

Genomic organization of a novel victorivirus from the rice blast fungus *Magnaporthe oryzae*

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Abstract Two double-stranded RNA (dsRNA) mycoviruses were found in isolate QSP5 of the rice blast fungus *Magnaporthe oryzae*. Sequence analysis of the two dsRNA mycoviruses revealed that one is closely related to Magnaporthe oryzae virus 2 (MoV2), and the other one is related to Magnaporthe oryzae chrysovirus 1-A (MoCV1-A). Therefore, they were named Magnaporthe oryzae virus 3 (MoV3) and Magnaporthe oryzae chrysovirus 1-C (MoCV1-C), respectively. In this paper, the molecular and structural characteristics of MoV3 were analyzed in detail. The full genome sequence (5181 bp) of MoV3 was obtained by cDNA cloning. Sequence analysis indicated that MoV3 has two overlapping open reading frames (ORF1 and ORF2). The 5'-proximal ORF1 encodes a putative coat protein (CP) with a molecular weight of 80,939 Da; the 3'-proximal ORF2 encodes a putative RNA-dependent RNA polymerase (RdRp) with a molecular weight of 90,506 Da. The stop codon of ORF1 overlaps

the start codon of ORF2, with the tetranucleotide sequence AUGA, which is characteristic of members of the genus *Victorivirus* of the family *Totiviridae*. Phylogenetic analysis of RdRp and CP further supported the view that MoV3, a novel mycovirus, belongs to the genus *Victorivirus* of the family *Totiviridae*.

Introduction

Magnaporthe oryzae, a filamentous heterothallic ascomycete, is the most destructive pathogen of rice worldwide and the principal model organism for determining the molecular basis of fungal disease of plants. *M. oryzae* depends on its asexual spores (conidia) for disease establishment in nature. The conidia colonize leaves and produce necrotic lesions, or attack other parts of the plant at any stage of growth [10, 15]. Mycoviruses have been reported in many fungi [1, 4, 5]. Most mycoviruses found in filamentous fungi have double-stranded RNA (dsRNA) genomes; however, some novel single-stranded RNA (ssRNA) and single-stranded DNA (ssDNA) mycoviruses also have been reported [2, 18]. Four distinct viruses in the family *Totiviridae* and *Chrysoviridae* have been found in *M. oryzae*: Magnaporthe oryzae virus 1 and 2 (MoV1 and MoV2), belonging to the family *Totiviridae*, and Magnaporthe oryzae chrysovirus 1-A and 1-B (MoCV1-B and MoCV1-A), belonging to the family *Chrysoviridae*. The complete sequences of these viruses have been determined [8, 12–14, 17]. In this study, we report a novel mycovirus from strain QSP5 that was isolated from Hubei province in China. Sequence comparison and phylogenetic analysis revealed that MoV2 is closely related to members of the genus *Victorivirus*, which mainly infect filamentous fungi.

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Materials and methods

The *M. oryzae* strain QSP5 was isolated from a lesion of a rice leaf collected in Hubei province, China, in 2011. The strain was stored on filter paper at $-20\text{ }^{\circ}\text{C}$ and cultured on potato dextrose agar (PDA) at $28\text{ }^{\circ}\text{C}$. dsRNA was extracted from the mycelia of strain QSP5 using cellulose (Sigma-Aldrich, Dorset, England) column chromatography [6, 7] and was detected using electrophoresis on 1.0 % agarose gels. Two dsRNA elements present in strain QSP5 were designated as Magnaporthe oryzae virus 3 (MoV3) and Magnaporthe oryzae chrysovirus 1-C (MoCV1-C)

(Fig. 1a). To obtain the full dsRNA sequence of MoV3 in strain QSP5, the following methods were used: first, the purified dsRNA was used as a template for reverse transcription PCR (RT-PCR) amplification using a cDNA Synthesis Kit (Fermentas) with tagged random primer dN6 (5'-CGATCGATCATGATGCAATGCNNNNNN-3') and a single specific primer (5'-CGATCGATCATGATGCAATGC-3'), which was based on the tagged random primer-dN6. The amplified PCR products were sequenced, analyzed and assembled using DNAMAN version 5.2.9 and the BLASTP program on the NCBI website [9, 16]. Second, regions corresponding to gaps in the sequences

