

Grapevine resistance to downy mildew: the innovative role of subtilisin-like proteases

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Objectives: to perform a functional characterization of the grapevine resistance associated subtilase VviSBT4.19; to achieve a comprehensive analysis of gene expression and lipid modulation in grapevine VviSBT4.19 transgenic plants after inoculation with the downy mildew pathogen; to access the link between subtilases and lipid signalling events and to propose a model of action

Methodology: Grapevine is one of the major crops grown in temperate climates. The cultivated *Vitis vinifera* is highly susceptible to downy mildew caused by the obligatory oomycete *Plasmopara viticola* leading to severe losses. A great effort has been conducted on the characterization of the resistance mechanism on this pathosystem. On this field we are one of the leading groups and we have already identified, through an 'Omics approach, key processes and mechanisms leading to grapevine resistance. Subtilisin-like proteases (subtilases) appear as key players in grapevine resistance to downy mildew. In model plants such as tomato and *Arabidopsis*, subtilases were already described as being associated to resistance mechanisms against pathogens and immune priming events, although their action mechanism remains unknown. Moreover, very recently subtilases were linked to jasmonic acid (JA) signalling events. As we have previously shown JA involvement in *P. viticola* resistance we believe that subtilases may be linked to this signalling mechanism. So far we have characterized grapevine subtilase gene family and selected a candidate for functional studies, VviSBT4.19.

This PhD is integrated in the project FCT PTDC/AGR-PRO/2438/2014 (associated to IF/00819/2015) that aims at a characterization of grapevine subtilases involved in *P. viticola* resistance. This PhD project aims at achieving a deeper knowledge on the function and model of action of VviSBT4.19 as well as to exploit the link between subtilases and lipid signaling events through a systems biology approach. We have previously obtained transgenic grapevine plants overexpressing VviSBT4.19 in collaboration with Professor Günther Buchholz, the Head of grapevine biotechnology department at RLP AgroScience GmbH/ AlPlanta - Institute for Plant Research, Germany. It is intended to analyse transgene integration and to use leaf discs inoculation assays to evaluate *P. viticola* resistance. Gene expression analyses (using both high-throughput and targeted approaches) will be followed to monitor gene modulation in transgenic plants after inoculation with *P. viticola*. A lipidomics approach will also be conducted using several techniques such as Fourier transform ion cyclotron resonance (FTICR), Gas-chromatography (GC-FID) and Thin Layer Chromatography (TLC). In parallel it is intended to use the model plant *Arabidopsis thaliana* for subcellular location and protein interaction studies with GFP-constructs. These approaches will allow us to identify the key processes and genes linked to grapevine subtilases mechanism. New resistance associated candidates are expected to be identified and they will be further evaluated by our team in *Arabidopsis* mutant lines for orthologue genes. Both Msc and PhD students are already involved in this thematic aiming at a comprehensive analysis of the role of subtilases in grapevine resistance.

Type of fellowship: Mixed