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Powdery mildew and grapevine: which genes, enzymes and metabolites in resistant and susceptible cultivars?

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John Bunyan descreveu, no "O Caminho do Peregrino, parte 2": «"and though with great difficulty I am got hither, yet now I do not repent me of all the trouble I have been at to arrive where I am. My sword I give to him that shall succeed me in my pilgrimage, and my courage and skill to him that can get it. My marks and scars I carry with me, to be a witness for me that I have fought His battles who now will be my rewarder."». Para ele, Deus foi o fio condutor ubíquo. A transversalidade da obra aplica-se ao sacrifício colossal que é escrever uma tese. Neste, a sucesso da "caminhada" não é apenas o reflexo singular do "peregrino", mas, em grande parte, uma repercussão de quem o acompanha. Como tal, agradeço aos meus alicerces omnipresentes e impulsionadores: à minha mãe (Mami querida), que numa visão reducionista e humilde é o ser mais perfeito que existe; e à Inês Diniz (Cara colega), a tão aguardada pessoa que teimava em nunca chegar. Pelo altruísmo gratuito, pelo bem querer genuíno, pela sapiência e, (acredito) pela amizade, obrigada!

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Resumo detalhado

A espécie euroasiática *Vitis vinifera* L. encontra-se taxonomicamente reduzida, por ausência de características morfológicas demarcadas e de uma barreira evolutiva impeditiva de fluxo genético, em duas subespécies. *V. vinifera* spp. *vinifera* (comumente denominada *V. vinifera*), como consequência da relação simbiótica estabelecida com o Homem, desde há 10,000 anos, é dos produtos hortícolas com maior impacto económico, à escala mundial, estando o seu cultivo primariamente direcionado para a produção de vinho, uvas de mesa e passas. Mais, o elevado teor em antioxidantes nos variados órgãos introduz ainda um valor fitoquímico na espécie. Apesar da detida superioridade agronómica e adaptativa, pela ausência de uma coevolução conjunta com stresses bióticos naturais, é altamente suscetível a uma diversidade de agentes patogénicos.

As fitopatologias fúngicas, pela sua representatividade mundial, nível de incidência em todas as cultivares e capacidade de infeção no anteceder ou preceder da colheita dos bagos, detêm o maior impacto associado à exploração hortícola da espécie. Dentro destas, o oídio, pelas particularidades biológicas, ecológicas e epidemiológicas do seu agente etiológico, *Erysiphe necator*, dispõe da maior expressividade. Apresentando uma estratégia de vida baseada na biotrofia obrigatória, *E. necator* diferencia estruturas de penetração (apressório) e recolha de nutrientes (haustório) com o intuito de perpetuar a infeção e completar o seu ciclo de vida. Nas diversas etapas do processo epidemiológico, é estabelecida uma interação dinâmica que culminará, aquando da presença de condições propícias, numa incompatibilidade (resistência) ou compatibilidade (doença) dos tecidos de videira à presença do fungo. Para ambas as interações, tanto em espécies de videira resistentes como suscetíveis, ainda que em ordens de magnitudes ou escalas temporais distintas, encontra-se reportada uma reprogramação do transcriptoma e proteoma referente às respostas imunitárias, vias de sinalização e metabolismo hormonal. Neste último, respostas de defesa com base no metabolismo e sinalização da via do ácido salicílico têm sido associadas à resistência contra *E. necator*. Contudo, o desencadear das respostas de defesa não é o resultado de uma ação unitária, mas de uma atuação conjunta e coordenada, sinergética ou antagonista, entre todo o hormonoma. Com o intuito de dissecar a rede hormonal reguladora das respostas de defesas que decorrem durante as interações incompatível/compatível com *E. necator* e, formular um modelo putativo associado à resistência e suscetibilidade em videira, procedeu-se à quantificação, por LC-MS/MS, de várias hormonas em folhas de um híbrido resistente, *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset e de uma espécie suscetível, *V. vinifera* cv. Aragonez. Para tal, cinco réplicas biológicas de folhas não infetadas e infetadas com *E. necator*, foram agrupadas em quatro tempos de infeção: 0, 6, 24 e 96 horas. Apesar da latente variabilidade das folhas, verificou-se uma composição hormonal constitutiva diferencial entre as duas espécies. Nas folhas da espécie resistente, evidenciou-se um maior conteúdo de ácido salicílico (SA), da sua forma glicosilada (12-*O*-Glc-JA) e de auxinas (sobre a forma de ácido indolacético; IAA). Contrariamente, nas folhas da espécie suscetível evidenciou-se um maior conteúdo de jasmonatos (JAs) específicos e ácido abscísico (ABA). Assim, o tipo de interação desencadeada aquando da infeção com *E. necator* parece ser modulada pela composição hormonal constitutiva e, particularmente, pelo balanço entre as diferentes hormonas. Mais ainda, em folhas infetadas, as vias de síntese, pelo aumento das respetivas hormonas às 6 e/ou 24 horas, parecem estar ativadas, corroborando a sua plausível ligação à resistência ou suscetibilidade.

Na literatura, vários genes têm sido funcionalmente caracterizados como estando associados a respostas de resistência ou suscetibilidade na interação entre *E. necator*-videira. Uma vez que as respostas de defesa são o resultado de multiprocessos paralelos e interligados, alguns dos descritos genes estão integrados em vias hormonais ou de transdução do sinal. Como tal, no presente trabalho, foi igualmente delineado e analisado, por PCR quantitativo em tempo real, o perfil de expressão de genes putativamente envolvidos nos mecanismos de síntese, sinalização e resposta das vias hormonais em estudo. Para o SA foram escolhidos dois reguladores positivos da via de síntese, enhanced disease susceptibility 1 *(EDS1)*

e phytoalexine deficient 4 *(PAD4*) e um gene de resposta à hormona, pathogenesis-related protein 1 (*PR1*). Na via das auxinas foram escolhidos os genes IAA-amido synthetase GH3-2 (*GH3-2)* e auxin-induced in root cultures protein 12 (*AIR12*), associados ao catabolismo e resposta à hormona, respetivamente. Na via dos JAs foi escolhido um regulador positivo da via de síntese, allene oxidase synthase (*AOS)*. Por último, na via do ABA foram escolhidos os genes sucrose non-fermenting-1 *(*SNF1)-related protein kinase 2 (*SnRK2*) e *HVA22C,* que codificam para uma proteína envolvida na transdução de sinal e de resposta à hormona, respetivamente. Para todos eles, o respetivo perfil de expressão não corroborou a descrita função, nem foi concordante com o perfil da hormona, demonstrando que, para ambas as interações, a regulação das vias hormonais é mais complexa do que o previamente descrito noutros modelos biológicos com oídio.

Os estudos do patossistema videira-*E. necator* ocorrem maioritariamente em folhas, existindo um desconhecimento sobre as respostas de defesa que ocorrem nos bagos. Em paralelo com um estudo mais amplo do transcriptoma e metaboloma, e tal como em folhas, foi quantificado o conteúdo hormonal em bagos suscetíveis de *V. vinifera* cv. Carignan. Para tal, foram colhidos cachos não infetados e infetados com *E. necator*, em dois estágios de desenvolvimento: EL33 (verde tardio) e EL35 ("pintor"). Neles, verificou-se uma putativa envolvimento das vias do SA e JA em resposta à presença de *E. necator*. A modulação das vias hormonais, na presença de infeção, sugere uma capacidade de induzir respostas de defesa, por parte dos bagos suscetíveis, mas que, devido a uma ativação temporal tardia torna-se incapaz de impedir o efetivar da infeção. A analise do perfil de expressão de genes relacionados com as respetivas vias hormonais foi mais conclusiva do que em folhas. Particularmente, na via do SA, *EDS1* encontrou-se diferencialmente expresso nos bagos infetados e em concordância com o perfil da hormona. Na via das auxinas, *AIR12* registou um aumento de expressão com a infeção. Conjuntamente com os dados das folhas, é possível identificar uma resposta diferencial entre os órgãos de videira baseada numa preferência por interações hormonais específicas e a ativação de diferentes isoformas, de um mesmo gene, para regular as respostas de defesa durante a infeção com *E. necator*.

Vários estudos reportam ainda uma reprogramação do metabolismo secundário durante a interação videira-*E. necator*. Dentre deste, a via dos fenilpropanóides concentra a maior porção de carbono redirecionado do metabolismo primário e dispõe de elevada importância, pela síntese de um heterogéneo conjunto de metabolitos secundários. Estes, ainda que constitutivamente presentes em alguns casos, integram a maquinaria induzível das células em resposta à infeção, sendo os compostos fenólicos o grupo mais representativo. Com o intuito de analisar o impacto de *E. necator* na via dos compostos fenólicos, foi quantificada a composição total destes, por espectrofotometria, em folhas e bagos. Comparativamente com a condição não infetada, em ambos os órgãos, o conteúdo em fenóis totais ficou inalterado durante a infeção. Nos bagos, por espectrofotometria, foi ainda quantificada a atividade bioquímica da fenilalanina amónia-liase (FAL), enzima integrada na via dos fenilpropanoides e transversalmente induzida nos cenários de infeção. Para esta, e tal como os fenóis totais, evidenciou-se uma ausência na alteração da sua atividade enzimática aquando da presença de *E. necator*. Ainda que não expectável, estes resultados sugerem que a reprogramação da síntese dos compostos fenólicos exprime-se através da síntese de compostos fenólicos específicos e mediante o tipo de interação. Tal foi corroborado, em bagos, através da análise do metaboloma. Mais, sugere ainda diferentes funções, dentro da via dos fenilpropanoides, para diferentes isoformas de FAL ou um possível impacto na tradução da FAL pela infeção. Os supramencionados resultados servem como conhecimento inicial que, após explorado e validado noutras espécies resistentes e suscetíveis, pode ser utilizado em programadas de melhoramento das cultivares de *V. vinifera* no sentido de aumentar a sua tolerância ao *E. necator*.

Palavras-chave: resistência e suscetibilidade; *Erysiphe necator*; quantificação hormonal; expressão génica; quantificação de fenóis totais

Abstract

The Eurasian *Vitis vinifera*, one of the most cultivated horticultural crops, has its economic impact derived from the fruits which are utilized to produce mainly wine, table grapes, raisins. The process of domestication originated a superior species with desirable agronomic traits and highly adaptable to external environment but prevented a coevolution with natural biotic stresses. In result, *V. vinifera* is highly susceptible to several pathogens. *Erysiphe necator* is one of the most threatening pathogens, being the etiologic agent of powdery mildew (PM). As an obligate biotrophic fungus, *E. necator* differentiates specialized infection (appressorium) and feeding (haustorium) structures to invade grapevine cells and acquired resources to complete his life cycle. At these stages of infection, a complex pathogen-host interaction occurs, and the outcome is the establishment of an incompatible (resistance) or compatible (disease) interaction. In grapevine, constitutive defences will avoid adhesion, germination and penetration. Induced defences organized in a two-layered system will block the endophytic growth and infection propagation. In these, in an intricate network, hormones act as secondary messengers to trigger downstream structural and biochemical changes. Based on additional undergoing studies and previous reports transcriptome and proteome reprogramming related with signalling and hormonal metabolism occurs; our study was performed to give some clues about the phytohormone network upon *E. necator* infection. For that, through LC-MS/MS, was quantified the content of some hormones in non-infected and *E. necator*-infected leaves of the resistant hybrid *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset and the susceptible *V. vinifera* cv. Aragonez. Quantitative real time-PCR (qPCR) was performed to analyse the expression profiling of genes putatively involved in the chosen phytohormones pathways. At constitutive levels, a higher content of salicylic acid (SA) and auxins (mainly indole acetic acid; IAA) and a specific selection of jasmonates (JAs) and abscisic acid (ABA) were present in resistant and susceptible species, respectively. Upon infection, an accumulation of these hormones in each species was noticed. Also, a putative phytohormonal network based on a blend between SA/IAA and JAs/ABA was present in resistant and susceptible species, respectively. Altogether, these results suggest a role of hormones, and particularly the balance among them, in modulating the type of interaction and a possible involvement in response to *E. necator*. Nevertheless, the gene expression analysis revealed a more complex regulation of hormonal pathways than in others reported plant-PM interactions. Research on grapes to understand the defences responses upon *E. necator* infection have been neglected. In parallel with a broader transcriptomic and metabolomic-based project, and as for leaves, it was quantified the hormonal content and analysed the gene expression of susceptible grapes of *V. vinifera* cv. Carignan. In these, even delayed, a putative involvement of SA and JA pathways seems to respond to *E. necator* infection. Together with the gene expression analysis, the study pointed a different network of phytohormones and activation of different gene isoforms among leaves and grapes in response to *E. necator* infection. Reports also indicate a reprogramming of metabolism upon *E. necator* infection. Regarding the secondary metabolism, the phenylpropanoid pathway produce a plethora of phenolic compounds and phytoalexins important in defence responses against *E. necator*. Through spectrophotometry, was performed a quantification of total phenolic content in leaves and grapes. Additionally, in grapes, the phenylalanine ammonia lyase (PAL) biochemical activity was determined. In both organs, no change was noticed in total phenolic content upon *E. necator* infection nor in PAL activity in grapes. This suggest a reprogramming of phenylpropanoid pathway in order to accumulate specific phenolic compounds. The new obtained data in leaves and grapes of grapevine upon *E. necator* infection, once fully dissected and validated, will be useful for breeding programmes in order to improve tolerance against this fungus.

Keywords: resistance and susceptibility; *Erysiphe necator*; hormonal quantification; gene expression; total phenolic quantification

Declaração

De acordo com o disposto no artigo n.º 19 do Regulamento de Estudos de Pós-Graduação da Universidade de Lisboa, Despacho n.º 2950/2015, publicado no Diário da República, 2.ª série — N.º 57 — 23 de março de 2015, foram incluídos nesta dissertação os resultados apresentados em:

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Poster

Amaro, R., Diniz, I., Santos, H., Pimentel, D., Rego, C., Mithofer, A., Fortes, A. Constitutive high levels of salicylic acid and IAA together with constitutive low levels of jasmonates and ABA may provide tolerance against infection with *Erysiphe necator* in grapevine leaves. Poster presented at Plant Biology Worldwide Summit 2020; 2020 July 27-31;
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Em cumprimento com o suprarreferido despacho, confirma-se ser da minha total responsabilidade (exceto quando indicado em contrário) a execução das experiências que estiverem na base dos resultados apresentados, bem como a interpretação e discussão dos mesmos.

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Abbreviations and acronyms

AAO, Aldehyde oxidase
ABA Abscisic acid
ABF ABA-responsive element binding factor
AIR12 Auxin-induced in root cultures protein 12
AFB Auxin signaling F-Box
AMI1 Indole-3-acetamide hydrolase
AOC Allene oxide cyclase
AOS Allene oxide synthase
AREB ABA responsive element binding protein
ARF Auxin response factor
AUX/IAA Auxin/indole-3-acetic acid
AVR Avirulence
BA2H Benzoic acid-2-hydroxylase
BCH1/2 β-carotene hydroxylases 1/2
BSA Bovine serum albumin
BZL Benzoyl-CoA ligase
cDNA Complementary DNA
CNL Coiled-coil (CC)-NBS-LRR
CTAB Hexadecyltrimethylammonium bromide
COI1 Coronatine insensitive1
C_T Threshold cycle
cv. Cultivar
12-COOH-JA-Ile Dicarboxy-JA-Ile
DAMP Damage-Associated Molecular Pattern
ddH_2O Distilled water
DEPC Diethyl pyrocarbonate
DHBA Dihydroxybenzoic acid
DNA Deoxyribonucleic acid
DNaseI Deoxyribonuclease I
DTT Dithiothreitol
dNTP Deoxynucleotide
DW Dry weight
EDL EDS1-like
EDTA Ethylenediaminetetraacetic acid
EDS1 Enhanced disease susceptibility 1
EF1α Elongation factor 1-alpha
ET Ethylene
ETI Effector-triggered immunity
ETS Effector-triggered susceptibility
FW Fresh weight
GAE Galic acid equivalents
gDNA Genomic DNA
GH IAA-amido synthetase
HDA6/9 Histone deacetylase 6/9
hpi Hours post inoculation
HR Hypersensitive response
H_2O_2 Hydrogen peroxide
IAA Indole acetic acid
ICSI Isochorismate synthase 1
IPL Isochorismate pyruvate lyase
ISR Plant-induced systemic resistance
KCl Potassium chloride
K_2HPO_4 Dipotassium phosphate
KOAc Potassium acetate
JA Jasmonic Acid
JA-Ile Jasmonoyl-isoleucine
JAs Jasmonates
JAZ Jasmoante ZIM-domain
LiCl Lithium chloride
LOX Lipoxygenase
MAMPs Microbe-Associated Molecular Patterns
MAPK Mitogen-activated protein kinases
MeJA Methyl jasmonate
miRNA microRNA
MgCl2 Magnesium chloride
MYC2 bHLHzip transcription factor MYC2
MJE Methyl jasmonate esterase
MTI Microbial-associated molecular patterns-triggered immunity
mRNA Messenger RNA
NBS-LRR Nucleotide-binding site leucine-rich receptors
Na_2CO_3 Sodium carbonate
NaOAc Sodium acetate
NaOH Sodium hydroxide
NCED 9-cis-epoxy carotenoid dioxygenase
NINJA Novel interactor of jaz
NIT1/2/3 Nitrilase 1/2/3
NPR1 Non-expressor of pathogenesis-related
YUCCA Flavin monooxygenase-like
12-OH-JA-Ile 12-hydroxy-JA-Ile
12-*O*-Glc-JA 12-*O*-glucosyl-JA
12-OH-JA 12- hydroxy-JA
$O_2^{\cdot-}$ Superoxide
OPDA 12-oxophytodienoic acid
OPR 12-oxophytodienoate reductase
PAD4 Phytoalexine deficient 4

PAL Phenylalanine ammonia lyase
PAMP Pathogen-Associated Molecular Pattern
PCD Programmed cell death
PEN1 Penetration 1 protein
PM Powdery mildew
PMSF Phenylmethylsulfonyl fluoride
PR Pathogenesis-related
PRR Pattern Recognition Receptors
PVPP Polyvinyl polypyrrolidone
PTI Pattern-Triggered Immune response
PR Pathogen related proteins
Pst DC3000 *Pseudomonas syringae* pv. tomato DC30000
PYR/PYL/RCAR Pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABA receptors
R Resistance
RLK Receptor-like kinases
RLP Receptor-like protein
RNA Ribonucleic acid
RNA-seq RNA sequence
RNI Reactive nitrogen intermediate
qPCR Quantitative real time-polymerase chain reaction
ROS Reactive oxygen species
SA Salicylic acid
SA-glucoside Salicylic acid-β-D-glucoside
SAG101 Senescence associated gene 101
SAGT SA glucosyltransferase
SAMT SA methyltransferase
SAR Systemic acquired resistance
SAUR Small auxin up-regulated RNA
SCF Skp-Cullin-F-box
SDR Alcohol dehydrogenase/reductase
SDS Sodium dodecyl sulfate
SNARE Soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SnRK Sucrose non-fermenting 1 (SNF1)-related protein kinase
SGT1 Salicylic acid glucosyltransferase
SSTE Sodium dodecyl sulfate–Tris-HCl–EDTA
TAA Tryptophan aminotransferase of Arabidopsis
TSB1/2 Tryptophan synthase beta-subunit 1/2
TIR Transport inhibitor response
TNL Toll/interleukin-1 receptor (TIR)-NBS-LRR
TPL Topless-related
Tris-HCl Tris hydrochloride
UBC Ubiquitin conjugating enzyme
VAG Vacuolar ATPase subunit G
VDE Violaxanthin de-epoxidase
Xoo *Xanthomonas oryzae pv. Oryzae*
ZEP Zeaxanthin epoxidase

Units

ºC Celsius degrees
g Earth's gravitational acceleration (RCF)
mg Miligram
mL Millilitre
mM Milimolar
ng Nanogram
Ta Annealing temperature (ºC)
Tm Melting temperature (ºC)
µg Microgram
µL Microlitre

1. INTRODUCTION

1.1.Grapevine – general contextualization and economic importance

About 90 million years ago, in central India, the first Vitaceae exemplar appear (Gerrath et al., 2015). The family comprise around 15 genera and 700 species of climbing lianas vines with a widely geographic and ecological range (I. Chen & Manchester, 2007; Manchester et al., 2013). *Vitis* L., the most relevant Vitaceae genus, has approximately 60 interfertile species spread mainly through temperate zones, with few species reaching pantropical areas (Bacilieri et al., 2013; Keller, 2010; X. Q. Liu et al., 2016).

The woody perennial *Vitis vinifera* L., due to an absence of demarcated morphological characteristics and an evolutive barrier to constrict the genetic flux, is subdivided into spp. sylvestris and spp. vinifera. *V. vinifera* spp. sylvestris is the ancestral of *V. vinifera* spp. vinifera (synonymously referred here as *V. vinifera*) (Arroyo-García et al., 2006). Regarding the last one, as a consequence of the domestication process during the last 10,000 years is nowadays present in all commercial vineyards being one of the most cultivated horticultural crop covering around 7.4 million hectares of land in 2018 and producing 77.8 million tons of harvest grapes (OIV & International Organisation of Vine and Wine, 2019). The primary use of harvest grapes is wine production (57%), followed by table grapes (36%), raisins (7%) and, in minor extent, grape juice, jelly, or others derivative products (Fasoli et al., 2012; OIV & International Organisation of Vine and Wine, 2019). Also, grapevine organs (mainly leaves and berries) contain natural antioxidants with health and nutritional value for pharmaceutical industries, cosmetic and food (Fasoli et al., 2012; Ana M. Fortes & Pais, 2015).

During the process of domestication, the artificial selection originated a superior species with desirable agronomic traits and highly adaptability to external environment (Hadacek, 2002). However, domestication prevented the species to coevolve with natural biotic stresses. As such, it is highly susceptible to a large range of pathogens that are responsible for several diseases (Myles et al., 2011).

1.2. Erysiphe necator – the etiologic agent of grapevine powdery mildew

Fungal diseases has the major economic impact in grapevine due to their representativeness worldwide, incidence in all *V. vinifera* cultivars and capacity to infect pre- and postharvest grapes (Armijo et al., 2016). Grey mold and downy mildew are fungal diseases with high impact and are caused by *Botrytis cinerea* and *Plasmopara viticola*, respectively. They are only overcome in impact by powdery mildew (PM) (Armijo et al., 2016; Gadoury et al., 2012). In general, PM is a widespread disease in Angiosperms affecting economical important staple and horticultural crops, such as wheat, barley, tomato and, also ornamental plants, such as roses (Wu et al., 2018). The etiologic agents of PM are ascomycetes fungi belonging to the monophyletic order Erysiphales. All of them are obligate biotrophic parasites indicating a physiological adaptation and specialization to theirs host in order to feed from live cells and avoiding cell death (Glawe, 2008; P. Spanu & Kämper, 2010).

In grapevine, *Erysiphe necator* [syn. *Uncinula necator* (Schwein) Burr.] is the etiologic agent of PM and is adapted to some Vitaceae genus, such as *Cissus*, *Parthenocissus*, *Ampelopsis* and *Vitis* (Braun, 2011; Takamatsu, 2013). The nomenclature of the fungus depends on the considered phase of his life cycle: *E. necator*, as commonly referred, correspond to the sexual phase (teleomorph) and *Oidium tuckeri* (Berk) to asexual phase (anamorph). Based on historical records, eastern North American is considered the origin centre of the first *E. necator* population introduced in Europe (England), by mid-1845 (Gadoury et al., 2012). Through the movement of vines commerce, two genetically distinct populations (called haplotype A and B) of *E. necator* are nowadays present in all viticultural regions (Brewer & Milgroom, 2010; L. Jones et al., 2014). In most of them, *E. necator* overwinters as cleistothecia (sexual structure consisting in an ascocarp with ascospores) within the bark of the vine. Also, in moderate winters, mycelium is an adopted form in dormant infected buds (flag shoots). In cold

winters, however no flag shoots are found regarding the reduce winter hardiness of dormant infected buds (Gadoury et al., 2012). At spring, despite the optimal development at 26ºC and relative humidity of 85%, temperatures above 15ºC and relative humidity exceeding 25% are favourable conditions for initiation of the epidemiologic process (Gadoury et al., 2012; Wilcox, 2003). As such, in grapevine tissues, the attached ascospores or conidia start to germinate. At the end of the elongate hypha is formed an appressorium. This highly specialized infection structure allows the penetration of host tissues (Deising et al., 2000; Ridout, 2009). For this, a penetration peg at the end of appressorium will impose a physical pression and release hydrolytic enzymes, such as cutinases, esterases and lipases, in order to degrade the cuticle and epidermal cell wall (Abera Gebrie, 2016; Armijo et al., 2016). Later, the formation of haustorium, a feeding structure, allows the invagination of plasma membranes and the establishment of a dynamic interaction with grapevine cells. The interaction allows *E. necator* to capture needed resources to complete his life cycle (Gadoury et al., 2012; Qiu et al., 2015). Secondary hyphae will be formed and branch across all the infected tissues (Armijo et al., 2016). Ultimately, simple, hyaline and multiseptate conidiophores (with single, hyaline, cylindro-ovoid conidia) and cleistothecia (with asci with hyaline, ovate to subglobose ascospores) are differentiated from mycelium and will infect new tissues, renewing the cycle (Gadoury et al., 2012; Karthick et al., 2019). *E. necator* infects all green grapevine organs above ground. In symptomatic leaves and berries, patches with white dusty appearance appear on surfaces. Heavily infected leaves became dry and drop prematurely. Young leaves become distorted as they expand. Depend on environmental conditions and cultivar, berries may also crack or splitting. In stems, later infection appears as dark necrotic lesions. This impacts the structure and functionality of grapevine organs and is responsible for losses in production yield, quality and create conditions for opportunistic pathogens. Ultimately, affects vines growth and winter hardiness (Pearson & Gadoury, 1992; Ridout, 2009; Y. Xu et al., 2009).

