Molecular Characterization of Fusarium Graminearum Virus 2 Isolated from Fusarium graminearum Strain 98-8-60

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Fusarium graminearum virus 2 (FgV2) infects Fusarium graminearum strain 98-8-60 and has at least five segments of double-stranded RNAs (dsRNAs), denoted as dsRNA-1 to dsRNA-5. In this study, the genome of FgV2 was sequenced and its phylogenetic relationship with other mycoviruses was analyzed. The lengths of FgV2 dsRNAs 1–5 ranged from 2414 to 3580 base pairs (bp). The 5’ and 3’ untranslated regions (UTRs) are highly conserved, and each dsRNA segment had 78–105 and 84–306 bp of 5’ and 3’ UTRs, respectively. Each dsRNA segment contained a single open reading frame (ORF). Computer analysis of dsRNA-1 revealed a putative open reading frame (ORF) that shows high sequence identity with an RNA-dependent RNA polymerase (RdRp) containing eight conserved motifs. dsRNAs 2–5 also each contain one putative ORF coding for products of unknown function. The sequences of FgV2 dsRNA-2 and dsRNA-3 have significant sequence identity with Magnaporthe oryzae chrysovirus 1 (MoCV1) dsRNA-3 and -4, respectively. When compared to other dsRNA mycoviruses in a phylogenetic analysis of the putative RdRp protein, FgV2 was found to form a distinct virus clade with Aspergillus mycovirus 1816 and MoCV1 in the family Chrysoviridae.

Keywords: Chrysoviridae, Fusarium graminearum virus 2, Mycovirus

The Fusarium graminearum clade [Teleomorphs: Gibberella species] is an important plant pathogens that cause head and seedling blight of small grains such as wheat and barley, stalk and ear rot of corn, and stem rot of carnation and thus cause enormous economic losses and mycotoxin contamination in cereals (Cook, 1981; Lee et al., 2010). In previous research, we determined the diversity and incidence of mycoviruses that infect F. graminearum clade based on the presence of dsRNAs (Chu et al., 2004). Among the viruses that infect the F. graminearum clade, Fusarium graminearum virus 1-DK21 (FgV1-DK21) has been reported to be associated with reduced mycelial growth, increased pigmentation, and reduced virulence on wheat plants (Chu et al., 2004). In contrast, the following three dsRNA viruses, which were isolated from F. graminearum strains 98-8-60 and DK3, infect their host asymptotically: Fusarium graminearum virus 2 (FgV2), Fusarium graminearum virus 3 (FgV3), and Fusarium graminearum virus 4 (FgV4) (Chu et al., 2004; Yu et al., 2009). Complete genome sequence analysis and molecular characterization of FgV1-DK21 indicated that its putative genomic organization and gene expression are similar to those of the plant potex-like ssRNA virus group, although the sequence similarity was low for both the RNA-dependent RNA polymerase (RdRp) domain and the full-length ORF1 (Kwon et al., 2007). In contrast, analysis of both genome organization and phylogeny indicated that FgV3 was closely related to members of the families Totiviridae and Chrysoviridae whereas FgV4 formed a distinct clade with the family Partitiviridae (Yu et al., 2009).

A similar analysis has not been performed for FgV2 because the complete genome sequence of FgV2 has not been reported. Here, we describe the nucleotide sequence of FgV2 infecting F. graminearum strain 98-8-60. We report that FgV2 has at least five dsRNA segments. Sequence analysis revealed that each dsRNA segment encodes a single open reading frame (ORF) and contains highly conserved sequences at both 5’ and 3’ ends. F. graminearum strain 98-8-60 containing dsRNAs was obtained from barley (Chu et al., 2004). Viral dsRNAs were extracted from mycelia using CF-11 cellulose chromatography (Chu et al., 2004). The number and presence of dsRNAs were determined by 1% agarose and 6% polyacrylamide gels. cDNA synthesis and amplification using random primers were conducted as previously described (Kwon et al., 2007; Urayama et al., 2010). The amplified polymerase chain reaction (PCR) product was visualized on 1% agarose gel by ethidium bromide (EtBr) staining. PCR products were purified using a QiAquick PCR purification
kit (Qiagen, Hilden, Germany) and subsequently cloned into the pGEM-T Easy Vector (Promega, Madison, WI, USA). Based on the dsRNA sequences obtained, dsRNA-specific primers were designed to amplify overlapping fragments and were used for RT-PCR. The 5' and 3' untranslated regions (UTRs) of each dsRNA segment were identified by the RACE method (Cold Spring Harbor Laboratory Press, 2005). All clones were sequenced at least three times in both orientations using universal and specific PCR primers at the National Instrumentation Center for Environmental Management, Seoul National University, Korea. Each dsRNA segment was identified by Northern hybridization using the cloned cDNAs as probes. Nucleotide sequence data were assembled using the Seqman program in DNASTAR. A sequence similarity search of viral RNAs was conducted with the NCBI BLAST program. The complete nucleotide sequences of dsRNA segments 1 to 5 of FgV2 were deposited in GenBank (accession numbers HQ343295 to HQ343299, respectively). The alignment of putative RdRp amino acid sequences of FgV2 dsRNA-1 and selected dsRNA mycoviruses was performed using BioEdit sequence alignment editor (Version 7.0.9) (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and GeneDoc programs (http://www.nrbsc.org/gfx/genedoc/). Phylogenetic analysis of RdRp regions of dsRNA mycoviruses including FgV2 were carried out by the MEGA program, version 4.0 (Kumar et al., 2008).

Previously, we reported that dsRNA mycovirus infecting F. graminearum strain 98-8-60 contained two similar sized dsRNAs of ca. 3.5 and 2.5 kbp with occasional occurrence of one additional smaller dsRNA of approximately 2.3 kbp based on separation on a 1% agarose gel (Chu et al., 2004).

In the current study, however, at least five dsRNA segments were always observed on a 6% polyacrylamide gel, and one or two additional smaller dsRNAs were infrequently observed (data not shown). Because these smaller dsRNAs are unstable in both repeated subculturing and single spore isolation, it is possible that these dsRNAs may be satellite RNAs, and thus they were not regarded as genome segments of FgV2. Accumulation of smaller satellite dsRNA(s)