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Molecular characterization of a novel potyvirus infecting noni

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Abstract
The complete genomic sequence of a novel potyvirus from a noni plant in China (*Morinda citrifolia*) with foliar mosaic and chlorotic symptoms was determined. The genomic RNA consists of 9645 nucleotides (nt) excluding the poly(A) tail, containing the typical open reading frame (ORF) of potyviruses and encoding a large putative polyprotein of 3077 amino acids (aa). Pairwise comparisons showed that the virus shares 48.8%–58.5% sequence identity at the genome sequence level, and 38.5%–53.4% identity at the polyprotein sequence level with other members of the genus *Potyvirus*. Phylogenetic analysis indicated that the virus is most closely related to jasmine virus T and plum pox virus in the genus *Potyvirus*. These results suggest that this virus should be considered a distinct member of the genus *Potyvirus*, and it was tentatively named "noni mosaic virus" (NoMV).

Introduction

Noni (*Morinda citrifolia*), commonly known as great morinda, Indian mulberry, nhau (yor), beach mulberry, cheese fruit, or duppy soursop, is a small tropical woody evergreen shrub belong to the family Rubiaceae. Noni is native to South Asia, Southeast Asia or Micronesia and has been used as a Polynesian medicine to cure or prevent a variety of illnesses for more than 2000 years [1]. Modern medicine demonstrated that noni is rich in phytochemicals with analgesic, antioxidant, anticancer, anti-inflammatory and anti-angiogenic properties [2]. The plant is now commercially cultivated in the South Pacific, the Caribbean, South America, the West Indies, and tropical and subtropical regions of China such as Hainan, Fujian, Taiwan and Yunnan provinces. However, several pathogenic fungi [3], nematodes [4, 5] and phytoplasma [6] have been reported to infect noni and cause significant yield loss.

Identification of a novel noni potyvirus

In 2017, symptoms of foliar mosaic and chlorosis were observed on a noni plantation in the city of Jinghong, Yunnan province, China (Fig. 1A). Symptomatic leaf samples from four diseased trees were collected, and viruses in crude sap samples from two of them were negative stained with 2% phosphotungstic acid (pH 6.8). Using transmission electron microscopy, viral particles with flexuous filaments about 800 nm in length and 12 nm in width were found (Fig. 1B) in both samples, indicating that the diseased noni plants were infected by a filamentous virus. The diseased leaf samples were then sent to Vazyme Biotech Co., Ltd (Nanjing, China) for NGS RNA-Seq sequencing after depletion of the rRNAs. An Illumina HiSeq X-ten platform with PE150 bp and CLC Genomic Workbench 9.5 (QIAGEN) was used

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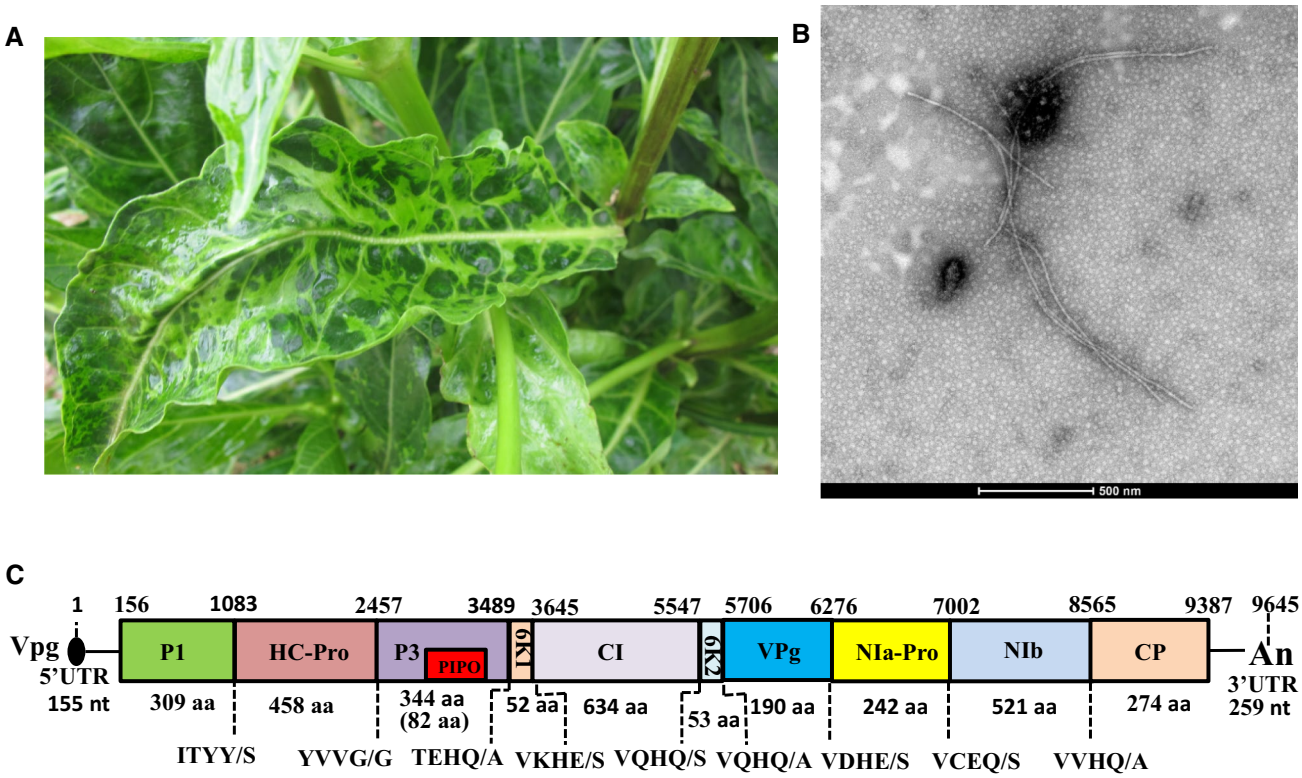