Nowadays, several management practices have been adopted in commercial vineyards for PM control. Cultural practice like grapevine planting spaced and pruning the canopy by elimination of leaves surrounding the clusters, decrease the favourable infection conditions by *E. necator* (Caffi et al., 2013). Nevertheless, the PM control is mainly based on seasonal application of sulphur-based and synthetic fungicides. Besides the low effectiveness due to high virulence and gain of resistance of *E. necator*, the economic impact for grape growers and detrimental impact for environment, imposed pressure to reduce the dependence on agrochemicals as a measure control of PM (Gadoury et al., 2012; Wilcox, 2003). Also, these chemicals have been also detected in wine incrementing their negative impact (Calhelha et al., 2006). Finally, the change of global temperatures and seasonal patterns may impact the ecology and epidemiology of *E. necator* (Caffarra et al., 2012) leading the necessity to exploitation and add the natural resistance of grapevine in breeding programmes in order to produce more resistant grapevine cultivars. Also, the information from genome sequencing of several trains of *E. necator* (L. Jones et al., 2014) will deeper our knowledge grapevine-*E.necator* pathosystem.

1.3.Plants defences against biotrophic fungal pathogens

Being sessile organisms, plants are in a continuum association with a broad array of microorganisms and rely exclusively on the innate immunity of each cells and systemic signals to combat the pathogens (J. D. G. Jones & Dangl, 2006).

The plant defence responses are shaped by the life and infection strategies of pathogens. Fungal pathogens are divided into three classes regarding to their life strategies. Necrotrophic fungi derive nutrients from dead or senescence tissues and during colonization produce phytotoxic metabolites in order to kill host cells. Biotrophic fungi derive nutrients from living tissues and establish parasitic facultative or obligate interactions with host cells. Hemibiotrophic fungi displayed a biotrophic and necrotrophic strategy in early and late infection, respectively (Glazebrook, 2005; X. Wang et al., 2014).

At the spore germination and penetration stage of infection process, fungi interact with the first line of plants innate immunity, the constitutive defences. At structural level, they correspond to pre-invasive barriers as waxy cuticle, rigid cell walls and toughness epidermal layers. Together, by creating a hydrophobic, repellent and impenetrable barrier, they avoid adhesion, germination and penetration of fungi spores. At biochemical level, correspond to antimicrobial compounds, toxic inhibitors and phytoanticipins. Their main function is inhibiting fungal enzymes and/or growth (JH, 2015; Muthamilarasan & Prasad, 2013).

If fungi successfully invade host tissue, the plant can discriminate self and nonself and elicit post-invasive defences which are organized in a two-layered system. The first one is the microbial/pathogen-associated molecular patterns (MAMP/PAMP)-triggered immunity (MTI/PTI) or formerly called basal/horizontal resistance (Boller & Felix, 2009). On surface of host cells, plasma-membrane-bound pattern recognition receptors (PRRs) recognized pathogen-associated molecular patterns (PAMPs). PRRs family is composed by receptor-like kinases (RLK) and receptor-like protein (RLP) (Muthamilarasan & Prasad, 2013). PAMPs are pathogens essential and conserved components which function as elicitors, triggering PTI. The polysaccharide chitin and β-glucans are main component of fungi cell wall and function as PAMPs (De Wit et al., 2009). PTI is also triggered by damage-associated molecular patterns (DAMPs) which are endogenous elicitors. They function as a warning self-signal since are mainly fragments of cuticle and cell wall degradation (Muthamilarasan & Prasad, 2013). PTI is an early slow, weaker and non-specific defence responses. However, plants can evaluate the effectiveness of early PTI and, by positive feedback loops, amplify it into a strong late defence response (Katagiri & Tsuda, 2010). Usually is a sufficient non-host resistance against non-adapted fungi (Lipka et al., 2008).

Regarding adapted fungi, after invading the host tissues, the formation of feeding structures, allow them to acquire nutrients and modulate host immunity (Dodds et al., 2009). This last one occurs by secretion of extracellular effectors [also called avirulence (AVR) proteins] at the apoplast or translocate cytoplasmatic effectors into the host cells (De Wit et al., 2009). The effectors are proteins with no conserved motifs, even among closest species, which disrupt PTI at PRRs or signalling level and lead to effector-triggered susceptibility (ETS) (J. D. G. Jones & Dangl, 2006; X. Wang et al., 2014). In resistant species, a second layer of post-invasive defences, the effector-triggered immunity (ETI) or formerly called R gene-derived/vertical resistance is activated. (Boller & Felix, 2009). According to gene-to-gene interaction, the effectors are recognized directly or indirectly, through association with accessory-proteins, by intracellular receptors, the R proteins which are codifying by resistance (R) genes (Muthamilarasan & Prasad, 2013). This will lead to an incompatible (resistance) interaction between the host-fungus. The major class of R protein are the nucleotide-binding site (NBS) leucine-rich receptors [(LRR); NBS-LRR]. Two subclasses are present in plants based on N- terminal domain, namely coiled-coil (CC)-NBS-LRR (CNL) and toll/interleukin-1 receptor (TIR)-NBS-LRR (TNL) (Shao et al., 2019). Both PTI and ETI extensively shared defence events which indicate an intrinsic network. Nevertheless, they differ in magnitude, being the speed and robustness of signalling a mark of ETI effectiveness (Katagiri & Tsuda, 2010). Recently, Ngou et al. (BIOARXIV) report a distinct perspective where different outputs are associate with PTI and ETI and a role of ETI in potentiate PTI.

The perceived of invading fungi at PRRs or R proteins levels triggers a downstream signal transduction (Y. Shen et al., 2017). The early signalling events include phosphorylation of proteins and alkalinisation of cytoplasm by changing the fluxes of several ions across the plasma membrane (Muthamilarasan & Prasad, 2013). Among them, the spaciotemporal accumulation of Ca^{2+} (called Ca^{2+} signature) at cytosol mediate several defence responses (Aldon et al., 2018). The oxidative and nitrosative burst through accumulation of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNIs), respectively, is another early event. Being a highly reactive molecules, ROS dispose a direct antimicrobial effect. Nevertheless, and like Ca^{2+}, ROS, mainly superoxide ($O_2^{\cdot-}$) and hydrogen peroxide

(H_2O_2), function as diffusible secondary messengers (Abera Gebrie, 2016; Heller & Tudzynski, 2011). Activation of mitogen-activated protein kinases (MAPK) cascade is one of ROS-mediated response (Verma et al., 2016). The core of MAPK cascade consist of three conserved kinases (MAP3K, MAPKK and MAPK) and through sequential phosphorylation transduce the biotic stress signal at intercellular level (Plotnikov et al., 2011).

Altogether, in an orchestrate organization, the early events promote a gene expression reprogramming which lead to secondary events. They correspond, at cellular level, to reinforcement of cell wall in order to slowing the invasion and enclose the feeding structure in a toxic and impermeable environment. At penetration site, this occurs through deposition of papillae enriched with callose, toxic and structural phenolic compounds like lignin, ROS, peroxidases and cell wall structural proteins (Liesche et al., 2015; Underwood, 2012). As a consequence of obligate biotrophy, several metabolic pathway were lost during evolution (P. D. Spanu, 2012). As such, at penetration site, the primary metabolism of those cells is manipulated in order to suppress photosynthesis, induce carbohydrate metabolism and accumulate hexoses, a preferred fungi source of carbon (H. Kuhn et al., 2016). To counter, plants remove the carbon source in order to starve fungi. Also, carbon flux is reallocating from primary to secondary metabolism in order to produce *de novo* a heterogenous pool of secondary metabolites (Bolton, 2009; Tenenboim & Brotman, 2016). Among them, phytoalexins and phenolic products, mainly from the phenylpropanoid pathway, dispose an antimicrobial activity and are pivotal in defences (Ahuja et al., 2012; JH, 2015). Regarding the protein synthesis induction, pathogenesis-related (PR) proteins will accumulate locally. The most prevailing are β-1,3-glucanases (PR2), chitinases (PR3), thaumatin-likes/osmotins (PR5) are usually related with resistance regarding theirs hydrolytic activity (Christensen et al., 2002; L. Dai et al., 2016; Narasimhan et al., 2009).

Induction of phytohormone biosynthesis is another event that follows pathogen attack. Present at low concentration in cells and acting as secondary messengers, phytohormones function as signalling molecules triggering specific events (Pieterse et al., 2012; Shigenaga & Argueso, 2016). Being associated with defence, a classical view relates salicylic acid (SA) and jasmonic acid (JA) with resistance against biotrophic and necrotrophic fungi, respectively (Glazebrook, 2005). Also, an antagonistic crosstalk between both signalling pathways is accepted (Derksen et al., 2013). Upon biotrophic infection, SA, a phenolic compound resulting from phenylpropanoid pathway, accumulated at penetration sites and mediates downstream responses usually by action of non-expressor of pathogenesis-related genes (NPR1), a main regulator of SA signalling (Fig. S2.1) (Bürger & Chory, 2019). Among them, the hypersensitive reaction (HR) is a specific plant type of programmed cell death (PCD) occurring in infected and surrounding cells and is an effective R-mediated resistance against biotrophic fungus (Balint-Kurti, 2019; Muthamilarasan & Prasad, 2013). Once triggered the HR, a distal SA mobile signal [volatile ester methyl salicylate (MeSA)], in parallel with PRs accumulation, will induce a system acquired resistance (SAR) in distant cells. SAR is broad-spectrum resistance against several pathogens and is maintained during a period of time (Bürger & Chory, 2019; JH, 2015; Staal & Dixelius, 2007). Nevertheless, the outcome of SA-signalling pathway is tightly modulated by synergetic or antagonistic interactions with all hormonome (Berens et al., 2017). Recently, the JA signalling pathway was related to resistance against biotrophic oomycetes, a class of fungus-like microorganisms (Fawke et al., 2015). Jasmonates were also reported to be involved in response against *Plasmopora viticola* together with SA (Polesani et al., 2010). As such, this contradicts the classical view and corroborate the new idea of SA-JA synergism interaction in a concentration-depending manner (Glazebrook, 2005; Yang et al., 2015). In addition, new roles in defence have been proposed for hormones classical associated with growth, development and adaptation to environment. Abscisic acid (ABA), hormone classically associated to response to abiotic stress, has been described as regulator of disease depending on the interaction and phase of plant defences (L. Chen & Yu, 2014; J. Xu et al., 2013). In *Arabidopsis thaliana-Golovinomyces cichoracearum*, the causal agent of PM, ABA was

reported to negatively impact post-penetration resistance through repression of SA signalling pathway (Xiao et al., 2017). Also, ABA may display an impact in callose deposition by regulating soluble N-ethylmaleimide-sensitive factor attachment protein receptor (*SNARE*) genes, as shown against necrotrophic fungus (Ton et al., 2009; Ton & Mauch-Mani, 2004). SNAREs are involved in trafficking of secretory vesicle to plasma membrane during papillae formation (Qiu et al., 2015). Regarding auxins, the main hormonal regulator of development, a role in susceptibility during biotrophic interaction has been reported, but only in bacteria (Fu & Wang, 2011). Infection of rice with the *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) lead to activation of auxin signalling. One of the downstream mediated responses is the activation of expansin genes. As such, the modulation of host defence creates an easier way to bypass the constitute barriers, since expansins are involved in cell wall loosening (Fu et al., 2011; González-Lamothe et al., 2012). The auxins role as virulence factor was also observed in Arabidopsis-*Pseudomonas syringae* pv. tomato DC30000 (Pst DC3000). In the presence of the PAMP-induced miR393, a microRNA (miRNA), repression of auxins receptors, mainly transport the inhibitor response 1 (TIR1) and auxin signaling F-Box proteins 2 and 3 (AFB2/3) (Fig.S2.5) lead to resistance against Pst DC3000 in a SA-independent manner (Ma & Ma, 2016; Navarro et al., 2006; D. Wang et al., 2007). This suggest that resistance in a biotrophic interaction may not always depend on SA-mediated defences. Also, for necrotrophic fungi, like *B. cinerea* in interaction with grapevine, an involvement of auxins in response was reported (Coelho et al., 2019). Altogether, the above association of hormones with resistance and susceptibility corroborate the idea that the actual role of hormones is unique for each pathogen-plant interaction (Derksen et al., 2013), emphasizing the significance of hormonomics-based studies.

1.4. Grapevine defences against Erysiphe necator

The resistance or susceptibility of *Vitis* species to *E. necator* is related to their evolutionary history. Since the fungus is endemic of North America, the spaciotemporal coevolution with North American wild *Vitis* species, as *V. labrusca*, *V. rupestris*, *V. riparia*, *V. aestivalis* and *Muscadinia rotundifolia* make them to dispose different levels of resistance. Nevertheless, the resistance is not restricted to North American and is also present in some non-vinifera accessions of wild Chinese *V. amurensis*, *V. romanetti, V. pseudoreticulata, V. piasezkii* and two Near Eastern *V. vinifera* cv. Kishmish Vatkana and cv. Dzhanzhal Kara. Contrary, and related to a short period of time exposed to *E. necator*, the Eurasian *V. vinifera* display several degrees of susceptibility due to a lack of genetic mechanism to annul the fungus virulence (Brewer & Milgroom, 2010b; Feechan et al., 2011; Riaz et al., 2011; Pimentel et al., under review).

In grapevine tissues, at constitutive level, an ontogenic or age-related resistance is present. Both resistant and susceptible species display an early susceptibility window. After that, aged tissues became resistant or tolerant and the epidemiologic process of *E. necator* in less effective (Ficke et al., 2002; Gadoury et al., 2003). In berries, this is reached during ripening, 2-3 weeks after bloom, while in leaves, is associated with maturation. However, the timing to acquire the resistance is different among grapevine tissues, cultivars and between species, being shorter for resistant ones (Gadoury et al., 2003; Gee et al., 2008). Besides the previously association with cuticle thickness, papillae formation or constitutive expression of PRs, the mechanism for ontogenic resistance is still elusive in grapevine (Ficke et al., 2004).

In cases were *E. necator* successfully penetrate and invade grapevine tissues, PTI is activated in order to block the endophytic development. The penetration resistance is associate with papillae formation at penetration site (Angela Feechan et al., 2011). The regulation of secretory vesicles trafficking with the cargo to cell wall is made by penetration 1 (PEN1) protein (Qiu et al., 2015). In Arabidopsis, PEN1 from grapevine accumulate under the *Erysiphe cichoracearum* (etiologic agent of PM in Arabidopsis) penetration site and is inhibit by endomembrane trafficking inhibitors. Also, Arabidopsis *pen1* mutants

complemented with the grapevine gene recover the phenotype. PEN2 and PEN3 proteins also intervene in Arabidopsis, but no grapevine orthologs were identified (A. Feechan et al., 2013; Qiu et al., 2015).
Being an adapted pathogen of *V. vinifera*, *E. necator* can bypass PTI. In resistant species, an ETI defence is than triggered in order to induce a PCD-associated resistance (Angela Feechan et al., 2011). In grapevine genome, R genes, namely NBS-LRR are highly represented and clustered in tandem repetitions in genomic regions as revealed by genome sequencing (Jaillon et al., 2007). To date, several loci with resistance gene analogue markers (RGAs) have been describe. In *M. rotundifolia* cv. Thomas, resistance to *Uncinula necator* 1 (*RUN1*) locus has 7 RGAs encoding TNLs on chromosome 12 and confer resistance against *E. necator*, approximately 24-48 hours post infection (hpi), by triggering PCD mostly in infected cells. However, *RUN1* promote an incomplete resistance, being broken by *E. necator* isolate (Musc4) from southeastern region of North America (Dry et al., 2008; Qiu et al., 2015). In *M. rotundifolia* cv. Trayshed and cv. Magnolia, two allelic variants of *RUN2*, *RUN2.1* and *RUN2.2* were identified on chromosome 18. Although still elusive, preliminaries studies indicate a PCD-mediated resistance to block fungal progression, but less strong than RUN1 (Angela Feechan et al., 2015; Qiu et al., 2015; Riaz et al., 2011). *Erysiphe necator* 1 (*REN1*) locus located in chromosome 13 was identified in *V. vinifera* cv. Kishmish Vatkana and cv. Dzhanzhal Kara. *REN1* confers partial resistance by restricting hyphae growth and decreasing conidiophore density and sporulation. However, results in a lower frequency and slow PCD compared with *RUN1* (Hoffmann et al., 2008; Pap et al., 2016). This is also true for *REN2* located at chromone 14 in *V. cinerea* cv. Illinois 547-1 (Pap et al., 2016). In *V. romanetii*, *REN4* locus is located on chromosome 18. Initially, *REN4* partial resistance was reported as penetration resistance. However, a complete post-penetration resistance was described by associated with PCD on infected cells or trapping of *E. necator* haustorium with deposition of callose (Qiu et al., 2015; Ramming et al., 2011). *REN6 and REN7*, on chromosome 9 and 19, respectively, was identified on *V. piasezkii*. Both induced resistance through PCD in penetrated cells, but the speed and magnitude of induction is higher in *REN6* compared with *RUN1* and *REN7* (Pap et al., 2016).
This type of resistance is also marked by accumulation of phytoalexins and secondary metabolites, mainly derived from phenylpropanoid pathway. In this, the stilbene pathway synthetized several defence biomarkers of grapevine regarding their antimicrobial activity at penetration site. Particularly, the levels of *trans*-revesterol, *trans*-piceid, α-, β-, ε- viniferin and pterostilbene are related with resistance (R. Dai et al., 2012; Viret et al., 2018). Enzymes involved in phenylpropanoids or stilbene pathways, like stilbene synthase (STS) and phenylalanine-ammonium lyase (PAL) are also induced (Welter et al., 2017). Accumulation of PRs is an additional defence response of grapevine against *E. necator*. In literature 17 PRs are describe, but only a few have been detected in grapevine (Enoki & Suzuki, 2016). Some PRs integrate the constitutive defences. Particularly, in grape juice and wine, depending on cultivar, several isoforms of PR2 and PR5 were detected (Hayasaka et al., 2001; Pocock et al., 2000). Also, both were described to accumulate during berries ripening and associated with ontogenic resistance (Ficke et al., 2004; Robinson et al., 1997; Tattersall et al., 1997). Upon infection, PTI and ETI in leaves and berries induce the expression of PRs, such as PR2, PR3 and PR5 and their synergetic action result in inhibition of fungal growth (Fung et al., 2008; Giannakis et al., 1998). Although occurs in both interactions, the accumulation of PRs is higher in incompatible and specific isoforms seems to be induced (Gomès & Coutos-Thévenot, 2009). Other PRs, as PR10, a protein with ribonuclease (RNase) activity, PR14, a lipid transfer proteins with antifungal activity or PR15 and PR16, germins (oxalate oxidases) and germin-like proteins (oxalate oxidase-like proteins) were associated with grapevine resistance, although the mechanism still elusive (Enoki & Suzuki, 2016). Synthesis of small molecules, namely phytohormones, is a parallel response (M. Gao et al., 2012). The activation of SA biosynthetic and signalling pathway is reported in incompatible and compatible interaction of grapevine-*E. necator* although with different magnitude or time of occurrence, respectively (Fekete et al., 2009; Fung et al., 2008). Also, in *V. pseudoreticulata*, PM-induced defence and immune responses and SAR were

associated with SA (Weng et al., 2014). In *V. aestivalis* cv. Norton, a resistant species, SA was constitutively higher than in the susceptible *V. vinifera* cv. Cabernet Sauvignon (Fung et al., 2008). Genes involved in SA pathway, like enhanced disease susceptibility 1 (*EDS1*) and phytoalexine deficient 4 (*PAD4*) were activated during infection evidencing an involvement in plant defences. However, this knowledge is mainly derived from transcriptomic studies and usually with information regarding one interaction (Borges et al., 2013; Jiao et al., 2015; Weng et al., 2014). If performed, the hormonal quantification occurs only for SA (Fung et al., 2008). Nevertheless, since the defence responses are the outcome of an intricate network among hormones (Katagiri & Tsuda, 2010), the hormonome profiling in incompatible and compatible interactions with grapevine-*E. necator* serves as valuable knowledge in breeding programmes.

1.5. Objectives and thesis outline

The grape growers demand for cultivars with desirable agronomic traits with durable resistance against the *E. necator* led to the development of breeding programs based on the identification of natural resistance to *E. necator* in germoplasm of wild grapevines. To this accomplishment, the sequencing of *V. vinifera* genome was a valuable tool by allowing the identification of potential chromosome regions with R genes for *E. necator*. One adopted strategy was the introgression of several R genes (p.e., *RUN1* and *REN1* or *RUN1*, *REN3* and *REN3.2*) into *V. vinifera* cultivars genetic background. The pyramiding of R genes in a same cultivar presupposed a correlated resistance. Contrary, several levels of resistance were identified. As such, the absence of resistant cultivars in conjugation with increasing of pathogens in commercial vineyards, climate changes and the highly evolutionary potential of *E. necator* lead to a necessity to find and functionally characterize new R genes. Also, the biochemical and molecular basis of grapevine ontogenic and inducible resistance is not fully deciphered.

Several transcriptomic-based studies in leaves from resistant or susceptible species revealed the effectiveness of SA-mediated responses against *E. necator* and identify some involved genes. However, defences responses are triggered by an unveiled hormonal blend, being fundamental the study of the grapevine hormonome. Also, the comparison of defence mechanisms between resistant and susceptible species allow the formulation of a more complete putative model of resistance and may reveal some clues regarding the susceptibility.

Therefore, this study aims to characterize the resistance of the hybrid *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset to PM through a molecular and biochemical approach, while susceptible *V. vinifera* cv. Aragonez to PM was used for comparation. The studies of grapes defences response against *E. necator* have been neglected and is still unclear if is admissible to predict their resistance or susceptibility based on leaves. Based on a similar approach used for leaves, the study aims to characterize the defence response of susceptible grapes of *V. vinifera* cv. Carignan against *E. necator*.

To accomplish the above mentioned, following specific research objectives were outlined:

1. Quantification, through LC-MS/MS, of hormonal content in non-infected and *E. necator*-infected leaves at 0, 6, 24 and 96 hpi and non-infected and *E. necator*-infected grapes at EL33 (late green) and EL35 (*veraison*) developmental stages;
2. Analyses of expression profiling by quantitative real-time PCR, of selected genes putatively involved in biosynthesis, signalling and downstream responses of the previously quantified phytohormones;
3. Quantification, through spectrophotometry, of total phenolic content in non-infected and *E. necator* infected leaves at 0, 6, 24 and 96 hpi and non-infected and *E. necator*-infected grapes at EL33 (late green) and EL35 (*veraison*) developmental stages;

4. Biochemical characterization of phenylalanine ammonia lyase (PAL) in non-infected and *E. necator*-infected grapes at EL33 (late green) and EL35 (*veraison*) developmental stages.

2. MATERIALS & METHODS

2.1. Plant material

In the present study, leaves were collected from five-years-old plants of the *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset and from Iberian *Vitis vinifera* cv. Aragonez maintained in the greenhouse at the Instituto Superior de Agronomia, University of Lisbon, Portugal (38º42'44.54''N 9º11'08.44''W). The interspecific hybrid and cv. Aragonez were selected due to their resistance and susceptibility to *Erysiphe necator*, respectively.

