the dsRNAs were determined by RLM-mediated rapid amplification of cDNA ends. The complete sequences of the two RNAs (RNA1 and RNA2) were
deposited in the GenBank database under the accession numbers MW553845 and MW553846, respectively.

RNA1 and RNA2 are 2008 and 1906 nucleotides in length, respectively. RNA1 contains an open reading frame (ORF) initiating at nt 94 and terminating at nt 1944, whereas RNA2 has an ORF spanning the region from nt 119 to 1585 (Fig. 1B). The 5' non-translated regions (NTRs) of RNA1 and RNA2 are almost identical, with the exception that the latter is 25 nucleotides longer than the former (Fig. 1C). They also show notable similarity to the 5' NTRs of vicia cryptic virus (VCV) [10], beet cryptic virus 1 (BCV1) [11], and carrot cryptic virus (CCV) [12] (Supplementary Fig. S2). The 3' NTRs differ substantially in size; whereas that of RNA1 consists of 64 nucleotides, that of RNA2 consists of 321 nucleotides. Nevertheless, both 3' NTRs have a long stretch of A (Fig. 1C), which mimics the poly(A) tract of eukaryotic mRNAs, and this seems to be a common feature of alphapartitiviruses [1, 2].

BLASTp searches of the NCBI non-redundant protein sequence database were performed. The protein encoded by RNA1 showed 80%-83% amino acid sequence identity to the RdRps of plant partitiviruses belonging to the genus Alphapartitivirus. This suggests that RNA1 codes for the RdRp of ICV1. In line with this, a conserved domain search found an RNA-dependent RNA polymerase domain (cl02808, E-value, 3.97e-14) spanning the region from amino acids 243 to 548 [13]. The protein encoded by RNA2 shared 48%-60% amino acid sequence identity with the CPs of some alphapartitiviruses. This suggests that RNA2 codes for the CP of ICV1.

To determine the taxonomic position of ICV1, a phylogenetic tree was constructed using the aligned RdRp amino acid sequences of selected partitiviruses (Fig. 2). As expected, the partitiviruses were separated into five major clades, each corresponding to a distinct genus of the family Partitiviridae [1, 2]. ICV1 was placed in a clade comprised of alphapartitiviruses. Within this clade, ICV1 formed a subclade together with four plant alphapartitiviruses, namely BCV1, CCV, VCV, and WCCV1 (Fig. 2).

Given the observations 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 a novel partitivirus of the genus Alphapartitivirus (www.ictv.global/report/partitiviridae). To determine whether ICV1 is commonly present in I. balsamina, I. balsamina seeds were obtained commercially from four different suppliers from Beijing, ShaoYang (Hunan Province China), Shenyang (Liaoning Province of China), and Hohhot (the Nei Monggol Autonomous Region of China). Twenty-eight seedlings from the seeds from each supplier were tested for ICV1 by RT-PCR using two specific primer pairs (Supplementary Table S2). The results showed that 100% of the seeds from Beijing, ShaoYang, and Shenyang contained ICV1. However, the seeds from Hohhot tested negative for ICV1.

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

**Fig. 2** 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 with 10,000 ultrafast bootstrap replicates [14]. The full names of the viruses are presented in Supplementary Table S1. The bar represents the number of substitutions per site.
Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s00705-022-05537-0.

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).

Declarations

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.

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