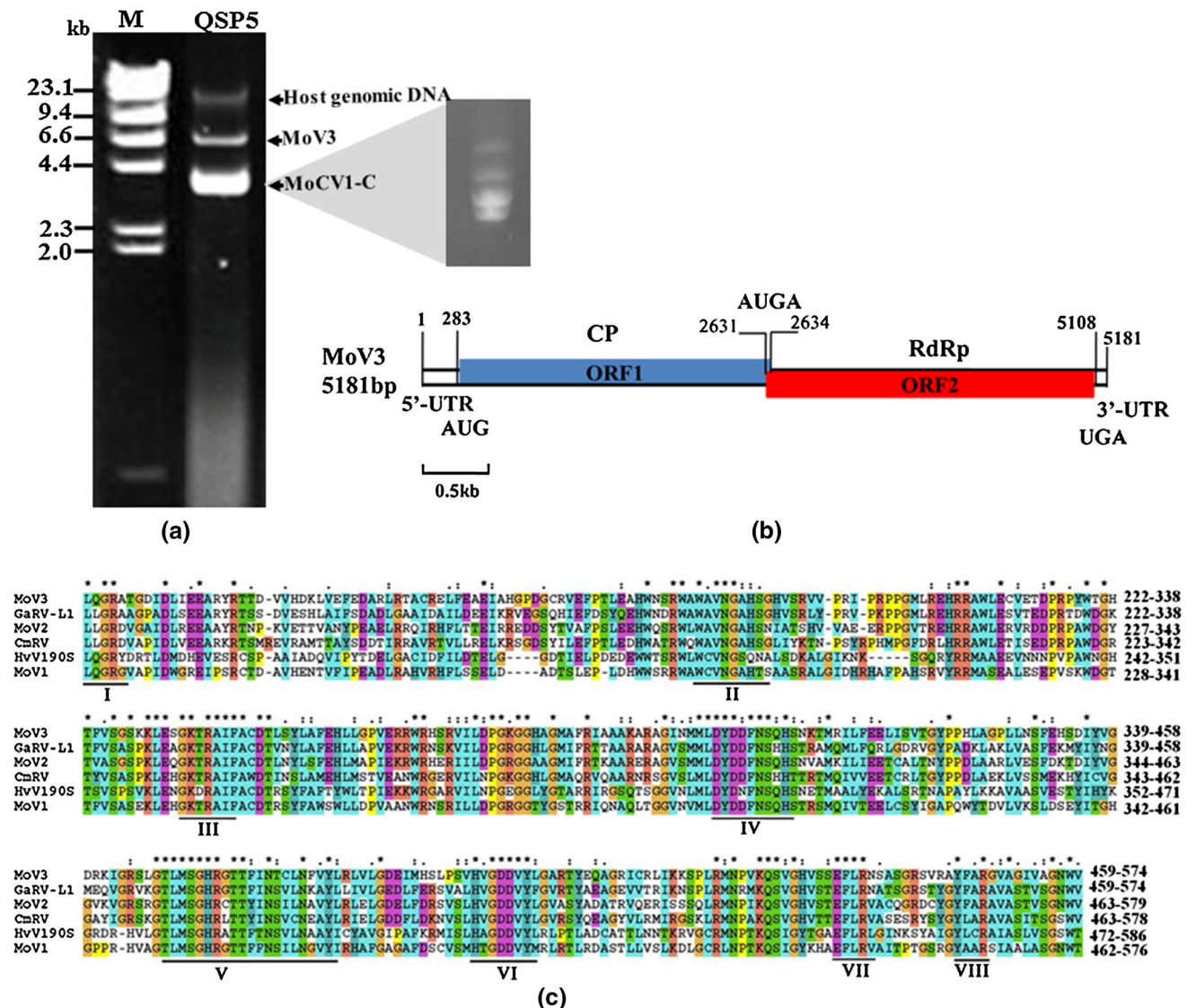


Fig. 1 (a) dsRNAs in *M. oryzae* strain QSP5. Total dsRNA extracted from mycelia of strain QSP5. dsRNA samples were fractionated on a 1.0 % agarose gel and stained with ethidium bromide. The positions of host genomic DNA and viral dsRNAs (MoV3, MoCV1-C) of strain QSP5 are indicated. Lane M, Hind III-digested λ DNA marker. (b) Diagrammatic representation of the genomic organization of