Grape clusters from Spanish *Vitis vinifera* cv. Carignan from a commercial vineyard located in Torres Vedras, Portugal (39º04'43.2''N, 9º20'58.9''W), were collected in 2017 at two developmental stages: EL33 (late green) and EL35 (*veraison*) [E-L refers to the modified Eichhorn and Lorenz developmental scale as described by Coombe et al. (1995)] (COOMBE, 1995). This cultivar was selected based on is high susceptibility to *E. necator*.

2.2. Fungal inoculation and sample collection

Since *E. necator* is an obligate biotrophic fungus, naturally infected grapevine leaves showing fully developed PM symptoms were collected from a field-grown *V. vinifera* and used as an inoculum source (Fig. S2.2, A). Thirty-five grapevine *per* species were randomly selected and, for each one, adaxial and abaxial leaf surfaces were water-treated prior to inoculation, in order to increase surface humidity to potentiate conidia germination (Schaechter, 2009). The inoculation occurs by direct contact between adaxial epidermis of the second - fifth fully expanded leaves beneath the apex and the surface of source leaves containing sporulating colonies (Fig S2.2, B and C). Mock-inoculated leaves were only water-treated and used as control (non-infected). Non-infected and infected leaves from each plant were collected separately and constituted a biological replicate in a total of five biological replicates for each condition and time point. Based on the proposed *E. necator* infection cycle (Fung et al., 2008), non-infected and infected leaves were harvest at 0, 6, 24 and 96 hours post-infection (hpi) and immediately frozen in liquid nitrogen and stored at -80ºC until further use.

Healthy and naturally infected grape clusters at EL33 (late green) and EL35 (*veraison)* were collected and categorize as "in presence" or "absence" of symptoms, and therefore considerer as infected and non-infected samples, respectively, after visual inspection. Clusters were harvested, immediately frozen in liquid nitrogen, transported in dry ice to the laboratory, and stored at -80ºC until sequent use. Four to five clusters were harvested, and each was considered as one biological replicate for each condition and developmental stage.

2.3. Assessment of Erysiphe necator infection

For infection confirmation, non-infected and infected leaves from resistant and susceptible species were harvested at 24 and 96 hpi, cut into portions of 1-3cm^2, immersed in 95% ethanol and stored at 4 ºC until photosynthetic pigments were completely removed. The presence of fungal structures in adaxial leaves surfaces was confirmed through staining with 250 µg/mL trypan blue in a solution of lactic acid, glycerol, and distilled water (1:1:1, v/v/v). After emersion in staining solution for 15 minutes, the cleared-stained leaves were rinsed rapidly with the same solution and mounted on microscope slides in 50% glycerol. Trypan blue was chosen since it stains chitin and glucans preferably, two major constituents of fungal cell walls and, therefore, allow the identification of fungal structures (Liesche et al., 2015; Waring, 1984). To detect callose deposition, cleared leaves were firstly rinsed in 50% ethanol followed by water and then stained for 30 minutes in 150 mM dipotassium phosphate [(K_2HPO_4); pH 9,5] containing 0,01% aniline blue. Staining leaves were mounted in 50% glycerol (Vogel and

Somerville, 2000; Rate *et al.*, 1999). Aniline blue, a commercial heterogeneous mixture, was chosen since contains a fluorochrome with callose-specificity which allow the detection of callose deposits as refractive deposits that fluoresced bright yellow in tissues (Silva et al., 2002). Acquired pictures were taking by a BX51 microscope (Olympus, Tokyo) and Zeiss stereo Lumar V.12 (Oberkochen, Germany).

2.4. Gene expression analysis

2.4.1. Optimization of total RNA extraction protocol from leaves

Prior to total ribonucleic acid (RNA) extraction, petioles and midrib were removed from non-infected and infected leaves of the resistant and susceptible species. The remain tissues were ground with mortar and pestle in the presence of liquid nitrogen, transferred to *falcon* tubes and stored at -80 ºC until used. To ensure absence of contaminants or RNases, grind utensils were sterilized overnight with sodium hydroxide (NaOH), followed by extensive washing with tap water and diethyl pyrocarbonate (DEPC)-treated water and heat sterilization at 180 ºC for a minimum of 2 hours. Several protocols commonly used for RNA extraction from leaves or grapes were tested and modified to ensure best results: *Protocol 1* includes Reid et al., (2006) extraction buffer and first protocol steps until chloroform: isoamyl alcohol (v/v) addition, followed by washing steps (without purification) from Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, USA); *Protocol 2* includes (Gambino et al., 2008) fully extraction protocol with modifications. For 1g of powdered samples, 1 mL of not pre-warmed extraction buffer was added following incubation at 55 ºC for 10 minutes. An equal volume of chloroform:isoamyl alcohol (24:1, v/v) was added. This procedure was repeated twice. The resulting supernatant was incremented with 1 volume of 6 M lithium chloride (LiCl) following 1 hour on ice and sequent transference to *Corex* tubes. *Pellet* resuspension occurs in 2 mL of 65 ºC pre-warmed sodium dodecyl sulfate–Tris-HCl–EDTA (SSTE) buffer and 1 volume of chloroform:isoamyl alcohol (24:1, v/v). The final washing steps were identical to Gambino et *al.* (2009) protocol; *Protocol 3* and *4* were similar to Protocol 2 with the increase of extraction buffer volume (final 4 mL) and without incubation at 65 ºC for 10 minutes. Moreover, Reid et *al.* (2006) and Gambino et *al.* (2009) extraction buffers were used in Protocol 3 and 4, respectively; *Protocol 5* and *6* are similar to Protocol 1 and 2 but with an additional step of sodium dodecyl sulfate (SDS) treatment of electrophorese material, during 45 minutes; *Protocol 7* uses Gambino et *al.* (2009) extraction buffer into the integral Spectrum™ Plant Total RNA Kit protocol (Sigma-Aldrich, USA) and *protocol 8* into RNeasy® (Qiagen Inc., USA) protocol; *Protocol 9* consist in total Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, USA) protocol; *Protocol 10* consist in previous steps of Fortes et *al.* (2011) until LiCl overnight precipitation follow Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, USA) purification steps. *Protocol 11* is considered the final and optimized protocol (extensively described below); *Protocol 12* includes an additional polysaccharides precipitation of Protocol 11; *Protocol 13* was similar to Protocol 11 with an increase of leaves macerated material (900 mg).

The *Protocol 11* was a total RNA extraction method based on hexadecyltrimethylammonium bromide (CTAB) method and according to Coelho et al., 2019 with slights modifications. The grinded leaves were homogenized at 600 mg *per* 5 mL in Gambino et *al*. (2008) extraction buffer [2% (w/v) CTAB, 2.5% (w/v) polyvinylpolypyrrolidone (PVP-40), 2 M NaCl, 100 mM Tris hydrochloride (Tris-HCl) pH 8.0, 25 mM pH 8.0 ethylenediaminetetraacetic acid (EDTA) and 2% (v/v) β-mercaptoethanol added just before use] previously heated to 60 ºC (Gambino et al., 2008). To potentiate sample-buffer contact and increase yield extraction, samples were mixed manually and by a vortex and incubated for 10 minutes at 60 ºC. To the sample lysates, it was added 1 volume of chloroform:isoamylic alcohol (24:1, v/v), followed by a vigorous agitation, transfer to *Corex* tubes, and centrifugation at 6,900×*g*, for 15 minutes at 4 ºC. The recovered aqueous phase was supplemented with 0.1 volume of 3 M sodium acetate (NaOAc) and 0.65 volumes of cold isopropanol, *Corex* tubes were sealed with parafilm, mixed by

inversion and left at -80 ºC for 30 minutes to proceed with nucleic acid precipitation. Samples were then centrifuged at 6,900×*g*, for 45 minutes at 4ºC and *pellet* was washed twice with cold 70% (v/v) ethanol, after which the tubes were left drying in the fume chamber for 15 minutes to ensure total ethanol evaporation and an easy *pellet* resuspension, avoiding RNA degradation. The *pellet* was later resuspended in 250 µL of DEPC-water. For RNA differential precipitation, 0.75 volumes of 6 M LiCl were added, and samples were kept at -20ºC for 1 week to obtain higher RNA concentration, followed by centrifugation at 10,000×*g*, for 15 minutes at 4 ºC. *Pellets* were washed twice with 70% (v/v) cold ethanol to remove polysaccharides and proteins contaminants, left inside fume chamber for 15 minutes, resuspended in 30 µL of DEPC-water and stored at -20ºC for a short period. Before treatment with DNase in *protocol 11* (Table S1.1), as well as for all the other total RNA extraction protocols total RNA (Table S1.2), RNA purity and concentration were measured by NanoDrop™ 1000 spectrophotometer (Thermo Scientific). RNA integrity was ascertained visually in a 1.2% agarose gel electrophoresis stained with SYBR safe (Invitrogen™, Thermo Scientific, USA) (Fig. S2.3).

2.4.2. Total RNA extraction from grapes

Total RNA from non-infected and infected grapes in EL33 and EL35 developmental stages was previously extracted (PhD student Diana Pimentel; Msc student João Coelho), according to Coelho et al., 2019. The same protocol was performed personally on *Vitis vinifera* cv. Trincadeira and cv. Syrah grape grapes infected with *Botrytis cinerea* (causal agent of grey mould), although these samples were not analysed in the context of this thesis. In the first step of total RNA protocol, to a 16.5 mL previously grinded grapes samples, approximately 12 mL of extraction buffer was added [2% (w/v) CTAB, 0.8% (w/v) PVPP, 2 M NaCl, 1 M Tris-HCl pH 9.0, 25 mM pH 8.0 EDTA, 1% (w/v), SDS and 5% (v/v) β-mercaptoethanol added just before use. Due to the high sample amount, vigorously mixing was performed to increase the contact with extraction buffer followed by 1 minute in ice. One volume of chloroform:isoamylic alcohol (24:1, v/v) was added to the homogenate, following vigorously mixing and centrifugation at 7300×*g* for 10 minutes at 4ºC. This procedure was repeated twice. Recovered aqueous phases were supplemented with 2 M potassium chloride (KCl), left on the ice for 1 hour to increase proteins precipitation and centrifuged at 10000×*g* for 15 minutes at 4ºC. The resulting supernatant was treated with 0.1 volume of 3 M NaOAc and 0.8 volume of cold isopropanol to precipitate nucleic acids, mixed, centrifuged at 10000×*g* for 15 minutes at 4ºC and the respective *pellet* was washed twice with 70% (v/v) ethanol. After 15 minutes inside the fume chamber, the dry *pellet* was dissolved in 1.4 mL of DEPC-treated water, added 0.1 volume of 2 M potassium acetate (KOAc) and incubated in ice for 60 minutes to precipitate polysaccharides and sequent centrifuged at 10000×*g* for 15 minutes at 4ºC. Followed by overnight precipitation with 1 volume of LiCl at 4ºC and centrifugation at 10000×*g* for 15 minutes at 4ºC, the *pellet* was washed with 70% (v/v) ethanol, left inside fume chamber for 30 minutes, resuspended in 50 µL of DEPC-treated water and stored at -20ºC until used.

2.4.3. Samples purification

Prior to RNA purification, leaves samples with a yield less than 1 µg/µL were considered unsuitable, and a RNA re-precipitation was performed according to Rio et al., 2010 with modifications in order to increase RNA concentration. Therefore, poor concentrated samples were pooled and considered as one biological replicate, leaving at least 2 replicates *per* condition (Table S1.3). Subsequently, for each replicate 0.1 volumes of 3 M NaOAc and 2.7 volumes of 100% cold ethanol were added, and mixed gently by pippeting. Afterwards the samples were kept at -20ºC overnight, to enhance re-precipitation of nucleic acids. In the following day, after centrifuging at 10,000×*g* for 20 minutes at 4 ºC, 150 uL of 70% cold ethanol was added to the samples, and a second centrifugation was repeated for 8 minutes. The supernatant was discarded, the *pellet* was dried in the fume chamber for 15 minutes, 20 µL DEPC-

water was added, and samples were allowed to stay on ice for 1 hour to promote solubilization before mixed carefully by pipeting. Ultimately, 1 µL of Ribboblock (Thermo Scientific, USA) was added to ensure RNA integrity by RNAse inibition. RNA quantification was performed in a Nanodropo 2000 (Thermo Scientific, USA) and samples were kept a -20 ºC until purification step (Table S1.3).

For RNA purification, the residual genomic DNA was digested using the TURBO DNA-free™ kit (Invitrogen, Thermo Scientific, USA). Ulterior measurement of final volume for each sample and when RNA concentration was not equal/higher to 200 µg/mL, 0.1 vol of 10× TURBO DNase Buffer, 1 µL of Ribiblock (Invitrogen, Thermo Scientific, USA) and 0.8 µL of TURBO DNase were added up to a final volume of 20 µL. Incubation at 37 ºC for 20 minutes without agitation was performed, and 0.2 volumes of resuspended DNase Inactivation Reagent was added to which sample, followed by a quick vortex and incubation at room temperature for 5 minutes. Due to the structural nature of DNase Inactivation Reagent, to increase the inhibition reaction and avoid its own precipitation, the tubes were inverted 2-3 during the procedure. After centrifugation at 10,000×*g*, for 1.5 minutes at room temperature of all samples, the supernatants were transferred to new tubes and RNA purity and concentration was again evaluated in Nanodropo 2000 (Thermo Scientific, USA) and absence of genomic DNA was assessed visually in a 1.2% agarose gel electrophoresis stained with SYBR safe (Invitrogen™, Thermo Scientific, USA) (Table S1.4).

2.4.4. Reverse transcriptase protocol

For leaves, complementary DNA (cDNA) was obtained from previously purified RNA by Thermo Scientific RevertAid™ H Minus Reverse Transcriptase (Fermentas, CA) as described by the manufacturer. For a minimum of 3 biological replicates *per* experimental condition, technical or biological replicates were pooled together to constitute a biological replicate with a concentration of 1 µg/µL. When only one biological replicate was available due to prior RNA degradation, two technical replicates with 1 µg/µL concentration each were pooled. The first-stranded cDNA was synthesized from 1 µg of total RNA and primed with 1 µL of synthetic Oligo$(dT)_{23}$ primer (Fermentas, CA) after which DEPC-treated water was added to make 12.5 µL of final reaction volume. The tubes with the previous mixture were mixed gently, briefly centrifuged at 10,000×*g* at room temperature, incubated at 65ºC for 5 minutes and put on the ice. To obtain the final reverse-transcription reaction mixture, 4.4 µL of 5x reaction buffer, 0.6 µL of RiboLock, 2.2 µL of 10 mM of dNTP mix and 1.1 µL of RevertAid H Minus reverse transcriptase were added to each sample. A second centrifugation as the previous one was performed, and the synthesis reaction occurred through incubation at 42ºC for 60 minutes.

2.4.5. Quantitative real-time PCR

Prior to primer design, nine genes were selected due to their putative involvement in hormone biosynthesis, signalling and response and are listed in Table S1.5. For salicylic acid (SA) pathway were chosen two positive SA-regulators enhanced disease susceptibility 1 (*EDS1*) and phytoalexine deficient 4 (*PAD4*), and a marker of SA-pathway pathogenesis-related protein 1 (*PR1*) (Dempsey et al., 2011). For auxins pathway were chosen the genes coding for an enzyme responsible for IAA conjugation with amino acids, IAA-amido synthetase GH3-2 (*GH3-2*) (Jiang et al., 2017) and auxin-induce enzyme in root cultures protein 12 (*AIR12*) (Gibson & Todd, 2015). For the jasmonates pathway was chosen the positive regulator of JA synthesis allene oxidase synthase *(AOS*) (Zhai et al., 2017). For the abscisic acid (ABA) the pathway the genes for sucrose non-fermenting-1 *(*SNF1)-related protein kinase 2 (*SnRK2*) (Kulik et al., 2011) and an ABA-induced LEA protein *HVA22C* (Gomes Ferreira et al., 2019) were chosen.

The primers were designed using the PrimerSelect software (DNAStar, Madison). Primer specificity and efficiency was ensured and the formation of secondary structures was avoid, since primers were designed by the following rules: amplicon length between 80-200 nucleotides; primer T_m between 58-61 ºC; no more than 1 ºC between primers pairwise; GC content at 40-60%; primers length between 18-25 nucleotides. Moreover, the primers sequences were blasted against *Vitis vinifera* genome database present in NCBI (http://www.ncbi.nlm.nih.gov/) to ensure primer specificity for coding sequence and null mismatched. cDNA was 1:35 and 1:40 diluted with ultrapure distilled water (ddH_2O) from leaves and grapes, respectively, to ensure optimal cDNA concentration for quantitative real-time (qPCR). For cDNA from cv. Carignan grapes, three to four biological replicates for each condition (non-infected or infected) and both developmental stages, were included. Additionally, 2 technical replicates, were admitted. For cDNA from leaves from both species, ideally 4 biological replicates were considered. However, in presence of 2 biological replicates, 2 extra technical replicates were formed by pooling both biological replicates. qPCR reactions were performed in a final volume of 20 µL containing 10.0 µL SYBR® Premix Ex *Taq*™ II (Applied Biosystems, CA), 4.0 µL of cDNA, 0.7 µL of forward and reverse primer (10 µM), and 4.6 µL ddH_2O and reaction were performed in a StepOne™ Real-Time PCR System (Applied Biosystems, CA). Thermal cycling started with a denaturation step at 95˚C for 10 minutes, followed by 42 cycles of denaturation at 95˚C for 15 seconds and annealing at the respective temperature for each gene for 40 seconds, an extension step at 95ºC for 15 seconds and an elongation step at 60ºC for 1 minute. Dissociation curves were used to analyse non-specific PCR products. Each set of reactions included a negative control with no template. For each gene of interest, the amplification efficiency was determined using the LinRegPCR version 2013.0 The relative gene expression in cv. Carignan grapes was normalized using *actin* and elongation Factor 1 α **(***EF 1α* gene) (Agudelo-Romero et al., 2015). The relative gene expression in leaves of both species, was normalized using vacuolar ATPase subunit G (*VAG)* and ubiquitin conjugating enzyme (*UBC)* (Borges et al., 2014). Relative gene expression was calculated according to $2^{\Delta\Delta}$ CT method (Livak & Schmittgen, 2001).

2.5. Extraction of phenolic secondary metabolites

Total phenolic compounds were extracted from leaves of resistant and susceptible species and grapes, in both developmental, for all hpi and conditions. Preferentially, 4-5 biological replicates were used, except for grapes in which only 3-4 biological replicates were considered due to previous selection regarding the degree of infection (Pimentel et al., under review). For total phenolic quantification, the Folin-Ciocalteu colorimetric method was adopted (Waterhouse, 2003), with minor modifications. Lyophilized leaves and grapes samples were homogenized at approximately 50 mg *per* 1.5 mL of ultra-pure water and centrifuged at 18,000×*g*, at room temperature, for 30 minutes. To the enriched phenolic extracts, 20 μL for each was mixed with 1080 μL of ultra-pure water and 100 μL of Folin reagent, following vigorously agitation and incubation on the dark, for 10 minutes. 800 μL of 2 M sodium carbonate [Na_2CO_3; 7,5% w/v] was added and a second incubation, identical to the previous one, was accomplished for 30 minutes, following absorbance measurement at 743 nm. Prior to spectrophotometrically characterization, total phenolic concentration was calculated using a gallic acid calibration curve. The calibration curve was obtained considering the relationship between peak areas *versus* standard concentrations at four concentration (n = 4) and dispose a linear fitting with values of the R squared (R^2) = 0.99. The concentration of total phenolic compounds was determined as µg of gallic acid equivalents [GAE] *per* mg of extract (µg GAE/mg).

2.6. Enzyme assays

2.6.1. Enzyme extraction

Total protein extraction from lyophilized grapes was extracted as described by Stoop and Pharr (1993), with minor modifications. Lyophilized samples were mixed with extraction buffer in an approximately 1:1 (v/v) sample:buffer extraction ratio. The extraction buffer was composed by 50 mM Tris-HCl pH8.9, 5 mM magnesium chloride ($MgCl_2$), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM dithiothreitol (DTT) and 0.1% (v/v) Triton X-100. The homogenised samples were centrifuged at 18,000×*g* for 20 minutes at 4ºC, and the supernatants were kept on ice. Total protein content was determined spectrophotometrically by the Bradford method (Bradford, 1976), with bovine serum albumin (BSA) as standard.

2.6.2. Phenylalanine ammonia lyase enzymatic assay

After acquiring the enzymatic extracts, phenylalanine ammonia lyase (PAL) biochemical activity was determined in relation to cinnamic acid production at 41 °C, in a total volume of 2 mL *per* reaction. The reaction mixture contained 0.2 mL of enzymatic extract, 3.6 mM NaCl, 25 mM L-phenylalanine in 50 mM Tris-HCl pH 8.9. To initiate the reaction was added to reaction mixture L-phenylalanine. The rate of conversion of L-phenylalanine to cinnamic acid was monitored spectrophotometrically at 290 nm.

2.7. Hormonal profiling analysis by LC-MS/MS

Several hormones and metabolized forms were quantify, namely salicylic acid (SA) and salicylic acid-*β*-D-glucoside (SA-glucoside)**,** jasmonates [12-oxophytodienoic acid (OPDA); jasmonic acid (JA); jasmonoyl-isoleucine (JA-Ile); 12-hydroxy-JA-Ile (12-OH-JA-Ile); dicarboxy-JA-Ile (12-COOH-JA-Ile); 12-*O*-glucosyl-JA (12-*O*-Glc-JA)], abscisic acid (ABA) and auxins (indole acetic acid; IAA). For leaves, three to five lyophilized samples for which time point, condition and *per* species were included. For grape, three to four lyophilized samples were considered. An approximately 30 mg *per* biological replicate was sent to Max Planck Institute for Chemical Ecology (Jena, Germany). Here, stock solutions of each individual phytohormone standard were prepared at 1 mg/mL in MeOH and deuterated compounds stock solutions were prepared in acetonitrile at 100 µg/mL. Working solutions of original phytohormones standards were prepared by diluting stock solutions in MeOH:water (7:3), at different concentration for each phytohormone depending on the range of the calibration curve: ABA and IAA at 100 µg/mL; JA and SA at 200 µg/mL; OPDA at 50 µg/mL; and JA-Ile at 40 µg/mL. The internal standard stock solutions (d5-JA, d6-ABA, d4-SA, and d5-IAA) were combined, and diluted in MeOH:water (7:3) ratio, resulting in the extraction solution. The final concentrations were 10 ng/m for both d4-SA and d5-IAA, and 20 ng/mL for both d5-JA and d6-ABA. To each sample, 1 mL of extraction solution containing the internal standards (d5-JA, d6-ABA, d5- IAA, and d4-SA). The samples were briefly mixed with a vortex and spiked with phytohormones standards as described in Trapp *et al*. (2014). The spiked samples were shaken for 30 minutes and centrifuged at 16,000g and 4°C for 5 minutes. The supernatant was transferred into a new micro-centrifuge tube and dried in a speed-vac concentrator. After drying, 100 µL of MeOH was added to each sample, which was them mixed with a vortex and centrifuged at 16,000×*g* and 4 °C for 10 minutes. The supernatant was analysed by liquid chromatography-mass spectrometry (LC-MS/MS).

2.8. Statistical analysis

Data from hormone quantification, gene expression, total phenolic content and enzymatic activity of PAL, was statically treated to identify significance. Firstly, the Shapiro-Wilk test was applied to test the normality of data. Then, the Dixon's Q-test was used to identify outliers, which were excluded. To

identify whether data presented variability or not an ANOVA-two way was performed, except for data concerning leaves at 0 hpi, where a Student's *t*-test was used to identify variability. These test were chosen since data presented a normal distribution and regarding number of independent variables: at 0 hpi only one independent variable was considered - "species"; for the remaining data, two independent variables were considered - "developmental stage" and "condition" for grapes and "species" and "condition" for leaves. After ANOVA-two way, to identify specifically where the significance relies, a Tukey post-hoc test was performed. For all statistics test a 95% of significance ($p\text{-}value \leq 0.05$) was considered and were executed on RStudio version 1.0.136 (RStudio, PBC).

3. RESULTS

3.1. Assessment of Erysiphe necator infection

In leaves from resistant cv. 101-14 Millardet et de Grasset and susceptible cv. Aragonez, the infection by *E. necator* was cytologically assessed at 24 and 96 hpi, by discolouring portions of infected leaves followed by identification of fungal structures and callose deposits. It was observed germinated conidia fixed on leave surface with ramified hyphae forming a mycelium (Fig.3.1, A). As show in Fig.3.1, B and D, callose deposits were observed at the infection sites in infected leaves from resistant and susceptible species. Callose deposition on papillae is a common defence response with the purpose to create a physical barrier around the hyphae and prevent fungus growth (Underwood, 2012). Also, due to a nonspecific capacity of aniline blue fluorochrome to labelling to any β-1-3-glucan (Heinlein, 2014), fungal structures were as well stained, therefore conforming the fungus colonization in grapevine leaves (Fig.3.1, C and E).

