Diseases Caused by Viruses

First Report of Grapevine Asteroid Mosaic Associated Virus in Grapevine in Spain

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Grapevine asteroid mosaic associated virus (GAMaV) is a member of the genus *Marafivirus*, family *Tymoviridae*. GAMaV was initially found to infect grapevine (*Vitis vinifera*) in California and was also reported in Japan, Canada, Uruguay, France, Hungary, and Italy (Candresse et al. 2017; Nakaune et al. 2008; Porceddu et al. 2018; Vargas-Asencio et al. 2017). In July 2019 a grapevine sample from cultivar Tempranillo (TS1), collected in a random survey from a vineyard in a Spanish grapevine growing area (D.O. Utiel-Requena), showing chlorotic mottling and leaf deformations, was analyzed by high-throughput sequencing (HTS). Total RNA extracted from leaves was sequenced after ribodepletion (Ribo-Zero Plant kit, Illumina) using TrueSeq Illumina technology (150 nt pair-end reads). Data analysis was performed by CLC Genomics Workbench 10.1.1. After quality control and host genome subtraction, 2,410,654 reads were used for de novo assembly. BLAST analysis of the 13,303 contigs obtained revealed the presence of four contigs (2,736, 1,448, 1,285, and 954 nt in size) related to GAMaV, indicating the presence of this virus in TS1 sample. Contigs related to other viruses/viroids were also found, in particular grapevine rupestris stem pitting-associated virus, grapevine leafroll-associated virus 3, grapevine virus A, grapevine fleck virus, grapevine red globe virus, grapevine rupestris vein feathering virus, and hop stunt viroid. For the assembly of the full-length GAMaV genome, contigs were extended by mapping the reads against the contigs using Geneious Prime 2020 software. This mapping step allowed the recovery of the GAMaV genomic sequence (635 reads, average coverage per nucleotide 10.0) with the exception of a small gap of 147 nt in the helicase region of the polyprotein. The gap in the genomic region was covered by

reverse transcription polymerase chain reaction (RT-PCR) using two newly designed primers overlapping the flanking regions (GAMaV-3755-F, 5'-ATCCTCACCAACTCCC-3'; GAMaV-3985-R, 5'-GTTGGAAGTGGTGTG-3'). The nearly complete sequence of the isolate TS1 (6,692 nt, MT459830) showed 87.7% nucleotide identity with the isolate 16GVP031 (MK253012) from France. The phylogenetic analysis performed on the available GAMaV full-length genomes showed that the Spanish isolate was positioned in a distinct clade. The presence of GAMaV in Spain was further evaluated by RT-PCR. Specific GAMaV primers, GAMaV-F3 and GAMaV-R3 previously reported by Candresse et al. (2017), were used without any success, due to primer mismatching. Based on TS1 sequence, two primers (GAMaV-6010F, 5'-CCCTCCTCCTAGCGACGACC-3'; GAMaV-6426R, 5'-GGGTTGAGACGGCGGAGATC-3') were designed and used to amplify a fragment of 417 nt in the CP region. Sanger sequencing of the obtained RT-PCR product confirmed the HTS recovered sequence. A total of 52 randomly collected samples from the same grapevine growing area were analyzed by RT-PCR using the newly designed primers. One sample bearing similar symptoms, TS7 (MT770919, cv. Tempranillo), and eight symptomless samples, MS1, MS2, and MS3 (MT770911, MT770917, and MT770918, cv. Macabeo) and TS2, TS3, TS4, TS5, and TS6 (MT770912, MT770913, MT770914, MT770915, and MT770916, cv. Tempranillo), tested positive for GAMaV, thus confirming its presence in Spanish vineyards. The nucleotide identity between these partial sequences and the homologous region of TS1 ranged from 94.7 to 98.8%, 0.04 being the mean diversity among isolates at the CP genomic region estimated by MEGA X software. To our knowledge, this is the first report of GAMaV in grapevine in Spain. The presence of other viruses/viroids in TS1 sample and the finding of asymptomatic GAMaV-infected plants make it difficult to associate this virus to the observed symptomatology. Other latent or semilient GAMaV infections have been previously reported (Candresse et al. 2017; Martelli 2014).

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