

Identification and molecular characterization of a novel species of the genus Totivirus from *Areca catechu* L.

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Abstract

In previous work, RNA-seq was applied to identify the causal agent of yellow leaf disease (YLD) of areca palm (*Areca catechu* L.), besides a YLD related areca palm velarivirus 1 (APV1), a totivirus-like virus annotated as *Panax notoginseng virus A* (PnVA) was identified. Because the virus was also detected in many asymptomatic areca palm, it was therefore tentatively named Areca palm latent virus 1 (APLV1). The complete sequence of APLV1 was determined to be 4754 base pairs (bp) in length. Two deduced proteins encoded by APLV1 have 55% and 69 % amino acid (aa) sequence identity with CP and RNA-dependent RNA polymerase (RdRp) of *Bursera graveolens* associated totivirus 1 (BgAT1), respectively. Phylogenetic analysis based on alignment of the CP and RdRp sequences clustered APLV1 with other members of the genus *Totivirus*, suggesting that APLV1 might be a novel species of the genus *Totivirus*, family *Totiviridae*.

Full Text

Totiviridae is a family containing a non-segmented dsRNA genome with at least two open reading frames (ORFs) on the positive strand. ORF1 usually encodes the capsid protein (CP), while ORF2 encodes the RNA-dependent RNA polymerase (RdRp). Some *Totiviridae* viruses' genomes encode three ORFs [7]. To date, 28 species of totiviruses have been ratified by ICTV and classified into five genera, namely, *Giardiavirus*, *Leishmanivirus*, *Trichomonasvirus*, *Victorivirus* and *Totivirus* [9]. Members of two genera (*Victorivirus* and *Totivirus*) of the family *Totiviridae* infect fungal host [5, 8]. So far, all of the ICTV-ratified members of the genus *Totivirus* infect fungi [9]. However, recent reports have shown that many novel members of genus *Totivirus* have been detected in wide range of plants other than fungi [1, 2, 4, 6, 11, 12].

Areca palm (*Areca catechu* L.) is an economically important crop widely cultivated in South-east Asia area. Yellow leaf disease (YLD) is a destructive disease serially threaten the plantation of areca palm. In order to identify the potential causal agent of YLD, RNA-seq and differentially expressed genes (DEGs) were applied to analyze the YLD samples, leading to the discovery of areca palm velarivirus 1 (APV1) and the association of APV1 with YLD [10]. The RNA-seq has also identified a unigene annotated as *Panax notoginseng virus A* (PnVA), a double-stranded RNA (dsRNA) virus isolated from *Panax notoginseng* [6]. Since it was detected in both YLD samples and health control, suggesting that it was likely not involved in YLD. Further investigation revealed that this virus could be detected in the vast majority of the asymptomatic areca palm in Hainan, China (data not shown), it was therefore tentatively named as Areca palm latent virus 1 (APLV1).

The genome of APLV1 was determined by RT-PCR and 5' and 3' RACE. The complete sequence is 4754 base pairs (bp) in length (GenBank accession no. ??). APLV1 has a G + C content of 37.7 %. Two open reading frames (ORF1 and ORF2) was identified in APLV1 genome by ORF finder function of the SnapGene software (<https://www.snapgene.com>). The 5'-proximal ORF starts from nucleotide (nt) 47 and ends at nt 2131, encoding a protein of 78.96 kDa. The 3'-proximal ORF begins from nt 2530 and terminates at nt 4722, encoding a protein of 83.63 kDa. Two predicted ORFs were separated by 398 nt

and two untranslated regions (UTR), e.g. a 46 nt 5'-UTR and a 32 nt 3'-UTRs (Fig. 1A). If the AUG start codon of the second ORF was ignored, the reading frame of ORF 2 could be extended upward to nt 2002. Correspondingly, ORF 1 and ORF 2 would be overlapped by 130 nt with each other. Furthermore, heptanucleotide ₂₀₄₄GGGTTTT₂₀₅₀, a putative slippery site, was found within the overlapped sequence. If a -1 frameshift occurs here, a fusion protein with 1557 aa and estimated molecular weight of 178.17 kDa would be translated (Fig.1A). Similar ribosomal frameshift mechanism was reported in other *Totivirus* species, such as PnVA, tea-oil camellia-associated totivirus 1 (TOCaTV1) and maize-associated totivirus (MATV) [4, 6, 12].