Susceptible grape clusters from cv. Carignan were previously collected at two developmental stages, EL33 (late green) and EL35 (*veraison*), according to the modified E-L system (COOMBE, 1995) and designated as "non-infected" or "infected" clusters by visual observation of fungal infection sites. The infection was confirmed by performing a qPCR with primers specifics for *E. necator* (Pimentel et al., under review).

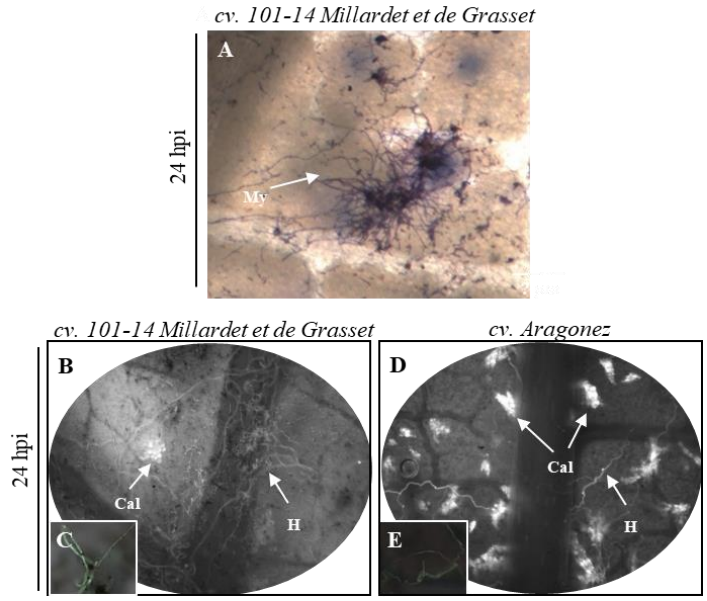


Figure 3.1 - Assessment of *Erysiphe necator* infection during incompatible or compatible interaction in leaves. Resistant *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset and susceptible *Vitis vinifera* cv. Aragonez were inoculated with *E. necator* and pathogen development and callose deposition were monitored at 24 and 96 hours post infection using fluorescent microscopy after staining. (A) Fungal mycelium and hyphae stained with trypan blue at 24 hours post infection. (B, D) Callose deposits in fungal penetration sites stained with aniline blue at 24 hours post infection. (C, E) Hyphae stained with aniline blue at 24 hours post infection. Abbreviations: My, mycelium; H, hyphae; Cal, callose.

3.2. Protocol optimization for extraction of RNA from leaves

The expression analysis by qPCR of genes related to hormonal pathways underlying grapevine - *E. necator* interaction, relies in RNA of high quality and integrity. Several protocols for RNA extraction from grapevine leaves and grapes were tested, with or without modifications. However, some extraction protocols produced unsatisfactory results regarding RNA yield and/or quality and for these reasons they were disregarded (Table S1.1 and S1.2; Fig. S2.4): in Protocol 1-4, 7 and 8 a smear with indistinct bands or a blob at the running edge of the 1.2% agarose gel indicate RNA degradation; Protocol 1, 2 and 8 had

low RNA extraction yield (15.58–31.85 ng/100 mg FW). The same inadequate yield was obtaining in Protocol 5, 6, 9 and 10 (3.54–11.3 ng/100 mg FW), but the presence of two defined rRNA bands (28S and 18S) with a higher intensity of the upper band corresponding to 28S indicates a non-degraded RNA. With the exception of protocol 7, in all the other protocols, RNA extraction presented poor ratios (A260/A280 and A260/A2309), suggesting a principal contamination with polysaccharides and polyphenols (A260/A230: 0.66–1.66) and less evidence with proteins (A260/A280: 1.70–1.99).
In order to solve the problems evidenced on previously tested protocols, an ulterior optimized protocol (Protocol 11) was formulated. To guarantee RNA integrity, standard procedures were adopted to inhibit RNases activities, namely heat sterilization of material used during extraction, water treatment with DEPC and SDS treatment of electrophorese material. Even more, to all purified samples RiboLock, was further added to ensure RNA quality by its RNase inhibitor activity.

Table 3.1 - Yield and purity of total RNA extracted from leaves with the optimized extraction protocol 11**.** Total RNA was extracted from non-infected and *E. necator*-infected leaves of resistant cv. 101-14 Millardet et de Grasset and susceptible cv. Aragonez and all time points (0,6,24,96 hours post-infection) were included in which condition.
[a] Values expressed are mean ± SD (standard deviation) of all biological/technical replicates for individual condition.

Species	**Conditions**	**RNA yield (ng/100 mg FW)[b]**	**A_{260}/A_{280}[b]**	**A_{260}/A_{230}[b]**
cv. 101-14 Millardet et de Grasset	Non-infected	185.18 ± 367.66	1.98 ± 0.08	1.57 ± 0.23
	Infected	45.01 ± 19.71	1.92 ± 0.11	1.54 ± 0.28
cv. Aragonez	Non-infected	22.88 ± 22.16	1.81 ± 0.14	1.29 ± 0.73
	Infected	9,18 ± 9.88	1.87 ± 0.49	0.86 ± 0.37

The presence of degraded RNA in some biological replicates may also be related to physiological conditions of leaves, and with greater representativeness of older leaves in those samples. The usually low yield of RNA extraction may be the consequence of low RNA content in grapevine leaves (Loulakakis et al., 1996). To null this, Reid et al., (2006) defined an optimum ratio of 1:5 (v/v) between leaves:extraction buffer. Due to small amount of leaves for all biological replicates, a compromise of 600 mg *per* 5 mL dictated a reasonable total yield (73±35.4 ng/ 100 mg FW) when contemplated both species (Table S1.2). However, the increase of tissue amount or volume of extraction buffer did not produce a proportional yield increase. Protocol 13 produced a high RNA yield than Protocol 11. However, it was only tested in non-infected leaves from resistant species. In Protocol 11, an additional step of overnight RNA precipitation with 6 M LiCl was implemented to increase total RNA extraction yield. Polysaccharides, and polyphenols are contaminants in RNA extractions and its abundance in grapevine leaves seems to be species-specific (Vasanthaiah, 2008). RNA extraction from cv. Aragonez produced a yellowish and highly viscous *pellet* indicating a high content of polysaccharides and polyphenols when compared with the one extracted from resistant species. These contaminates co-precipitated with RNA and are responsible for low yield and purity of extracted RNA (MacKenzie et al., 1997). To minimized polysaccharides contamination, it was performed a precipitation with 2 M KOAc. However, this last step had minimal effect in RNA purity with significant loss of yield, and therefore, it was discarded (Table S1.2). Nevertheless, contaminant removal was substitute by double washing steps with 70% (v/v) of cold ethanol. All the steps result in an optimized protocol that could extract RNA with quality and integrity and with high yield. However, as shown in Table 3.1, these parameters differ between species and conditions. Yield is higher in resistant species rather than susceptible ones and upon infection decreases in both species. These results occur probably due to changes in physiological conditions after infection. Also, susceptible species had more polysaccharides and polyphenols than the resistant one and these compounds can have an impact in the yield.

3.3. Hormonal metabolism with powdery mildew

To understand the impact of *E. necator* infection in grapevine hormonome, several hormones classically related to plant defences (SA, JA), abiotic stress (ABA) and development (IAA) (Kazan & Lyons, 2014), were analysed through LC-MS/MS. Additionally, the expression profiling was evaluated for nine genes related to the biosynthesis, signalling transduction and response to the selected hormones. Particularly, the selected genes are known to be involved in resistance or susceptibility against biotrophic fungi and were also differentially expressed in the RNA-seq database of cv. Carignan grapes infected with *E. necator* (Pimentel et al., under review).

The hormonal quantification and gene expression were analysed in leaves of resistant cv. 101-14 Millardet et de Grasset and susceptible cv. Aragonez, in non-infected and infected conditions at 0, 6, 24 and 96 hpi and, also in cv. Carignan non-infected and infected grapes at EL33 (green late) and EL35 *(veraison)*.

3.3.1. Hormonal content in leaves

3.3.1.1.Content in salicylic acid salicylic acid-β-D-glucoside

The hormonal profiling of SA and SA-glucoside, an storage form of SA (Fig. S2.1, A) (Dempsey et al., 2011), was similar in both species and between conditions (Fig. 3.2). For both hormones, the constitutive and upon infection levels were significantly higher in resistant species when compared with the susceptible one starting at 6 hpi. Furthermore, at 24 hpi is was observed a significant hormonal increase upon infection only for the resistant species. Concerning SA, it was also observed a hormonal accumulation upon infection starting at 6 hpi, that was not observed in infected leaves from the susceptible species. These results suggest that SA and SA-glucoside are induced upon infection and are putatively involved in resistance.

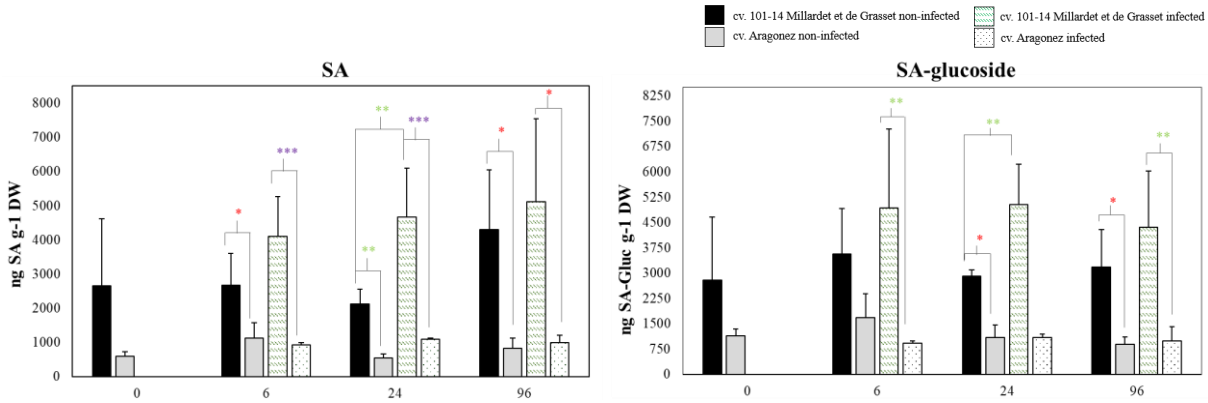


Figure 3.2 - Metabolism of salicylic acid in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed mock-inoculated and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi). Hormonal quantification of salicylic acid (SA) and salicylic acid-*β*-D-glucoside (SA-glucoside) in ng of SA/ SA-glucoside *per* g of dry weight (DW). Bars indicate standard errors of means (three to five for each condition and time point). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.3.1.2.Content in auxins

At 0 hpi, constitutive level of IAA, the most abundant auxin, IAA (Fig. S2.5, A) (Jiang et al., 2017), was significantly higher in resistant species when compared with susceptible one (Fig. 3.3). Also, at 6 hpi IAA level in infected leaves from resistant species was significant higher regarding the susceptible

one. These results suggested that IAA is putatively involved in resistance and is induced upon *E. necator* infection.

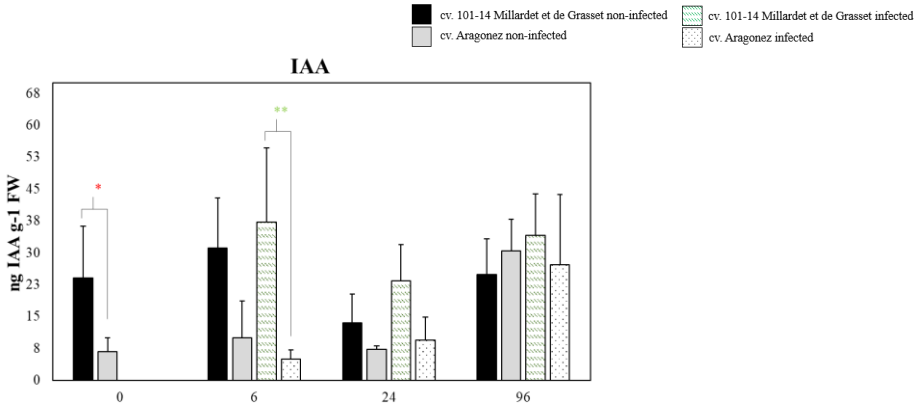
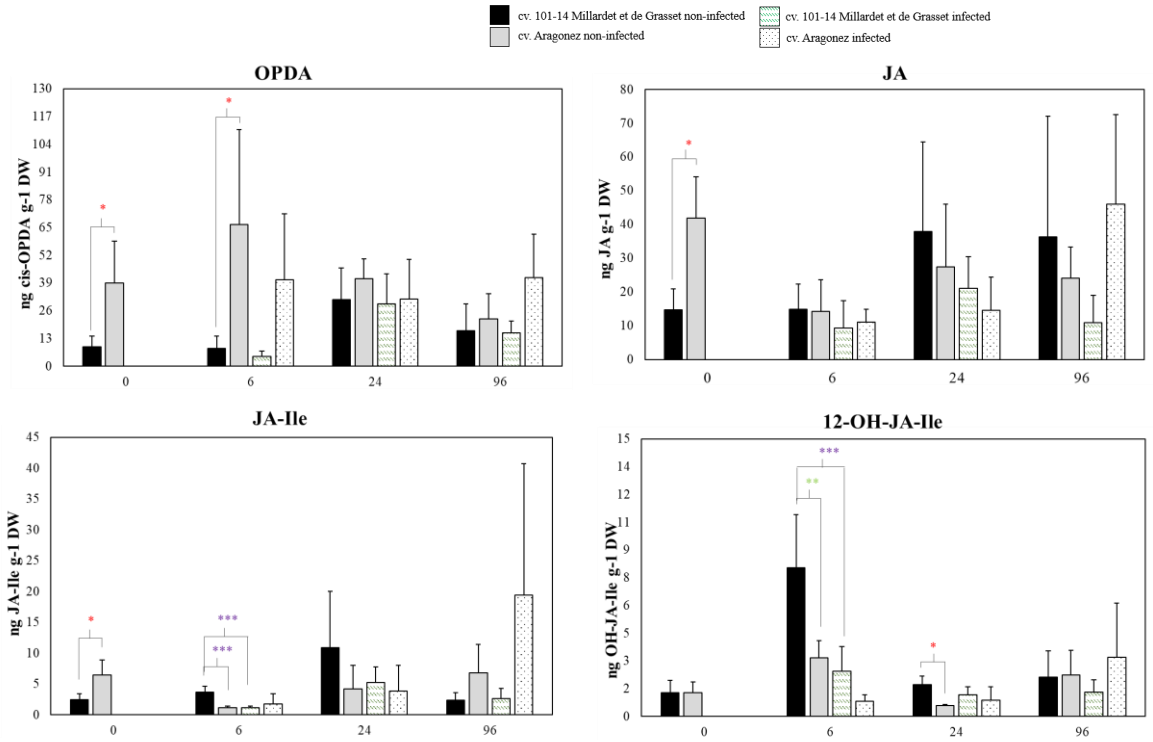


Figure 3.3 - Content in IAA in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected (mock-inoculated) and infected (PM-disease) leaves at four time points (0, 6, 24 and 96 hpi). Hormonal quantification of indole-3-acetic acid (IAA) in ng of IAA *per* g of dry weight (DW). Bars indicate standard errors of means (three to five for each condition and time point). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.3.1.3.Content in jasmonates

At 0 hpi, the constitutive level of OPDA, JA, JA-Ile and 12-*O*-Glc-JA was significantly higher in susceptible species when compared with resistant ones (Fig. 3.4). Contrarily, at 6 hpi, constitutive levels of JA-Ile, 12-OH-JA-Ile and 12-COOH-JA-Ile were significantly higher in leaves from resistant species when compared with susceptible ones. Also, in infected leaves from resistant species, levels of JA-Ile and 12-OH-JA-Ile decrease significantly at 6 hpi and 6-24hpi, respectively. Only for 12-*O*-Glc-JA a significant increase upon infection was observed in susceptible species when compared with resistant species at 96 hpi. These results suggested that constitutive and specific JAs metabolites (Fig. S2.6, A) are putatively involved in susceptibility upon *E. necator* infection.



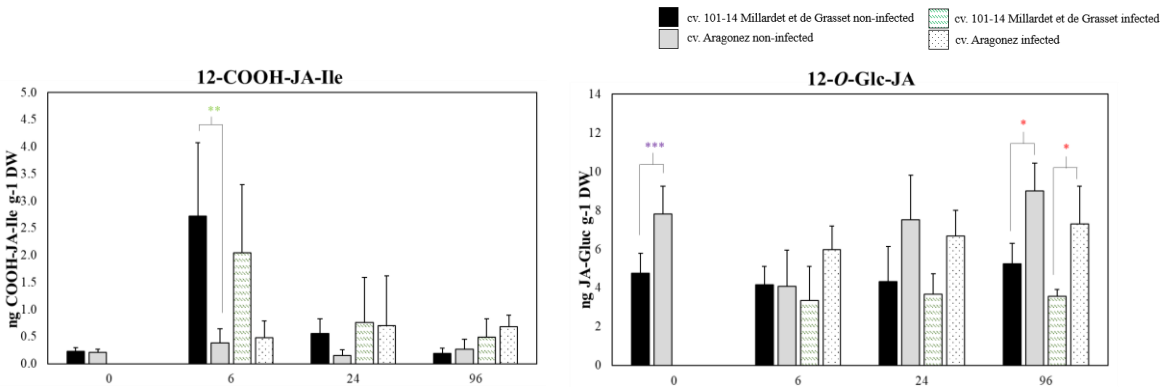


Figure 3.4 - Metabolism of jasmonates in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected (mock-inoculated) and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi). Hormonal quantification of jasmonates in ng of OPDA/JA/12-OH-JA-Ile/12-COOH-JA-Ile/12-*O*-Glc-JA *per* g of dry weight (DW). Bars indicate standard errors of means (three to five for each condition and time point). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.3.1.4.Content in abscisic acid

Constitutive levels of ABA (Fig. S2.7, A) were significant higher in leaves of susceptible species when compared with resistant ones at 0, 24 and 96 hpi (Fig. 3.5). It was also observed at 24 hpi an increase of ABA content in infected leaves of susceptible species. These results suggested that ABA is putatively involved in susceptibility in leaves and induced upon *E. necator* infection.

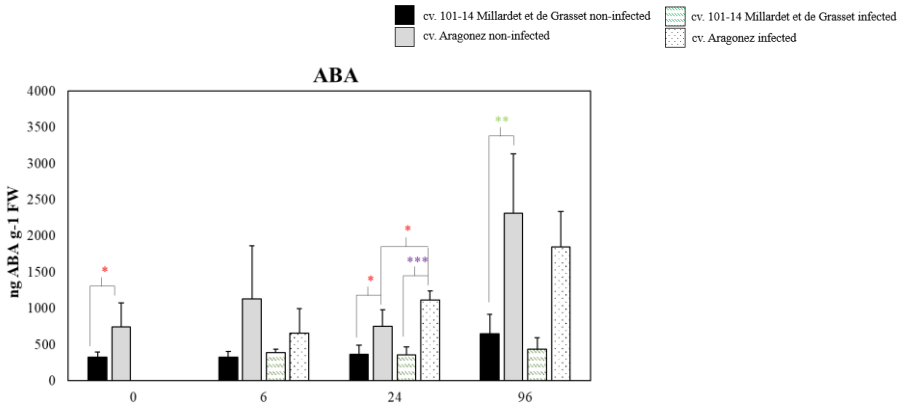


Figure 3.5 - Metabolism of abscisic acid (ABA) in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed mock-inoculated and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi). Hormonal quantification of abscisic acid (ABA) in ng of ABA *per* g of dry weight (DW). Bars indicate standard errors of means (three to five for each condition and time point). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.3.2. Gene expression profiling involved in hormonal metabolism in leaves

3.3.2.1.Expression of genes related with Salicylic Acid pathway

Only for *PR1*, a SA-response gene (Fig. S2.1,B) (Wiermer et al., 2005), it was observed a significant increase of constitutive expression at 6 and 24 hpi for susceptible species when compared with the resistant one (Fig. 3.6). Also, *PR1* expression was significant higher at 24 hpi upon infection in susceptible species when compared with the resistant one. For *EDS1* and *PAD4*, regulators of SA signalling (Cui et al., 2017), no significant changes were observed in gene expression between species and conditions for all time points. The results of gene expression are not in agreement with the hormonal data.

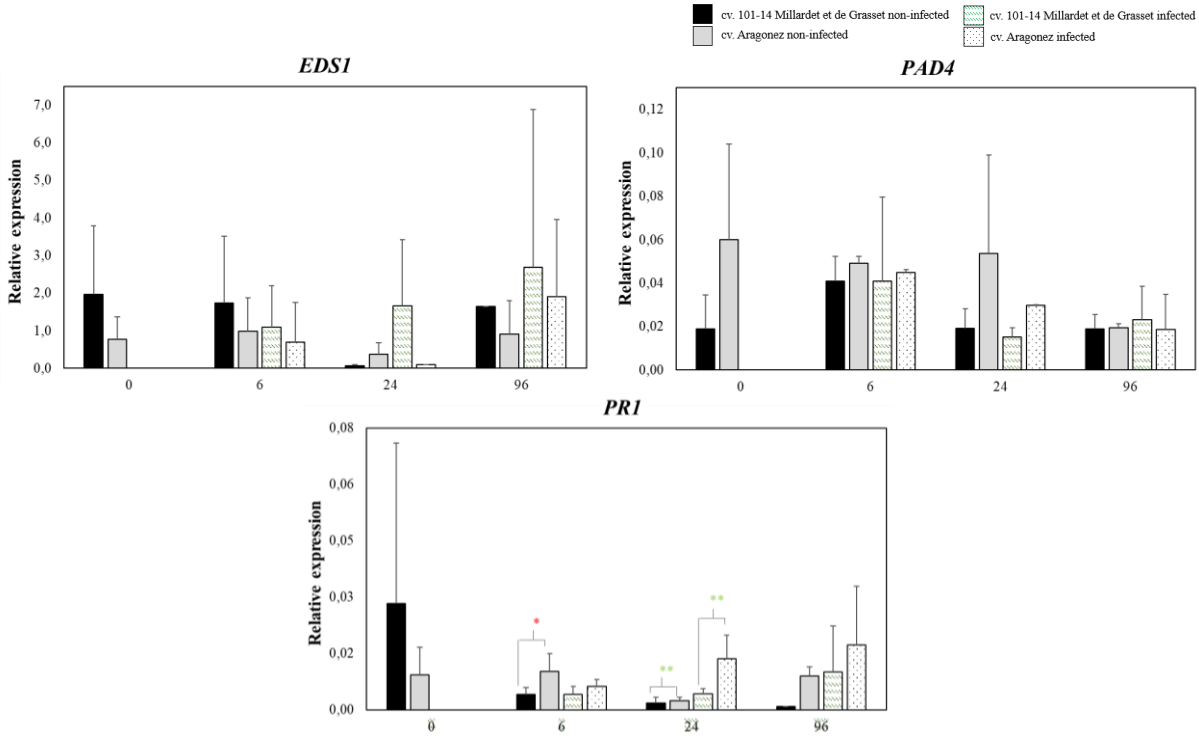


Figure 3.6 - Expression of genes involved in salicylic acid metabolism in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected (mock-inoculated) and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi): enhanced disease susceptibility 1 (*EDS1*), phytoalexine deficient 4 (*PAD4*) and pathway pathogenesis-related protein 1 (*PR1*). Relative expression was obtained applying ΔΔCT method. Bars indicate standard errors of means (two to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.1.1.Expression of genes related with Auxins pathway

No significant changes in gene expression was observed for *GH3-2*, an enzyme responsible for IAA conjugation with amino acids (Ludwig-Müller, 2011), and *AIR12*, auxin-responsive gene (Fig. S2.5, B), between both species and conditions for all time points (Fig. 3.7). These results suggest that these genes do not play a major role in IAA pathway upon *E. necator* infection.