Fig. 1 (A) Symptoms on the diseased noni plants. (B) Morphology of noni mosaic virus (NoMV) particles. (C) Schematic representation of the genome organization of NoMV. The 5'- and 3'-untranslated regions (UTR) are represented by a solid lines, and the open reading frame (ORF) is depicted by an open box with solid line. The putative protein PIPO is indicated within P3 protein by a small box in red. The putative protein 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 ORF

for sequencing and data analysis [7]. A total of 89,008,119 paired-end reads were obtained, and 94,149 contigs (200 to 21,219 bp) were generated *de novo* and compared with sequences in the GenBank virus database using BLASTn or BLASTx. A large contig of 9646 nt sharing the highest sequence identity in the polyprotein region (52.14%) with kalanchoe mosaic virus (accession number APX54983) was obtained, suggesting that this virus was a distinct potyvirus.

To verify the presence of the potyvirus in field plants, specific primers corresponding to the 104- to 172-nt overlapping regions of the ends of the amplification products were designed to cover the entire genome sequence according to the contig (Table S1). Total RNA from the original samples was extracted using TRIpure Reagent (Bioteke Corporation, Beijing, China), and RT-PCR was performed using a PrimeScript™ One-Step RT-PCR Kit Ver. 2 (TaKaRa Biotechnology Co. Ltd., Dalian, China). The PCR products were cloned into the pMD19-T vector (TaKaRa Biotechnology Co. Ltd., Dalian, China), and at least three clones were sequenced for each amplicon (BGI, Guangzhou, China). A 3'-terminal sequence of approximately 0.6 kb was obtained using a specific forward primer and the primer viral8 (GAC CACGCGTATCGATGTCGACTTTTTTTTTTTTTTTV), which was designed to recognize the poly(A) tail of potyviruses, while the 5'-end sequence was obtained using SMARTer®RACE 5'/3' kit (TaKaRa Biotechnology Co. Ltd., Dalian, China). The genome sequence was assembled using SeqMan in the Lasergene v7.1 package (DNASTAR Inc., Madison, WI, USA). Pairwise comparisons of nucleotide (nt) and amino acid (aa) sequences were performed using EMBOSS Needle Pairwise Sequence Alignment at http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html. Phylogenetic analysis was performed in MEGA7 using 1000 bootstrap replicates [8].