MoV3. (c) Amino acid sequence alignment of RdRps between MoV3 and selected viruses (GaRV-L1, MoV2, CmRV, HvV190S and MoV1) of the genus *Victorivirus*. Conserved motifs are motif I to VIII, asterisks represent identical residues, and colons represent similar residues

were obtained by RT-PCR using specific primers. Third, to obtain the 5'- and 3'-terminal sequence of dsRNA, an adaptor, pC3-T7loop (P-GGATCCCGGAATTCGGTA ATACGACTCACTATATTTTTATAGTGAGTCGTATT A-OH), was added to the terminal sequence of the purified dsRNA segments. The dsRNA with pC3-T7loop was then used as a template for RT-PCR using the specific primer pC2 (5'-CCGAATTCCCGGGATCC-3'), which was based on pC3-T7loop. Sequence analysis, alignments, phylogenetic analysis and identification of protein motifs was done by using the DNAMAN, CLUSTALX, MEGA5.0 and BLAST programs at the National Center for Biotechnology Information website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) [3, 9].

Sequence data

The full-length cDNA sequence of MoV3 has been deposited in the GenBank databases with accession number KP893140. The complete genome of MoV3 in strain QSP5 was 5181 bp, with a GC content of 61 %. Sequence analysis indicated that MoV3 has two overlapping open reading frames (ORF1 and ORF2). The 5'-proximal ORF1 (nt 283-2634) encodes a putative coat protein (CP) of 783 amino acid residues with a molecular weight of 80,939 Da that shows high similarity to the CPs of viruses in the family *Totiviridae*, particularly to those of *Gremmeniella abietina* RNA virus L1 (GaRV-L1, 73 % identity) [11], *Magnaporthe oryzae* virus 2 (MoV2, 67 % identity) [8], and *Coniothyrium minitans* RNA virus (CmRV, 63 % identity) [3]. Moreover, there is an Ala/Gly/Pro (A/G/P)-rich region near the C-terminal sequence (amino acid position from Gly⁷¹¹ to Gln⁷⁸³) of MoV3 CP, which is a unique molecular feature of members of the genus *Victorivirus* in the family *Totiviridae* [19]. The 3'-proximal ORF2 (nt 2631-5108) encodes a putative RNA-dependent RNA polymerase (RdRp) of 824 amino acid residues with a molecular weight of 90,506 Da, which showed a high similarity to the RdRps of viruses in the family *Totiviridae*, such as GaRV-L1 (58 % identity), MoV2 (52 % identity) and CmRV (49 % identity). According to the ICTV rules for species demarcation in the genus *Victorivirus*, sequence identity less than 60 % in either the CP or the RdRp protein indicates that the viruses belong to different species [19]. Although the CP sequence of MoV3 shares 67 % identity with MoV2, the RdRp sequence shares only 52 % identity with MoV2, which is less than the 60 % cutoff for species delimitation. Thus, MoV2 could be a new species in the genus *Victorivirus*. The stop codon of ORF1 overlaps the start codon of ORF2 with the tetranucleotide sequence AUGA, which is characteristic of the genus *Victorivirus* of the family *Totiviridae*. The 5' untranslated region (UTR)

and 3'-UTR contains 282 and 73 nucleotides, respectively (Fig. 1b).

In addition, a multiple sequence alignment of the deduced amino acid sequences of the RdRps of MoV3 and five other victoriviruses (GaRV-L1, MoV2, CmRV, HvV190S and MoV1) in family *Totiviridae* revealed that MoV3 has eight conserved motifs that are typical of members of the family *Totiviridae*. In addition, the eight conserved regions exhibited high sequence similarity among these viruses (Fig. 1c).