The conserved domains of two ORFs of APLV1 were searched online by domain finder of NCBI (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). A conserved domain named LA-virus_coat super family (pfam09220, evalue = 6.29e-89) was found in APLV1 ORF 1. The putative coat protein is most similar to the coat protein of BgAT1 (GenBank accession no. WEL36414.1) with a 55 % aa sequence identity (query coverage 98 %, E-value 0.0) and coat protein of Loquat associated totivirus 1 (LAT1, GenBank accession no. UUY86182.1) with a 45 % aa sequence identity, respectively. Another conserved domain named RT_like superfamily (cl02808, RNA-dependent DNA polymerase) was found in APLV1 ORF 2 (E-value: 1.06e-65). The putative RdRP protein shares as high as 69 % aa identities (69%) and query coverage (100%) with RdRp of BgAT1 (GenBank: WEL36415.1) and Koper totivirus 1 (GenBank: UTQ50479.1). However, these genetically similar totivirus have not been ratified by ICTV. The members of ICTV approved species of the genus *Totivirus*, such as *Saccharomyces cerevisiae* virus L-A and *Tuber aestivum* virus 1, share only as low as 40 % identity of RdRp aa sequence with ORF 2 of APLV1.

Using aa sequences of CP proteins from representative viruse members of five genera of the family *Totiviridae*, a phylogenetic tree was constructed in the MEGA 6.0 program using the maximum-likelihood method based on the LG+G model. Sequence comparisons and phylogenetic analysis clearly demonstrate that APLV1 was most closely related with BgAT1 and Koper totivirus 1. APLV1 was clustered with all the members of the genus *Totivirus* and obviously separated from members of other four genera of family *Totiviridae* (Fig. 1B). Similar clustering was revealed by the phylogenetic trees based on the amino acid sequences of RdRP (Fig. S1), suggesting that APLV1 is a novel member of the genus *Totivirus*.

Totiviruses have been identified in a wide range of fungal hosts. At present, all of the ICTV-authorized members of the genus *Totivirus* infect fungi [9]. The possibility that the host of APLV1 is a fungus infected areca palm could not be ruled out. However, no indicative symptoms of a fungal infection were observed on the leaf samples used to isolate APLV1. Additionally, many novel totiviruses have been identified from plants, including PnVA from *Panax notoginseng* [6], Black raspberry virus F (BrVF) from black raspberry (GenBank accession no. NC_009890), MATV from maize [1, 2, 4], TOCaTV1 from tea oil trees [12], Ribes virus F (RvF) from gooseberry (GenBank accession no. ACA61232), Papaya meleira virus (PMeV) from papaya [3]. A number of totiviruses cause disease symptoms on plant hosts [3, 6]. Furthermore, the phylogenetic tree has clustered APLV1 more closely with totiviruses identified from the plant rather than the totiviruses from fungi. Thus, APLV1 is presumed to be a plant virus. Recently, a full-

length infectious cDNA clone of a totivirus-like virus, the Omono River virus (OmRV), was successfully constructed and transformed into host cell, presenting strong cytopathic effects, infectivity and similar morphology [11]. Therefore, constructing an infectious clone of APLV1 and transforming into protoplast, on the one hand, could verify whether APLV1 is plant virus, on the other hand, could create a transient expression vector for areca palm.

Declarations

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Competing Interests

The authors declare that they have no conflict of interest.

Ethical standards

This article does not contain any research involving human or animal participants.