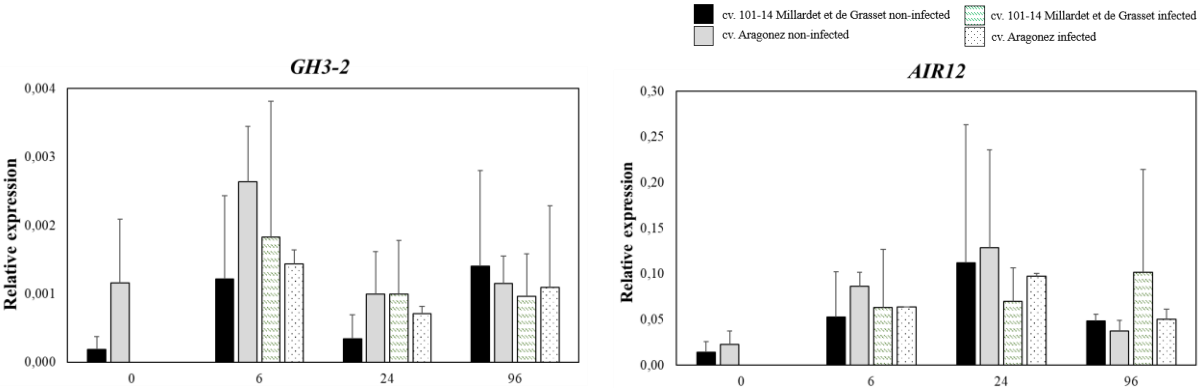


Figure 3.7 - Expression of genes involved in auxins metabolism in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected (mock-inoculated) and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi): IAA-amido synthetase GH3-2 (*GH3-2*) and auxin-induced in root cultures protein 12 (*AIR12*). Relative expression was obtained applying ΔΔCT method. Bars indicate standard errors of means (two to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test.

3.1.1.2.Expression of genes related with Jasmonates pathway

AOS is a positive regulator of JA synthesis (Fig. S.2.6, A) (Farmer & Goossens, 2019). *AOS* expression (Fig.3.8) had no significant changes between species and conditions for all time points though expression levels seem to decrease throughout the time points. These results suggest that this gene do not play a major role in JAs pathway upon *E. necator* infection.

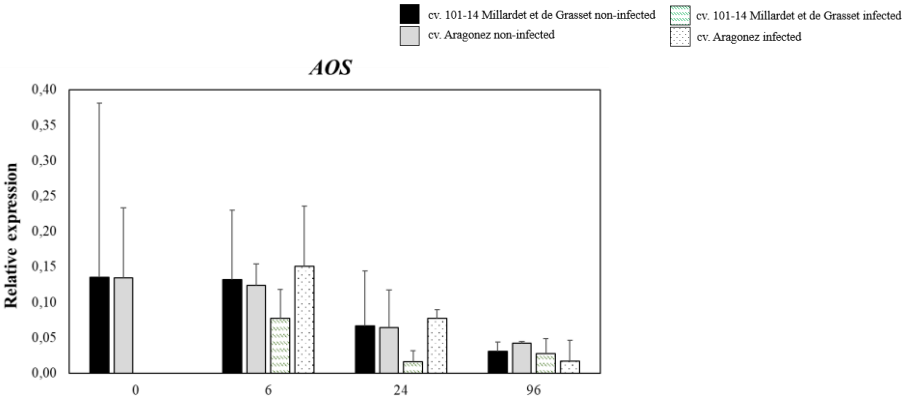


Figure 3.8 - Expression of gene involved in jasmonate metabolism in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected (mock-inoculated) and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi): allene oxidase synthase *(AOS)*. Relative expression was obtained applying ΔΔCT method. Bars indicate standard errors of means (three to five for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test.

3.1.1.3.Expression of genes related with Abscisic acid pathway

Only for *SnRK2*, gene involved in ABA signalling (Fig. S2.7, B) (Kulik et al., 2011), at 6 hpi it was observed a significant higher constitutive expression in susceptible species when compared with resistant ones (Fig. 3.9). Also, a significant decrease in *SnRK2* expression was observed at 6 and 96 hpi when non-infected leaves of susceptible species were compared with the infected ones. For *HVA22C*, an ABA-response gene (Fig. S2.7,B) (Q. Shen et al., 2001), no significant expression were observed between species and conditions for all time points. These results indicate that these genes are not strongly modulated in leaves under infection with *E. necator*.

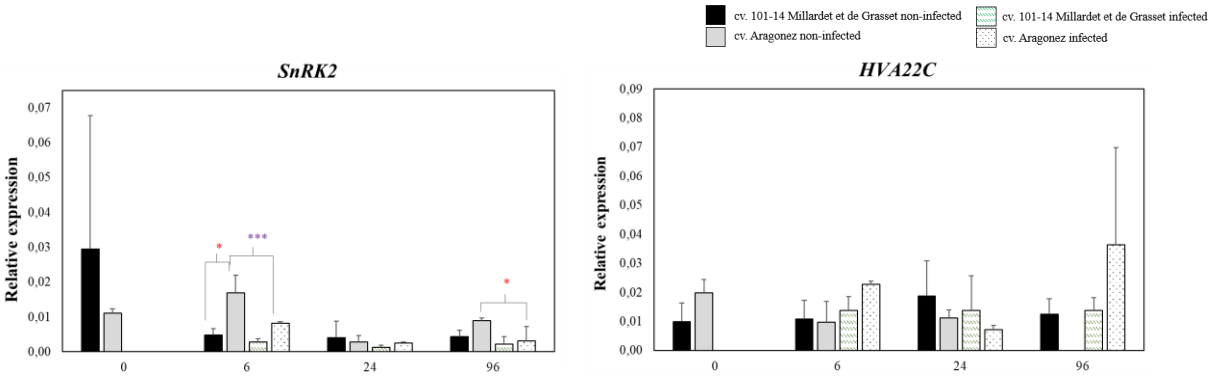


Figure 3.9 - Expression of gene involved in abscisic acid (ABA) metabolism in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected (mock-inoculated) and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi): sucrose non-fermenting-1 *(*SNF1)-related protein kinase 2 (*SnRK2*) and *HVA22C*. Relative expression was obtained applying ΔΔCT method. Bars indicate standard errors of means (three to five for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.2. Hormonal content in grapes

3.1.2.1.Content in salicylic acid **and** *salicylic acid-β-D-glucoside*

Similar hormonal profiling was observed for SA and SA-glucoside (Fig. S2.1, A) when developmental stage and conditions were compared (Fig. 3.10). Concerning SA, only in EL33 a significant accumulation was observed in infected grapes when compared with non-infected ones. Also, a significant decrease was observed in infected grapes when EL33 was compared with EL35 while no significant differences were observed between SA constitutive levels when the two developmental stages were compared. Concerning SA-glucoside, a significant accumulation was observed in infected grapes when compared with non-infected in EL33 and EL35. Furthermore, it was observed a significant decrease of SA-glucoside in infected grapes at EL35 when compared with EL33. These results suggest that SA and SA-glucoside are induced upon *E. necator* infection.

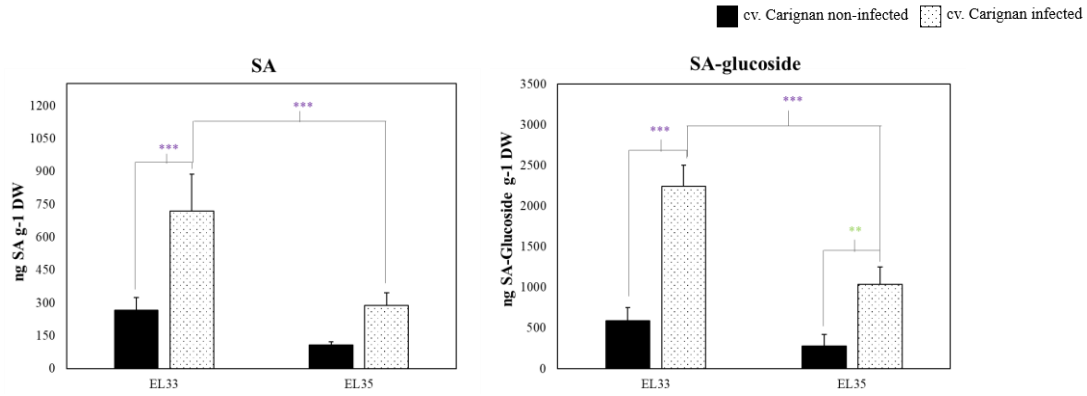


Figure 3.10 - Metabolism of salicylic acid in cv. Carignan grapes upon infection with *E. necator* at two developmental stages: EL33 (late green) and EL35 (*veraison*) and two conditions: non-infected and infected (PM-disease). Hormonal quantification of salicylic acid (SA) and salicylic acid-*β*-D-glucoside (SA-glucoside) in ng of SA/ SA-glucoside *per* g of dry weight (DW). Bars indicate standard errors of means (three to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.2.2.Content in auxins

In cv. Carignan grapes, no significant changes were observed in the content of IAA (Fig. S2.5, A) when non-infected and infected grapes were compared in each and between developmental stages (Fig. 3.11). These results indicate that auxins metabolism is not induce in grapes upon infection with *E. necator*.

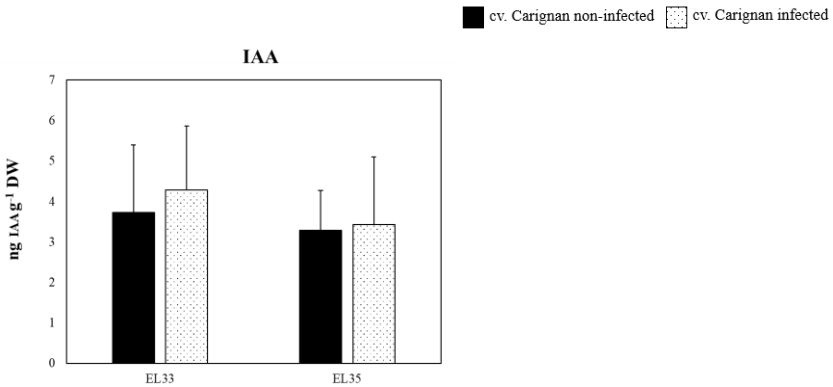


Figure 3.11 - Metabolism of auxins in cv. Carignan grapes upon infection with *E. necator* at two developmental stages: EL33 (late green) and EL35 (*veraison*) and two conditions: non-infected and infected (PM-disease). Hormonal quantification of indole-3-acetic acid (IAA) in ng of IAA *per* g of dry weight (DW). Bars indicate standard errors of means (three to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test.

3.1.2.3.Content in jasmonates

Concerning jasmonates, no significant changes were observed in the content of OPDA, JA, JA- Ile, and 12-COOH-JA-Ile when non-infected and infected grapes were compared for EL33 and EL35 and between developmental stages, respectively (Fig. 3.12). However, for 12-OH-JA-Ile and 12-*O*-Glc-JA a significant accumulation was observed in infected grapes when compared with non-infected ones at EL33. Furthermore, for 12-*O*-Glc-JA a significant decrease in accumulation was observed in EL35 when compared with EL33. These results indicate that jasmonates´ metabolism (Fig. S2.6, A) selectively changes upon *E. necator* infection.

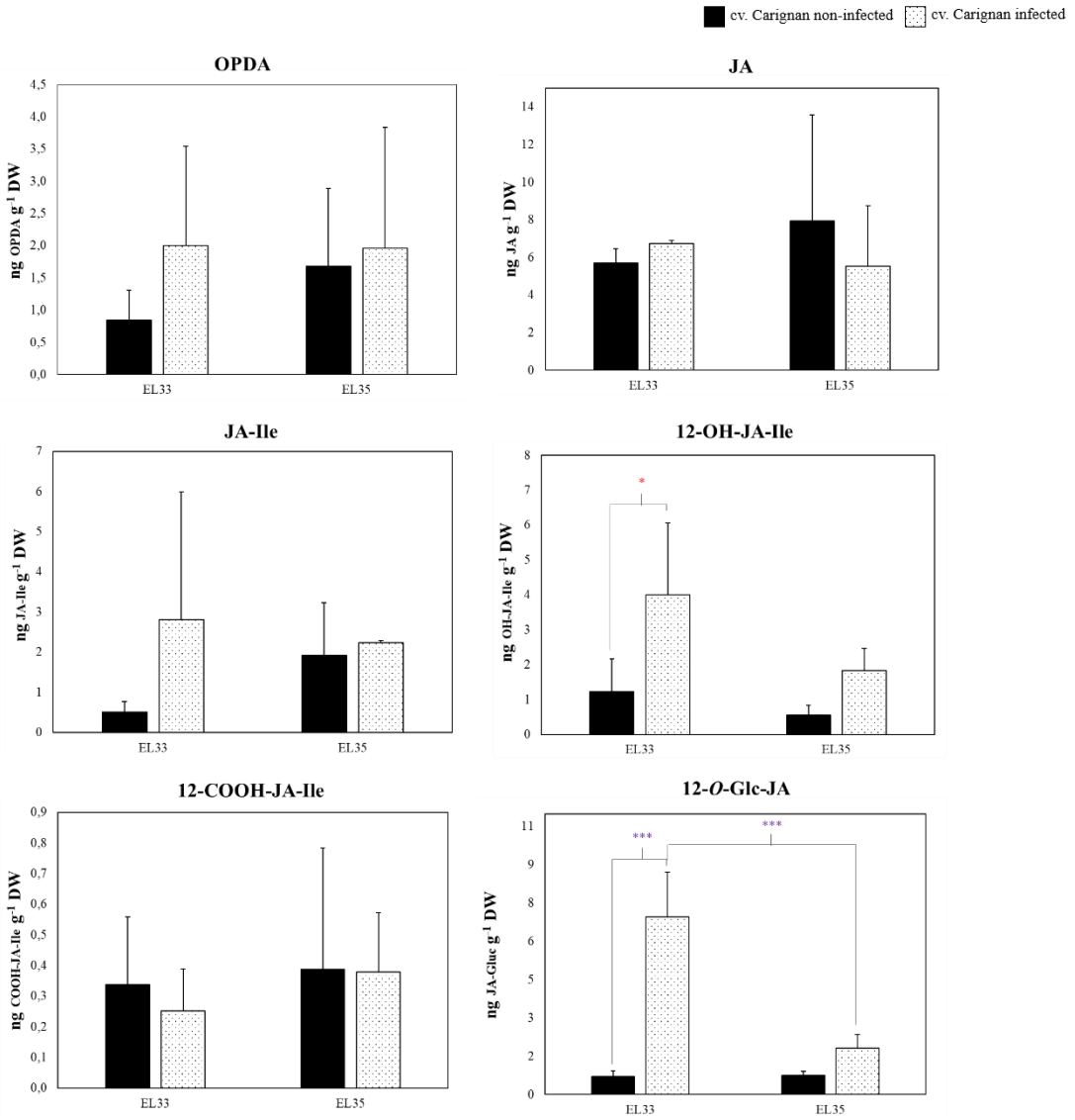


Figure 3.12 - Metabolism of jasmonates in cv. Carignan grapes upon infection with *E. necator* at two developmental stages: EL33 (late green) and EL35 (*veraison*) and two conditions: non-infected and infected (PM-disease). Hormonal quantification of jasmonates in ng of OPDA/PA/12-OH-JA-Ile/12-COOH-JA-Ile/12-*O*-Glc-JA *per* g of dry weight (DW). Bars indicate standard errors of means (three to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.2.4.Content in abscisic acid

Concerning ABA (Fig. S2.7, A), a significant increase was observed for non-infected and infected grapes, respectively, when developmental stages are compared (Fig. 3.13). However, for each developmental stage, there were no significant differences between conditions. These results suggest that ABA is involved in the onset of ripening but not in defence response against *E. necator*.

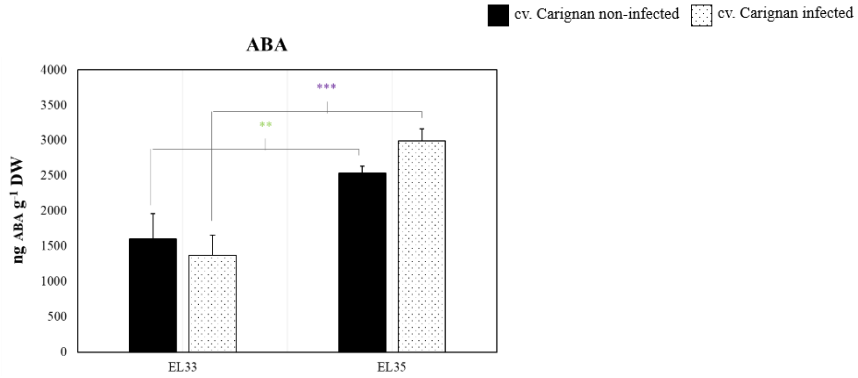


Figure 3. 13 - Metabolism of abscisic acid (ABA) in cv. Carignan grapes upon infection with *E. necator* at two developmental stages: EL33 (late green) and EL35 (*veraison*) and two conditions: non-infected and infected (PM-disease). Hormonal quantification of abscisic acid (ABA) in ng of ABA *per* g of dry weight (DW). Bars indicate standard errors of means (three to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.3. Gene expression profiling involved in hormonal metabolism in grapes

3.1.3.1.Expression of genes related with Salicylic Acid pathway

Only for *EDS1* it was observed an increase of expression upon infection at EL35. For *PAD4* and *PR1* no significant changes were observed in gene expression between conditions and developmental stages (Fig. 3.14). These results suggest an activation of SA signalling, through EDS1, upon *E. necator* infection.

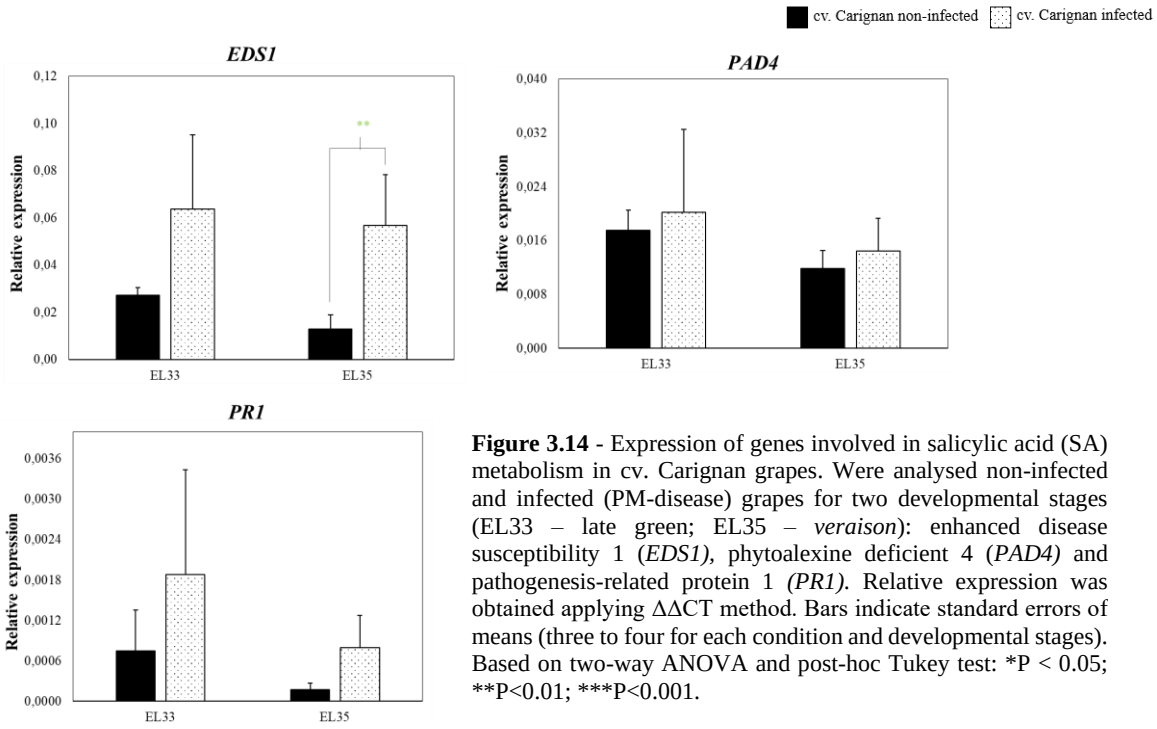


Figure 3.14 - Expression of genes involved in salicylic acid (SA) metabolism in cv. Carignan grapes. Were analysed non-infected and infected (PM-disease) grapes for two developmental stages (EL33 – late green; EL35 – *veraison*): enhanced disease susceptibility 1 (*EDS1),* phytoalexine deficient 4 (*PAD4)* and pathogenesis-related protein 1 *(PR1).* Relative expression was obtained applying ΔΔCT method. Bars indicate standard errors of means (three to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.3.2.Expression of genes related with Auxins pathway

For gene coding for *GH3-2* no significant differences were observed upon infection for EL33 and EL35 and between developmental stages (Fig 3.15). However, for *AIR12* (Fig. S2.5, B), a significant increase of expression upon infection was observed in both developmental stages. As such, these results suggest an absence of GH3-2 role in IAA pathway upon *E. necator infection*, but a possible role of AIR12 in this compatible interaction.

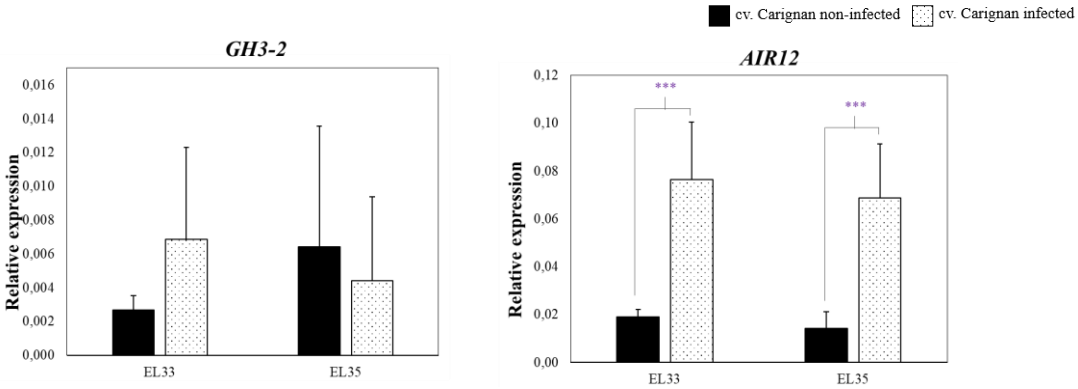


Figure 3.15 - Expression of genes involved in auxins (IAA) metabolism in cv. Carignan grapes. Were analysed non-infected and infected (PM-disease) grapes for two developmental stages (EL33 – late green; EL35 – *veraison*): IAA-amido synthetase GH3-2 *(GH3-2*) and root cultures protein 12 (*AIR12*). Relative expression was obtained applying ΔΔCT method. Bars indicate standard errors of means (three to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.3.3.Expression of genes related with Jasmonates pathway

In cv. Carignan grapes, it was observed an increase of constitutive and induced gene expression in non-infected and infected grapes, respectively, where compared between developmental stages (Fig. 3.7). Furthermore, for EL35 was observed a significant decrease in AOS gene expression when infected were compared with non-infected. These results indicate that expression of this AOS isoform (Fig. S2.6, A) is not in agreement with JAs hormonal data.

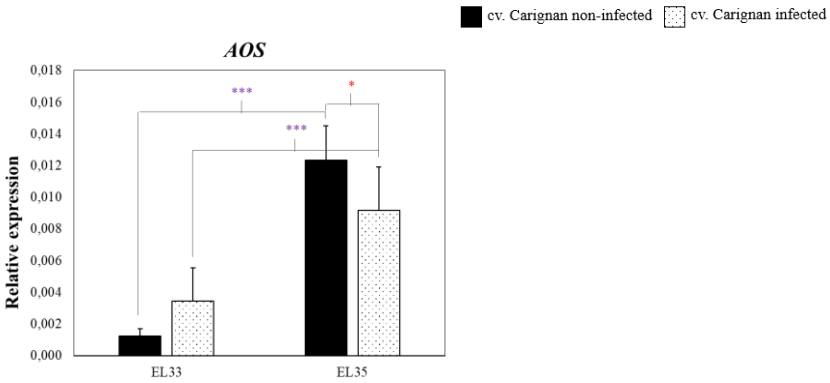


Figure 3.16 - Expression of genes involved in jasmonates metabolism in cv. Carignan grapes. Were analysed non-infected and infected (PM-disease) grapes for two developmental stages (EL33 – late green; EL35 – *veraison*: allene oxidase synthase (*AOS*). Relative expression was obtained applying ΔΔCT method. Bars indicate standard errors of means (three to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.3.4.Expression of genes related with Abscisic acid pathway

The expression of two *SnRK2* and *HVA22C* (Fig. S2.7, B) were studied upon *E. necator*-infection (Fig. 3.8). For *SnRK2* no significant changes were observed in gene expression between conditions and

between developmental stages. However, for *HVA22c*, a significant decrease was observed only in EL33 upon infection. These results suggest that *SnRK2* do not play a role in ABA pathway upon *E. necator* infection. Also, suggest a downregulation of ABA-responsive genes.

