Identification and characterization of a novel potyvirus infecting *Paris yunnanensis*

Pingxiu Lan
Yunnan Agriculture University: Yunnan Agricultural University
Peng He
Yunnan Agriculture University: Yunnan Agricultural University
Mengji Cao
Southwest University
Guohua Zhou
Chinese Association of Chinese Medicine
Li Chenrong
Yunnan Agricultural University
Guanlin Tan
Yunnan Agricultural University
Xiaojiao Chen
Yunnan Agricultural University
Jie Yang
Yunnan Agricultural University
Taiyun Wei
Fujian Agriculture and Forestry University
Fan Li (fanlikm@126.com)
Yunnan Agricultural University https://orcid.org/0000-0002-4394-2431

Research Article

Keywords: sequence, potyvirus, virus, level, genomic, paris, sequencing

Posted Date: September 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-895232/v1

Abstract

The complete genomic sequence of a novel potyvirus from *Paris yunnanensis* was determined by high-throughput sequencing then confirmed by Sanger sequencing. Its genomic RNA consists 9600 nucleotides (nt) excluding the 3'-terminal poly (A) tail, containing a typical large open reading frame (ORF) of potyviruses and encoding a putative polyprotein of 3098 amino acids (aa). Pairwise comparison analysis showed the virus shares sequence identity with other members of *Potyvirus* was 53.0–57.8% at genome sequence level, and 39.3–51.2% at polyprotein sequence level. Phylogenetic analysis indicated that the virus was clustered as a single clade within the genus *Potyvirus* both using nt and aa level. These results suggest that the virus should be considered as a distinct species within the genus *Potyvirus*, and it was tentatively named as "Paris mottle virus" (PaMoV).

Introduction

Paris yunnanensis (Chonglou in Chinese), previously recognized as a conspecific variety of *P. polyphylla* (*P. polyphylla* var. *yunnanensis*), is a perennial herb in genus *Paris* of family *Melanthiaceae* in order Liliales [1]. *P. yunnanensis* is an important Chinese traditional herb and major component material for at least 49 Chinese patent medicines, such as Yunnan Baiyao Powder and Snake-bite Therapeutics. The plant of *P. yunnanensis* was naturally growing in the 1400–3200 altitude of shady place in Southwest China, but it had been excessively excavated to the edge of extinction and commercially planted to meeting the market demand since 1980s. In 2017, the annual planting area of *P. yunnanensis* in China was beyond 10000 hm^2 with a production of 50 thousand tons, and the profit of selling the *P. polyphylla* related drugs and products got approximately 10 billion CNY (ca. 1.6 billion USD) [1]. However, virus disease became daily serious with its extension of planting years and expansion of planting area. several viral pathogens, including Paris polyphylla virus X (PPVX) [2], Paris mosaic necrosis virus (PMNV) [3], pepper mild mottle virus (PMMoV) [4], Paris virus 1 (ParV1) [5], Paris virus 2 (ParV2) [6] and chilli veinal mottle virus [7] had been reported infecting the cultivation plants of *P. yunnanensis* and caused production declining. This paper investigated the viral disease of *P. yunnanensis* in Yunnan province, determined and characterized the complete genome sequence of a novel potyvirus from plants with leaf mottle.

Materials And Method

In August 2017, symptoms of foliar mosaic, mottle and yellowing were observed in a *P. yunnanensis* commercial plantation of Mangshi, Dehong autonomous prefecture of Yunnan Province (Fig. 1A). Nine leaves samples were collected and pooled into one sample for total RNA extraction and high-throughput sequencing (HTS) by Vazyme Biotech Co., Ltd (Nanjing, China). The qualified total RNA was depleted ribosomal RNA and then subjected to HTS RAN-seq on the Illumina HiSeq X-ten platform with PE150 bp. The sequence data were analyzed by CLC Genomic Workbench 9.5 (QIAGEN). A total of 64,278,370 paired-end reads were obtained after removing the failed reads, with which 105,208 contigs larger than 200 bp were assembly generated by *de novo*. BLASTx analysis of the assembled contigs against the

NCBI databases indicated that one large contigs of 9504 nt in length with several uncertain base temporarily marked as “N”, was represented the preliminary genome scaffold of a potyvirus and shared the highest amino acid (aa) sequence identity of 51%-52% with Kalanchoe mosaic virus (APX54983).