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Figures

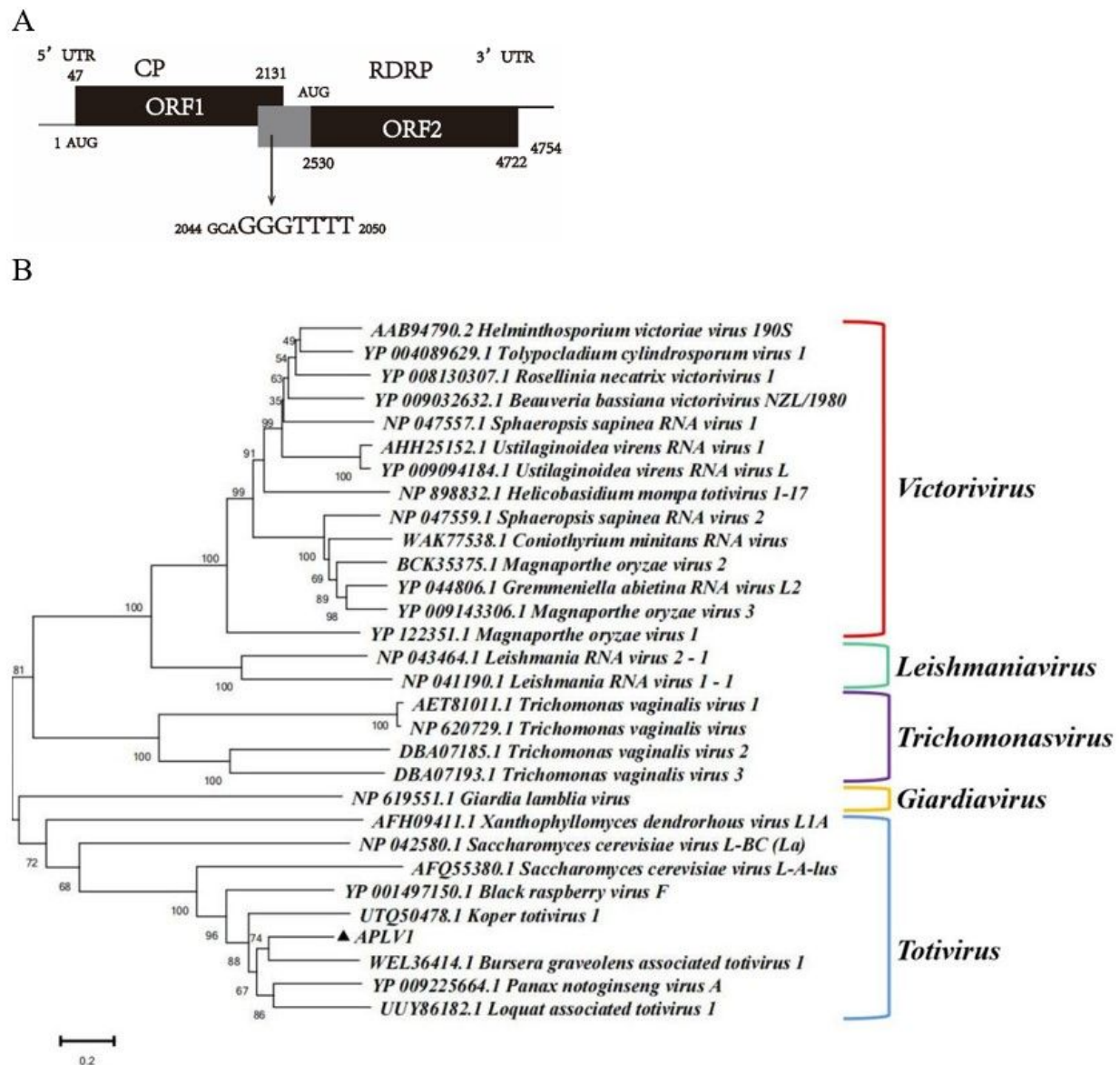


Figure 1

(A) Genomic organization of Areca palm latent virus 1 (APLV1). The open reading frames encoding the CP and RdRp proteins of the virus are represented by rectangular boxes, two conserved domains are indicated by gray boxes, and an arrow indicates the putative slippery heptamer where the -1 frameshift is predicted to occur. (B) Phylogenetic trees based on sequences of APLV1 and selected members of the family Totiviridae, generated by the maximum-likelihood method based on the LG+G model. Phylogenetic trees are shown for the amino acid sequences of CP. The solid triangle dot indicates the virus characterized in this study.

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