

**GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS
INFLUENCES EXPRESSION OF miRNAs AND RESILIENCE TO
DROUGHT IN *VITIS VINIFERA***

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Grapevine rupestris stem pitting-associated virus (GRSPaV) is a virus that infects grapevines and establishes a compatible interaction in *Vitis vinifera* without the development of macroscopic phenotypic alterations. Some unexpected responses are induced in *V. vinifera* by GRSPaV, including overlapping responses to drought and salinity stress. In the frame of a CNR project (Progetto Premiale AQUA), we hypothesised an interaction between GRSPaV and drought and we subjected GRSPaV-free and infected plants to water stress under controlled conditions. By investigating ecophysiological parameters, we show that GRSPaV activates in *V. vinifera* a physiological state that induces resilience to drought. Indeed, infected plants under controlled water stress conditions show (i) a high rate of photosynthesis and stomatal conductance; (ii) low hydraulic resistance to water transport; and (iii) more growth than GRSPaV-free plants. The molecular basis of these virus-grapevine-drought interactions is still poorly understood; however, it is reasonable to hypothesise an involvement of RNA silencing, which is a natural defence against invading viruses in plants. Micro(mi)RNAs have fundamental roles in plant development and adaptation to stresses through post-transcriptional control of several physiological pathway intermediates. The analysis of four libraries of small RNAs (i.e., from GRSPaV-free and infected plants, both in well watered and stress conditions) allowed us to highlight some known miRNAs and novel miRNA candidates that could be correlated with the physiological modifications in plants upon water stress treatment. Targets of known (i.e. miR396, miR164, miR156, miR3633) and novel miRNAs (miRC121, miRC129, miRs409712_2) annotated as players in drought stress adaptations were indeed validated. The relevance of miRNAs in the interaction between GRSPaV infection and water stress is not defined only by their tissue abundance but mainly by their activity on targeted mRNAs. In infected plants, the high photosynthesis reported above was accompanied by higher stomatal conductance, which was also linked to modifications in leaf morphology (high stomatal density and cell number). In infected grapevines, we observed the regulation of several miRNAs and their targets involved in leaf development (miR156, miR164, miR319, miR394, miR396).

The grapevine is a woody plant that has been cultivated and selected together with its viruses for centuries. Our data support the idea of mutual adaptation between GRSPaV and grapevine,

resulting in beneficial effects for the host under water stress conditions. Therefore, GRSPaV might represent the first example of a plant virus that is more appropriately defined by the categories of 'conditional mutualism' and 'beneficial virus' proposed by Roossinck (2011).