Molecular characterization of a novel cytorhabdovirus infecting Plumbago indica L.

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Research Article

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Abstract

In 2021, *Plumbago indica* L. plants showing leaves necrotic spots were observed in Beijing, China. Through high-throughput sequencing (HTS), we discovered a putative novel *Cytorhabdovirus*, which was provisionally named plumbago necrotic spot associated virus (PNSaV). The full-length genome of negative-sense single-stranded RNA comprises 13,180 nucleotides and contains eight putative open reading frames (ORFs), in the order of 3' leader-N-P'-P-P3-M-G-P6-L-5' trailer. The phylogenetic analysis and pairwise comparisons suggested that PNSaV is closely related to chrysanthemum yellow dwarf associated virus (CYDaV), with 57.18% nucleotide sequence identity in the complete genome level and 53.00% amino acid sequence identity in the L protein level. These findings suggest PNSaV to be considered as a new member of the genus *Cytorhabdovirus*.

Main Text

Cytorhabdovirus belongs to the family *Rhabdoviridae*, with negative-sense, single-stranded RNA. The genomes of *Rhabdoviridae* are approximately 10-16 kb in size, which can infect both plants and animals [1]. Generally, the genomes of cytorhabdoviruses have 12.2-14.5 kb nucleotides and encode five canonical proteins, including nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase protein (L) in the order of 3' leader-N-P-M-G-L-5' trailer. In recent years, a few novel cytorhabdoviruses, including chrysanthemum yellow dwarf associated virus (CYDaV), sambucus virus 1 (SaV1), rudbeckia virus 1 (RudV1), and patchouli chlorosis-associated cytorhabdovirus (PCaCV), have been reported in various plant species using high-throughput sequencing technology (HTS) [2-5].

Plumbago indica L. belongs to the family Plumbaginaceae, which is used as garden ornament and traditional Chinese herb for medication. In April 2021, *P. indica* plants showing symptoms with suspected viral infection were observed in Beijing, China. Compared with the healthy plant (Fig. 1a), the leaves of suspected plants showed purple necrotic spots symptoms (Fig. 1b). In addition, the newly emerged leaves displayed mild symptoms with small necrotic spots (Fig. 1b left), while the older leaves developed severe symptoms (Fig. 1b right). Total RNA of the symptomatic leaves was extracted by hot-borate method and then sent to Biomarker Technologies for HTS on an Illumina HiSeq X-10 platform [6].

A total of 22,272,428 pairs of clean reads were obtained by HTS. The obtained original data were spliced via megahit (MEGAHIT v1.1.3 Copyright (c) The University of Hong Kong & L3 Bioinformatics Limited). Errors in assembly were corrected by cap3 (VersionDate: 12/21/07), contigs with overlapped sequences were spliced meanwhile [7]. After that, highly similar contigs in cap3 results were clustered by cd-hit-est (CD-HIT version 4.6) with 95% similarity [8]. Bioinformatic analysis showed that a contig, comprising 13,168 nts, has similarity with cytorhabdoviruses. We provisionally named this novel virus as plumbago necrotic spot associated virus (PNSaV).

6 pairs of specific overlapping primers were designed (Table S1) to confirm the nearly complete viral sequence based on the viral contig (Fig. 1c). RT-PCR for the diseased *P. indica* plants was performed to verify the HTS result, subsequently, 5'- and 3'-RACE reaction (SMARTer® RACE 5'/3' Kit, Takara, 634858) were used to determine the terminal sequences of the genome. Each of the fragments was cloned into vector and purified using a Plasmid Mini Kit (OMEGA) for Sanger sequencing (Tsingke Biotechnology Company, China).

