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Characterization of the Phytopathogenic Fungus' Mycovirome.

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Abstract

Neofusicoccum parvum is a fungus that belongs to the Botryosphaeriaceae family and is one of the most aggressive causes of Botryosphaeria dieback, also known as grapevine trunk disease (GTD). The mycovirome of a single N. crassa strain was examined in this investigation. High-throughput sequencing of total RNA and subsequent bioinformatic analyses were used to describe parvum (COLB). We were able to describe six new mycoviruses from four different viral families using contig annotations, genome completions, and phylogenetic studies. Two victoriviruses from the Totiviridae family, one alphaendornavirus from the Endornaviridae family, two mitoviruses from the Mitoviridae family, and one narnavirus from the Narnaviridae family make up the virome. Sequencing the RT-PCR results obtained from total nucleic acids verified the existence of the co-infecting viruses.

Keywords: ***Mycovirus; High throughput sequencing; Fungi; Grapevine trunk disease; Botryosphaeriaceae; Neofusicoccum parvum***

Introduction

Grapevine trunk diseases (GTDs) are one of the most damaging diseases of the grapevine and a major source of concern for the global viticulture industry. Eutypa dieback, Esca, and Botryosphaeria dieback are the three primary illnesses caused by fungal pathogen complexes. Several species of the Botryosphaeriaceae (Ascomyceta) family have been linked to Botryosphaeria dieback, with Diplodia seriata, Diplodia mutila, and Neofusicoccum parvum being the most frequent in France. The latter is the most aggressive, with Lasiodiplodia viticola invading the cells and tissues of grapevine wood to develop an interior canker. The fungal infection can also induce foliar necrosis and chlorosis, which causes the affected plants to decline and possibly die. Several research have looked at the interactions between Botryosphaeriaceae species and grapevine cultivars, identifying several virulence factors involved in the disease process and highlighting differences between Botryosphaeriaceae species. Furthermore, some investigations have revealed intra-species virulence differences, demonstrating that there is no clear and stringent association between isolate genetic grouping and virulence.

Mycoviruses, sometimes known as fungal viruses, are found in all major fungal taxa, and their existence could be one of the causes contributing to the virulence variability shown in some Botryospheriaceae species. Even though many mycoviruses are thought to have no influence on their hosts' biology or fitness, some have been shown to have a major impact on the fungal host's biological features. In Botryosphaeria dothidea, an important phytopathogenic fungus, numerous viruses that give hypovirulence to infected strains have been discovered. Hu et alstudy .'s also sheds light on the molecular mechanisms underpinning B. dothidea's hypovirulence in pear caused by infection with two mycoviruses, botryosphaeria dothidea chrysovirus 1 and botryosphaeria dothidea partitivirus 1. Mycoviruses infecting Botryosphaeriaceae species that cause grapevine disease are less well studied, although a few viruses have been identified, particularly in Neofusicoccum luteum, N. parvum, and D. seriata. Two mycoviruses from Neofusicoccum luteum have been identified: neofusicoccum luteum mitovirus 1 and neofusicoccum luteum fusarivirus 1, which belong to the Mitoviridae and Fusariviridae families, respectively. Nerva et al. discovered a new endornavirus from D. seriata (diplodia seriata endornavirus 1), as well as six new viruses infecting N. parvum and belonging to the families Chrysoviridae (neofusicoccum parvum chrysovirus 1), Mitoviridae, Narnaviridae (neofusicoccum parvum narnavirus 1 (NpNV1) (neofusicoccum parvum ourmia-like virus 1). The virome of an N. parvum strain from Chardonnay grapevine plants collected in Burgundy (France) in 2009 was studied in this work, and six novel mycoviruses belonging to four different viral families were discovered and their complete or near-complete genomic sequences determined. In 2009, N. parvum COLB (S-116) was discovered on Chardonnay trees in nurseries, indicating a decline (Burgundy region, France). It was grown at 22 °C on malt agar medium (MA, 20 g/L malt, 15 g/agar) as described before [9]. The isolate's mycelium was scraped and freeze-dried after three days at 22 °C on MA media coated with a film (Hutchinson, Chalette/Loing, France). DNA was isolated as previously described [23]. After centrifugation, DNA was precipitated in isopropanol at 20 °C, the pellet was washed with 70% ethanol, and then solubilized in ultra-pure water. As previously stated, fungal identification was established by amplifying and sequencing the Internal transcribed spacer region (ITS). The TRI Reagent technique was used to extract total RNA from growing mycelium (Sigma-Aldrich, Lyon, France). Fresh mycelium from a half plate was ground in a precooled mortar in the presence of liquid nitrogen and sterile sand. The powder was homogenised in 2 mL of TRI Reagent and incubated for 5 minutes at room temperature. The manufacturer's protocol was followed for the next steps. Following the manufacturer's instructions, total RNAs were treated with DNAase using the Turbo DNA-free kit (Ambion, Thermo Fisher Scientific, Illkirch, France). Purified and concentrated RNAs were subsequently stored at –80 °C (RNA clean and concentrator kit, Zymo Research, Ozyme, Saint-Cyr-l'Ecole, France). Genewiz used the Illumina HiSeq platform (2*150 nt) to examine the sequencing library, which was made from rRNA-depleted RNA (Illumina Ribo-Zero rRNA removal kit) (Takeley, United Kingdom). The readings were assembled from scratch using CLC Genomics Workbench version 11.0 after a quality trimming phase. BlastN and BlastX comparisons with nucleotide and protein GenBank databases were used to annotate the resultant contigs. Using ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) (Accessed on 26 January 2021), sequences were screened for open reading frames (ORF) using either the conventional genetic code or the yeast mitochondrial code. The conserved domain search software on the NCBI website was used to find conserved protein domains in deduced amino acid sequences. Phylogenetic analyses included nucleotide (nt) and deduced amino acid (aa) sequences of viral contigs, as well as the equivalent sequences of related viruses. The ClustalW tool, as implemented in