To confirm the presence of the virus and amplify its whole genome sequence, morphology of the novel potyvirus was observed under transmission electron microscopy (TEM) by negative stain technology. Specific primers covering the entire genome sequence were designed according to the 9504 nt contig. All primers have an overlapping region of ~ 115 to 151 nt at the ending of the contiguous amplicons (Table S1). Total RNAs from the initial 9 samples (0.1 g each) were extracted using EasyPure Plant RNA kit (TransGen Biotech, Beijing, China). RT-PCR was performed using a PrimeScript™ One-step RT-PCR Kit ver. 2 (TaKaRa Biotechnology Co., Ltd., Dalian, China). The 5’-end sequence was obtained using SMARTer®RACE 5’/3’ kit (TaKaRa Biotechnology Co. Ltd., Dalian, China), while the 3’-terminus was amplified using a virus-specific forward primer as well as degenerated primer of viral8 and viral9 designed for the viruses with poly (A) tail [8]. The amplicions expected to the primers designed were purified then cloned into pMD19-T vector [TaKaRa Biotechnology (Dalian) Co. Ltd., Dalian, China], at least three overnight culture clones for each amplicion were selected for sequencing (BGI, Guangzhou, China) and analysis. The genomic sequences were assembled by DNASTAR 7.0 package (DNASTAR Inc., Madison, WI, USA). Pairwise comparisons of nucleotide and polyprotein sequences were performed using the EMBOSS Needle Pairwise Sequence Alignment at https://www.ebi.ac.uk/Tools/psa/emboss_needle/. Multiple alignment was performed using the Clustal Omega Mutiple Sequence Alignment at https://www.ebi.ac.uk/Tools/msa/clustalo/. Nine highly conserved proteolytic cleavage sites in its polyprotein were predicted using a multiple alignment of relevant potyviruses polyprotein according to the criteria proposed by Adams et al [9]. Phylogenetic trees were constructed using maximum likelihood (ML) method in the MEGA 7 software [10].

Results And Analysis

Flexuous, filamentous particles of 700 ~ 800 nm in length were observed by Electron microscopy using crude sap from the virus infected plants (Fig. 1B). The complete genomic sequence of the virus from mangshi (designed as YMSh-CL isolate) was determined to be 9600 nucleotides (nt) excluding the 3’-terminal poly (A) tail (GenBank accession No. OK073904), flanked by 5’ and 3’ untranslated regions (UTRs) of 136 nt and 170 nt, respectively. The major putative ORF encodes a polyprotein of 3098 amino acid (aa) residues which starts at nt 137 and ends at nt 9432. Nine highly conserved proteolytic cleave sites are identical to those of other potyvirus and bioinformatically yield ten putative mature proteins of P1 (316 aa), HC-Pro (458 aa), P3 (378 aa), 6K1 (51 aa), CI (636 aa), 6K2 (53 aa), VPg (187 aa), NIa-Pro (243 aa), NIb (515 aa) and CP (261 aa), respectively (Fig. 1C). The small ORF (PIPO) within the P3 cistron of potyviruses, was also identified by the presence of ^{3007}GGAAAAA^{3014} encoding a protein of 73 aa residues. Most conserved motifs of potyviruses with known function are identified in the polyprotein of the virus [11], such as ^{6}I-T-F-G^{9} and ^{228}H-X$_{12}$-D-X$_{29}$-S-G-X$_{18}$-R-G^{292} associate with protease activity in P1 protein, the putative zinc finger metal-binding motif of ^{27}C-X$_{8}$-C-X$_{18}$-C-X$_{2}$-C^{58} related to aphid