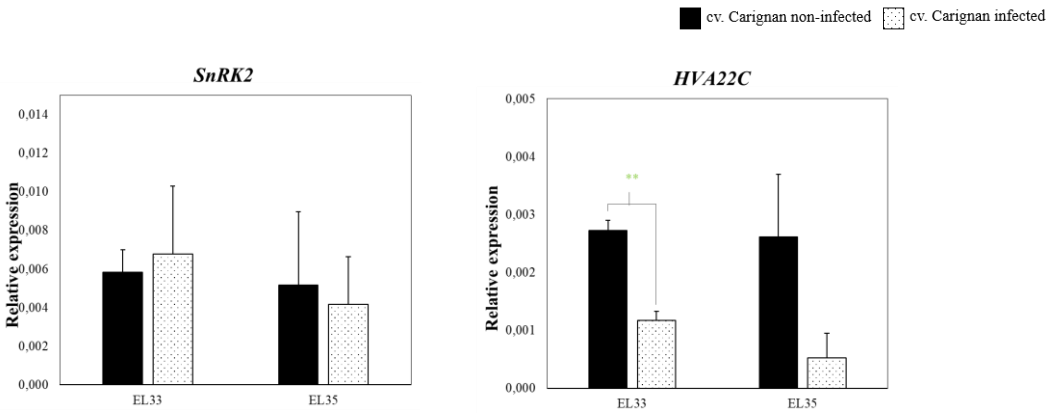


Figure 3.17 - Expression of genes involved in abscisic acid (ABA) metabolism in cv. Carignan grapes. Were analysed non-infected and infected (PM-disease) grapes for two developmental stages (EL33 – late green; EL35 – *veraison*): Sucrose non-fermenting-1 (SNF1)-related protein kinase 2 (*SnRK2*) and *HVA22C*. Relative expression was obtained applying ΔΔCT method. Bars indicate standard errors of means (three to four for each condition and developmental stages). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.2.Total phenolic content and enzymatic activity of phenylalanine-ammonia synthase upon powdery mildew infection

The ongoing work on transcriptome and metabolome in cv. Carignan grapes evidenced a reprogramming of secondary metabolism upon infection, namely the phenylpropanoid pathway (Pimentel et al., under review). It was observed that this reprograming implies a transcriptional modulation of genes coding for enzymes associated with the phenylpropanoid pathway. Concretely, several *PAL* genes were upregulated (Pimentel et al., under review). Due to these results, the total phenolic content was quantified with Folin-Ciocalteu (FC) method and the biochemical activity of *PAL* was measured according to Bradford method (Bradford, 1976).

The quantification of total phenolic contents was performed in two grapevine tissues: cv. Carignan non-infected and infected grapes at green late EL33 (green late) and EL35 (*veraison)*; and in leaves of resistant cv. 101-14 Millardet et de Grasset and susceptible cv. Aragonez, in non-infected and infected conditions and at 0, 6, 24 and 96 hpi. The biochemical activity of PAL was measured only in cv. Carignan grapes.

3.1.1. Total phenolic content in leaves

At 0 and 96 hpi (Fig. 3.18) it was observed a significant higher content of total phenolic is susceptible species when compared with the resistant ones. Also, at 96 hpi upon infection was observed a higher phenolic content in susceptible species when compared with resistant ones, suggesting that the total phenolic content is eventually involved in susceptibility.

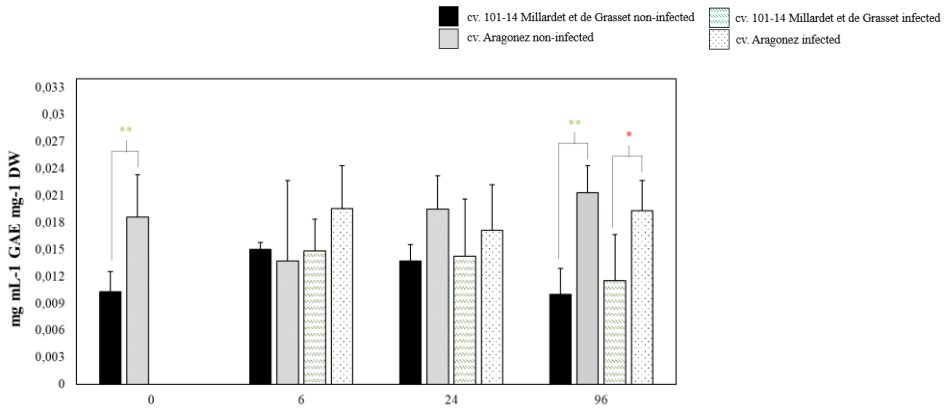


Figure 3.18 - Total phenolic content in two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi). Total phenolic compounds concentration is represented as mg mL^{-1} of gallic acid equivalents (GAE) *per* mg of dry weight (DW). Bars indicate standard errors of means (three to five for each condition and time point). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.1. Total phenolic content in grapes

In grapes, no significant differences were observed in total phenolic content between developmental stages and conditions (Fig. 3.19). This result indicates that total phenolic content does not change across the developmental stages and upon *E. necator* infection.

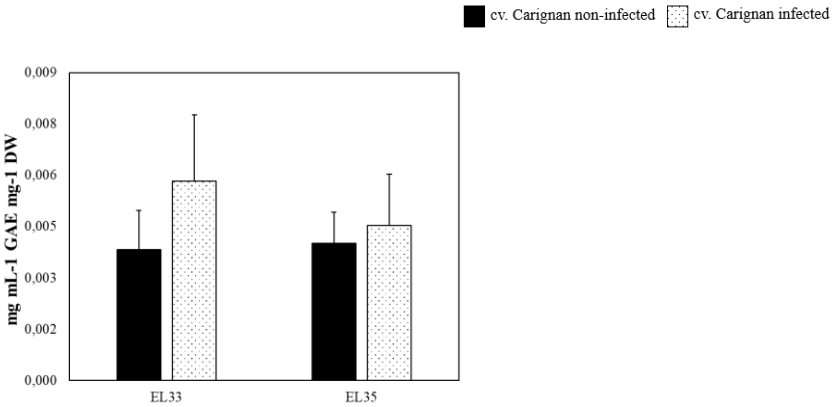


Figure 3.19 - Total phenolic content in cv. Carignan grapes. Were analysed non-infected and infected (PM-disease) grapes at two developmental stages: EL33 (late green) and EL35 (*veraison*). Total phenolic compounds concentration is represented as mg mL^{-1} of gallic acid equivalents (GAE) *per* mg of dry weight (DW). Bars indicate standard errors of means (three to four for each condition and developmental stage). Based on two-way ANOVA and post-hoc Tukey test: *P < 0.05; **P<0.01; ***P<0.001.

3.1.2. Enzymatic activity of phenylalanine-ammonia lyase in grapes

The biochemical activity of PAL showed no significant changes between developmental stages and conditions (Fig. 3.20). This result suggest that PAL activity doesn't changes across developmental stages and upon *E. necator* infection.

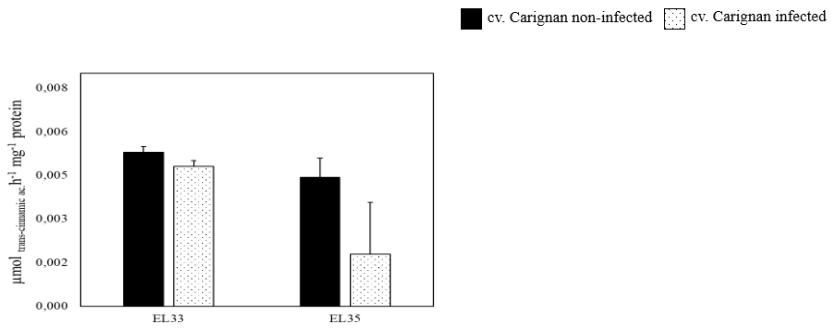


Figure 3.20 - Phenylalanine ammonia lyase (PAL) specific activity in cv. Carignan grapes. Were analysed non-infected and infected (PM-disease) grapes at two developmental stages: EL33 (late green) and EL35 (*veraison*). Biochemical activity is represented as µmol of trans-cinnamic acid *per* hour *per* mg of protein. Bars indicate standard errors of means (three to four for each condition and developmental stage). Based on two-way ANOVA and post-hoc Tukey test.

4. DISCUSSION

The Eurasian *V. vinifera* is a widely planted grapevine that has an economic impact derived from its fruits produced mainly for wine, table grapes and raisins (X. Q. Liu et al., 2016). However, grapevine cultivation is greatly affected by a large number of pathogens (Fung et al., 2008). The biotrophic ascomycete *E. necator*, is one of the most threatening pathogens in grapevine, being the causal agent of one of the widespread disease, the PM (Gaforio et al., 2011). PM affects all green organs above ground and symptoms in leaves and grapes are mainly patches with white dusty appearance appear on surfaces (Ridout, 2009). As such, PM affects the structure and functionality of grapevine organs with significant losses in production yield and quality (Y. Xu et al., 2009).

Recently advances on (in) compatible grapevine-*E. necator* interaction reveal a reprograming of transcriptome and proteome of grapevine leaves, related to immune responses, secondary metabolism, signalling pathways and hormonal metabolism (Fung *et al.*, 2008; Marsh *et al.*, 2010; Fekete *et al.*, 2009). However, regarding the hormonal metabolism and the balance among different hormones upon *E. necator* infection is still unknown and emphasizes the significance of our hormonomics-based study. In our work, grapevine-*E. necator* incompatible and compatible interactions seem to be modulated in infected leaves by the constitutive content of hormones, and by a possible synergism/antagonism between them. Also, gene expression analysis revealed a more complex regulation of hormonal pathways than in others plant-PM interactions (F. Gao et al., 2010). Furthermore, in parallel with a broader project in susceptible grapes with PM, our work unravelled the presence of a distinct defence responses among distinct grapevine-organs by means of different hormonal content and gene expression. Also, suggested that a delayed defence response may be associated with susceptibility. Finally, in both organs, our work showed that the reprograming of secondary metabolism did not impact the total phenolic content.

Resistance against Erysiphe necator is putatively associated with high constitutive levels of SA and IAA and additional induction upon infection in leaves

In our work, constitutive levels of SA in leaves tend to be higher in resistant species compared with the susceptible one. This genotypic variability may impact the type of SA-mediated responses (Isah, 2019). Regarding biotic stress, SA has been commonly associated with defence against biotrophic and/or hemibiotrophic pathogens (Pieterse et al., 2012; Seyfferth & Tsuda, 2014). Upon infection, host basal and R gene-derived defences are triggered and induce SA biosynthesis (Seyfferth & Tsuda, 2014). Isochorismate synthase (ICS) pathway, and not the alternative phenylalanine ammonia synthase (PAL) pathway (Fig S2.1, A) is mainly responsible for SA biosynthesis in several pathogenic interactions (X. X. Huang et al., 2018; Vlot et al., 2009). ICS pathway is positively regulated by two putative lipases, EDS1 and PAD4 (Gao et al., 2014; Rietz et al., 2011). Also, both integrate a positive feedback loop with SA in order to potentiate SA signalling and amplify defences (Jirage et al., 1999; Wiermer et al., 2005). In our work, a significant SA accumulation at 24 hpi in resistant species suggests an activation of SA biosynthesis upon *E. necator* infection. This was absent in susceptible species. *EDS1* and *PAD4* expression profiling was not in agreement with the hormonal profiling for both species. In leaves from American resistant *Vitis aestivalis* cv. Norton – *E. necator*, *EDS1* expression was constitutive and upon infection higher than in susceptible cv. Cabernet Sauvignon and it was related to SA content (Fung et al., 2008; F. Gao et al., 2010). Also, in grapevine, EDS1-like (*EDL*s) were identified as SA and PM responsive genes, increasing the complexity of SA regulation (F. Gao et al., 2014). As in our work, Fung et al., 2008 also reported the absence of changes in *PAD4* expression in both interactions. This suggest that defences against *E. necator* may not depend on PAD4-SA signalling amplification. Senescence associated carboxylesterase 101 (SAG101), another co-regulator with EDS1, is a possible candidate in

our interaction due to its partially redundancy with PAD4 (Wiermer et al., 2005) and should be further analysed.
In order to modulate SA-mediated processes and defence, *de novo* SA is metabolized. In plants, glycosylation is the major process of SA modification (Y. Chen et al., 2013). Through SA glucosyltransferases (SAGTs), SA is preferably glycosylated into SA-glucoside (Fig S2.1, A) in cytosol and is further stored in vacuoles, in a stable but inactive form (X. X. Huang et al., 2018; Ross et al., 2001). In our work, a higher constitutive SA-glucoside content and an accumulation at 24 hpi, upon infection, was present in resistant species. The similarity to SA hormonal profiling indicates a redirect of SA to its glucoside. This suggests a role of SA-glucoside not only in modulating SA levels but also in response against *E. necator*. In Arabidopsis interaction with Pst DC3000, glycoside forms of dihydroxybenzoic acid (DHBA), other SA metabolite, accumulate and integrate a positive feedback loop to induce SA synthesis (X. X. Huang et al., 2018). SA signalling transduction is mediated, in part, by ROS. Kawano et al., 2004 report a function of SA-glucoside as a slower inductor of oxidative bust. Associated with resistance to *E. necator*, SA-glucoside may function in a non-toxic and controlled way to induce the oxidative bust and trigger SA-mediated responses rather than SA (Loake & Grant, 2007).
One of the downstream responses is the accumulation of PRs (Lipka et al., 2008; Mérillon, 2018). PR1 is the most abundant and conserved in PR-families and its antimicrobial activity is related to the sequestering of sterols from fungal membranes (Breen et al., 2017; Shin et al., 2014; van Loon et al., 2006). In our work, *PR1* expression was not coincident with the SA hormone profiling in both species upon infection. PR1 is considered a marker of SA pathway (Ali et al., 2018). However, this was not evident in our interactions. A marker of a hormonal pathway vary regarding species, organs, and even within the species regarding the interaction (Papadopoulou et al., 2018). As such, others PR1 isoforms or even different PRs could be markers of SA pathway in our interaction. For example, the well documented SA-responsive PR3, PR2 and PR5 are worth it of further analysis. Also, PR10, PR15/16 or PR17 has been reported to be induced in several PM interactions and establishment of hypersensitive reaction (HR) and systemic acquired resistance (SAR) (Christensen et al., 2002; Enoki & Suzuki, 2016; Fung et al., 2008; Y. Xu et al., 2009).

Auxins, predominantly in the form of IAA (Fig S2.5, A), are a major coordinating signal responsible for the regulation of a plethora of growth and developmental processes in plants organs (Teale, Paponov and Palme, 2006; Ludwig-Müller, 2011). As for SA, IAA quantification pointed a difference depending on species and was constitutive higher in resistant species.
Besides the classical reference as 'plant growth regulator', a possible role of auxins in response to biotic stress has been admitted (Ma & Ma, 2016; Saini et al., 2017). Particularly, auxins are considered pivotal for establishment of pathogenic relations, since their imbalance is used by pathogens to promote their own growth or virulence and/or modulate host defences in order to facilitate the progression of disease (Kunkel & Harper, 2018; Mutka et al., 2013). In our work, along with the constitutive higher levels, a significant IAA level upon infection at 6 hpi in resistant species suggests a role of IAA in resistance against *E. necator*.
Auxins-regulated processes are controlled by auxins gradients *in planta* (Čarná et al., 2014). The maintenance of auxin homeostasis is the result of a tight arrangement between biosynthesis, transport, degradation and conjugation (Böttcher et al., 2012; Meents et al., 2019; Park et al., 2007). As such, disturbance will change auxins regulatory functions in a pathogenic-beneficial manner. To counter, a host-defence based on GH3 action has been reported (Kunkel & Harper, 2018). *GH3-2* encodes an IAA acyl-amido synthetases which preferably conjugate IAA with aspartate (Asp) (Park et al., 2007). This resulting in an inactive catabolism conjugate (González-Lamothe et al., 2012). In rice and Arabidopsis, *GH3-2* overexpression induce resistance against several pathogens and *gh3-2* mutants display compromised resistance and low IAA-Asp content (Ding et al., 2008; Fu et al., 2011). Also, *GH3-2* is a primary auxin-responsive gene regulated by a positive loop with IAA content (Peat et al., 2012; Ruiz

Rosquete et al., 2012). Our work suggests that *GH3-2* was not involved in IAA role in resistance nor the *GH3-2* expression profiling was concordant with the IAA hormone profiling. Nevertheless, others GH3, like GH3-8 may be related to our IAA pathway and be associated with IAA role in resistance as has been suggested in rice interaction with *Xoo*, a biotrophic bacteria (Ding et al., 2008; D. Wang et al., 2007). The second chosen gene, *AIR12* was firstly described as an auxin-responsive gene (Fig S2.5, B) in lateral root formation and *air12* mutants were less sensitivity to IAA (Gibson & Todd, 2015). Also, AIR12 is described as an ascorbate-reducible b-cytochrome (Preger et al., 2009). Ascorbate controls ROS levels at apoplast, and the interaction with AIR12 to modulate oxidative signals between apoplast and cytosol seems to promote susceptibility in *B. cinerea* infection (Costa et al., 2015). In our work *AIR12* expression profiling was not related to IAA hormone profiling or to susceptibility. Therefore, AIR12 is neglectable as marker of IAA pathways in our interactions and additional functional characterization of this gene is needed.

Susceptibility against Erysiphe necator is putatively associated with high constitutive levels of specific jasmonates and ABA in leaves

Jasmonates (JAs), a class of lipid-derived hormones, are integrated in oxylipin group and include JA and its derivatives (Fig S2.6, A) (C. Yan & Xie, 2015; Y. Yan et al., 2013). All JAs derive from the biosynthetic octadecanoid pathway (Fig. S2.5, A) which starts at chloroplast and ultimately produces OPDA, the direct precursor of JA (Jia et al., 2016). From there, OPDA is exported to peroxisome and convert into JA (Mei et al., 2006; Zhai et al., 2017). At cytoplasm, JA is metabolized into several derivatives. JA-Ile, the most important JA metabolite, is formed by JA conjugation with isoleucine (Claus Wasternack & Strnad, 2018). Through the ω-oxidative pathway, JA-Ile is hydroxylated into 12-OH-JA-Ile and further oxidized into 12-COOH-JA-Ile (A. J. K. Koo & Howe, 2012; Widemann et al., 2016). Conjugation of ω-oxidative with further deconjugation pathway convert JA-Ile or 12-OH-JA-Ile into 12-hydroxy-JA (12-OH-JA) (Farmer & Goossens, 2019). In parallel, JA is also directly oxidized into 12-OH-JA. From 12-OH-JA, through glycosylation, 12-*O*-Glc-JA is produce (Haroth et al., 2019). Emerging as growth and developmental regulators, JAs content and composition vary according to organs, developmental stage and among species (Delker et al., 2006). In our work, the constitutive levels of OPDA, JA, JA-Ile and 12-*O*-Glc-JA were higher in susceptible species. JAs also vary according to environmental stimuli and are associated with abiotic tolerance (Farhangi-Abriz & Ghassemi-Golezani, 2019). In our resistant species, the higher content of JA-Ile, 12-OH-JA-Ile and 12-COOH-JA-Ile at 6 hpi in non-infected leaves, suggests a different extent of response to abiotic stress among both species.
Regarding biotic stress, JAs are classically associated with resistance to necrotrophic pathogens and susceptibility to biotrophic pathogens (Antico et al., 2012; Glazebrook, 2005). The higher constitutive content of specific JAs, in our susceptible species, suggest a role of JAs in susceptibility against *E. necator*. This may be the result, as in rhizobacteria-mediated induced systemic resistance (ISR), of a JAs-sensitized state where previous synthetized signalling proteins are activated in a faster and effective way to induce JA-mediated defences (Pozo et al., 2004; Van der Ent et al., 2009; Zhang et al., 2017). JA-Ile is the bioactive signalling mediator of JAs (Widemann et al., 2016). The intensity and duration of JA-responses reflects primarily the free pool of JA-Ile which is controlled by the fine-tuning of JA-metabolism (Campos et al., 2014; Zhai et al., 2017). By the ω-oxidative pathway, JA-Ile is further catabolized into an inactive 12-COOH-JA-Ile form. However, 12-OH-JA-Ile is still perceived by SCF^{COI1}–JAZ (Coronatine Insensitive 1 and Jasmonate ZIM-domain-containing protein) complex, not in a weaker manner, but similar to JA-Ile (A. J. Koo et al., 2014; Poudel et al., 2019). Our resistant species showed a significant decrease of JA-Ile and 12-OH-JA-Ile, upon infection at 6 hpi. Altogether, this may indicate a possible role of JAs signalling in susceptibility. Also, possibly reveals a different way to modulate host defence and promote susceptibility beyond JA-Ile. Alongside with conjugation, glycosylation modulates JAs signalling (Widemann et al., 2016). 12-*O*-Glc-JA is reported in various

plants organs and species (Miersch et al., 2008). In development, its activity is related to leaf movement. Also, was described to accumulate upon wounding. Nevertheless, the role of 12-*O*-Glc-JA in defences is still not clear (C. Wasternack & Hause, 2013; Claus Wasternack, 2014). Possibly, since glycoside forms are more soluble, 12-*O*-Glc-JA may be a way to transport 12-OH-JA (Haroth et al., 2019; C. Wasternack & Hause, 2013). 12-OH-JA, a derivative of JA-Ile, retain partial JA-Ile activity since induce several JA-responsive genes (Delker et al., 2006). Mutants for enzymes responsible for JA-Ile conversion to 12-OH-JA increase resistance to *B. cinerea* (Farmer & Goossens, 2019). As such, 12-OH-JA and its glycoside form may dispose a role in susceptibility. Related, in our susceptible species, in addition to constitutive significance of 12-*O*-Glc-JA, at 96 hpi was also significant higher upon infection. However, further studies are needed to understand the function of JAs metabolites in plant defence.

The selected gene for analysis, *AOS*, showed no expression differences for both species at constitutive level. AOS, a plastid-localized enzyme, produce an intermediate compound which is converted into OPDA (Fig S2.6, A). Reports indicate a positive induction of *AOS* upon infection (Mei et al., 2006; C. Wasternack & Hause, 2013). Also, as key regulator of JA synthesis, a regulatory mechanism based on substrate availability, dictates that JA accumulation only occur after a stimulus, through AOS (Delker et al., 2006; Y. Yan et al., 2013). In our work, *AOS* expression profiling was not coincident with JAs hormonal profiling. These results suggest a necessity to dissect JAs biosynthetic pathway in more detail and evaluate the expression of different genes from the same gene family. Moreover, other genes involved in JAs biosynthetic pathway, like AOC or OPRs are possibly better choice to characterize the pathway.

ABA regulates plant growth and development but has a prominent role in response to abiotic stresses (Fig. S2.7, A) (Fan et al., 2009). In our work, as for JAs, ABA quantification was distinct between both species and higher in the susceptible one. In plant defence, ABA also display an ambivalent role (Asselbergh et al., 2008; Ton, Flors, et al., 2009). In parallel with the high constitutive levels, the significant increase of ABA content upon infection at 24 hpi in susceptible species suggest a role of ABA in susceptibility against *E. necator*. ABA-induced susceptibility is a post-penetration event in several biotrophic interaction (Xiao et al., 2017). Further cytological studies in our (in) compatible interaction may clarify this assumption. ABA-mediate responses dependent on SnRK2 activation (Fig S2.7, B). In presence of high ABA levels, SnRK2 is de-repressed and will positively regulated the ABA signalling (Saddhe et al., 2017; Umezawa et al., 2010). However, the significant decrease of SnRK2 expression at 6 and 96 hpi in infected leaves of susceptible species was not in agreement with the high ABA levels in the same time points. SnRK2 family is divided into three subclasses and SnRK2 belongs to subclass I that are not ABA responsive (Saddhe et al., 2017). Recently, overexpression of SnRK1 in rice shown an increase in resistance to several pathogens (Filipe et al., 2018; Hulsmans et al., 2016). This suggests a need for further functional studies. However, since the *SnRK2* expression profile is like ABA hormonal profiling at 6 and 96hpi, the downregulation may be related with the biphasic response of susceptible species when infected. The second selected gene, HVA22C is a downstream ABA-responsive gene (Fig S2.7, B). In barley, HVA22C higher expression in aleurone layer is related to the increased levels of ABA (Gomes Ferreira et al., 2019). Also, has been associated with tolerance to desiccation (Brands et al., 2002). However, in our work, HVA22C gene expression had no significant changes at the constitutive level or upon infection, and therefore, seems not to be related to ABA hormone profiling. As reported by Shen et al. (2001), this suggests that HVA22C expression is tissue specific.