MEGA version 7.0 [26], was used to achieve multiple alignments. The neighbor-joining technique was used to reconstruct phylogenetic trees with stringent aa distances. A randomised bootstrapping test was used to assess branch validity (1000 replicates). MEGA version 7.0 was used to calculate genetic distances (p-distances based on nt and aa identity). HTS was used to examine ribo-depleted RNAs from a culture of N. parvum COLB. A total of 115,166,554 cleaned reads were employed in the bioinformatic analysis after quality control and trimming processes. Long contigs (between 2064 and 13,816 nt) were identified using de novo assembly and BLAST-based annotation, indicating sequence similarities with known mycoviruses from the families Endornaviridae (one contig), Totiviridae (two contigs), Mitoviridae (two contigs), and Narnaviridae (one contig) (Table 2). The mapping of reads on the constructed contigs revealed that viral readings made up 47.4 percent of the total, distributed unevenly among the six contigs representing suspected viruses. Contigs 1 and 2, which are connected to mitoviruses, integrated the majority of the viral readings (93 percent), while the remaining four contigs only constituted 0.5 percent to 1% of the total reads. When comparing FpNV2, NpNV2, and AfNV1 RdRp, the pairwise aa identity values range from 53.5 percent (NpNV2 vs AfNV1) to 67.2 percent (NpNV2 vs AfNV1) (FpNV2 vs NpNV2). These three species, particularly NpNV2 and FpNV2, have been deemed unique despite the fact that they have not been officially approved by the ICTV. As a result, NpNV3 appears to be a separate species from FpNV2 and NpNV2, and we suggest the name neofusicoccum parvum narnavirus 3 for this new species.

Conclusion

Despite the fact that 71 narnaviruses have been recorded in the GenBank database, the ICTV has only authorised two species in the Narnavirus genus, Saccharomyces 20S narnavirus and Saccharomyces 23S narnavirus, complicating the taxonomic position of putative novel species, such as NpNV3. Nonetheless, for species demarcation, sequence criteria (less than 50% aa identity in the RdRp) and biological criteria (stable maintenance of multiple species in the same host) have been proposed. The disparity between the two accepted species and the enormous number of described —but not yet approved—species shows that the genus, and maybe the species demarcation criteria, are in desperate need of revision. In fact, the SHU levels found in this study were 30–50 times lower than those found in conventional hot peppers like habaneros (about 300,000 SHU). This reflects the Mediterranean diet's gastronomic and cultural applications. The high production paired with larger fruit weight is undoubtedly an advantage in hand harvesting, which is still extremely frequent with chillies, resulting in a higher final product yield for both the fresh and processed markets. As a result, the environment had little impact on the manipulation of these fruit characteristics. We created a more precise approach for shape attributes based on fruit scans to account for probable bias in hand measurements due to the curvature of fruits in the longitudinal section (in particular horn-shaped types). The fruit scan observations gave more detailed information, allowing for the measurement of more attributes than those that were visually recorded and manually collected. This demonstrates the utility of scans for phenotyping chilli pepper fruit. We identified similar trait correlations across locations, though BP had a higher number. This could be related to the different pedoclimatic conditions of the cultivation locations, as well as the quantitative nature of the qualities that are affected by environmental changes.

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