Uncovering gene silencing mechanisms in grapevine and their response to abiotic stress.

Part of plant defense reactions to biotic mechanisms involves post-transcriptional gene silencing (PTGS). During a viral infection, cleavage of double-stranded RNA (dsRNA) viral RNA molecules by DICER-LIKE (DCL) enzymes forms primary siRNAs. These siRNAs are supported by the Argonaute proteins that, in turn, cleave the viral RNA. The cleavage products are converted into dsRNA by RNA-dependent RNA polymerases (RDR), and then cleaved by DCL enzymes to form secondary siRNAs (Vaucheret, 2006; Voinnet, 2006). This amplification step of small RNAs is particularly critical for conferring resistance to virus (Wang et al, 2009). In *Arabidopsis* and *Nicotiana benthamiana*, this defence mechanism works very well at temperatures above 20°C but is inactive at 15°C (Szittya et al, 2003). This is presumably due to the temperature sensitivity of the primary RNA polymerase RNA-dependent PTGS: *RDR6* (Wu et al, 2008, Guo et al, 2010, Niu et al, 2006). Grapevine is a perennial plant exposed to very large temperature amplitudes. It is therefore possible that the defense strategies based on PTGS are inactive due to the exposure of the grapevine at low temperatures. The aim of this project is to develop plant material and functional assays to characterize PTGS in grapevine. We will study the response of these mechanisms to drastic temperatures changes, with a particular focus on the Vv*RDR6* gene expression control.

Characterization of gene silencing mechanisms in grapevine will be performed on the 40024 line, derived from Pinot Noir, whose genome was sequenced (Jaillon et al, 2007). We have obtained a transgenic line expressing GFP from 40024 line. Our preliminary studies suggest that transgenic and non-transgenic plants develop similarly at high and low temperature (≤12°C and ≥30°C) and transgenic plants that retain a normal fluorescence. This line is being re-transformed with a construct expressing a sequence that can form a GF-FG stem-loop in order to produce siRNAs corresponding to the GF portion of the GFP gene (Moissiard et al, 2007). Fluorescence studies coupled with molecular analysis to detect GF and P-derived siRNAs will be informative about gene silencing preservation in plants.

In addition, several 40024 lines transformed with the stem-loop GF-FG were obtained and are under molecular characterization. Graftings between lines GFP and GF-FG will be conducted to determine whether gene silencing is transmitted from stock to scion as shown in tobacco or *Arabidopsis* (Palauqui et al, 1997, Voinnet and Baulcombe, 1997). The series of grafts GFP / GF-FG and GF-FG/GFP will be grown at different temperatures in order to evaluate the temperature sensitivity of PTGS and its transmission. The same plant material will be challenged with GFLV infection to determine whether this grape virus encodes a putative PTGS suppressor. In parallel, the orthologous gene *AtRDR6* will be identified *in silico* in thegenome of the grapevine, and its expression will be characterized by RT-PCR from 40024-derived RNAs.
The *VvRDR6* and *AtRDR6* gene promoters will be cloned upstream of the GUS reporter gene. Transgenic *Arabidopsis* and grapes will be produced for both constructs. A thorough comparison of *RDR6* expression under temperature stress and GFLV infection will be conducted at histochemical and molecular levels. Results obtained in the frame of this project shall help defining strategies to protect grapevine against its major viruses.