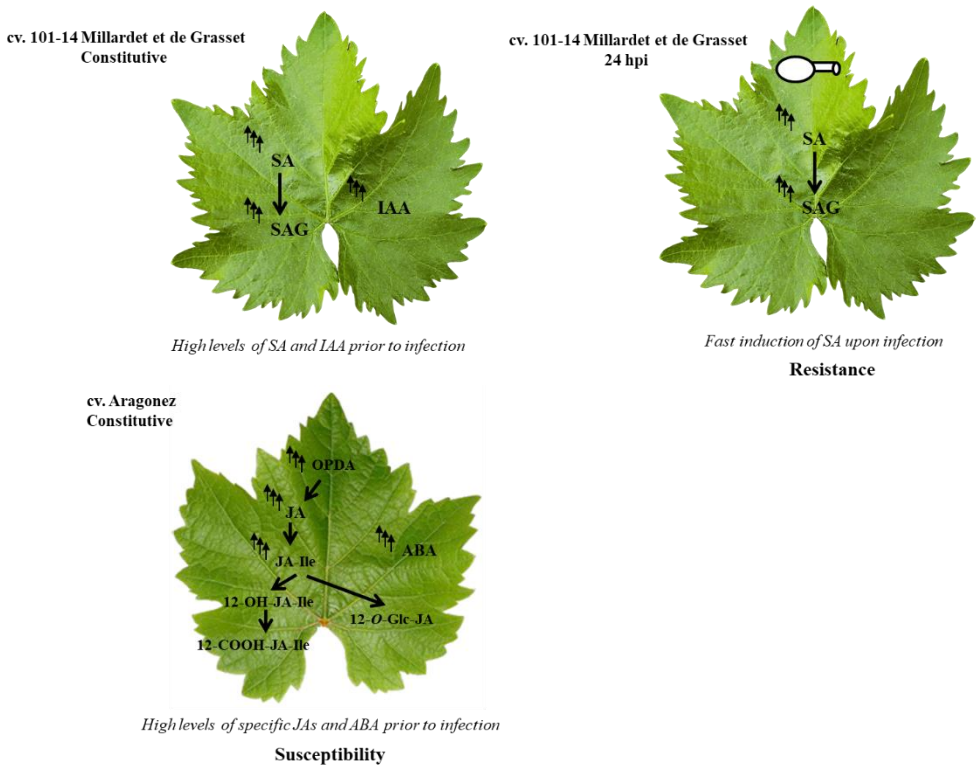


Figure 3.21 – Propose model for hormonal metabolism in leaves from two *Vitis* species: *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). At constitutive level, a higher content in SA, SA-glucoside and IAA is present in resistant species. Upon *E. necator* infection, at 24 hpi, resistant leaves accumulate SA and SA-glucoside. Contrary, in susceptible species, specific JAs (OPDA, JA, JA-Ile, 12-OH-JA-Ile, 12-COOH-JA-Ile, 12-*O*-Gluc-JA) and ABA are highly accumulated at constitutive level. No changes were noticed upon infection. Abbreviations: SA, salicylic acid; SAG, salicylic acid-*β*-D-glucoside; IAA, indole acetic acid; OPDA, 12-oxophytodienoic acid; JA, jasmonic acid; JA-Ile, jasmonoyl-isoleucine; 12-OH-JA-Ile, 12-hydroxy-JA-Ile; 12-COOH-JA-Ile, dicarboxy-JA-Ile; 12-*O*-Gluc-JA, 12-*O*-glucosyl-JA; ABA, abscisic acid.

Susceptibility in grapes against Erysiphe necator seems putatively related with a delayed and specific hormonal response

As a developmental regulator, SA has been described to delay grapes ripening (Lo'ay, 2017). However, in non-infected susceptible grapes, the transition between EL33 and EL35 was not associated with a significant decrease in SA content. Upon infection, the significant increase of SA content in EL33 and trend in EL35 (*p-value* = 0.056) suggest an activation of SA biosynthesis. In accordance, regarding the ICS pathway (Fig. S2.1, A), *ICS1* (VIT_17s0000g05690) was upregulated in both developmental stages (Pimentel et al., under review). Also, the SA signalling seems to be induced regarding the expression of *EDS1*, a positive SA regulator, which showed a tendency at EL33 (*p-value* = 0.065) and significant higher expression at EL35. The expression of co-regulator *PAD4* did not change upon infection in both developmental stages. Contrary, most SAG101 isoforms were upregulated (Pimentel et al., under review), suggesting a preference of EDS1-SAG101 interaction in grapes-*E. necator* interaction. SA-glucoside (Fig. S2.1, A), in both conditions, shared a similar profiling as SA. This suggest a redirect of SA to SA-glucoside and a possible role in response to infection. Several reports indicate an accumulation of glycoside forms of SA in pathogenic interaction. SATGs has been indicated to respond to pathogen infection (Fig. S2.1, A) (Ross et al., 2001; Tiwari et al., 2016). In bean, increase hypersensitive reaction associated (*Hra25*) expression, an UGT, suggest an activation upon PST DC3000 infection (Sullivan et al., 2001). In our infected grapes, *Hra25* (VIT_17s0000g07080) was also upregulated upon infection (Pimentel et al., under review). Downstream of SA signalling (Fig. S2.1, B), *PR1* showed a similar

expression profiling as *EDS1* and appears to respond to SA upon infection. Nevertheless, due to the highly variability of grape clusters, gene expression was not statistically significant when control and inoculated grapes were compared. As such, is not admissible to consider PR1 as a SA-marker of our interaction. Other PRs, as PR3 (VIT_06s0061g00120), PR2 (VIT_00s0540g00050) and PR5 (VIT_02s0025g04230) were upregulated at both developmental stages (Pimentel et al., under review). All of them are SA-induced and with antimicrobial activity, and in cooperation, interact to hydrolyse and increase permeability of fungal cell wall (Enoki & Suzuki, 2016; Wanderley-Nogueira et al., 2012). Altogether, the results indicate a response in grape against *E. necator* based on SA.

Regarding auxins, IAA (Fig. S2.5, A) content was low and constant in both conditions and between developmental stages. In literature, the onset of ripening is marked by a decrease in auxins content and a maintenance of low IAA levels at harvest (Ana Margarida Fortes et al., 2015; Ma & Ma, 2016). However, and as in our work, low and constants auxin levels throughout berry development has also being described (Böttcher et al., 2010; N. Kuhn et al., 2014; Symons et al., 2006).
The control of auxins content occurs through GH3 action. Analysis of *GH3-2* expression showed an absence of change between both developmental stages. However, in others cultivars GH3-2 is described to participate in berry development (Böttcher et al., 2013). Upon infection, and like IAA hormonal profiling, *GH3-2* expression did not change within or between developmental stages. This suggest an absence of induction of IAA signalling in response to *E. necator* infection. In agreement, in EL33, topless-related (TPL1; e.g., VIT_04s0008g06350), a repressor of auxins signalling, and transport inhibitor response 1 (TIR1; VIT_14s0083g00890), which integrate SCF^{TIR1} complex for perceiving auxins (Jiang et al., 2017), were up- and downregulated, respectively (Fig. S2.5, B) (Pimentel et al., under review). Also, several primary auxin-responsive, like small auxin up-regulated RNA (SAUR) or auxin/indole-3-acetic acid (AUX/IAA) (Fig. S2.5, B) were downregulated (Pimentel et al., under review). In others interactions, the repression of auxins signalling is associated with resistance (Fu & Wang, 2011).
The other analysed gene, *AIR12*, an auxin-responsive gene (Fig. S2.5, B) (Gibson & Todd, 2015) had a significant expression upon infection in both developmental stages. In grapes infected with *B. cinerea*, *AIR12* was early induced (Agudelo-Romero et al., 2015) and *air12* mutants displayed a resistance to infection (Costa et al., 2015). In accordance, in our data, *AIR12* expression profiling seems to respond to *E. necator* infection and therefore, may display a role in susceptibility.

JAs function has been associated with the onset of ripening in non-climacteric fruits since a higher accumulation in the early development, precedes a sharply decrease in ripe fruits (Ana Margarida Fortes et al., 2015; Jia et al., 2016). In our work, no significant changes at JAs constitutive level were associated with the transition between developmental stages. Since this study is based on two closely developmental stages, differences in hormone content may be diluted. Also, in terms of cellular events and berry composition, both developmental stages differ from early or ripening stages.
Genes involved in JAs biosynthesis are reported to be downregulated prior and during *veraison* (Fig. S2.6, A) (Ana M. Fortes et al., 2011; Jia et al., 2016). Our *AOS* expression was significant higher at EL35 when compared with EL33. Also, several lipoxygenase isoforms [(*LOX2*); VIT_06s0004g01500] and 12-oxophytodienoate reductases [(*OPR1*); VIT_18s0041g02050] (Fig. S2.6, A) were upregulated in *EL35* (Pimentel et al., under review). Since all these genes integrate the octadecanoid pathway, this presupposes a higher accumulation of JAs at *EL35*. However, in order to maintain JAs homeostasis, other processes, such as conjugation and degradation, occurs parallel to biosynthesis and annul possible differences between the developmental stages (Tran & Pal, 2014). Upon infection, the biosynthetic pathway was also induced (Pimentel et al., under review), regarding our absence of changes of *AOS* expression in developmental stages which may indicate a different activation of *AOS* homologous sequences.

The biosynthetic products seem to be redirected to conjugated and glycoside forms, at EL33, as a response to *E. necator* infection. This may be a strategy to interference in JA-Ile signalling by affecting the type of JA-mediated responses (Aubert et al., 2015; Widemann et al., 2016). Particularly, since has been attributed a role of 12-OH-JA-Ile and 12-*O*-Glc-JA in susceptibility, accumulation of both metabolites may be a *E. necator* strategy to bypass grape defences. JA-Ile signalling controls JA-mediated defences in a COI1-dependent manner. Upon infection, COI1 (Fig. S2.6, B) was downregulated at EL35 (Pimentel et al., under review), suggesting also a blocking in JA-Ile signalling. Nevertheless, reports also mention defences responses in a COI1-independent manner, usually through OPDA (Zhang et al., 2017).

ABA is considered the main regulator of non-climacteric fruit ripening, like grapes (Pilati et al., 2017). In our work, a significant increase in ABA content was associated with the transition between EL33 and EL35. This increase at EL35 was also describe in others cultivars and is in agreement with the a role of ABA in triggering ripening (Ana M. Fortes et al., 2011). Genes in ABA biosynthetic pathway, like 9-cis-epoxycarotenoid dioxygenases (NCED1; e.g., VIT_02s0087g00930) (Fig. S2.7, A) were upregulated at EL35 indicating an activation of ABA biosynthesis (Pimentel et al., under review). The significant increase in ABA content between both developmental stages does not seem to reflect an induction due to infection. In fact, seems to be related to the increase involved in the onset of ripening. Regarding ABA signalling (Fig. S2.7, B), *SnRK2* expression seem not to be associated with ripening process. However, SnRK2 and SnRK2.8 were upregulated in others cultivars at EL35 (Ana M. Fortes et al., 2011). Absence of changes in SnRK2 expression was also present upon infection. In agreement, J. Y. Liu et al., 2016 report an absence of induction in PM for all SnRK2, independent of subclasses. *HVA22C*, an ABA-induced gene (Fig. S2.7, B), showed a significant decrease of expression upon infection at EL33. However, ABA levels did not change upon infection. This suggests other regulations of *HAV22C* and a need to further analysis.

Infection with Erysiphe necator does not affect total phenolic content in leaves and grapes

Phenolic compounds are the most widely occurring class of secondary metabolites (Deng & Lu, 2017). In our work, the constitutive content of total phenolics was higher in susceptible leaves when compared with the resistant ones. Regarding defence, accumulation of phenolic compounds at penetration sites creates a toxic environment (Koornneef & Pieterse, 2008). Resistance mechanisms are, in part, associated with an investment on preformed and/or induce phenolic compounds in order to inhibit or constrict infection (Lattanzio et al., 2008). This was reported in leaves infected with *E. necator* from others resistant species (Welter et al., 2017; Weng et al., 2014). Even considering the reported variance in total phenolic content between species (Atak et al., 2017), the lower constitutive content in resistant species and the absence of accumulation upon infection was an unexpected result. In grapes, phenolic compounds contribute to the organoleptic and antioxidants properties which have being used for human benefits and impacts wine composition (Andjelkovic et al., 2013; Schoedl et al., 2012; Teixeira et al., 2013). In our work, in both developmental stages and conditions, the total phenolic content did not change. However, the metabolic profiling of our grapes upon infection revealed a stimulation of specific secondary metabolism, namely the phenylpropanoid metabolism (Pimentel et al., under review). Phenylpropanoid pathway starts with conversion of phenylalanine into cinnamic acid by (PAL) action (Deng & Lu, 2017). Integrated in a multigenic family, *PAL*s expressions are induced during fungal infection (J. Huang et al., 2010; Pillet et al., 2016). In our infected grapes, several PALs isoforms were upregulated (Pimentel et al., under review). Nevertheless, biochemical activity of PAL did not change between developmental stage nor conditions, which may be explained by distinct functions, inside phenylpropanoid pathway, for different PAL isoforms or issues involving transduction efficiency. Altogether, in both grapes and leaves, total phenolic content was not affected by *E. necator* infection.

As in others susceptible cultivars (Fung et al., 2008) this could be explained by the possible reorientation of the metabolism by the *E. necator* in order to accumulate specific phenolic compounds and promote disease (Atak et al., 2017; Kedrina-Okutan et al., 2018). Usually, reports indicate that *E. necator* induce specific branches of phenylpropanoid pathways and lead to increase of gallic acid and SA (phenolic acid pathway), catechins and epicatechins (flavonoids pathway), lignin (monolignol pathway) and resveratrol (stilbene pathway) (Vogt, 2010; Pimentel et al., under review). Those may be considered possible markers of infection.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

V. vinifera is highly susceptible to *E. necator*, the causal agent of PM. In contrast, American species have different levels of resistance due to a coevolution with the fungus.

Most studies of grapevine-*E. necator* interaction occurs separately in leaves from resistant or susceptible species. Also, the hormonal quantification occurs mainly for SA, if performed. Our work integrates a comparison between the hormonal profiling of leaves from resistant and susceptible species infected with *E. necator*. The resistance and susceptibility seem to be related with high constitutive levels of SA and IAA and specific JAs and ABA, respectively. Upon infection, the induction of these hormones in each interaction indicate a possible role in response to *E. necator*. Also, we revealed a putative phytohormonal network were a synergetic balance between SA/IAA and JAs/ABA is present in resistant and susceptible species, respectively. Nevertheless, further dissection of this crosstalk is needed in order to be validated. Also, to formulate a fully representative model, other hormones and respective pathways, such as brassinosteroids, ethylene, gibberelling, cytokinins and several metabolites, as MeSA, need to be quantified and dissected. Our gene expression analysis reveals a more complex regulation of the hormonal pathways than previous reported. Nevertheless, is still an ongoing work. Particularly, we will enrich our analysis with more genes related to the biosynthetic and signalling pathways and downstream induced responses for each hormone. Also, we will introduce genes involved in hormonal crosstalk in order to confirm our propose network model for both interactions.

We also analysed the hormonal profiling of susceptible grapes infected with *E. necator*. Susceptible grapes had a response against *E. necator* based on a putative involvement of SA and JA pathway. However, it was ineffective to block the fungus penetration and infection. Supported with information from RNA-seq, the analysis of gene expression was more conclusive in grapes, even considering the variability of grape clusters. This shows a different network of phytohormones and activation of different enzyme isoforms among leaves and grapes in response to *E. necator* infection and highlights the importance of studies based on different grapevine organs.

In both organs, *E. necator* did not change the total phenolic content but possibly reprograms the phenylpropanoid pathway in order to accumulate specific phenolic compounds. A further metabolic study will be conducted in grapevine leaves of our species and will allow a characterization of phenolic profile for each interaction and possible find specific markers of infection. Functioning as fingerprints of infection, these markers may be used in disease diagnosis. The comparison of leaves phenolic profile with the obtained for grapes will allow to understand if grapevine organs are also differently metabolically reprogrammed by *E. necator*.

Further cytological studies focused on the PM-infected grapevine leaves or grapes will be needed as they will be associated with molecular, metabolomic and others results to better understand the pathosystem grapevine-*E. necator*.

Finally, the output of our study are clues related with the phytohormonal network associated with resistance and possible markers of resistance. By comparison, we also increased our knowledge related with susceptibility to PM. Nevertheless, ultimate conclusions can only be made after characterizing the interaction of *E. necator* with more resistant and susceptible genotypes which will be included in our work. After that, the knowledge may be applied to improve the resistance of grapevine cultivars. Regarding the hormones, the tolerance may be increased by means of exogenous application. However, hormones function in a dosage-manner and imbalance of hormones impacts the phytohormonal network. Since is difficult to define which hormone content is absorbed, other technologies may be considered. Benefiting from the sequencing of whole grapevine genome, gene editing may be of use, rather than traditional breeding. Insertion of genetic components by means of CRISP-Cas9 may be used. In this case, genes involved in SA pathway or downstream responses, such as PRs, are possible candidates.

Particularly, through CRISP-Cas9 is also possible to knockdown certain genes related with susceptibility.

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7. ANEXES

Appendices I – Supplementary Tables

Table S1.1 - Total RNA quantification after protocol optimization and without DNase I treatment. Total RNA was extracted with an optimized protocol (Protocol 11) based on (Coelho et al., 2019) protocol with several modifications. Extraction occur in non-infected and infected (PM-disease) leaves from cv. 101-14 Millardet et de Grasset (resistant species) and cv. Aragonez (susceptible species) at 0, 6, 24 and 96 hours post-infection. Abbreviations: C, control (non-infected); I, infected; hpi, hours post-infection.

Species	Sample name	Concentration (ng/µL)	A_{260}/A_{280}	A_{260}/A_{230}
cv. 101-14 Millardet et de Grasset	C_1 0 hpi	353.1	1.82	1.26
	C_3 6 hpi	620.4	2.06	1.6
	C_5 24 hpi	472.6	1.94	1.74
	C_2 96 hpi	440.94	1.92	1.5
cv. Aragonez	I_3 6 hpi	366.3	2.02	1.92
	I_4 24 hpi	256.2	1.94	1.66
	I_1 96 hpi	61.1	2.04	1.76
	C_6 0 hpi	103.7	1.765	3.89
	C_3 6 hpi	86.6	2.04	2.11
	C_2 24 hpi	50.7	1.58	0.69
	C_1 96 hpi	36.5	1.76	0.89
	I_1 6 hpi	27.15	1.54	0.67
	I_2 24 hpi	25.4	1.72	0.88
	I_5 96 hpi	37.2	1.85	0.82

Table S1.2 - Comparison of yield and purity of total extracted RNA with thirteen extraction protocols. For RNA extraction several reported protocols were tested: (1) Reid *et al.*, (2006) first steps with Spectrum™ Plant Total RNA Kit washing steps; (2) Gambino *et al.*, (2009) protocol with modifications; (3)(4) similar to Protocol 2 with the increase of extraction buffer volume and with Reid et *al.* (2006) and Gambino et *al.* (2009) extraction buffers, respectively; (5)(6) similar to Protocol 1 and 2 but with an additional step of SDS treatment of electrophorese material; (7) integral Spectrum™ Plant Total RNA Kit with Gambino et *al.* (2009) extraction buffer; (8) RNeasy® protocol with Gambino et *al.* (2009) extraction buffer; *(9)* integral Spectrum™ Plant Total RNA Kit; *(10)* Fortes et al. (2011) steps until LiCl overnight precipitation follow Spectrum™ Plant Total RNA Kit; *(11)* optimized protocol; *(12)* Protocol 11 with additional polysaccharides precipitation; *(13)* Protocol 11 with increase of leaves material. Extraction occur in non-infected and infected (PM-disease) leaves from cv. 101-14 Millardet et de Grasset (resistant species) and cv. Aragonez (susceptible species) at 0, 6, 24 and 96 hours post-infection. Abbreviations: C, control (non-infected); I, infected; hpi, hours post-infection.
[a] – number of total extraction (RNA degraded included);
[b] – number of extraction (only integrate RNA included);
[c] – Yield is given ng RNA *per* 100 mg of fresh weight;
[d] – Values expressed are mean ± SD (standard deviation) of all replicates for individual condition.

			Absorbance	
Method	**n**	**RNA yield (ng/100mg FW)[c,d]**	**260/280**	**260/230**
Protocol 1	13[a]	17.92±28.03	1.75±0.35	1.60±0.71
Protocol 2	4[a]	31.85±37.22	1.99±0.05	1.66±0.24
Protocol 3/4	4[a]	-	-	-
Protocol 5/6	4[a]	-	-	-
Protocol 7	2[a]	109.98±47.84	2±0.03	2.03±0.1
Protocol 8	2[a]	15.58±3.22	1.85±0.1	0.66±0.1

Protocol 9	3[a]	11.3±8.14	1.70±0.05	0.71±0.13
Protocol 10	3[b]	3.54±2.04	-	-
Protocol 11	63[b]	73±35.4	1.89±0.25	1.33±0.53
Protocol 12	5	161.71±81.66	1.93±0.06	1.60±0.17
Protocol 13	2	147.58±37.87	1.90±0.12	1.66±0.03

Table S1.3 - Pools of biological samples formed by mixing samples with insufficient RNA concentration. Pools include cv. Aragonez (susceptible) non-infected and infected (PM-disease) leaves at 0, 6, 24 and 96 hours post-inoculation. RNA concentration was measured after samples mixture and before re-precipitation. Abbreviations: I, initial (after samples mixture and before re-precipitation); f, final (after re-precipitation).

Sample Pools	**Samples**	**Concentration$_i$ (ng/µL)**	**Concentration$_f$ (ng/µL)**
cv. Aragonez I_{POOL1} 6 hpi	I_1 (7.6 ng/µL); I_2 (46.7 ng/µL); $I_{3.1}$ (5.9 ng/µL); $I_{3.2}$ (2.3 ng/µL);	94.7	62.7
cv. Aragonez I_{POOL2} 6 hpi	$I_{3.1}$ (68.1 ng/µL); $I_{3.1}$ (68.7 ng/µL); $I_{4.1}$ (82.4 ng/µL); $I_{4.2}$ (46.7 ng/µL);	211.7	118.1
cv. Aragonez I_{POOL3} 6 hpi	$I_{4.1}$ (82.4 ng/µL); $I_{4.2}$ (77.6 ng/µL);	152.2	90.9
cv. Aragonez I_{POOL4} 6 hpi	I1 (46.7 ng/µL); I2 (59.8 ng/µL); I3 (68.1 ng/µL); I5 (27.1 ng/µL);	66.2	
cv. Aragonez I_{POOL5} 24 hpi	$I_{1.1}$ (11.8 ng/µL); $I_{1.2}$ (45.5 ng/µL); $I_{2.1}$ 24 (14.3 ng/µL); $I_{2.1}$ (41.5 ng/µL);	136.2	87.1
cv. Aragonez I_{POOL6} 24 hpi	$I_{3.1}$ (14.3 ng/µL); $I_{3.2}$ (41.5 ng/µL);	59.1	36.9
cv. Aragonez I_{POOL7} 24 hpi	I_4 (12.1 ng/µL); $I_{5.1}$ (41.5 ng/µL); $I_{5.2}$ (12 ng/µL); $I_{5.3}$ (14.3 ng/µL); $I_{5.4}$ (41.7 ng/µL);	47.7	31.6
cv. Aragonez C_{POOL8} 96 hpi	$C_{2.1}$ (5.2 ng/µL); $C_{2.2}$ (59 ng/µL); $C_{2.3}$ (59 ng/µL); $C_{2.4}$ (21.3 ng/µL);	31.5	21
cv. Aragonez C_{POOL9} 96 hpi	$C_{4.1}$ (15.8 ng/µL); $C_{5.1}$ (24.9 ng/µL); $C_{5.2}$ (37.2 ng/µL); $C_{1.1}$ (36.5 ng/µL);	50.9	36.8
cv. Aragonez C_{POOL10} 24 hpi	$C_{1.1}$ (85.3 ng/µL); $C_{2.1}$ (50.7 ng/µL); $C_{5.1}$ (30.9 ng/µL);	246.3	184.9
cv. Aragonez C_{POOL11} 6 hpi	$C_{2.1}$ (66.4 ng/µL); $C_{3.1}$ (86.6 ng/µL); $C_{5.1}$ (29.1 ng/µL);	146.6	109.4
cv. Aragonez C_{POOL12} 0 hpi	$C_{1.1}$ (40 ng/µL); $C_{2.1}$ (6.3 ng/µL);	31.6	67.6

cv. Aragonez C_{POOL13} 0 hpi	$C_{3.1}$ (78.6 ng/µL); $C_{6.1}$ (201.1 ng/µL);	125.9	90.1
cv. Aragonez I_{POOL14} 96 hpi	$I_{2.1}$ (37.3 ng/µL); $I_{2.2}$ (41.3 ng/µL); $I_{5.1}$ (37.2 ng/µL); $I_{3.1}$ (49.9 ng/µL);	158.3	47.6

Table S1.4 - Concentration of total RNA after DNase I treatment. RNA was extracted according to Protocol 11. Extraction occur in non-infected and infected (PM-disease) leaves from cv. 101-14 Millardet et de Grasset (resistant species) and cv. Aragonez (susceptible species) at 0, 6, 24 and 96 hours post-infection. Abbreviations: C, control (non-infected); I, infected; hpi, hours post-infection.

