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**Post 'OMICS.  
Contribution for a sustainable  
viticulture**

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# Post 'OMICS. Contribution for a sustainable viticulture

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## ABSTRACT

The reduction of phytochemicals used to control pests and diseases is one of modern agriculture's demands. Grapevine is one of the most important crops grown in temperate climates where Europe's wine industry represents 40% of the world production. The cultivated grapevine, *Vitis vinifera* is prone to several diseases, being downy mildew one of the most devastating. Preventive fungicide applications are used on each growing season to control disease incidence with major environmental and economic constrains. A deeper knowledge on the grapevine – *P. viticola* interaction is needed to define alternative disease control strategies. A first approach based on 'OMIC technologies, allowed us to fully characterize this interaction and to identify several resistance associated candidates and mechanisms that should be further exploited. Of them, lipid-associated signaling mechanisms linked to the modulation of chloroplast associated lipids is important for photosynthetic machinery protection and jasmonic acid (JA) biosynthesis leading to the establishment of the incompatible interaction. Also, subtilisin-like proteases (subtilases) appear as key players for pathogen recognition and immune priming activation. All the knowledge generated by the 'OMIC approaches also highlighted the need to explore natural grapevine biodiversity, to identify novel inducers of disease resistance (priming or elicitor agents) in order to enhance grapevine defense mechanisms and ultimately to identify biocontrol agents. All of these approaches will allow us to define new phytochemical-free disease control measures for a greater sustainability of viticulture practices.

**Keywords:** grapevine, downy mildew, serine proteases, Lipid signaling, microbiome

## VITIS VINIFERA L.

Grapevine (*Vitis vinifera* L.) represents a great agricultural and economic value worldwide, with deep ties to human culture for more than 5000 years. In 2017, grapevine plantation areas reached 7.6 mha, with Spain, Italy and France leading the grapevine plantation areas in Europe (OIV report, 2018). Its fruit is mostly processed into wine, but is also consumed fresh (grape) and dried (raisins). With lower economic expression, it is also processed into nonalcoholic juice, and distilled into spirits (OIV report, 2018).

Fungi and oomycetes are considered to be responsible for major crop losses in all grapevine-growing countries. One of the most threatening diseases is caused by the obligate biotrophic oomycete *Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni, the grapevine downy mildew disease. This pathogen was

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introduced in Europe in the 19<sup>th</sup> century becoming one of the most devastating diseases for today's viticulture. The main specie cultivated in Europe for wine and berry production is the domesticated grape, *Vitis vinifera* subsp. *Vinifera* with around 10 000 varieties being selected for their quality and adaptation to different climatic conditions and developed by vegetative propagation and by crosses (OIV report, 2018). By evolving without pathogen pressure, *V. vinifera* is highly susceptible to pathogens, particularly to downy mildew. Disease management relies widely on the extensive use of synthetic chemicals, bearing high economic and environmental costs (Gessler *et al.*, 2011). American grapevine species exhibit natural host resistance against this pathogen and have been used in breeding programs resulting on *V. vinifera* varieties such as 'Regent' and 'Solaris' (Vitis International Variety Catalogue 2011) that harbor high resistant traits to grapevine pathogens. However, the defense mechanisms of these newly bred hybrids are being overcome by new *P. viticola* resistance-breaking isolates in Europe (Peressotti *et al.*, 2010).

Plants employ sophisticated mechanism to defend themselves from pathogens (reviewed in Jones and Dangl, 2006). On a first level, plants recognize conserved molecules and/or molecular patterns from pathogens activating the Pathogen Associated Molecular Patterns (PAMP) – Triggered Immunity (PTI). This first response is sufficient to overcome the invading pathogen, but in some cases, pathogens are able to counteract PTI through effector molecules secretion, manipulating host mechanism. These effector molecules may be specifically recognized by plant resistance proteins receptors (R) and a second line of defense occurs (reviewed in Bozkurt *et al.*, 2012). This interaction between plant R proteins and pathogen effectors triggers an immunity based on effectors (ETI). To overcome ETI, pathogens evolve their effectors and counteracting plants evolve new receptors – “zigzag” model (Jones and Dangl, 2006).

New strategies for plant improvement rely on the accurate characterization of grapevine resistance mechanisms against *P. viticola*.