The complete genomic sequence of PNSaV comprises 13,180 nts (GenBank accession No. OR335651) and eight ORFs were predicted in the negative strand, according to the ORF Finder from the National Center for Biotechnology Information (NCBI) (Fig. 1d). ORF1 encodes a 455 amino acid (aa) protein, which is predicted to be the N protein. ORF2 encodes a 115 aa protein, with no significant similarity to the proteins of other rhabdoviruses. We predicted the ORF2 to be the P', since three transmembrane alpha-helices (TMHs) at aa position 31-51, 66-82, 93-109 were predicted using DeepTMHMM program (https://dtu.biolib.com/DeepTMHMM), which are also predicted to contain in P' of other cytorhabdoviruses [2, 9-12]. ORF3 encodes a 318 aa protein, predicted to be the P protein and overlaps ORF2 with 271 nts. ORF4 encodes the putative P3 protein of 219 aa, which can function as a movement protein [13]. ORF5 encodes a 166 aa protein, predicted to be the M protein. ORF6 encodes a 576 aa putative G protein. ORF7 encodes a 96 aa protein, predicted to be P6. A TMH domain of P6 was predicted between aa 62 and 79, which was also identified at similar ORF in other cytorhabdoviruses [2, 9-12]. ORF8 predicted to be the L protein, which is an RNA-dependent RNA polymerase (RdRp) with 2,091 aa. These eight ORFs place in the order of 3' leader-N-P'-P-P3-M-G-P6-L-5' trailer, which is similar to CYDaV, SCV, RVCV and alfalfa dwarf virus (ADV) [2,10-12]. Genes encoded by PNSaV are separated by gene junction regions (Fig. 1e), which is conservatively when comparing with other cytorhabdoviruses. Like other cytorhabdoviruses, the terminal sequences of the 3' leader and 5' trailer shows highly complementary (Fig. 1f).

A maximum-likelihood phylogenetic tree was constructed using MEGA-11 software based on the L protein amino acid sequences of PNSaV and 24 other cytorhabdoviruses, as well as 4 representative members of the genus *Nucleorhabdovirus*, used as outgroups (Fig. 2a). Phylogenetic analysis showed that PNSaV grouped closely with CYDaV, with 57.18% nucleotide sequence identity in the complete genome and 53.00% amino acid sequence identity in the L protein. The similarity of other proteins is also far less than 80% (Fig. 2b), which is a demarcation criterion when establish a new species in the genus *Cytorhabdovirus* [14]. These results indicate that PNSaV should be classified as a new member of the genus *Cytorhabdovirus*.

The incidence of the PNSaV was determined as well. The total RNA of symptomatic leaf samples from 8 different plants was extracted and subjected to RT-PCR using specific primers PNSaV-2-F/R (Table S1). The PNSaV fragments with the expected size were amplified from 7 different samples and confirmed by Sanger sequencing of the PCR products (Fig. 3c), indicating that this virus may be related to this disease. Biological studies are needed to assess effects of PNSaV on *P. indica* in the future.

Declarations

Statements & Declarations

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were mainly performed by Zhangyao Nie and Xiuqi Zhang. HTS data analysis was performed by Yingxi Li, Xiuqi Zhang and Zhangyao Nie. The manuscript was written by Zhangyao Nie, Xiuqi Zhang and Ying Wang, assisted by Chenggui Han and Zongying Zhang. All authors read and approved the manuscript.

Data availability The virus genome sequences were deposited in the GenBank database. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Walker PJ, Freitas-Astúa J, Bejerman N, Blasdell KR, Breyta R, Dietzgen RG, Fooks AR, Kondo H, Kurath G, Kuzmin IV, Ramos-González PL, Shi M, Stone DM, Tesh RB, Tordo N, Vasilakis N, Whitfield AE, ICTV Report Consortium (2022) ICTV Virus Taxonomy Profile: *Rhabdoviridae* 2022. J Gen Virol 103. https://doi.org/10.1099/jgv.0.001689
2. Liu Q, Jin J, Yang L, Zhang S, Cao M (2021) Molecular characterization of a novel cytorhabdovirus associated with chrysanthemum yellow dwarf disease. Arch Virol 166:1253–1257. https://doi.org/10.1007/s00705-021-04987-2
3. Šafářová D, Candresse T, Navrátil M (2022) Complete genome sequence of a novel cytorhabdovirus infecting elderberry (*Sambucus nigra* L.) in the Czech Republic. Arch Virol 167:1589–1592. https://doi.org/10.1007/s00705-022-05444-4
4. Lee D-S, Kim J, Jun M, Shin S, Lee S-J, Lim S (2022) Complete genome sequence of a putative novel cytorhabdovirus isolated from *Rudbeckia* sp. Arch Virol 167:2381–2385. https://doi.org/10.1007/s00705-022-05556-x
5. Kauffmann CM, de Jesus Boari A, Silva JMF, Blawid R, Nagata T (2022) Complete genome sequence of patchouli chlorosis-associated cytorhabdovirus, a new cytorhabdovirus infecting patchouli plants in Brazil. Arch Virol 167:2817–2820. https://doi.org/10.1007/s00705-022-05594-5
6. Ma N, Tan H, Liu X, Xue J, Li Y, Gao J (2006) Transcriptional regulation of ethylene receptor and *CTR* genes involved in ethylene-induced flower opening in cut rose (*Rosa hybrida*) cv. Samantha. J Exp Bot 57:2763–2773. https://doi.org/10.1093/jxb/erl033
7. Huang X, Madan A (1999) CAP3: A DNA sequence assembly program. Genome Res 9:868–877. https://doi.org/10.1101/GR.9.9.868