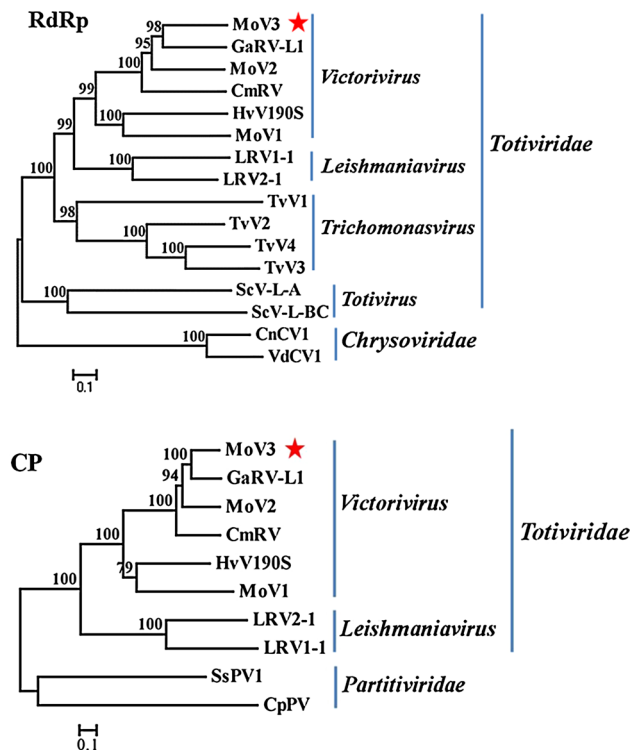


Fig. 2 Phylogenetic analysis of the RdRp and CP genes of MoV3 and related viruses in the families *Totiviridae*, *Chrysovirus* and *Partitiviridae*. The unrooted phylogenetic tree was constructed by the neighbor-joining method using the program MEGA 5.0. Bootstrap values (1000 replicates) are shown at the nodes, and the scale bar (0.1) corresponds to genetic distance. The position of MoV3 is indicated by a red star. The sequences of the RdRp and CP were obtained from the GenBank database. The mycoviruses are as follows (abbreviation; GenBank accession no.): *Gremmeniella abietina* RNA virus L1 (GaRV-L1; AAK11656.1), *Coniothyrium minitans* RNA virus (MoV2; YP_001649206.1), *Coniothyrium minitans* RNA virus (CmRV; YP_392467.1), *Helminthosporium victoriae* virus 190S (HvV190S; NP_619670.2), *Magnaporthe oryzae* virus 1 (MoV1; P_122352.1), *Leishmania* RNA virus 1-1 (LRV1-1; NP_041191.1), *Leishmania* RNA virus 2-1 (LRV2-1; NP_043465.1), *Trichomonas vaginalis* virus 1 (TvV1; ABF57713.1), *Trichomonas vaginalis* virus 2 (TvV2; AED99806.1), *Trichomonas vaginalis* virus 4 (TvV4; AED99794.1), *Trichomonas vaginalis* virus 3 (TvV3; AED99800.1), *Saccharomyces cerevisiae* virus L-A (ScV-L-A; NP_620495.1), *Saccharomyces cerevisiae* virus L-BC (ScV-L-BC; NP_042581.1), *Cryphonectria nitschkei* chrysovirus 1 (CnCV1; ACT79256.1), *Verticillium dahliae* chrysovirus 1 (VdCV1; ADG21213.1)

Phylogenetic analysis of the RdRps between MoV3 and selected viruses in the families *Totiviridae* and *Chrysoviriidae* showed that the putative RdRp of MoV3 clusters closely with the members of genus *Victorivirus* (GaRV-L1, MoV2, CmRV, HvV190S and MoV1). Furthermore, phylogenetic analysis of the CP of MoV3 and that of viruses in the families *Totiviridae* and *Partitiviridae* also indicated that the putative CP of MoV3 clusters with members of genus *Victorivirus* (Fig. 2).

Our present results showed that MoV3 is a new member of the genus *Victorivirus* of the family *Totiviridae*. Our report provides preliminary evidence that two distinct viruses, MoV3 and MoCV1-C, from the families *Totiviridae* and *Chrysoviriidae*, could exist in the same host, which is similar to what has been reported previously for *Helminthosporium victoriae*, which can be co-infected by the victorivirus *Helminthosporium victoriae* virus 190S and the chrysovirus *Helminthosporium victoriae* virus 145S [5].

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