Complete Genome Sequence of a Novel Vitivirus Isolated from Grapevine

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A novel virus-like sequence from grapevine was identified by Illumina sequencing. The complete genome is 7,551 nucleotides in length, with polyadenylation at the 3′ end. Translation of the sequence revealed five open reading frames (ORFs). The genomic organization was most similar to those of vitiviruses. The polymerase (ORF1) and coat protein (ORF4) genes shared 31 to 49% nucleotide and 40 to 70% amino acid sequence identities, respectively, with other grapevine vitiviruses. The virus was tentatively named grapevine virus F (GVF).

To date, four different viruses, all members of the genus *Vitivirus* (family *Betaflexiviridae*), are reported to be associated with diseases in grapevine. These viruses have been referred to as grapevine virus A (GVA), grapevine virus B (GVB), grapevine virus D (GVD), and grapevine virus E (GVE) (1). These grapevine-infecting vitiviruses are reported to be associated with the rugose wood complex, which includes several important diseases that produce woody cylinder modifications.

We have encountered a novel *Vitivirus* during the characterization of black grape accession AUD46129. Bioassay of that accession resulted in death within 1 to 2 years of Cabernet Sauvignon plants propagated on Freedom, 420A, 3309C, and 101-14 rootstocks. In order to understand the pathological reaction, we extracted double-stranded RNA (dsRNA) from the source of the virus and used the dsRNA as a template for DNA library construction (2). The library was subjected to high-throughput sequencing using the Illumina platform (Eureka Genomics, Hercules, CA).

BLAST analysis of the assembled reads against the GenBank database (3) revealed 15 contigs, ranging in size from 114 to 988 nucleotides, and showed a distant relationship with grapevine vitiviruses. Using total RNA from the original vine as a template, PCR was used to reconfirm the presence of the novel sequences and to generate sequence information to fill in the gaps between the 15 contigs. The exact 5′- and 3′-end sequences of the putative new virus were obtained using the FirstChoice RLM-RACE kit (Life Technologies, Grand Island, NY).

The complete genome was 7,551 nucleotides in length, plus a polyadenylate 3′ tail. The genome structure revealed five open reading frames (ORFs) organized similarly to other vitiviruses. ORF1 encoded a polypeptide of 1,727 amino acids with a calculated molecular mass of 196.9 kDa and containing conserved domains characteristic of methyltransferase, RNA helicase, and an RNA-dependent RNA polymerase. ORF2 encoded a 17.9-kDa hydrophobic protein of unknown function. ORF3 encoded a 30.4-kDa movement protein homolog. ORF4 encoded a 21.7-kDa capsid protein-like gene. ORF5 encoded a 12.3-kDa protein similar to an RNA binding protein.

ORFs 1 through 5 shared 31 to 49%, 8 to 26%, 28 to 47%, 40 to 70%, and 19 to 51% homologies, respectively, with other previously known grapevine vitiviruses.

Nucleotide sequence accession number. We have tentatively named the novel virus described here grapevine virus F (GVF). The GenBank accession number for the sequence of this virus is JX105428. We are currently pursuing field surveys and biological studies that appear to show the involvement of this novel virus in graft incompatibility reactions.

REFERENCES

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