Sample name	**Concentration$_i$ (ng/µL)**	**Concentration$_f$ (ng/µL)**
cv. 101-14 Millardet et de Grasset C_2 0 hpi	688.0	334.4
cv. 101-14 Millardet et de Grasset $C_{5.1}$ 0hpi	300.8	100.2
cv. 101-14 Millardet et de Grasset C_3 0hpi	1014.7	603.1
cv. 101-14 Millardet et de Grasset $C_{5.2}$ 0hpi	417.2	254.8
cv. 101-14 Millardet et de Grasset $C_{4.1}$ 0 hpi	717.5	305.8
cv. 101-14 Millardet et de Grasset C_5 6 hpi	647.5	295.9
cv. 101-14 Millardet et de Grasset $C_{4.2}$ 6 hpi	596.0	277.3
cv. 101-14 Millardet et de Grasset $C_{1.1}$ 6 hpi	87.2	62.4
cv. 101-14 Millardet et de Grasset C_3 6 hpi	620.4	253.3
cv. 101-14 Millardet et de Grasset $C_{1.2}$ 6 hpi	656.1	328.6
cv. 101-14 Millardet et de Grasset C_2 24 hpi	615.1	277.9
cv. 101-14 Millardet et de Grasset C_5 24 hpi	724.0	54.8
cv. 101-14 Millardet et de Grasset C_1 24 hpi	176.5	160.7
cv. 101-14 Millardet et de Grasset C_1 96 hpi	231.4	43.3
cv. 101-14 Millardet et de Grasset I_3 6 hpi	366.3	83.8
cv. 101-14 Millardet et de Grasset I_5 6 hpi	185.3	98.2
cv. 101-14 Millardet et de Grasset I_2 24 hpi	421.8	149.3
cv. 101-14 Millardet et de Grasset I_3 24 hpi	168.6	109.9
cv. 101-14 Millardet et de Grasset I_5 24 hpi	410.1	233.2
cv. 101-14 Millardet et de Grasset I_2 96 hpi	131.6	62.7
cv. 101-14 Millardet et de Grasset I_4 96 hpi	261.0	100.4
cv. 101-14 Millardet et de Grasset I_5 96 hpi	87.2	106.1
cv. 101-14 Millardet et de Grasset I_3 96 hpi	258.6	137.9
cv. Aragonez C_6 0 hpi	201.1	104.4
cv. Aragonez C_{POOL12} 0 hpi	31.6	67.6
cv. Aragonez C_{POOL13} 0 hpi	125.9	90.1
cv. Aragonez C_1 6 hpi	661.5	27.0
cv. Aragonez C_4 6 hpi	214.6	133.8
cv. Aragonez C_{POOL11} 6hpi	146.6	109.4
cv. Aragonez C_4 24 hpi	156.7	123.4
cv. Aragonez C_{POOL10} 24 hpi	246.3	184.9
cv. Aragonez C_3 96 hpi	214.0	99.3
cv. Aragonez C_{POOL8} 96 hpi	31.5	21.0
cv. Aragonez C_{POOL9} 96 hpi	50.9	36.8

cv. Aragonez C_4 96 hpi	363.6	38.0
cv. Aragonez I_{POOL1} 6 hpi	94.7	62.7
cv. Aragonez I_{POOL2} 6 hpi	211.7	118.1
cv. Aragonez I_{POOL3} 6 hpi	152.2	90.9
cv. Aragonez $I_{1.1}$ 6 hpi	80.0	19.2
cv. Aragonez I_2 6 hpi	59.8	45.9
cv. Aragonez $I_{1.2}$ 6 hpi	27.15	8.2
cv. Aragonez I_5 6 hpi	27.1	35.3
cv. Aragonez I_1 24 hpi	60.4	30.1
cv. Aragonez I_{POOL5} 24 hpi	136.2	87.1
cv. Aragonez I_{POOL6} 24 hpi	59.1	36.9
cv. Aragonez I_{POOL7} 24 hpi	47.7	31.6
cv. Aragonez I_4 24 hpi	12.1	21.7
cv. Aragonez I_5 24 hpi	19.95	4.0
cv. Aragonez $I_{3.1}$ 96 hpi	56.4	38.8
cv. Aragonez $I_{3.2}$ 96 hpi	123.8	21.7
cv. Aragonez $I_{3.3}$ 96 hpi	49.9	29.6
cv. Aragonez I_1 96 hpi	241.6	86.4
cv. Aragonez I_{POOL14} 96 hpi	158.3	47.6
cv. Aragonez I_2 96 hpi	39.3	106.1
cv. Aragonez I_5 96 hpi	37.2	19.3

Table S1.5 - List of primers used as reference genes or target genes, accession number and reference (DOI). Primers for vacuolar ATPase subunit G (*VAG)* and ubiquitin conjugating enzyme (*UBC)* genes were used as reference genes for leaves. Primers for *actin* and elongation factor 1 α (*EF1α)* genes were used as reference genes for grapes. Primers for enhanced disease susceptibility 1 (*EDS1*), phytoalexine deficient 4 (*PAD4*), pathogenesis-related protein 1 (*PR1*), IAA-amido synthetase GH3-2 (*GH3-2*), auxin-induced in root cultures protein 12 (*AIR12*), allene oxidase synthase *(AOS*), sucrose non-fermenting-1 *(*SNF1)-related protein kinase 2 (*SnRK2*) and *HVA22C* genes were used for leaves and grapes.
a – Reference (DOI) - 10.1371/journal.pone.0111399; b - Reference (DOI) - 10.1016/j.plantsci.2019.01.024; c - Reference (DOI) – (Pimentel et al., under review).

Annotation-Identity based on genomic annotation	**Accession number**	**Primer sequence (5'→ 3')**
Vacuolar ATPase subunit G (*VAG*)[a]	XM_002281110.1	Fw: TTGCCTGTGTCTCTTGTTC Rev: TCAATGCTGCCAGAAGTG
Ubiquitin conjugating enzyme (*UBC*)[a]	EE253706	Fw: CATAAGGGCTATCAGGAGGAC Rev: TGGCGGTCGGAGTTAGG
Actin (*Actin*)[b]	VIT_04s0044g00580	Fw: GGTCAACCATGTTCCCTGGTATT Rev: GGAGCAAGAGCAGTGATTTCCTT
Elongation Factor 1 α (*EF1α*)[b]	VIT_06s0004g03220	Fw: CGTCATAGTTTTCTGCCTTCTTCC Rev: TGCCACCGCCTATCAAGC
Enhanced disease susceptibility 1 (*EDS1*)[b]	VIT_17s0000g07420	Fw: GAGCTTCCGGTGTCTTCTGATG Rev: TTTCGCTTCTCCAACTCTCCTG

Phytoalexine deficient 4 (*PAD4*)[b]	VIT_07s0031g02390	Fw: GGCTAGCTGGGCAGGAGTCAA Rev: AGGTGTGGCGGTAACGGATTCA
Pathogenesis related protein 1 precursor (*PR1*)[b]	VIT_03s0088g00700	Fw: TGCCTACGCCCAGAACTATGC Rev: TGCCTGTCAATGAACCACTGC
Indole-3-acetic acid-amido synthetase 3-2 (*GH3-2*)[c]	VIT_07s0129g00660	Fw: GAGGCCATTCTTTGCGTTGACT Rev: CGACTCGGAGGACTTCTTTGTG
Auxin-responsive protein AIR12 (*AIR12*)[c]	VIT_12s0057g00420	Fw: GTGAACTCCAGTGCCCCCTATG Rev: TCTCACCGTTGCTGTTCTCTGC
Allene oxide synthase (AOS)[c]	VIT_18s0001g11630	Fw: GCCCGATATTTGCCTCTGTTTC Rev: ACTGGTGTTTCTGGGCTCTGGA
SNF1 protein kinase 2-3 AKIP OST1 (*SnRK2*)[c]	VIT_07s0197g00080	Fw: CAAGGACTTCCGAAAAACAATAC Rev: CTCCAGGGTCAGCAACAAATA
HVA22C[c]	VIT_01s0026g02190	Fw: AGCCCGCAAACTTCTCAAATC Rev: CTTCTGTCCCGTGCTCTTCAATA

Appendices II – Supplementary Figures

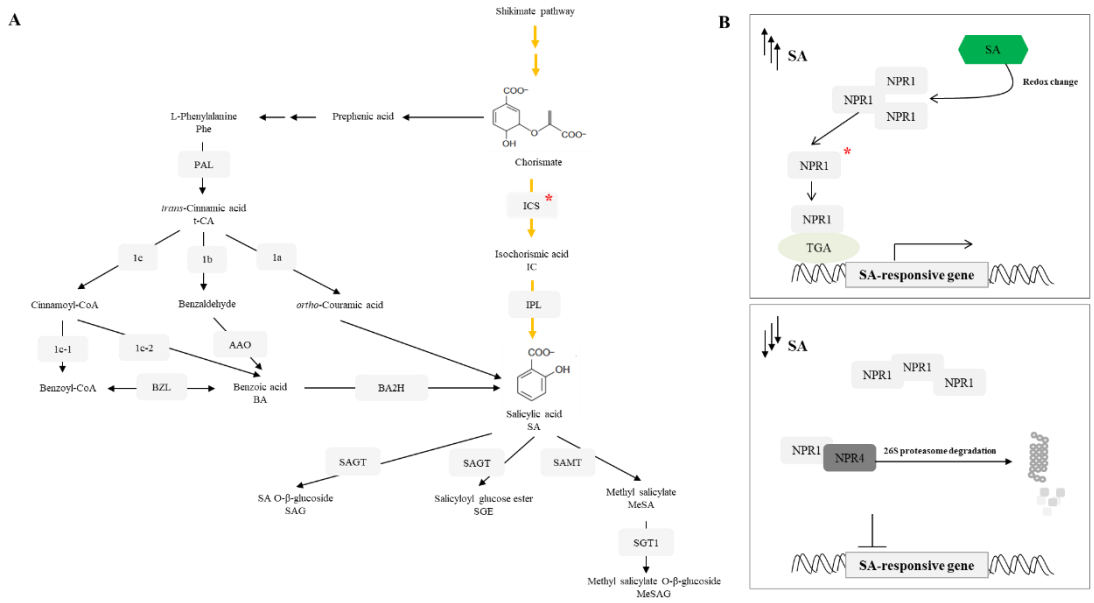

Figure S2.1 - Simplified pathway of salicylic acid (SA) adapted from Vlot et al., 2009 and Kumar et al., 29014. (A) Biosynthesis and metabolism of SA. (B) Signalling pathway of SA. In absence of SA, NPR1 is oligomerized in cytosol. By interaction with NPR4, NPR1 is subject to degradation at 26S proteasome. Upon infection, through ICS or PAL pathway, SA accumulate at cytosol. A redox change disrupts NPR1 oligomers and monomers of NPR1 migrate to the nucleus. Here by interaction with TGAs transcription factor, NPR1, as a major regulator of SA pathway, will activate SA-responsive genes like PR1. Abbreviations: ICS, isochorismate synthase; PAL, phenylalanine ammonia lyase; IPL, isochorismate pyruvate lyase; BA2H, benzoic acid-2-hydroxylase; AAO, aldehyde oxidase; BZL, benzoyl-CoA ligase; SAGT, SA glucosyltransferase; SAMT, SA methyltransferase; SGT1, salicylic acid glucosyltransferase 1; SA, salicylic acid; NPR1/4, non-expressor of pathogenesis-related genes 1 and 4.; Pathogenesis-related protein 1. (*) indicate selected genes for further analysis.

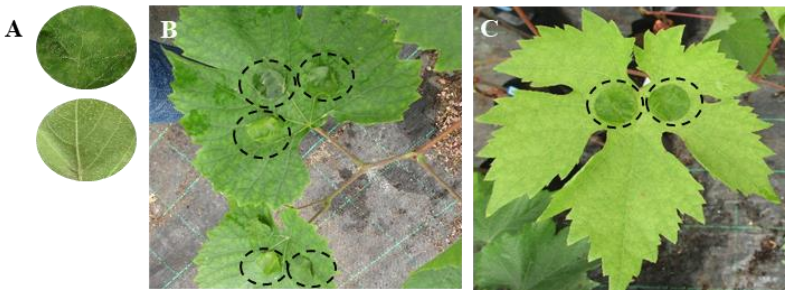

Figure S2.2 - Scheme of leaves inoculation. (A) naturally infected leaves from *Vitis vinifera* used as inoculum source of *Erysiphe necator*; (B) Leaves from cv. 101-14 Millardet et de Grasset (resistant species) and (C) from cv. Aragonez (susceptible species) were inoculate by direct contact between adaxial epidermis of the second - fifth fully expanded leaves beneath the apex and the surface of source leaves containing sporulating colonies.

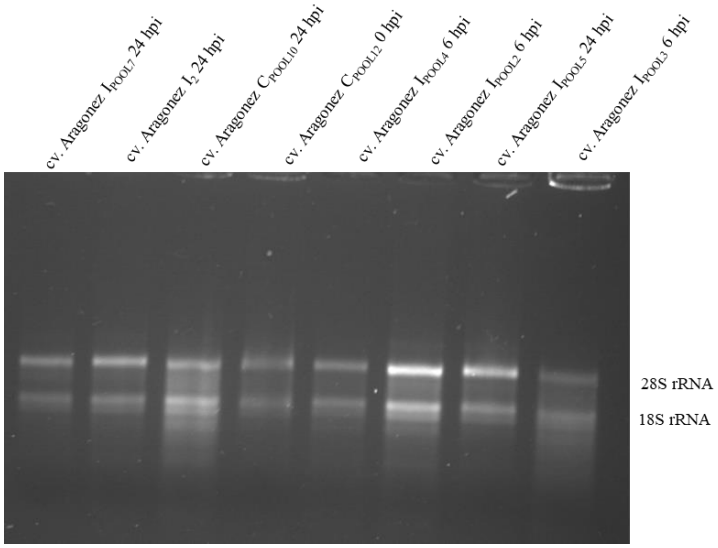

Figure S2.3 - RNA integrity with the optimized extraction protocol (Protocol 11). RNA was extracted from *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi) and visualized using 1.2% agarose gel electrophoresis stained with SYBR safe.

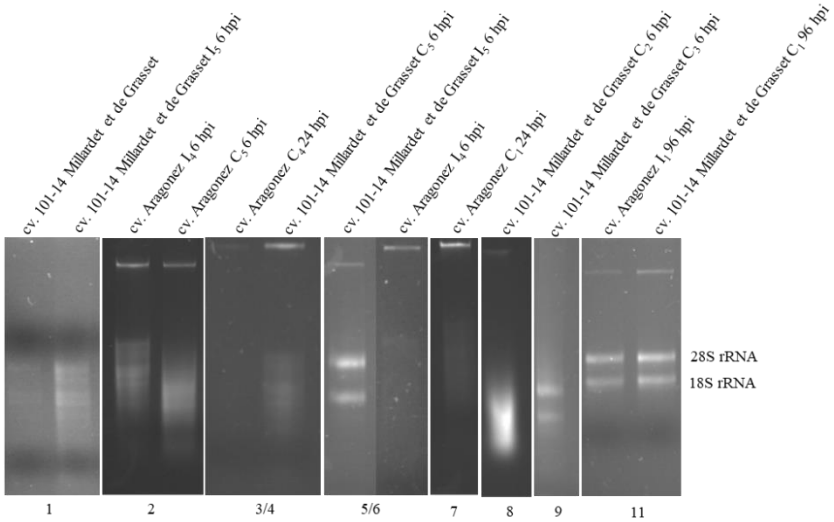

Figure S2.4 - RNA integrity obtained with different tested extraction protocols. RNA was extracted from *Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset (resistant) and *Vitis vinifera* cv. Aragonez (susceptible). Were analysed non-infected and infected (PM-disease) leaves for four time points (0, 6, 24 and 96 hpi) and visualized using 1.2% agarose gel electrophoresis stained with SYBR safe.

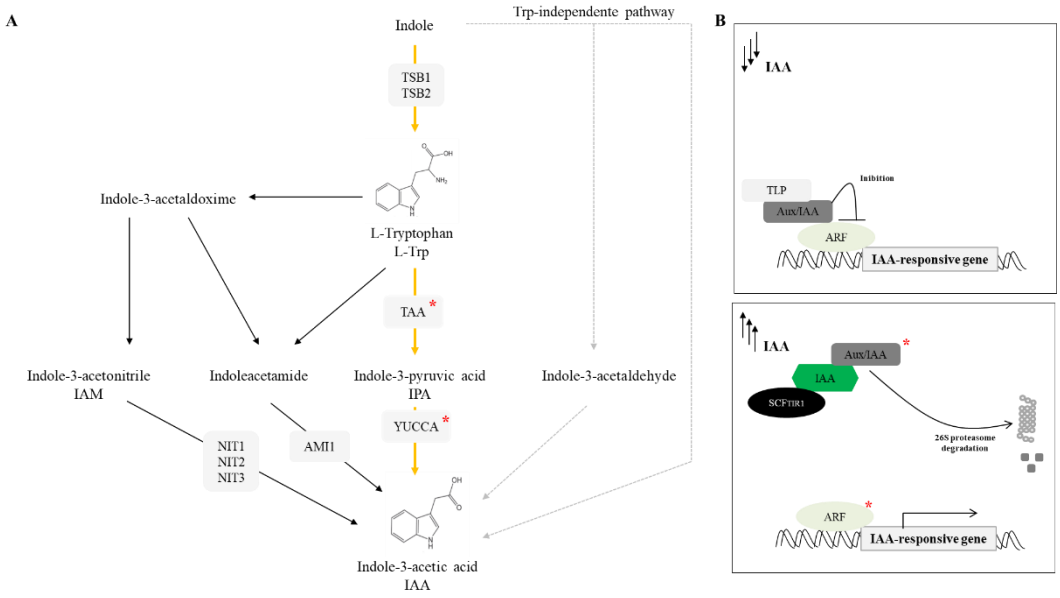

Figure S2.5 - Simplified pathway of auxins (IAA) adapted from Leyser, O., 2018 and Korasick, et al. (2013). (A) Biosynthesis of IAA. (B) Signalling pathway of IAA. In absence or in presence of low levels of IAA, Aux/IAA repressors recruit TLP co-repressor and interact with ARF transcription factors at promotor site. This will repress the expression of Auxin-responsive genes. If high levels of IAA are present, they are precepted by SCF_{TIR1} which will further interact with Aux/IAA. This will subject Aux/IAA to degradation at 26S proteasome and release ARF at promotors. Free ARF will activated or repress auxins-responsive genes. Abbreviations: TSB1/2, tryptophan synthase beta-subunit 1/2; TAA, tryptophan aminotransferase of Arabidopsis; AMI1, Indole-3-acetamide hydrolase; YUCCA, flavin monooxygenase-like; NIT1/2/3, Nitrilase 1/2/3; IAA, indole-3-acetic acid; TLP, topless; SCF, Skp-Cullin-F-box; TIR1, transport inhibitor response 1; ARF, auxin response factor; Aux/IAA, auxin/indole-3-acetic acid. (*) indicate selected genes for further analysis.

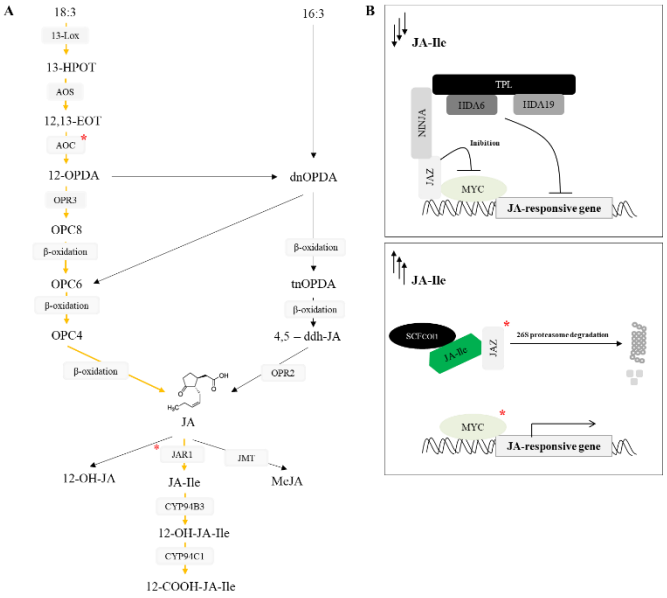

Figure S2.6 - Simplified pathway of jasmonates (JAs) adapted from Claus Wasternack & Strnad, 2018. (A) Biosynthesis and metabolism of JAs. (B) Signalling pathway of JAs. In absence or low levels of JA-Ile, JAZ repressors recruit the co-repressor NINJA, TLP, HDA6/19 and together block MYC and repress JA-responsive genes expression. Upon infection, high levels of JA-Ile are perceived by SCF_{COI}-JAZ. JAZ is than subject to degradation at 26S proteasome. MYC, being a transcriptional activator, will induce the gene expression. Abbreviations: 13-LOX, 13-lipoxygenase; AOC, allene oxide cyclase; AOS, allene oxide synthase; OPR2/3, OPDA reductase 2/3; JAR1, JA-amino acid synthetase; COI1, coronatine insensitive1; MYC2, bHLHzip transcription factor MYC2; NINJA, novel interactor of jaz; JAZ, jasmonate-ZIM domain; TLP, topless; SCF, Skp-Cullin-F-box; hda6/9, histone deacetylase 6/9. (*) indicate selected genes for further analysis.

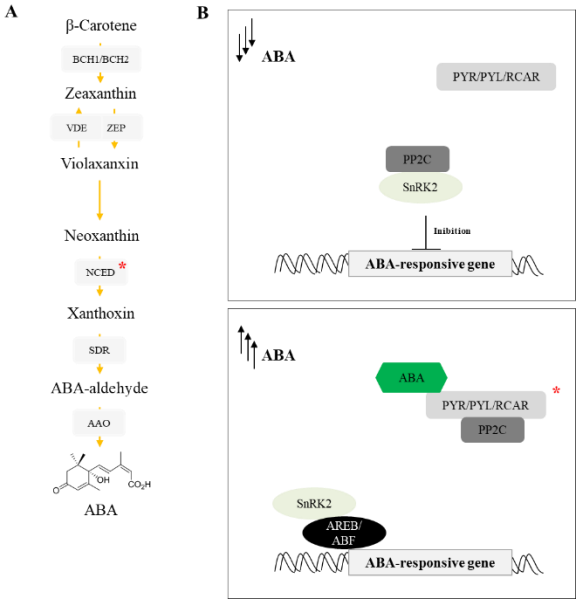

Figure S2.7 - Simplified pathway of abscisic acid (ABA) adapted from Sah et al., 2016. (A) Biosynthesis of ABA. (B) Signalling pathway of ABA. In absence of ABA, PP2C block SnRK2 activation and induction of ABA-responsive genes. In ABA presence, PYR/PYL/RCAR receptor binds to ABA and interact with PP2C. This step release SnRK2. When auto-phosphorylated, the activated SnRK2 binds to AREB/ABF transcription factor. These will interact with ABA-responsive genes promotors, regulated them. Abbreviations: BCH1/2, β-carotene hydroxylases 1/2; VDE, Violaxanthin de-epoxidase; ZEP, zeaxanthin epoxidase; NCED, 9-cis-epoxy carotenoid dioxygenase; SDR, alcohol dehydrogenase/reductase; AAO, abscisic aldehyde oxidase; ABA, abscisic acid; PYR/PYL/RCAR, pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABA receptors; PP2C, protein phosphatase 2C; SnRK2, (Sucrose non-fermenting) SNF1- related protein kinase 2; AREB, ABA responsive element binding protein; ABF, ABA-responsive element binding factor. (*) indicate selected genes for further analysis.