transmission, ^{181}F-R-N-K-X$_{12}$-C-D-N-Q-L-D^{202} for symptomatology, ^{215}H-A-K-R-F-F^{220} possible for cell-to-cell movement and ^{344}C-X$_{72}$-H^{417} for protease activity in HC-Pro,the potential helicase activity motifs of ^{106}V-L-M-V-E-P-T-R-P-L^{115}–^{175}D-E-C-H^{178}, ^{202}K-V-S-A-T-P-P^{208}, ^{253}L-V-Y-V^{256}, ^{304}V-A-T-N-I-I-E-N-G-V-T-L^{315} and ^{348}G-E-R-I-Q-R-L-G-R-V-G-R^{359} in CI cistron, proteolytic activity related motif of ^{46}H-X$_{34}$-D-X$_{67}$-G-X-C-G-X$_{14}$-H^{167} in NIa-Pro, ^{169}S-L-K-A-E-L^{174} for RNA polymerase activity, ^{188}F-T-A-A-P-I-D^{194}, ^{202}C-V-D-D-F-N^{207}, ^{244}F-D-A-D-G-S^{249}, ^{306}G-N-N-S-G-Q-P-S-T-V-V-D-N-S-L-M-V^{322} and ^{350}G-D-D^{352} for RNA-dependant polymerase in NIb. Besides, the highly conserve motif K-I-T-C that located in the N terminal of HC-Pro involved in aphid transmission, was taken place by ^{52}R-I-T-C^{55} in PMoV-MShi, while ^{310}P-T-K^{312} in HC-Pro and ^{7}D-A-G^{9} in CP with similar function, both were present in its corresponding proteins.

The complete sequences of the virus were pairwise compared with other members of genus *Potyvirus* available in the GenBank Database, the result showed the virus shares sequence identity with other members of *Potyvirus* was 53.0% (onion yellow dwarf virus, OYDV, Accession number NC_005029) to 57.8% (Narcissus yellow stripe virus, NYSV, Accession number NC_011541) at nt sequence level, and 39.3% (OYDV, NC_005029) to 51.2% (plum pox virus, PPV, NC_001445) at deduce aa sequence level (table 1). Phylogenetic analyses were conducted using the deduced polyprotein sequence and selected members of the genus *Potyvirus*. The virus was clustered as a single clade between the subgroup of turnip mosaic virus (TuMV) and that of PPV (Fig. 2A). A distinct phylogenetic relationship was maintained when the complete nt was used (Supplementary Fig. 1), suggesting that the virus should be a divergent species in the genus *Potyvirus*.

Crude sap from symptomatic leaves infected by the virus were injected into solanaceaes plants of *Nicotiana benthamiana*, *N. tobacum*, *N. glutinosa*, *N. rustica*, *N. tabacum* var. Xanthi nc, and *Capsicum annuum*, or mechanically inoculated plants of *Solanum lycopersicum* and *Vigna unguiculata*. All inoculation plants were neither symptomatic nor proved by RT-PCR indication they are non-host. A batch of 132 leaf samples collected in 2017 to 2019 were performed RT-PCR using the specific primers of PMoVDF/ PMoVDR (TGCGGACGATGGAACGATAG/ CGAGGGAAAGGTGGGAAGTC), 25.0% detection rate indicated the virus was common in the planting area. The symptoms induced by virus were mainly showed mild or heavy mottle and we tentatively named it as “Paris mottle virus” (PaMoV).

Declarations

Acknowledgments: This study was funded by the National Science Foundation of China (31660509) and the Yunnan Academician Expert Workstation (202005AF150040).

Compliance with ethical standards:

Conflict of interest: The author declares no competing interests.