Genomic properties

The complete genome of a Jinghong isolate of the virus (designated as NoMV-YJh) was determined to be 9645 nt excluding the poly (A)tail and flanked by 5' and 3' untranslated regions (UTRs) of 155 nt and 259 nt, respectively (GenBank accession no. MN114634). Its genomic organization and structure is typical of members of the genus *Potyvirus*, containing a large open reading frame (ORF) encoding a polyprotein of 3077 aa residues. Nine highly

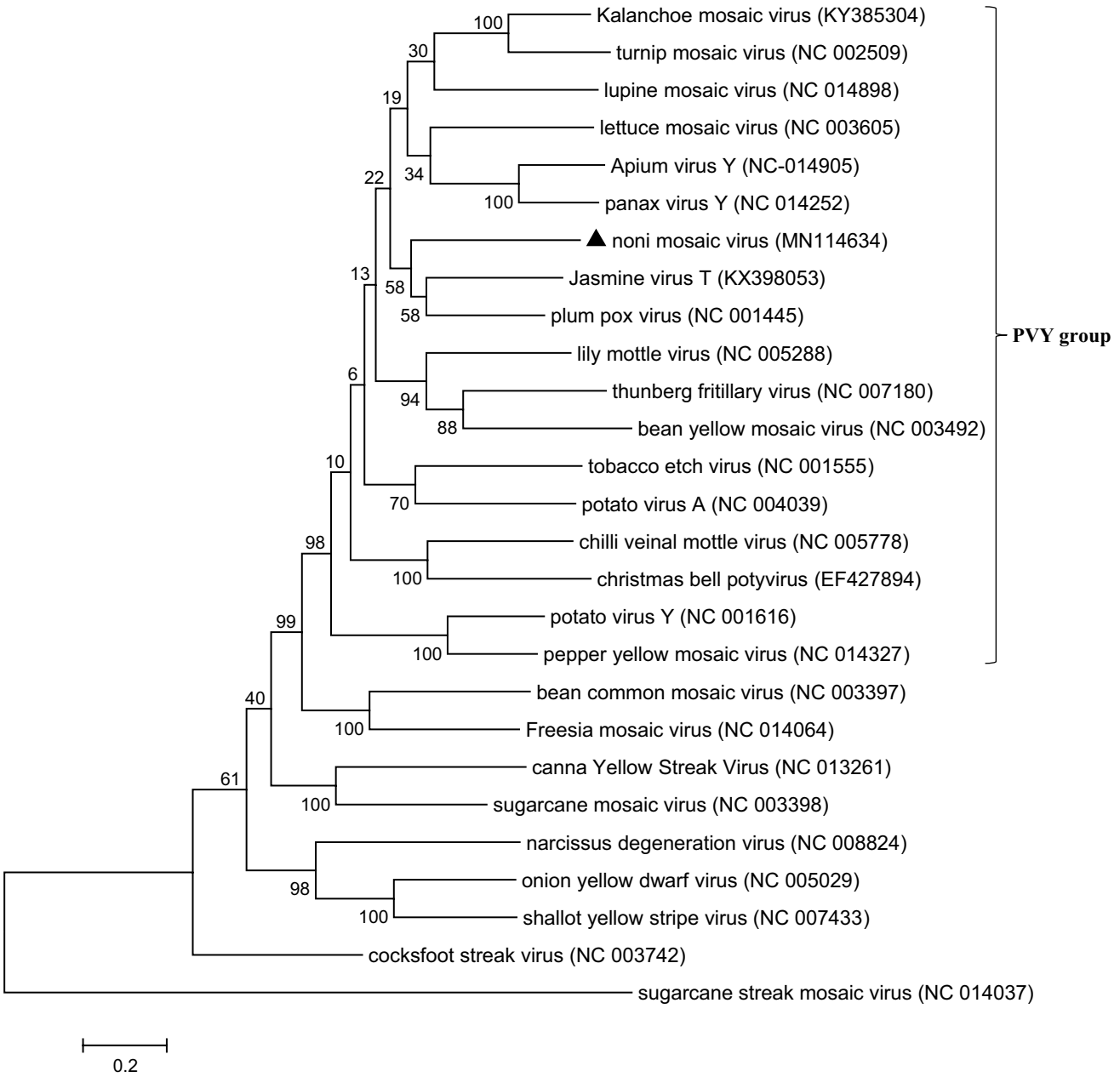

Fig. 2 Maximum-likelihood tree based on the deduced polyprotein sequence of NoMV and the representative members of genus *Potyvirus*. Bootstrap analysis was applied using 1000 bootstrap replicates. The scale bar representing a genetic distance of 0.2. Sugarcane streak mosaic virus, a member of genus *Poacevirus*, was used as an outgroup. Solid triangle indicated the NoMV-YJh isolate characterized in this study

