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O25: Detection and molecular characterization of newly emerging viruses in Greek vineyards

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INTRODUCTION

Grapevine is one of the most important crops in Greece, associated with wine and table grape production. It is affected by a variety of pathogens, among which viruses and viroids play a crucial role, causing important losses in vineyard production (Martelli, 2014). Recently, three newly identified viruses were detected in Greek vineyards: *Grapevine Roditis leaf discoloration-associated virus* (GRLDaV, genus *Badnavirus*, family *Caulimoviridae*), *Grapevine Pinot gris virus* (GPGV, genus *Trichovirus*, family *Betaflexiviridae*) and *Grapevine Syrah virus 1* (GSyV-1, genus *Marafivirus*, family *Tymoviridae*) (Maliogka et al., 2015; unpublished data). In Greece, studies that involve newly discovered virus agents of grapevine are rather limited. Thus the objective of this research was to investigate the incidence of GRLDaV, GPGV and GSyV-1 in Greek vineyards as well as to molecularly characterize the obtained isolates.

MATERIALS AND METHODS

During 2009-2017, 192 samples originating from local and foreign varieties were collected from 11 vineyards (7 locations) of Greece (Table 1). Total RNA isolation was conducted according to Chatzinasiou et al. (2010). For the detection of GRLDaV, an RT-PCR was conducted using the primer set BADNAUP/BADNADO (5'-GAA GGA ATT GAA TCT CCA GCA GCA GG-3', 5'-CTC TGC TAC ACC AAG TGA TAG ATT GTT GAG-3') (Maliogka et al., 2015) that amplifies a 261 bp part of the ORF2 gene, while for GPGV and GSyV-1 an RT-PCR was employed using the primers GPG-5637F/GPG-5939Do (5'-ATT GCG GAG TTG CCT TCA AG-3', 5'-CTG AGA AGC ATT GTC CCA TC-3') (Glasa et al., 2014) and GSyV1F/GSyV1R (5'-CCA CCA TCT TCA CCG TYG ATC C-3', 5'-CCA TDG GRG AGG TTT CAG ATT TG-3') that target a 295 bp fragment of the movement protein and a 347 bp part of the coat protein (CP) of each virus, respectively. Ten isolates of GRLDaV, 5 from GPGV and 10 from GSyV-1 were selected for sequencing analysis. Amplified PCR products were purified from agarose gel and sequenced. The sequences were aligned with CLUSTALW available in MEGA7 and subjected to phylogenetic analysis using the Maximum Likelihood (ML) method. Sequence identities were calculated using Geneious (Biomatters Ltd., Auckland, New Zealand).

Ribosomal RNA depleted total RNA from two grapevine samples, that were found positive to GRLDaV, GSyV-1 (A2-1, grafted) and GPGV (GpFd, self-rooted), were further subjected to NGS using Illumina platforms (NextSeq, HiSeq2500) in order to acquire the full sequences of the viruses. The obtained paired-end read data were trimmed and deduplicated using PrinSeq-lite, and subjected to *de novo* assembly, after the removal of host reads, using the MIRA assembler plugin implemented in Geneious. The resulting *de novo* contigs and reads were mapped to the reference genomes to produce the complete genome of the isolates.

RESULTS AND DISCUSSION

Results showed the presence of GRLDaV, GPGV and GSyV-1 in both self-rooted and grafted local varieties (Table 1). Interestingly, GRLDaV was found in high incidence in the Greek varieties and especially in the self-rooted ones (44.9%) from the islands of Cyclades. On the contrary the virus was not detected in any of the foreign varieties tested thus indicating that it is endemic in Greece and probably in some neighboring countries. This notion is further reinforced by the fact that it was recently identified in local varieties in Italy and in Turkey. On the other hand, GPGV was found in high incidence in the foreign varieties tested (37.2%) and in lower in the local ones, a fact that highlights its putative introduction through the imported plant material. Finally, GSyV-1 was also identified in higher incidence in the foreign (15.7%) rather than the local varieties. Overall, GSyV-1 was identified in lower frequency compared to the other two viruses.

Table 1: Virus species incidence in Greek vineyards

	GRLDaV	GPGV	GSyV1
Foreign Varieties (%)	0/51 (0%)	19/51 (37.2%)	8/51 (15.7%)
Greek Grafted Varieties (%)	16/63 (25.4%)	5/63 (8%)	2/63 (3.2%)
Greek Self-Rooted Varieties From Cyclades Islands (%)	35/78 (44.9%)	1/78 (1.3%)	2/78 (2.3%)
TOTAL (%)	51/192 (26.6%)	25/192 (13%)	12/192 (6.25%)

Similarity analysis of Greek GRLDaV isolates revealed nucleotide (nt) identities between 78%-98%. Two of the isolates, from the self-rooted varieties, were the most divergent showing an identity between 78-88% in nt with already characterized isolates. Phylogenetic analysis of GRLDaV showed that the self-rooted varieties from Cyclades were clustered together, with the exception of the two divergent ones which formed a different group, while sequences from the Greek grafted varieties were closer to the Italian and the W4 isolates. Analysis of GPGV sequences revealed high identity ranging between 95-98% in nt. In the phylogenetic analysis the Greek GPGV isolates were clustered with the Slovakian and Turkish isolates (SK56, 11MP). Similarity analysis of the CP gene of GSyV-1 revealed high nucleotide identity among the Greek isolates as well as between Greek and foreign isolates deposited in GenBank (89-100% in nt). Subsequent phylogenetic analysis clustered the obtained Greek GSyV-1 isolates in two major groups according to the classification by Glasa et al. (2015).

Recovery of full genome sequences from GRLDaV and GPGV was made possible using the NGS data. However, only few contigs could be obtained from GSyV-1 possibly due to the low concentration of the virus, which could be also correlated with its low frequency detection. Full genome analysis of the GPGV isolate (GpFd) showed 99% identity with a Slovakian isolate (SK704) further confirming the low diversity of the virus. On the other hand, the GRLDaV isolate (A2-1) was 89.4-89.8% similar to the Italian Bobino Nero and the Greek W4 isolates, respectively. Interestingly, whole genomes analysis of the full sequences of GRLDaV also revealed the presence of a highly variable region with an indel polymorphism. Further analyses are currently underway in order to better analyse the diversity and evolutionary history of GRLDaV.

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