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

References

1. Ji YH (2021) A Monograph of Paris (Melanthiaceae) [M]. Science Press (Beijing) and Springer Press (Singapore): 69–81
2. Dong JH, Ding M, Fang Q, Luo YQ, Zhang ZK, Li Z, Yang B, Li XM (2007) Molecular identification of a Potexvirus isolate infecting *Paris polyphylla var. yunnanensis* and analysis of 3'terminal sequence. Acta Phytopathologica Sin 37(3):237–241
3. Lan PX, Zhao JR, Zhou YL, Li YY, Shen DC, Liao QC, Li RH, Li F (2018) Complete genome sequence of *Paris mosaic necrosis virus*, a distinct member of the genus Potyvirus. Arch Virol 163(3):787–790
4. Wen GS, Yang LY, Anane R, Chen Z, Yang Y, Chen L, Sun Y, Zhao M (2019) First Report of Pepper Mild Mottle Virus in *Paris polyphylla var. yunnanensis* in China. Plant Dis 103(12):3289
5. Chen L, Anane RF, Wang Z, Yang L, Chen ZL, Wen GS, Zhao MF (2020) Whole-genome sequence analysis of paris virus 1: a novel member of the genus *Potyvirus* infecting *Paris polyphylla var. yunnanensis*. Arch Virol 165(4):985–988
6. Chen L, Anane RF, Wang Z, Chen ZL, Gao L, Wen GS, Zhao MF (2021) Characterization of a novel Tombusviridae species isolated from *Paris polyphylla var. yunnanensis*. Arch Virol. https://doi.org/10.1007/s00705-021-05191-y
7. Yang J, Ma QZ, Meng Y, Huang D, Li CR, He P, Li F, Lan PX, Tan GL (2021) First report and partial genomic sequence analysis of ChiVMV in *Paris yunnanensis*. Acta Phytopathologica Sin. https://doi.org/doi:10.13926/j.cnki.apps.000573
8. Lan PX, He P, Zhang YK, Zhang S, Zhang ZB, Chen XJ, Tan ST, Luo HM, Cao MJ,Li Fan (2019) Molecular characterization of a novel potyvirus infecting noni. Arch Virol, 2019, 164(12):3099–3102
9. Adams MJ, Antoniw JF, Beaudoin F (2005) Overview and analysis of the polyprotein cleavage sites in the family *Potyviridae*. Mol Plant Pathol 6(4):471–487
10. Kumar S, Stecher G, Tamura K (2016) $MEGA_7$: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874
11. Worrall EA, Hayward AC, Fletcher SJ, Mitter N (2019) Molecular characterization and analysis of conserved potyviral motifs in bean common mosaic virus (BCMV) for RNAi-mediated protection. Arch Virol 164(1):181–194