We have previously used a system biology based on 'OMICS approaches, transcriptome, proteome and metabolome analysis (Figure 1), to identify mechanisms and resistance-associated candidates differentiating two grapevine cultivars were selected as our study model, 'Regent' a resistant hybrid and 'Trincadeira', a Portuguese traditional variety highly susceptible to *P. viticola*. Both genotypes are inherently different at a constitutive level, both at metabolite level, where 'Regent', presents higher accumulation metabolites related with primary and secondary metabolism (Figueiredo *et al.*, 2008) and at transcriptome level, where several genes associated to plant immunity are constitutively expressed, as the case of those encoding subtilisin-like proteases (Figueiredo *et al.*, 2012; Monteiro *et al.*, 2013). In the first hours of interaction with the pathogen, several transcripts and proteins related to lipid signaling events are more accumulated in 'Regent' (Figueiredo *et al.*, 2012; Ali *et al.*, 2012; Figueiredo *et al.*, 2015; Guerreiro *et al.*, 2016; Figueiredo *et al.*, 2017a). At the metabolome level the response of the resistant genotype is also characterized by accumulation of phenolic metabolites such as trans-caftaric acid (caffeoyl derivative), trans-fertaric acid (feruloyl derivative) and quercetin-3-O-glucoside (Ali *et al.*, 2012). Moreover, lipid signalling events and the activation of the octadecanoid cascade leading to jasmonic acid production seem to be a key event on the establishment of the incompatible interaction. We have recently shown that after *P. viticola* challenge 'Regent' presents modulation of chloroplastidial lipids (MGDG and DGDG) leading to the increase of C18:3 fatty acid content which may be either associated to photosynthetic membranes protection or JA synthesis (Laureano *et al.*, 2018).

Moreover, recent research has pointed out the potential of alternative control methods together with the exploitation of plant immunity. These may be associated to the use of inducers of disease resistance (elicitors or priming agents) that lead to the production of antimicrobial proteins, accumulation of secondary metabolites, generation of reactive oxygen species (ROS), reinforcement of cell wall, callose deposition, HR-like cell death or to the use of biocontrol agents. Both rhizosphere and phyllosphere microbiomes are known to provide protection against plant pathogens through a variety of mechanisms (Marssat *et al.*, 2016). One of the most attractive aspects of the use of microbial biocontrol agents as alternatives to chemical fungicides is related to their multiple modes of action, thus preventing or at least delaying the build-up of fungicide-resistant strains.

We will further focus on our Post 'OMICS studies associated to the key players associated to resistance traits against *P. viticola* and on the soil metagenomics approach to identify biocontrol agents.