8. Li W, Godzik A (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659. https://doi.org/10.1093/bioinformatics/btl158
9. Fránová J, Přibylová J, Koloniuk I (2019) Molecular and biological characterization of a new strawberry cytorhabdovirus. Viruses 11:982. https://doi.org/10.3390/v11110982
10. Koloniuk I, Fránová J, Sarkisova T, Přibylová J (2018) Complete genome sequences of two divergent isolates of strawberry crinkle virus coinfecting a single strawberry plant. Arch Virol 163:2539–2542. https://doi.org/10.1007/s00705-018-3860-4
11. Jones S, McGavin W, MacFarlane S (2019) The complete sequences of two divergent variants of the rhabdovirus raspberry vein chlorosis virus and the design of improved primers for virus detection. Virus Res 265:162–165. https://doi.org/10.1016/j.virusres.2019.03.004
12. Bejerman N, Giolitti F, de Breuil S, Trucco V, Nome C, Lenardon S, Dietzgen RG (2015) Complete genome sequence and integrated protein localization and interaction map for alfalfa dwarf virus, which combines properties of both cytoplasmic and nuclear plant rhabdoviruses. Virology 483:275–283. https://doi.org/10.1016/j.virol.2015.05.001
13. Wang Q, Ma X, Qian S, Zhou X, Sun K, Chen X, Zhou X, Jackson AO, Li Z (2015) Rescue of a Plant Negative-Strand RNA Virus from Cloned cDNA: Insights into Enveloped Plant Virus Movement and Morphogenesis. PLOS Pathog 11:e1005223. https://doi.org/10.1371/journal.ppat.1005223
14. ICTV (2021) https://ictv.global/report/chapter/rhabdoviridae/rhabdoviridae/cytorhabdovirus. Accessed 23 July 2023