Tables

Table 1 is not available with this version

Figures

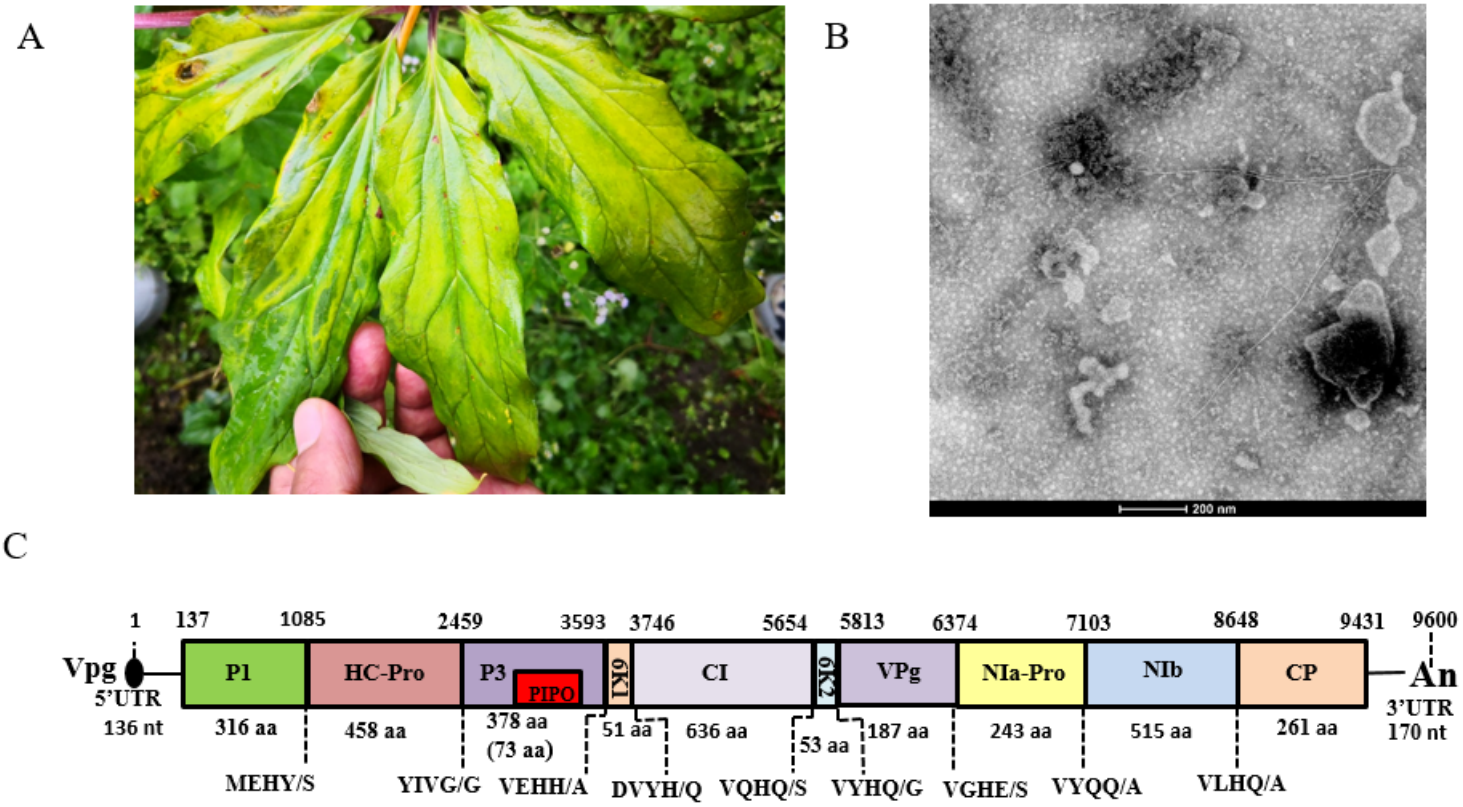

Figure 1

Symptoms, morphology and genome organization of Paris mottle virus (PaMoV). (A) Symptoms induced by PaMoV. (B) Morphology of PaMoV particles. (C) Schematic representation the genome organization of PaMoV. The 5'- and 3'-untranslated regions (UTR) are represented by a solid lines, and the open reading frame (ORF) is depicted by an open box with solid line. The putative protein PIPO is indicated within P3 protein by a small box in red. The putative proteolytic cleavage sites in the polyprotein and the length in amino acids of each protein is indicated below the genome, whereas the numbers above the genome indicate the start for each region.