conserved proteolytic cleavage sites were predicted in the NoMV-YJh polyprotein based on a multiple alignment of related potyviruses polyprotein sequences and using the criteria proposed by Adams et al. [9], resulting in ten putative mature protein: P1 (309 aa), HC-Pro (458 aa), P3 (344 aa), 6K1 (52 aa), CI (634 aa), 6K2 (53 aa), VPg (190 aa), NIa-Pro (242 aa), NIb (521 aa) and CP (274 aa) (Fig. 1C). A small ORF (PIPO) embedded within the P3 cistron of potyviruses, encoding a protein of 82 aa residues, was also identified by the presence of ^{2913}GAAAAAA^{2919}. Most of the conserved motifs that are typical of potyviruses [10], such as ^{209}H-X_8-D-X_{28}-G-X-S-G^{261} (proteolytic activity) in P1, ^{27}C-X_8-C-X_{18}-C-X_2-C^{58} (putative zinc finger binding motif), ^{181}F-R-N-K-X_{12}-C-D-N-Q-L-D^{202} (symptomatology) and ^{292}C-C-C-V-T^{296} (long-distance movement) in HC-Pro, ^{84}G-A-V-G-S-G-K-S-T^{92} (NTP-binding motif) in P3, ^{200}K-V-S-A-T-P-P^{206}, ^{251}L-V-Y-V^{254}, ^{302}V-A-T-N-I-I-E-N-G-V-T-L^{313}and ^{346}G-E-R-I-Q-R-L-G-R-V-G-R^{357} (potential

helicase activity) in CI, ^{46}H-X$_{34}$-D-X$_{67}$-G-X-G-G-X$_{14}$-H^{167} (proteolytic activity) in NIa-Pro, and ^{172}S-L-K-A-E-L^{177}, ^{205}C-V-D-D-F-N^{210}, ^{309}G-N-N-S-G-Q-P-S-T-V-V-D-N-T-L-M-V^{325}, and ^{353}G-D-D^{355} (RNA-dependent polymerase activity) in NIb were identified. Furthermore, three motifs involved in aphid transmission, ^{52}K-I-T-C^{55} and ^{310}P-T-K^{312} in HC-Pro and ^{7}D-A-G^{9} in CP, were found to be present in the polyprotein of NoMV-YJh.

Pairwise comparisons of the complete genome sequence of NoMV-YJh with those of other members of the genus *Potyvirus* available in the GenBank database showed that NoMV-YJh shared 48.8% (freesia mosaic virus, accession number NC_014064) to 58.5% (jasmine virus T, accession number NC_029051) nt sequence identity, and 38.5% (shallot yellow stripe virus, accession number NC_007433) to 53.4% (plum pox virus, accession number NC_001445) aa sequence identity with other potyviruses. A maximum-likelihood tree was constructed using the deduced polyprotein sequences of NoMV-YJh and selected members of genus *Potyvirus*, which clearly placed NoMV-YJh, JaVT (accession number NC_029051) and plum pox virus (accession number NC_001445) into a subgroup within the potato virus Y supergroup [11] (Fig. 2). These results indicate that this virus should be considered a member of a novel species in the genus *Potyvirus*, and it was tentatively named "noni mosaic virus" (NoMV).

Sites of possible recombination events within the genome sequence of NoMV-YJh were identified using RDP4 version 4.97 package with default settings [12]. The complete genome sequence of NoMV-YJh and 92 full-length genome sequences of 84 potyviruses were aligned using MEGA7 and then analyzed using the programs RDP, Chimaere, BootScan, 3Seq, GENCONV, MaxChi, and SiScan, and the highest acceptable *P*-value was set at 0.05. A likely recombination site by five programs was detected that suggested recombination between tobacco vein mottle virus (accession number U38621, major parent) and tobacco etch virus (accession number NC_001555, minor parent) (Fig. S1), and two or three other possible recombination sites were also identified (Fig. S2). These results suggest that interspecies recombination occurred during the evolutionary history of NoMV-YJh.

The presence of three aphid-transmission-associated motifs in the NoMV-YJh polyprotein suggests that NoMV might be aphid transmissible. Crude sap from symptomatic leaves of a noni plant were mechanically inoculated onto healthy *Nicotiana benthamiana*, *N. rustica*, *N. tabacum* var. Xanthi nc, *Glycine max*, *Vicia faba* and *Pisum sativum*; however, none of them were found to be infected with NoMV. More biological characteristics, such as the mode of transmission and other properties, need to be examined in the future in order to understand how to effectively control NoMV. To our knowledge, this is the first report of a virus that naturally infects *Morinda citrifolia*.

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Compliance with ethical standards

Conflict of interest As the corresponding author, Fan Li declares that there is no conflict of interest involving in the authors of this paper.

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

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