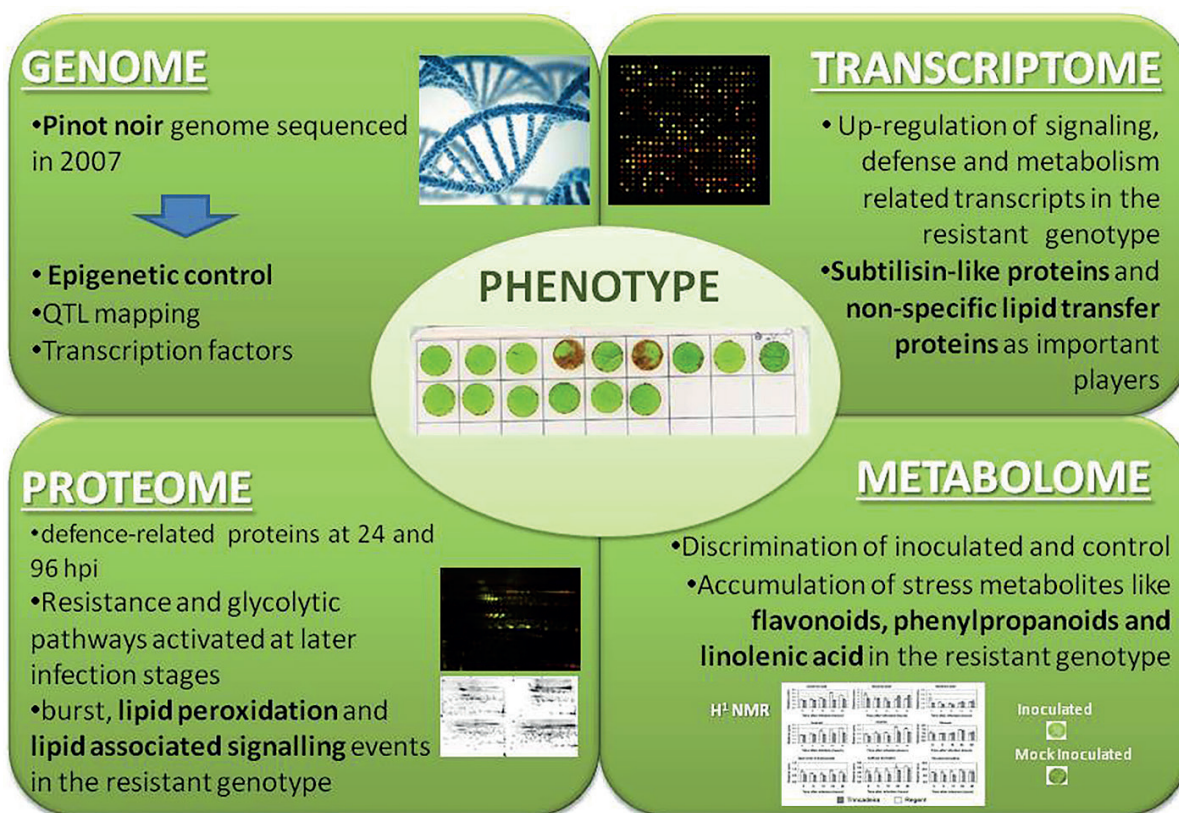


Figure 1 – 'OMICS approaches transcriptome, proteome that enable the identification of tolerance mechanisms and resistance-associated candidates in *V. vinifera* cv Regent.

## LIPID SIGNALING AND SERINE PROTEASES AS KEY PLAYERS ON GRAPEVINE DEFENCE AGAINST *P. VITICOLA*

The function of phytohormones in plant defense mechanisms has received considerable attention, it is assumed that salicylic acid (SA) is involved in the activation of defense responses against biotrophic and hemi-biotrophic pathogens and the establishment of systemic acquired resistance, jasmonic acid (JA)

is generally assumed to be involved in defense against leaf-chewing insects and necrotrophic pathogens (Glazebrook, 2005). However, we have previously shown that JA is also involved in the signaling events against *P. viticola*, a biotroph pathogen (Figueiredo *et al.*, 2015; Guerreiro *et al.*, 2016). Several enzymes involved in JA biosynthesis and signaling are up-regulated on the first hours of *P. viticola* challenge together with JA, JA-ILE and SA accumulation (Guerreiro *et al.*, 2016). Lipid transport also occurs as suggested by the early accumulation of non-specific lipid transfer proteins and fibrillins (Figueiredo *et al.*, 2017). A study by Polesani and co-workers (2010) also corroborates our results on this pathosystem by showing an increase of jasmonates and their biosynthetic enzymes in the resistant grapevine specie *V. riparia* after inoculation with *P. viticola*.

Moreover, our results point out to a preponderant role of serine proteases (subtilases) highlighting a possible participation of some subtilases on lipid signaling events. Subtilases are serine proteases that are synthesized as inactive proteins, suffer glycosylation during protein maturation and are secreted to the plant extracellular matrix (ECM) where they accumulate and presumably recognize and process substrates (reviewed in Figueiredo *et al.*, 2017). Over the past five years, subtilases have been associated to immune priming events in plants, namely an Arabidopsis thaliana, an extracellular subtilase, SBT3.3, was identified and associated with enhanced innate immune response. Indeed, SBT3.3 expression promotes the chromatin remodelling in a durable auto-induction mechanism and activates a salicylic acid-dependent mechanism for amplified response of defence genes (Ramirez *et al.*, 2013). In grapevine, the first clues highlighting subtilase participation in defence mechanisms were reported by Figueiredo and co-workers (2008), where a subtilase, XM\_010660203.1, was shown to be highly expressed on the first hours after *P. viticola* inoculation (Figueiredo *et al.*, 2008, 2012; Monteiro *et al.*, 2013). We have also characterized the subtilase gene family and analysed the gene expression modulation of some members after inoculation with *P. viticola* (Figueiredo *et al.*, 2016; Figueiredo *et al.*, 2017c). We have found that the same subtilase was 300-fold enhanced in the resistant grapevine genotype 6 hours post inoculation (hpi) with *P. viticola* in 'Regent' and in the susceptible grapevine genotype, the expression induction of this subtilase suffers a time delay (Figueiredo *et al.*, 2016). This subtilase is predicted to be an extracellular protein that shares high sequence identity with other plant defence-associated subtilases, particularly with the pathogenesis-related protein 69 (P69) from tomato (Tornerio *et al.*, 1996) and SBT3.3 from *A. thaliana* (Ramirez *et al.*, 2013), described as being associated to an effective resistance response and priming events.