Figures

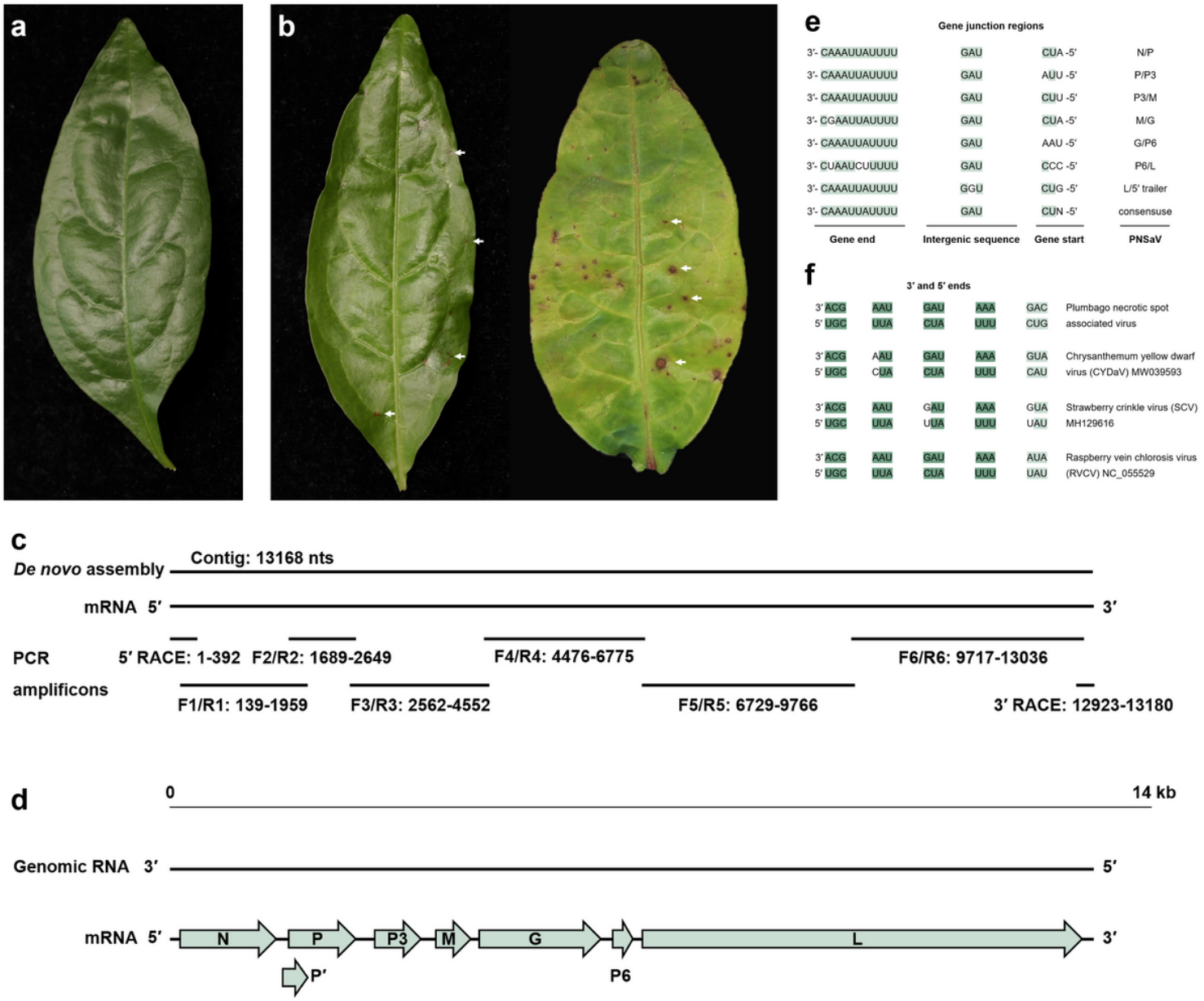
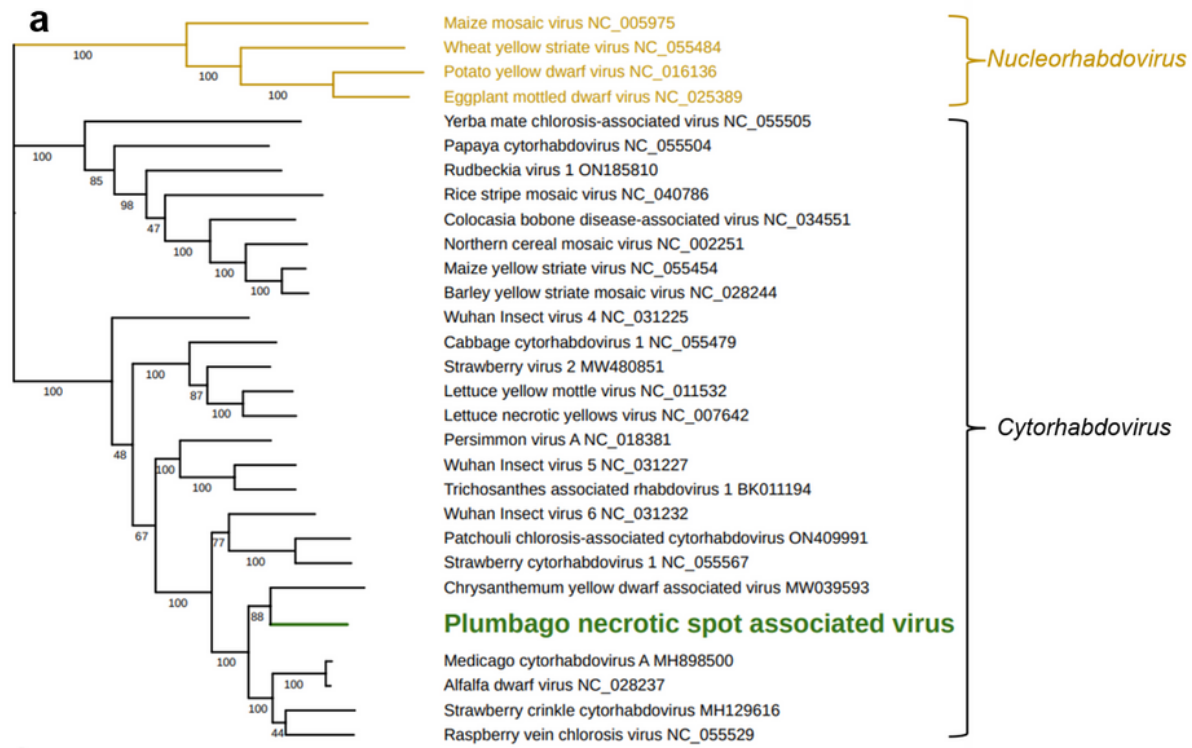