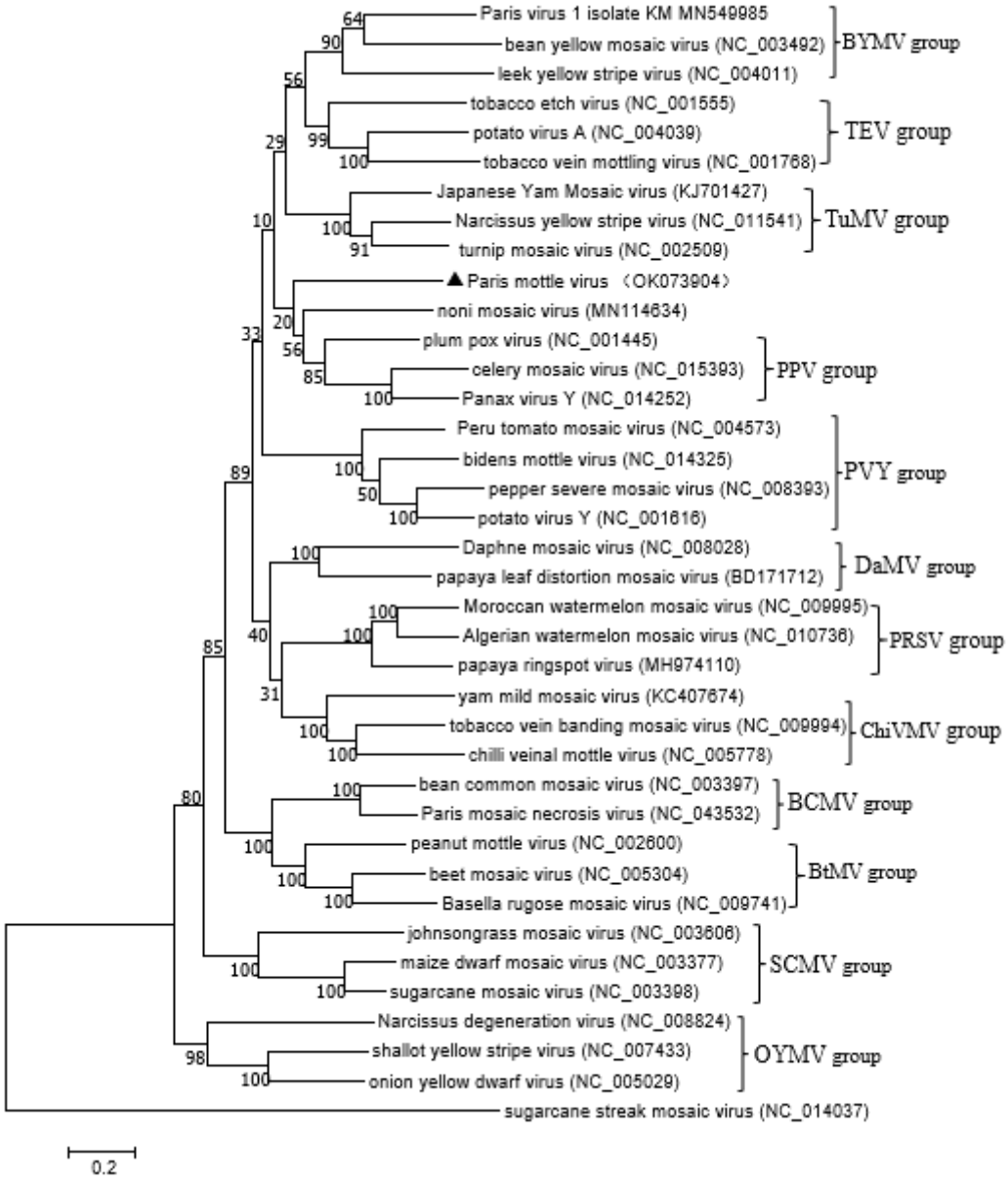

Figure 2

Maximum-likelihood tree based on the deduced polyprotein of PaMoV-YMSh-CL as well as those of the representative members of genus Potyvirus. Bootstrap analysis was applied using 1000 bootstrap replicates. The scale bar representing a genetic distance of 0.2. Sugarcane streak mosaic virus, a member of genus Poacevirus, was used as an outgroup. Solid triangle indicated the PaMoV-Mshi isolate characterized in this study

Supplementary Files

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

- Summplmenttable.docx

- Supplementaryfigure.pptx