Complete Genome Sequence of a Novel Latent Tobamovirus Infecting *Hevea Brasiliensis* in China

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Research Article

Keywords: Hevea brasiliensis, Tobamovirus, rubber tree latent virus 1 (RTLTV1), genome sequence, China

Posted Date: April 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-359778/v1>

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Abstract

In this work, we describe the complete sequence and genome organization of a novel tobamovirus detected in rubber tree (*Hevea brasiliensis*) by high-throughput RNA-seq. The infected plants show no identifiable symptoms, therefore this virus is tentatively named rubber tree latent virus 1 (RTLTV1). The full genome of RTLTV1 is 9,422 nt in length and contains three open reading frames (replicase, movement, and coat proteins) with 157 nt 5' UTR and 316 nt 3' UTR. The replicase encoded by ORF1 shares only 33.29% amino acid sequence identity with that of the closest related Frangipani mosaic virus. Phylogenetic analysis using the ORF1 amino acid sequence clusters RTLTV1 with members of genus Tobamovirus (family Virgaviridae) at relative high bootstrap, suggesting that RTLTV1 is a novel member of Tobamovirus.

Background

Rubber tree (*Hevea brasiliensis*) is an important crop in tropical area and a major commercial source of natural rubber. Vegetative propagation in traditional rubber tree plantation is prone to accumulating viruses, but only a few viruses have been identified in rubber tree so far [4, 8]. Through RNA-seq, de novo assembly and annotation as previous description [8], several assembled unigenes identified in latex samples of rubber tree were annotated as tobacco mosaic virus (TMV), one of which was 9,361nt long, suggesting that the rubber tree sample was infected by a TMV-like virus. TMV is a member of tobamoviruses genus of family Virgaviridae. TMV has had an illustrious history for more than 100 years and been known to infect members of nine plant families, and at least 125 individual species, including a number of nightshades, cucurbits, beans and ornamental flowers. However, TMV has never been reported in rubber tree. The identified virus might be a novel member of tobamovirus.

Virgaviridae is a plant virus family that currently includes 59 types of viruses in 7 genera: Furovirus, Goravirus, Hordeivirus, Pecluvirus, Pomovirus, Tobamvirus and Tobravirus [1, 2]. Virgaviridae was named because its members have rod-shaped virions (Latin virga means rod). The seven genera are classified according to their distinct genome organisations and other features such as transmission modes. Tobamovirus has no natural vector and the transmission is usually mechanical, whereas other six genera have biotical vectors such as pollen (Goravirus and Hordeivirus), or seed (Hordeivirus), or nematode (Tobravirus), or plasmodiophorid (Furovirus, Pomovirus and Pecluvirus). The genome of Tobamovirus is monopartite, linear, single stranded and positive sense RNA, which is distinct from multi-partites genome of other 6 genera [1, 2].

In order to clone the entire genome of the virus, eight fragments nearly covering the complete genome were amplified by RT-PCR for subsequent Sanger sequencing, and the terminal fragments were obtained by 5' and 3' rapid amplification of cDNA ends (RACE) using a RACE 5'/3' kit, 2nd Generation (Roche, Indianapolis, IN) according to the manufacturer's instructions (Fig. 1). The ORFs were predicted using the ORF Finder (www.ncbi.nlm.nih.gov/orffinder) and the conserved domains were analyzed online with default settings (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The complete genome consists of

9,422 nucleotides (Nt) (submission number: 244064), and the G/C content is 39%. RTLTV1 has a typical tobamovirus organization with a 5' untranslated region (UTR, 157 nt) and 316-nt 3' UTRs (Figure 1). The viral genome has three open reading frames (ORFs). ORF1 (nt 158-7963) encodes a 297.9 kDa replicase. Conserved domain analysis showed that ORF1 (nt 158-7963) is putative replicase containing four known domains: methyltransferase (307-670 aa), 2OG-Fe(II) oxygenase (1406-1508 aa), RNA helicase (1782-2049 aa), and RNA-dependent RNA polymerase (RdRP; 2139-2584 aa) (Figure 1). ORF2 (nt 7976-8635) encodes a putative 24.5kD movement protein, and ORF3 (nt 8639-9106) encodes a putative 17.82kD coat protein (155aa). Blastp of ORF1 against non-redundant proteins sequences (nr) revealed the most related replicase protein (APD13852.1) of tobamovirus Frangipani mosaic virus, but amino acid sequence identity is as low as 33.29% with only 46% coverage. The deduced amino acid sequences of putative MP and CP show less sequence homology with those of members of recognized tobamovirus species, suggesting that RTLTV1 is novel member of Virgaviridae virus. To further examine the relationship between RTLTV1 and other Virgaviridae viruses, the amino acid sequences of replicase proteins from 36 members of seven genera in the family Virgaviridae were selected for phylogenetic tree construction and analysis (Figure 2). The phylogenetic tree was constructed with MEGA6. RTLTV1 was clustered in Tobamovirus with relative high bootstrap, suggesting that RTLTV1 is much distantly related to other member and is a novel member of Tobamovirus.

Agrobacterium tumefaciens-mediated transformation has been widely used in plant genetics research [10, 11] and has been successfully applied in rubber tree [5], but *Agrobacterium*-mediated transformation has rarely been used in the research of rubber tree due to the low transformation efficiency and time cost. Virus-induced gene silencing (VIGS) allows the analysis of gene functions in species that are not amenable to stable genetic transformation, such as many non-model organisms including rubber tree [3]. Very recently, tobacco rattle virus (TRV) was reported to induce VIGS in rubber tree [7], which provides a mean to analyze the functions of candidate genes identified in rubber tree. However, rubber tree is not the natural host of TRV, whether serious side effects could be caused by TRV infection still requires further investigation. RTLTV1 was detected in many “healthy” rubber trees without any visible symptoms; therefore RTLTV1 was regarded as a latent virus of rubber tree. Like Apple latent spherical virus (ALSIV) [6, 9], RTLTV1 has the potentiality to be developed as a vector for expression of foreign proteins, or VIGS, or virus-based vaccines in rubber tree.

Declarations

Acknowledgments

This study was supported by the Chinese National Natural Science Foundation (no. 31860194 and 31860222).

Compliance with ethical standards

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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Figures

Figure 1

Schematic representation of RT-PCR fragments and the genome organization of rubber tree latent virus 1 (RTLTV1). MTR, RNA methyltransferase; HEL, RNA helicase; RdRp, RNA-dependent RNA polymerase; CP, coat protein; MP, movement protein.

Figure 2

Phylogenetic tree based on the deduced amino acid sequence of the RTLTV1 ORF1 and that of 36 members of the family Virgaviridae. Multiple alignment was carried out with CLUSTALX 1.8.1 (<http://www.clustal.org>), and a phylogenetic tree was constructed using MEGA7.0.20. Accession numbers and virus names are given directly in the phylogenetic tree.

Supplementary Files

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