Figure 1

(a) The healthy leaf of *P. indica*. (b) Mild (left) and severe (right) necrotic spot symptoms on the leaves of *P. indica*. White arrows indicate the purple lesion. (c) The assembled contig from HTS and RT-PCR products amplified by specific primers. (d) The genome organization of plumbago necrotic spot associated virus (PNSaV). (e) Conserved gene junction sequences of PNSaV. (f) The complementary terminal sequence of PNSaV and other cytorhabdoviruses.



b

Viruses	Genome nt identity	N aa identity	P aa identity	M aa identity	G aa identity	L aa identity
CYDaV MW039593	57.18	52.62	35.47	31.74	42.07	53.00
SCV MH129616	57.50	47.96	32.20	29.34	37.98	54.59
RVCV NC_055529	56.86	45.36	32.51	34.71	38.84	54.02

maximum

minimum

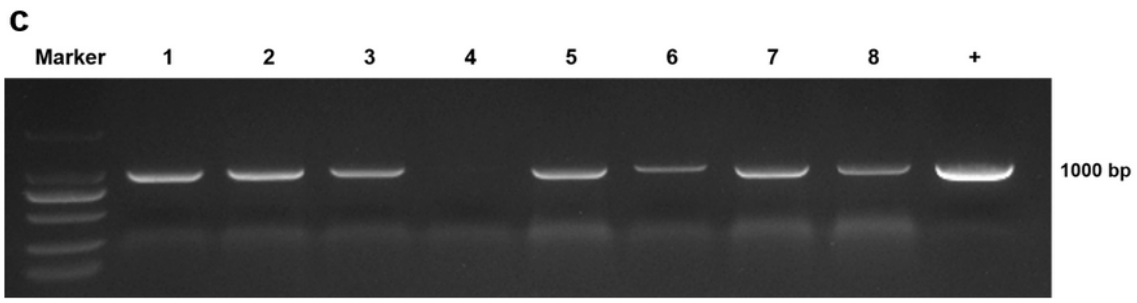


Figure 2

(a) Maximun-likelihood tree based on L protein amino acid sequences of PNSaV and other representative members of genus *Cytorhabdovirus* and *Nucleorhabdovirus*. (b) Genome nucleotide sequence and amino acid sequence identity (%) alignment between PNSaV and other cytorhabdoviruses. The genome sequences and aa sequences were compared using MAFFT. Pairwise sequence identity was calculated

using Jalview. (c) The incidence of the PNSaV. Lanes 1~8 represent leaf samples from different plants, + is the plasmid of the gene *P* of the PNSaV.

Supplementary Files

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- Plumbagonecroticspotassociatedvirus.docx
- TableS1.docx