Further work must be conducted to validate this hypothesis, and particularly in the grapevine – *P. viticola* pathosystem, where both subtilases and JA seemed to be key players in the establishment of an incompatible interaction.

## BIOCONTROL AGENTS

To control the major fungal diseases, synthetic fungicides are intensively applied on each growing season, leading to severe environmental and health costs. Moreover, emerging of new fungal strains with increased resistance to different active ingredients should also be taken into account. Producers and consumers are gaining more conscience and new viticulture trends are appearing.

Bacteria and fungi live in complex co-associations with plants and have important roles in shaping the quality of the soil and in promoting the productivity and health of the plant itself. Plant microbiome has both direct and indirect relationships with its host, from transforming the availability of organic matter and essential nutrients in the soil, including nitrogen fixation, mitigating the impact of environmental stresses (such as drought or the presence of phytotoxic contaminants), to preventing the growth or activity of plant pathogens through competition for space and nutrients, antibiosis, production of hydrolytic enzymes, inhibition of pathogen-produced enzymes or toxins, and through systemic induction of plant defense mechanisms (Turner *et al.*, 2013).

The viticulture industry has been selectively growing grapevine cultivars with different traits for millennia, and small variations in soil composition, water management, climate, and the aspect of vineyards have long been associated with shifts in these traits. As our understanding of bacterial and fungal influence on plant characteristics improves, it is possible that also microbial flora that coexists with the plant may be one of the key factors that influence these traits. Recent advances on DNA sequencing technologies allowed vineyard microbiome characterization illustrating that different wine-growing regions maintain different microbial communities, with some influences from the grape variety and the year of production (Bokulich *et al.*, 2014). Also, the concept of soil as a source of microorganisms inhabiting grape surfaces was pointed out by the work of Zarraonaindia *et al.*, (2015). This study has shown that bacterial communities associated with grape leaves, flowers, and fruit shared a greater proportion of taxa found in soil compared with each other, which they suggested as evidence of soil serving as a bacterial reservoir in vineyards (Zarraonaindia *et al.*, 2015).

Understanding microbiome effect may help to determine sustainable production strategies. Soil microbiome manipulation can improve soil quality and, hence, crop productivity. This practice can also be used to help improve the wine terroir or even reproduce those terroirs in sites a priori not suitable for generating a wine with such characteristics. It is expected that climatic factors could constrain the biogeographic distribution of grape microbiome, the soil characteristics (e.g., availability of nutrients for the plant) and soil structure still has a great impact on constraining the microbiota that could colonize the remaining plant niches (reviewed in Gilbert *et al.*, 2013). Moreover, microbial biocontrol agents (BCAs) could be an alternative tool in organic grapevine downy mildew management. Microorganisms used in biocontrol are fungi, yeast and bacteria which, in addition to their antagonistic activity, can also be able to induce plant growth increase by either controlling minor pathogens or producing growth-stimulating factors (Vecchione *et al.*, 2007). We have started exploiting soil microbiome and the association soil-plant on a phytochemical-free vineyard in the Portuguese Douro region. So far, following a metagenomics approach based on a 4<sup>th</sup> generation sequencing technology, Oxford nanopore technology, we were able to sequence up to 1 million reads and to discriminate between soils of different locations (terroir effect) and different depths. The majority of the taxa found were identified as Bacteria, 6% and Eukaryota with <1% representation as Archaea and Viruses (unpublished data).

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